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AGRO PRODUCTIVIDAD

The association between MITES and the *Agave* L. snout weevil, more than phoresis

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Contenido

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Evaluation of the use of glyphosate and legumes in valencia orange (*Citrus sinensis* L. Osbeck), in the north of Veracruz: case study

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ABSTRACT
Evaluation of the use of glyphosate and legumes in valencia orange (*Citrus sinensis* L. Osbeck), in the north of Veracruz: case study. The objective of the case study was to contribute to the knowledge of herbicides (mainly glyphosate) and legumes of valencia orange producers in northern Veracruz. The study was carried out in the year 2021, with producers from three municipalities located in the north of Veracruz: Papantla, Álamo Temapache and Ixhuatlan de Madero. A total of 55 interviews were carried out. The interview that was conducted was structured around three sections: personal information of the interview respondent, information about herbicides, and information about legumes. The characteristics found in this study indicate that the majority of producers continue to use herbicides, 80% do not know the term glyphosate even though it turns out to be the main active ingredient of several of the herbicides that they apply and despite knowing that these can be very dangerous due to various reasons, they continue to use them without any protective equipment, due to how easy, fast and cheap their application is. However, regardless of everything, the producers are in the best disposition to know and cultivate leguminous plants in their plots to observe their characteristics, benefits and advantages in weed control.

Keywords: glyphosate, legumes, presidential decree, valencia orange, weeds.

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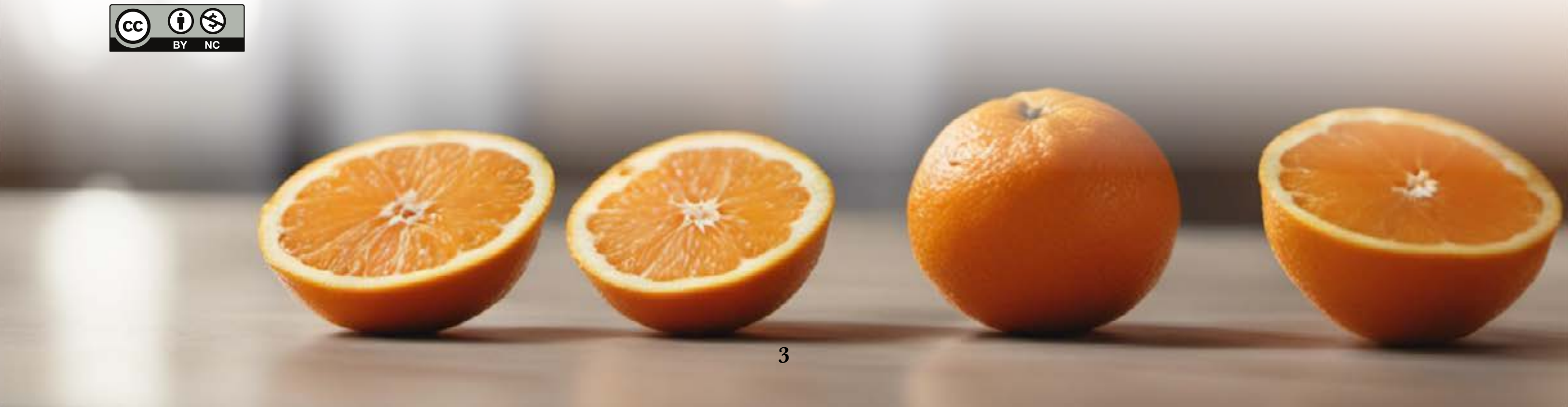
INTRODUCTION

Glyphosate is the name of the active ingredient of a total or non-selective herbicide, which means that it has the capacity to kill all types of plants, both of narrow leaf and of wide leaf; it is of foliar action, meaning that it is not absorbed by the roots, so its application is done post-emergence (Ramírez, 2021) [1] (p. 24).

It is the herbicide of greatest use in the world, its commercialization began in 1974 by the Montsanto company, with its most widely known formulation as ROUNDUP. The uses given to it are agricultural, livestock, urban, gardening and industrial, in addition to also being used as a dissecating agent, in crops such as sorghum or soy to dry the grain



and hasten the harvest, or in sugarcane as maturing agent to increase the degree of sucrose (Bejarano, 2017) [2] (p. 86) and is authorized in 32 agricultural crops, in addition to grasses, forest plantations and non-agricultural areas, with 96 different formulations (Ramírez *et al.*, 2017) [3] (pp. 59-72).

In Mexico, glyphosate is used in the cultivation of different species in the agricultural sector, among which its application in corn stands out, with 35% of the total national use, followed by citrus production with 14%, grasses 11%, and sorghum 10% (SEMARNAT, 2020) [4] (w/p). In this sense, in addition to citrus production being one of the most important activities in Mexico, it is also the second crop of highest use of glyphosate (Gómez *et al.*, 2021) [5] (pp. 5-6).

Although glyphosate and other herbicides are used to eradicate agrestal plants, herbs or weeds under the argument that they reduce the quality and the productivity of crops (between 13% and 30%) (Menalled, 2010) [6] (pp. 73-78), when competing for water, space, light and nutrient; the reality is that they are largely affecting the natural cycles: organic matter, microorganisms, minerals, fauna and human health, and can cause congenital malformations, alterations in the renal, hepatic, endocrine and gastrointestinal system, infertility and various types of cancer (non-Hodgkin's lymphoma), mutagenesis, encelopathy, autism, Parkinson's, and damage to the liver, to intestinal bacteria and to the immune system (Watts *et al.*, 2016, cited by Bejarano, 2017) [2] (p. 87).

It is important to mention that in 2015 glyphosate was classified by the World Health Organization (WHO) as probable carcinogen in humans, which is why it is considered a highly dangerous pesticide (HDP) for health and this has been confirmed in 1,108 scientific articles (Rossi, 2020) [7] (p. 8).

Facing this situation and with the aim of decreasing the impact of glyphosate, president of Mexico issued a decree that went into force on December 31, 2020, which suggests, among other issues: "[…to establish the actions to gradually substitute the use, acquisition, distribution, promotion and import of the chemical substance denominated Glyphosate for sustainable and culturally adequate alternatives, which allow maintaining the production and which are safe for human health, biocultural diversity of the country, and the environment]" (DOF, 2020) [8] (p.1).

In addition to this, and with the aim of contributing to the national crusade, as well as in function of what the third article of the presidential decree mandates[1], CONACYT agrees to carry out a project between regions; Yucatán-Campeche, Michoacán and the north of the state of Veracruz, key regions in the production of three agriculture and livestock products of utmost importance, corn, citrus and avocado, crops that together represent 53% of the total use of glyphosate in Mexico with 35, 14 and 4%, respectively. When it comes to the total national corn production, Michoacán occupies the 3rd place and Veracruz the 7th, with 7.52 and 4.71% respectively. Regarding orange production,

[1] **Article Three**. – The National Council of Science and Technology, in the scope of its competence, will coordinate, articulate, promote and support scientific research, technological development and innovations that allow sustaining and proposing to SADER and SEMARNAT alternatives to glyphosate.

which figures as one of the main crops in the north of Veracuz, this state is the 1st place and Yucatán the 7th with 51 and 3% of the total national production. Finally, regarding the avocado production, Michoacán occupies the 1st place, Yucatán the 10th and Veracruz the 11th place with 75.19, 0.42 and 0.31% of the total national production.

The project is titled: "MANAGEMENT OF PLANT COVER FOR THE CONTROL OF WEEDS IN MEXICO" (*MANEJO DE COBERTURAS VEGETALES PARA EL CONTROL DE ARVENSES EN MÉXICO*) and its main objective is to promote and disseminate the use of plant covers (legume and non-legume species) for the management of weeds that help substitute the use of herbicides in Mexico, through the systematization of successful experiences and a broad and intense effort of technology transfer and awareness, to be able to reach the goal of eliminating the use of herbicides based on glyphosate by January 2024.

In response to that project, a case study was conducted titled **Evaluation of the use of glyphosate and legumes in valencia orange (*Citrus sinensis* L. Osbeck), in the north of Veracruz**, to contribute to the knowledge that producers have with regards to glyphosate and legume plants.

The state of Veracruz was chosen because it is the main orange-producing state at the national level with 51% of total production, equivalent to 2,361,612.31 tons (SIACON, 2020) [9] (w/p).

The municipalities that were visited to carry out the interviews were found within the first 10 municipalities according to SIAP, 2020 [10] (w/p), that concentrated 96% of the total production with 2,263,526.05 tons and 95% of the total production value, equivalent to 6,075,138,545.83 pesos. Álamo Temapache was found as the main orange producer, in terms of tons and production value with 31 and 32%, Papantla was the third place with 17% and Ixhuatlán de Madero in ninth place with 2%. The last two municipalities with the same percentage compared to the total production value and the total of tons produced.

MATERIALS AND METHODS

Type of study

The study is a qualitative interpretation through 55 interviews where the degree of knowledge that producers have is analyzed, in their perception about herbicides and the use of legumes in the management of weeds in the north zone of Veracruz.

Study location

The interviews were carried out in three municipalities of Veracruz; the municipality of Álamo Temapache with coordinates 20° 55' latitude North, 97° 41' longitude West at an altitude of 40 masl; the municipality of Papantla with coordinates 20° 27' latitude North, 97° 19' longitude West at an altitude of 180 masl; and the municipality of Ixhuatlán de Madero with coordenates 20° 41' latitude North and 98° 01' of longitude West at an altitude of 260 masl (INEGI, 2022) [11] (w/p).

Structure of the interview

The interview conducted with producers is constituted by the following three sections:

1. General information. This section includes the most important data, such as: Name of the interview respondent, age, sex, number of hectares they have, as well as which crops they produce.
2. Information about knowledge of herbicides. In this section, the following questions were asked: Do you know, what are herbicides? Do you know what they are used for? Do you use herbicides? What herbicides do you use? How do you apply the herbicides? Do you use any type of protection? Do you know, what is glyphosate? Do you consider that herbicides are harmful to health?
3. Information about the knowledge of legumes. This section included the following questions: Do you know legumes? Do you grow or currently use legumes? Would you like to learn more about how legumes can help to control weeds in your plot without using herbicides? Would you be willing to test them in your plot?

RESULTS AND DISCUSSION

Next, the results obtained from the interviews applied are presented.

General information of interview respondents:

Of the producers interviewed, 80% are men, which indicates that the masculine sex is prevalent, although a significant increase of the participation of women in the field is increasingly reflected, in this case of 20%. Of the producers, 76% are 50 years or older.

Regarding the marital status, 78% of the producers are married or in civil union, and the remaining 22% are single or widows. Regarding schooling, 67% have basic education (preschool, primary or secondary), and the remaining 33% have high school or university education (bachelor's, master's or PhD).

Of the producers, 91% have less than 5 hectares, which indicate that they are mostly small-scale producers, which agrees with Gómez *et al.*, (2021) [12] (p. 114), regarding the variation of the surface in the majority group of producers of valencia orange in the north of the state of Veracruz, which is 3 to 7 hectares.

Of the producers, 69% have been citrus producers for more than 10 years, which indicates that they have experience in the trade, mainly in the production of valencia orange.

Knowledge of glyphosate among interview respondents:

All of the producers (100%) know what is the function of herbicides, although they are not familiar with the term herbicide, so producers refer to herbicides as "liquids", "burners" or "weed killers"; therefore, it is fundamental to use the common terms that producers use in order to avoid biases in the results due to mistakenly describing the information. For example, in the sensitizing campaign carried out by the Ministry of the Environment and Natural Resources (*Secretaría de Medio Ambiente y Recursos Naturales*, SEMARNAT), Glyphosate is mentioned and not the terms that producers understand.

The producers who are currently using herbicides represent the largest part, with 94% of the total producers surveyed. Among the herbicides that are most often mentioned due to their generalized or extensive use, there are: faena, la fam and glifos (glyphosate);

dragocson, gramoxone (paraquat), and fitoamina (2,4D). It is fundamental to mention that producers use herbicides alternately; thus, for example, in their first application they can use glyphosate and for the next application they use the same or one of the others, such as paraquat or 2,4D. In this sense, the herbicide that predominates the most is glyphosate, since 100% of the producers who apply herbicides use it, in contrast with the herbicides paraquat and fitoamina which are used by 75 and 45% of the producers, respectively. The season of application depends on the stage of growth of the weeds. Glyphosate, paraquat and 2,4-D are applied before sowing the crop and only glyphosate and paraquat after emergence of weeds and the crop (CEDRSSA, 2020) [13] (p. 10).

Glyphosate is a herbicide of total action, that is, it kills all types of plants; its application is post-emergence and systemic; it is transported internally from the point of contact in the plant towards some others of its parts (Ramírez, 2021) [1] (p. 11). According to the results, only 20% mentioned knowing what glyphosate is for, and they commonly refer to it as "glifos".

Similar to glyphosate, within the group 2,4D there are herbicides of systemic action that move fast which combat weeds selectively and post-emergence, although they are only used to control weeds of broad leaf.

On the other hand, paraquat is another one of the relavant herbicides due to its use in citrus crops; it is of non-selective contact, functions for all types of weeds, and only acts on the green organs of the plants, by causing an immediate necrosis, withering and total dissecting after three days post-application (Martínez, 2017) [14] (p. 10).

Of the total producers who continute to use herbicides, only 8% uses some protection equipment when they carry out the application through a sprinkler backpack and the most common are boots and goggles. This shows that aside from being a small percentage, the equipment they use is not enough, since according to the PROJECT of the Norma Oficial Mexicana PROY-NOM-003-STPS-2016, Agricultural Activities-Safety and Health Conditions at Work, the personal protection equipment (PPE) that should be used during the application of agrichemicals (in this case herbicides) are: "[impermeable gloves, long-sleeve clothes, impermeable boots, facial protection, respiratory protection equipment, and impermeable wide-brimmed hat]" (STPS, 2006) [15] (w/p).

It is important to mention and highlight the fact that 100% of the producers consider that herbicides cause harm to health, although 94% continues to use them because of the low costs that they generate by not requiring too much labor and because they are of easy and fast to handle.

Table 1 shows three of the indicators already mentioned before: surface, age, sex, with the aim of showing in function of them and the parameters established the percentage of producers that apply herbicides.

Table 1 shows that both small-scale (<5) and medium and large scale producers (>10) incur in the use of herbicides, since they are not less than 75% of the producers. When it comes to age, the percentages are also high, since 95% of the producers with an age of 50 years or more use herbicides and the percentage in those who are less than 50 years is 92%. Finally, regarding the last indicator, 100% of the women producers apply herbicides and of the masculine sex the percentage is 92%.

Table 1. Percentage of producers who use herbicides per indicator of surface, age and sex.

Indicator	Parameter	Use of herbicides (%)
Surface (hectares)	>10	100
	5-10	75
	<5	96
Age (years)	≥50	95
	<50	92
Sex	Masculine	93
	Feminine	100

Source: Prepared by the authors.

Similarly, this indicates that from all the parameters established for each indicator, there is not a single one where producers do not use herbicides, but rather on the contrary: they do use it and the percentages are rather high.

Knowledge of legumes among interview respondents:

Of the producers, 60% identify legume plants, but only those plants that are common to the region, such as bean, peanut, northern bean, year-round bean, pea and lentil. The remaining 40% mentioned not being familiar with them.

Only 4% of the producers cultivate some type of legume, but they are not aware of their use in weed control.

The legumes grown by 4% of the producers are bean, broad bean and lentil, addressing self-supply for their families.

Of the producers, 96% who do not cultivate legumes are interested in cultivating them and in performing the trial on their plots, and likewise the remaining 4% since they are already sowing bean, broad bean or lentil, and they were very interested in knowing more types of legume plants and in sowing them in their orchards.

Table 2 maintains the same indicators from Table 1 with the objective of sustaining the essence of the characterization or typification of producers, although now it is with relation

Table 2. Percentage of producers who are interested in legumes by indicator of surface, age and sex.

Indicator	Parameter	Interest in legumes (%)
Surface (hectares)	>10	100
	5-10	
	<5	
Age (years)	≥50	100
	<50	
Sex	Masculine	100
	Femenine	

Source: Prepared by the authors.

to the interest there is over legume plants. As can be seen, 100% of the producers in the different parameters established are interested in these plants.

In addition to this, 100% of the producers mentioned that they would like to begin with the most recommendable legume plants in terms of their functionality with regards to the type of soil, climate and other important factors, so that in this sense they can observe the benefits they provide. Most of the interview respondents are unaware of most of these species: [Gandúl bean (*Cajanus cajan*), Fodder soy (*Neonotonia wightii*, *Glycine* sp.), Mucuna (*Mucuna pruriens* ó *Stizolobium deeringianum*), Canavalia (*Canavalia ensiformes*), Crotalaria (*Crotalaria juncea*)] and they are not aware of the benefits and different uses that they can provide.

However, legumes play a fundamental role by virtue of their qualities and the diversity of benefits they offer at the level of the dietary system (Voisin *et al*., 2014) [16] (pp. 361-380); for human and animal consumption, as a source of plant protein and with growing importance in the improvement of human health (Tharanathan *et al*., 2003) [17] (pp. 507-518); at the level of production system; with biological fixation of atmospheric nitrogen, the mitigation of Greenhouse Effect Gases (GEGs) such as carbon dioxide (CO_2) and nitrous oxide (N_2O) (Lemke *et al*., 2007; Reckling *et al*., 2014) [18] (pp. 1719-1725) [19] (pp. 54-55); the emission of GEGs of around 5 to 7 times less per area unit compared to other crops (Jeuffroy *et al*., 2013) [20] (pp. 1787-1797); improvements in the structure and organic matter of the soil (Hernanz *et al*., 2009) [21] (pp.114-122); the mobilization of phosphorus (Shen *et al*., 2011) [22] (pp. 997-1005); and the retention and availability of water in the soil (Angus *et al*., 2015) [23] (pp. 523-552); and at the level of cultivation system as diversification crops in agroecosystems based on few principal species, breaking the pest cycles, and reducing the pressure from diseases and bad weeds (Robson *et al*., 2002) [24] (pp. 369-427).

Among the most important criteria for the adoption of legumes in agricultural systems according to the analysis in 140 distinctive agricultural systems, which involve 41 species, there is the fact that the crop must have an additional use for the producer and not only weed control or green manure; in particular for human or animal diet (for example, gandúl bean, ayocote bean, year-round bean), the installation costs should not be high (being important that the same farmer grow his own seed); and the demand for work should be low (Bunch, 2004) [25] (pp. 11-12).

Some desirable characteristics in a legume would be: easy establishment, vigorous growth, capacity of covering weeds quickly, and also of fixing nitrogen or concentrating phosphorus, being resistant to insects, diseases, resistant to drought, having multiple uses, and producing viable seed for its future cultivation (Bunch, 2004) [25] (p. 13).

CONCLUSIONS

Although the sample that was considered for the study is small, it provides a representation of the knowledge and use of herbicides and legumes that producers have in three municipalities in the north of Veracruz, characterized mainly by belonging to the first ten most important producing municipalities of valencia orange.

It should be highlighted that citrus producers from the north of Veracruz, and probably producers from the country in general, do not know the terms glyphosate and legumes, and any official decree, research, specific action, etc., that is undertaken should use the words and concepts that are comprehensible for the people with whom they will work, interview or sensitize, so that there can be an impact or comply in the best way with the actions taken.

Likewise, it is important to clarify that from the agroecological point of view, the producers that are using herbicides (from small, medium, large scale, older and younger than 50 years old, and feminine and masculine) are not really aware of the damage that these cause to health and biodiversity, since they only center their attention on the results that are obtained in the short term, but not in the long term, where it has been proven that herbicides are affecting largely and strongly the natural cycles, from microorganisms, fauna and flora to human health. This confirms that the actions of the Presidential Decree from December 31, 2020, are correct, directed towards eradicating the use of glyphosate in Mexico in a transition period that ends on January 31, 2024.

Finally, it is necessary to emphasize that regardless of the number of hectares, of the age or of whether they are men or women, all the producers are interested in knowing and using legume plants and in this sense, it is fundamental to direct strategies that allow transferring the knowledge there is regarding these plants for the management of weeds by substituting the use of herbicides.

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Income variability and agricultural policy

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ABSTRACT
Objective: To compare producer income volatility under two types of policies (support prices and direct payments).
Design/Methodology/Approach: Producer income is understood as the result of multiplying price by yield; therefore, income is the product of two random variables modeled with a lognormal distribution, accounting for the covariance. After the subtracting trend, the cyclical component is subjected to a volatile analysis under each policy studied.
Results: Income volatility is systematically higher for support price programs than for the direct payment policy.
Study Limitations/Implications: Government programs have recently taken up support prices again; therefore, income variability should be reviewed.
Findings/Conclusions: Government programs aim to increase producer income by different means. However, they overlook possible volatility implications. By taking up support price programs again, producer income may be at risk of rebounding.

Keywords: Support prices, PROCAMPO, lognormal distribution, trend, cycle.

INTRODUCTION

Since the second half of the 20th century and up to the present (2022), the government has applied, in separate but continuous periods, two alternate policies regarding the prices of selected agricultural products that affect the magnitude and stability of producer income: 1) the support price policy (PG) and 2) the direct payments to producers, better known as PROCAMPO (PC).

The first, as its name indicates, is focused on the price of agricultural output in the market; meanwhile, the second focuses on a complement to the producer income and is granted based on the area in which the selected crops have been planted (García *et al.* 2011). Each of these policies has a different effect on the producer income level. Hernández and Martínez (2009) have studied this phenomenon; however, they do not mention the problem caused by the variability or stability of the producer income. Income variability is important because it affects the welfare of resource recipients in the face of uncertainty. This phenomenon is the result of price variations in the market or the presence of factors that affect yield (pests, diseases, and other climatic or production process factors). In particular, a risk-averse decision-maker would be willing to pay a risk premium to trade an allocation under uncertain conditions for a risk-free allocation (Friedman and Savage, 1948).

Income variability has been studied from several points of view. Brambila *et al.* (2014) point out that, when prices are experiencing a downwards trend, income growth can only be achieved via intensive, but lower risk methods. Barry *et al.* (2000) studied the

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factors that can explain the movement of agricultural income (such as farm size and yield), in a cross-sectional and time series context, with divergent results for the type of adjustment employed. Mishra and Sandretto (2002) correlated the stability of producer income with off-farm income, concluding that it helps to stabilize agricultural income. Severini *et al.* (2016) found that direct producer support contributes to the reduction of agricultural income variability in Italy. Nitta *et al.* (2022) determined that a direct payment policy has helped to stabilize the income of Japanese rice farmers. Delgadillo *et al.* (2016) have separately established the trend behavior of yield the aforementioned staple crops in Mexico; this component of agricultural income can back up the support price policy. This research reviews agricultural income as the result of price times yield, when both are subject to uncertainty. Therefore, the objective is to determine which type of policy (PG or PC) produces a higher (lower) variability of agricultural income.

On the one hand, a PG policy only affects price, but has no relationship with yield; therefore, it eliminates price uncertainty, but not yield risk. On the other hand, a direct payment does not have a relationship, neither with price nor yield; consequently, price and yield uncertainty remain a problem, although it certainly has an additive and positive effect on producer income. The scale or coverage effect of the policy on producer income depends on the government resources allocated to each policy in question; therefore, policies can have a greater or lower effect on income level, depending on each situation. The specific objective is to research the effect of each policy on the variability of agricultural income, taking its variance as a measure. The analysis considers the following approach: producers are risk-averse and, therefore, greater variability harms their welfare. The hypothesis is whether income variability is or is not the same in each policy arrangement. This research is justified given the recent return to a support price policy in Mexico; therefore, it would be possible to infer the effects of the policy on income variability (Flores *et al.*, 2022).

MATERIALS AND METHODS

In order to have more information (*i.e.*, replications), the price and yield for wheat and corn in each state were determined, using the average rural price reported by SIAP (2021). Wheat and corn were chosen because both products were subjected to the two policies under study. Their price was likewise used to obtain information on yield and quantity produced, enabling the estimation of the value of the total production and therefore the discrimination of those states to be included in the study. The average rural price was used to quantify the variability. This price is lower under a support price policy. However, in terms of variability, the wheat and corn support price used for the five states under study has approximately a 99% correlation with the said price and, therefore, no information is lost in this regard. The five producing states that contribute most to the value of national production were used in each case. Consequently, five replicates were chosen for both corn and wheat, which generates robust results. The period under study (1980-2018) was divided as follows: PG period (1980-1994) and PC period (1995-2018). Later years were not taken into consideration, because support prices were reinstated. According to the crop produced, the states were divided into two groups: 1) Sinaloa, Jalisco, Estado de México, Michoacán, and Chiapas (corn); and 2) Sonora, Baja California Norte, Guanajuato, Sinaloa, and Michoacán (wheat). Both groups were chosen under the criterion of greatest share of the value of the national production.

Using variance as a measure of variability, the objective is to contrast the following set of hypotheses:

$$Ho = \sigma^2_{PG} = \sigma^2_{PC} \quad vs \quad Ha : \sigma^2_{PG} \neq \sigma^2_{PC}$$

Specifically, this research seeks to determine whether the variance of income under GP is the same as the variance for PC (direct payments). On the one hand, it is well known that variance measurement is sensitive to scale; therefore, the scale effect (trend) is separated beforehand and the variability about that trend is used. In order to measure income, the average rural price (P) is multiplied by the yield per hectare (R), in order to determine the income per hectare. However, this is a product and, provided that income and yield are not independent, the covariance between both variables must be considered to calculate income variance. The distribution of the product of random variables can be difficult to obtain; therefore, a logarithmic transformation is employed to make the income a sum of variables in the logarithm, as suggested by Roy in the 1950s (Heckman and Sattinger, 2015). On the other hand, given their trend, the price and yield data as reported in the official information cannot be used in the analysis. The trend affects the scale of price or yield and therefore the measure of its variability (*i.e.*, variance). To address this problem, the Hodrick-Prescott filter for annualized series —*i.e.*, the frequency of the data in use. This filter decomposes a series in such a way that its value at time t is expressed as the sum of a trend component, a cyclical component, and an irregular component: $Y_t = YT_t + YC_t + \varepsilon_t$ (Cedillo and Martinez, 2019). The analysis interest is the YC_t component, which is trend-free and summarizes the variability of the series around the YT_t trend component. Therefore, the procedure consisted of estimating the cyclical component of both price and yield, based on their logarithmic transformations. With these estimates and by means of the sum, the logarithmically detrended income is determined. This income was fitted to a lognormal distribution. A random variable Z is said to have a lognormal distribution or Galton distribution, if its density function is:

$$f(Z) = \frac{1}{Z\sqrt{2\pi\sigma^2}} Exp\left[-\frac{(\ln(Z)-\mu)^2}{2\sigma^2}\right]; \quad Z \geq 0$$

If the random variable Z has lognormal distribution, then the $Y = \ln(Z)$ random variable has a $Y \sim N(\mu, \sigma^2)$ normal distribution The lognormal distribution is important because it can be fitted to variables with a multiplicative origin, such as income (Lubrano and Ndoye, 2016). Therefore, once the lognormal distribution of income was fitted, the variance homogeneity was tested between the PG period and the PC period.

RESULTS AND DISCUSSION

For reasons of space, the graphical results for the trend and cyclical components are presented for only one state. Figure 1 and 2 show the trend component and the cyclical component of the average rural price logarithm and the corn yield logarithm, respectively, in the state of Sinaloa. Greater volatility can be detected around the trend in both price and yield in early periods, which correspond to the time during which the support price policy was applied.

The sum of the cyclical components in logarithms was used to obtain a measure the of the income per hectare without trend. Since data from five states per crop was used, the same treatment was applied ten times for prices and yields. The Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling tests were used to confirm whether the logarithm of income per hectare fitted or not the normal distribution (Table 1). The laxest fit was recorded in the Estado de México for corn, while the best fit occurred for wheat in Baja California Norte.

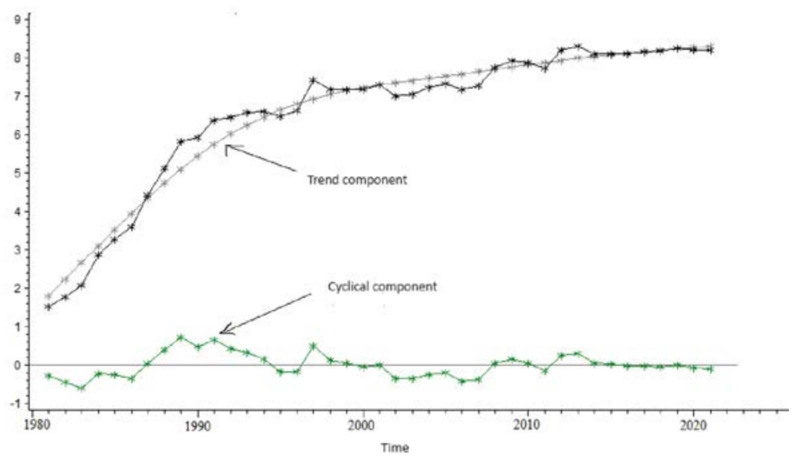

Figure 1. Cyclical component and trend of the average rural price of Sinaloa corn (logarithms).

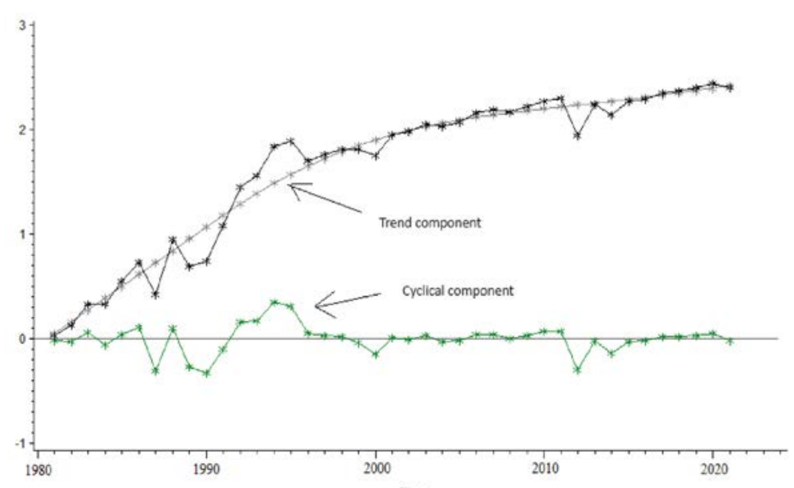

Figure 2. Seasonal and cyclical components for the yield of Sinaloa corn (logarithms).

Table 1. Fit of the cyclical component of the logarithm of income to the normal distribution.

State-Crop / Test	**Test statistic**		**Lowest fit**	
Edo. Méx-Corn			P. value	
Shapiro-Wilk	W	0.945856	Pr<W	0.0501
Kolmogorov-Smirnov	D	0.108594	Pr>D	>0.1500
Cramer-von Mises	W-Sq	0.098738	Pr>W-Sq	0.1156
Anderson-Darling	A-Sq	0.697095	Pr>A-Sq	0.0670
Baja Calif. N-Wheat			Highest fit	
Shapiro-Wilk	W	0.98679	Pr<W	0.9081
Kolmogorov-Smirnov	D	0.06155	Pr>D	>0.1500
Cramer-von Mises	W-Sq	0.02784	Pr>W-Sq	>0.2500
Anderson-Darling	A-Sq	0.19212	Pr>A-Sq	>0.2500

Table 1 shows that, overall, the fit to the normal distribution of the logarithm of income per hectare (of cyclical components) is not rejected. With this information, the homogeneity of variance is checked for each period, using a variance homogeneity test for normally distributed random variables (Table 2).

In conclusion, the income variability is systematically higher and statistically significant for the support price policy (Table 2). Implicitly, a risk-averse producer would prefer a more stable policy in terms of income volatility. Although it was obtained with a different methodology, this result matches the findings of Severini *et al.* (2016) and Nitta *et al.* (2022).

Using wheat in Sonora as example, by recovering the variance of the cyclical income in its original units (and therefore the standard errors), the change in standard deviation is 0.493302913 for the support price period. In comparison, the change during the

Table 2. Income variability per hectare.

Corn cultivation					
	Sinaloa	**Jalisco**	**Estado de México**	**Michoacán**	**Chiapas**
Variance PG	0.182734558	0.206162311	0.181956029	0.15515793	0.12452835
Variance PC	0.047700009	0.031134217	0.062842767	0.033933596	0.04078124
F statistic	3.830912472	6.621727891	2.895417193	4.572398746	3.05356942
P value	0.002436299	4.65892E-05	0.012569486	0.000751056	0.00940477
Wheat cultivation					
	Sonora	**Baja California norte**	**Guanajuato**	**Sinaloa**	**Michoacán**
Variance PG	0.171150811	0.202103682	0.184970044	0.195872507	0.20139651
Variance PC	0.071175447	0.063224239	0.079733283	0.082057536	0.05813431
F statistic	2.404632755	3.196617076	2.319859869	2.387014242	3.46433136
P value	0.031897241	0.007266644	0.037625700	0.03300812	0.00453571

PG: Support price; PC: PROCAMPO payments.

direct payments period was 0.274600885. These changes result in a >55% variability in the support price period. Nevertheless, this does not imply that one of the two policies generated higher (lower) income, because the income scale effect has been subtracted. This analysis implies that the support price policy was characterized by higher income volatility. This volatility is a key factor, since a producer (as a credit subject) can be affected by a risk premium —*i.e.*, the credit they can have access to will be more expensive or those that seek agricultural insurance will also be charged a risk premium, resulting in a higher insurance cost.

CONCLUSIONS

One of the objectives of agricultural policy is to enhance producer income, through the implementation of support price policy and direct payment policy. However, its effect on income variability has been ignored. With price and yield data, this research concludes that, based on previous experience, a support price policy results in higher income volatility, a phenomenon which may discourage risk-averse producers. Nevertheless, now that this type of policies has been taken up again, this information will be useful.

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Colegio de Postgraduados

Preliminary study on the reproductive phenology of *Eucalyptus urophylla* in Huimanguillo, Tabasco (Mexico)

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ABSTRACT

Objective: to describe the reproductive phenology of *Eucalyptus urophylla* in Huimanguillo, Tabasco.

Design/Methodology/Approach: this is a preliminary, descriptive, and non-experimental study of the different stages of the reproductive phenology cycle of *E. urophylla* conducted in a 16-year-old provenance-progeny trial with 63 half-sib families. The study was done during 2019, 2020 and 2021.

Results: the reproductive cycle of *E. urophylla* lasts approximately one year. There was a lag of about a month and a half for the beginning of flowering among genotypes that flowered during the three years of evaluation.

Study Limitations/Implications: the results are limited to a single site condition; so, it is recommended to make evaluations in other locations. This is, to observe plant behavior in different site conditions to evaluate the potential of the species development in southeastern Mexico.

Findings/Conclusions: the beginning of the reproductive cycle of *E. urophylla* coincided with that of other species of the genus from tropical and subtropical climate. However, the duration differed compared to other genotypes of the same species growing in other parts of the world under similar environmental conditions. This study allowed to know relevant information of the species in Huimanguillo, Tabasco, which will contribute to improve the process of domestication of this species in the southeast of Mexico.

Keywords: eucalyptus, flowering, controlled pollination.

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INTRODUCTION

Eucalyptus is one of the most planted genera with around 30 million hectares worldwide (Payn *et al*., 2015). The genus comprises about 700 species, however, only nine of them belonging to the *Symphyomyrtus* subgenus concentrate around 90% of all eucalyptus plantations in the world (Harwood, 2011). *Eucalyptus urophylla* is part of this select group and has been planted in various tropical and subtropical regions around the world.

In Mexico, *E. urophylla* plantations have great potential for the development of the forestry industry due to their rapid growth and the quality of their wood for various purposes. Currently, most of the production of this species is used for the elaboration of medium density fiberboard (MDF). Previous research in our country has made progress in the genetic improvement of *E. urophylla*. Studies have been carried out on wood density (Ortega-Ramírez *et al.*, 2020), pollen management (Ortega-Ramírez *et al.*, 2021) and estimation of genetic parameters to model growth (Sánchez-Vargas *et al.*, 2004; Ignacio-Sánchez *et al.*, 2005).

However, aspects of the sexual reproductive cycle are still unknown in order to carry out controlled crosses, which is an activity necessary for the continuity of genetic improvement programs. With a better understanding of the stages of the reproductive cycle, it will be possible to program more efficient crosses, which increase the quantity, and genetic and physiological quality of the seeds (Mora and Ferreira, 1978).

For this reason, the objective of this study was to describe the phenological stages of the sexual reproductive cycle of *E. urophylla* in populations of Huimanguillo, Tabasco.

MATERIALS AND METHODS

For the study, 114 (16-year-old) trees were observed for three consecutive years; selected from 63 families in a provenance-progeny trial of the company "Forestaciones Operativas de México SA de CV" (FOMEX) in Huimanguillo, Tabasco.

Trees were evaluated for 10 years and selected due to proven records of good growth and cylindrical stem. These trees will be part of a second stage of improvement, so it was essential to obtain seeds and pollen to assess their genetic superiority. In addition, scions were obtained to graft them in a controlled pollination orchard of the company. As part of the breeding process, these trees will be crossed to obtain progenies of high genetic quality.

During the first stages of flower development, observations were made to the trees weekly or once every 15 days. Since the appearance of the flower bud until the anthesis stage, monitoring was done at ground level with binoculars. Climbing was made on some chosen trees to obtain buds and flowers for the photographic record. In those cases, climbing started once the flower opened to collect pollen and seeds.

After the period of natural pollination, record collections were prolonged, in order to monitor the development of the fruit. To describe the different stages, the approach of Loewe *et al.* (1996) for *Eucalyptus* species. In addition, virtual herbarium collections worldwide that had records of flowers and fruits of *E. urophylla* were reviewed to compare with the phenology observed at the study site.

Despite the fact that the collections are quite extensive, few herbaria have precise information on the phenology of *E. urophylla*. The virtual collections of the Global Biodiversity Information Facility —GBIF (GBIF, 2022); of the herbaria Missouri Botanical Garden (MO, 2022), The New York Botanical Garden (NYBG, 2022), Museum National d'Histoire Naturelle (MNHP, 2022), REFLORA (REFLORA, 2020) and University of South Florida Herbarium (USF, 2022) supplied information on the presence of flowers and fruits. As well as the date of collection to classify the possible phenology state in which the tree was found at the time of collection.

During the years of evaluation, records of precipitation, relative humidity and solar radiation were kept with a portable weather station of Pessl Instrument Ges® located approximately 30 km from the selected trees to see if those variables could explain the reproductive phenology of *E. urophylla* during the evaluation years.

RESULTS AND DISCUSSION

The inflorescence of *Eucalyptus urophylla* is axillary, it mainly developed in odd number umbels of three to seven flower buds; the most abundant were groups of five to seven. Groups of buttons were also found in pairs of four or six, though these are rarer. Flowers do not have typical petals, but petals merge to form the operculum (Loewe *et al.*, 1996) characteristic of the species of the *Symphyomyrtus* subgenus (Junior and Garcia, 2021). The operculum protects the male (stamens) and female (stigma) reproductive system.

Flowering began in some genotypes in May, and it extended throughout June. This floral asynchrony of *E. urophylla* has already been reported in other species of the genus such as *Eucalyptus dunnii* (Sousa and Higa, 1991) and *Eucalyptus grandis* (Aguiar and Kageyama, 1987; Chaix *et al.*, 2007). According to Chaix *et al.* (2007) this characteristic may be linked to the origin of the parents. Species with a wide natural distribution have been subject to different site-specific evolutionary processes, which differentiate the adaptation processes of the species.

Spencer *et al.* (2020) found that the provenance effect also modified the number of flowers per tree in *Eucalyptus loxophleba*. Floral asynchrony plays a role for and against in eucalyptus improvement programs. In open-pollination orchards, a much extended floral asynchrony can be a problem, since it would reduce panmixia. Since mating would take place just between the few genotypes that were blooming together, so decreasing the genetic variability of the progenies.

In orchards with controlled pollination, foliar asynchrony can be managed differently. The pollen of those genotypes that bloom early is stored to apply it to the genotypes that bloom later. In a similar way, it is recommended to store the pollen of the late-blooming genotypes for use it in the following season with the genotypes that flower first (Molina *et al.*, 2014); thereby ensuring an increase in the genetic variation of the progenies in breeding programs.

In accordance with the approach of Loewe *et al.* (1996), nine stages of reproductive phenology were observed in eucalyptus at Huimanguillo, Tabasco (Table 1). The beginning of flowering in the first week was visible by the emergence of the flower bud covered by the bract (Figure 1a). Starting at the third week (Figure 1b) the buds lost the outer operculum, they remained as such, until their complete development (Figure 1e).

During the three years of the study, the beginning of flowering coincided with the end of the dry season (May and June) and the longest days of the year in the Northern hemisphere (Figure 2).

The first flowers matured at the end of July and the ripening period extended by a month up to September, what gave an average of 90 days from the start of flowering until the flower is fully mature. The flower of *E. urophylla* is hermaphroditic with a staminophore that supports numerous white stamens and the stigma. On top of the stamens are the anthers

Table 1. Reproductive phenology stages of *Eucalyptus urophylla* in Huimanguillo, Tabasco.

Phenophase	Week	Description	J	F	M	A	M	J	J	A	S	O	N	D
0	0	The floral bud emerges in the leaf axils of the plant, only the bract that protects the flower is visible (Figure 1a)					X	X						
1	1	The green bract darkens, opens, dries up and falls, the flower bud emerges.					X	X						
2	3	The flower bud grows, the outer operculum detaches (Figure 1b)					X	X						
3	4-12	Full development of the flower bud					X	X	X	X				
4	12	The operculum changes to a yellow color and detaches, leaving the stamens and stigma visible (Figure 1f).							X	X				
5	13-14	The flower is in anthesis, the anthers release pollen, four to six days after the flower opens, the stigma slightly thickens and releases a crystalline nectar with a sweet scent							X	X	X			
6	14-15	The stamens change to dark brown color and detach, the stigma loses turgidity and undergoes gradual color changes, from green to brown. When it is completely brown, it detaches from the flower (Figure 1g).									X	X		
7	16-50	Fruit growth, the green color is characteristic of this phenophase (Figure 1h).	X	X	X	X	X			X	X	X	X	X
8	45-55	The ripe fruit releases the seed, the capsule turns dark brown and acquires a woody appearance and texture						X	X					
9	>55	The empty capsules fall, some remain on the tree for a longer time						X	X	X	X	X		

that store pollen, the anthers are pale yellow (Figure 1F). The flowers are protandrous, this means that the male sexual organs mature before the female ones. This behavior has been observed in other *Eucalyptus* species (Sousa and Higa, 1991; Rojas, 2014).

According to Mora and Ferreira (1978) this characteristic is a strategy to avoid self-fertilization and encourage panmixia in natural populations. Spencer *et al.* (2020) found that the highest sum of hot temperature in one year advanced the flowering in *E. loxophleba*, which shows that flowering, in addition to genetic control, is also affected by environmental conditions and their interaction. Mora and Ferreira (1978) studied the reproductive phenology of *E. urophylla* in Brazil, those authors found different results from those observed in Huimanguillo, especially in fruit development time.

In the study by Mora and Ferreira (1978) the flowering season began in the months of November and December; the fruit matured in May, six months after the start of the reproductive cycle. Such a mentioned period was shorter than that observed in Huimanguillo; however, the beginning of flowering coincided with the end of spring in

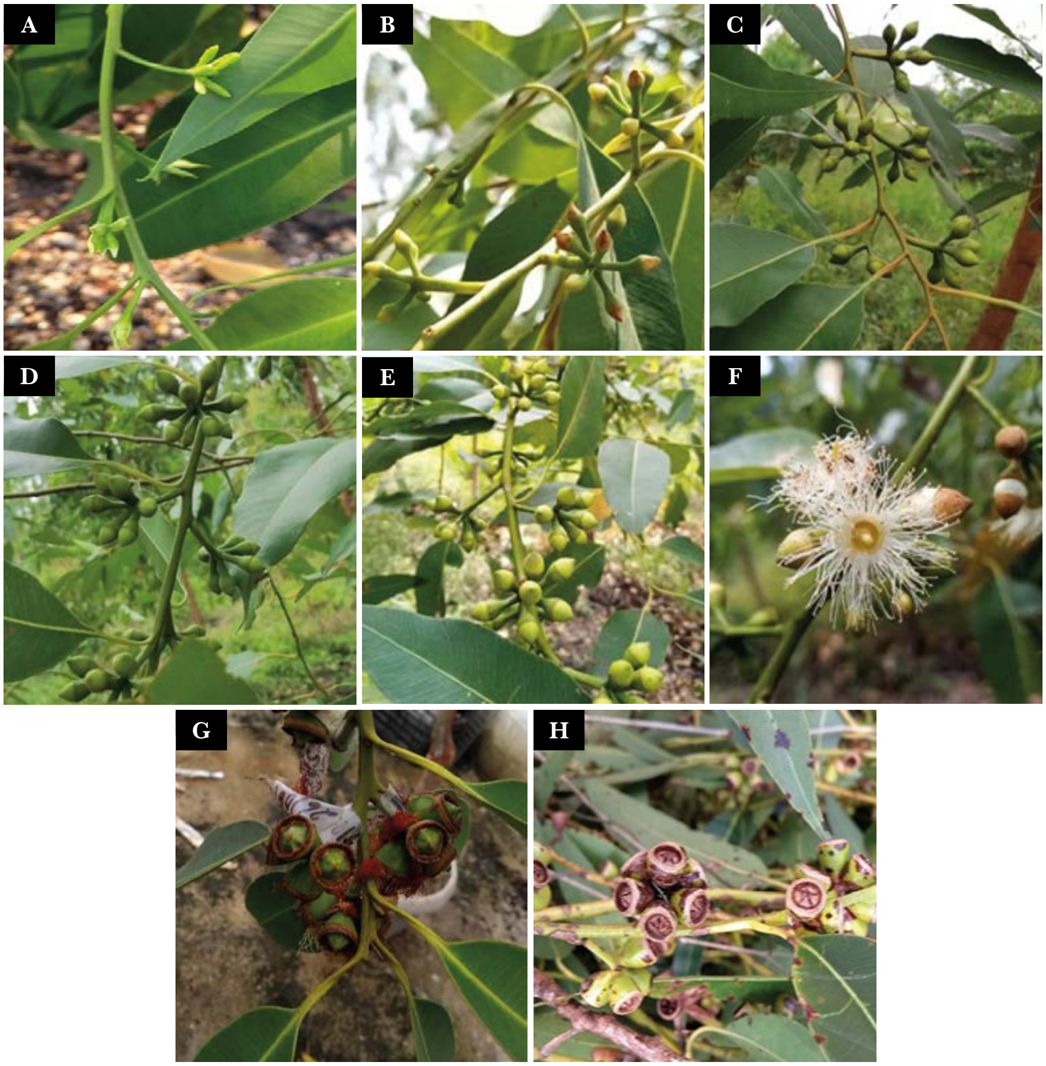

Figure 1. Reproductive phenology stages of *Eucalyptus urophylla* in Huimanguillo, Tabasco (Mexico). A: week 1, B: week 3, C: week 7, D: week 10, E: week 12, F: week 13, G: week 15, and H: week 50.

both Mexico (May-June) and Brazil (November-December). Flowering in both places coincided with the beginning of the rainy season (Figure 2); also did with the longest days and those that receive the greatest amount of light in the year (Figure 2). These are the main environmental factors that promote flowering (Molina *et al*., 2014).

The release of pollen occurred between weeks 14 and 15, in the months of August and September (Table 1). Between four and six days later the stigma became receptive. A typical feature to distinguish receptivity is the slight thickening and the release of crystalline nectar at the tip of the stigma. Between week 16 and 17, once the flower has been fertilized, the stamens change from white to a reddish color, the stigma loses turgidity and begins to change from green to dark brown to later detach (Figure 1G).

The fruit matured between weeks 45 and 50, approximately, this stage is easy to identify because the fruit turns brown, with a woody structure (Figure 1H). The reproductive cycle of *E. urophylla* in Huimanguillo was shorter than that observed in other species such as

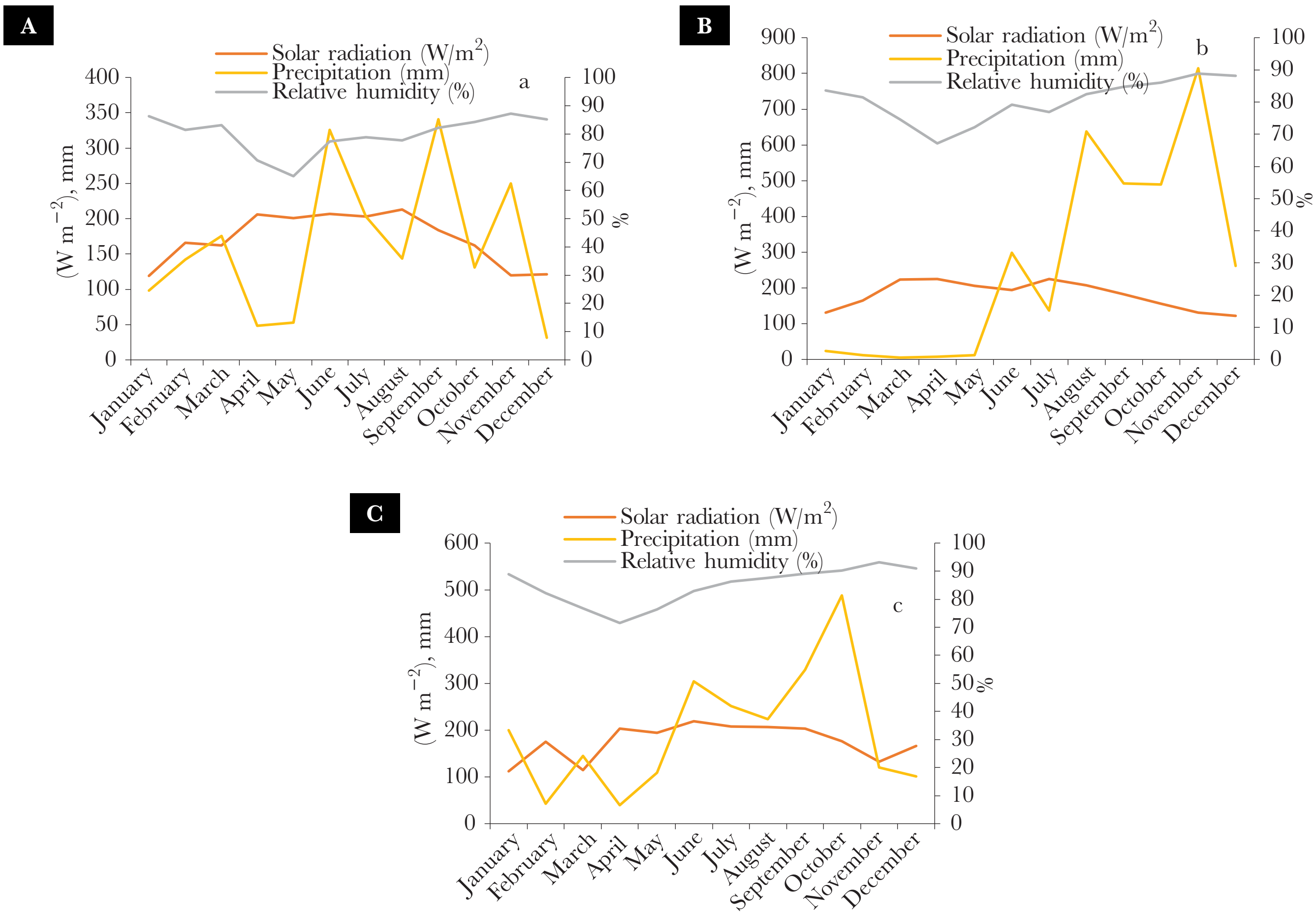

Figure 2. Environmental variables in the study site in A: 2019, B: 2020 and C: 2021.

Table 2. Collections of *Eucalyptus urophylla* reviewed virtually in herbaria located worldwide, with data of their reproductive phenology stage.

Herbarium	Collection site	Collection date	Phenophase
REFLORA	Itatinga, Sao Paulo, Brazil	28/02/2007	6
REFLORA	Itatinga, Sao Paulo, Brazil	28/02/2009	5
REFLORA	Rio Grande do Sul, Brazil	04/03/2010	3
REFLORA	Piracicaba, Sao Paulo, Brazil	03/06/2010	8, 5, 4, 6
The New York Botanical Garden	Central Mountain Range, Dominican Republic	04/07/1986	9
Herbarium of the Paris Museum	Timor Island, Indonesia	16/06/1968	9
Herbarium of the Paris Museum	Kourou, French Guiana	7/03/1987	3
Herbarium of the Paris Museum	Flores Island, Indonesia	20/10/1936	3
Herbarium of the University of South Florida	Paramaribo, Suriname	24/03/1968	3
Herbarium of the University of South Florida	Lake Yojoa, Honduras	21/04/1985	8
Herbarium of the University of South Florida	Miami, Florida, USA	22/02/1945	8

Eucalyptus dunnii, which has a reproductive period of almost two years from the moment the flower bud emerges until the seed is released. It was much shorter even than that observed in *Eucalyptus delegatensis* or *Eucalyptus fastigata*, which have a reproductive cycle of up to three years (Sousa and Higa, 1991). Table 2 shows different collections of *E. urophylla* in the herbaria reviewed virtually. Based on the photographs obtained, those were assigned a tentative reproductive phenology stage for comparison with the phenology observed in Huimanguillo, Tabasco.

The preliminary results observed in this study are different from those observed in the collections of the herbaria reviewed. The season of initiation and maturation of the flowers found in the herborized collections extended from January to July, with the release of seeds and fruit drop in the months of November and December. Facts that coincide very little with those observed in Huimanguillo.

CONCLUSIONS

Although the study is preliminary, it allows us to know important information regarding the reproductive phenology of *Eucalyptus urophylla* in Huimanguillo, Tabasco. This is an important aspect for the conservation and reproduction of the species given its economic importance in the forestry industry in Mexico.

The phenology did not change strongly during the three years of evaluation. Facing an environment of climate change these stages of reproductive phenology might be altered within longer evaluation periods. Therefore, it is recommended to continue implementing phenological studies in order to specify the information regarding *E. urophylla* for conservation and management in Southeastern Mexico.

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Colegio de Postgraduados

Dynamics and structure of research in swine health in Mexico: A methodological approach

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ABSTRACT
Objective: To characterize the socio-structural dynamics in the field of swine health research in Mexico.
Design/methodology/approach: The data used were obtained from proceedings of IPVS international conferences from 2010-2018. The study unit was the result of co-authorships. The Social Networks Analysis (SNA) was used to understand the working dynamics of the research groups through the UCINET 6 software for Windows.
Results: In the area of swine health and veterinary science, 63% of the research has depended on higher education institutions and research centers and the rest on the private sector. The most active universities with research are Universidad Nacional Autónoma de México (34%), Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (9%), Universidad Autónoma del Estado de México, and Universidad Autónoma de Yucatán. The main research areas in swine diseases were PRRS, Circovirus, Influenza, Porcine Epidemic Diarrhea, among others.
Limitations on study/implications: This study was done considering only scientific information about swine health contained in proceedings of IPVS Conferences from 2010-2018. To visualize these working networks allows detecting spaces for possibility in terms of creation of research policies.
Findings/conclusions: The research about swine health in Mexico is concentrated in five working groups, which is why understanding these key stakeholders will allow greater dissemination of the information.

Keywords: Social networks analysis, universities, laboratories.

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INTRODUCTION

Pork meat production in Mexico represented a volume of 1,652,362 TM in 2020, 41% more than in 2010 and 60% more than in 2000, with a value of $2.20, 2.84 and 3.50 trillion dollars and an inventory of 15.39, 15.43 and 18.79 million heads, respectively (SIACON-NG, 2020).

To produce and feed this number of animals, large amounts of natural resources, inputs and farming lands are required, involving diverse processes and procedures for their transformation into food for human beings (Samán *et al.*, 2020).

One of the most important characteristics in the Mexican swine farming system has been its tendency towards the concentration of animals in few farms (Callejas-Juárez *et al.*, 2020) partly due to the intent to increase productivity and as a response to an economic concentration model with huge pressures for agrifood systems (Ploeg, 2010; Delgado, 2010).

This structural weight of swine farming in Mexico, in terms of volume of kg, heads produced and its economic value, means broad technological and scientific subsystems implicated in trying to solve the contradictions that are inherent to the sector's growth, primarily the contradictions of health in such massive herds, which are susceptible to epidemics and zoonosis, something that reflects in the concerns of research systems in the sector.

In relation to this, the agroindustrial systems of swine farming in Mexico, as well as the homogenization they have developed in their levels of modernization and biosafety, and local and international transport systems for loads of swine products and byproducts resulting from globalization processes, have presented an issue of urgent analysis: the risk factors over the increasingly common presence of pathogens and diseases that circulate in such porcine systems (Pradal-Roa *et al.*, 2010; Vial-Rigo *et al.*, 2018). Some of the most prominent diseases in the production system are: Porcine Reproductive and Respiratory Syndrome (PRRS); Circovirus-PCV2; Swine flu; Porcine Rubulavirus; Parvovirosis and Porcine Epidemic Diarrhea.

This allows us to consider wide gaps in research and social intervention about the health-market pairing, primarily due to some of these zoonotic and epizoonotic scenarios. In face of this, the presence of broad social structures made up by people and legal stakeholders that have built a communication ecosystem that underpins, one way or another, the technological process of animal production and health systems in Mexico in the swine sector; although, at the same time, they generate structural omissions in these animal research and production systems. This makes it possible to attempt to categorize the organization patterns that predominate in the scientific-technological and swine health sector, as well as the social dynamics in the regional and national structures responsible for it.

In this order of ideas, swine farming faces challenges specific to its area such as safety, low productivity, nutrition, etc., and they are inclined towards a structural framework of different specialty and scientific areas that must be addressed by different experts, private, government and academic institutions, which safeguard the functioning of this productive aggregate (Núñez-Espinoza *et al.*, 2022). Therefore, the objective of this study was to characterize the socio-structural dynamics in the research field of the swine health sector in Mexico. Although the dimensions of this task could be immeasurable for the limits of this study, a condensed and partial exercise on such structures is proposed, in order to achieve the aforementioned.

MATERIALS AND METHODS

The data used that allowed this analysis were obtained directly from the proceedings of international conferences of the International Pig Veterinary Society (IPVS) from the year 2010 to 2018 (IPVS 2010, 2012, 2014, 2016 and 2018).

The social unit used for this study came from two elements: a) social dyads and b) ordered pairs contained in co-authorships of conference presentations, which allowed defining and typifying the relationships of collaboration in the research subsystem involved in this analysis. According to prior analyses (Nuñez-Espinoza *et al.*, 2022), this set of scientific collaborations makes it possible to gain access to a measurable and topological structural expression allowing to distinguish different qualities: exploring possible access to research funds, opportunities of scientific intellectual capital management, and access to subsystems of trust between research teams.

To access these agglomeration patterns and the social dynamics present within the scientific and research structures in the area of swine health, the Social Networks Analysis (SNA) was used, which considers the complexity and topological dimensionality of the social structures, allowing for establishing a variety of measures and delimitations, under specific circumstances, to conceptualize and measure the diameter of the social network structure.

The analysis of the resulting social structure was conducted through the mathematical equalities of centrality and grouping: degree of centrality, intermediation, social density and cliques.

The data were analyzed with the UCINET 6 software for Windows.

RESULTS AND DISCUSSION

Mexico occupied the sixth place of the total of scientific contributions in the IPVS with 5.82% (Núñez-Espinoza *et al.*, 2022). A total of 283 studies were presented with participation from 642 researchers.

Swine farming has increased in practically the entire world (OCDE-FAO, 2021) and the demand for pork meat is increasing. Mexico, according to SADER (2022), was among the main consumers and producers in Latin America, with an estimated production of 1.73 million tons at the end of the year 2022. Because of this, the production of pork meat is strategic in the country (Sosa-Urrutia *et al.*, 2017), although currently this sector presents various challenges such as safety, low productivity, nutrition, profitability, sustainability, design, and development of intervention strategies, among others.

Table 1 summarizes the concentration of studies by institution and laboratory. The participation of UNAM stands out, which concentrates a fifth of the Mexican production.

Presently, in Mexico, 51 institutions teach undergraduate studies in Veterinary Medicine and Zootechnics, each with its objectives, missions and visions; however, all of them have contributed to the improvement of productive sectors through research, technical training with producers, expositions and attendance to debates, both nationally and internationally.

In the area of swine health and veterinary science, the study has depended primarily on higher education institutions and agencies from the public and the private sectors participate actively, to a lesser degree. During the conferences analyzed in this study, 283 studies were

Table 1. Main institutions in swine farming research in Mexico.

Source	Studies	%
Universidad Nacional Autónoma de México	72	25
Laboratorio Avi-mex	26	9
INIFAP	17	6
Universidad Autónoma del Estado de México	16	6
Universidad Autónoma de Yucatán	13	5
Boehringer-Ingelheim Vetmedica, Inc.	11	4
Others	128	45
Total	283	100

presented, of which 178 (63%) by universities or public research centers and the remainder by the private sector. Of the studies, 68.55% were generated by 8 institutions: 5 public and 3 private; 96 studies were generated by the scientific cluster of Universidad Nacional Autónoma de México (33.92% of the total), 26 (9%) by a laboratory, 17 by INIFAP (public research institution), 16 by Universidad Autónoma del Estado de México, and 13 by the Universidad Autónoma de Yucatán.

The main research areas in swine diseases in Mexico were PRRS, Circovirus, Influenza, Rubulavirus, Porcine Epidemic Diarrhea and to a lesser extent actinobacillocis, provovirosis and almonelosis, among others.

According to the units analyzed in the period covered (2010-2018), these populations of researchers were clustered in a structure of scientific collaboration, with particular dynamics and areas: a) a significantly condensed central area; b) areas of dispersion or social expansion; and c) peripheral areas independent from the central component. These particularities showed a variety of groups and social connections through the collaboration process analyzed, as well as a social hierarchy, in function of intellectual and economic resources handled by each group, expressing a concrete symbolic and political differential: some have greater power than others to make and/or change the information that circulates within the network (Núñez-Espinoza and Martínez-Castañeda, 2020). In the case of this general structure, the mean values of centrality and intermediation were 3.85 and 96.75. The degree of centrality of the network was 1.12% which indicated certain paths through which the information is distributed, but also a structure fed with circumstantial connections. A socio-centric behavior was seen (Figure 1), although with areas directed at centralization processes.

The importance of the working groups observed in the graphs of the period analyzed (2010-2018) has concentrated the information in a few stakeholders, and two positions emerge from this: a) they are stakeholders that have prominence in the information flow, centralizing it, which places them in a strategic situation as intermediary stakeholders between institutions, producers, and public policy designers for the swine farming sector; and b) this behavior indicates a centralized structure in a scientific and technological sector that develops immersed in a complex agrifood concept: that of health; so this centralization, instead of generating diverse solutions to the complex epizootic and zoonotic schemes at

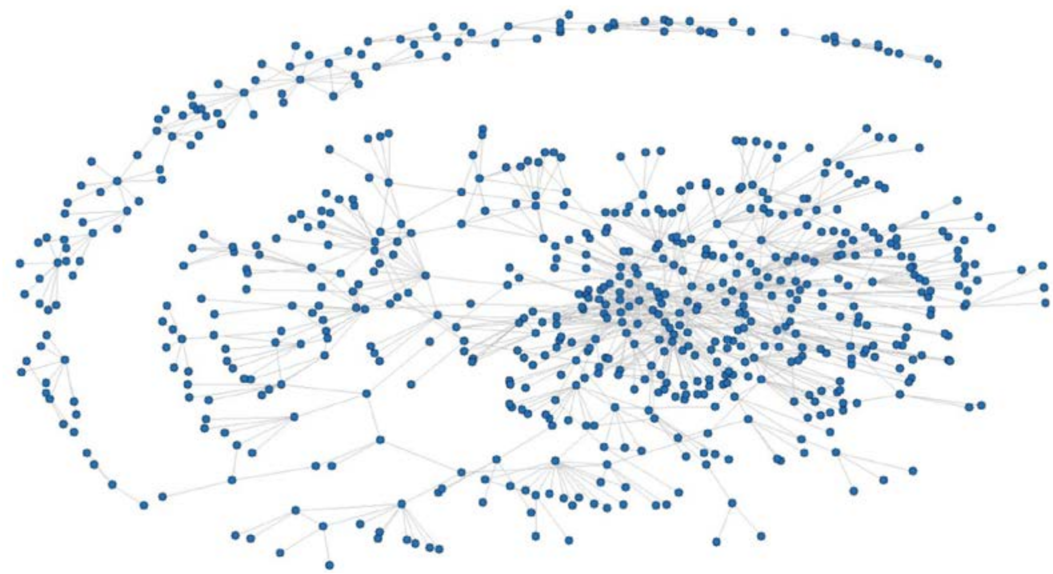

Figure 1. General structure of the Mexican research network on swine health.
Source: IPVS (2010-2018).

the level of large livestock production systems, generates solutions that are unavoidably and problematically partial.

Mexico Network 2010

Considering the analysis of such a structure for each of the periods analyzed, it was seen that the structure expressed in 2010 (Vancouver, Canada), presented an average degree of centrality of 3.23, a maximum of 53 and a standard deviation of 5.42. This behavior suggests variation in the number of direct connections and a distribution of the moderately uniform social prominence (Figure 2). For its part, the average degree of intermediation was 3.88 although with deviation of 16.66.

Mexico Network 2012

This research structure suffered changes by the year 2012 (Jeju, South Korea), showing a degree of centrality of 2.31, with a maximum of 13 (40 less than in 2010) and a deviation of

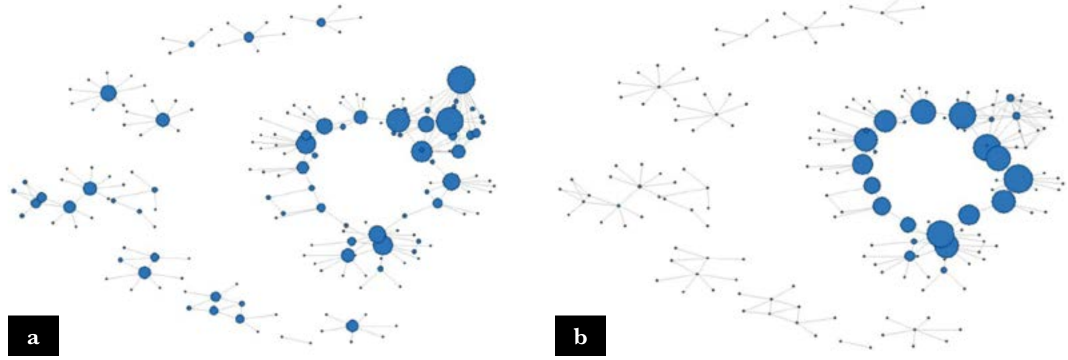

Figure 2. Degree of centrality (a) and intermediation (b) of Mexican collaborators in IPVS 2010.

2.57. In addition, a disconnect was evidenced of the structure for research and collaboration, presenting ego networks with a reduced number of components and one main component (which could suggest a differential in terms of economic resources to present studies in a conference of this type, although evidence about it is still lacking) (Figure 4). Compared to the 3.88 of intermediation from 2010, and due to the low connection between areas and/or research groups, in 2012 it was only possible to record a value of 0.611 with a deviation of 3.21 (Figure 3).

Mexico Network 2014

The structural behavior found in 2014 (Cancún, Mexico) was characterized by a more socio-centric behavior. The degree of centrality reported was 3.08, with a maximum of 43 and a deviation of 4.10, and these values were higher than those reported for 2012, although lower than those of year 2010. The number of observations was the highest, with a total of 383, 270 more than in 2012 and more than in 2010. Despite this, it is possible to identify a higher number of nodes and relationships, and two integration areas were observed: one main component and an area of peripheral groups disconnected between each other (Figure 4). The intermediation was 5.10, but it was still concentrated in central groups.

Mexico Network 2016

The behavior observed for the social structure of the year 2016 (Dublin, Ireland) was very similar to that of year 2012. A central component and a periphery made up of ego networks, perhaps evidencing groups with better resource management to participate in this type of international events. The degree of centrality was 2.21 with a deviation of 2.29 (Figure 5). This conference was where the least observations by the Mexican delegation were recorded, with only 103. The degree of intermediation was 0.21, suggesting a concentration of the power of information in few stakeholders.

Mexico Network 2018

The structural behavior of the year 2018 (Chongqing, China) was similar to that observed in 2012 and 2016, although with some slight variations: a more articulate central

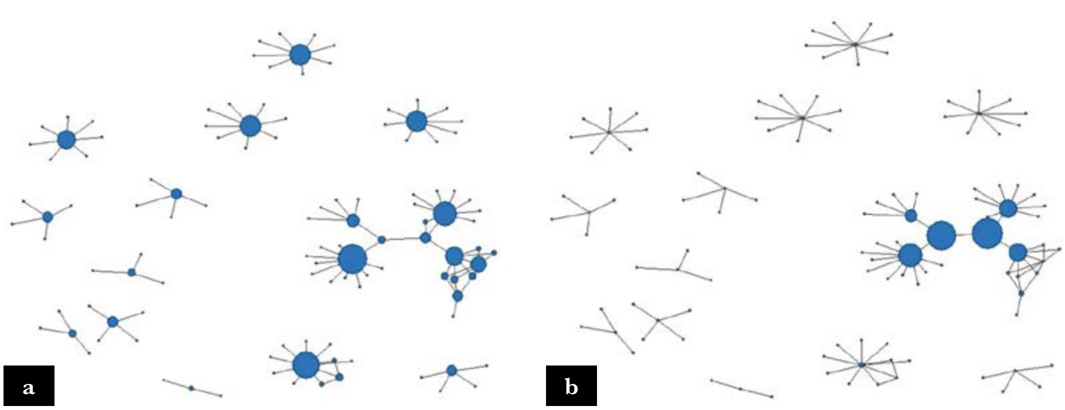

Figure 3. Degree of centrality (a) and intermediation (b) of Mexican collaborators in IPVS 2012.

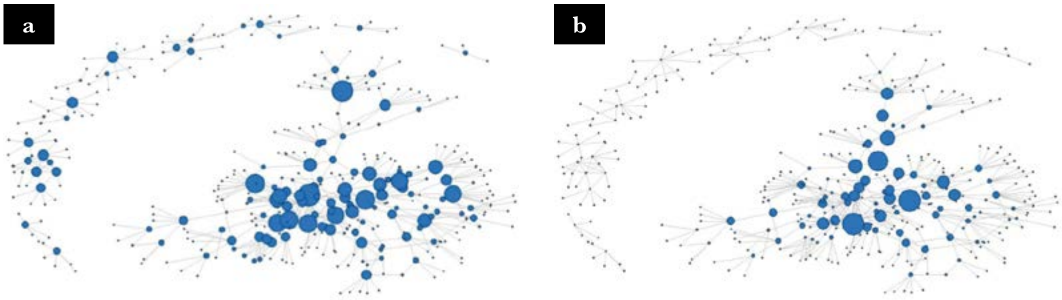

Figure 4. Degree of centrality (a) and intermediation (b) of Mexican collaborators in IPVS 2014.

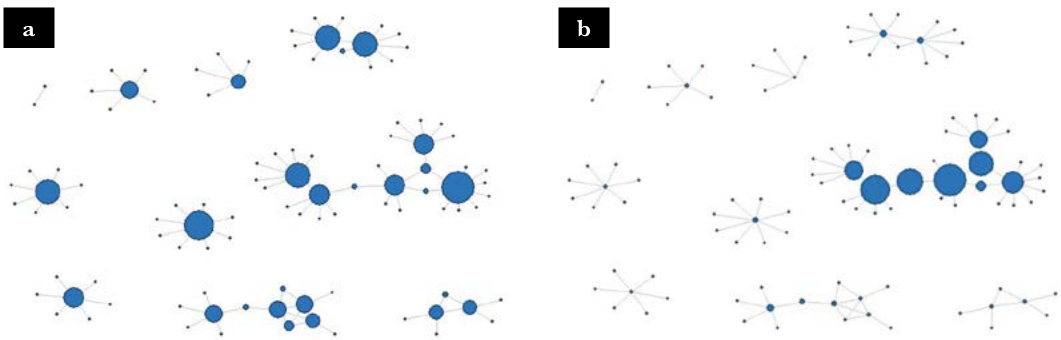

Figure 5. Degree of centrality (a) and intermediation (b) of Mexican collaborators in IPVS 2016.

component and with egos in the process of connecting between each other. The values of centrality and intermediation were 2.85 and 0.88, and the deviations 3.53 and 5.56. The information flow was concentrated in the central component (Figure 6).

Network by gender

When this research structure in swine health was addressed, dividing the values by gender (Figure 7), it was observed that its composition was determined by 53.58% men, 30.37% women, and 16.04% whose gender it was not possible to determine.

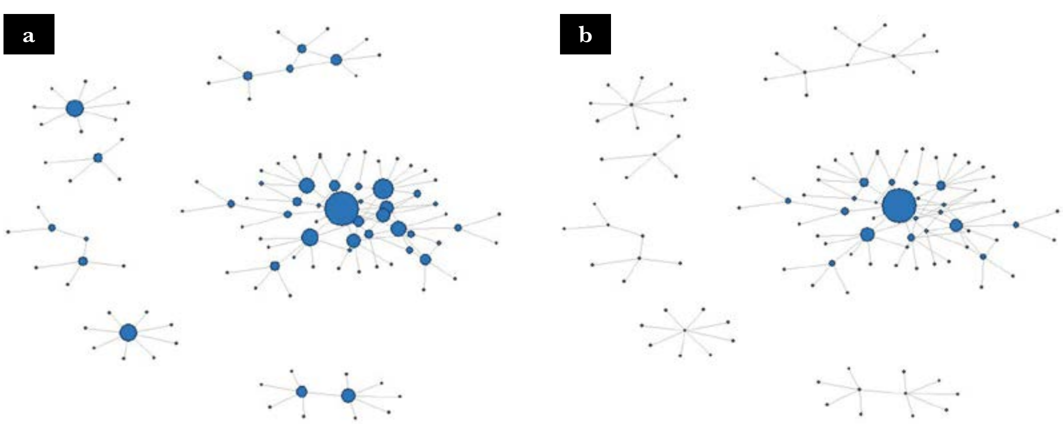

Figure 6. Degree of centrality (a) and intermediation (b) of Mexican collaborators in IPVS 2018.

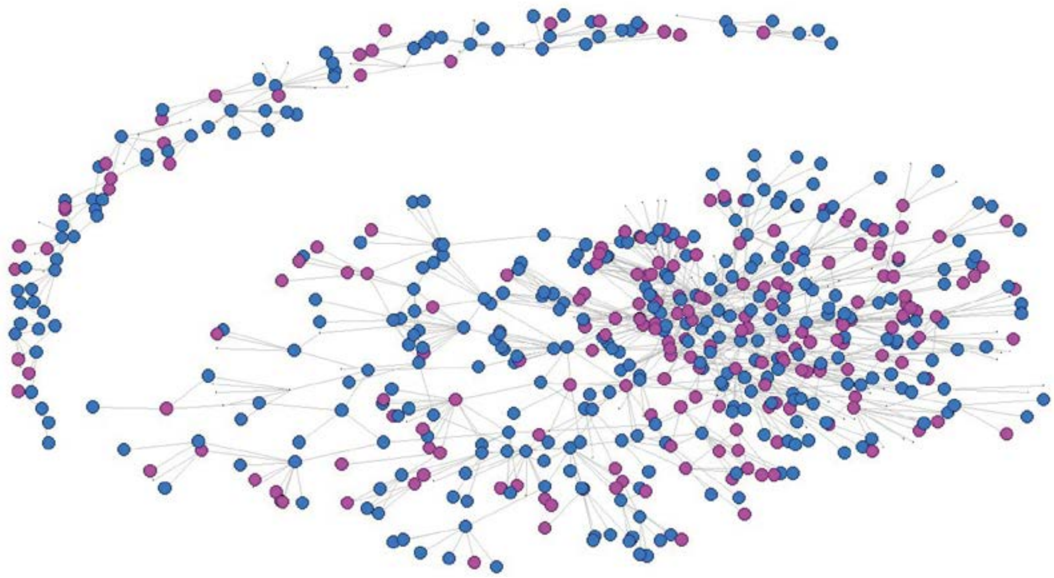

Figure 7. General structure of the Mexican research network in swine health divided by gender.

When analyzing the centrality of the structure, it was seen that both the nodal degrees and the intermediation degrees were managed and centralized primarily by women, indicating a strategic position to gain access to the information circulating in the network, as well as to connect nodes and areas that are not directly connected between each other. This indicates a prominence in the determination of research themes in the area analyzed (Figure 8). The means of centrality and intermediation by gender were 2.51 and 45.45.

With a more introverted analysis of the information flow in this structure and gender network, focusing on the central component, informed by recurrences, strengths, continuity and reciprocity in the information flow between stakeholders, the decision was made to discriminate the circumstantial values of correlation, with a nodal value=1. Therefore, it was possible to gain access to a more condensed social structure, with a more horizontal distribution of social prominence. In this case, both the values of degree and intermediation were managed by feminine stakeholders (Figure 9).

The asymmetrical relationship that traditionally exists between men and women who do research on swine in Mexico is notable, given that as Lechuga Montenegro *et al.*, (2018) mentions, education in Mexico is influenced by the customs and traditions of the place where it develops, and for the case of livestock production, because of how heavy animals are and the maneuvers necessary, it has been regarded as an area of masculine action. However, the analysis presented here detected that although there are less women participating, they are the ones who have highest degree of intermediation, which indicates that they are key stakeholders to connect with the network. There is still much to do to achieve a real education with gender perspective; however, it is worth highlighting that currently there is an important feminine representation in the sector of research on swine health and veterinary science.

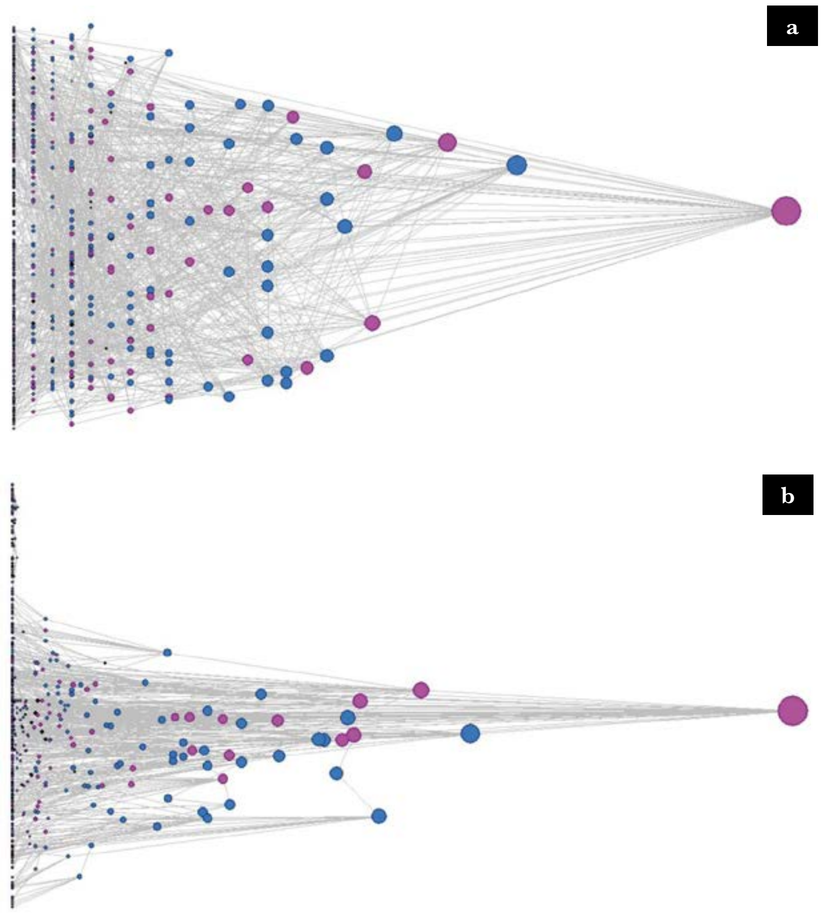

Figure 8. Degree of centrality (a) and intermediation (b) by gender. Pink (women), blue (men).

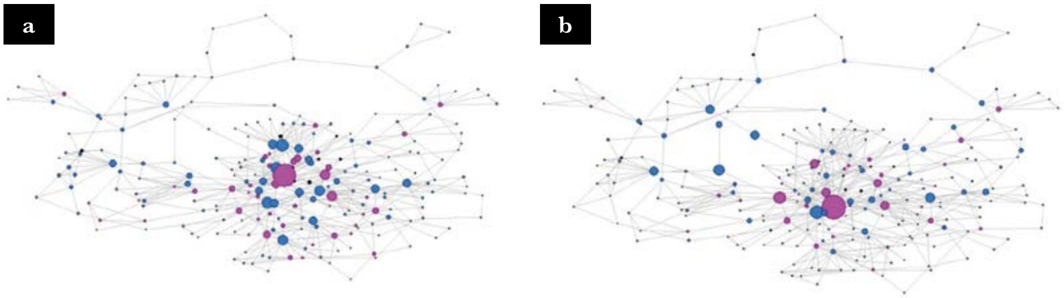

Figure 9. Degree of centrality (a) and intermediation (b) in the structure of the Mexican research network on swine health, divided by gender, excluding the nodal value=1.

CONCLUSIONS

Public universities are integrated into the current quandary that affects swine farming in Mexico and they have some connection with the swine-producing industry and some private sectors.

The degree of centrality shows that throughout the IPVS events, the nuclei of researchers became centralized from more to less, ending up with less than five research groups in the country that disseminate through these means the scientific results that support swine farming in the country, which without a doubt contribute to the resolution of problems present in swine farms, since without a doubt the porcine livestock inventory has increased, as well as the productive efficiency and per capita consumption.

Mexican women researchers are leaders in the research sector in the country, through the generation and intermediation of research projects connecting to a certain degree between each other and in lower proportion with the private sector. Understanding who the key stakeholders are in these working networks between universities will allow an adequate dissemination of information.

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Colegio de Postgraduados

Methods for the control of whitefly (Aleyrodidae) in citrus: a systematic review

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ABSTRACT
Objective: To carry out a systematic review of original articles published from 1950 to 2021 about the methods used to control whitefly on citrus crops and the biological control organisms.
Design/Methodology/Approach: The study was carried out following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses. The reference works were retrieved from electronic databases. The Mendeley Bibliography Manager was used to create a new data base which, in its turn, was used to analyze the information.
Results: Two groups were identified as control methods: 1) chemical control, and 2) biological control. The latter was used with greater frequency than the former. Several genera of the family Aphelinidae were the parasitoids most frequently used for biological control. *Aleurothrixus floccosus* was the most frequent pest in citrus orchards.
Study Limitations/Implications: The information is limited and scattered. There is no information about the efficiency of the methods.
Findings/Conclusions: The use of antagonistic organisms used as a biological control method is of utmost importance, given the impact those organisms have on pests. They make a contribution to the natural regulation of the population levels of harmful insects in citrus crops.

Keywords: Whitefly parasitoids, biological control, citrus pests.

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INTRODUCTION

Citrus are one of the main crops worldwide; they are in high demand and are grown in tropical and subtropical regions, across more than 140 countries (Vu *et al.*, 2018). The main producers are Brazil, China, India, Spain, the USA, and Mexico (FAO, 2017). In 2019, 9,898,643 ha were used for the worldwide production of citrus, reaching a production of 99,410,000 t —*i.e.*, a 17.78% increase in production from 2012 to 2019 (FAOSTAT, 2022; Knoema, 2021). Nevertheless, the crop faces phytosanitary problems, as a result of pests and diseases. The following diseases have particularly devastating effects: huanglongbing

(citrus green disease), leprosis, and variegated chlorosis. They are transmitted by the Asian citrus psyllid (*Diaphorina citri*), mites from the genera *Brevipalpus* spp., and the whitefly (*Aleurothrixus floccosus*). Whiteflies include approximately 1,556 species, divided into 161 accepted genera (SENASICA, 2020).

Whiteflies cause direct and indirect damage to the plants, hindering respiration and reducing photosynthesis and nitrogen levels. Nymphs produce molasses and a waxy secretion, weaking buds, causing defoliation, and enabling the development of *negrilla* (sooty mold) and other saprophytic fungi that have a direct repercussion on citrus production per surface unit and its commercial value (Sáenz-Pérez *et al.*, 2019). Mexico is the fourth largest citrus producer in the world (Knoema, 2021) and the losses in lemon, orange, and grapefruit cultivation caused by the said pest can reach up to 40% of the total harvest (Agroasemex, 2019).

The scientific texts that discuss the methods used to control whitefly in citrus orchards are scarce and scattered. Therefore, the objective of this work was to carry out a systematic review of the said methods, along with the number of whitefly species recorded, and the organisms used for their biological control. This research was based on original articles published from 1950 to 2021. The purpose of this analysis was to group the existing information, make contributions to the subject matter, and lay the foundations for future research. As far as it can be determined, no previous systemic reviews have been carried out regarding this issue.

MATERIALS AND METHODS

Search strategy and reference management

The work complied with the conditions established by the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) (Urrútia y Bonfill *et al.*, 2010). To guarantee its exhaustiveness, a bibliographical search was made on the Scopus y ScienceDirect electronic databases, as well as in Google Scholar, Dialnet, and SciELO (Figure 1). The starting point was the research question: What methods are used to control whiteflies in citrus? A general search route was established combining terms through Boolean operators and the time period was limited to the years 1950 to 2021.

Inclusion and exclusion criteria

The structure of the information source of the study was managed based on original articles published in Spanish or English. Only studies from the time range established

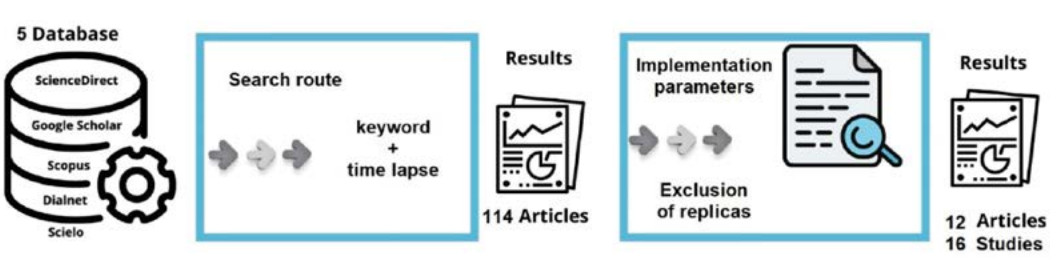

Figure 1. Methodology. Figure developed by the authors.

and which included the description of the control method, the whitefly species, and the control organisms were considered to be eligible. Duplicate articles were excluded through the Mendeley Reference Manager. An Excel database was developed and it included the information to be evaluated. Percentages were calculated and graphs and tables were developed for descriptive purposes.

RESULTS AND DISCUSSION

One-hundred-fourteen articles were published during the established time range. Having applied inclusion and exclusion criteria and eliminated duplicates, the resulting sensitivity was 12 publications, based on which 16 studies were extracted and used in the systematic review.

Spatial and Temporal Distribution of the Publications

Regarding their point of origin, 37.5% of the studies were developed in Europe, while the Americas, Africa-Asia, and Oceania accounted for 31.2%, 12.5%, and 6.5%, respectively. Most researches were carried out in Spain, the USA, and Chile (Figure 2). Regarding the publication period, over 40% of the studies were carried out from 1998 to 2002, which matches the increase in whitefly reports in citrus orchards in the early 1990s, resulting from the lack of efficiency of the usual pesticides. The *Parabemysia myricae* populations diminished in Spain from 1995, but *Dialeurodes citri*, *Aleurothrixus floccosus*, and *Trialeurodes vaporariorum* decreased slowly and their levels were still considerable in 1999 (Soto *et al.*, 2001).

The introduction and establishment of *Eretmocerus debachi* in Spain's citrus zones, along with the natural control exercised by the abundant population of *Cales noacki* (a parasite of *Aleurothrixus floccosus*), drastically diminished the populations of *Parabemisia myricae*. This phenomenon could be related to the decrease in publications about the subject, since it was the only fly species that damaged citrus orchards during those periods (Soto *et al.*, 1999). Santaballa *et al.* (1980) published the first study about the application of a method to control whitefly in citrus.

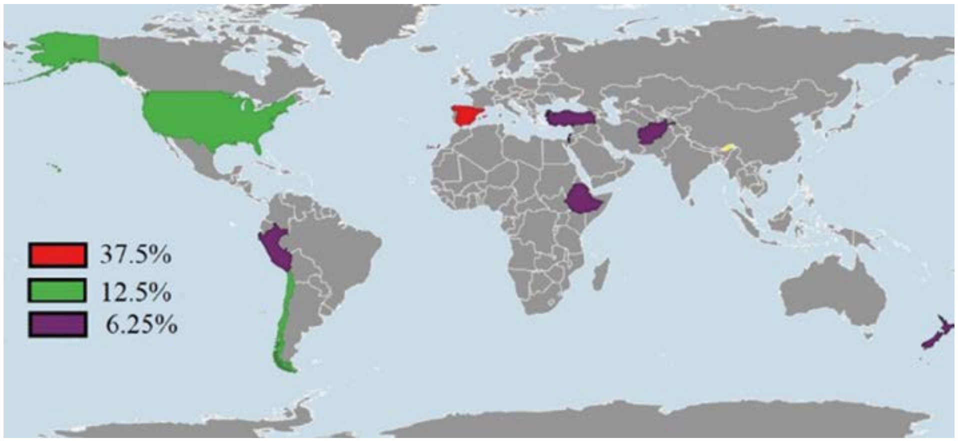

Figure 2. Spatial distribution of publications per country. Figure developed by the authors.

Objectives of the studies

One-quarter (25%) of the studies evaluated the effectiveness of insecticides —12.5% of which were contact products, while the rest were systemic or applied on the leaves. The evaluation of agrochemicals is a major element in pest control. Employing an adequate control, applying the product only where pests have been detected, using adequate doses, time, and schedule, providing a good cover technique, and rotating active ingredients with other modes of action improve the quality of the crop production, as these practices reduce sooty mold and avoid resistance or efficiency loss (Ripa *et al.*, 2006). The effectiveness of parasitoids was evaluated in 18.75% of the studies. In most cases, parasitoids were used to control whiteflies, because this "key pest" does not only colonize whiteflies on the release site; it can also scatter to up to 60 km away from the original area. Therefore, it can cover a wider area of the citrus cultivation zone, achieving a greater impact in the control of whitefly (Santaballa *et al.*, 1980). Other objectives include the evaluation of the effectiveness of predators and of the infestation degree on the whitefly stages (12.5%). The remaining studies had various objectives, represented a lower percentage, and were grouped as "others."

Identification of control methods

Two control method groups were identified: 1) chemical control and 2) biological control. The latter group was the subject of the highest percentage of reports (68.75%), revealing the interest in the use of methods with a lower environmental impact (Table 1). Biological control is a major strategy that not only helps to reduce the population density of pests, but also diminishes environmental degradation and the imbalance in the natural control of agricultural pests resulting from the use of agrochemicals (SENASICA, 2020).

The use of parasitoids is an outstanding biological control method (Table 1). These natural antagonists have a major impact on whiteflies, causing damage that conditions any intervention on the remaining pests and diseases that attack citrus (Santaballa *et al.*, 1980). Several authors have reported the suppression of whiteflies populations in citrus as a result of the introduction and release of parasitoids and their subsequent establishment and spreading (Ulusoy *et al.*, 2003). However, there are remarkable variations in the abundance indexes and the infestation rate by larvae and adults, which mainly depend on the growth of foliage and the regional meteorological conditions (Mahmoudi *et al.*, 2018). Entomopathogenic fungi were also used, despite their sensitivity to weather conditions —which impact their stability and persistence— and their slower action rate (Table 1). They are an alternative for the intensive application of agrochemicals to combat citrus pests —including the Asian citrus psyllid, the major citrus pest worldwide (Pucheta-Diaz *et al.*, 2006).

Chemical control accounted for 31.25% of the methods employed, and they were divided in insecticides and detergents (Table 1). Two sulfonate-based commercial detergents were used: SU-120 (liquid presentation) and SU-143 (powder). Overall, organic insecticides were the most frequently used chemical control method (53%, out of which organophosphates account for 40%); systemic insecticides and pyrethrum were used in a lower rate (27% and 13%, respectively), while natural control methods were the least used. Agrochemicals are

Table 1. Methods used for whitefly control.

Control	Used Method	Usage (%)
Biological	Parasitoids	43.75
	Entomopathogenic fungi	12.5
	Predators	6.25
	Plantation management	6.25
Chemical	Detergents	6.25
	Contact insecticides	25

the most efficient and quick way to control a pest. However, the constant use of insecticides made from chemical synthesis disturbs and destabilizes the agroecosystem, potentially affecting populations that act as a natural pest control (Castresana, 2016). Nevertheless, when biological control fails, a chemical intervention or using a strategy that includes chemicals is recommended (Santaballa *et al.*, 1980; Xu *et al.*, 2013).

Biological control organisms

Several genera of the family Aphelinidae were some of the parasitoids used for biological control. The two most represented genera were *Eretmocerus* (41%) and *Cales* (33%). *Cales noacki* and *Eretmocerus debachi* were the species with the highest use percentage (Table 2). Since it was introduced to citrus orchards, *Cales noacki* has controlled the nymphs of *Aleurothrixus floccosus*, significantly decreasing the application of phytosanitary treatments. This hymenopteran parasitoid was introduced in Spain in 1970, achieving a better adaptation than the two other genera of the same family that were introduced at the same time. For their part, *Cales noacki*, *Eretmocerus paulistus*, and other species had been used in Mexico and the USA before they were introduced in Spain, obtaining good results as a biological control (Myartseva *et al.*, 2017). In 25% of the analyzed sources, the genus *Encarsia* (25%) was found to be less efficient as a pest control (Foltyn and Gerling, 1985; Gerling *et al.*, 2001). The use of parasitoids is an efficient method for the control of *Aleurothrixus floccosus* in citrus; however, its action must not be disturbed by the application of incompatible phytosanitary treatments.

Beauveria bassiana was the only entomopathogenic fungi species reported. Its entomopathogenic function has been known since 1835 (Commonwealth Mycological Institute, 1979). It has been used to control members of the Aleyrodoidea superfamily,

Table 2. Employment of organisms as biological control against whitefly.

Organism	Specie	%	Organism Type	Specie	%
Parasitoid	*Cales noacki*	20	Parasitoid	*Eretmocerus* spp.	5
Parasitoid	*Eretmocerus debachi*	15	Predator	*Conwentzia psociformis*	10
Parasitoid	*Encarsia lahorensis*	5	Predator	*Coccinella seprempunctata*	5
Parasitoid	*Encarsia strenua,*	5	Predator	*Clitostethus arcuatus*	5
Parasitoid	*Encarsia lutea*	5	Predator	*Chrysoperla carnea*	5

including *Bemisia tabaci* (Genn.), *Dialeurodes citri* (Ashm.), and *Trialeurodes vaporariorum* (Westw.) (Ruiz *et al.*, 2009; Santamaría *et al.*, 1998). The most frequently used predator was *Conwentzia psociformis*, from order Neuropetara (Table 2). Predators can reduce pest populations. Nevertheless, there are used less frequently than parasitoids as an exclusive control method, because being generalists they feed on various phytophagous species in citrus, hindering an efficient control of the whitefly (García-Marí, 2012).

Whitefly species

Seventy-five percent of the studies were carried out with hybrids from the genus *Citrus*: *Citrus maxima* (31.5%), *Citrus medica* (31.2%), and *Citrus reticulata* (12.5%). Out of this total, 25% were *Citrus*×*sinensis* and a similar percentage was reported for a non-specified lemon hybrid. Five whitefly species were detected (Figure 3). *Aleurothrixus floccosus* was reported in 54.5% of the studies and in the 3 citrus species. Table 3 shows that orange was the citrus most affected by *A. floccosus*, which was found in the two reported hybrids (22.7%), mainly in *Citrus*×*sinensis*. The non-identified citrus group was affected in a similar percentage, along with the non-specified lemon hybrid, although the latter recorded a lower percentage (Table 3). In countries like Spain, *Aleurothrixus floccosus* is considered the most important fly species, because it has colonized the greatest surface and causes major health and economic problems to the citrus industry (Garrido, 1994; Xu *et al.*, 2013).

The frequency of the *A. floccosus* reports could be related to its apparent high potential in areas with favorable weather conditions, mainly where winter temperature is higher than 14 °C a condition which allows a constant oviposition throughout the period, severely damaging the harvest (Tello-Mercado and Zarzar-Maza, 2021). Weather variations in the environment favor the acclimatization of the fly to the environmental conditions of places that it had found formerly unfavorable (Beingolea, 1959).

Citrus×*sinensis* is a hybrid that is usually affected by whiteflies (including *A. floccosus*). Whiteflies feed and lay their eggs on the abaxial surface of young leaves (Giliomee and Millar, 2009; Ripa *et al.*, 2006), taking advantage of weather conditions, particularly high temperatures (Tello-Mercado and Zarzar-Maza, 2021). The molasses and wax secretion produced during the advanced nymph stages have a significant impact on the yield and quality of the product; they protect such pests as woodlouse and mites

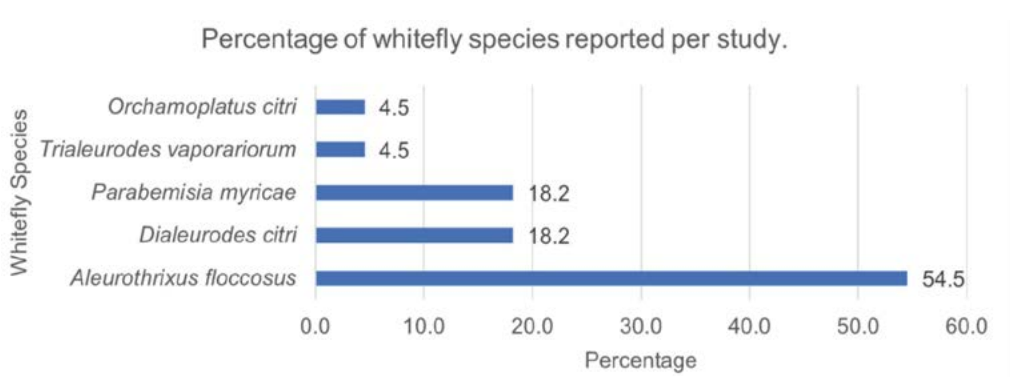

Figure 3. Whitefly species detected. Figure developed by the authors.

Table 3. Percentage of whitefly species by citrus in the studies.

Whitefly species	Plant	%
Aleurothrixus floccosus	*Citrus* spp.	18.2
Dialeurodes citri		13.6
Parabemisia myricae		13.6
Aleurothrixus floccosus	Limón N/E	9.1
Dialeurodes citri		4.5
Parabemisia myricae		4.5
Trialeurodes vaporariorum	*Citrus limon*	4.5
Orchamoplatus citri	Mandarina N/E	4.5
Aleurothrixus floccosus	*Citrus reticulata* blanco	4.5
Aleurothrixus floccosus	*Citrus sinensis*	18.2
Aleurothrixus floccosus	*Citrus aurantium* L.	4.5

against phytosanitary treatments and the action of their natural enemies, consequently encouraging their development. Molasses and wax secretion also generate diseases (*e.g.*, sooty mold), which cover the leaves and interfere with photosynthesis (Riceña-Durán *et al.*, 2017). Other species with similar report percentages were identified, including *Dialeurodes citri* and *Parabemisia myricae* (Figure 3). They mainly affected the group made of non-identified species (Table 3). Both whitefly species were found in an expansive state in Spain in 1994; since their detection, they have become resistant to a wide range of insecticides, including organophosphates (OP) and carbamates (CBs) (Garrido, 1994; Xu *et al.*, 2013).

The three fly species with the greatest presence in this review cause major problems to citrus orchards. Severe yearly attacks by *Dialeurodes citri* can affect fruit quality, because sooty mold —which grows as a result of the molasses produced during the immature phases— indirectly influences plant vigor and, consequently, its productivity. Severe attacks by *Parabemisia myricae* do not only lead to the development of sooty mold, they also, as a consequence of the darkening of the tree, prevent an adequate photosynthesis; this phenomenon stops the growth and fruition of the tree, which has a direct influence on production (Garrido, 1994).

CONCLUSIONS

The use of antagonistic organisms to combat the various whitefly species (whether native or introduced to the environment) is a highly important matter. Given the effect they have on pests, they contribute to the natural regulation of the population levels of harmful insects in citrus orchards. This study lays the foundations for the development of new research that evaluate the effect and efficiency that beneficial organisms used as biological control methods have on the various Aleyrodidae species that affect citrus.

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Colegio de Postgraduados

Agricultural drought in the context of climate change: a bibliometric analysis

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ABSTRACT
Objective: To perform a systematic review of the scientific studies carried out about agricultural drought in the context of climate change reported in the last ten years.
Design/methodology/approach: The study was based on the bibliographic review of the referential database Scopus® using the concepts "agricultural drought", "vulnerability" and "climate change".
Results: In the last ten years (2014-2022) the United States, China and United Kingdom stood out in publications on the topic, which are focused on Environmental sciences (33%), Agricultural sciences (22%), Earth sciences (13%), and Social sciences (12%).
Findings/conclusions: The studies published are isolated and there is no conection between the large topics of drought, climate change and vulnerability, thus detecting an area of opportunity to carry out research that unifies these topics.

Keywords: Drought monitor, extreme events, water resources.

INTRODUCTION

Drought is defined as a decrease or absence of rainfall, or else, as meteorological drought (Scarpati and Capriolo, 2016). Concerning the annual index, it happens in a cyclical manner in every climate zone in the world, although with greater intensity and recurrence in arid and semiarid zones (Esquivel, 2002). Drought in Mexico takes place approximately every 20 years and the period can last from one to three years, which alters the water cycle provoking its insufficiency or hydrological drought. This period ends when rainfall normalizes and the normal precipitation index and the functioning of water bodies are recovered (Cerano-Paredes *et al.*, 2009).

Mexico's government, through the Mexican Institute of Water Technology (*Instituto Mexicano de Tecnología del Agua*, IMTA, 2019) points out that droughts are inevitable, unpredictable, without an established trajectory, without well-defined start and finish, recurrent yet not cyclical, and potentially catastrophic.

The causes for drought involve natural factors represented by modifications in atmospheric circulation patterns, variations in solar activity, and phenomena of interaction between the ocean and the atmosphere (Velasco *et al.*, 2005), in addition to anthropogenic factors such as global warming.

According to Article 1 of the United Nations Framework Convention on Climate Change (UNFCCC), it is defined as the modification of climate attributed directly or indirectly to human activity that alters the composition of the world atmosphere and which happens in addition to natural climate variability during comparable periods of time (IPCC, 2013).

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The North American Drought Monitor (*Monitor de Sequía de América del Norte*, SMN-CONAGUA, 2022) classifies drought into: Abnormally dry (D0), Moderate drought (D1), Severe drought (D2), Extreme drought (D3) and Exceptional drought (D4).

Countries such as Canada, the United States and Mexico have worked through the Commission for Environmental Cooperation with the aim of creating greater awareness and expanding the access to early alert systems for drought events, such as the North American Drought Monitor (NADM), which has the objective of describing the drought conditions in North America to allow those responsible for decision-making and citizens equally to understand the vulnerabilities when facing drought and to create resilience in the presence of this phenomenon (Ambiental, 2022).

In Mexico drought is a meteorological phenomenon that has affected agricultural production, impacting primarily the South-Southeast Region of the country. Figure 1 shows the states of the Mexican republic most affected by drought, which are Colima, Michoacán, Guerrero, Oaxaca, Chiapas and Yucatán. These data were determinant for the National Water Commission (*Comisión Nacional del Agua*, CONAGUA) to issue the general agreement for emergency start for the occurrence of severe, extreme or exceptional drought in basins for the year 2022, since out of the 2,471 municipalities of the country, 770 are in some level of drought and 972 are in the stage prior to some level of drought. Consequently, the users of national waters in the municipalities under any drought condition are encouraged to implement Preventive and Mitigation Measures, with the aim of having an efficient use of water (DOF, 2022).

In addition to contamination, it has been seen that water scarcity places food production systems at risk globally. Something else to be considered is that agriculture in Mexico consumes 70% of the total use of fresh water, so steps should be taken to manage this resource sustainably and intelligently and to help agriculture adapt

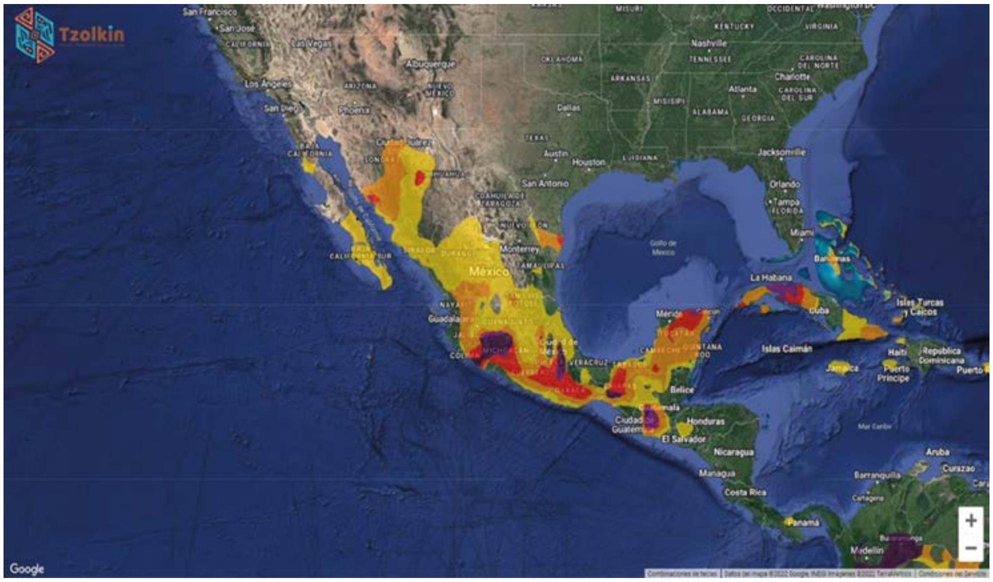

Figure 1. Drought monitor in Mexico, January 1, 2017, to September 30, 2022. Source: Servicio Meteorológico Nacional-CONAGUA 2022.

to climate change (CEPAL, 2012), unlike the water use for domestic use which only represents 10% (FAO, 2013).

Based on this, it is important to revise the theme of drought in Mexico within the context of climate change, in order to reduce the vulnerability and to develop strategies as part of an adaptation process with the aim of making decisions for management and efficiency. Because of this, a bibliometric analysis was carried out with the purpose of identifying and analyzing the studies from the last ten years in the topic of drought in the context of climate change and vulnerability of the agricultural sector.

MATERIALS AND METHODS

A bibliographic review was conducted using the database of Scopus®, exploring the studies carried out around drought in the context of climate change and related to the vulnerability of the agricultural sector.

For this purpose, keywords such as: "*agricultural drought*" were used, and their association with the keywords "*vulnerability*" and "*climate change*".

The search was limited to articles and reviews carried out in the period of 2014 to 2022, and all the documents with this theme in Scopus® were tracked, analyzed and visualized, developing clouds with principal authors, country of origin, and area of knowledge of the scientific journals where these studies have been published.

The data obtained were recorded in a database in Excel and then analyzed with the VOSviewer® 1.6.14 software (Van Eck and Waltman, 2010), through which co-occurrence maps were created, and connectivity of keywords to identify trends and connections of dominions according to the themes to approach drought.

RESULTS AND DISCUSSION

For the period indicated from 2014-2022, 1,221 publications were identified that refer to the keyword "*agricultural drought*"; 6,236 to "*vulnerability*"; and 41,965 to "*climate change*". A total of 435 publications was obtained with the combination of these three keywords (Figure 2).

In 2021 the highest number of publications was found for agricultural drought, 1,050 for vulnerability, and the topic of climate change with the highest scientific production, reaching 7,639 articles. Nevertheless, it must be considered that 2022 had not concluded and the number of articles could possibly be similar. However, when the three themes are related, the production of citations decreases notably reaching only 72 articles, which indicates a low relation of the theme of drought with climate change and vulnerability of the agricultural sector, so there should be more research into the theme although in a context of vulnerability of the drought agricultural sector in the context of climate change.

The three journals with the highest number of scientific articles on agricultural drought are: Water Switzerland, Agronomy, and Frontiers in Plant Science. Concerning the theme of vulnerability, they were: Water Switzerland, Plos One, and Frontiers in Marine Science; for the theme of climate change: Water Switzerland, Forests, and Plos One. And, finally, for the relationship of these three themes, that is, vulnerability of agricultural drought in face of climate change, there were: Climatic Change, Science of the Total Environment,

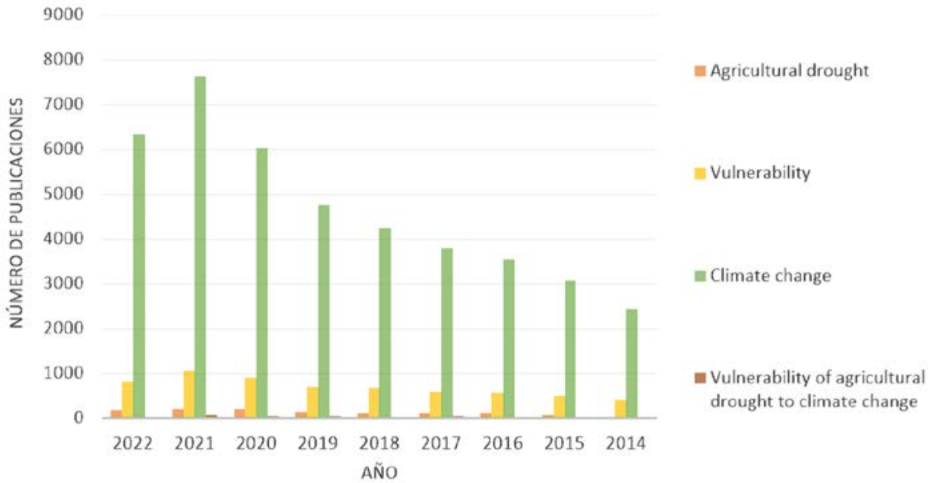

Figure 2. Number of articles on the themes "*agricultural drought*", "*vulnerability*" and "*climate change*" published in the period from 2014 to 2022, consulted on the database Scopus®. Source: Prepared by the authors with data from the search results in Scopus®.

and Climate Change Management. As can be appreciated, these are research themes that are usually published in journals with high impact factor, as well as high impact on the citation of the articles (Table 1).

Evidence shows that the journals that publish these studies are of a high impact factor (IF), led by Remote Sensing of Environment with IF of 13.850 and the journal with lowest IF was Ecosphere with 1.151. Likewise, it was found that the journal with most citations from the contributions performed are Ecology and Evolution with a score of 22.3, and with the highest number of citations, the journal Proceedings of the Royal Society B Biological Sciences with 1.31. This reflects the need for Latin American journals to have a more leading role in publishing scientific studies in this theme, for them to gradually become positioned within this international ranking.

The United States, China and the United Kingdom are the countries with the most articles published on agricultural drought, vulnerability and climate change with 53% of the total (Figure 3), focalized primarily in the environmental sciences, earth and planetary sciences, as well as agricultural and biological sciences.

Figure 3 presents the list of countries that publish scientific articles that connect the three concepts, of which none is Latin American, so this denotes an area of opportunity for Mexican or Latin American JCR journal publications. The main authors that have been published on agricultural drought regarding climate change are presented in Figure 4, as well as their citation rate.

Figure 4 shows that the most cited authors are Opitz-Stapleton, S. from the United States, Trnka, M. from China and Wang, J. from Germany; however, the co-occurrence of the most cited authors are Wang, J. for his article, "Impact of green financing on carbon drifts to mitigate climate change: mediating role of energy efficiency" published in Climate Change Management; Zhang, X. for the article "Sub-diffraction-limited optical imaging with a silver superlens" published in American Association for the Advancement of Science;

Table 1. Main scientific journals with publications about agricultural drought, vulnerability, climate change and vulnerability of agricultural drought in face of climate change obtained from Scopus® from 2014 to 2022.

Theme	Publication	Number of publications	Impact Factor	CiteScore
Agricultural Drought	Water Switzerland	163	3.53	4.8
	Agronomy	53	3.949	3.9
	Frontiers in Plant Science	53	6.627	8.0
	Agricultural Water Management	43	6.611	8.8
	Plos One	42	2.776	5.3
Vulnerability	Water Switzerland	429	3.530	4.8
	Plos One	419	2.776	5.3
	Frontiers in Marine Science	145	5.247	5.2
	Ecological Indicators	136	6.263	8.4
	Forests	116	3.282	4.0
Climate Change	Water Switzerland	2602	3.530	4.8
	Forests	1437	3.282	4.0
	Plos One	1292	2.776	5.3
	Frontiers in Marine Science	1136	5.247	5.2
	Ecology and Evolution	1087	3.167	22.3
Agricultural Drought Vulnerability due Climate Change	Climatic Change	22	4.743	7.1
	Science of the Total Environment	17	10.753	14.1
	Forests	14	3.282	4.0
	Regional Environmental Change	13	4.704	7.4
	Sustainability Switzerland	12	3.889	5.4

CiteScore: Measures the average of citations received by document published in the journal. Source: Prepared by the authors with data from results from the search in Scopus®.

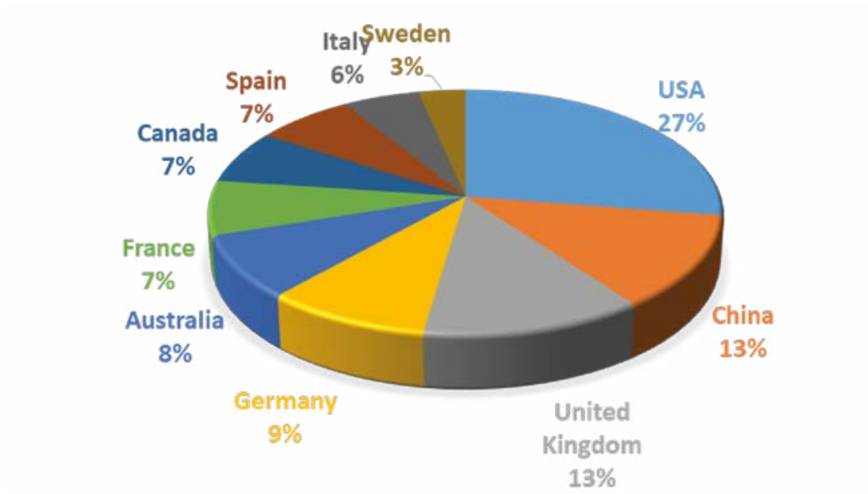

Figure 3. Main countries that publish studies on agricultural drought, vulnerability and climate change. Source: Prepared by the authors with data from results of the search in Scopus®.

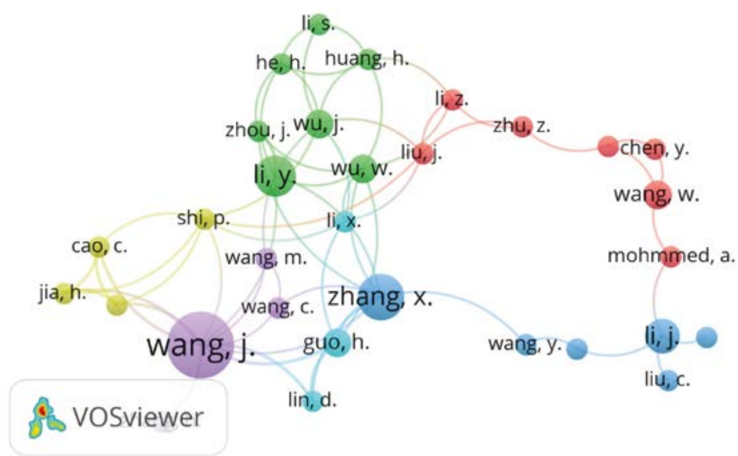

Figure 4. Citation of authors of scientific articles on the vulnerability of drought in the presence of climate change. Source: Prepared by the authors with support from VOSviewer® sotfware.

and Li, Y. for his article "Global Monsoon Dynamics and Climate Change" published in The Annual Review of Earth and Planetary Science.

The financial institutions to carry out research in the theme of vulnerability and drought in face of climate change are represented primarily by the National Sciences Foundation, the National Natural Sciences Foundation in China, and the Chinese Academy of Sciences (Figure 5).

Figure 5 shows that within the financing institutions there is not a Mexican one, although there is one institution from Latin America, the Conselho Nacional de Desenvolvimento Científico e Tecnológico del Ministerio de Ciencia, Tecnología e Innovación from Brazil. The co-occurrence map was obtained after the search in the Scopus® database, combining the three keywords (vulnerability, agricultural drought and climate change), obtained a

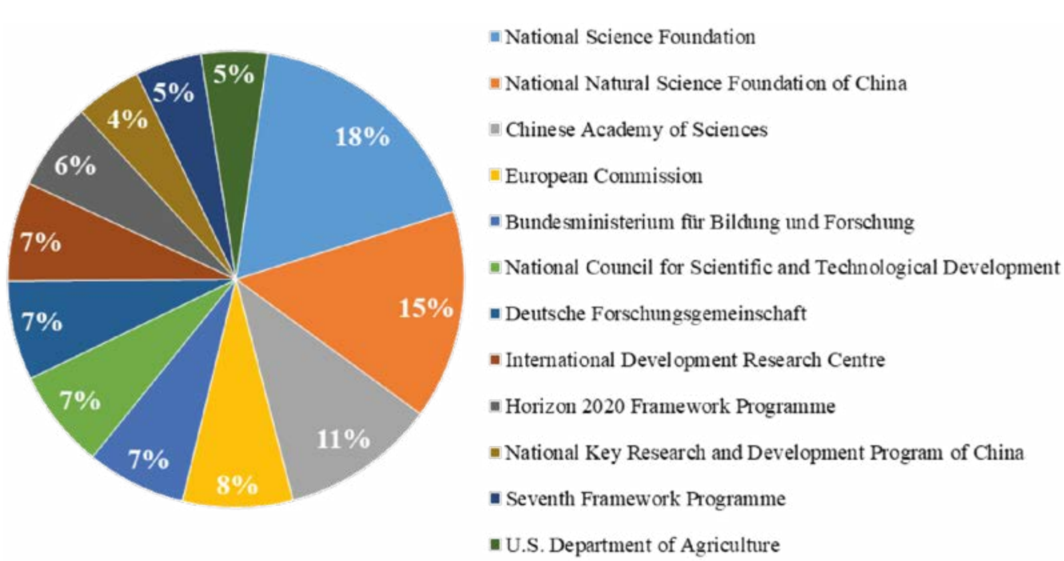

Figure 5. Financing institutions to perform research on the theme of vulnerability of drought in face of climate change. Source: Prepared by the authors with data from search results in Scopus®.

total of 435 articles. An area of opportunity was found to continue with the research on agricultural drought with relation to other themes, and that this is a scientific area of global interest.

In the co-occurrence map, nodes can be seen that determine the importance of keywords within the network. As the nodes are larger, the co-occurrence or citation within a set of data are higher. The distance between two circles represent affinity, and the closer they are the higher affinity. The lines represent the level of co-citation. It can be seen that there is a great affinity of climate change and vulnerability and agriculture (Figure 6).

Figure 6 denotes that the "climate change" and "drought" nodes are the largest. However, it stands out that the studies of seasonal agriculture are related with themes such as food security, sustainability, and vulnerability; and also that the social analysis (psychology) is at its height, the same as themes of technology development for water supply, among others.

When only the keyword vulnerability was used, 6,236 articles were obtained; then, the co-occurrence analysis was conducted and it could be seen that there is a great affinity between vulnerability and climate change, although it is far from keywords such as drought and agriculture (Figure 7). This indicates that the drought is related to the phenomenon of climate change.

Figure 7 shows that there is a principal node, that of vulnerability, which is related with resilience, sustainability, and decision-making. In turn, it stands out that the decision-making node is directly related to climate models and assessment methods. Regarding the keyword agricultural drought, 1,221 articles were obtained and from the co-occurrence analysis it was seen that there is great affinity with agriculture and climate change. However, great affinity was not found with the word vulnerability (Figure 8).

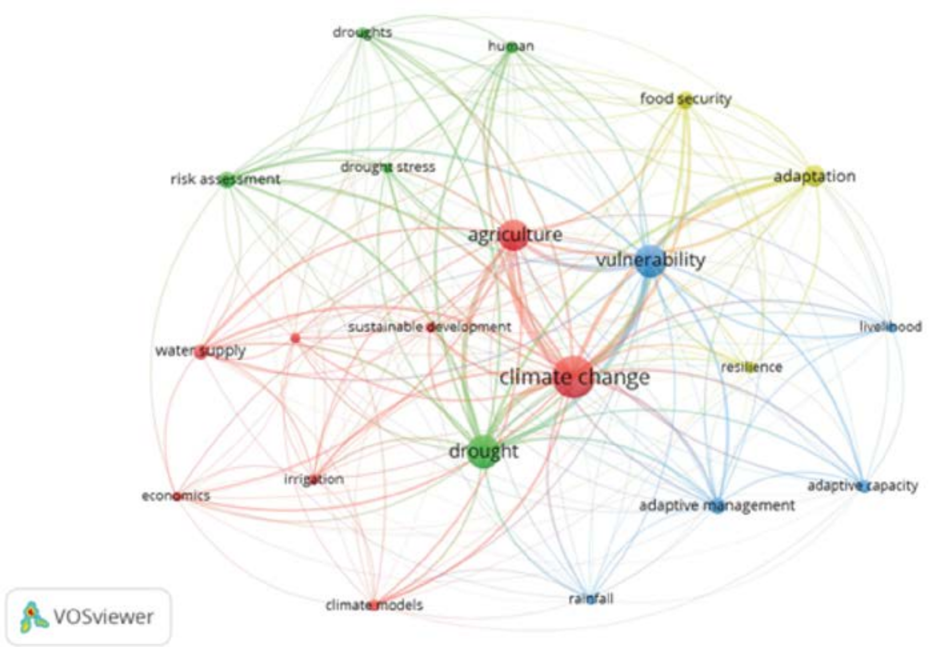

Figure 6. Co-occurrence network from the database of drought vulnerability in face of climate change. Source: Prepared by the authors with support from the VOSviewer® Software.

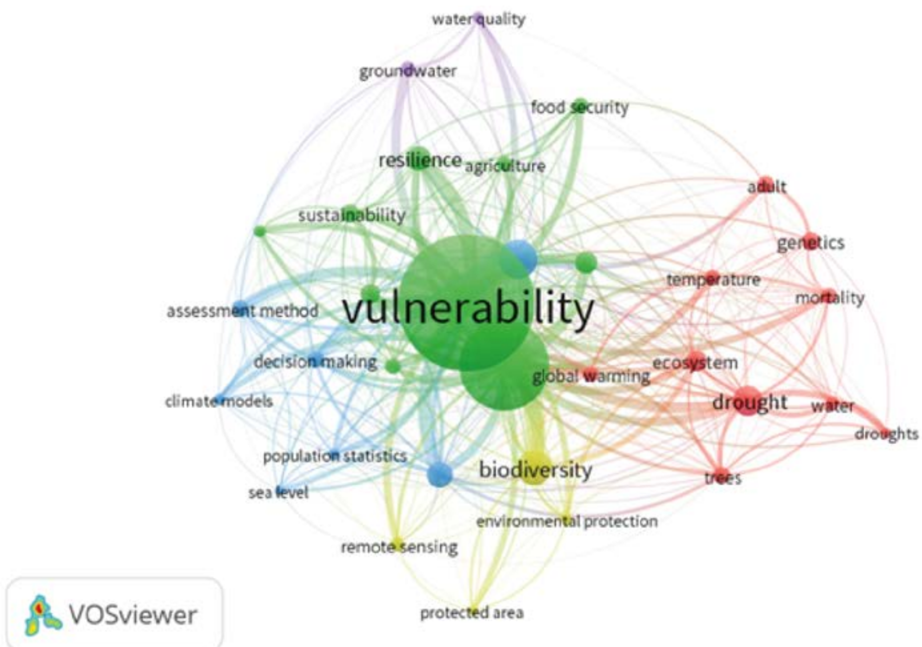

Figure 7. Co-occurrence network of the vulnerability database. Source: Prepared by the authors with support from the VOSviewer® software.

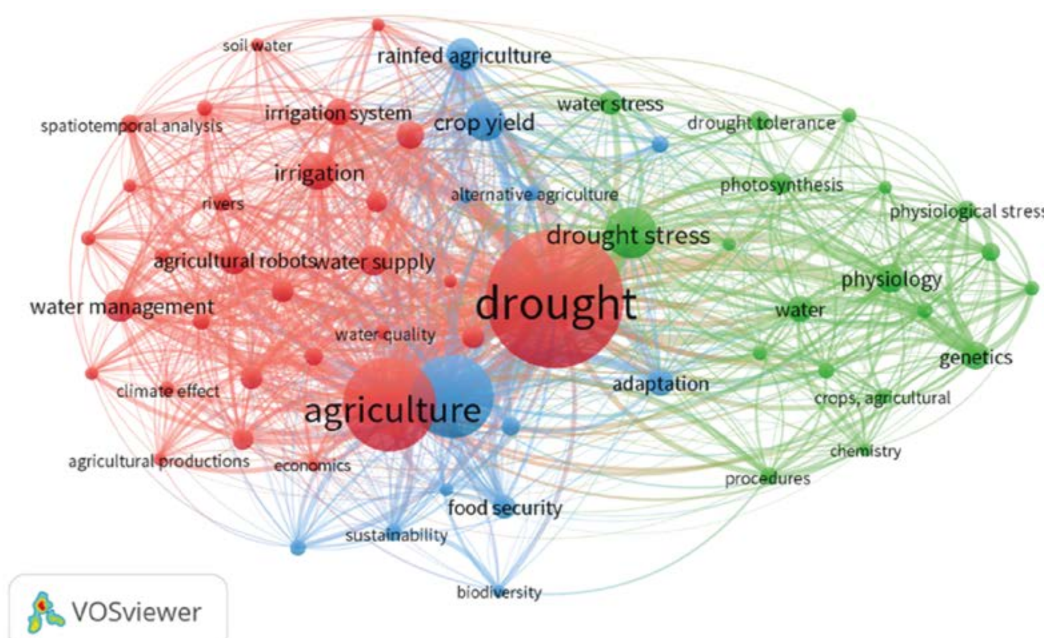

Figure 8. Co-occurrence network of the agricultural drought database. Source: Prepared by the authors with support from the VOSviewer® software.

Figure 8 shows that the principal node of drought is related with agriculture, climate change and stress from drought. These studies are developed with multidisciplinary approaches since the most outstanding node of drought is directly related with the effect of climate, economy, irrigation, water management, agricultural production, water qualities, supply, climate effect, and space-time analysis. Based on this, there is a great field to carry out studies with inter- and trans- disciplinary approach in order to generate a theoretical-conceptual background to cover more drought scenarios.

Finally, 41,965 citations were obtained for the word climate change and from the co-occurrence analysis it was seen that there is great affinity with drought and agriculture. However, there was not great affinity with the word vulnerability (Figure 9).

Figure 9 shows that the climate change node is related with drought, climate models, and biodiversity. However, when the vulnerability node is located, it is found far from the network, showing more relation with themes such as ecosystem service, agriculture and food security. One of the great attributes of this Vosviewer® tool is that it can identify the areas of opportunity for research development. Therefore, further research is suggested in themes of drought vulnerability in the context of climate change.

CONCLUSIONS

During the last ten years, few scientific studies have been developed on the vulnerability of agricultural drought in the context of climate change, which has been identified through the bibliometric analysis. The leading countries in the development of this theme are the United States, China and the United Kingdom. Therefore, it is an area of opportunity for researchers and scientific journals from Latin American countries so they can enter the ranking of the ten countries that address this global problem.

As strategy for Mexico to be strengthened as one of the main countries to develop this type of studies, as well as managing the financing that fund these studies, it is advisable to

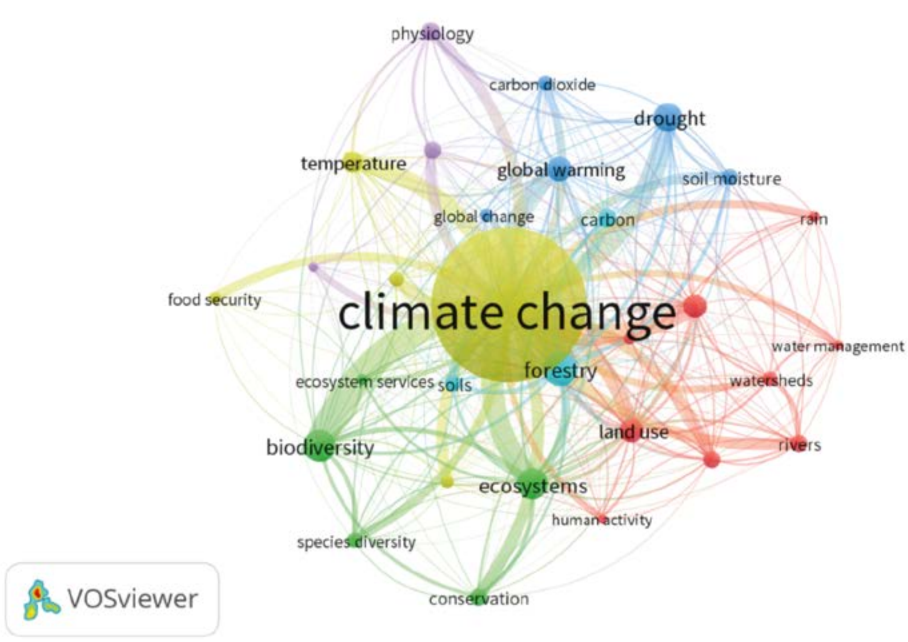

Figure 9. Co-occurrence network of the climate change database. Source: Prepared by the authors with support from the VOSviewer® software.

follow the trends from the United States, taking advantage of the relationship and binding monitoring that arises from the North America Drought Monitor. That is, if Mexico supports the recommendations in the practice about establishing the state of emergency from the occurrence of drought, but it also participates in scientific studies to gradually have a part in the global participation.

Presently, the theme of vulnerability from agricultural drought in the context of climate change integrates multidisciplinary studies such as earth and planetary sciences, biochemistry, genetics, molecular biology, computer sciences, engineering, neuroscience, economic and social sciences; therefore, it is advisable to reach inter- and trans- discipline and to broadly understand these concepts and problems.

As a challenge, it is considered that the periods of drought increase due to climate change, which is why it is necessary to carry out studies that delve into aspects such as vulnerability, resilience, and adaptation actions; in order to adopt practices and strategies to manage the water resource, and as consequence, to reduce the vulnerability in food production in the country without compromising the deterioration of the water resource, which is increasingly scarcer due to its high demand.

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Colegio de Postgraduados

Determination of production costs of vanilla (*Vanilla planifolia* Jacks ex Andrews) in Huehuetla, Puebla, Mexico

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ABSTRACT
Objective: To identify the production costs of vanilla, as a primary link within the value chain, to determine the profitability of the crop in Huehuetla, Puebla, Mexico.
Design/methodology/approach: The information was obtained from applying N=40 surveys to producers in nine communities, with the methodology of financial evaluation of agricultural projects in the long term.
Results: The diagnosis showed that vanilla is a highly profitable crop, indicating an average production cost of 0.1 ha^{-1} of $54,471.90 and the average total sale price of 0.1 ha^{-1} of $187,500.00 which has an impact on a high profitability (B/C R=2.54), even under conditions of small cultivation surfaces, in addition to farmers combining it with other perennial and annual species that generate income in the short and medium term to support families.
Limitations on study/implications: Vanilla producers are located within a region with characteristics of marginalization due to their geographic location, which makes access to communication difficult; they do not have a government agency to represent them, and therefore, the information of production costs is limited and dispersed.
Findings/conclusions: Vanilla is a crop of great productive potential in the Totonacapan region, and particularly in Huehuetla, due to their high commercial value. It is advisable to promote programs that favor its expansion with improvements in cultivation techniques that reflect higher yields, quality and commercialization to improve the standards of living of the farmers.

Keywords: vanilla, costs, production, profitability, value chain.

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INTRODUCTION

Vanilla (*Vanilla planifolia*) (Orchidaceae) is a tropical crop originally from Mesoamerica and very important commercially. Currently it is one of the most demanded products in the refreshment and culinary industry (Rocha *et al.*, 2018), considered one of the most profitable crops after saffron (Luis *et al.*, 2020). The distribution of vanilla has been reported since colonial times in Mexico, all of Central America, Jamaica, Tobago, Colombia, Venezuela, Guyana, Ecuador and Peru, with Mexico being the place where more studies have been conducted (Gamboa *et al.*, 2014).

Authors like Porras (2013) mention that this orchid is cultivated in many parts of the world, although, the main producing countries are Madagascar, Indonesia, China, Mexico and Turkey. Production of the vanilla with best quality in the world is in Mexico, in the region of Totonacapan, which includes 20 municipalities in the state of Veracruz and 19 in the state of Puebla with a contribution of 76.8% of the production. Its main use is in the food industry and in the cosmetic industry, among others (Damirón, 2004; OMPI, 2009; Díaz *et al.*, 2018).

In the National Agricultural Plan 2017-2030 in Mexico, an increase is estimated in the global demand of 7,307.14 ton to 10,515 t (accumulated growth of 43.9%), while the national production of vanilla has the ability of increasing from 512.78 to 856.71 t, which represents an accumulated growth of 67.07%. To convert vanilla into something useful and finally deliver the finished product to the consumer, it has to go through a process from the acquisition of the plant material, the crop's growth and development, incorporating inputs, cultural tasks and agronomic management, harvest, processing, packaging and commercialization of the product. In terms of production, given the importance of the first link of the production chain, it is necessary to understand which elements are integrated in its analysis process, taking into account key elements that are conjugated in their productive systems, specifically the cost factor, where its use today is not only for transformation and service businesses, but rather for the agricultural business or company (Molina, 2017).

Based on the criterion by Sánchez (2013), the production process is defined as "a series of steps and actions that people take with the help of tools and machinery to achieve the transformation of raw materials into finished products or providing services of a different nature". Therefore, the importance of knowing the profitability margin of a business demands having accurate records about the processes developed, that is, quantifying the activities so that accurate information can be available in quantitative terms, on which decision making could be sustained or supported (Melean and Torres, 2018). As for Ochoa (2015), the author mentions that production costs have become quite a transcendental variable for farmers, considering them as the fundamental basis for planning, control and decision making.

In terms of agricultural production cost, it is conceptualized as the cost in which they incur to convert seeds and inputs into finished products (Molina *et al.*, 2009); the author describes the basic elements (direct raw material, direct labor, and indirect production costs) as a fundamental part for the quantification of costs. Barrera *et al.* (2011) and Espinosa *et al.* (2015) suggest that it is necessary to conduct studies about profitability and productivity for decision makers, as well as for producers; therefore, performing an analysis of technical and economic viability of an agricultural activity is important. Therefore, the objective was to identify the production costs of vanilla as primary link within the value chain to determine the profitability of the crop in Huehuetla, Puebla, Mexico.

MATERIALS AND METHODS

The study was carried out in Huehuetla, Puebla, which benefits from the denomination of origin of vanilla (20° 02' 10" N and 97° 35' 97" W) of the Northeastern Sierra in Puebla, located at an altitude of 517 masl, in the climate transition zone of the temperate Northern

Sierra to the warm zone of the Gulf slope, with a single climate that is semi-warm sub-humid with rain throughout the year, and where there are around 18,803 inhabitants (SNIM, 2015). Huehuetla is eminently rural, reporting that 89% of the population, in addition to having a broad traditional agricultural knowledge, conserves the native language, which is the dialect variant known as Totonaca from the Sierra (Mackay and Trechsel, 2015). It is one of the rural populations characterized by presenting a very high degree of marginalization, and currently it occupies the first place in the state (SNIM, 2015).

The target population was made up of n=40 farmers, based on the list of beneficiaries from the program "Productive reconversion for crops of high commercial value" of the Ministry of Rural Development in the state of Puebla. Through the key informants, the nine communities devoted to vanilla production were located: Cinco de Mayo, Putlunichuchut, Kuwikchuchut, Xonalpu, Huehuetla, Leacaman, Putaxcat, Lipuntahuaca and Chilocoyo El Carmen. A study with qualitative and quantitative approach was conducted. The technique used for the application of the instrument in the field is sampling by reference chain or snowball (Mendieta, 2015). The field work was carried out in the month of May, 2022, and consisted in the application of a structured survey for the diagnosis of the vanilla production system in the municipality, specifically the production costs emphasizing the cost of preparing the land (cleaning, cutting or felling trees, weeding, establishing tutor trees, treating soil or substrate, and boxing), establishing the crop (planting, cuttings, replanting, organic and inorganic fertilizers, pest and disease control, and live tutors), farming tasks (tying cuttings, safety pruning, pruning for vines and tutors, channeling, pollination, and manual weeding), organic and inorganic fertilization, application of insecticides and fungicides, irrigation, harvest, gathering and commercialization.

Each of the members of the sample was surveyed directly on his plot with the aim of corroborating and complementing the information from the interview respondents. The profitability of the crop was determined by applying the methodology of financial evaluation of agricultural projects in the long term (Gittinger, 1982). In the financial evaluation, the flow of production costs (*CF*) and flow of income (*IF*) were estimated for a time horizon of 10 years, since this is the period when the plantation achieves stable production. To estimate production costs, the following formulas were applied (Espinosa *et al.*, 2015):

$$FC = TCoE_1 + \left(\sum_{i=1}^{N=10} FPCo + VPCo \right)$$

Where: *TCoE*=Total cost of establishment of the plantation on year 1, calculated by the sum of the amount of inputs used for the establishment of a surface of 0.1 ha of vanilla by its respective market average in 2021; *FPCo*=Fixed production cost of year 1 to year 10, calculated by the sum of the depreciation costs of the plantation (*TCoE* minus the rescue value, divided by the 10 years of useful life); and *VPCo*=Variable production cost from year 1 to year 10, calculated by the sum of the different amounts of inputs for the operation and maintenance of the plantation for its respective average market price. The Income flow was estimated using information about the sale price of the vanilla producer (*Px*) and the yield obtained from the pod (*Rx*) in a surface of 0.1 ha. Mathematically, it is obtained with the following formula:

$$FI = \left(\sum_{i=1}^{N=10} Px * Rx \right)$$

The production and income costs correspond to the useful life of the project, which is why they were taken to present value by applying an updating rate of 12%. Finally, the Net Present Value (NPV), the Internal Return Rate (IRR) and the Benefit-Cost Rate (B/C R) were estimated.

To compile data, an Excel registry format was designed on the costs of establishment and the costs of maintenance for a surface of 0.1 ha of vanilla; likewise, information was gathered about the sale prices of the producer and yield for that surface.

RESULTS AND DISCUSSION

The producers surveyed have surfaces of less than 0.5 ha, which is why 95% have surfaces of 100 to 1000 m^2, which agrees with what experts from Universidad Autónoma Chapingo, Colegio de Postgraduados and the Sistema Producto Puebla describe, who mention that 79% of the vanilla producers from the Totonacapan region devote surfaces of less than 0.5 ha (smallholding) for vanilla production (Santillán *et al.*, 2019). Of the farmers, 93% sow under rainfed conditions and 100% have the type of land tenure of private property, which is a result similar to that obtained by Torres (2020), where the author mentions that in the municipality of Huehuetla, land tenure obeys the scheme of private smallholding, reason why some peasants opt for having lands for other basic crops such as corn and bean.

The vanilla production system in Huehuetla shows that 77% manages the crop using the *cocoite* tutor (*Gliricidia sepium*) intensively. Under this system of high-density plantation, the distance between tutors recommended for plantations is 2×1 m, and two cuttings per tutor, so that the plant density is 1000 plants 0.1 ha^{-1}, compared to the production system in shade mesh characterized by having 1862 cuttings 0.1 ha^{-1} on average compared to another mean of 123 cuttings 0.1 ha^{-1} in production systems under orange trees (Barrera, 2009). As Sánchez (2001) mentions, the number of tutors in a vanilla plantation will depend on the tutor canopy, since the regulation of shade is very important, so that the vanilla is left exposed to the sun in 50%. Of the farmers, 75% use the commercial variety that is *Vanilla planifolia* J., as mentioned by Herrera *et al.* (2022) who describe that vanilla production in Mexico is through *V. planifolia*, with the Totonacapan region being one of the most profitable.

This orchid is planted in the spring-summer cycle and fall-winter cycles under the system mentioned before. Land preparation for sowing is diverse, it takes place in two periods or dates; it begins with weeding, cleaning, in the months of January and March. After these activities, cutting or felling trees is done, the establishment of live tutors, the treatment of land or substrate, some cleaning activities and some other task to prepare the land, although all the farmers do not necessarily carry out the activities or in the order mentioned.

Once the land is in optimal conditions for the establishment of the cuttings and their good growth and development, there is a period of four months for sowing, which happens in the months of May and August. An activity called auxiliary irrigation is carried out, generally performed in the flowering season or in the month of May when the temperature is intense and higher than 35 °C.

In the study region, only chemical fertilization is applied based on the formulation N46-P00-K00 (urea). In the case of organic fertilization, it is based on the use of organic fertilizers such as bokashi (1 kg plant^{-1}) and manure (1 kg plant^{-1}) applied each year.

Weed management in vanilla cultivation is of great importance, and their control is carried out since the moment when the crop is established, twice per year. Among the main weeds found there are *Simsia amplexicaulis* (*acahual*), Commelinaceae (*oreja de ratón*), *Ipomoea purpurea* (*quiebraplatos*) and *Bidens odorata* (*aceitillo*). Regarding the impact of pests and diseases, there are *Tentecoris confusus* (red bug) and diseases from *Fusarium oxysporum* (basal rotting), *Puccinia* sp. (rust), and *Colletotrichum* spp. (canker), which agrees with Mata *et al*. (2007) cited by Barrera *et al*. (2011), who mention that the red bug, *Fusarium*, and canker are the most common in the vanilla crop, so farmers resort to applying chemical and natural products for their prevention and control such as copper sulfate, cypermethrin pyrethroid, calcium hydroxide, extract of chili pepper, onion, garlic and soap (FAOSTAT, 2017).

The harvest is carried out during the fall, once the pods reach their state of physiological maturity; the indicator to perform this activity is when the apex or tip of the fruit changes from a green to a yellow color, description that is similar to what was exposed by Cervantes *et al*. (2019), and it happens six or eight months after pollination (March and May). The harvest is carried out manually and up to two workdays are considered for this activity. The production costs for the establishment of vanilla under the production system in cocoite (*Gliricidia sepium*) and in high densities with the variety *Vanilla planifolia* Jacks Ex Andrews were $48,920.00, including auxiliary irrigation which is applied four times per month with a total of 48 irrigation events per year, tools and other activities of workforce (Table 1).

Table 1. Initial production costs and maintenance of the *Vanilla planifolia* Jacks Ex Andrews crop produced with cocoite tutors in high densities in Huehuetla, Puebla.

Concept	Vainilla
Establishment of the plantation	$ 0.1 ha^{-1}
Module maintenance	$ 48,920.00
Variable costs	$ 49,378.90
Fixed costs	$ 5,093.00
Costs of production	$ 54,471.90

Source: Prepared by the authors with information obtained in the field 2022.

On average, the total production cost to produce vanilla in a surface of 0.1 ha is $ 54, 471.90, where all the activities or practices recommended for the management of vanilla are contemplated.

To estimate the Income flow, the yield and sale price of the green pod was required. Under the production system in high densities with *Gliricidia sepium* tutors, the yield was 250 kg, which contrasts with the research carried out by Barrera *et al*. (2011) who evaluated the shade mesh system and estimated a yield of 100 kg on a surface of 6000 m^2. The increase in yield is one of the indicators of the crop's productivity, but when the unitary cost is analyzed this is confirmed, as shown in Table 2. For a farmer, it costs $191.2 to produce one kg of green vanilla, which means that the yield obtained allows covering the production costs. The gross income obtained was $187,500.

Regarding the indicators of the profitability of the vanilla crop, under this production system in high densities under the cocoite tutor (*Gliricidia sepium*), a NPV higher than zero was obtained, which indicates that the vanilla production generates profits, so it can be deduced that it is a good investment since it generates economic benefits. The IRR was higher than the discount rate of 12%, which indicates that the investment is convenient and profitable (Table 3).

To calculate the profitability, it is necessary to take into account the benefit/cost (B/C) rate as indicator of its economic viability. In this sense, the profitability analysis of the vanilla crop revealed that the B/C rate was 2.54 (Table 3), so that vanilla production in Huehuetla is considered a crop of commercial value, highly profitable, since the investment is recovered and there is a result of nearly double of the profits compared to the investment made; that is, for each peso that is invested in this crop, a utility of 1.54 is expected.

Table 2. Unitary cost and income from vanilla in the municipality of Huehuetla, Puebla.

Variable	Vanilla
Unit cost ($ 0.1 t)	192.2
Yield (kg 0.1 t^{-1})	250
Sales price ($ kg)	750
Gross income ($ 0.1 t^{-1})	187,500
Net income ($ 0.1 t^{-1})	139,450

Source: Prepared by the authors with information obtained in the field 2022.

Table 3. Indicators of profitability and economic feasibility of the vanilla crop in Huehuetla, Puebla.

Indicator	Vanilla
GO	515,391
B/C ratio	2.54
IRR (%)	88

Source: Prepared by the authors, 2022.

According to Borja *et al.* (2016), when the benefit/cost rate is higher than the unit, it indicates that there is profitability. Other authors such as Herrera *et al.* (2022) mention that the value of the vanilla value chain is important both in general and in the profitability of each link, so it is an opportunity in employment and welfare for local communities.

CONCLUSIONS

Vanilla is a crop of very high productive potential in the Totonacapan region, and quite importantly in the municipality of Huehuetla, which has the denomination of origin in addition to its great diversity and uses, and because it is a crop with high commercial value, it is a profitable alternative for the farmers. It is recommended to firmly support with programs that promote its expansion, to support the development of strategies and technologies that improve cultivation techniques which result in higher yields and better quality, and to ensure a commercialization that benefits producers in these vanilla-producing zones of the northeastern region of the state of Puebla and surrounding area, in order to detonate regional development through crops that are potentially profitable such as vanilla.

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Colegio de Postgraduados

Effect of agronomic characteristics of hybrid and Creole corn using native plant growth-promoting bacteria to reduce the production cost

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ABSTRACT

Objective: To design and to evaluate an environmentally-friendly biofertilizer based on plant growth-promoting bacteria (PGPB), assessing the agronomic characteristics of two genotypes of C/Acceleron A7573 hybrid and Creole corn.

Design/methodology/approach: A biofertilizer based on PGPB was designed and assessed in a completely random experimental block design with nine treatments and four repetitions in *C/Acceleron A7573* hybrid and Creole corn in a plot at El Pericón, municipality of Tecoanapa, Guerrero, Mexico. The microorganisms *Rhizobium* sp., *A. brasilense* and *A. vinelandii* were used.

Results: The use of PGPB has greater effectiveness in all the agronomic variables and better yields because they are adapted to the environmental and soil conditions, with it being an excellent alternative to the use of fertilizers.

Limitations on study/implications: The demonstrative experimental plot had 5000 m^2 and it was the main limitation.

Findings/conclusions: Bacteria of the genus *A. brasilense* YOM9 and *A. vinelandii* YOC4 contributed to the higher yield of the *C/Acceleron A7573* hybrid corn seed, and *Rhizobium* sp. R01 and *A. vinelandii* YOC4 in the Creole grain of the olotillo race compared to the T9 fertilizer and the absolute control. A biofertilizer for corn is obtained based on results from this study, as an ecotechnology based on PGPB.

Keywords: Corn, plant growth-promoting bacteria, biofertilizer.

INTRODUCTION

Corn (*Zea mays* L.) is the most important agricultural crop in the Mexican economy and the most consumed cereal for the diet; it is produced for subsistence by farmers in rural communities. Corn occupies more productive surface than any other crop; although it is cultivated in more than 160 countries, Mexico stands out among the eight countries that concentrate 75% of the total global consumption (Haytowitz *et al.*, 2018). In Mexico, corn is cultivated annually in nearly 8,500,000 ha, which produce around 24 million tons, while the annual demand is around 28 million tons (FIRA, 2020; SIAP, 2020). The Agrifood and Fishing Information Service (Servicio de Información Agroalimentaria y Pesca, SIAP) informed that Guerrero destines a cultivated surface annually of around 481,523.05 ha, 96% under rainfed conditions with 85% of native seeds, with annual state production of around 1,271,850.89 t (SAGARPA, 2018).

Presently, Mexico faces serious production problems due to low fertility and loss of soils that are becoming exhausted and making the production of crops of economic interest more expensive, derived from the excessive use of fertilizers and other agrichemicals (Reyes *et al.*, 2018), which affect the environment negatively.

These problems entail the need to seek efficient alternatives that ensure the sustainable production of the grain, through the application of biofertilizers or bioinnoculants that contribute in a high percentage to the development, nutrition, improvement and fertility of the soils, reducing the use of synthetic fertilizers in up to 50% (FAO, 2019). With great environmental and social impact, thanks to the reduction of costs and the balance in agroecosystems (Reyes *et al.*, 2018), the advantages attributed to the PGPB whose ability to fix atmospheric nitrogen, solubilize phosphate and produce phytohormones undoubtedly favor good results in growth and development. The main objective of this study was to design and to evaluate a biofertilizer that is environmentally friendly with PGPB from the genera *Rhizobium* sp., *A. brasilense* and *A. vinelandii*.

MATERIALS AND METHODS

The demonstrative plot is located in the community of El Pericón, municipality of Tecoanapa in the region of Costa Chica, Guerrero, Mexico (Figure 1) (INEGI, 2019).

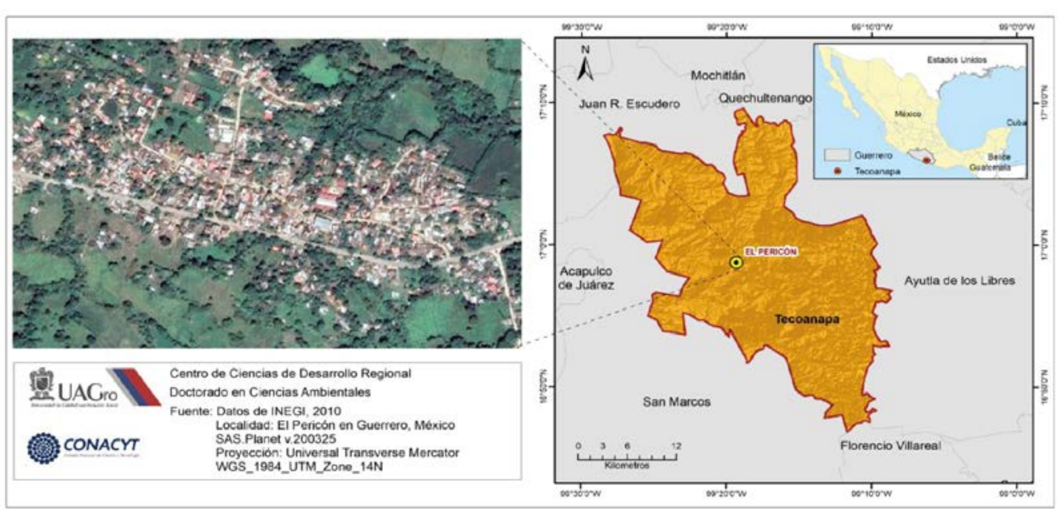

Figure 1. Geographic location of El Pericón, Municipality of Tecoanapa, Guerrero (INEGI, 2019).

Analysis of soil, genetic material, experimental design and economic analysis

The soil was analyzed physicochemically according to number 7 of the regulation NOM-21-RECNAT-2000, which establishes the specifications of fertility, salinity and classification of soils.

The corn genotypes used were the Creole race Olotillo and the *C/Acceleron A7573* hybrid. The experiment was established based on the completely random blocks with four repetitions with the following dimensions: 5 m of length by 0.81 m of width with nine furrows per block.

Experiment estabishment. The experiment was conducted in the period of February to July, 2021. Sowing was done with three seeds per sowing site at 0.40 m, and from these three, it was adjusted to two plants per shrub during the stage of vegetative growth (VG) that included 25 plants per treatment and 225 plants per block, equivalent to 900 plants per genotype of corn in four repetitions.

Farming activities in the experiment. Irrigation was conducted by gravity every 48 h, according to the hydric requirement of the plant. During the phenological stage of vegetative growth (VG), 1200 mL were added; in stage V1-V3 the amount was increased to 2400 mL of water; and finally 8400 mL of irrigation was applied in stage V7 to R1. Weed control was done manually.

Description of the bacteria strains. The strains that were used were donated by the biobank of the Laboratory of Molecular Microbiology and Environmental Biotechnology of the School of Chemical-Biological Sciences at the Universidad Autónoma de Guerrero, reactivated in NYDA agar and incubated at 37 °C during 24 h. The previously isolated strains of corn root, reed, bean nodules and rhizosphere, were confirmed *in vitro* as PGPB and labeled as: *Rhizobium* sp., R01, *A. brasilense* YOM9 and *A. vinelandii* YOC4.

Treatments

Combinations of the following treatments were made: T1 (Absolute control), T2 (*Rhizobium* sp. R01), T3 (*A. brasilense* YOM9), T4 (*A. vinelandii* YOC4), T5 (*Rhizobium* sp. R01 + *A. brasilense* YOM9), T6 (*Rhizobium* sp. R01 + *A. vinelandii* YOC4), T7 (*A. brasilense* YOM9 + *A. vinelandii* YOC4), T8 (*Rhizobium* sp. + *A. brasilense* + *A. vinelandii*), T9 (chemical fertilizer).

Table 1. Identification and qualitative characterization of 6 strains from corn root, bean, reed and rhizosphere.

Strain No.	Identification	FBN	Solubilization (PO_4^{-2})	Production (AIA)	Production (Gibberellins)	Production (enzymes)
R01		+++	+++	+++	+++	+
AYM9		+++		+++	+++	+
YOC4		+++	+++	++	++	++

+++: High concentrations of Auxins, Gibberellins, IS, FBN and PE.
++: Moderate concentrations of Auxins. Gibberellins, IS, FBN and PE.
+: Minimal concentrations of Auxins, Gibberellins, IS, FBN and PE.
-: Without production of Auxins, Gibberellins, IS, FBN and PE.

Preparation of inoculates

The inoculates were prepared in solid based on molasses at 2% at a concentration of 1-2$\times 10^8$ UFC/mL of each bacterial strain in nutritional agar (MCD LAB). In addition, the following inputs were used: two kilos of rice at a volume of 30 L (2 kg of sterile rice, 40 mL of molasses, 10 mL of saline solution at 0.9% and 100 mL of each concentrate). The final inoculates were left resting during three days for the strains to activate metabolically (Orbe-Díaz *et al.*, 2020).

Inoculation of plants and variables evaluated

The first inoculation was made 20 days after sowing of the Creole (olotillo race) and *C/Acceleron A7573* hybrid corn genotypes, and for that purpose deposits of 20 L were used per treatment; with the support of a graduated cylinder of 250 mL, the stem base of each corn plant was inoculated, following the same procedure, and inoculations were carried out at 20, 34, 48 and 62 d with the same conditions and concentrations of PGPB.

The chemical fertilizer N80-P23-K15 and N10-P46-K30 was added to the side of the stem base of the corn plant (Martínez-Reyes *et al.*, 2018). Per treatment, 25 plants were selected to measure plant height (PH), stem diameter (SD), and leaf area (LA). The harvest of cobs was done at 135 days, and then data of the number of rows per cob (NRC) and cob length (CL) were taken, and the grain yield (GY) was estimated.

The data recorded and ordered were processed through analysis of variance and Tukey's test at 0.01% of probability through the support of the statistical software (SAS).

Economic analysis

The data recorded in the irrigation experiment logbook were subjected to analysis with the CEZACA software (Carpio *et al.*, 2022).

RESULTS AND DISCUSSION

The physicochemical properties of the soil in the study region are loam-clay-sand with evident deterioration that affects the grain yields due to bad practices. The soil analysis of the sample PF2 is loamy sandy texture, with a moderately acid pH of 5.64; according to López-Báez *et al.* (2019), corn adapts to all types of soils, where the optimal value of pH is 6.0 to 7, the soil presents Electric Conductivity 1:2 (H_2O) (mS/cm) of 0.08 free of salts, Electric Conductivity in Saturation Extract (mS/cm) 0.31 free of salts and is moderately rich in organic matter with 1.68; based on the NOM-021-RECNAT-2000 (Table 2).

In this sense, López-Báez *et al.* (2018) reported 64% of the soils with evident deterioration, among them generalized exchangeable acidity and a pH of 5.2 that evidences the need to design strategies that are environmentally friendly with the aim of increasing the productivity and the profitability in corn crops under sustainable agriculture. One year later, López-Báez *et al.* (2019) related the amendments in organic matter characteristic of the region as an alternative for degraded soils in response to the bad practices of burn, tilling and grazing and the non-incorporation of other sources of microorganisms to nourish the soils, as seen in the results from the soil analysis of the El Pericón sector (Table 2).

Table 2. Results from the physicochemical analysis of El Pericón sector (PF2).

<table>
<tr><th rowspan="2">Sector</th><th rowspan="2">Sample</th><th colspan="3">Texture</th><th rowspan="2">Textural clss</th><th rowspan="2">pH</th><th rowspan="2">C.E ds/m</th><th rowspan="2">M.O. %</th></tr>
<tr><th>Ao</th><th>Li</th><th>Ar</th></tr>
<tr><td>Pericón</td><td>PF2</td><td>19.64</td><td>15.64</td><td>64.72</td><td>Sandy loam</td><td>5.64</td><td>0.08</td><td>1.68</td></tr>
<tr><td colspan="3">Phosphorus (Bray) (mg/kg)</td><td colspan="2">ppm. 72.17</td><td colspan="4">Sample level: Medium</td></tr>
<tr><th colspan="9">Cations (+)</th></tr>
<tr><th colspan="3">Elements</th><th colspan="3">μg/ml</th><th colspan="3">Interpretation</th></tr>
<tr><td colspan="3">Sodium (Na^{+})</td><td colspan="3">0.07</td><td colspan="3">Under</td></tr>
<tr><td colspan="3">Potassium (K^{+})</td><td colspan="3">0.14</td><td colspan="3">Medium</td></tr>
<tr><td colspan="3">Calcium (Ca^{2+})</td><td colspan="3">6.98</td><td colspan="3">Medium</td></tr>
<tr><td colspan="3">Magnesium (Mg^{2+})</td><td colspan="3">1.63</td><td colspan="3" rowspan="2">Moderately low</td></tr>
<tr><td colspan="3">Catión Exchange Capacity</td><td colspan="3">8.82</td></tr>
<tr><th colspan="9">Microelements</th></tr>
<tr><th colspan="3">Elements</th><th colspan="3">μg/ml</th><th colspan="3">Interpretation</th></tr>
<tr><td colspan="3">Iron</td><td colspan="3">136.41</td><td colspan="3">Moderadately high</td></tr>
<tr><td colspan="3">Zinc</td><td colspan="3">2.12</td><td colspan="3">Medium</td></tr>
<tr><td colspan="3">Copper</td><td colspan="3">0.57</td><td colspan="3">Under</td></tr>
<tr><td colspan="3">Manganese</td><td colspan="3">2.82</td><td colspan="3">Very low</td></tr>
<tr><td colspan="3">Boron</td><td colspan="3"><0.02</td><td colspan="3">Very lowo</td></tr>
</table>

Figure 2. Demonstrative plot. A) Start of cultivation, B) Irrigation, C) Fertilization and D) Biofertilization.

The basic elements (Na, K, Ca and Mg) present low, medium and moderately low viability in the soil analysis with 0.07, 0.14, 6.98 and 1.63 for Na, K, Ca and Mg, respectively, as shown in Table 2, based on the NOM-021-RECNAT-2000. In terms of micronutrients (Fe, Zn, Cu, Mn and B), iron and zinc presented adequate levels compared to copper, manganese, and boron which show deficient and marginal levels (Table 2).

Physical-chemical analysis according to numeral 7 of NOM-021- RECNAT-2000, which establishes the specifications of fertility, salinity and soil classification, El Pericón sector (PF2).

Throughout the experiment, a minimum temperature of 17 to 21 °C was recorded, and a maximum of 36 to 38 °C.

Agronomic variables of both corn genotypes

The analysis of variance detected statistically significant differences for the agronomic variables stem height and diameter between treatments ($p \leq 0.01$) (Tabla 3). It was seen that treatments T4 (*A. vinelandii* YOC4), T2 (*Rhizobium* sp. R01), and T9 (chemical fertilization) showed greater consistency in terms of PH, while the lowest height was obtained from the absolute control.

After analyzing the statistically significant data ($p \leq 0.01$) through Tukey's test (Table 3), it was found that treatments T3 (*A. brasilense* YOM9), T2 (*Rhizobium* sp. R01), and T8 (*Rhizobium* sp. R01 + *A. brasilense* YOM9 + *A. vinelandii* YOC4) showed greater consistency in terms of SD and the smallest stem diameter was obtained in T1 (absolute control); in terms of LA, the treatments T2 (*Rhizobium* sp. R01), T6 (*Rhizobium* sp. R01 + *Azotobacter vinelandii* YOC4), and T8 (*Rhizobium* sp R01 + *A. brasilense* YOM9 + *A. vinelandii* YOC4) showed better results compared to T1 (control); in the case of the CL, the treatments T2 (*Rhizobium* sp. R01), T6 (*Rhizobium* sp. R01 + *A. vinelandii* YOC4), and T8 (*Rhizobium* sp. R01 + *A. brasilense* YOM9 + *A. vinelandii* YOC4); and lastly, in NRC, treatments T2 (*Rhizobium* sp. R01), T4 (*A. vinelandii* YOC4), and T6 (*Rhizobium* sp. R01 + *A. vinelandii* YOC4).

Treatments T2, 3, 4, 6 and 8 showed greater biological effectiveness in corn growing when they were inoculated based on native PGPB, since they produce adhesive substances, excretion of substances that promote the growth and development of the plant, and indirect mechanisms, which agrees with Caiza *et al.* (2019). Creole corn (olotillo race) exceeded the plant height in comparison to the *C/Acceleron A7573* hybrid genotype. Authors like Zulueta-Rodríguez *et al.* (2020) showed statistical significance in plant height after confirming notable differences in their treatments with *A. brasilense*, catalogued as PGPB with great impact within sustainable agriculture due to the multiple promotion mechanisms attributed to them. However, Yousef *et al.* (2019) reported that *Rhizobium* sp. is associated to grasses as endophytes, and therefore, when the cereals are inoculated with PGPB they accumulate the nutrients P, K, Ca, Mg and even Fe.

Peréz-Peréz *et al.* (2021) reported that the inoculation of *Rhizobium* sp., in its 20 treatments, evidence that the height of the corn plants was statistically higher than the control in four out of the five variables, which is related to the mechanisms of plant growth promotion that they show. Therefore, a sustainable biofertilizer based on these bacteria genera is undoubtedly one efficient alternative for the producers. Caiza *et al.* (2019) reported statistically significant differences in the leaf area when inoculating PGPB of the genera *Azotobacter* and *Azospirillum* and, therefore, they conclude that the advantages from PGPB play an important role in the crops.

Pérez-Pérez *et al.* (2021) evaluated PGPB in corn and reported a significant increase in the stem diameter and root length so they highlight the importance of applying biofertilizer.

The analysis of variance and Tukey's test on the number of lines per cob showed significant differences between the *C/Acceleron A7573* hybrid and the Creole-olotillo, according to what is reported by Martínez-Reyes *et al.* (2018), who point out that the treatments based on *Azotobacter* sp., *A. brasilense* + *C. violaceum* + TQ 160-46-30, A. *brasilense* + FQ 80-23-15 increase the number of lines per cobs, evidencing the biotechnological potential of PGPB.

In the case of the results of cob length, the effectiveness of the PGPB is confirmed in this study compared to what was reported by Martínez-Reyes *et al.* (2018), where they quantified the best averages and determined that *Azospirillum* exerts a better effect since they attribute the capacity to fix nitrogen and the production of gibberellins, while the control shows a shorter length (Table 3). For their part, the purpose of Jaraba *et al.* (2020) of using *Rhizobium* sp., *A. brasilense* and *A. vinelandii* as biofertilizers was to substitute up to 60% the nitrogenous fertilizers, increasing the production, yields and cost-benefit rate, showing that their application replaces 20 to 60 kg N ha^{-1}. The results of this study give an advantage to future research to adopt sustainable agriculture with the aim of reducing the dependency of fertilizers in up to 50% using biofertilizers based on native PGPB.

Grain yield

The results point to a significant increase among corn genotypes, where the *C/Acceleron A-7573* hybrid presented higher grain yield of 7.69 t ha^{-1} with treatment T7 (*A. brasilense* YOM9 + *A. vinelandii* YOC4), and of 7.21 t ha^{-1} with T6 (*Rhizobium* sp. R01 + *A. vinelandii* YOC4); concerning the Creole corn, the yield of 4.48 t ha^{-1} with treatment T6 (*Rhizobium* sp. R01 + *A. vinelandii* YOC4) and of 4.16 t ha^{-1} with treatment T2 (*Rhizobium* sp. R01); and the lowest production for both genotypes was obtained in T1 (Table 4).

According to Beltrán-Pineda *et al.* (2022), the problems faced in farming regarding the excessive use of chemical fertilizers has given place to researchers seeking alternatives that are environmentally friendly with the aim of decreasing the dependency of producers on these toxic inputs, and this agrees with López-Báez *et al.* (2018), who report the effect of *Azotobacter* sp. on corn and the excellent results in the grain yield of 5.88 and 5.65 k ha^{-1} respectively.

Results show that *Rhizobium* sp., *A. brasilense* and *A. vinelandii* increase the yield, attributable to a greater absorption of nutrients.

Yousef *et al.* (2019) obtained similar results with a yield of 33.78 t ha^{-1} using *Rhizobium* sp., *A. brasilense* and *A. vinelandii*, attributing them as a sustainable alternative of biofertilizer for corn. In the same year, Hernández-Reyes *et al.* (2019) obtained grain yields of 1.79 and 2.07 t ha^{-1} attributable to *Azospirillum* sp., while Ayvar-Serna *et al.* (2020) obtained yields

Table 4. Corn grain yield expressed in t ha^{-1}.

Grain yield of hybrid genotype A7573 C/Acceleron and irrigated landrace-Olotillo.		
Treatments	**A7573 C/Acceleron Hybrid**	**landrace-Olotillo**
T1 Absolute witness	2.2 b	1.6 c
T2 *Rhizobium* sp. R01	**6.9 a**	**4.1 a**
T3 *Azospirillum* M9	5.4 a	3.4 b
T4 *Azotobacter* C4	5.7 a	3.5 b
T5 *Rhizobium* sp. R01 + *Azospirillum* M9	6.1 a	3.5 b
T6 *Rhizobium* sp. R01 + *Azotobacter* C4	**7.2 a**	**4.4 a**
T7 *Azospirillum* M9 + *Azotobacter* C4	**7.6 a**	**3.4 b**
T8 *Rhizobium* sp. + *Azospirillum* M9 + *Azotobacter* C4	6.6 a	3.8 a
T9 FQ 21-00-24 y 46-00-00	5.0 b	2.4 b
Media (Tukey = p≤0.01)	**5.9147 a**	**3.4034**

MH=maize hybrid A7573 C/Acceleron irrigation; MC=maize criollo-olotillo irrigation. Values are expressed as means ±SD for each treatment of the independent experiment. One-way ANOVA, Tukey's multiple comparison test; different letters in the bars indicate significant differences between experiments (P≤0.01).

of 14.13 Mg t ha^{-1} in DK357 hybrid corn, based on biofertilizers with *G. intraradices* + *A. brasilense*, and therefore, they conclude that using biofertilizers based on microbial consortiums increase the grain yields from 5 to 30%; thus, it is a field of opportunities focused on improving the soils with similar or better yields than those obtained with the use of fertilizers.

González-Mateos *et al.* (2018) relate the positive effect of microorganisms in corn grain yield with the capacity for atmospheric nitrogen fixation, solubilization of phosphates and production of phytohormones. Reyes *et al.* (2018) showed that *A. brasilense* and *A. vinelandii* strengthen the grain yield and, therefore, the results from this study agree slightly with Pérez-Luna *et al.* (2021) and Beltrán-Pineda *et al.* (2022).

This corroborates the effectiveness and the impact that PGPB have on the development of biofertilizers and the fundamental role they play within sustainable agriculture.

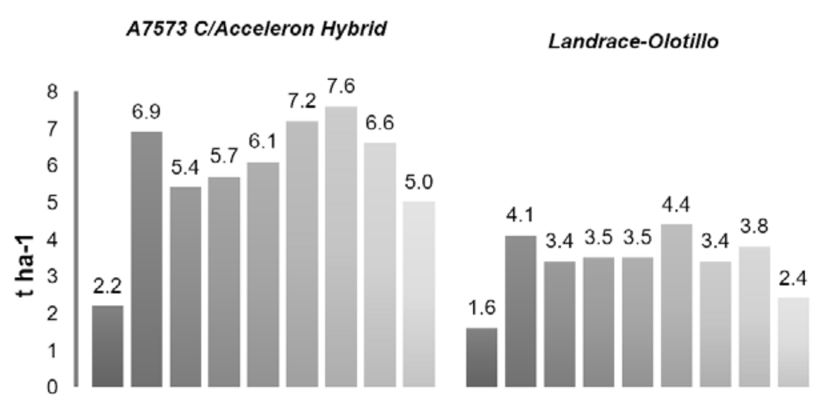

Figure 1. Corn grain yield assessed in nine treatments.

It is fundamental to specify that irrigation systems for rural development increase 60% of the territory destined to irrigation farming, since the superiority in yields of t ha^{-1} in comparison to rainfed farming is attributable. Therefore, the SHCP (2019) stated that the national average yield with irrigation is 7.5 t ha^{-1} and with natural precipitation it is 2.2 t ha^{-1}, which agrees with what was reported in this study, where yields of 7.6 t ha^{-1} were obtained using PGPB. Instead, Zepeda *et al.* (2021) state that irrigation farming generates more surfaces cultivated since a decisive factor in production is controlled and, therefore, the profits for small-scale and large-scale farmers increase.

Economic analysis for hybrid and Creole corn production

The results from the profitability analysis indicate that the treatments that contributed most to the grain yield are profitable for decision making, in the case of the *C/Acceleron A-7573* hybrid genotype with treatments T7 (*A. brasilense* YOM9 + *A. vinelandii* YOC4) and T6 (*Rhizobium* sp. R01 + *A. vinelandii* YOC4), with 7.69 t ha^{-1} and 7.21 t ha^{-1} of corn, respectively.

In this sense, the analysis indicates the total production cost of irrigation corn as $14,559.00, where the net income was $24,678.00, which is higher than the total cost, giving positive results of profitability by reflecting significant profits in grain production and yield. It should be highlighted that the sale price in the production cycle was stable and it remained at $18.00 L^{-1} with a production reached of 75. 341 (Table 5).

An important piece of data reflected in the analysis is the free net profit of $10,219.00, which is positive for the producers since it reflects their salary as worker within the production costs. The sum of profits is analyzed based on the production cycle in the six months, giving as a result a monthly profit of $1,703.00. This indicator of profitability is of utmost importance for the farmers in decision-making for their crops (Table 5).

Meanwhile, Ayvar-Serna *et al.* (2020) conducted a profitability analysis between corn genotypes, chemical NPK fertilization and biofertilizers, where they observed an increase per peso invested in the IT, IN and GPI obtained which fluctuated from $2.00 with NPK fertilization to $2.91 with biofertilizers (*G. intraradices* + *A. brasilense*). In comparison, Zepeda *et al.* (2021) conducted a study in irrigation corn and reported that their profits in irrigation exceed the profits from rainfed farming, from the production costs that have lower social and environmental cost, but the importance lies in that income with irrigation is a trigger that favors the region's economy due to the demand for goods and services.

However, Carpio *et al.* (2022) reported through an economic analysis the results from their experiment based on three corn genotypes, using a biofertilizer and a nitrogenated fertilizer, where they highlighted that for each investment they recovered what they invested despite the excessive costs of fertilizer and inputs. It is important to mention that corn farming is risky because of the excessive prices in the market and the social, environmental and economic problems that are attributed to the grain. Therefore, it is important to carry out a profitability analysis with the goal of generating important income and adopting new environmentally-friendly ecotechnologies.

Table 5. Cost structure and profitability indicators.

Concept	Unit	Quantity	Cost structure (%)
Surface area	(m^2)	5,000	
Production cycle	month	6	
Cycles per year	cycle	1.0	
Variable costs		7,738.00	
Manual tillage	($)	1,800.00	12.45%
Mechanized tillage	($)	300.00	2.07%
Seeds or seedlings	($)	568.00	3.93%
Biofertilizer	($)	200.00	1.38%
Chemical fertilizer	($)	240.00	1.66%
Energetics	($)	3,030.00	20.96%
Fees	($)	600.00	4.15%
Taxes	($)	1,000.00	6.92%
Fixed costs		6,721.00	
Land	($)	1,000.00	6.92%
Machinery and equipment	($)	4,209.00	29.11%
Tools and materials	($)	648.00	4.48%
Maintenance	($)	864.00	5.98%
Total, costs		14,459.00	
Production		1,371.00	
Average price	($)	18.00	
Revenues		24,678.00	
First	($)	24,678.00	
Profit	($)	10,219.00	
Cycle rate of return	(%)	7.6%	
Annual rate of return	(%)	7.7%	
Total, unit costs	($)	10.55	
Unit variable costs	($)	5.64	
Unit fixed costs	($)	4.90	

CONCLUSIONS

Bacteria from the genera *A. brasilense* YOM9 and *A. vinelandii* YOC4 contributed to the higher grain yield of *C/Acceleron A-7573* hybrid corn, and *Rhizobium* sp. R01 and *A. vinelandii* YOC4 in the Creole grain of the olotillo race, regarding the T9 fertilizer and the absolute control. Therefore, based on the results from this study to obtain a biofertilizer for corn as ecotechnology that plays an important role in the absorption and stabilization of the necessary essential elements, they are a good environmentally-friendly alternative to reduce the excessive use of fertilizers within farming. The use of biofertilizers by farmers with limited capital is recommended, because they are more economic, contribute nutrients in a natural way to the soil, and are highly profitable within sustainable agriculture.

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Colegio de Postgraduados

Agronomy and chemical composition of sunflower (*Helianthus annuus* L.) as a forage option in a warm-humid intertropical environment

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ABSTRACT
Objective: To determine the phenology of sunflower and its potential as forage for ruminants in a humid-warm environment.
Design/Methodology/Approach: Six treatments were evaluated during the 2016 dry season, according to the number of cutting days after sowing (DAS): 65 (T1), 72 (T2), 79 (T3), 86 (T4), 93 (T5), and 100 (T6). The SYN3950 HO hybrid was planted in 5×40 m plots with a completely randomized block design and three replications. The following variables were measured: days to reach reproductive stages (Ri), plant height (PH) in cm, stem perimeter (SPM) in cm, flower diameter (FD) in cm, and dry matter production in stem, peduncle, leaf, flower, and total (DM t ha^{-1}), as well as the content of dry matter (DM), crude protein (CP), ash (A), neutral detergent fiber (NDF), and acid detergent fiber (ADF). Analysis of variance were carried out, as well as correlation between variables, mean comparison tests (Tukey, $P<0.05$), and regression between variables and DAS.
Results: There were significant differences ($P<0.05$) between PH, FD, and DM production in stem, peduncle, flower, and total DM (t ha^{-1}), as well as in DM, CP, NDF, and ADF. The highest biomass yields were obtained at 100 DAS (16 t ha^{-1} DM) with 26% DM, 12.7% CP, 21.9% A, 44.2% NDF, and 30.9% ADF. It is concluded that sunflower is an option for feeding ruminants in the study area.

Keywords: Phenology, reproductive stages, humid tropic, nutritional value, forage.

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INTRODUCTION

Livestock production is one of the most important economic activities in the warm-humid intertropical region of Mexico. The main food sources for cattle are pastures (Poaceae) and forages (Rubio *et al.*, 2015); however, pasture and forage production is a seasonal activity (Gray *et al.*, 1987). In areas such as the state of Tabasco, Mexico, the growing seasons are known as lluvias (the rainy season, from June to September), nortes (the windy and rainy season, from October to January), and secas (the dry season, from

February to May) (Ruíz-Álvarez *et al.*, 2012). During the dry season, the production of pastures and forages decreases, as a consequence of the scarcity of water and the high temperatures, resulting in a lower protein and high fiber content, low digestibility, loss of animal body weight, and reduced stocking rate (*i.e.*, animal carrying capacity) (Mello *et al.*, 2006b).

Sunflower (*Helianthus annuus* L.) is an oleaginous crop used as forage, bird feed, ornamental plant, and fuel (Gomes *et al.*, 2017). It adapts to temperate, tropical, and subtropical climates (Debaeke *et al.*, 2021) and it requires little manure or fertilizers (Peniche *et al.*, 2008). It tolerates drought and high temperatures (Granados *et al.*, 2004; Blamey *et al.*, 2009; Sainz-Ramírez *et al.*, 2020). All these characteristics make it an alternative for forage production in the warm-humid region of Mexico. The objective of this study was to determine its phenology, chemical characteristics, and potential for feeding ruminants in a humid-warm intertropical environment.

MATERIALS AND METHODS

The study was conducted at the Colegio de Posgraduados, Campus Tabasco, located at Km. 21 of the Cárdenas-Coatzacoalcos Federal Highway, in Cárdenas, Tabasco, Mexico (18° 00' N and 93° 30' W, 9 m.a.s.l.). The average annual precipitation is 2,163 mm, while the average annual temperature is 25.9 °C (Köppen, modified by García, 1988).

Sowing took place on January 21, 2016, after harrowing and with minimum tillage. One seed per plant was sown every 0.25 m, with 0.80 m between rows. The SYN3950 HO hybrid was rotated with corn, under residual fertilization, and rain fed dependent. The crop was protected with two manual weed controls, two applications of Engeo® at commercial doses (to control Coleoptera, Lepidoptera, and Homoptera), and one application of Priori Xtra® (to control anthracnose).

The variables evaluated in this study covered the reproductive stages based on the Schneiter and Miller (1981) scale, and the following phenological traits: plant height (PH), measured in cm from the ground to the apex; stem perimeter (SPM), measured in cm at the base of the plant; flower diameter (FD), measured in cm; and weight of stem (SW), leaf (LW), peduncle (PW), and flower (FW), measured in g of dry matter.

Likewise, moisture (MC), ash (A), and crude protein (CP) content were measured following the methods established by AOAC (2012). For their part, neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined according to Van Soez *et al.* (1991). Dry matter (DM) was calculated substracting the moisture percentage from 100. The bromatological analyses were conducted in the animal science laboratory of the same institution.

The experimental units were established within three 5×40 m plots, using a randomized complete block design with three replications. The phenological variables were measured in five 2 linear m random sites within each plot. The treatments were assessed on six cutting dates with weekly intervals (65, 72, 79, 86, 93, and 100 DAS). To obtain a helpful biomass sample, four 2 linear m rows were selected from every ten rows of each block. Four representative plants were separated from the material harvested in each block and their stem, petiole, leaf, and flower were measured. The remaining plants were grounded to acquire a sample for the bromatological analysis. The data obtained for each variable was subjected to an analysis of variance, mean comparison test (Tukey, $P<0.05$), regression of variables regarding the DAS, and correlation analysis among variables using the SAS software, version 9.4 (2013).

RESULTS AND DISCUSSION

Significant statistical differences were recorded in all variables, except SPM and A (Table 1).

The R-4, R-5.1, R-5.5, R-6, R-7, and R-8 reproductive stages occurred at 61, 65, 72, 79, 86, 93, and 100 DAS, respectively (Figure 1). They became more evident at the start of R-4 (61 DAS), flowering earlier than the 72 DAS reported by Sainz-Ramírez *et al.* (2020) and after the 50 DAS registered by Gai *et al.* (2020). Complete flower exposure, anthesis, pollination, and grain fill followed a concentric pattern (R-5.1, R-5.5, and R-6, respectively) (Figure 1); meanwhile, R-6 occurred at 79 DAS. The plant began to wither at 93 DAS and its bracts began to show a typical brown color (R-7 and R-7.5), before it finally reached physiological maturity (R-8) at 100 DAS (Schneiter and Miller, 1981).

The highest PH (188.6 cm) was recorded at 100 DAS and it showed no statistical difference with those mean values observed at 86 and 93 DAS, which were 179 and 183.6 cm long, respectively. Therefore, the PH stabilized at 86 DAS (Figure 2a). These values exceed the observations of Martínez *et al.* (2017), who recorded a maximum of 183 cm at 120 DAS (Table 2), and the findings of Escalante-Estrada *et al.* (2008), who reported an average height of 102.3 cm. The highest FD value was reached at 93 DAS (20 cm) (Table 2), a similar result to the one reported by Martínez *et al.* (2017) (19.75 cm, at 120 DAS), and had a significant correlation with SW ($P<0.0001$, $R^2=0.98$) (Table 3).

Table 1. Mean squares and significance level of different variables evaluated in sunflower (Cárdenas, Tabasco, 2016).

Variable	Block	Treatment	Error
PH	5125.2525	32857.0759 ***	591.6228
SPM	29.1495116	37.2716883 NS	17.826693
FD	1.113875	1120.434584***	20.75948
SW	2852.3002	110101.9463***	28751.598
PW	540.40785	12583.63135***	2855.6023
LW	1143.3374	24121.5302**	7359.5645
FW	383.023	312636.793***	12337.441
DM	0.0096167	122.1743967***	0.0534433
CP	3.4482583	39.4346183***	7.1789310
A	26.0101194	50.5004494 NS	53.739872
NDF	18.0739361	86.2141161*	11.7308635
ADF	9.2363194	66.9879778 *	10.6172998

PH: plant height, SPM: stem perimeter, FD: flower diameter, SW: stem weight, PW: peduncle weight, LW: leaf weight, FW: flower weight, DM: dry matter, CP: crude protein, A: ashes, NDF: neutral detergent fiber, ADF: acid detergent fiber, *: $P\leq0.05$, **: $P\leq0.01$, ***: $P\leq0.001$, NS: not significant.

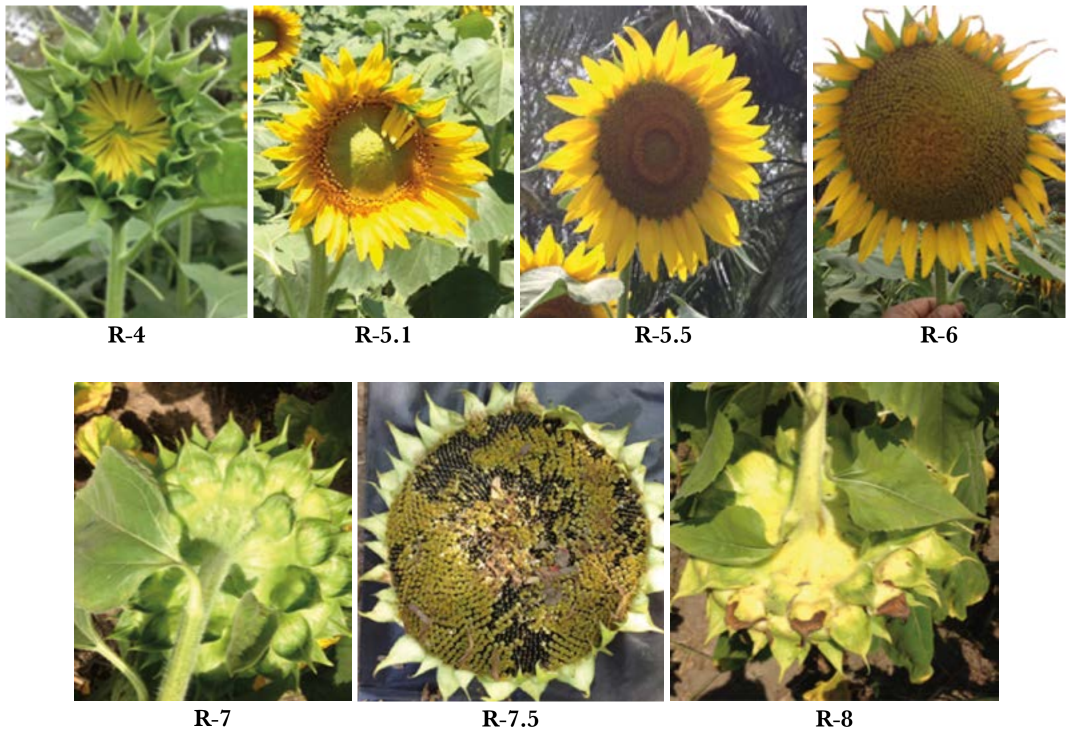

Figure 1. Phenological classification of the different reproductive stages, based on days after sowing (Schneiter and Miller, 1981).

Table 2. Plant height, stem perimeter, and flower diameter of the sunflower on different days after sowing.

Days after sowing	Plant height (cm)	Stem perimeter (cm)	Flower diameter (cm)
65	131.97 c	-	-
72	143.36 c	6.89 a	11.62 c
79	164.02 b	7.25 a	16.54 b
86	179.04 a	7.08 a	17.79 b
93	183.14 a	7.71 a	20.96 a
100	188.62 a	8.55 a	19.90 a
EE	1.13	0.20	0.22

Means with different letters in the same column indicate significant differences (Tukey, $P<0.05$).

Table 3. Pearson correlation coefficients among plant height, stem perimeter, and flower diameter of sunflower.

Variable	Height	Stem perimeter	Flower diameter
Height	1.00000	0.10684	0.14725
Stem perimeter	0.10684	1.00000	0.97549
Flower diameter	0.14725	0.97549	1.00000

Regarding the dry weight (DW) of structures, the highest stem (91 g), leaf (54 g), and flower (163 g) weight were recorded at 100 DAS, with no statistical difference in PW (Table 4). SW and LW showed a quadratic trend (R^2=0.73, R^2=0.84), while FW showed a linear trend (R^2=0.96) (Figure 2d). The highest DS plant and forage weights were recorded at 100 DAS: 323 g and 16.18 t ha^{-1}, respectively (Table 5); these mean values exceed the 11.41 t ha^{-1} recorded by Mello *et al.* (2006a).

The bromatological analyses showed differences ($p<0.05$) in all the variables, except in A (Table 6). The highest DM content (26.0%) was obtained at 100 DAS, while the lowest (7.4%) was recorded at 65 DAS. This structural accumulation of DM had a similar behavior to that observed by Romero *et al.* (2009): the most notable changes were recorded in flowers during the last stages and there were significant differences between samplings within each variable (Table 7). This may be caused by the accumulation of carbohydrates, proteins, and oil as the grain develops (Sainz-Ramírez *et al.*, 2020).

The DM values observed were lower than the 35% recommended for the optimal fermentation of forage (Mello *et al.*, 2006b). However, a low ADF and NDF content favors the digestibility of sunflowers (Table 7), increasing their potential as food for ruminants (Mello *et al.*, 2006b; Romero *et al.*, 2009; Sainz-Ramírez *et al.*, 2020).

Table 4. Dry weight (DW) of the structures of sunflower at different cutting days after sowing (DAS).

Days after sowing	Stem weight	Peduncle weight	Leaf weight	Flower weight
65	46.250 b	10.961 a	22.629 b	6.120 e
72	78.291 ab	13.674 a	32.272 ab	22.133 de
79	83.220 ab	14.373 a	36.984 ab	46.680 cd
86	76.150 ab	13.212 a	35.765 ab	74.120 c
92	82.040 ab	10.112 a	37.916 ab	117.03 b
100	91.595 a	13.797 a	54.490 a	163.878 a
EE±	8.14	13.60	47.44	222.91

Means with different letters in the same column indicate significant differences (Tukey, $P<0.05$).

Table 5. Biomass yield of sunflower per plant and per hectare on a dry basis at different cutting days after sowing.

Days after sowing	Plant weight (g)	Forage yield t ha^{-1}
65	85.96 d	4.298 d
72	146.37 cd	7.318 cd
79	181.26 bcd	9.063 bcd
86	199.24 bc	9.962 bc
93	247.10 ab	12.355 ab
100	323.76 a	16.188 a
EE±	287.31	14.36

Means with different letters in the same column indicate significant differences (Tukey, $P<0.05$).

Table 6. Chemical composition of the whole sunflower plant (%) on different cutting days after sowing.

Days after sowing	Dry matter	Crude protein	Ashes	Neutral detergent fiber	Acid detergent fiber
65	7.4 f	17.5 ab	21.1 a	55.1 a	35.8 ab
72	14.1 e	18.2 a	17.2 a	51.0 a	36.4 ab
79	16.1 d	12.9 bc	17.2 a	50.1 a	41.3 a
86	18.2 c	13.7 abc	20.2 a	50.5 a	37.1 a
93	21.5 b	12.5 c	14.3 a	50.0 ab	37.2 a
100	26.0 a	12.7 bc	21.9 a	44.2 b	30.9 b
EE	0.054	0.54	1.22	0.57	0.54

Means with different letters in the same column indicate significant differences (Tukey, P<0.05).

The highest CP content was 18.2% at 72 DAS, while the lowest was recorded at 93 DAS (12.5%). Other studies have also documented this decreasing trend in CP (Mello *et al.*, 2006b; Romero *et al.*, 2009). In this study, CP was negatively correlated with DM (Figure 3a), while its minimum and maximum values were higher than those observed by Peireti and Meineri (2010) and by Pereira *et al.* (2014), who recorded 5.9% at 66 DAS and 6.8% at 101 DAS, respectively.

CP increased from R-4 to R-5, then experienced a linear descent until R-8. Mean CP values exceed those of corn as the main silage source (Guney *et al.*, 2012; Osuna and Martínez, 2017) and favor the integration of sunflowers in the diet of ruminants. The lowest NDF content (44.2%) was observed at 100 DAS, while the highest value (55.1%) was reported at 65 DAS, without statistical difference at 72, 79, and 86 DAS. The lowest ADF content was 30.9% at 100 DAS, while the highest was 41.3% at 79 DAS. The NDF and ADF values were higher than those reported by Pereira *et al.* (2014), who reported means of 43.9, 43.9, 45.7, 44.6, and 45.0% for NDF and 33.8, 33.9, 36.4, 35.5, and 35.5% for ADF, at 66, 73, 80, 87, 94, and 101 DAS, respectively.

Table 7. Percentage of dry matter in sunflower structures at different cutting days after sowing.

Treatment	Stem (%)	Peduncle (%)	Leaf (%)	Flower (%)
65	7.2 f	7 f	8.2 f	7.2 f
72	14.5 e	11.9 e	14.2 e	12.8 e
79	18.6 d	17 c	18.4 d	14.3 d
86	20.2 c	15.3 d	22.6 c	18.5 c
92	21.8 b	18.7 b	27 b	24.9 b
100	21.9 a	20.3 a	29.7 a	30.3 a

Means with different letters in the same column indicate significant differences (Tukey, P<0.05).

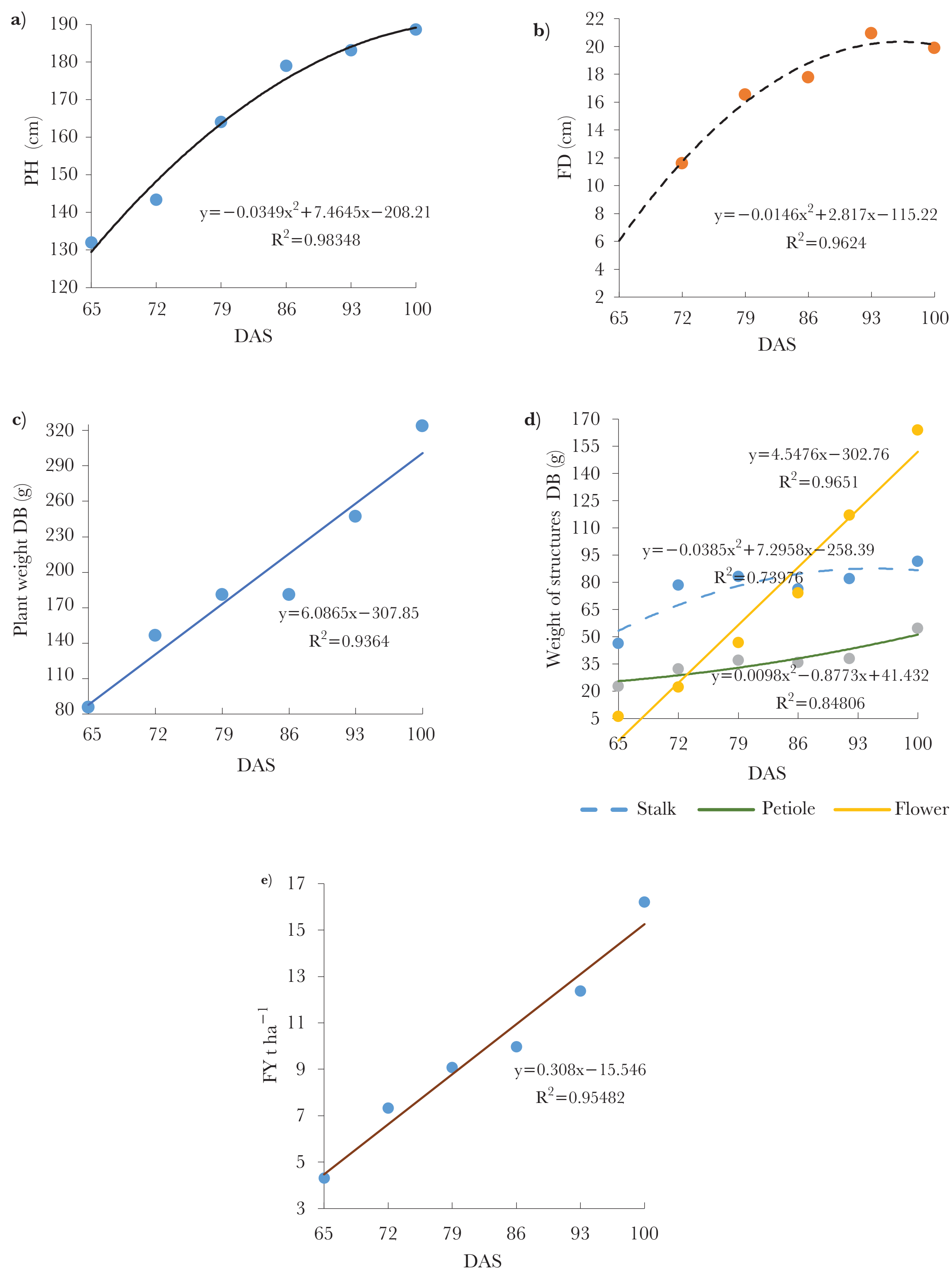

Figure 2. Regression lines on days to cut after sowing (DAS) of: a) Plant height (PH), b) Flower diameter (FD), c) Plant weight (Dried Base, DB), d) Structure weight (Dried Base, DB), and e) Forage yield (FY t ha^{-1}).

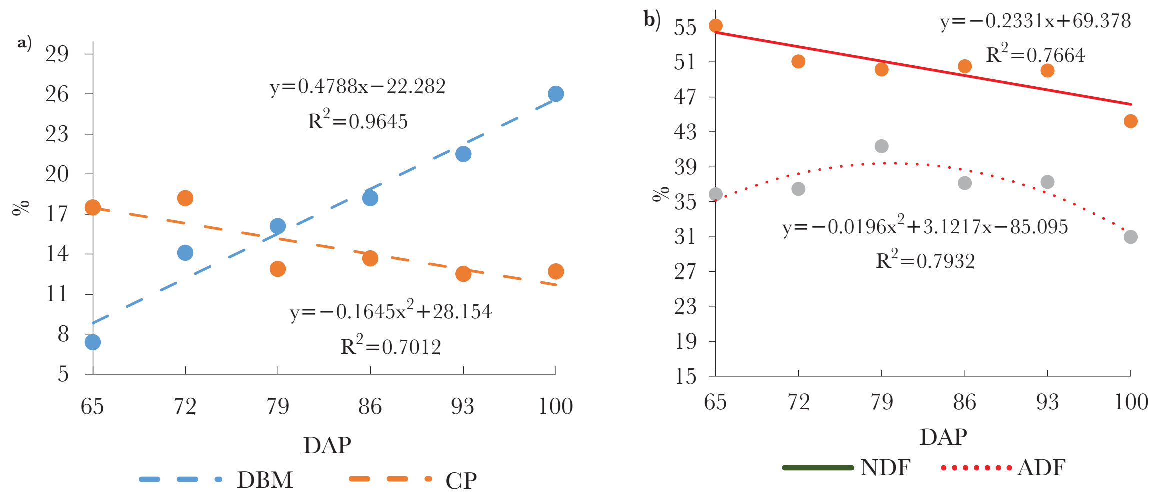

Figure 3. Regression lines of % of Dry Biomass (DBM) and Crude Protein (CP) (a), and Neutral Detergent Fiber (NDF) and Acidic Detergent Fiber (ADF) (b) in sunflower, at different cutting days after planting (DAP).

CONCLUSIONS

Production and nutritional quality of sunflower forage are closely related to cutting dates. The later the date, the higher the DM, although the CP decreases. In terms of forage yield, the best cutting was made at 100 DAS, when the following results, pertaining to the R-6 phase, were obtained: 16.7 t ha1 yield, a nutritional value of 26% DM, 12.7% CP, 21.9% A, 44.2% NDF, and 30.9% ADF. Based on these values, sunflower is a suitable alternative for forage production, destined to ruminant feeding in the warm-humid environment where the study was made.

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Colegio de Postgraduados

Approximation to the social structure of swine health and production research in Colombia

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ABSTRACT

Objectives: To characterize the organizational patterns in the swine health and production research systems in Colombia.

Design/methodology/approach: The social networks approach was used to analyze the associative pairing of a prominent community of researchers in the field of swine health and production in Colombia, considering scientific studies generated between 2010 and 2018.

Results: The swine research network in Colombia has a circumstantial growth, incipient behavior, and is highly centralized, delimiting the research narrative and generating a normal distribution in the transitivity of the network.

Limitations on study/implications: The sample analyzed was by convenience and determined to a number of scientific studies, so it would be necessary to broaden considerably the population and the period analyzed. Visualizing and measuring this social structure could allow managing risks and opportunities for the local swine farming sector.

Findings/conclusions: The social density and the values of structural centrality obtained reflect an organizational model that replicates the global production model: limited information flows and partially connected structures, highly centralized and with low variability in their information channels and knowledge exchange in a highly strategic sector.

Keywords: Social networks analysis, swine farming research systems, agrifood sector.

INTRODUCTION

The high nutritional and protein value of pork meat has made it the second most consumed globally, after chicken meat [1, 2, 3]. In 2020, nearly 110 million tons of pork meat were produced in the world [4], and this will increase 13% by 2030 [5]. In 2019, swine farming in Latin America and the Caribbean was the one that increased most in the

world, with a weighted average of 4.6%; in 2020, it was 8.9 million tons: Brazil contributed 50%; Mexico, 18.4%; Argentina, 7.3%; Chile, 6.4%; and Colombia, 4.9%. The latter increased its pork meat production the most [6], which means an exponential increase in size, yield, productivity and specialization of the farms [5], as well as the consolidation of a globalized agroindustrial production model based on the intensification and concentration of the activity, expulsion of animals from the farmlands, establishment of industrial farms with mechanized productive methods, and genetically improved animal breeding, in confinement under specific dietary and pharmacological requirements [7]. According to Shahbandeh [8], the global herd is composed of 784.2 million pigs and to feed them, the following is required: intensive use of industrial inputs and biotechnology for large-scale monocrop production, hoarding and concentration of land, as well as entrepreneurial expansion and financial capital [9].

This also depicts swine farming systems linked to ecological problems of public health and food security, since massive confinements have been built under homogeneous conditions, reduced spaces, and constrained genetic diversity, accelerating the rotation of animals and amplifying the risk of various diseases [10, 11, 12]. This is worsened since the globalized swine farming model promotes the interregional transport of inputs for production and consumption (pig carcass, cold meats, etc.); therefore, complex social networks are articulated of commercial branches and international supply chains with high interconnectivity, which makes them more vulnerable to systemic interruptions: wars, economic and environmental crises, epidemics; pandemics; epizootics, etc. [13, 14].

In this sense, the propensity of the global swine farming system to the transmission and dissemination of trans-border diseases of the animals is logical: African swine fever (ASF), classical swine fever (CSF), Aujeszky disease, foot and mouth disease, or porcine reproductive and respiratory syndrome, among other pathologies [15]. On the other hand, the global and annual emission of greenhouse gases associated to swine farming has been estimated to be in the order of 700 million tons of CO_2, where the production of concentrated feed for animals is the main contributor, with nearly 60% of the emissions of the supply chain, while manure management, post-farming processing, transport, use of energy in production and enteric fermentation add the remaining 40% [16]. These potentialities and vulnerabilities have been accentuated regionally.

In Colombia, the swine agribusiness grew during the decade of the 1980s, as a result of globalization and trade liberalization processes in Latin America [9]. The participation of the World Trade Organization promoted the liberalization and a greater privatization of the agrifood sector [17].

In 1983, the Colombian Pig Producers' Association was created with the aim of modernizing and strengthening the production of pork meat. In 1996, the creation of the National Swine Farming Fund (*Fondo Nacional de la Porcicultura*, FNP), promoted a greater investment in technical, sanitary, economic, commercial, research and technological transference programs for the development of the swine farming activity in Colombia [18, 19]. These organizational dynamics implied a clear expansion of productive processes, greater integration of the chain, innocuousness, formalization of the activity, improvement of the perception of quality, etc. [20], allowing the significant increase in the production and consumption of pork meat [21], which made Colombia a competitive regional producer [22]: from 2010 to 2019, the activity grew on average 8.7%; by 2021, 491,233 tons of pork meat were produced, which corresponded to an additional offer of 22,805 tons compared to 2020 [23].

The Colombian swine farming system went from slightly over 2 million heads in 1961, to reaching 6 million heads in 2019 (Figure 1). However, this also meant high degrees of productive homogenization and contradictions in animal health.

According to Rincón [25], in 2006-2009 there was an epizootic outbreak in Colombia of the porcine post-weaning multi-systemic wasting syndrome (PMWS) caused by type 2 porcine circovirus (PCV2n), in the herds of industrial swine farms, causing important economic losses. The presence of classical swine fever (CSF) [26], respiratory syndrome (PRRS), porcine parvovirus (PPV) [27], virus of porcine epidemic diarrhea (PED) [28] has also been detected; high presence of toxoplasmosis [29]; in addition to a large variety of parasites in swine [30]. These problems are derived from deficient practices in herd management, causing significant mortality rates [31]. On the other hand, the Colombian swine farming system is a globalized consumer of livestock inputs, so some of their problems come from the interregional swine transport system (disseminator of PED among various regions of Colombia [32]), and from inputs imported for the production. According to Rincón [33], the prevalence of the porcine reproductive and respiratory syndrome virus (PRRSV) in intensive swine farms (agroindustrial) is associated to the import of live animals and seed from Canada and the United States.

All of this suggests transversal organizational processes in the animal market-production-health trinomial. In face of the development of massive swine farming systems, the institutional systems of scientific-technical research have broadened their capacities to measure, prevent, analyze, stop and mitigate these scenarios in local productive chains. These systems involve community dynamics whether as societies, trade unions, communities,

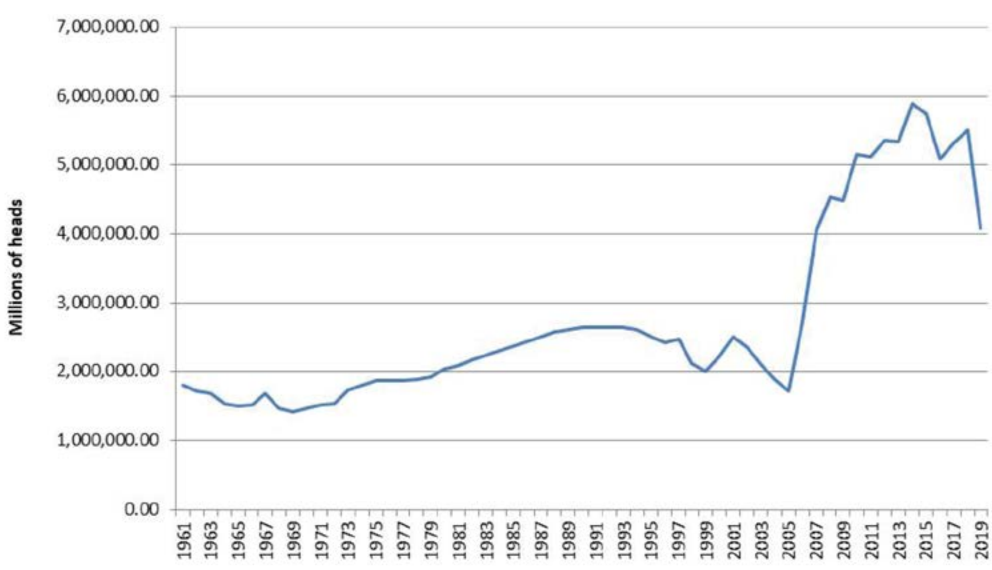

Figure 1. Evolution of swine farming in Colombia, 1961-2019. Source: [24].

etc., generating flows and exchanges of specialized information and knowledge [34], which beg the following question: What are the organizational patterns that predominate in the technical-scientific structures responsible for the animal health and production of the Colombian swine farming system? Concerning this, the objective established was to recognize and characterize the organizational patterns in the field of swine health and production research in Colombia, considering the processes of social agglomeration generated in the technological and scientific research structures of that productive chain. As a result, a partial approach to an otherwise complex system is proposed, with the aim of starting to generate strategic information about the organizational patterns that prevail in these social systems.

MATERIALS AND METHODS

The analysis of the social structures of swine health and production research allows gaining access to the dynamics of correlation and management of specialized knowledge between diverse academic and agribusiness sectors. This analysis was made based on a mixed methodological approach using two instruments of analysis:

1. The proceedings from international conferences carried out by the International Pig Veterinary Society (IPVS) conducted in 2010 to 2018 [35-39], and selecting the studies that refer to Colombia (67 researchers). The IPVS was founded in 1969 with the objective of "…sharing knowledge related to swine health and production… and fostering the potential cooperation between swine veterinary societies, scientists, swine veterinarians, and pork meat producers…" [40]. Thus, IPVS represents a historical model for the integration of porcine veterinary research globally. This has generated a growing participation of specialists in the subject matter. For example, in 1969 there were 500 participants; in 2010 there were 2716 and in 2018, 5599. The IPVS is not the sole scientific information exchange forum about swine farming, but it has generated, globally, spaces for the exchange and generation of specialized knowledge, through dialogue and discussion of scientific information and ideas [41]; and it allows having the certainty that whatever is proposed has been measured and justified methodologically, making its experimental replication possible [42], and fostering the construction of collaboration networks [34] as well as the possibilities of analyzing these connections.
2. Social networks analysis (SNA). This is a structuralism approach centered on the relationships established by social stakeholders [43, 44], exposing structures of correlation generated by the diversity of relationships found between these stakeholders (individuals, institutions, organizations, etc.). In the case of this study, the connections between various scientific stakeholders in Colombian swine farming were analyzed, involving the co-authorship in scientific works circumscribed to Colombia. This analysis used measures of centrality (nodal degree and intermediation) and cohesion (social density), which belong to the SNA. The first refer to the position that each stakeholder occupies in the network [45], and

they are a way of measuring the dominance and influence of the stakeholders [44]. The second describes the structural efficacy to manage information [45].

- Nodal degree: It is the number of direct connections (co-authorships) that each author has [44, 46]. Its calculation [47]:

$$C_g\left(n_i\right)=\sum{}^{A} L\left(n_i,n_j\right)/\left(A-1\right)$$

Where: $Cg\ (n_i)$: number of nodes with which n_i is connected; $(A-1)$: Amplitude of the network.

- Degree of intermediation: It is the number of times that a stakeholder connects to a pair of stakeholders not connected directly [48]:

$$C_I\left(n_i\right)=\sum g_{jk}\left(n_i\right)/\, g_{jk} \forall j<k$$

Where: $C_I(n_i)$: degree of intermediation; $g_{jk}(n_i)$: number of geodesics between nodes j and k that pass by node i; g_{jk}: number of geodesics that connect nodes j and k [47].

- Density: Percentage of existing relationships between the possible relationships [49]:

$$\Delta=\frac{L}{g\left(g-1\right)}$$

Where: L: number of existing arches; $g\ (g-1)$: possible number of arches [46].

These measurements allowed characterizing topological qualities of the set of connections between Colombian researchers, and therefore potentialities and vulnerabilities. The software used was UCINET 6 [50].

RESULTS AND DISCUSSION

The network of swine production and health research in Colombia has an incipient behavior between 2010 and 2018, although with a clear growth: some disconnected areas are observed and two sub-groups stand out that expose a socio-centric and hierarchical behavior, although one is broader, with a higher number of connections, inputs of trust (thickness of arches) and defined reciprocity (arch in red), although centralized; this evidences a more cohesive group and with more operation time (Figure 2). The social density of the structural set is 2.46%. In turn, the average in nodal degree is 3.06, with a standard deviation of 4.13, pointing to a circumstantially communicated structure. This pattern of centralization is replicated at the level of intermediation: the average values

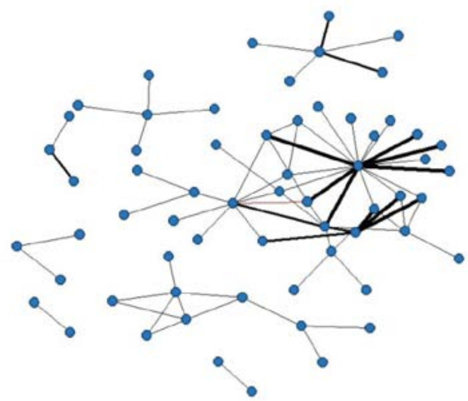

Figure 2. Swine research network in Colombia 2010-2018. IPVS 2010-2018.
Source: [35-39].

were 0.953, with a deviation of 4.58, indicating communication channels and limited information exchange.

This centralization has influenced the temporary dynamics of the social structure itself, which has gone from limited patterns of communication, correlation and transitivity (2010), to intermediate stages of greater transitivity (2012-2014), although evolving towards structures that are clearly more centralized (2016), more limited and reduced (2018) than those found in the first year of the period reviewed (Figure 3).

In general terms, the agglomeration trends in this research network have moved towards decreasing their information and correlation exchange channels. Although in 2018 there was a limited recovery at the level of intermediation, compared to 2016 and 2010, it is still a

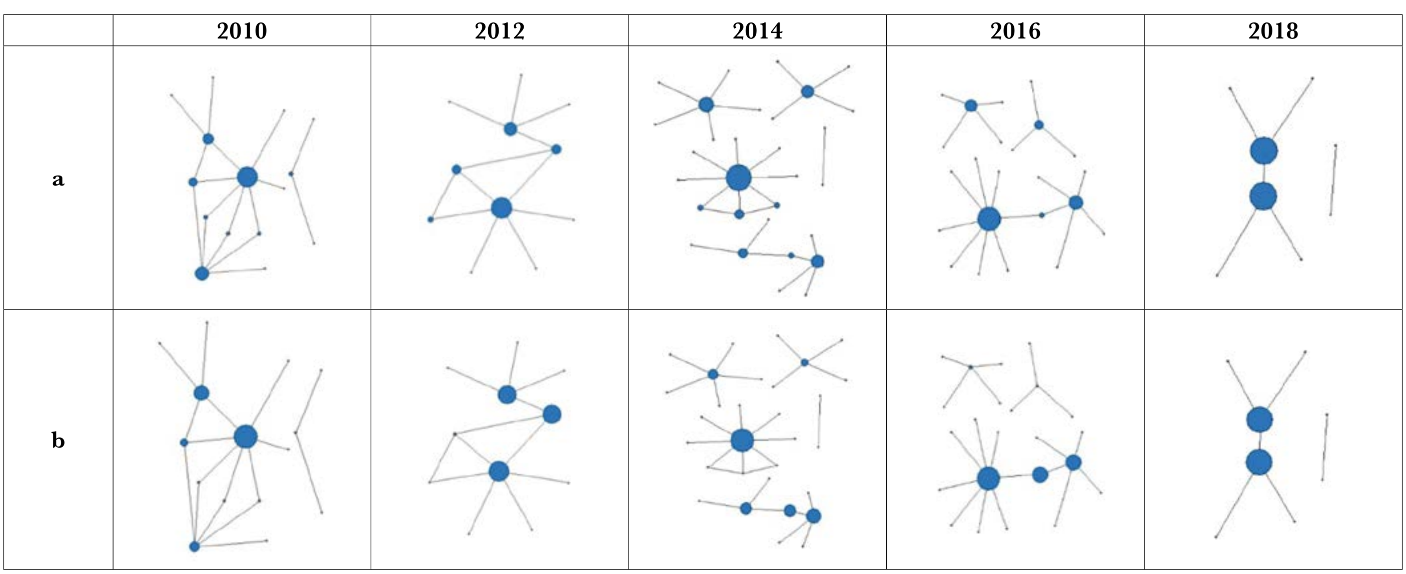

Figure 3. Evolution in Degree (a) and Betweenness (b) in the swine farming research network in Colombia, 2010-2018
Source: [35-39]

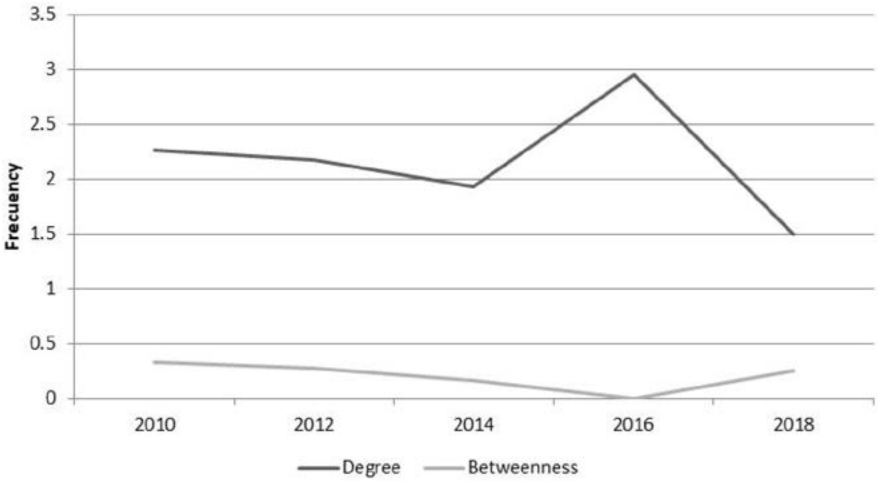

Figure 4. Structural trends in the swine farming research network in Colombia. Source: [35-39]

lower value (Figure 4). For its part, the capacity for communication increased significantly in 2016 although it fell by 2018.

It should be mentioned that the patterns of social prominence observed in time are the expression of the information management itself (and the power of correlation) inside the structure analyzed, and the flow of this information allows observing a structure connected from the first year analyzed (2010) to the last (2018), confirming a continuum of correlation/communication between research groups in swine health and production. In this case, defining the capacity for intermediation in the graph, it is evident that this capacity is managed by a limited group of stakeholders, primarily since the first year (Figure 5).

For its part, the gender composition of the research structure is mainly masculine (56.45%), although the feminine factor is highly significant (43.55%) and holds the main values of communication and correlation of this research structure (Figure 6).

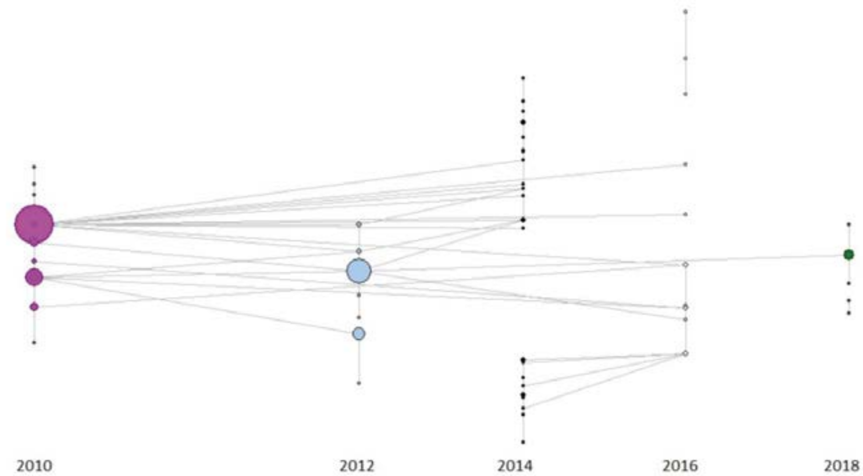

Figure 5. Swine farming research network in Colombia from a five-year period (2010-2018). Source: [35-39]

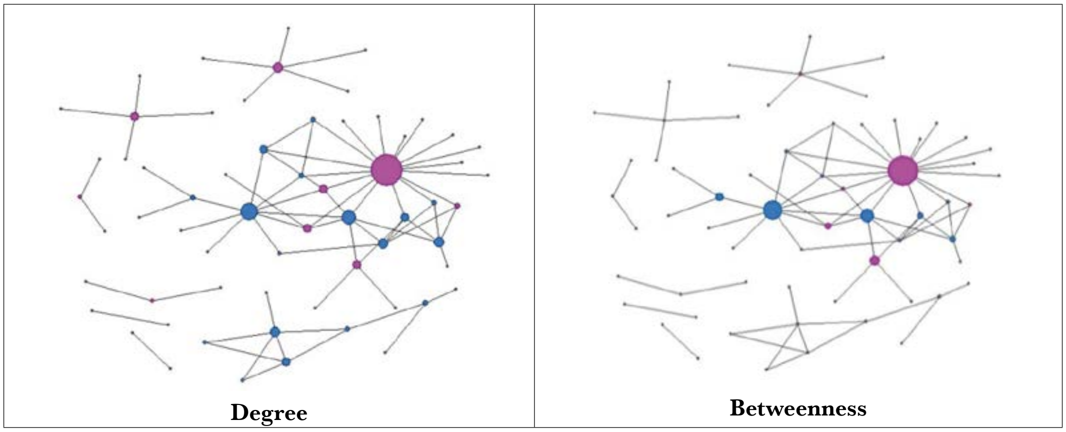

Figure 6. Gender in the swine farming research network in Colombia (2010-2018). Source: [35-39]

CONCLUSIONS

The scientific and technological system involved in swine farming in Colombia presents an incipient and circumstantial organizational behavior. The social density and values of structural centrality found reflect a partially-connected organizational model, highly centralized and with low variability in the information/exchange channels of knowledge in the branch. However, the size of the network itself presents a specific potential to reformulate the information flows in animal health and production with the aim of strengthening the structural value of the network; the latter, primarily, because the complexity of the contradictions inherent to swine farming tend to spill over to the local systems of research and safety control. The zoonotic scenarios, increasingly present in our societies, indicate the need for research networks in agrifood and livestock sectors that are capable of innovation in their organization models.

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Colegio de Postgraduados

Traditional knowledge and actions of the Pisel Nek-mej (*Scaptotrigona mexicana*) stingless bee honey production in Cuetzálan, Puebla

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ABSTRACT

Objective: During the last few years, the production of honey from stingless bees has become significant, as a result of its therapeutic properties. However, its production and commercialization are limited by the low yield per colony and its high sale price. This study seeks to recover the lore and actions that a group of producers from Cuetzálan, Puebla, Mexico has about the production of honey from the *Scaptotrigona mexicana* species of stingless bees. The objective was to collect information about the handling, production, and commercialization of tis bee species, as well as the challenges that producers face in the Cuetzálan region.

Design/Methodology/Approach: This research was carried out using a qualitative approach. Semi-structured interviews were conducted to gather the information. The participants were chosen using the snowball sampling technique, contacting stingless bee honey producers in Cuetzálan, Puebla, Mexico.

Results: The lore about the handling of the stingless bees is passed on from generation to generation. This traditional handling is understood as a cultural symbol. This type of honey is renowned for its medicinal properties; however, its production is scarce, and the resulting product is very expensive. Consequently, the theft and adulteration of honey has become a major problem, creating mistrust among consumers.

Study Limitations/Implications: More producers should be interviewed, and additional proof of the handling processes used to identify adulteration should be gathered. This information would be used to develop recommendations that could be applied in other producing regions of the country.

Findings/Conclusions: The social and scientific recognition of the properties of stingless bee honey shows the importance of adopting innovations; however, including organizational processes to improve the commercialization channels and to minimize unfair honey production practices is also very important.

Keywords: honey, stingless bees, Cuetzálan, *Scaptotrigona mexicana*.

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INTRODUCTION

In Mexico, meliponiculture was practiced way before the arrival and settlement of the Spanish conquistadors (Alquisira-Ramírez, 2019; Ayala *et al.*, 2013). The archaeological

evidence suggests that the Mayans carried out meliponiculture about 2,000 years ago and that stingless bees played an important role in their social, religious, economic, and politic activities (Christoph, 2020; Vit *et al.*, 2013). Plenty of information about this subject can be found in the illustrations of the Madrid Codex (Źrałka *et al.*, 2014). Despite the lack of detailed information about this subject in other regions of México, the diverse ethological and ecological lore —which has been passed on from generation to generation— is living proof that the stingless bees handling took place in other regions of México as well (Chan Mutul *et al.*, 2019). Out of the 46 recorded stingless bee species, 19 are exploited in México (Ayala *et al.*, 2013). In the Yucatán Península, the Mayans still handle the *Melipona beecheii*, while meliponiculture activities are still carried out with the *Scaptotrigona mexicana* species in the Sierra Norte of Puebla (Cuetzalan), the Huasteca Potosina, and the Totanacapan region in Veracruz. Meanwhile, in Sierra de Atoyac (Guerrero), stingless beekeeping is carried out with the *Melipona fasciata* and *Scaptotrigona hellwegerii* species (González Acereto, 2012). In 2002, because of the growing interest in "miel virgen", meliponiculture was revaluated in Cuetzálan. At that point, although the activity was just a fading memory in the minds of some producers, meliponiculture activities were taken up again (Padilla-Vargas and Vásquez-Dávila, 2013). Cuetzálan became the main producer of *S. mexicana* honey in México and is now considered to be one of the main producers of "miel virgen" in the country (Guzmán Díaz *et al.*, 2011). Consequently, the objective of this study was to provide information about the handling, production, and commercialization of the *Scaptotrigona mexicana* stingless bees, as well as the challenges faced by the producers of the Cuetzálan region, México.

MATERIALS AND METHODS

A qualitative method was used for this study. Additionally, interviews were conducted to gather information. The interviews included 45 questions, arranged in four categories: the first category included general information; the second category focused on the practices used to harvest the honey from the stingless bees; the third category referred to the commercialization practices; and the fourth category tried to identify production problems. The following criteria were used to select the participants: they had to be stingless bee honey producers from Cuetzálan and they should be willing to freely take part in this research. Eight producers were contacted using the snowball sampling (*i.e.*, chain reference) described by Navarrete *et al.* (2022). They agreed to be part of an approximately 40-minute-long interview. In addition to the interviews, articles, books, and other scientific communications on this subject were reviewed. A 6-axis trend analysis was carried out, including: a) meliponiculture in Cuetzálan; b) honey harvest process; c) medicinal properties; d) commercialization; e) cultural and environmental value; and f) problems faced by meliponiculture in the study area.

Study area

The research was carried out in the Cuetzálan municipality, located in the Sierra Nororiental of Puebla (19° 57' 00" and 20° 05' 18" N and 97° 24' 36" and 97° 34' 54" W), in the meridional region of the Sierra Madre Oriental (INEGI, 2010) (Figure 1). The

Figure 1. Location of the Cuetzálan Puebla, México.

climate is mostly temperate; however, as a result of its closeness to the tropical climate of the Gulf of Mexico, it has a semi-warm subhumid climate. Rain falls all year long and the area has an uneven topography, as a consequence of which two types of vegetation can be found in this area: cloud forests in the highlands and medium semi-deciduous forests in the low parts (Rzedowski, 2006).

RESULTS AND DISCUSSION

Meliponiculture in Cuetzalan, Puebla

In the Cuetzálan region, the Nahuas call the *S. mexicana* stingless bees *pisilnekmej* ("small bees") (Castillo Hernández, 2020). They handle these bees using two clay pots attached by their rim s, commonly known as *mancuernas* ("dumbbells") (Crane, 1999). Meanwhile, in the Yucatan Peninsula, hollow trunks (called *jobones*) are used to breed the stingless bees (Christoph, 2020; Źrałka *et al.*, 2014). These strategies are part of the indigenous stingless bees breeding legacy in both regions and they are still in use today. Although stingless beekeepers have tried to introduce wooden boxes to improve honey production In Cuetzalan, their attempts have been fruitless because, according them: "*bees tend to abandon artificial hive boxes, because the boxes do not have the appropriate climate characteristics for the region*" (Carmen, personal communication, November 18, 2021). Meliponaries are placed under the overhang of the house (to avoid direct sunlight) or under roof structures nearby the house of the producer (to avoid rainfall). These structures are called *casa de las abejas* ("beehouse") and are usually located near the area of foraging activity: "*we place the bees near coffee, vanilla, banana, orange, jonote, litchi, chalahite, and several sweet flowers*" (Martín, personal communication, November 18, 2021). Consist of clay pots placed on rustic wood, bamboo, or rods: "*we use ashes to attach the mouths of the clay pots or mancuernas, but we must leave a hole for the bees, so they can make the 'trumpet'*. This is the door of the bee colony"

(Margarita, personal communication, November 19, 2021). According to five interviewees: "*the care of the meliponaries is easy. We spend approximately 15 minutes per day, every morning, checking them. We clean the place where they are placed, to protect them from spiders, lizards, birds, and big ants, which sometimes invade the mancuernas*" (Martin, Margarita, Mateo, Julián, and Carmen, personal communications, November 18 and 19, 2021).

Nahua and Totonaco stingless beekeepers are the main stingless bee honey producers in Cuetzalan. Some of the regional stingless beekeepers are independent producers; however, most of them belong to the Sociedad Cooperativa Agropecuaria Regional Tosepan Titaniske. This organization is very important to the community and to the region. Paredes and Rodríguez (2014) pointed out that Tosepan was created in 1980 to deal with the needs of coffee and pepper crops (Garza and Garcés, 2009). However, the interest in stingless bee honey came later (Medina, 2015), leading to the foundation of Tosepan Pisilnekmej in 2017. This organization is fully dedicated to the production of honey from *S. mexicana* (Meza, 2017).

The interviewees said that they owned 10-200 meliponaries; however, other producers of the region own more than 400 stingless bee colonies and have more than 40 years of experience as Melipona stingless beekeepers (Castillo-Hernández, 2020). They have different practices and ideas about their handling. For instance, one of the interviewees said: "*we burn incense before we open the clay pots. This is a small ritual in which we burn some honey, beeswax, and propolis to thank the pisilnekmej*" (Mateo, personal communication, November 19). This is a frequent practice, which has been described in detail by Dávila-Vargas *et al*. (2014). Harvests are usually carried out in the morning; however, some producers prefer to harvest in the evening or at night: "*I harvest at night because at that time the bees are not so attracted by the honey and I also prevent bigger bees [Apis species] from taking the honey*" (Toño, personal communication, November 17, 2021). Nevertheless, producers share some technical aspects, as a result of the meliponiculture information and practices that have been passed on from generation to generation in Cuetzálan: "*our parents learned from their parents. We teach these practices to our sons; we teach them to our daughters before they get married. We also learn from other Melipona stingless beekeepers. At the Tosepan cooperative we participate in courses about the breeding of stingless bees*" (Mateo, Carmen, Toño, Margarita, and Martín, personal communications, November 17, 18, and 19, 2023).

Honey harvest process

In Cuetzálan, *mancuernas* or *nekomit* (*nektsin* —bee and *komit*— pot, from the Náhuatl), are used as beehive box for *S. mexicana* and they are two pots joined s mouths (Padilla-Vargas and Vásquez-Dávila, 2013). The lower pot works as a breeding chamber, while the upper pot is used to store the honey and pollen reserves. This honey is harvested from April to June (Guzmán Díaz *et al*., 2011). Some interviewees told us that: "*You can also harvest in July and August, but not everybody does it because bees need to feed and it is not right to take it all away from them*" (Martín and Carmen, personal communication, November 18, 2021).

Some stingless beekeepers pointed out that they use cloths to protect their faces, a machete or a knife to separate the *mancuernas*, and ash or clay to seal them again, after the harvesting. This process is also mentioned by Castillo-Hernández (2020). "*we clean the pots* *with a cloth before we separate them, we look for the honey balls [the storage posts] in the upper pot. After that, we squeeze them and we use a net to filter the honey, to remove pollen or wax*" (María, personal communication, November 17, 2021). The filtering process is very important because it removes all the impurities that produce a dark amber mass on the surface of the honey, making the product less attractive for the consumers. The identification of the storage posts is fundamental for the harvesting process. The interviewees told us that: "*it's easy, if the balls are soft, then the honey is ready. If the balls are hard, they are filled with pollen [this is called a flower ball]*" (Margarita and Mateo, personal communication, November 19). Two of the interviewees pointed out that some of the stingless beekeepers recently chose to extract the honey with a syringe because this technique is cleaner and preserves the purity of the honey. However, it is a longer process: "*we must carefully wash our hands, the tools, and the clothes or the bees will be frightened by the strong smell*" (Mateo and Julián, personal communication, November 18 and 19).

Medicinal properties

As a result of its medicinal properties, stingless bee honey was very valuable for some pre-Columbian cultures (Christoph, 2020; Vit *et al.*, 2013) and this is the main reason why it is still harvested and commercialized nowadays. The honey produced by *S. mexicana* in Cuetzálan is known for its medicinal attributes: "*our honey is quite good; it helps with the cough, sore throat, burning eyes, gastritis, and it heals wounds*" (Carmen, Martín, Mateo, and Toño, personal communication, November 17,18, and 19). The stingless beekeepers believe that the medicinal properties of the honey come from the sources of foraging activity because the bees collect pollen from healing plants (Dávila, Vargas *et al.*, 2014).

Most of the ethnopharmacological properties that stingless beekeepers associate with the honey of *S. mexicana* have been tested in research carried out with the different species of stingless bees. Some of this research proved that stingless bee honey has anti-inflammatory (Borsato *et al.*, 2014; Vit *et al.*, 2004), antioxidant (Ávila *et al.*, 2018; Biluca *et al.*, 2016), antimicrobial (Boorn *et al.*, 2010; Kimoto-Nira and Amano, 2008; Zamora *et al.*, 2017), and wound-healing (Jalil *et al.*, 2017) properties. Additionally, it can also help to treat cataracts (Pedraza *et al.*, 2015) and foot ulcers caused by diabetes (Grajales-Conesa *et al.*, 2018).

Overall, these therapeutic qualities are the result of its high sugar content, its acidity, the presence of hydrogen peroxide (Mandal and Mandal, 2011), and its vitamin and mineral content, as well as their phenolic compounds (Abu Bakar *et al.*, 2017; Jalil *et al.*, 2017), which are the cause of its antioxidant activity (Aljadi and Kamaruddin, 2004). The phenolic compounds identified include phenolic acids (*e.g.*, salicylic, p-coumaric (Biluca *et al.*, 2016, 2020), pherulic, and ellagic acids) and flavonoids (*e.g.*, myricetin, catechin, and rutin (Sousa *et al.*, 2016), aromadendrin, and taxifolin (Biluca *et al.*, 2020).

Commercialization

In the Sierra Norte of Puebla, the value of stingless bee honey during the 1980s accounted for only one-third of the cost of *Apis mellifera* honey (Medina, 2015). However, the stingless bee honey or "miel virgen" currently has a higher sale price: "*the purchase price* *of our honey has increased, because many people want it, now that its medicinal properties are known*" (Julián, Carmen, and Toño, personal communication, November 18 and 19, 2021). The additional income that stingless bee honey can provide to their families has encouraged communities to take care of *S. mexicana* (Escobedo Ávila, 2021).

A 250-mL jar of stingless bee honey is sold at $250.00 Mexican pesos. A liter of this honey costs approximately $800.00-$1,000.00 Mexican pesos. Costs can change, because "*some producers lower the price when buyers go directly to their home. Producers that sell their product through a cooperative must adjust their prices to the price established by the cooperative*" (María and Toño, personal communication, November 17, 2021). Nevertheless, the interviewees pointed out that when the cooperative sells the honey at higher prices than the cost price, the producers receive "*a couple of extra bucks-*" Regardless of this situation, the current sale price cannot be compared with the sale price in countries such as Malaysia, where the stingless bee honey reaches a sale price of up to $100.00 American dollars per kilogram (Shadan *et al.*, 2017).

Taking into account the outstandingly high price of stingless bee honey, stingless beekeepers told us that clients are reluctant to buy this product, especially if they compare the price with the price of *A. mellifera* honey. Meanwhile, commercialization is slowed down as a consequence of the low production per stingless bee colony, which amounts to one liter per year (Chuttong *et al.*, 2014; González-Acereto *et al.*, 2006). Consequently, meliponiculture in Cuetzálan is considered a complementary economic activity. Stingless honey producers mainly work as farmers, day laborers (Guzmán Díaz *et al.*, 2011), traders, and artisans, selling their products in parks and small markets. Nevertheless, meliponiculture has opened possibilities for an alternative source of employment: "*those of us with the greatest experience provide our services during the honey harvesting to new producers or to those that don't have the time to harvest the honey*" (Mateo, personal communication, November).

Cultural and environmental value

Meliponiculture is the practice of breeding stingless bees in artificial hives to obtain products such as honey, beeswax (Lemlin, 2020), pollen, or propolis (Guzmán Díaz *et al.*, 2011). In Cuetzálan, stingless bee breeding is also perceived as a cultural symbol and some stingless beekeepers believe that "*bees stay in those households where love lives*" or that "*when a household lacks harmony, people stop caring for the bees and they leave*" (Carmen and Martín, personal communication, November 18, 2021). Therefore, stingless beekeepers believe that bees can perceive if there is balance in a household. These ideas are part of the regional cultural worldview: stingless beekeeping is the legacy of their ancestors and, consequently, stingless bees and their handling are so much more than an economic exploitation.

As a consequence of their pollination services, stingless bees provide sustenance and guarantee the food safety of the farmers (Alquisira-Ramírez, 2019; Castillo Hernández, 2020). These bees have shared a long evolutionary history with the plants and crops of their place of origin; therefore, their role as pollinators of native crops is considered a yield benefit that cannot be taken lightly (Christoph, 2020; Vit *et al.*, 2013).

Nevertheless, stingless bees face several problems, such as the presence of pesticides and the competition with exotic species (*A. mellifera*). However, one of the most important problems is felling and the fragmentation of the forests and jungles where these bees feed and naturally build their nest. In Cuetzálan (like in many other regions of México), natural ecosystems such as the cloud forest and the medium semi-deciduous forest have been fragmented, as a consequence of the soil use change caused by agricultural activities, until only relics of the native vegetation remain (INEGI, 1996).

The main crop grown in Cuetzálan is Arabiga bean coffee. In order to provide shade to the crops, producers grow trees such as banana (*Musa sapientum*), lemon (*Citrus limón*), orange (*Citrus sinensis*), or lichi (*Litchi chinensis*). Benítez-García *et al.* (2015) pointed out that, according to the observations of stingless beekeepers, these are some the trees that *S. mexicana* visit on a regular basis; additionally, stingless bees also visit allspice (*Pimenta dioica* (L.) Merr.), lobster claw (*Heliconia rostrata* Ruiz & Pav.), macaw flower (*Heliconia bihai* (L.) L.), holy basil (*Ocimum* spp.), great bougainvillea (*Bougainvillea spectabilis* Willd.), huichin (*Verbesina persicifolia* DC.), American elder (*Sambucus canadensis* L.), chalahuite (*Inga vera* subsp. *Spuria* (Willd.) J. Leon), chocolate pudding fruit (*Diospyros nigra* (J.F. Gmel.) Perrier), mamey sapote (*Pouteria sapota* (Jacq.) H.E.Moore & Stearn), capulín agrio (*Ardisia compressa* Kunth) (Dávila, Vargas, *et al.*, 2014), cinnamon (*Cinnamomum* sp.), scarlet bush (*Hamelia patens*), jonote (*Heliocarpus appendiculatus*), and some wild yellow flowers akin to Devil's beggarticks (*Bidens* sp.). Unfortunately, these food sources of stingless bee do not substitute the food resource from native vegetation; consequently, stingless beekeepers have witnessed how honey production has diminished from one generation to the next. Currently, during low flowering times, they face a major problem since they obtain less than 250 mL per *mancuerna*.

Problems faced by meliponiculture

Adulteration is the main problem caused by low honey production. Stingless bees honey producers mentioned that "miel virgen" can be adulterated with piloncillo (also known as panela), water, "*honey from big bees*" (*i.e.*, *Apis mellifera*), and sugarcane —which, after some time, accumulates at the bottom of the jar. Stingless beekeepers pointed out that adulterated honey "*goes off faster*," resulting in mistrust among consumers. Ávila *et al.* (2018) mentioned that this problem is growing, given the lack of official regulation and quality standards.

Meanwhile, *mancuernas* are stolen. In Cuetzálan, some stingless beekeepers consider *mancuernas* as an inheritance for their children or grandchildren and other stingless beekeepers have bought a stingless bee colony as a consequence of their interest (Padilla-Vargas and Vásquez-Dávila, 2013). This demand for stingless bee colonies promotes the theft and clandestine sale of the *mancuernas*. Some producers told us that "*stolen mancuernas are sold for approximately $400.00-$500.00 Mexican pesos, while owners sell them for approximately $1,000.00 Mexican pesos*" (Toño, personal communication, November 17, 2021). To protect their bees against this well-known activity, several stingless beekeepers prefer to keep the number of *mancuernas* that they possess and their location a secret or to keep guardian dogs near the honeycombs.

Additionally, the lack of government support limits the growth of meliponiculture. Many stingless beekeepers told the interviewers that "*Meliponiculture in Cuetzálan needs* *support; the government should take care of the small producers of "miel virgen".* This activity has been carried out for years and it is still waiting for its moment to shine. Producers need support to offer a competitive sale price and to find the right market for their product. And even if this support is provided, many stingless beekeepers do not have computers or cell phones with internet access and, consequently, the information they receive is limited to mouth-to-mouth promotion.

Additionally, the commercialization of "miel virgen" in Cuetzálan faced an unexpected challenge: the COVID-19 pandemic of 2020. This pandemic is known by the Nahuas as *kokolis uejueyinemamaualis kaxtol uan naui* or "the contagious disease from 2019". The health restrictions and the isolation imposed in the whole country drastically reduced the arrival of tourists to the area (Castillo Hernández, 2021). This situation caused a stagnation in the sales of honey because producers sell most of their production to tourists. However, stingless beekeepers are optimistic and hope that tourism will soon be normal again.

CONCLUSIONS

In Cuetzálan, meliponiculture is linked to the indigenous culture. It is not just a way to earn an additional income, it is also a symbol and a part of the regional historical legacy. This legacy has not remained unchanged, because meliponiculture includes lore and beliefs that change and whose understanding changes through time. Cuetzálan has become a place of great interest, as a result of the honey produced by stingless bees; however, it faces several difficulties. Small producers from indigenous communities require support in their fight to turn meliponiculture into a praiseworthy commercial activity.

The social and scientific acknowledgment of the properties of the honey shows the importance of using innovations that enhance the production. A horizontal approach is required, because it encourages producers and researchers to develop alternatives together. Acknowledging lore can be an opportunity to make the most of successful practices and to give a new cultural value to the production of this type of honey. Consequently, organizational processes that improve the commercialization channels and reduce unfair practices in honey production must also be included in this approach.

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Silkworm (*Bombyx mori* L.) fed with mulberry (*Morus alba* L.) leaves and cow's milk

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ABSTRACT

Objective: To evaluate the effect of cow's milk as a protein supplement to improve growth and expression of productive parameters in the silkworm (*Bombyx mori* L.).

Design/Methodology/Approach: Four treatments with mulberry foliage supplemented with milk were used, plus a control (which was fed with fresh leaf). Feeding was determined for the last two larval instars, resulting in the following groups: Treatment 1: V instar-TD, Treatment 2: V instar-DI, Treatment 3: IV and V instar-TD, and Treatment 4: IV and V instar-DI.

Results: Larvae fed with foliage supplemented with milk obtained an increase in growth rate and larval weight and formed their cocoons faster than the control. Additionally, there were differences in cocoon weight, silk percentage, and pupal weight, relevant traits for silk production.

Study Limitations/Implications: Very few works have been published about silkworms fed with mulberry foliage supplemented with milk.

Findings/Conclusions: Supplementation has a positive effect on growth and the evaluated traits, improving the yield of silk production.

Key words: Silkworm, milk, mulberry, economic parameters, supplementation.

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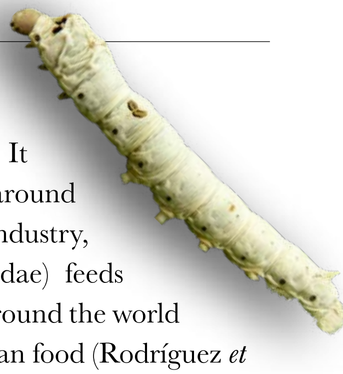
INTRODUCTION



Mulberry (*Morus alba* L.) is native to temperate zones of Asia. It was first cultivated in regions of China, Japan, and the Himalayas around 4,500 years ago. It is the agronomic element of the sericulture industry, because the silkworm (*Bombyx mori* L.) (Lepidoptera: Bombycidae) feeds exclusive on its leaves. It is mainly used in more than 42 countries around the world in sericulture, for ecosystem improvement, and as animal and human food (Rodríguez *et al.*, 2012). Mulberry has one of the highest nutritive values among forages from plant origin; additionally, it has good biomass production and an important agronomic versatility, with an excellent potential to improve feed quality and increase animal production (Camayo *et al.*, 2021; Elizondo 2004). It is highly digestible and has an excellent crude protein (CP) content that reaches levels of 20 to 24% (Mejía, 2019; González and Milera, 2000). Therefore, the cell wall content —along

with the structural carbohydrates and ashes found in the foliage— has been considered as an excellent feed for high-yielding animals and can be offered fresh or dried as part of compound rations (Boschini, 2002). The crop is also valued both for its fruit (consumed fresh, juiced, or canned) and its medicinal properties as an infusion (mulberry leaf tea) (Kumar *et al.*, 2015).

The main use of mulberry is as food for the silkworm (*Bombyx mori* L.) (Lepidoptera: Bombycidae). This monophagous insect —considered as a model system among the lepidopterans, given its easy management and low cost— does not represent a biological hazard, and it is economically important for the sericulture industry, since it is vital for silk production worldwide (Konala *et al.*, 2013).

Insect nutrition plays a fundamental role in sericulture, since it improves the expression of the commercial characters of *B. mori* (Ruiz *et al.*, 2020; Prieto *et al.*, 2016), influencing the quality and quantity of production at an almost individual level (Ruiz *et al.*, 2020). Currently, efforts have been focused on research trials related to the enrichment of mulberry foliage, using proteins, carbohydrates, amino acids, vitamins, hormones, and antibiotics (among other nutrients) to encourge better productive responses (Mohamed and Helaly, 2018). In this sense, Bentea *et al.* (2011) point out that the use of these nutrients can improve the expression of such characteristics as larval and cocoon weight. Konala *et al.* (2013) and other authors also suggest that their inclusion in the diet of insects (particularly of *B. mori* L.) is important. Cow's milk can simultaneously provide bioactive compounds —*i.e.*, proteins, carbohydrates, fatty acids, minerals, and other nutrients that favor the growth and development of the life cycle— and peptides, polyamines, and enzymes that can potentially modulate several regulatory processes of the organism (Haug *et al.*, 2007). Therefore, this study evaluated the effect of milk as a protein supplement that improves growth and expression of productive parameters in silkworm (*B. mori*).

MATERIALS AND METHODS

The trial was conducted in 2019 at the Silkworm Laboratory of the Universidad Politécnica de Francisco I. Madero (UPFIM) (20° 15' 2" N and 99° 00' 1" W). Located at 1,995 m.a.s.l., the location has a temperate-cold climate, with 17 °C annual average temperature and 540 mm average annual precipitation (Rodríguez *et al.*, 2013).

Biological material. The biological material (Figure 1) was made up of populations of *B. mori* (kinshu×showa hybrid / Chinese×Japanese race). Larvae were reared in 40×40 cm plastic containers, at 24±4 °C and with a 55-65% relative humidity.

Rearing method. Mulberry (Kanva variety) leaves were dipped in milk and dried at room temperature before the first feeding of the day. Rations were offered three times a day (7:30 am, 12:00 pm, and 5:00 pm). Beddings and brood containers were cleaned daily to avoid health issues, eliminating feces and mulberry residues.

Milk composition. Ultra-pasteurized whole milk with added vitamins (A and D) was used. The nutritional composition per 250 ml was as follows: 7.8 g of protein, 8.8 g of lipids, 5.4 g of saturated fat, 13 g of carbohydrates, 13 g of sugars, 116 mg of sodium, 290 mg of calcium, 166 μg of vitamin A, 1.25 μg of vitamin D, and 162 kcal of energy content. Experimental design. For the purposes of the comparative analysis, larvae were divided

Figure 1. Larva of *Bombyx mori* L. kinshu×showa hybrid / Chinese×Japanese race.

into five groups. Group 1 was fed with fresh mulberry leaves every day, during the fifth instar, and was considered as the control. Group 2 was fed with mulberry leaves dipped in milk every day, during the fifth instar (Treatment 1: V instar - TD). Group 3 was fed with mulberry leaves dipped in milk on alternate days (1, 3, and 5) of the fifth instar (Treatment 2: V instar - DI); whereas, on days 2, 4, and 6, they were fed with fresh mulberry leaves. Group 4 was fed with mulberry leaves dipped in milk every day, during the fourth and fifth instar (Treatment 3: IV and V instar - TD). Group 5 was fed with mulberry leaves dipped in milk on alternate days (1, 3, and 5) from the fourth to the fifth instar (Treatment 4: IV and V instar - DI); whereas, on days 2, 4, and 6, they were fed with fresh mulberry leaves. Weights were recorded from day 1 to 5. A completely randomized design was used, with three replicates per treatment (with n=50 larvae each). ANOVA assumptions were verified individually for each variable. The independence of experimental errors was ensured through the random assignment of populations to each experimental unit. The assumption of normal distribution was analyzed using the Shapiro-Wilk test. The statistical software used was SPSS v. 25. The "F" value was considered significant at a 0.05 probability level and Tukey's multiple comparison test was performed at 5%.

Productive traits evaluated. Cocoon weight (g), bark weight (g), bark percentage (%), pupal weight (g), and fecundity (number) were defined as productive parameters related to silk quantity and relevant for silkworm rearing.

RESULTS AND DISCUSSION

Effect of fresh mulberry and mulberry supplemented with milk on larval growth

Larvae fed with mulberry foliage supplemented with milk showed a tendency to gain weight after the first day of handling; the highest growth and weight (3.175 g) was observed in Treatment 4. Additionally, in treatments where mulberry leaves were treated with milk during the IV and V instars, larvae decreased their consumption by the fifth day and matured early. The behavior observed with Treatment 1 was also expressed in larval weight, which was equal to the control on the fourth day of the V instar. Treatment 1 was the first group to initiate cocoon formation and the weight was reduced by the fifth day, the same as in Treatment 3 (Figure 2).

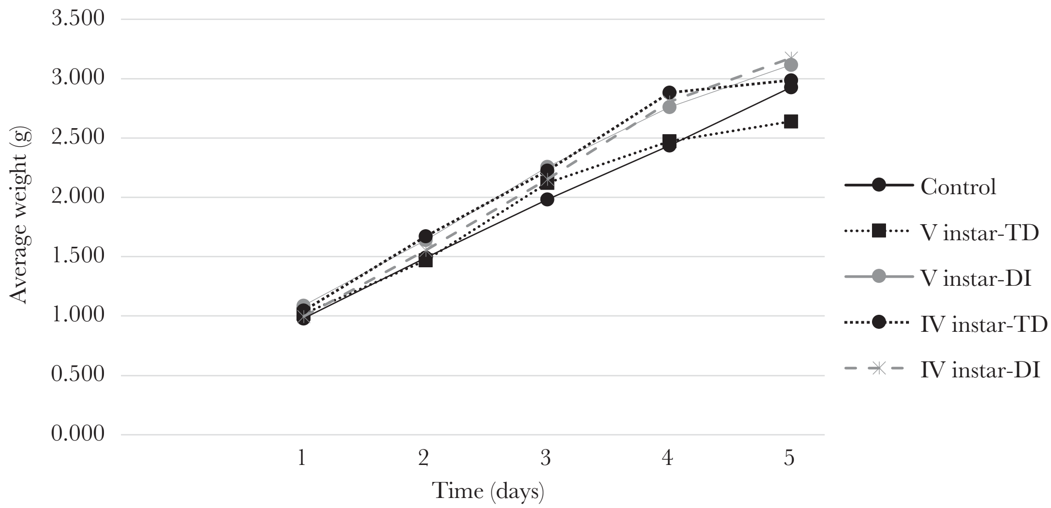


Figure 2. Relationship between the weight of larvae fed with fresh mulberry during the fifth instar days.

In general terms, the growth and weight of larvae fed with enriched foliage showed an increased trend compared to the control and, in this sense, Guevara *et al.* (2014) indicate that casein (the main protein component of milk) has a high content of essential amino acids. Casein has proven to be beneficial for the development of *B. mori*, probably because it contains large amounts of glutamic acid, which, just like aspartic acid, is required for the proper growth of larvae (Konala *et al.*, 2013). Other authors, including Mohamed and Helaly (2018), suggest that the increased growth and development could be caused by the high lipid and protein content of milk. Likewise, Hossain *et al.* (2015) and Byeon *et al.* (2005) point out that, during the fourth and fifth instars, nutrients used to enrich the foliage can have a positive influence on this trend.

Effect of treatments on productive traits

The results of the trial showed that cocoons formed by larvae fed with foliage supplemented with milk weighed more than those fed with fresh mulberry leaves (Table 1). Compared with control worms, the cocoon weights for Groups 3 (Treatment 2) and 5 (Treatment 4) increased by 27.5 and 24.7%, respectively; meanwhile, Group 4 (Treatment 3) increased by 18% and Group 2 (Treatment 1) increased by 12.5%.

Supplementation could induce an increase in larval weight. In this regard, Ramesh *et al.* (2018) points out that cocoons are selected by weight, because it functions as an indicator that predicts the approximate amount of silk that can be obtained —an aspect that largely depends on the quality and quantity of the mulberry foliage supplied.

Significant differences were found in the silk percentage variable. According to Tukey's multiple comparison test (5%), Treatment 1 and the control were grouped and showed similar results, while treatments 2, 3, and 4 —whose means did not represent differences, suggesting a similar behavior— formed the second group

These results suggest that milk has a high nutritional value, which could influence cocoon weight and directly affect silk percentage. In this context, Hossain *et al.* (2015) and Byeon *et al.* (2005) point out that foliage supplemented with milk does not hinder the

Table 1. Effect of milk on the productive traits of silkworms.

Treatments	Cocoon weight (g)	Bark weight (g)	Silk percentage (%)	Pupal weight (g)	Fertility (No.)
1	1.572 bc (±0.176)	0.279 a (±0.033)	17.831 a (±2.004)	1.293 bc (±0.158)	594.1 a (±90.5)
2	1.782 a (±0.153)	0.292 a (±0.024)	16.452 b (±1.336)	1.490 a (±0.143)	556.7 a (±37.3)
3	1.652 ab (±0.228)	0.274 a (±0.026)	16.759 b (±1.935)	1.378 ab (±0.211)	526.6 a (±35.5)
4	1.742 ab (±0.155)	0.293 a (±0.029)	16.945 b (±2.197)	1.448 ab (±0.189)	520.7 a (±62)
Control	1.397 c (±0.221)	0.275 a (±0.048)	19.819 a (±2.519)	1.121 c (±0.189)	521.1 a (±78.7)

Note: a, b, c: means in each column with different letters show significant differences, according to Tukey (5%). Fecundity: number of eggs oviposited by a moth.

digestion of *B. mori*. Although lactose is the main carbohydrate component in milk, *B. mori* has the beta-glucosidase enzyme, active for cellobiose and lactose. Consequently, there are no negative effects to the metabolism of *B. mori*.

Significant differences were found in pupal weight. Treatment 2 had the best response. In this regard, Mohamed and Helaly (2018) and Sekar *et al.* (2016) indicate that feeding the larvae of *B. mori* L. in the fifth instar mulberry foliage supplemented with milk or other nutrients improves some biological characteristics, possibly as a result of the high content of nutrients (proteins and lipids) that increases their growth and development. Manuelian *et al.* (2018) and Haug *et al.* (2007) report that casein contains fatty acids, cholesterol, sugars, vitamins, and minerals (Ca, Mg, P, K, and Na) and, according to Konala *et al.* (2013), they stimulate the feeding efficiency of *B. mori* L. Regarding bark weight and fecundity, no significant differences were found between the control and the treatments used.

CONCLUSIONS

This study determined that foliage supplemented with milk has a positive effect on the growth and weight of the larvae of *B. mori*. In addition, it promoted the early maturation of the larvae —*i.e.*, it accelerated the beginning of cocoon construction, an indicator of the growth rate acceleration. This is a highly relevant aspect, since it would reduce costs as a consequence of the decrease in the amount of foliage supplied, therefore reducing the labor used per brood. Likewise, cow's milk induced a significant increase on productive characters such as cocoon weight, silk percentage, and pupal weight, which are directly related to the increase in yield for silk production. Therefore, the results suggest that *B. mori* larvae can be fed with mulberry foliage supplemented with bovine milk and, in this way, increase silk production. However, the offspring should be fed constantly and its effect should be evaluated for each generation.

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Colegio de Postgraduados

Phenols as an alternative, neuroprotective, and preventive strategy for Alzheimer's disease: (mini-review and bibliometric analysis)

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ABSTRACT

Objective: This study aimed to perform a bibliometric review focused on secondary metabolites of phenolic origin in the context of Alzheimer's disease. Analyzing the research trend in this field during the last two decades, highlighting the chemical-computational (*in silico*) perspective.

Design/methodology/approach: Publications from the last two decades (2001-2023) were examined using the academic search engine Dimensions. The focus of our analysis was on identifying co-occurrence networks of terms present in the titles and abstracts of these publications, setting a minimum threshold of 100 co-occurrences for inclusion in the study (VOSviewer v. 1.6.19,2023).

Results: The literature consulted suggests phenolic compounds as metabolites with preventive capacity, derived from their antioxidant, anti-inflammatory, and neuroprotective properties.

Limitations on study/implications: However, it is essential to highlight the limitations observed in each area so that an integral vision is encouraged in future research.

Findings/conclusions: The integration of epidemiological studies, *in vitro* investigations, *in silico* analysis, and *in vivo* experiments will advance the development of therapeutic strategies based on phenolic compounds for the care of multifactorial Alzheimer's disease.

Keywords: Alzheimer; bibliometric analysis; *in silico*; Phenols.

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INTRODUCTION

Secondary metabolites (MeS) represent a diverse group of biomolecules derived from natural sources, including plants, bacteria, fungi, and algae. These substance's roles complement primary or essential metabolism (nucleic acid and protein synthesis) with functions such as interspecies interactions, quorum sensing, and tissue differentiation. Moreover, MeS are involved in several critical functions, such as defense mechanisms against predators, adaptation to stress conditions (either biotic or abiotic), but also growth regulation, pollination, interspecies competition, and act as chemical messengers enabling intra- and interspecific communication (Böttger *et al.*, 2017; Garcia-Mier *et al.*, 2018).

The diversity of mechanisms involving MeS is directly due to its wide chemical range. In plants, for instance, there are more than 200,000 different MeS reported (Rasmann *et al.*, 2012). These molecules are synthesized and distributed at various levels, tissues, or phenological stages, being present in a limited way between taxonomic groups and representing 1% of the total weight of their organisms. (Akula & Ravishankar, 2011; Caretto *et al.*, 2015). Moreover, the diversity of biological activities exhibited by secondary metabolites (Gibney *et al.*, 2019; Kim *et al.*, 2021) underscores the significance of research on the chemistry of MeS, such as phenols, triterpenoids, flavonoids, among others.

The phenolic compounds are widely distributed in the cell walls of plants, forming part of polymers such as p-hydroxybenzoic acid, suberin, and lignin. In the case of lignin, a cross-arrangement of phenols is observed, with diverse spatial and structural distributions, adopting ortho-, meta- and para-positions (Li *et al.*, 2023). This structural variability is due to the susceptibility of the benzene ring in phenols to be modified by hydroxyl groups (Hithamani *et al.*, 2022). Such modifications lead to the formation of different phenolic compounds, each with its own distinct bioactivities. The phenolic compounds possess a versatility that makes them indispensable in our daily lives and essential both in scientific research and in different industries, including food, agriculture, cosmetics, and pharmaceuticals (Silva *et al.*, 2020; Bondam *et al.*, 2022). Hence, the importance of the identification of phenolic compounds as active principles in herbal drugs due to their medicinal and pharmacological properties (Sá *et al.*, 2017; Xu *et al.*, 2022).

With increasing life expectancy and the growing concern to address multimorbidity with more effective pharmacological strategies, there has been an increasing focus on the development of drugs with diverse biological properties (Zhang *et al.*, 2017; Elansary *et al.*, 2019; Foscolou *et al.*, 2021). These include antioxidant capacity, antiproliferative effects, vasodilators, and enzyme modulators that are progressively relevant in the context of healthy aging toward addressing age-associated and chronic-degenerative diseases. (Zhang *et al.*, 2017; Elansary *et al.*, 2019). Among these diseases are heart disease, chronic obstructive pulmonary disease (COPD), diabetes mellitus, and Alzheimer's dementia (AD) (Kovacic, 2017; Luna-Guevara *et al.*, 2018).

Alzheimer's disease, the most common form of dementia worldwide with approximately 55 million cases, is listed as the third most costly disease according to data recorded by the Alzheimer's Association and the International Classification of Diseases (Gauthier *et al.*, 2021; ICD-11, 2023). The reported incidence in Mexico is 27.3 persons per thousand inhabitants yearly (Arrieta-Cruz & Gutiérrez-Robledo, 2015). Moreover, the number of cases is estimated to triple in the next 30 years (Hernández-Reyes *et al.*, 2012; Mejía-Arango *et al.*, 2020). This dementia is a progressive multisystemic terminal neurodegenerative disease of unknown etiology. It is mainly characterized by memory loss, language impairment, decreased motor coordination, and gradual intellectual decline (Sherman *et al.*, 2021).

Concerning pharmacotherapy, medications approved by the Food Drug Administration (FDA) offer temporary symptomatic relief tailored to clinical needs at different progressive stages of dementia. During the initial therapeutic approach, acetylcholinesterase inhibitors, monoclonal antibodies, and NMDA receptor antagonists are administered. In more advanced stages of the disease, a combination therapy incorporating antidepressants, anxiolytics, and antipsychotics is usually chosen (Gustavsson *et al.*, 2022; Cammisuli *et al.*, 2022). These therapies often lead to adverse effects such as confusion, edema, and microhemorrhages, in addition to showing reduced efficacy (Huang *et al.*, 2020; Alhazmi & Albratty, 2022). It has been observed that naturally occurring molecules of phenolic origin present a bioactive potential in neurodegenerative diseases, which can be exploited in the search for new strategies for the treatment of Alzheimer's disease (Rojas-García *et al.*, 2023). This study aims to conduct a bibliometric review focused on secondary metabolites of phenolic origin in the context of Alzheimer's disease. The trend of research in this field during the last two decades will be analyzed, highlighting the chemical-computational (in silico) perspective.

MATERIALS AND METHODS

The aim of this research is to conduct a bibliometric review of studies related to Alzheimer's disease with particular emphasis on in silico studies concerning bioactive phenolics that might be useful as lead compounds in diverse drug development stages.

Construction of a bibliographic database

The database was obtained using the next-generation academic search engine Dimensions. It indexes more than 10 million datasets from more than 1000 repositories, including sources such as multidisciplinary publications, scientific, grants, datasets, clinical trials, patents, and policy documents (https://app.dimensions.ai) (Van Eck & Waltman, 2010; Arruda *et al.*, 2022).

The systematic search for publications was performed by enriching the thesaurus "Alzheimer," "phenols," "*in silico* analysis," and "computer-designed drugs." The Boolean operators used were "AND" and "OR". All publications in a time interval of the last two decades (2001-2023), were considered valid hits. The information obtained was classified, with special curation of unrelated topics to the study.

Bibliometric analysis

For the bibliometric analysis, the software tool VOSviewer version 1.6.19,2023 was used. The software tool allows the construction and visualization of bibliometric networks based on queries in databases such as Web of Science, PubMed, Scopus, and Dimensions. In this work, our aim was to analyze the components of the bibliometric network generated by VOSviewer. The generated networks derived from the analysis of the co-occurrence of terms found in the titles and abstracts of the documents, with a minimum threshold of co-occurrences set at 100. Nodes and links represent the semantic elements of the network. In this representation, nodes correspond to terms that co-occur, while links represent the relationships between these terms (Van Eck & Waltman, 2010; Arruda *et al.*, 2022). The node size within the network reflects the frequency with which term appears, the distance between nodes indicates proximity, and colors group terms into independent clusters within the network.

RESULTS AND DISCUSSION

Chemotaxonomy of secondary metabolites

The biosynthesis of secondary metabolites (MeS) is a complex and diverse process involving distinct metabolic pathways that sometimes become species-specific. However, several authors describe MeS biogenesis through four main pathways: 1) The mevalonic acid pathway used by plants, bacteria, fungi, and animals for isoprenoid synthesis (Bach & Weber, 1989; Thompson *et al.*, 2018). 2) The methylerythritol phosphate pathway used by archaea, protozoa, plants, and algae in the synthesis of terpenoids (González-Cabanelas *et al.*, 2016; Rodriguez-Concepcion, 2016). 3) The acetate-malonate pathway mainly employed by bacteria, fungi, and algae to synthesize polyketides (Niu *et al.*, 2019). 4) The shikimic acid pathway mainly used in plants to synthesize aromatic compounds such as alkaloids and phenols (Santos-Sánchez *et al.*, 2019). Furthermore, these secondary metabolites are classified into four main groups: alkaloids, terpenes, cyanogenic glycosides, and phenolic compounds (Chomel *et al.*, 2016). It is relevant to mention that phenols are the most prevalent secondary metabolites found in plants, with more than 8,000 molecules reported (Bhuyan & Basu, 2017). Phenols are classified according to their molecular weight, *i.e.* low molecular weight or simple phenols and high molecular weight phenols, also known as complex phenols (Chomel *et al.*, 2016; Carregosa *et al.*, 2022). Both can be subclassified based on their constituent carbon structure (Table 1). This classification provides a swift approximation to understand the diversity and mechanisms of action of these compounds in the plant kingdom as well as their potential use in biomedicine.

The structural diversity of phenolic compounds can explain, in part, the heterogeneous biological activities that these compounds can display, suggesting their role as potential bioactive agents. Several clinical and preclinical studies have shown that phenols can help from the prevention to the treatment of neurodegenerative diseases, such as Parkinson's and Alzheimer's. Therefore, as progress is made in natural products as alternative therapies in treating chronic degenerative diseases, current challenges are addressed, and future perspectives for using phenols in neurodegenerative diseases are outlined.

Phenols and their therapeutic potential in chronic degenerative diseases

Due to their quasi-ubiquitous presence in different organisms, phenols play an essential role in developing cosmetic additives, herbal products, functional foods, and active ingredients in pharmaceutical treatments (Ammar *et al.*, 2020). The therapeutic activity of phenols was evidenced in various diseases (Rigacci & Stefani, 2015; Essa *et al.*, 2016), where different metabolites, such as anthocyanins, flavanols, procyanidins, flavanols hydroxycinnamates and ellagitannins exhibited antiplatelet aggregation actions, oxidation

Table 1. Classification of phenolic compounds according to molecular weight and carbon structure.

Molecular weight	carbon skeleton
Low molecular weight less than 500 Da (LMW)	Benzoquinones (C_6) Phenolic acids (C_6C_1) Acetophenones and phenylacetic acids (C_6C_2) Coumarins and hydroxycinnamic acids (C_6C_3) Naphthoquinones (C_6C_4) Xanthones ($C_6C_1C_6$) Stebilenes and anthraquinones ($C_6C_2C_6$) Flavonoids ($C_6C_3C_6$)
High molecular weight from 500 to 3000Da (HMW)	Lignans $(C_6C_3)^2$ Lignins $(C_6C_3)^n$ Catecholamines $(C_6)^n$ Tannins $(C_6C_3C_6)^n$

Lattazio, 2013; Jawal *et al.*, 2018.

of low-density lipoproteins, decrease in blood pressure, as well as, a lower association of mortality in cardiovascular diseases (Rodriguez-Mateos *et al.*, 2014; Behl *et al.*, 2020; Vetrani *et al.*, 2020). Meanwhile, in respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD), phenols have stood out for their antioxidant, anti-inflammatory, immunomodulatory, and bronchodilator effects (Sagar *et al.*, 2020; Beigoli *et al.*, 2021).

Regarding diabetes mellitus, resveratrol, and anthocyanins showed reduced blood glycemic index and improved functions in pancreatic βcells (Asgar, 2013; de Paulo Farias, 2021). Clinical studies in humans suggest that phenols present in grapes, such as catechin, epicatechin, anthocyanidin, and quercetin, after prolonged ingestion, tend to accumulate in the brain and show the ability to cross the blood-brain barrier, modifying the modulation of cell signaling and neutralization of the redox state in aged brains, and improve the cognitive activity. In addition, another study revealed that a diet rich in flavonoids, combined with physical activity, was associated with a lower risk of developing Alzheimer's disease (Luna Guevara *et al.*, 2018; Devi & Chamoli, 2020; Shishtar *et al.*, 2020). This evidence proposes phenols as bioactive molecules with a broad pharmacological potential in neurodegenerative-type diseases.

Epidemiological studies

Epidemiological studies focused on Alzheimer's disease (AD) have provided information on geographical prevalence (countries), age (dependent on aging rates), incidence (increases after 65 years), and risk factors (smoking, insomnia, stress, diet, and sedentary lifestyle in others). There are strategies designed for healthy mental aging (Yamada et al., 2015; Shahidi & Yeo, 2018), highlighting the adoption of healthy habits and incorporation of social, physical and cognitive activities (Makrakis *et al.*, 2022), which, in conjunction with an anti-inflammatory diet high in phenols, can lead to a lower incidence of AD (Tobias *et al.*, 2014; Gao *et al.*, 2019; Bermejo-Pareja *et al.*, 2016; Fernandez *et al.*, 2018.; Azar *et al.*, 2021; Chu *et al.*, 2023). Some phenol-rich foods, such as olive oil, red wine, coffee, tea, cocoa-derived products, a variety of fruits, culinary herbs, and vegetables, have been identified as important sources of polyphenols (Angeloni *et al.*, 2017; Holland *et al.*, 2020; Pintać *et al.*, 2022; Rivero-Pino *et al.*, 2023).

These foods contain phenols, such as resveratrol, curcumin, apigenin, caffeic acid, ferulic acid, chlorogenic acid, quercetin, and other compounds. These substances add flavor and color to foods and have shown health benefits, including possible preventive effects for Alzheimer's disease (Khan *et al.*, 2019; Dhingra & Chopra, 2023). However, these associative studies highlighting the beneficial properties attributed to phenols lack scientific validation to understand the mechanisms of action of these metabolites in AD.

***In vitro* and *in vivo* studies**

Due to the above, *in vitro*, and *in vivo* studies seek tangible evidence of the attributes ascribed to phenols in AD. In this regard, the research conducted by Vargas-Restrepo and coworkers (2018) suggests quercetin can act as an anti-inflammatory agent in AD, based on results obtained from a transgenic mice (3xTg-AD) animal model, identifying the decrease of reactive microglia as well as fluorescent intensity of Aβ aggregates, GFAP (glial fibrillary acidic protein), iNOS (nitric oxide synthase) and COX-2 (cyclooxygenase-2) immunoreactivity in the hippocampal area. All of them are factors associated with neuroinflammation present in AD brains. Phan *et al.* (2019), using an electrochemical approach, identified that the characteristics of aromatic rings and hydroxyl groups present in flavonoid-type polyphenols (gallocatechin gallate and theaflavin) and stilbene (resveratrol and piceid), allow interaction with Aβfibrils, leading to inhibition in structured aggregation of the same. On the other hand, in vivo studies were performed in the organism Caenorhabditis elegans as an AD model, through the generation of oxidative stress induced by AAPH (2,2'-azobis (2- methylpropionamidine dichlorohydrate) and then applying honey extracts from avocado multiflora (Romero-Márquez *et al.*, 2023), which is rich in phenols such as cafeic acid, ferulic acid, and protocatechuic acid (Zhang *et al.*, 2018). This research identified a positive regulation of the daf-16 gene associated with the oxidative stress response, effectively decreasing the accumulation of reactive oxygen species (ROS). There is also some clinical evidence in humans, regarding the beneficial effects of these phenols in treating dementia (Quinn *et al.*, 2004; Kovacic, 2017).

One of the most recent and extensive works was focused on 921 older adults with a mean age of 81.2 years. These individuals were subjected to a diet enriched with flavonoids, including kaempferol, quercetin, myricetin, and isorhamnetin. The results obtained after one year of treatment revealed that only 23.89% (220 cases) developed Alzheimer's disease, pointing to a significant correlation between phenol intake and reduction in the incidence of AD. However, the mechanisms of action of phenols as an alternative treatment for Alzheimer's disease are not yet fully understood, nor whether this preventive effect lasts in the long term (Hollan *et al.*, 2020). There is a need for more comprehensive research in this field. Furthermore, the implementation of standardized protocols that broaden the spectrum of analysis is essential. These protocols could include high-throughput strategies to address the chemical complexity of phenols, as allowed by *in silico* analyses (Carecho *et al.*, 2023).

In silico **studies**

In the last four decades, with the increase of computational resources and advancement in microprocessing technologies, *in silico* studies have occupied a central role in drug discovery workflows. Moreover, *in silico* analysis of phenolic compounds has been applied to find alternative strategies for AD. A systematic increase in the number of publications regarding Alzheimer's with chemical-computational tools is the principal metric that reveals the interest of the scientific community. For the last decade (2013- 2023), a 2.5-fold increase regarding in silico research is evident, as shown in Figure 1A. In 2001, only 32 studies were carried out in this field, but in 2023, this number increased to 1,638 investigations and continues to grow. It is worth noting that a maximum was recorded in 2008, with a total of 4,597 published papers. Overall, to date, a total of 17,312 research studies have been published in this field (Dimensions, 10/10/23).

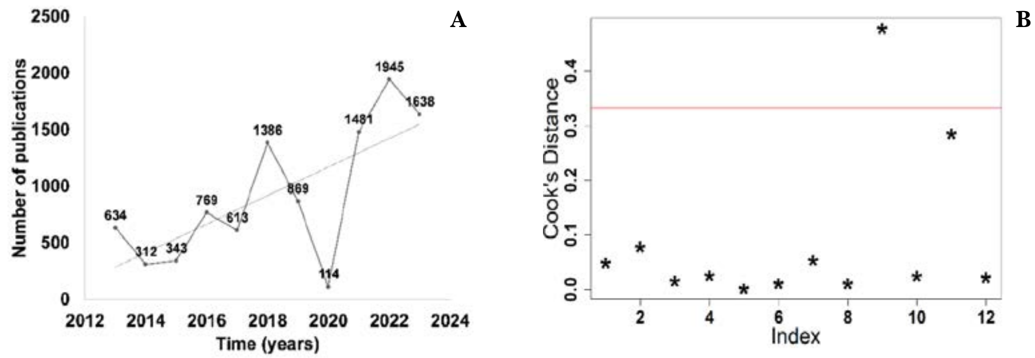

Figure 1. A) Scatter plot: sustained growth in academic contributions on Alzheimer's disease with chemical-computational tools in the last ten years (2013-2023). B) Cook's distance for annual publications from 2012 to 2023.

The sustained growth in academic contributions on Alzheimer's disease with chemical-computational tools in the last ten years (2013-2023) is summarized in Figure 1. Each point represents the influence of a specific year in the regression model. The red line denotes the overall threshold (4/n), where points above are considered potentially influential. Notably, the year 2020 (index 9) exceeds this threshold, indicating a significant influence on the model. The increase in the number of investigations is reflected in a positive upward trend, with a value of $R^2=0.48$ and $\alpha<0.05$. It is important to note that although the correlation value is not statistically significant, this is due to the leverage effect caused by the low scientific production in 2020.

Two methods were employed to determine the influence of the year 2020 on the observed correlation. First, a residual analysis to assess the discrepancies between the observed values and those predicted by the model annually, was performed. Subsequently, the specific impact of 2020 on the regression model was quantified using Cook's distance. This analysis yielded a value of 0.4782631, significantly exceeding the typically established threshold, illustrated by a red line (Figure 1B). Generally, those points in which Cook's distance exceeds 4/n are considered as influent or outliers. In this case, with n corresponding to the total number of observations (12 years), the threshold is 0.3333; since 0.4782631 exceeds this threshold, it is evident that the data corresponding to the year 2020 exerts a significant influence on the model, substantially affecting the estimates of its coefficients. The results mentioned above are supported by bibliometric network analysis of co-occurring terms.

Three clusters were identified in the first decade (2001-2011) (Figure 2A). Cluster I covered topics related to drugs and studies of a genetic nature, in addition to research exploring the action of peptide aggregates such as beta-amyloid and tau. Cluster II highlighted research aimed at therapeutic targets of protein origin with *in vitro* evaluations. Cluster III focused on work of genetic interest that explores the biological origin of Alzheimer's disease. During 2012-2023, four clusters were identified (Figure 2B): Cluster I group articles highlighting the biological properties of naturally occurring phenolic compounds. Cluster II complements the findings of Cluster I by highlighting the bioactivity of biomolecules in pathological events associated with Alzheimer's disease. As for Clusters III and IV, the outstanding inhibitory activity of phenols on various enzymes, including AChE, is highlighted. AChE

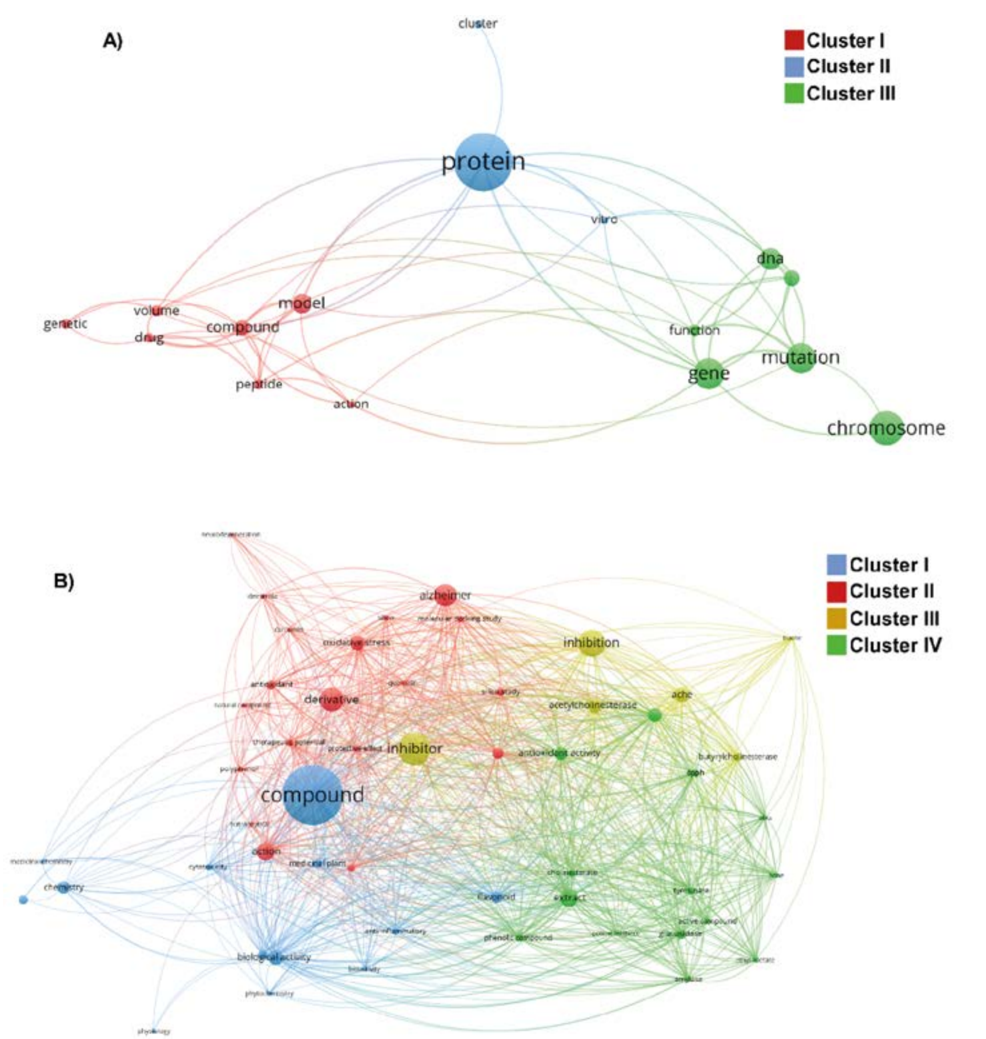

Figure 2. Bibliometric network analysis of co-occurring terms. A) most representative items used in Alzheimer's research from 2001-2011. B) most representative items of the last decade (2012-2023).

is essential in the degradation of the neurotransmitter acetylcholine and has been a critical therapeutic target since the early stages of Alzheimer's research. The *in silico* node shows a significant connection to this network's four previously mentioned clusters; the results are presented in Figure 2, where the contribution of computational-theoretical research to Alzheimer's disease is evident. This result differs from the network corresponding to 2001-2011, where the clusters focus on experimental (*in vitro*) approaches and place the items "cluster," which refers to *in silico* studies, in a distant position with low interconnection.

According to our results, the networks have allowed the definition of two key lines of research in recent years (Figure 3). The first of these lines focuses on predicting the bioactivity of phenolic compounds, with particular emphasis on the antioxidant and anti-inflammatory activity of flavonoids (Cluster I). In comparison, the second cluster focuses on the deciphering of the mechanisms of action of phenols in AD pathological events, such as oxidative stress, inflammation, and protein aggregation. Both clusters are

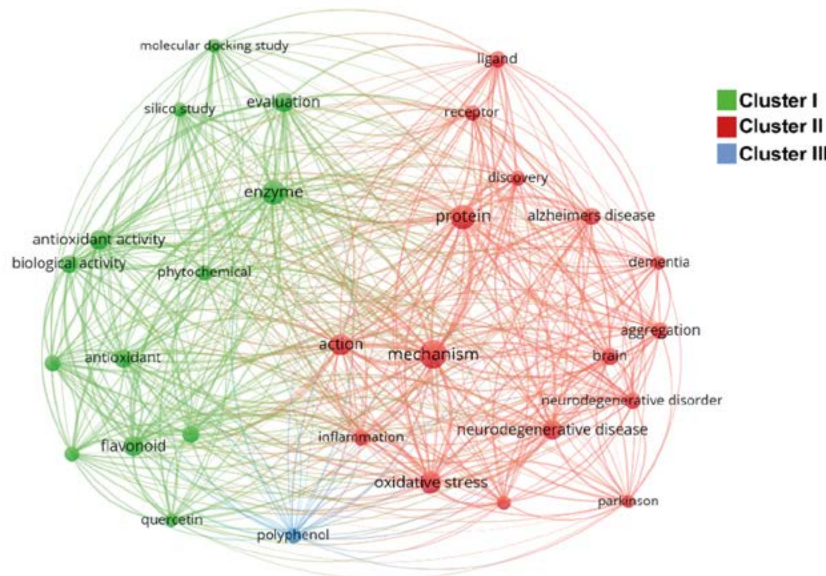

Figure 3. Generalized bibliometric network based on the most representative items of the last two decades (2001-2023). In the network, two apparent aspects can be identified that start from a common origin (polyphenols), which correspond to studies carried out on phenolic compounds, and the second to mechanisms of action on pathogenic events in Alzheimer's disease (green and red), respectively.

interconnected through the "polyphenol" node (Cluster II), representing a pivot in the chemical computational studies of the last two decades. This third network encompasses the studies analyzed in this research (2001-2023). It underlines the importance of evaluating these phytochemicals in therapeutic targets of AD with the assistance of computational theoretical analyses.

The above is reflected in several reports, such as Benchikha and collaborators (2022), which describe an experimental model to evaluate different biological activities, *i.e.* antioxidant, hypoglycemic, and cholinesterase inhibition, enriched with chemical-computational analyses implementing molecular docking techniques. Moreover, these in silico analyses can approximate the binding affinity of the phenols present in the extract of zamarilla (*Teucrium polium* L) against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes, which are first-order therapeutic targets in the progression of AD. Another in silico report sought to evaluate 3150 phytochemicals of diverse origins, including phenolic compounds, to identify secondary metabolites with inhibitory potential against the BACE-1 (β-secretase) enzyme. The pipeline included a first screening phase based on pharmacokinetic profiles to select metabolites that met the predefined ranges in ADME processes (absorption, distribution, metabolism, and excretion). Molecular docking analysis was performed to select metabolites by comparing their binding energies with reference drugs. Subsequently, electronic effects and reactivity at the active site of the BACE-1 enzyme were evaluated using hybrid density functional theory (DFT). Taken together, *in silico* / computational tools allowed the identification of seven compounds (shinflavanone, glabrolide, glabrol, prenillicoflavone A, macleanine, 3a-dihydro-cadambine, and volvalerelactone B) that exhibited inhibitory potential against BACE-1.

However, *in silico* results should be subjected to experimental validation (*in vitro* and *in vivo*), as suggested by Arif *et al.*, 2020. Barai *et al.*, 2018 showed that docking and molecular dynamics analyses are tools that can be employed as tools to predict, compare, and target inhibitory bioactivities of phenol bergenin towards specific therapeutic targets related to Alzheimer's disease, such as acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), Tau protein kinase 1 and β-secretase (BACE-1). The ability of bergenin to exhibit dose-dependent inhibitory bioactivity, specifically about AChE and BuChE enzymes, was addressed in this report. Interestingly, the results from animal models (*in vivo*), showed a significant reduction in neuro-inflammation, suggesting a remarkable improvement in cognitive function in bergenin-treated rats. Consequently, it is pertinent to suggest that *in silico* analyses facilitate the prediction of the bioactivity of phenolic molecules on specific molecular targets, such as enzymes related to dementia. Through both in vitro and *in vivo* assays, these compounds have demonstrated their capacity as bioactive molecules with promising pharmacological applications. Likewise, it is worth noting that *in silico* analyses enabled massive screening of secondary metabolites, resulting in the identification of specific phenols with activity on crucial Alzheimer's disease (AD) related targets. Furthermore, these computational approaches provided accurate predictions of anti-Alzheimer's bioactive activities and contributed to a deeper understanding of the mechanisms of action of phenols in this disease (Monteiro *et al.*, 2018; Cruz-Vicente *et al.*, 2021).

The impact of chemical-computational tools on drug Discovery

Drug development has undergone remarkable advances over the years. The traditional approach consists of evaluating the efficacy of secondary metabolites through a process that involves three fundamental stages, as shown in Figure 4. The first of these stages is discovery, in which the identification of candidate molecules and their subsequent synthesis is carried out. This process begins with a broad chemical space to illustrate the magnitude of the challenge to be considered, and according to other models, the starting point is 9,000 molecules, which will eventually require four to nine years of experimental research (De la Calle, 2009). The second stage is known as the preclinical stage and involves profiling a more manageable and economically viable set of molecules. In this phase, the number of molecules is significantly reduced to around 100 (less than 1%). These molecules are subjected to *in vitro* assays, which allows for the identification and discarding of those chemical units that lack biological activity. In this phase, screening is performed on *in vitro* models in line with their biochemical characterization, which allows for refining the selection of candidates with therapeutic potential in a time interval of three to four years of analysis (Hernández Cabanillas, 2020). In the clinical phase, ca. five molecules undergo rigorous evaluations in patients, and only one of them manages to meet the necessary regulatory requirements to be introduced into the market (Saldívar-González *et al.*, 2017). This final stage can extend over a period that can be around ten years of clinical explorations in animal models and, finally, be transferred to human candidates (Carranza-Aranda *et al.*, 2019).

This lengthy drug development process can take up to 15 to 18 years before a drug is approved (De la Fuente, 2009; Hernández Cabanillas, 2020). This extended duration poses significant challenges that include delayed response to public health emergencies, high costs that often fall on the consumer, limited access to treatments, disincentives for innovation, and the risk of treatments becoming obsolete, as has occurred in the case of Alzheimer's disease (Goldman *et al.*, 2018). Consequently, optimizing drug development processes and reducing costs without compromising drug quality have become imperative. In this area, pharmaceutical research, through computational chemistry, plays an essential role during drug development (Saldívar-González *et al.*, 2017) with computer-aided design (CADD). The CADD has become a relevant approach that streamlines this process and allows the execution of predictive and comprehensive analyses of secondary metabolites, significantly reducing periods that can hover between three and twelve years of research (Figure 4). Furthermore, as a result, these challenges lead to the efficient selection, design,

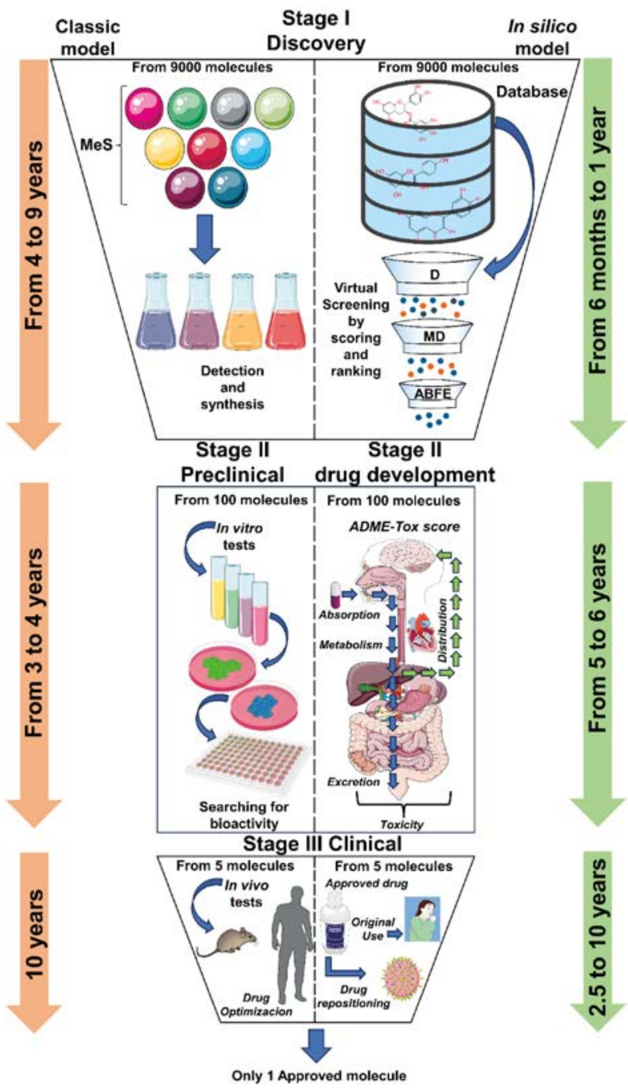

Figure 4. Comparative model of the classical (experimental) drug design versus the in silico model.

optimization, and repositioning of the most promising phytochemical candidates and their orientation toward fundamental molecular targets in Alzheimer's disease progression (Lin, 2022; Ece, 2023).

In summary, *in silico* assays have improved the efficiency and cost-effectiveness of drug design, minimizing investment in laboratory experimental resources, and optimizing the management of new drug alternatives (Shaker *et al.*, 2021). Using theoretical-computational approaches such as molecular docking and molecular dynamics has revolutionized the discovery of natural molecules by predicting how they bind and react in biological systems and improves efficiency in the identification of pharmacological compounds, accelerating the availability and reducing costs of effective treatments, offering an alternative to the classical method.

On the other hand, in stage I, or the discovery phase of the *in silico* approach, candidate molecules are obtained from open-access databases, mostly containing naturally occurring compounds (Önder *et al.*, 2023). Then, through computational execution, structural similarity studies are carried out, and affinities are calculated using techniques such as docking (D), molecular dynamics (MD), and absolute binding free energy (ABFE). These techniques allow the identification of potential ligands with the ability to interact and bind to therapeutic targets, as depicted in Figure 4. In stage II, corresponding to drug development (Geerts & Vander Heyden, 2011), theoretical-computational predictions are carried out to estimate and optimize ADME-Tox pharmacokinetic properties (Absorption, Distribution, Metabolism, Excretion, and Toxicity). These processes are fundamental in drug research and development (Cerny *et al.*, 2023), as they predict possible drug interactions with the organism through the calculation of ADME-Tox properties, thus providing a detailed profile of their efficacy and safety. Computational calculations play a crucial role by ruling out inefficient, nonspecific, toxic or unstable molecules (Bruno *et al.*, 2019; Więckowska *et al.*, 2020). With this, the allocation of resources and time to discover new natural molecules with pharmacological potential in neurodegenerative diseases can be optimized (Loele *et al.*, 2022). In Phase III, chemical-computational analyses have enabled the repositioning and optimization of drugs, with successful examples such as the case of lopinavir, a drug initially designed to treat HIV-1 infection. This drug was redirected to emergency health care to treat severe acute respiratory syndrome (SARS) caused by COVID-19 (Mohamed *et al.*, 2021; Ramirez Salinas *et al.*, 2023).

In this review, we have evaluated the scientific evidence related to the effects of phenols, a group of secondary metabolites of plant origin, in preventing and treating Alzheimer's disease, the most common form of dementia worldwide. The results of this analysis indicate that phenols present therapeutic potential in addressing Alzheimer's disease, given their remarkable antioxidant, anti-inflammatory, and neuroprotective properties and ability to modulate cell signaling. Despite these promising findings, it is worth noting that some reviewed studies have presented shortcomings and contradictions. Therefore, further research in this field is mandatory for a more complete understanding of the benefits and limitations of phenols in Alzheimer's disease.

Epidemiological studies reviewed have revealed an inversely proportional relationship between the consumption of phenol-rich foods, such as tea, coffee, wine, chocolate, and fruits, and the risk of developing Alzheimer's disease (Pintać *et al.*, 2022; Rivero-Pino *et al.*, 2023). These results support the hypothesis that phenols may prevent or delay the onset of this disease by protecting neurons from oxidative stress and inflammation, two critical pathogenic factors in Alzheimer's disease (Cherbuin *et al.*, 2022; Juiz & Lenarz, 2023). However, it is fundamental to recognize that these studies have certain limitations. These include indirect measures to assess phenol consumption, variability in diagnostic criteria, and the difficulty in establishing a robust causal relationship between exposure and effect. Therefore, there is a clear need for more rigorous and specific epidemiological studies. These studies should assess the consumption of individual phenols or groups of phenols, employ biomarkers for both exposure and effect, standardize models for the administration of phenols (Ohishi *et al.*, 2021), and conduct long-term follow-up of participants to confirm detected changes in the incidence and progression of Alzheimer's disease.

The *in vitro* and *in vivo* studies reviewed provide evidence of the molecular and cellular mechanisms through which phenols may exert a beneficial effect on Alzheimer's disease (Phan *et al.*, 2019). These investigations demonstrate that phenols can reduce the formation and aggregation of amyloid and tau proteins, which are responsible for the growth of senile plaques and neurofibrillary tangles, respectively. Such aggregates are distinctive histological features in AD brains (Barai *et al.*, 2018). In addition, phenols can improve mitochondrial function (Mthembu *et al.*, 2021; Flannery & Trushina, 2019), restore calcium homeostasis (Palmerini *et al.*, 2005;) and induce autophagy (Michałowicz *et al.*, 2018; Hung & Livesey, 2021). Moreover, phenols have demonstrated the ability to promote neurogenesis (Corona & Vauzour, 2017) and to modulate the activity of various cell signaling pathways, acting on transcription factors (CREB), insulin receptor substrate (IRS), signal transducers, and activator of transcription (STAT3), among others. These pathways play a significant role in the progression of Alzheimer's dementia and other neurodegenerative diseases. (Kooshki *et al.*, 2023). However, it is worth noting that these *in vitro* and *in vivo* studies have notable drawbacks, such as lack of specificity and selectivity of phenols, assessment of synergistic activity in crude extracts, variability in experimental models, lack of randomized controlled trials, and lack of translation of results to the clinical level (Karim *et al.*, 2020). Therefore, further experimental support in representative models under standardized conditions is always required. It also includes considering the activity of phenols in assays that reproduce the pathological conditions of Alzheimer's disease.

A clear trend shown in the scatter plot (Figure 1A) presents a steady increase in papers incorporating computational theoretical studies over time. In addition, through the generation of co-occurrence networks, the leading research approaches during the last two decades have been identified. From 2001 to 2011, the focus was on questions related to genetic diagnostics and causation (Cluster III) and exploring various protein hypotheses (Cluster II). Collectively, these investigations have moved to the development of an integrative model of Alzheimer's disease on set and progression (Cluster I). In the second period, which covered from 2012 to 2023, the co-occurrence network revealed an exponential increase, with the implementation of chemical-computational tools and the study of phenols as inflection points, leading to a radical change in the focus of research, which emphasizes the identification of protein targets of Alzheimer's disease (Cluster III), where *in silico* analyses allowed to model phenolic compounds' affinity and intermolecular interactions, *i.e.*, inhibition mechanisms of AChE and other enzymes (Cluster II and IV). The previous proposes a multidisciplinary approach that addresses different pathogenic events in Alzheimer's disease (Cluster I) by studying bioactive molecules such as phenols. The *in silico* publications reviewed highlight the applicability of theoretical, computational calculations as complementary tools for the design and optimization of new phenol-based drugs to treat Alzheimer's disease, with computational techniques such as virtual screening, molecular docking, molecular dynamics, and pharmacological modeling the selection of ligands that interact with enzymes involved in AD is possible (Barai *et al.*, 2018; Arif *et al.*, 2020), as well as, prediction and optimization of pharmacokinetic and pharmacodynamic properties of the potential phenolic drug (Kumar & Ayyannan, 2022; Sahadevan *et al.*, 2022). Integrating computational chemistry into the classical drug design model represents an opportunity to foster innovation and develop more effective and accessible treatments for patients suffering from multifactorial neurodegenerative diseases, such as Alzheimer's.

CONCLUSION

This review highlights the role of phenols as promising candidates in developing new drugs for Alzheimer's dementia due to their multiple beneficial effects on general health, particularly those reported in mitigating pathogenic progression. However, it is also clear that research in this field still needs improving since systematization and deepening must come from multidisciplinary and integrative studies (epidemiological data, molecular investigations, and computational studies). Epidemiological studies may lack precision and traceability in some cases. In contrast, *in vitro* and *in vivo* studies, although promising, need to address specificity issues and validate their findings in controlled clinical trials. On the other hand, in silico studies have demonstrated efficiency and cost-effectiveness but require experimental substantiation and validation. Consequently, collaborative epidemiological, *in vitro*, *in vivo*, and *in silico* studies should be conducted as an integral part of the research and development process for new drugs. This interdisciplinary collaboration will broaden the understanding of the effects of phenols in Alzheimer's disease and promote significant advances in the search for effective and accessible treatments for patients facing this multifactorial neurodegenerative disease.

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Colegio de Postgraduados

The association between mites and the *Agave* L. snout weevil, more than phoresis

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ABSTRACT

Objective: to identify the phoretic mites associated with the *Agave* (Maguey) snout weevil *Scyphophorus acupunctatus*.

Materials and methods: manual collections of *Scyphophorus acupunctatus* were carried out in Agave plantations. The daily behavior of mite-infested weevils was documented. The latter were observed and classified according to specialized taxonomic keys.

Results and Conclusions: there was an anomalous behavior of the agave weevil possibly favored by the infestation by mites, which can play an important role in the detriment of the vitality of that pest. *Scyphophorus acupunctatus* (Coleoptera: Curculionidae) interacted with three groups of mites; *Macrocheles merdarius* (Acari: Mesostigmata: Macrochelidae), *Tridiplogynium* sp. (Acari: Mesostigmata: Diplogyniidae) and *Curculanoetus* sp. (Acari: Sarcoptiformes: Histiostomatidae).

Implications: this study is the first to document the presence of phoretic mites on *S. acupunctatus*. It is the first report to document the association between the agave weevil and the aforementioned arachnids (Subclass Acari) that participate and play a desirable role in the biological control of *S. acupunctatus*.

Keywords: chemotaxis, predatory mites, new species, Mezcalero Agave, agave snout weevil, phoresis.

INTRODUCTION

The Agave snout weevil, *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Curculionidae), is considered the most important pest in plants of the genus Agave (Mezcalero maguey), since it is associated as the cause of the disease called “soft rot” of Agave plants; as well as the cause of more damage directly and indirectly (Cruz-Esteban *et al.*, 2021; Cuervo-Parra *et al.*, 2020). The control of this pest is done mainly by making use of synthetic chemical insecticides such as malathion, endosulfan, methomyl and fipronil. However, it is difficult to control this pest because the larvae, pupae and adults develop in the roots and the core of the Agave stem base, making it difficult for the products to contact insects (Terán-Vargas *et al.*, 2012).

For the biological control of the Agave snout weevil, some natural enemies of larvae, *Placodes ebeninus*, *Lioderma cacti* and *Phileurus valgus* have been reported, as well as the ants *Odontomachus bauri* and *Ectatomma ruidum* (Hymenoptera: Formicidae). With *Hololepta quadridentata* and *Hololepta yucateca* (Coleoptera: Histeridae) as the main predators with the highest incidence. *Alienoclypeus insolitus* and *Cyclaulacidea* sp. (Hymenoptera: Braconidae) are also known to be parasitoids of larvae and pupae respectively (Velazquez *et al.*, 2008). For the control of adult *S. acupunctatus*, the nematodes *Heterorhabditis bacteriophora*, *Steinernema feltiae* and *Steinernema websteri* (Rhabditida: Heterorhabditidae) and the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* (Ascomycota: Hypocreales) have been reported infecting both adults and larvae in field conditions (Aquino-Bolañoz *et al.*, 2006). Among the groups of mites used in biological control are those of the order Mesostigmata and Prostigmata of which there are commercially important species available (Castilho *et al.*, 2015; Hernandes *et al.*, 2015). However, there are no reports for the control of the Agave snout weevil through the use of mites of these orders.

Therefore, this is the first report of a consortium of mites that use the Agrave snout weevil as a vehicle; that in other species has been reported as phoresis. But which possibly play a role of greater importance pointing towards the biological control of *S. acupunctatus*. This suggests an agroecological management of the pest in wild and domesticated plantations with importance in forestry and agriculture.

MATERIALS AND METHODS

Collection and treatment of agave weevils

In June 2021, manual collections of *S. acupunctatus* were carried out in wild Agave plantations and plantations with some planting management in localities in the central area of the state of Guerrero, Mexico. The Agave snout weevil specimens were transferred to the Mezcal Quality Analysis Laboratory of the DES of Chemical, Biological and Biomedical Sciences of the Autonomous University of Guerrero for the establishment of a breeding stock for future bioassays. They were placed in glass boxes 11 cm×6 cm high and 8 cm deep feeding them with pieces of fresh Agave plant, their behavior was documented every day.

Observation and classification of mites

Each specimen of Agave snout weevil was observed under a stereo microscope at a magnification of between 0.67X and 5X (Nikon SMZ745T); the mites they carried on their outside surface were removed, using a soft bristle brush. Those mites were preserved in 70% ethanol for further analysis and classification.

The mites found in the snout weevils were mounted in the middle of modified Berlese (Schuster and Pritchard, 1963). Assembled specimens were incubated at 40 °C for 15 days and then identified using a phase contrast microscope (Olympus BX41, Olympus Corporation of the Americas, PA, USA). Several keys were used for the identification of genera (Azevedo *et al.*, 2017; Özbek *et al.*, 2015; Hunter, 1993; Kethley, 1977; Evans, 1956).

RESULTS AND DISCUSSION

After 4 days on average, the agave snout weevils associated with mites showed an anomalous behavior (lethargy) until they died. In the meticulous observation of the live and dead specimens under the stereoscopic microscope the consistent presence of a cluster of mites on the thorax of the weevil was evidenced without ruling out that the result of this association could be something more than a phoresis. The mites were identified as *Macrocheles merdarius* (Acari: Mesostigmata: Macrochelidae), *Tridiplogynium* sp. (Acari: Mesostigmata: Diplogyniidae) and *Curculanoetus* sp. (Acari: Sarcoptiformes: Histiostomatidae) (Figure 1). The average number of mites that an adult of *S. acupunctatus* carries on its armor was 30 individuals with no apparent numerical relationship between the infesting species.

The first two species of the aforementioned mites were found attached to both the head and the thorax and abdomen of the Agave snout weevil (Figure 2). It was also recorded that these mites might express potential chemotaxis to the Agave snout weevil because the specimens detached from their host began to search, quickly returning and climbing on the host body in their original locations. Finally, it was observed that the mite *Curculanoetus* sp. (Acari: Sarcoptiformes: Histiostomatidae) is found colonizing inside the elytra of the beetle where significant deterioration was observed in the wings of the Agave snout weevil, thereby limiting its natural mobility. Similarly, to this latter mite, the presence of hyaline eggs has been documented without learning at the moment to which species they correspond (Figure 3).

Findings on the associations between mites and beetles have been described. Francesco Porcelli *et al.* (2009) pointed out the possible introduction of new species of mites in Malta

Figure 1. Observation of specimens through a stereoscope. (a) *Macrocheles merdarius*, (b) *Tridiplogynium* sp., and (c) Deutonymph of *Curculanoetus* sp. The scale shown represents 1 mm, with divisions of 0.1 mm.

Figure 2. Mite-infested agave weevil (*Macrocheles merdarius*, *Tridiplogynium* sp. and *Curculanoetus* sp.); as observed in a stereoscopic microscope. The bar represents 0.5 cm

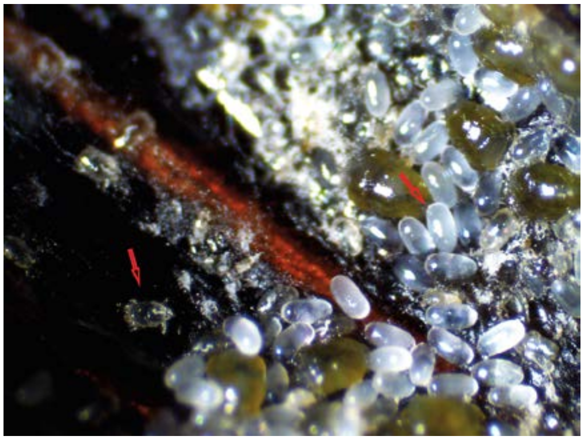

Figure 3: Dissection of the elytra of *S. acupunctatus*. Anterior surface of an elytra of the Agave snout weevil. The arrows indicate hyaline eggs not yet classified and individuals of *Curculanoetus* sp. in the deutonymph phase.

through the red palm weevil. Those authors also indicated that *Centrouropoda almerodai* and *Uroobovella marginata* were housed in the elytra of *Rhynchophorus ferrugineus* from the first moment of the observations. That differ to what was documented by Gómez-Marco *et al*. (2021), who pointed out that a close related taxon *Rhynchophorus palmarum* (the South American palm weevil), initially did not present development of mites under the elytra, but after a couple of years they began to colonize this part of the beetle. Those authors identified three species of mites moved by the beetle, within which *Centrouropoda* n. sp. and *Dinychus* n. sp. were considered as new species in the United States of America, where it was only recorded *Fuscuropoda marginata*. All these species were associated with the beetle as their causal disperser.

These associations have been described as an adaptation of the needs of the mite on its prospects of dispersion documenting a large number of arachnids on the exoskeletons of the hosts in a multispecies set where up to 14 species come to be represented in association. Or else, a large number of individuals that belong to fewer species, but always located on the outer surface of the host and even inside the elytra (Milan Pernek, 2012; Quintero-Gutiérrez, and Romero-García, 2014). To add to those records on the mobilization of mites within the aforementioned type of association, in another study by Mohammad Ali Al-Deeb *et al.* (2011) where they collected the red palm weevil *Rhynchophorus ferrugines* Oliver in the Arab Emirates; they reported for the first time that these exotic species of mites *Uroobovella* sp. *Curculansoetus* sp. and *Uropoda orbicularis* were presented in the country as representatives of three families. Those authors also pointed to the beetle as the responsible and means of transportation that facilitated the mobilization of the arachnids. In addition, those authors concluded suggesting that the presence of this consortium of mites implies something more than phoresis, then arguing that their numerical abundance is to the detriment of the host.

In Mexico the presence of *S. acupunctatus* as well as its economic importance in commercial and wild agave plantations (National Service of Health, Safety and Food Quality, 2016), and other types of vegetation (Servin *et al.*, 2016) has been widely reported. However, this study shows the first evidence that it can also be associated with mites as do other beetles previously cited. In addition, a behavioral change of the hosts was observed that make possible to infer that mites might be proposed as an interesting means towards the biological control of the Agave snout weevil pest.

CONCLUSIONS

The hypothesis raised on this bipartite interaction (insect-mites) is the existence of a natural enemy of *S. acupunctatus* in plantations of wild and semi-domesticated agaves where the applications of synthetic chemicals have been null, moreover, few losses of agaves are reported due to the damages attributed to *S. acupunctatus*. Nonetheless, this study describes for the first time, the natural interaction between the Agave snout weevil (pest of the Mezcalero maguey) and a group of mites that possibly means something more than just a phoresis, which also had not been described among these participants.

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Women in the labor market and economic growth in Mexico

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ABSTRACT

Objective: the objective of this study was to analyze the participation of women in the labor market and the effect on economic growth in Mexico, 2000-2021.

Design/Methodology/Approach: three multiple linear regression models were developed using the Ordinary Least Squares method.

Results: the most statistically significant variables were the exchange rate, wage and the employed female population. The most significant elasticities were found at an increase of 10.0% of the employed female population, the GDP would increase by 7.83%. For the model where only the employed female population was analized, at an increase of 10% in it, the GDP would increase by 10.01%.

Study limitations/Implications: the main limitation was that the information is not available from a single source and the figures vary depending on the official institution.

Findings/Conclusions: based on the results obtained, it is concluded that the participation of women in the labor market does increase economic growth.

Keywords: women, employment, labor market, general minimum wage, economic growth.

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INTRODUCTION

Women account for almost half of the world's working-age population. Of about 5 billion women, only 50% of them participate in the labor force, compared to 80% of men. Not only is labor participation lower, with paid work, but women are also overemployed in the informal sector. Especially in developing economies, where employers are under fewer regulations and workers are more vulnerable to low wages and job loss. Moreover, even in the formal sector, women with the same level of employment and educational background generally earn less than men (FMI, 2019).

Loss derived from the lack of economic female participation is estimated at 10.0% of GDP in advanced economies; at more than 30.0% in South Asia, the Middle East, and North Africa. This is because women and men may have the same potential, but bring distinct and economically valuable skills and ideas (Ostry *et al.*, 2018). Gender differences may reflect social norms and their impact on education, social interactions, risk attitude, and reaction to incentives. Therefore, gender diversity in the boards of banking supervisory agencies is also associated with greater financial stability (Sahay and Čihák, 2018 cited in FMI, 2019).

Women's participation in economic activity is essential for the economic growth of any country. For this, opportunities must be generated to ensure female insertion into the labor market with well-paid and quality jobs for the development of the country. Especially for those who were affected by the impact of the CovID pandemic who not only have lost their jobs, but also have had to dedicate themselves to the care of children or family even more. Women are overrepresented in some of the occupations most affected by the health crisis, such as small retail, tourism, and hosting, while men are more present in construction or manufacturing of goods according to World Bank (Banco Mundial, 2021).

Women's labour force participation globally has been declining from 51.22% in 1990, 47.76% in 2019, to 45.92% during the 2019-2020 pandemic. Women represent just over half of the world's population but their participation in economic activity valued at growth and well-being is below their potential, which has considerable macroeconomic effects. Despite progress in recent decades, global labour markets remain separated by gender and appear to be stuck towards equality.

Female labour force participation (PFFL) has remained below that of men; women perform most unpaid jobs, and when they have paid employment there face wage gaps; or are overrepresented in the informal sector and among the poor. In many countries, distortions and discrimination in the labour market restrict women's options for remunerative employment, and recruitment into senior positions and employers is low (Elborgh-Woytek *et al.*, 2013).

In the Latin American rural context, the Chilean labor market is characterized by the low insertion of women in paid activities (Caro, 2011; Contreras, Hurtado and Sara, 2012; Chong, Herrera, Chávez and Sánchez, 2015; National Institute of Statistics [INE], 2015; Valdés and Rebolledo, 2015; Tomaselli, 2017). Compared to cities, rural areas offer fewer stable jobs and less diversified and low-dynamic economic activity. All this added to lower educational level, cultural restrictions, and attitudinal profiles unfavorable to extra-domestic work that discourages female employability (Alario, Baraja and Pascual, 2008; Ballara and Parada, 2009; Economic Commission for Latin America and the Caribbean–ECLAC, 2010; National Women's Service– Sernam, 2010).

In addition, the discrimination and gender gap observed in the countryside define more precarious and lower-wage jobs for women, with the tendency of the employer to privilege male hiring because its workforce is less onerous (Abramo, 2004; Kay, 2009; Gisa and Rodriguez, 2009; Aninat, González and Szederkenyi, 2017: cited in Rodríguez Garcés; Padilla Fuentes; and Valenzuela Orrego, 2019).

Currently, Mexico has the highest level of informal employment among the most important economies in Latin America with 60.0% according to the OECD, which translates into a population with low wages and poor social security coverage, non-compliance with labor rights and no taxes. According to INEGI, informal employment impacts almost 30 million people working in the informal sector. Mexico is one of the countries with the lowest tax collection, according to the OECD, only 30% of what should be captured is collected. The sale of products through e-commerce supported by Mexican women adds more than 9 million pesos per day to the country's economy, according to a study by Martínez Cortés (2021), who states that the monthly contribution of this social stratum to the economy exceeds 285 million pesos. As long as the initiatives support the participation of women in the country's economic activity, the effects of the pandemic can be restored and a more favorable and inclusive Mexico can be ensured, according to World Bank economists (Forbes Women, 2021).

In 2018, 78% of men and 44% of women participated in economic activities. Despite the increase in women's participation in paid work in recent decades, it remains well below male participation due to many factors such as discrimination in recruitment, remuneration, mobility and promotion practices. As other inflexible working conditions, the inadequacy of services such as childcare, as well as the inadequate distribution of family tasks at home, among others. The participation rate of women and men in unpaid domestic work was 96.1 and 65.4%, respectively (2018). The average income per hour worked was 37.7 pesos for men and 38 for women; while the average hours of paid work per week by men was 45.8 and by women 37.9 hours. Within the EAP portion not employed or unemployed 3.4% corresponded to male population and 3.3% to female population.

As a result of the economic participation of men and women, in the elderly population (60 years and older) the retirement rate of the population was 27.3% of men and 11.5% of women (INMUJERES, 2022). After a gradual growth of several years, Mexico's gross domestic product (GDP) began to decline at the end of 2019 and then suffered the worst drop in the last decade during the second quarter of 2020 due to the health crisis caused by the COVID-19 pandemic. From April to June 2020, the total of goods and services produced in Mexico reached 15.11 trillion Mexican pesos, which represented a decrease of 18.6% compared to the value recorded in the same quarter of the previous year (INEGI, 2022).

In 2017, Mexico's average inflation rate registered its highest point in the last five years, standing above 6.0%. The inflation rate is expected to reach 6.8% in 2022, it is estimated that from 2023 it will continue around 3.0%. The new consumption habits in the first weeks of the confinement to prevent the spread of SARS-Cov-2, the pandemic motivated consumers to make more purchases online, mainly to not leave homes and avoid crowds. Products that were only purchased in physical stores, such as food and medicines, began to be requested online. According to respondents, they said they were interested in doing more online activities after the COVID-19 outbreak in Mexico (Statista, 2022a).

Based on the above, the objective of this study was to analyze the participation of women in the labor market and the effect on economic growth in Mexico during 2000-2021.

MATERIALS AND METHODS

This study consulted different sources such as the World Bank (WB), International Monetary Fund (IMF), the Economic Commission for Latin America and the Caribbean (ECLAC), Center for the Implementation of Public Policies for Equity and Growth (CIPPEC), Center for Macroeconomic Analysis (CAMACRO), Mexico's National Institute of Statistics and Geography (INEGI), Bank of Mexico (BM or Banxico), Statista Research Department (Statista), among others. From where quarterly data were obtained on the Gross Domestic Product, the inflation rate, the exchange rate, the general minimum wage, the employed population of women and men during 2000-2021 for each of the variables. Based on the theoretical elements, three multiple linear regression models were developed. To estimate the coefficients or parameters of each of the explanatory variables, the Statistical Analysis System (SAS) was used, using the Ordinary Least Squares (OLS) method, functions were expressed as follows:

$$PIB_t = \alpha_0 + \alpha_1 INF_t + \alpha_1 E_t + \alpha_2 W_t + \alpha_3 PocupH_t + \alpha_4 PocupM_t + \alpha_5 PdesocupM_t + \varepsilon_t \quad (1)$$

$$PIB_t = \beta_0 + \beta_1 INF_t + \beta_2 E_t + \beta_3 W_t + \beta_4 PocupM_t + \alpha_5 PdesocupM_t + \varepsilon_t \quad (2)$$

$$PIB_t = \gamma_0 + \gamma_1 INF_t + \gamma_2 E_t + \gamma_3 W_t + \gamma_4 PocupM_t + \varepsilon_t \quad (3)$$

where: coefficients to be estimated, α_0, α_1, α_2,..., α_n; β_1, β_2,..., β_n; γ_1, γ_2,...,γ_n; ε_t=error. PIB_t=Gross Domestic Product of Mexico (Millions of pesos at 2013 prices); *INF*=Inflation rate (%); E_t=exchange rate (MXN to USD); W_t=real general minimum wage (MXN pesos in July 2018); $PocupH_t$=the employed population of men (million people), quarterly; $PocupM_t$=Employed population of women (million people), quarterly; $PdesocupM_t$=unemployed population of women (millions of people). The main limitation was that the information is not available from a single source and the figures vary depending on the official institution.

RESULTS AND DISCUSSION

In this section, the statistical results were analyzed based on the parameters of the equations obtained; subsequently, the economic results according to the coefficients and their relationship with the estimators of economic theory. Finally, the elasticities were interpreted.

The statistical analysis was based on the coefficient of determination (R^2), the value of the calculated F (F_c), the mean square of the error, the value of the partial t's for each of the estimators, from the analysis of variance. To test the statistical significance of each of the fitted regression equations, the following set of hypotheses was considered, H_0: $\beta_1=\beta_2=...=\beta_n=0$ against H_a: -i≠0 for $i \geq 1$.

The results of the analysis of variance (Table 1) indicated that according to the statistical data that were collected, the value of the global test for equation 1 of the Gross Domestic Product (GDP_t), the null hypothesis (H_0) was rejected. The results of the analysis of variance (Equation 2), indicated that the value of the global test for the GDP equation, with a

Table 1. Analysis of variance of the structural model of GDP_t.

Dependent variable	Independent variables					
			Equation 1			
PIB$_t$	***INF$_t$***	***E$_t$***	***W$_t$***	***PocupH$_t$***	***PocupM$_t$***	***PdesocupM***
Coefficient	12898	44605	−28591	0.24249	0.69842	−1.81233
t_c	0.46	1.51	−7.37	2.82	7.90	−6.56
Pr>|t|	0.6474	0.1345	<.0001	0.0061	<.0001	<.0001
R^2=0.9750 F-value=514.09	Pr>F<.0001					
			Equation 2			
Dependent variable	Independent variables					
PIB$_t$	***INF$_t$***	***E$_t$***	***W$_t$***	***PocupM$_t$***	***PdesocupM$_t$***	
Coefficient	26009	81559	−29746	0.90645	−2.06183	
t_c	0.90	2.96	−7.40	17.85	−7.56	
Pr>|t|	0.3705	0.0040	<.0001	<.0001	<.0001	
R^2=0.9725 F-value=566.21	Prob>F<.0001					
			Equatión 3			
Dependent variable	Independent variables					
PIB$_t$	***INF$_t$***	***E$_t$***	***W$_t$***	***PocupM$_t$***		
Coefficient	61087	167912	−25136	0.63277		
t_c	1.65	5.15	−4.86	13.65		
Pr>|t|	0.1035	<.0001	<.0001	<.0001		
R^2=0.9529 F-value=409.43	Prob>F<.0001					

Source: elaborated by the authors, with the output of the statistics (SAS) results.

probability of 0.0001, so the null hypothesis (H_0) was rejected, which revealed that at least one of the parameters estimated by the least squares regression is non-zero. For equation 3, the overall test of Gross Domestic Product (GDP_t) was significant and the null hypothesis was rejected.

The coefficient of determination (R^2) for model 1 indicated that the variable Gross Domestic Product of Mexico was explained in 97.5% by the variables included in the equation. Regarding the individual test, the real general minimum wage, the employed and unemployed population of women turned out to be more significant with a value of t of −7.37, 7.9 and −6.56>1; the least significant according to the statistical results were the employed population of men and the exchange rate whose value of t was 2.82>1 and 1.51>1 respectively. The only one that was not significant was the inflation rate (0.46<1).

For equation 2, the coefficient of determination (R^2) for Mexico's Gross Domestic Product was explained by 97.2% by the variables included in the equation. Regarding the individual test, the real general minimum wage, the employed and unemployed population of women turned out to be more significant with a value of t of −7.40, 17.85 and −7.56>1;

the least significant according to the statistical results was the exchange rate whose value of t was 2.96>1. The only one that was not significant was the inflation rate (0.90<1).

Model 3, which had a coefficient of determination (R^2) for Mexico's Gross Domestic Product was explained by 95.2% by the variables included in the equation. For the individual test, the employed population of women, the exchange rate, and the real general minimum wage turned out to be more significant with a value of t of 13.65, 5.15, and −4.86>1, the least significant was the inflation rate whose value of t was 1.65>1.

In the three linear models, the inflation rate presented a positive relationship with the Gross Domestic Product and was not statistically significant (0.46<1, 0.90<1, and 1.65>1), the average inflation rate for the period 2000-2021 was 4.6%, which agrees with the study carried out by Sarel (1996) who found that for inflation levels above 8.0%, the relationship between economic growth and inflation was negative and statistically significant. Whereas for countries with inflations below 8.0%, the relationship between these two variables is practically nonexistent, and in some cases it is even positive, although not statistically significant.

This section presents the economic analysis of the estimated coefficients, according to economic theory:

$$\widehat{PIB_t} = -75959 + 12898 INF_t + 44605 E_t - 28591 W_t + 0.24249 PocupH_t + 0.69842 PocupM_t - 1.81233 PdesocupM_t + \varepsilon_t \quad (4)$$

$$\widehat{PIB_t} = 2441413 + 26009 INF_t + 81559 E_t - 2974 W_t + 0.90645 PocupM_t - 2.06183 PdesocupM_5 + \varepsilon_t \quad (5)$$

$$\widehat{PIB_t} = 3994212 + 61087 INF_t + 167912 E_t - 25136 W_t + 0.63277 PocupM_t + \varepsilon_t \quad (6)$$

In the estimated equations of the Gross Domestic Product (4, 5 and 6), the variables minimum wage, exchange rate, employed population of men, women, and unemployed women met the sign. That is, increasing employment will result in an increase in GDP according to economic theory. However, the inflation rate showed the opposite sign to that expected. According to the Bank of Mexico (Banco de México, 2016), inflation can lead to redistributive effects that increase inequality and impede economic development. It also produces an inefficient allocation of productive resources thus damaging the economy's capacity for growth. In addition, inflation limits the planning horizons of economic agents, negatively affecting their investment and savings decisions. For all the above, there is an inverse relationship between the inflation rate and economic growth.

For the analysis of the elasticities, the estimated parameters of the structural shape of the model were considered for each of the variables studied.

Table 2. Model elasticities in their structural form.

Equation 1		
$\varepsilon_{INF}^{PIB} = 0.003786618$	$\varepsilon_{E}^{PIB} = 0.03972681$	$\varepsilon_{W}^{PIB} = -0.15247176$
$\varepsilon_{PocupH}^{PIB} = 0.45838199$	$\varepsilon_{PocupM}^{PIB} = 0.78375703$	$\varepsilon_{PdesocupM}^{PIB} = -0.08439326$
Equation 2		
$\varepsilon_{INF}^{PIB} = 0.00763577$	$\varepsilon_{E}^{PIB} = 0.07263937$	$\varepsilon_{W}^{PIB} = -0.15863122$
$\varepsilon_{PocupM}^{PIB} = 1.01720535$	$\varepsilon_{PdesocupM}^{PIB} = -0.09601151$	
Equation 3		
$\varepsilon_{INF}^{PIB} = 0.01793403$	$\varepsilon_{E}^{PIB} = 0.14954845$	$\varepsilon_{W}^{PIB} = -0.13404674$
$\varepsilon_{PocupM}^{PIB} = 0.71008553$		

Source: elaborated by the authors, with the output of the statistics (SAS).

RESULTS AND DISCUSSION

The short-term elasticities, obtained from the estimators of the model in their structural form, are shown in Table 2, particularly those most relevant for the analysis. In Model 1, faced with an increase of 10.0% of the employed population of women and men, the economy would grow by 7.83%, and 4.58% on average respectively. On the other hand, with an increase of 10.0% of the unemployed population of women and the minimum wage GDP would decrease by 0.84% and 1.5% on average respectively. For equation 2, by using only employed women, GDP would increase by 10.17% and with unemployed women, it will decrease by 0.96% on average, ceteris paribus.

In the case of equation 3, when the unemployed population of women is eliminated to test their importance in economic growth, it was obtained that with an increase of 10.0% of the employed population of women, the economy would grow by 7.1%. For the minimum wage if it were increased by 10.0%, GDP would decrease by 1.34%, keeping the other factors constant, which agrees with what was reported by Corvera-Vergara (2021) where they considered the importance of employment as a fundamental part of all production processes of goods and services.

A study conducted in Chile by CLAPES UC (on the increase in female labor participation, estimating the effect on GDP; Rodrigo Cerda, González and Larraín, 2020), in which among other authors the current Minister of Finance participated, sought to answer what would happen in the economy of that country if female labor participation increased. To estimate the effect on GDP, it was calculated how closing the gap with the OECD would impact total employment; the share of employment in GDP is determined through a Cobb Douglas production function like the one used for the calculation of trend GDP).

With figures for 2019 (pre-pandemic) it was obtained that each point of increase in female labor participation represented the creation of 79 thousand jobs, so that closing the gap with the OECD would imply creating between 307 thousand and 558 thousand jobs depending on the definition used. In 2019, depending on how the participation rate is defined, this gap was between 3.9 and 7.1%. If women between 15 and 64 years old are considered, the gap is 7.1%, while those between 15 and more reaches 3.9%. On the other hand, each point of increase in participation signifies an increase in GDP of 0.5%, so closing the gap with the OECD would imply an increase in GDP ranging from 1.8 to 3.2%, depending on the definition. This study also calculates that considering the recovery of the fall in female labor participation to pre-pandemic levels plus the increase in participation to the OECD levels that were in force before the crisis, the total effect would be an increase in GDP ranging from 8.3 to 9.7%.

The estimates obtained suggest that expanding economic opportunities through an employment-focused growth would create an enabling macroeconomic environment for women's empowerment, without men and women competing with each other for forms of decent work (Robino and Tebaldi, 2018). Female labor force participation is important for different reasons. First at the macroeconomic level, low levels of collaboration in paid work and entrepreneurship represent a large loss of productivity and thus in GDP. At the microeconomic level, female work could be transformative for them and their households (Duflo, 2012).

When women control a larger part of the household budget, the benefits derived from food, health and education expenditures increase more than when men do so (Rubalcava, Teruel and Thomas, 2009). If women anticipate going to work, they can reduce desired fertility and increase their investments in human capital (Jensen, 2012). The work of women can also give them representation and voice within their societies. With such a low rate of female labor force participation, Mexico squanders a large proportion of its population (Banco Mundial, 2020).

CONCLUSIONS

The most significant variable was the employed population of women, obtaining 7.1% of economic growth. This value is consistent with other studies. The findings of this study show that in a macroeconomic environment conducive to women's work, there would be less competition between women and men, higher productivity and entrepreneurial intention, and a consequent increase in gross domestic product.

Other derivative effects include improvements in household budget management, a reduction in the fertility rate, and greater investment in human capital. By limiting women's labor force participation, Mexico is undermining the economic potential of this group.

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Colegio de Postgraduados

Yield evaluation of broiler carcasses from a poultry processing plant with automated processing conditions in the Colombian Eastern Plains

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ABSTRACT

Objective: To evaluate the yield of dry and hydrated broiler carcasses from two suppliers that manage technical poultry processing plants in the Colombian Eastern Plains, per department (Cundinamarca and Meta) and per sex (females and males).

Design/Methodology/Approach: A total of n=777 broilers were used for carcass yield analysis, n=398 and n=384 of which were provided by supplier A and supplier B, respectively, with an equal proportion of females and males. Inferential statistics (ANOVA test) and descriptive statistics were used to determine the behavior of the analyzed variables, with the aim of determining a significant difference between the variables.

Results: Supplier B recorded the highest yields: an 11.93% increase in carcass after pre-chiller and chiller. The plains area near the processing plant of Cumaral recorded better results: 2.79% higher than the live weights of the Cundinamarca area. In the comparison by sex, the males were dominant, since their live weights were 5.47% higher and the carcass weights were 6.67% higher than in females.

Findings/Conclusions: After the pre-chiller and chiller processes, the yield of the poultry meat production chain at AVIMA S.A. was >10% (weight increase of the dry carcass). In conclusion, the optimal stimulation of bird pores and the cooling generate profits in weight.

Keywords: Carcass, efficiency, broiler, production, yield.

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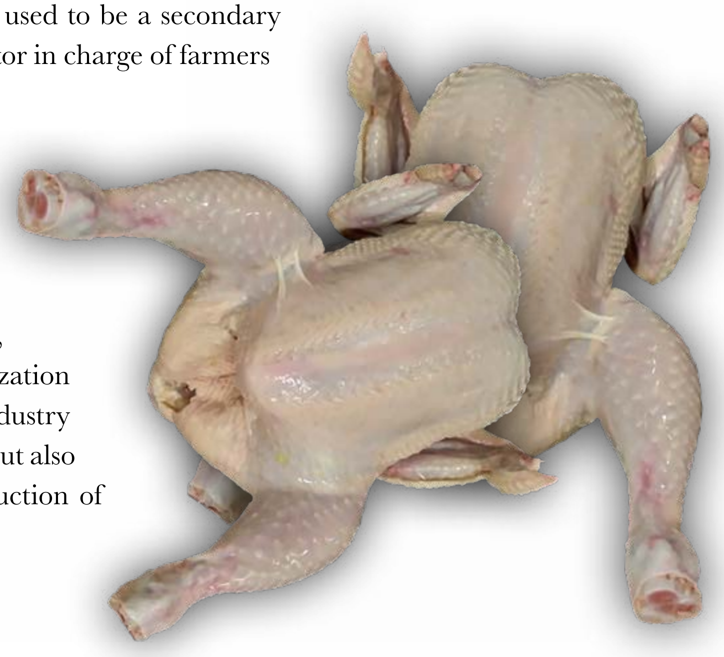
INTRODUCTION

Worldwide poultry production used to be a secondary activity within the agricultural sector in charge of farmers and has now become a true industry. According to the UN (2022), this sector continues to grow and industrialize in many parts of the world, as a consequence of the powerful boost of demographic growth, purchasing power, and urbanization processes. Furthermore, this industry includes not only egg production, but also the industrial and high-tech production of poultry meat (Saenz, 2022).



Poultry companies have made great efforts to identify new technologies in production systems, with the aim of generating greater productivity and reducing production costs (Montero, 2020). The yield of broiler carcasses is one of the most important economic profitability parameters (Sanz, 2021; García *et al.*, 2009). This parameter is generally measured in eviscerated broiler (*i.e.*, blood and feathers are considered losses). Processing companies choose to obtain high-range yields and consider the conditions that affect broiler carcasses (Gillén *et al.*, 2015).

According to the International Organization for Animal Health (OIE, 2010), an animal with adequate well-being fulfills the following parameters: it is healthy, it is well fed, and does not suffer from unpleasant sensations (pain and fear). In this regard, Cockram *et al.* (2004) mention the five freedoms that ensure animal well-being: they must not be hungry or thirsty; they must not experience an insufficient level of nutrition; they must not present pain, injuries, or illnesses; they must not feel fear or distress; they must not be uncomfortable; and, finally, they must show a natural behavior.

Regarding the Colombian animal protection and welfare legislation, the Ley N° 84 -Estatuto Nacional de Protección de los Animales includes wild or unbridled, domestic and domesticated species, both captive and in their natural habitat (FENAVI, 2019). However, the law was modified and Ley 1774 of 2016 is currently applied in the country. Indeed, Romero and Sánchez (2011) mention five explicit provisions in health legislation regarding primary production.

In this sense, this research compared the yield of dry and hydrated broiler carcasses under different conditions (by departments, by sex, and by geographical areas), in order to determine the incidence of these percentages, as reported in the bibliography. These conditions must be regularly measured and analyzed to ensure that production standards are being met. In addition, monitoring the capacity, flexibility, and sustainability of the processing plant is also essential to ensure efficient and sustainable long-term operation.

MATERIALS AND METHODS

The research was carried out at the La Balestra station of Avícola del Magdalena S.A. (AVIMA S.A.), located in the municipality of Cumaral, department of Meta, Vereda de Presentado lane, Km 10 of the Vía Yopal Casanare road (4° 17' 41.7" N, 73° 23' 58.9" W). The average ambient temperature was 23.6 °C and the relative humidity was 58%. This company processes poultry through the application of knowledge and updated technical systems.

Sampling Description

The 30-day work began with a total sampling of n=777 broilers from two main suppliers, supplier A and supplier B (n=393 and n=384 broilers, respectively). The birds were received, weighed, and marked in the hanging area (Figure 1).

Subsequently, the broiler samples were subjected to processing and cooling to confirm the weight of dry (after evisceration and before internal-external washing) and hydrated (after the chiller and draining process) carcasses. The weight data were recorded daily, classified by suppliers and by departments of origin (Cundinamarca and Meta), and tabulated in

Figure 1. Broiler weighing.

Excel worksheets for control purposes. A response variable was calculated, which was the weight of the birds under four different conditions: A) birds supplied by supplier A; B) birds supplied by supplier B; C) birds from the department of Cundinamarca; and D) birds from the department of Meta (Llanos Orientales).

Statistical analysis

With the aim of determining the behavior of the analyzed variables, inferential statistics (ANOVA test) were used to observe a significant difference between the variables. Descriptive statistics were applied to the analyzed weights, determining measures of dispersion (mean, standard deviation, sample variance, and coefficient of variation) for the proposed variables. The R statistical software was used for the analyses with a 95% significance level.

RESULTS AND DISCUSSION

The average of the broiler live weight, dry broiler carcass weight, and broiler carcass after the pre-chiller and chiller processes weight were tabulated in order to compare the yields of the carcasses from two suppliers (supplier A and B), per sex (female or male) and per department (Cundinamarca and Meta) (Figure 2).

Supplier classification

One of the indicators that affect suppliers is the yields of broiler carcass. Supplier B has better production, reaching yields of 73.94% in dry carcasses and 82.76% in cold carcasses (after pre-chiller and chiller). The carcass weights increase by 4.5%, after they are kept for 15 to 20 min in a chlorinated water solution of no more than 50 ppm, a pH of 6-7, and a temperature of 22 to 28 °C (Galarza, 2011). Taking into account the data graphed above (Figure 2), the weights of the dry carcasses increased in the pre-chiller and chiller by 11.55% (supplier A) and 11.93% (supplier B). The analysis of the results shows that the good manufacturing practices and operational actions of supplier B are better.

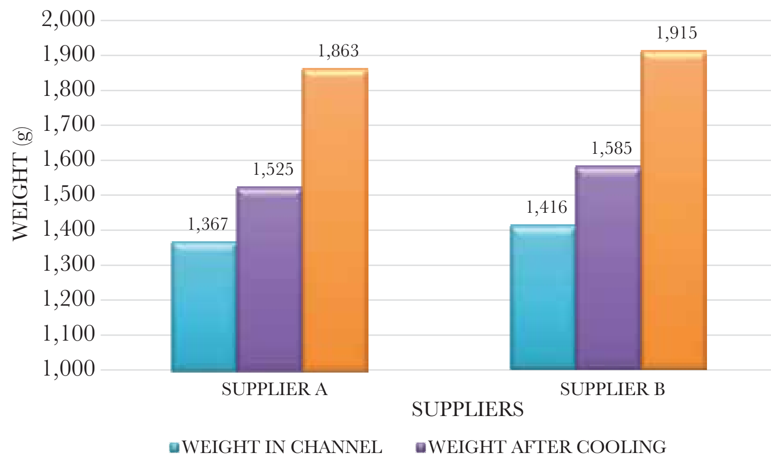


Figure 2. Comparison of carcass weight of poultry from suppliers A and B.

Classification by departments

The location of the producing farms and the transportation times of the animals can affect the yields. The following table shows the average yields of the farms located in two different areas (Cundinamarca and Llanos Orientales).

Since the processing plant is located in the city of Cumaral, Meta, transportation time affects the yield of the carcasses from Cundinamarca (poultry production farms are an average distance of 68 km from the processing plant). In their research about the well-being during transport and quality of meat, Ruiz and Manteca (2002) determined that broilers can lose between 5 and 10% of their live weight during the journey from the farm to the processing plants. This research determined that the live weight of the broilers from Cundinamarca is 2.79% lower than in Llanos Orientales (poultry farms are an average of 7 km away from the processing plant). This reduction is explained by the longer time elapsed between fasting and processing and the additional stress experienced

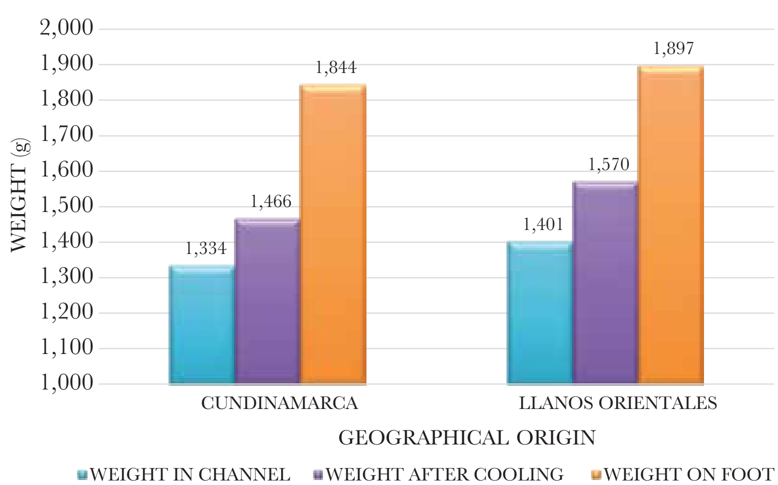


Figure 3. Average broiler carcass weights according to geographical area (Cundinamarca and Llanos Orientales).

by broilers from farms located in areas further away from the processing plant (in this case, in Cumaral, Meta).

Other factors that affect the carcasses are edaphoclimatic conditions. In their development stage, broilers have scarce ability to regulate their temperature; therefore, they need an average temperature of 30 °C and a relative humidity between 60-70% (Aviagen, 2009). However, these conditions are difficult to comply with in geographical areas such as Cundinamarca, where the average temperature ranges from 14 to 18 °C (IDEAM, 2020).

The Avima company carries out a three-stage process which includes: a scalding process; a cooling process with a pre-chiller and chiller and rinsing the carcass to remove excess blood. This threefold process seeks to ensure that the sudden changes in temperature allow water to enter the protein of the poultry, through the pores dilated during the scalding process (Fajardo, 2014), generating a significant increase in weight and a relative price gain for the company. For their part, Mir *et al.* (2017) mention that "water content and its distribution within the meat have a great influence on its quality and economic value." In conclusion, several factors affect the water retention capacity (WRC) of the muscle proteins of poultry; in turn, each of the factors clearly depends on the increase in the production of lactic acid resulting from bird stress.

The comparison by departments of the two main suppliers determined that stress is one of the primary aspects that significantly influences the yield of the carcasses. Figure 3 shows a significant difference between yields: the department of Meta has a higher percentage of both dry carcass yield and hydrated carcass yield than the department of Cundinamarca. According to Gillén *et al.* (2015) an animal shows stress when its movements are restricted and it is handled inadequately, it undergoes occasional fatigue, pain, or injuries, objects, or people foreign appear in its usual environment, or it suffers from hunger, thirst, or lack of thermal comfort.

Additionally, stress affects the yield of the poultry because the flocks in the sheds are usually transported to destinations located several hours away (Cundinamarca-Cumaral). In addition, stress is also generated by an inadequate harvesting management on the different farms, which affects the water retention capacity (WRC) in the processing stage, as a result of changes in lactic acid. As indicated by Perez (2018), the impact of WRC is caused by the increase in lactic acid and the decrease in pH in the meat.

Consequently, animal welfare is a major issue that affects the quality of meat in processing companies. As it moves away from the isoelectric point of proteins (5.1-5.5) (Moreno, 2005), the pH increases the WRC and improves the ability of the meat to retain water (Viteri, 2013). It is inferred that, being closer to the processing plant, the poultry from the Department of Meta did not have to undergo so many hours of transportation, which would have increased the lactic acid in their body (as a consequence of stress); the opposite phenomenon has been recorded with poultry from of the Department of Cundinamarca.

Classification by sex

De Obaldia and Perales (2015) studied the production of males and females of the Arbor Acres Plus® line and determined that, after 32 days, the males were 11.64% heavier than the females, based on the average weights shown in Figure 4.

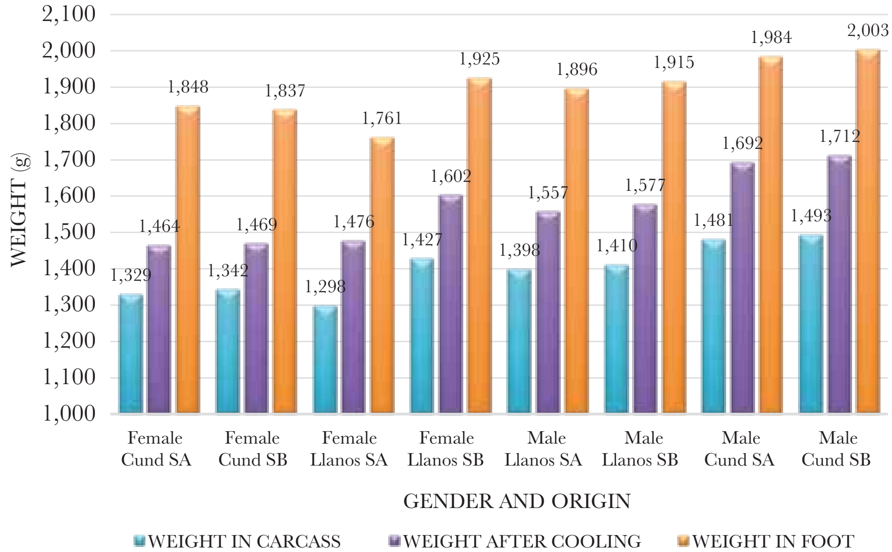


Figure 4. Average broilers carcass weight according to sex (male and female) and supplier (PA: supplier A; PB: supplier B), in the different geographical areas (Cundinamarca and Llanos Orientales).

The carcasses were compared by sex (female and male) and area of origin (Cundinamarca and Meta). When live weights were taken into account and the carcasses were compared before the cooling process, males exceeded the yield of females by 5.47%. Likewise, the males recorded a 6.67% higher carcass yield (average: 74.13%), resulting in a higher food/meat conversion. After the pre-cooling and chilling processes, carcass yields were 83.83% for males and 81.55% for females. On average, WRC yields in males were 1.39% higher. According to FENAVI (2019), the greater weight gain of males is also partially as a result of the considerable increase in their average daily consumption during the fattening or finishing stage. Males consume an average of 142.28 g of food per day for three weeks, with 15 to 20% increases in consumption each week, reaching a weight gain of 391.2 g poultry^{-1}. The external conditions of the well-being of an animal before it enters a technical processing plant play an important role in the yield of the carcasses. Those conditions are related to the water retention capacity and influence the increase or decrease in lactic acid in the animal's body. The poultry industry should take into account the external conditions to which animals are subjected (*e.g.*, transportation), if they are to prevent monetary loss resulting from the failure to achieve the expected yields in their daily production. The temperature and relative humidity of the farms greatly influence the performance of weight gain in broiler chickens. Finally, the viability of production in cold climate areas is related to the atmospheric conditions of the producing farms.

CONCLUSIONS

The external conditions that link animal welfare prior to entering a highly mechanized processing plant play a significant role in carcass performance, as the water retention capacity is closely associated with variations in lactic acid content in the animals' bodies.

It is imperative for poultry industry stakeholders to take into account external conditions, such as transportation, in order to prevent financial losses resulting from not achieving the expected daily production yields.

Temperature and relative humidity in poultry facilities have a significant impact on the weight gain of broilers and broiler hens, and the viability of production in cold climate areas is largely contingent on atmospheric control in the producing farms.

The poultry meat production chain in Avima has >10% yields (weight increase of the dry carcass) after the pre-chiller and chiller processes. In conclusion, the optimal stimulation of the pores of the poultry and the cooling generate an increase in weight.

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Emergence and growth of *huacle* chili seedlings (*Capsicum annuum* L.) with the use of biological formulations in commercial plot soil

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ABSTRACT

Objective: To evaluate the effect of biological control agents (BCA) and two biological formulations (Bio-Terra and Bio-CNPR) against the phytopathogens found in the soil of *huacle* chili (*Capsicum annuum*) plantations, in order to obtain healthy seedlings.

Design/Methodology/Approach: The following design variables were evaluated: germination (%), disease incidence (%), and survival (%). Stem height and root length were also evaluated. The experimental unit was a tray with n=30 seedlings per each treatments (T0, T1, T2, T3, and T4) and four repetitions. An analysis of variance (ANOVA) was used. Tukey's mean test was applied (using the Minitab Statistical program version 20.0) in units that presented differences ($p \leq 0.05$).

Results: The phytopathogenic microorganism *Fusarium* spp. was isolated from the soil of the plots cultivated with *huacle* chili. The T1 treatment recorded the best inhibitory effect against *Fusarium* spp.: it had 92.2% germination, 18.3% incidence of *Fusarium* spp., and 82% surviving seedlings (with an average height of 16 cm and a root length of 7.9 cm). The sterilization of the soil lead to T4 having the best results in germination, incidence, and survival, since *Fusarium* spp. did not damage *huacle* chili seedlings. In contrast, T0 did not prosper neither with BCA nor with biological products, since all the seedlings in non-sterile soil died.

Study Limitations/Implications: A plastic barrier was placed between the trays with the chili seedlings to avoid cross-contamination between treatments.

Findings/Conclusions: T1 (the BCA with nitrogen-fixing bacteria and *B. subtilis*) recorded the best counteracting results against the damage generated by *Fusarium* spp. Their antagonism allowed a high percentage of survival of *huacle* chili seedlings and encouraged plant development through the best root growth and height of the seedlings.

Keywords: *huacle* chili, biological product, *Fusarium*.

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INTRODUCTION

The *huacle* chili (*Capsicum annum* L.) is an endemic plant of the region of San Juan Bautista Cuicatlán, Oaxaca, where it is cultivated in small areas in the open field (Aguilar-Rincón *et al.*, 2010; García-Gaytán *et al.*, 2017). The critical stage of the crop takes place during the seedbed

—between 35 to 45 days (three days for germination, 12 days for emergence, and the rest as seedling) (López-López and Pérez 2015). Common diseases in nurseries or seedbeds can be caused by *Pythium* spp., *Fusarium oxysporum*, *Rhizoctonia* sp., or *Phytophthora capsici* in the preemergence and postemergence stages. The damages observed during germination include wet rot, neck decapitation of the seedling, fall of necrotic cotyledons or necrotic roots, all of which cause the death of the seedling (Barranco, 2016).

One way to counteract these phytosanitary problems is the application of antagonistic microorganisms. Species such as *Rhizobium* —which is obtained from rice (*Oryza sativa* L.) plants— produce high concentrations of indole compounds, polyhydroxybutyrate, and ammonium, and solubilize calcium phosphate (Hernández Forte and Nápoles García, 2019). Fungi of the genus *Trichoderma* and bacteria of the genus *Bacillus* compete for the substrate, parasitize organisms producing lytic enzymes that degrade the cell wall of phytopathogens (particularly, *Fusarium*), and stimulate the growth and development of plant roots, improving the uptake of nutrients and water (Martínez *et al.*, 2013; Vásconez *et al.*, 2022). *Bacillus subtilis* produces siderophores, indole compounds, and jasmonic acid, which promote stem height and a greater number of leaflets (Anguiano Cabello *et al.*, 2019). As for *Trichoderma harzianum*, it can potentially control vascular fusariosis, resulting in a high germination percentage in miahuateco chili (Miguel-Ferrer *et al.*, 2021) and mycoparasitism, through the production of antifungal enzymes with potential antibiotic characteristics. In addition, it increases the pod production (24%), root length (40%), and yield (23%) of chickpea crops (Martínez-Martínez *et al.*, 2020). Consequently, the effect of a set of biological control agents (BCA) and two biological formulations (Bio-Terra and Bio-CNPR) were evaluated, combining substrate with soil from an area cultivated with *huacle* chili, in order to obtain healthy seedlings.

MATERIALS AND METHODS

The experimental phase was carried out at the facilities of the Universidad de la Cañada, located in the municipality of Teotitlán de Flores Magón, Oaxaca, Mexico.

Sample collection

The experimental plot is located at 17° 79’ 47.8” N and 96° 96’ 49.8” W, and 588 m.a.s.l. Soil samples were collected, duly labeled (NOM-021-RECNAT-2000), and transported to the laboratory. *Huacle* chili plants were found to have been damaged by wilting or strangling at the base of the stem.

Isolation and identification of the causative agent

The samples of local cultivation soil were treated according to AS-01 of the NOM-021-RECNAT-2000. They were mixed until a 1-kg sample was obtained. Subsequently, 1.0 g of soil was extracted and mixed with 9 mL of distilled water in an Erlenmeyer flask and stirred for 20 min. Serial dilutions were made up to 10-4. Under aseptic conditions, 0.1 mL of the dilution were taken and inoculated in a Petri-dish with Bioxon® Potato Dextrose Agar (PDA) culture medium, which was prepared according to the product label (39 g L^{-1}) and sterilized in an All American® pressure cooker for 15 min at 15 Lb. The Petri-dishes with PDA were inoculated per streak with the serial dilutions of soil and incubated at 27 °C for seven days; the process was repeated until pure cultures were obtained (Samaniego-Fernández *et al.*, 2018).

The sticky tape technique with cotton blue staining (Díaz *et al.*, 1999) was used to identify the causative agent: fragments of mycelium were extracted gently pressing the sticky side of the tape against the pure cultures and placing the tape on the slide, for observation under an optical microscope at 100x with an Euromex® LCD monitor. The morphological characteristics were recognized according to the taxonomic keys proposed by Barnett and Hunter (1998). The purpose of the isolation and identification was to corroborate the presence of *Fusarium* in the soil for future assessments.

Germplasm

Six hundred *huacle* chili seeds were used for the experiment. Each biological product to be evaluated was applied to 120 seeds per treatment. The seeds were subsequently germinated. They were sprayed with a set of antagonists and nitrogen-fixing bacteria (biopolymer 1 g/L, *B. subtilis* 5.5×10^{8} UFC/L, and *Rhizobium* spp., 6.0×10^{8} UFC/L), Bio-Terra (1 g biopolymer, *B. subtilis* 5.5×10^{8} UFC, *B. thuringiensis* 6.0×10^{8} UFC, *T. harzanium* 5.0×10^{10} spores, and *Beauveria bassiana* 4.6×10^{10} spores in 500 g of inert wettable powder), and Bio-CNPR (1 g/L biopolymer, *B. subtilis* 5.5×10^{8} UFC/L, *Rhizobium* sp. 6.0×10^{8} UFC/L, *B. thuringiensis* 6.0×10^{8} UFC/L, *T. harzanium* 5.0×10^{10} spores/L, and *Metarhizium anisopliae* 2.08×10^{10} spores/L) (Hernández-Rosas, 2019).

Experimental design

A base mixture of the soil collected from the cultivation area was made with Peatmoss®, at a 1:1 v/v ratio. The resulting mixture was used to carry out the following treatments: T0=non-sterilized base mixture + chili seed, T1=non-sterilized base mixture + chili seed + *Rhizobium* + *B. subtilis*; T2=non-sterile base mix + chili seed + Bio-Terra; T3=non-sterile base mix + chili seed + Bio-CNPR; and T4=sterilized base mixture + chili seed.

The biological products were sprayed once a week with an atomizer to guarantee the inoculation in the substrate of each treatment (Gómez and Cruz, 2016). The following doses were applied: 1 L of BCA and Bio-CNPR in 200 L of water; and 500 g of Bio-Terra in 200 L of water. The treatments were placed at a height of 30 cm, with 50 cm between trays, and divided into blocks by a plastic barrier to avoid possible cross contamination.

Experiment response variables

The measurement of variables began when the 600 seeds were placed inside the trays (emergence). The germination percentage was obtained multiplying the number of germinated seeds/number of seeds sown by 100 (Prado-Urbina *et al.*, 2015). Disease incidence was calculated according to the formula proposed by Van der Plank in 1975 (Navarrete-Maya *et al.*, 2009): number of affected plants/number of total plants multiplied by 100. The percentage of survival was calculated based on the number of surviving plants/number of germinated seeds multiplied by 100 (Cóbar-Carranza *et al.*, 2015). The plant height and root length were measured from the base of the stem to the apex of the seedling and from the base of the stem to the apex of the root (Candelero *et al.*, 2015). Fifteen of the surviving seedlings per treatment were measured with a Vernier® (MetroMex).

Statistical analysis

The response variables were subjected to an analysis of variance (ANOVA) to determine the germination percentage, disease incidence percentage, survival percentage, plant height, and root length. The experimental unit was a tray with 30 seedlings and four repetitions per treatment. The response variables with significant differences were analyzed with Tukey's mean test ($p \leq 0.05$), using with the Minitab statistical software version 20.0.

RESULTS AND DISCUSSION

The soil samples showed white mycelial growth in the PDA culture medium with violet pigmentation, cottony mycelium, typically curved and pointed macroconidia, ellipsoidal (canoe-shaped) with three septa, septate hyphae, and thick-walled chlamydospores (Figure 1A-C). These findings match the description of the genus *Fusarium* spp. made by some authors (Barnett and Hunter, 1998; Rentería-Martínez *et al.*, 2019).

The analysis of variance of the germination percentage, disease incidence, survival, plant height, and root length were different ($p \leq 0.05$) (Figure 2).

With the T4 treatment, the *huacle* chili seeds recorded a high germination percentage (99.6%), as a result of the previous management, and no impact by *Fusarium* spp. However,

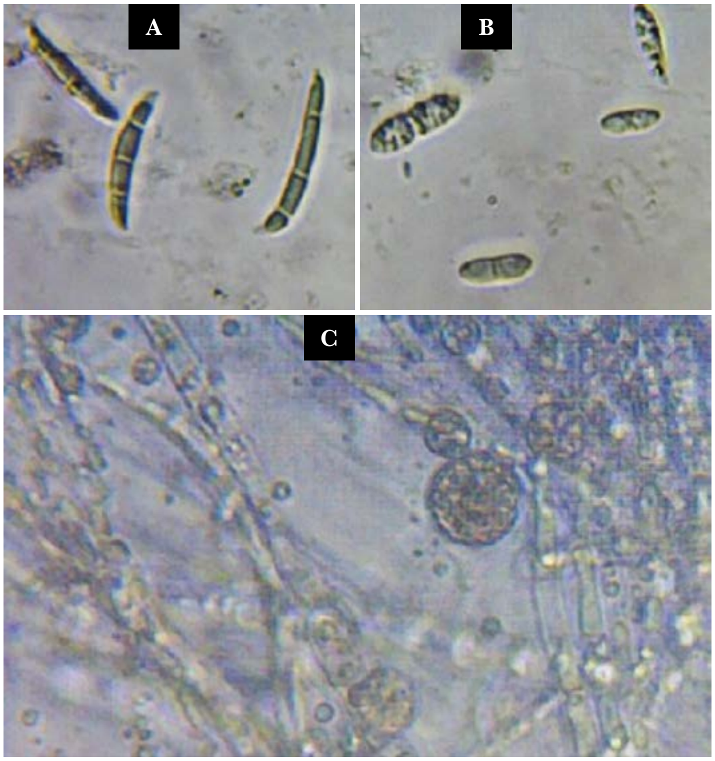

Figure 1. *Fusarium* spp. morphology observed at 100x. A: macroconidia; B: microconidia and C: chlamydospores.

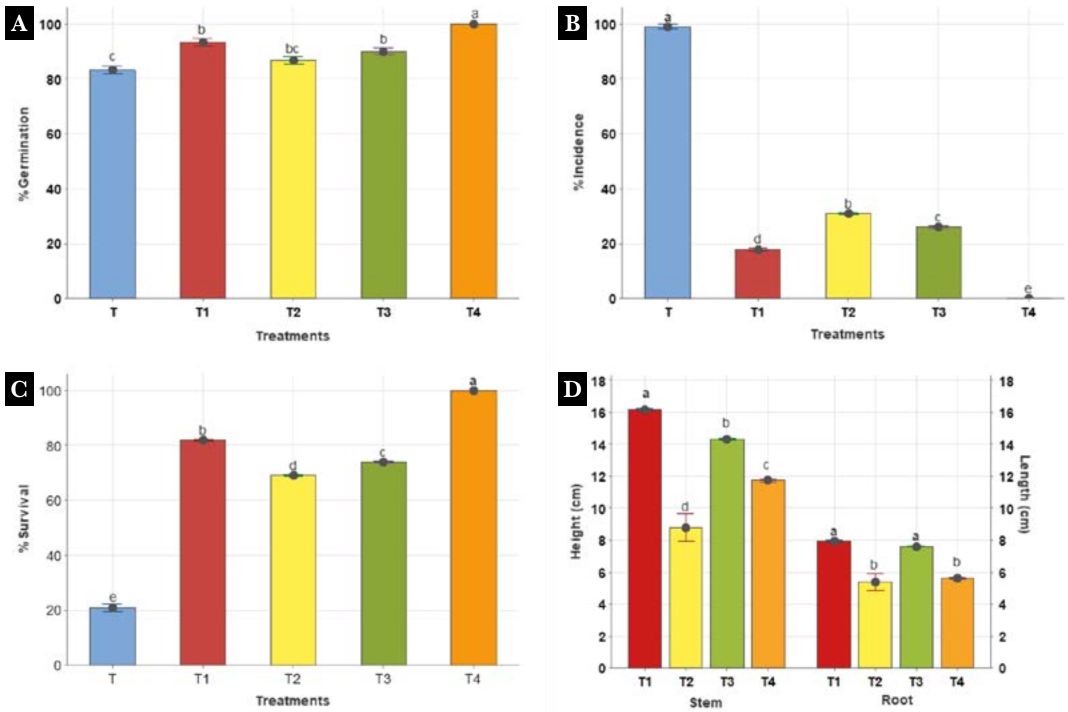

Figure 2. 2A: germination, 2B: incidence, 2C: survival, 2D: stem height and root length (± standard error) of *huacle* chili seedlings subject to different treatments. Letters A-C indicate significant differences (P<0.05) between the treatments, according to Tukey's multiple-comparison test.

treatments T1 and T3 —in which the biological products with *Rhizobium* – *B. subtilis* and Bio-CNPR were applied— had 92.2% and 90.9% germination, respectively (Figure 2A). Therefore, *B. subtillis* and *Rhizobium* encourage germination (Hernández Forte and Nápoles García, 2019; Anguiano Cabello *et al.*, 2019) and, as antagonists of *Fusarium* spp., they protect the *huacle* chili seeds (Martínez *et al.*, 2013; Rojas *et al.*, 2017, Pérez; García-Godos, 2019; Vásconez *et al.*, 2022; Miguel-Ferrer *et al.*, 2021).

Regarding the incidence of the disease (Figure 2B), unlike the other treatments, T4 did not show any symptoms, due to the use of the sterile substrate. Regarding the T1 treatment —which contained the BCAs (*Rhizobium* and *B. subtilis*)— only 18.3% of the seedlings showed signs of disease, because *B. subtilis* acted favorably by activating defense mechanisms (Anguiano Cabello *et al.*, 2019).

For their part, the T3 and T2 treatments had an incidence of 25.7% and 30.7% respectively, resulting from the reduction of the damage caused by *Fusarium*. Although these treatments include both *Trichoderma* and *Bacillus*, their effectiveness is lower than T1. Regarding the control (T0), all the seedlings were damaged by *Fusarium* spp. (Figure 3, A-C).

Regarding the survival rate (Figure 2C), all the *huacle* chili seedlings in T4 survived and reached a height of 11.7 cm and a root system length of 5.6 cm. Meanwhile, the BCA set of the T1 treatment inhibited *Fusarium* spp.; in this case, the survival percentage was 82%, the seedlings grew up to be 16 cm tall and the root system was 7.9 cm long (Figures 2D and 3D). It is likely that the inoculum of both bacteria encouraged synergy

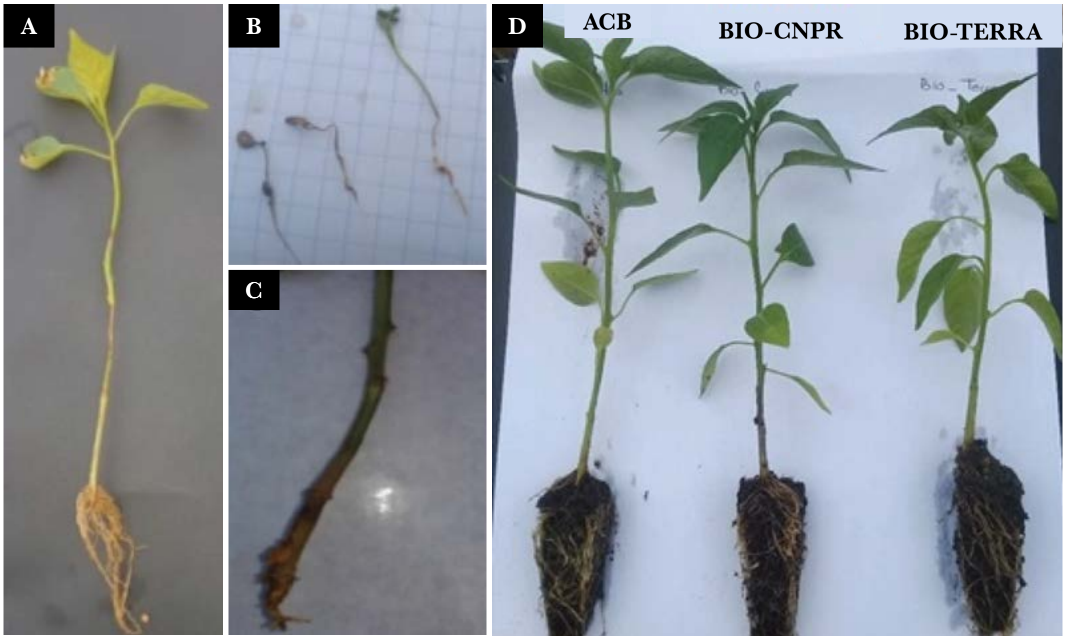

Figure 3. *Huacle* chili seedlings. A-C) damage caused by *Fusarium* spp.; and D) seedlings treated with BCA (ACB) and *Rhizobium* – *B. subtilis*, Bio-CNPR, and Bio-Terra.

among *Rhizobium* and *B. subtilis*, which resulted in healthy plants (Hernández-Forte and Nápoles-García, 2019; Anguiano-Cabello *et al*., 2019). In T3, 74% of *huacle* chili seedlings survived, reaching a height of 14.3 cm and a root length of 7.6 cm. Despite the complexity of microorganisms found in the content of the biological product (Bio-CNPR), a favorable performance was observed, while all the control chili seedlings (T0) showed symptoms of damage by Fusarium spp. and did not survive (Figure 3A).

CONCLUSIONS

The best treatment regarding germination, incidence, survival, stem height, and root length in *huacle* chili seedlings was recorded with biological control agents (BCA) of the *Rhizobium* + *B. subtilis* bacteria group. Their microbiological duality enabled the antagonistic activity against *Fusarium* spp. in this phase of the growth of the *huacle* chili, promoting the highest root growth and seedling height values. Antagonistic effects could be distinguished even with the survival levels recorded in the different treatments where the biological products were applied.

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Good aquaculture production practices and detection of pathogens in rainbow trout alevins

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ABSTRACT

Objective: To evaluate the compliance with Good Aquaculture Practices (GAPs) in the Health Compliance Units (UPAs) during the incubation of rainbow trout eggs and to identify the presence of *Aeromonas salmonicida* and *Aeromonas hydrophila* using the Polymerase Chain Reaction (PCR) technique.

Design/Methodology/Approach: Seven and three egg incubation UPAs were identified in Puebla and Veracruz, respectively. An structured questionnaire was applied to evaluate the compliance with the GAPs. Samples were collected from the trout avelin batches and were analyzed in search of *A. salmonicida* and *A. hydrophila* using the PCR technique. The results were compared with the health characteristics of the fish of each batch.

Results: The GAP-certified UPAs comply with the recommendations made by the authorities and do not show morbidity. UPAs that comply with less GAP points have health issues. *A. hydrophila* was detected in batches with a lower compliance with the GAPs. The presence of *A. salmonicida* was not identified.

Study Limitations/Implications: The lack of compliance with the GAPs can lead to infection by other trout pathogens (not taken into consideration in this study).

Findings/Conclusions: Compliance with the GAPs reduces the health risk at the rainbow trout egg incubation UPAs.

Keywords: *Aeromonas salmonicida*, *Aeromonas hydrophila*, PCR, trout incubation, aquaculture health.

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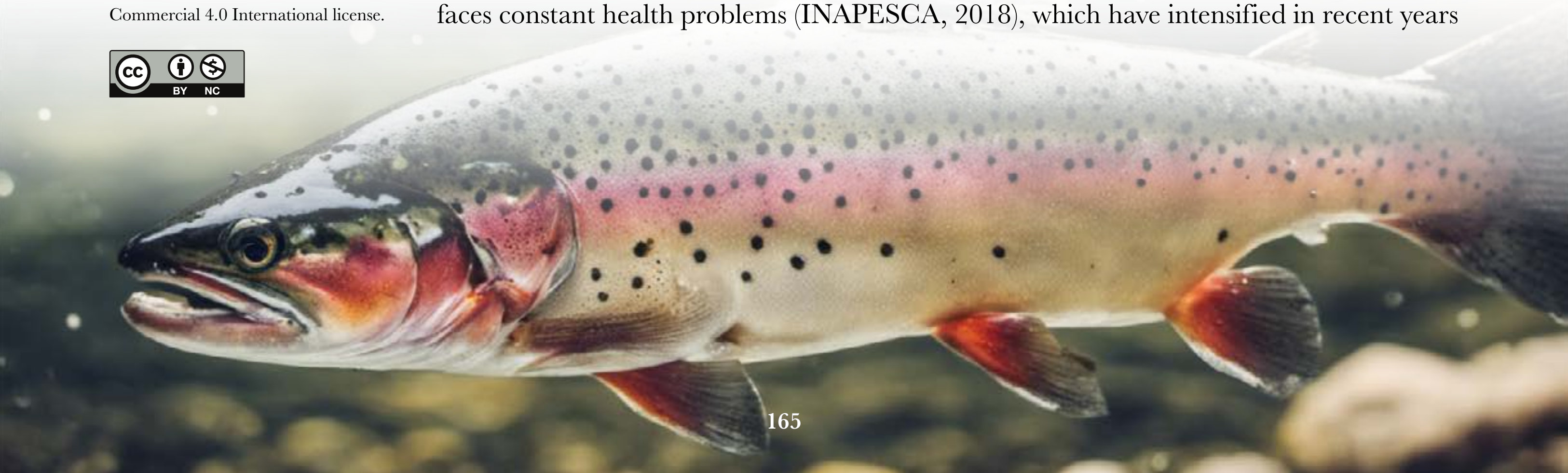
INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) is a fish species with high importance in Mexico. It is raised by small-scale producers in the mountainous regions of Mexico. This is the first species to be introduced as a freshwater species to repopulate rivers and for its commercial development. Small producers have successfully adopted management practices and technology in the fattening process; these producers obtain economic and social benefit from this practice. However, since this species is not native to the tropics, it faces constant health problems (INAPESCA, 2018), which have intensified in recent years



as a consequence of global warming and the vulnerability of the open production system (Carrillo-Longoria *et al.*, 2018).

Trouts are raised in 19 Mexican states and its production amounts to $876 millon pesos and 19,118 t. The states of Puebla and Veracruz produce 2,785.82 and 1,012.48 megagrams (Mg), respectively (Ontiveros-Córdova, 2022). Single sex alevin raising has not been successfully established in Mexico; therefore, production depends on the introduction of eyed eggs (Ortega *et al.*, 2011). Eggs are incubated in UPAs known as "incubators". Alevins are sold to fattening farms once they have reached a length of 5 cm. Three-hundred-and-five fattening farms have been registered in Puebla, while 186 have been reported in Veracruz (DOF, 2021). This is evidence of a reduced traceability and of the risk of distribution of water parasites.

Fish diseases are visually identified through changes in their behaviour and their physical characteristics, as well as through an increase in mortality. Nevertheless, high sensititivy techiques, such as the polymerase chain reaction (PCR), can be used to improve the accuracy of pathogen identification (Tufiño-Loza *et al.*, 2020).

In Mexico, the Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria (SENASICA) is in charge of following up epizootic diseases in aquaculture. However, scarce information has been published about the introduction or outbreak of pathogen bacteria in trouts (Alcántara-Jauregui *et al.*, 2022).

SENASICA encourages the use of Good Aquaculture Practices (GPAs). The purpose of these practices is to reduce health risk through the careful selection of the growing site, the supply of water and the control of its quality, the source of eggs or alevins, the reception and storage of the food, health criteria, use of chemicals and drugs, and harvest operations (García-Ortega and Calvario-Martínez, 2003).

Therefore, the compliance with GPAs in the UPAs where rainbow trout eggs are incubated is an important measure, along with the identification of the presence of *Aeromonas salmonicida* and *Aeromonas hydrophila*, using the Polymerase Chain Reaction (PCR) technique.

MATERIALS Y METHODS

Identification of the UPAs

Ten UPAs were identified in the states of Veracruz and Puebla, with the help of the Sistemas Producto Trucha (Table 1).

Sample collection

Avelin batches with $<$5-cm long specimens were visually inspected to detect integument and behavior anomalies. Productions logs were reviewed and the operators were subjected to non-structured questionnaires. Finally, samples were collected from each batch and put in 90% ethanol.

Evaluation of Good Aquaculture Practices

A structured questionnaire was applied to the operators of the UPAs, in order to obtain information regarding the management of the GAPs in each farm; additionally, in the case

Table 1. Aquaculture Production Units evaluated in the states of Puebla (P) and Veracruz (V).

Aquaculture Production Units	V1	V2	V3	P1	P2	P3	P4	P5	P6	P7
Municipality	X	X	TN	TH	TH	TH	H	H	H	TH
CBPA						*	*			
Origin of eggs	MEX	MEX	USA	MEX	MEX	MEX	USA	MEX	MEX	MEX

X=Xico, TN=Tlalnelhuayocan, H=Huauchinango, TH=Tlahuapan, GAPC=Good Aquaculture Practices Certificate, (*)=observed parameter, MEX=Mexico, USA=United States of America.

of GAP-certified units, the corresponding documentary evidence was requested. Following the proposal of García-Ortega and Calvario-Martínez (2003), a discriminatory table was developed to define the biosafey status of the sample UPAs. The operations were subjected to a visual evaluation. A value was assigned based on the compliance percentage, using a measurement unit called Health Compliance Unit (UCS). To obtain an overall assessment of the processes of each UPA, the UCSs of each processes were added together.

Sample treatment

A. salmonicida and *A. hydrophila* strains were requested from the Centro de Investigación en Alimentación y Desarrollo (CIAD), with reference keys CAIM 674 and CAIM 675, respectively. After their DNA was extracted; these strains were used as positives. Freeze-dried oligonucleotides were acquired (Table 2) and hydrated with ultrapure water, in order to adjust the concentration to 100 μMo.l.

In the case of *A. hydrophila*, the PCR test was carried out following the methodology proposed by Lee *et al.* (2000). The following conditions were established: a thermal cycler at 94 °C for 2 min, 35 amplification cycles (denaturation at 94 °C for 40 s, recooking at 60 °C for 40 s, and one extension at 72 °C for 60 s), and a final 5-min elongation period at 72 °C. In the case of *A. salmonicida*, the methodology proposed by Del Cerro *et al.* (2002) was used. The following conditions were established: thermal cycler with one 40-cycle amplification, 94 °C for 1 min, 35 °C for 2 min, and a 2-min elongación at 72 °C. The cycle was extended to 20 min.

Data analysis

The GAP results of the UPAs are shown as descriptive statistics, using the UCS as the measurement unit. The results of the PCR tests were expressed as the presence or abscence of the studied bacteria. Finally, the visual results of the batches are shown in a descriptive style, for their correlation with the results of previous tests.

Table 2. Oligonucleotides used to test the Polymerase Chain Reaction.

Sequence description	Base pairs	Sequence	Bacteria	Reference
AH-F	23	GAA AGG TTG ATG CCT AAT ACG TA	h (*Aeromonas hydrophila*)	Lee *et al.* (2000)
AH-R	21	CGT GCT GGC AAC AAA GGA CAG	h (*Aeromonas hydrophila*)	
PAAS1	19	CGT TGG ATA TGG CTC TTC T	S (*Aeromonas salmonicida*)	Del Cerro *et al.* (2002)

RESULTS AND DISCUSSION

Evaluation of Good Aquaculture Practices

The UPAs that show the highest compliance with the recommendations made by García-Ortega and Calvario-Martínez (2003) also have a GPA certification (Table 3). Farms V1, V2, P3, and P7 face high health risks, given their failure to comply with the expected health criteria.

All the UPAs under study are located in growing sites (GS) with the adequate altitude and temperature for the species. However, the supply and control of water quality (SCWQ) was inadequate, as a consequence of the sediments and the lack of filtration treaments. Sediments can damage gill cells, which may cause stress and be an entry point for pathogens (David-Ruales and Vásquez-Torres, 2010).

Pathogens such as *A. salmonicida* may enter the facilities through eyed eggs (Zepeda-Velázquez, 2015). The V1, V2, P3, and P7 UPAs produce eggs from their reproducers. However, they fail to provide the minimum health care to the incubation area and mortality reaches up to 100% of the batches.

A proper feeding program diminishes the vulnerability of fish against oportunistic pathogens (Velasco-Garzón and Gutiérrez-Espinoza, 2019). Although all UPAs use quality commercial food, not all of them have an adequate program for the productive stages. Farms V1, V2, and P3 do not protect the food from light, humidity, or pests, which may diminish its nutritional quality and pollute it.

The UPAs with the highest GPA compliance face a lower risk of pathogens entering and scattering in their production unit, consequently avoiding stress in the organisms and improving the response capacity during disease treaments.

Consequently, they diminish their losses, while also contributing to public health and environmwental wellbeing (Figueredo *et al.*, 2020). Biosafety criteria diminish the need to apply control chemicals (Fajer-Ávila *et al.*, 2017). The infrastructure and equipmen of farms P4 and P5 favours health; their staff is trained, and they have their biosafety protocols in writing.

Table 3. Result of the evaluation of the Good Aquaculture Practices, expressed in Health Compliance Units (UCS).

Activity	Veracruz (V)			Puebla (P)						
Aquaculture Production Unit	V1	V2	V3	P1	P2	P3	P4	P5	P6	P7
Cultivation site	100	100	100	100	100	100	100	100	100	100
Water supply and quality control	38	38	50	40	45	48	88	88	45	45
Origin of eggs	0	0	60	33	10	0	100	95	18	0
Reception and supply of food	53	53	75	67	67	50	100	100	67	67
Health criteria	0	0	50	64	0	0	100	100	14	0
Chemicals and drugs	0	0	25	85	25	0	85	85	0	0
Harvest process	30	30	75	100	60	60	100	100	30	30
Total UCS	51	51	110	138	67	53	192	190	72	58
Certified farm							*	*		

(*)=observed parameter.

Farms P1, P4, and P5 record the chemical products and drugs they provide; nevertheless, their health diagnostics are not carried out by specialists. As as standarized procedure, all the UPAs apply antibiotics to the fish from day 10, until day 20 after the hatching. González-Salas *et al.* (2021) explain that antibiotics are used as prophylactics in aquaculture, although bacteria develop resistance and their control is consequently more difficult.

García-Ortega and Calvario-Martínez (2003) and other authors described the harvest process (HP) in trout farms, under the consideration that stress and handling can cause diseases during this stage. In the analyzed UPAs, HP consists of counting, extracting, and selling on the farm itself 4- to 6-cm long alvins. UPAs V1, V2, P6, and P7 lack spoon nets that prevent mechanical injuries to the integument and fail to disinfect their equipment.

PCR test for the detection of Aeromonas

All PCR tests in search of *A. salmonicida* were negative. This bacteria can cause skin lessions, intestinal inflamation, typical furunculosis, severe septicemia, and over 100% mortality in fish batches (Zepeda-Velázquez, 2015). Since it is a non-mobile pathogen, it is passed on from fish to fish or from the reproducer to the egg. Trout egg importation is a critical point for Mexico. Castro-Escarpulli *et al.* (2003) have reported *A. salmonicida* cases in the country, especifically in tilapia; meanwhile, Salgado-Miranda *et al.* (2010) identified this species in seven samples from trout farms in the state of Chihuahua.

A. hydrophila is a mobile bacteria with flagella; it is found in bodies of water (*e.g.*, rivers) and can be an oportunistic pathogen. It is distributed all over the world and attemps to control it have led to the indiscriminate application of antibiotics in aquaculture (Perretta *et al.*, 2019). In this study, *A. hydrophila* was detected in 70% of the UPAs (Table 4). This finding matches the resutls of Salgado-Miranda *et al.* (2010) who identified this parasite as the most common bacteria that affects trouts.

Batches with positive results to *A. hydrophila* showed coincidences with the symptomatology described ty Fuentes and Pérez (1998), who linked the presence of the bacteria to exophthalmos, darkening of the skin, altered behaviour pattern, 80% sickness rate, and a 52% death rate. Nevertheless, these visual symptoms match other pathogens that also attack trouts (Alcántara-Jauregui *et al.*, 2022). However, the PCR technique has a 97.5% accuracy and the results regarding the presence of this bacteria can be therefore considered trustable (Chapela *et al.*, 2018).

CONCLUSIONS

Good Aquaculture Practices are inadequately implemented in most of the production units evaluated. Those farms with highest compliance with the biosafety points have less health problems. The presence of *Aeromonas salmonicida* should be monitored in imported trouts alevins and eggs The presence of *Aeromonas hydrophila* could be related to a defficient application of the Good Aquaculture Practices. The *A. hydrophila* bacteria can generate economic losses in trout farms, as a result of fish mortality and the cost of the supplies required for its control.

Table 4. Detection of *A. hydrophila* through the Polymerase Chain Reaction and visual evaluation of the health of fish batches in the evaluted Aquaculture Production Units (UPAs).

Batch	UPA	*A. hydrophila*	Natación Errática	Oscurecimiento de la Piel	Puntos Rojos en Abdomen	Exoftalmia	Mortality
1V1T	V1	+		*		*	A
1V2T	V1	+		*		*	N
1V3T	V1	+		*		*	A
2V1T	V2	+	*	*			MA
3V1T	V3	-					N
1P1T	P1	+	*	*			A
1P2T	P1	+	*	*			MA
1P3T	P1	+	*	*			MA
2P1T	P2	+	*	*	*		A
2P2T	P2	+	*	*	*		MA
2P3T	P2	+	*	*	*		MA
3P1T	P3	+	*	*	*		MA
3P2T	P3	+	*	*	*		MA
4P1T	P4	-					N
4P2T	P4	-					N
5P1T	P5	-					N
5P2T	P5	-					N
6P1T	P6	+	*	*	*		MA
7P1T	P7	+	*	*	*		MA
7P2T	P7	+	*	*	*		MA

(+)=Positive test; (-)=Negative test; N=normal; MA=moderately high; =high; (*)=observed parameter.

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Colegio de Postgraduados

Surface water balance at the Chapingo River basin: rainfall intercepted by vegetation and water infiltration into the soil

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ABSTRACT

Objective: To estimate the surface water balance in the Chapingo River microbasin in the years 2014, 2016, 2017, and 2018, based on information collected on rainfall and runoff, the use of interception simulation models, and the estimation of the of infiltration as a remainder of the balance.

Design/Methodology/Approach: During the 2014, 2016, 2017, and 2018 wet seasons, the water balance in water depth was estimated based on rainfall and runoff data, mathematical interception simulation models, and estimation of infiltration as a remainder of the balance.

Results: The highest interception rate was recorded in 2014, with a shorter range and lower dispersion of rainfall, while the lowest interception occurred in 2018 with opposite rainfall characteristics. A linear relationship was found, in two years, between rainfall and surface runoff with R^2 greater than 0.81. The interception rate was lower in the oyamel fir forest (7.7-9.3%), while the variation in interception between grasslands, pine forests, oak forests, and agricultural areas ranged from 20% to 23%. The remaining infiltration water represented between 85.5% and 88.2% of the rainfall.

Study Limitations/Implications: Determining the temporal evolution of the interception and humidity in the soil is necessary to specify the potential recharge to the aquifer.

Findings/Conclusions: Interception is the main vehicle by which water reaches the ground in areas covered by vegetation. Rainfall intensity has a negative impact on interception and infiltration. The basin under study offers a high recharge potential to the Texcoco aquifer.

Keywords: Hydrology, land-use, intensity, rainfall characteristics, aquifer.

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INTRODUCTION

The Water Balance (WB) is the evaluation of the water cycle variables (rainfall, evapotranspiration, runoff, infiltration, and interception) and is calculated at different observation scales (Sokolov and Chapman, 1974; Xu and Singh, 1998). Rainfall goes through two fundamental processes: interception by the canopy and infiltration into the soil. Runoff occurs when rainfall exceeds the retention capacity in these two processes. In basins with abundant vegetation, the joint study of rainfall and evapotranspiration allows the modeling of hydrological operations (Sun *et al.*, 2018). Although evapotranspiration (ET) accounts for approximately 40% of the annual accumulated rainfall in temperate climate basins (Shimizu *et al.*, 2003), it is not linked with other elements and, in the absence of rain, most of the water accounted through this method comes from interception (Savenije, 2004). Therefore, during a rain event, the ET can be omitted and the WB elements can be listed as: rainfall, interception, infiltration, and runoff.

At the beginning of a rain event, vegetation retains most of the water (Gerrits *et al.*, 2007; Savenije, 2004). In small rain events, water is stored in the leaves and afterwards evaporates and returns to the atmosphere. When it exceeds the storage capacity of the canopy, it falls to the ground as drops and flows downwards through the stems (Muzylo *et al.*, 2009; Sadeghi *et al.*, 2015); therefore, the water depth retained and conducted in these processes depends on the type of plant and the morphology of the canopy (Magliano *et al.*, 2022). Although the interception accounts for 20% to 50% of the total rainfall on average (Gerrits *et al.*, 2007), other processes are generally used for its estimation, given the lack of data and the complexity of this process. The interception rate is different between ecosystem components: in temperate forests, it varies from 18% to 60% of the total rainfall (Bolaños-Sánchez *et al.*, 2021; Chen *et al.*, 2013; Flores *et al.*, 2016), in grasslands it approaches 50% and even reaches 100%, depending on the intensity of the rain (Dyer *et al.*, 2022; Ochoa-Sánchez *et al.*, 2018), and in agricultural covers (specifically, corn) its value ranges from 12% to 45% of the total rainfall (Liu *et al.*, 2015; Ma *et al.*, 2016; Zheng *et al.*, 2018).

Both rainfall and runoff (which can be obtained more easily) and, to a lesser extent, interception and infiltration are variables that can be measured to establish explanatory relationships. The simplest approach to WB is to calculate runoff as a fraction of rainfall (Machado *et al.*, 2022). To improve the accuracy of the mathematical models, land cover and use variables are used, along with simplifications of the hydrodynamic characteristics of the soil. The use of these variables results in simple models, such as the curve number (NRCS, 2004), although they do not consider the effect of the interception.

Rain events consists of an initial phase where interception predominates, followed by runoff, which occurs out of phase in time but can be integrated on a daily basis. Therefore, the objective of this research was to estimate the surface water balance in the Chapingo River microbasin in the years 2014, 2016, 2017, and 2018, based on information collected on rainfall and runoff, the use of mathematical simulation models of canopy interception, and estimation of infiltration as a remainder of the balance.

MATERIALS AND METHODS

The Chapingo River microbasin is located in Texcoco, State of Mexico. Its limits are located at 525,057 E, 512,018 W, 2,155,257 N, and 2,147,639 S (UTM 14 N). It has a total area of 1,725.78 ha. The Series VI land-use and vegetation chart was used at a scale of 1:250,000 (INEGI, 2017). The said chart groups five representative sets by dominant vegetation type: rainfed agriculture (18%), pine (*Pinus hartwegii*) forest (25%), oak (*Quercus* *laurina*) forest (17%), oyamel fir (*Abies divino*) forest (7%), grassland (13%), and other coverage (20%). The last type includes human settlement polygons, mines, and water bodies.

In the wet season, rainfall records were obtained from two meteorological stations, located in the upper part (Davis® station) and the middle part (Hobo® station) of the study area. The information was integrated on a daily basis, determining the rainfall every 24 hours, grouped by year of observation.

Surface runoff was recorded at four gauging stations located in the main bed: one in the outlet, another in the lower part, and two more in the middle of the basin. The data were used at the same temporal resolution as rainfall.

Canopy rainfall interception models were used to calculate the interception fraction (%). In the formulations, the intercept (I) is expressed as a percentage (%) and the precipitation (P) in millimeters (mm).

The Zheng *et al.* model was used for agricultural coverage (2018):

$$I = 96.642P^{-0.733} \tag{1}$$

The model of the rainfall-interception ratio in a temperate pine forest —obtained under similar conditions by Bolaños-Sánchez *et al.* (2021)— was adapted to express the intercepted values as a fraction of the rainfall:

$$I = 32.065e^{-0.066P} \tag{2}$$

On the surface covered by oak trees, the model developed by Flores *et al.*, (2016) in the same microbasin was used:

$$I = -6.1571 \ln P + 31.83 \tag{3}$$

The interception simulation on the surface covered by oyamel firs was carried out based on an adaptation of the model developed by Bolaños-Sánchez *et al.* (2021) in the same biome:

$$I = 58.765e^{-0.094P} \tag{4}$$

The grassland interception was obtained with the annual grass model (Corbett and Crouse, 1968):

$$I = (\mathrm{P} + 58.7375)/\,31.25 \tag{5}$$

The results of the modeling of the interception fraction were converted to water depth (intercepted per event) to integrate the water balance into the temporal resolution of the analysis period.

The infiltration was estimated simplifying the continuity equation:

$$Inf = P - I - SR \quad (6)$$

Where: *Inf* is the infiltration (mm); *P* is rainfall (mm); *I* is the interception (mm); and *SR* is the surface runoff.

The components of the 24-hour water balance were integrated annually for their analysis. Information was used from some rainfall events during which surface runoff had been recorded in four years: 8 in 2014 (August-October), 15 in 2016 (May-August), 8 in 2017 (May-August), and 12 in 2018 (August), with a daily rainfall of 0.8-20.6 mm, 1.1-34.6 mm, 6.9-36.9 mm, and 0.5-45.2 mm, respectively.

RESULTS AND DISCUSSION

The rainfall events analyzed are representative of the rainfall regime of the study site; as shown in Figure 1, there was variability in daily precipitation, with water depths close to unity and extreme water depths similar to those reported by the SMN (2023). The average precipitated water depths of the 2014, 2016, 2017, and 2018 events were 11.5 mm, 14.1 mm, 16.5 mm, and 14.6 mm, respectively, while the standard deviations were 6.8 mm, 9.7 mm, 9.6 mm, and 13.6 mm for the same events.

The resulting water balance showed variations in the distribution of rainfall among infiltration, surface runoff, and canopy retention (Table 1). The rainfall interception fraction had variations of 9% to 12% in the four years, obtaining the highest value in 2014. Although the highest average rainfall in 24 h was recorded in 2017, the lowest interception rate (9.3%) was reported in 2018, as a consequence of the high dispersion of the rain events. Four events were above the 50% average and one reached 200%. Under these conditions, the rain saturated the retention capacity of the canopy and water was directly transferred to the soil surface.

The relative partitioning of rainfall was lower when rainfall intensity increased. These results are similar to the findings of Gerrits *et al.* (2007), given that the interception capacity of the vegetation remains constant. When there are high levels of rainfall in 24 h, the

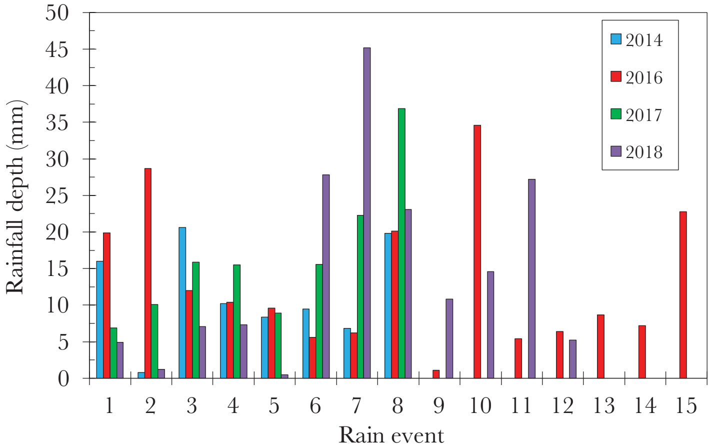

Figure 1. Studied rain events (mm).

Table 1. Elements of the water balance with percentage partitions of rainfall.

Component	2014		2016		2017		2018	
	(mm)	(%)	(mm)	(%)	(mm)	(%)	(mm)	(%)
Rainfall	92.0		198.7		132.1		174.9	
Infiltration	78.7	85.5	175.2	88.2	115.6	87.5	153.7	87.9
Surface runoff	2.0	2.2	2.0	1.0	3.7	2.8	5.2	3.0
Canopy Interception	11.3	12.3	21.6	10.9	12.8	9.7	16.3	9.3

canopy quickly reaches storage saturation and the interception ratio decreases. From that moment onwards, the water that enters the system is distributed only between low-density infiltration and runoff. In vegetation areas, if the intensity of rainfall exceeds the soil infiltration rate, water is lost through runoff. Under these conditions, infiltration depends on the canopy's capacity to transmit water to the soil surface; therefore, infiltration is dependent on interception. Infiltration only occurs at low rainfall rates when the soil is devoid of vegetation; meanwhile, when the intensity exceeds the infiltration capacity of the soil, runoff occurs regardless of the wetting of the soil profile. Shrubs and species with rough bark have a higher interception rate than trees and species with smooth bark (Magliano *et al.*, 2022). Therefore, the interception rate variation depends on the structure and composition of the land cover and use.

The relationship between rainfall and surface runoff had a decoupled behavior: the fraction of instantaneous runoff varied from 1% (2016) to 3% (2018). The differential behavior between rainfall and runoff can be taken as an indication of the spatial distribution of rainfall events. The rainfall and runoff data were analyzed with the linear regression method, finding in all cases a positive slope. However, the adjustment was low, since the R^2 for 2014, 2016, and 2018 was 0.21, 0.49, and 0.10, respectively. Meanwhile, the coefficient of determination for 2017 was high (0.92), mainly because the rains were of low intensity — apart from one event that was 100% higher than the average. In 2018, the extreme rainfall events of August 10 (27.8 mm) and August 11 (45.2 mm) produced low runoff rates; when they were omitted from the rainfall-runoff analysis, the correlation of these two variables reached an R^2 of 0.81. This behavior is a result of the spatial distribution of rainfall, since the information recorded by the two meteorological stations located inside the basin (upper part and middle part) probably did not represent the homogeneous spatial behavior of rainfall expected for a small basin.

Infiltration was estimated as a remnant of the effects of interception and surface runoff, recording an 85.5-88.2% average. Since the mathematical interception models used herein have high proportional values regarding small rain events, when the rainfall depth is greater than 20 mm, interception does no longer condition the balance and gives way to the infiltration. However, if the intensity per rain event is very high, most of the water is distributed by runoff from the initial stages of the event.

The pine forest (Figure 2) was the cover with the highest interception percentage (22.0-25.5%); these results are similar to the values reported by Bolaños-Sánchez *et al.* (2021) for a *Pinus hartwegii* forest (23.4%). Secondly, rainfed agriculture had values between 22.9%

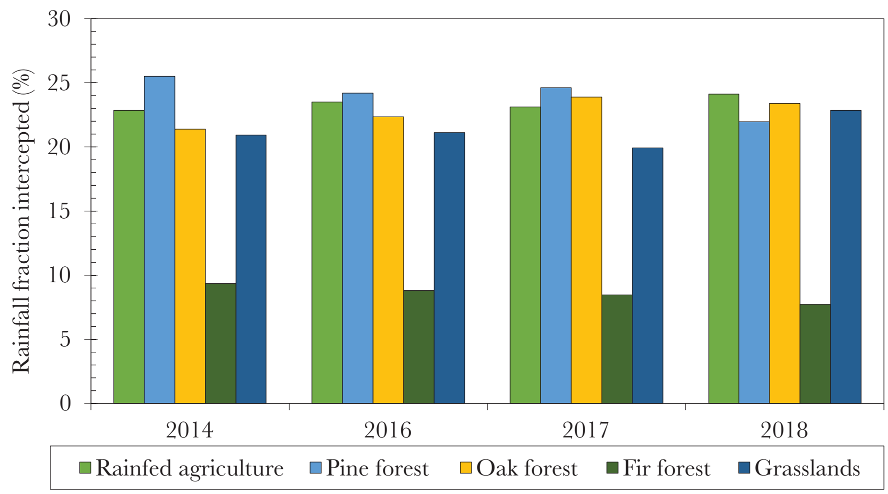

Figure 2. Fraction of rain interception by type of coverage.

and 24.1%, which contrasted with the findings of Zheng *et al.* (2018), who recorded 12.5% in corn crops, with an intensity of 23.1 mm per rain event and high dispersion of rainfall depths, which ranged from 1.9 mm to 87.8 mm per event. In third place was the oak forest with percentages ranging from 21.4% to 23.4%, which match the 21.7% reported by Flores *et al.* (2016) for an oak (*Quercus laurina*) forest. The grassland had interception percentages ranging from 19.9% to 22.8%, higher than the 12.6% annual value reported for grasslands (Corbett, 1968). The high interception values are the result of the structure and high density of leaves (Corbett & Crouse, 1968; Ochoa-Sánchez *et al.*, 2018). Finally, the oyamel fir forest was the land-use with the lowest interception percentage, with values (7.7-9.3%) below the 37.6% reported in a forest of the same kind in Mexico (Bolaños-Sánchez *et al.*, 2021). These results pertain only to rain events within the wet season; atypical rains outside this period have a different behavior, as a consequence of the variation in the composition of the canopy, especially in deciduous ecosystems. However, the models described in this study lack information for seasonal modeling.

The infiltration values were proportionally high and contributed to filling the storage capacity of the soils. However, in an annual water balance, evapotranspiration becomes a highly relevant element, particularly when the type of coverage is taken into consideration. Mature forests reach high evapotranspiration rates, mainly in the absence of rain events and in the dry season (Nicholls and Carey, 2021). Therefore, most of the water stored by ecosystems in the basins during rain events is consumed by vegetation, leaving a small portion for the recharge of deep aquifers.

The pine forest, oak forest, rainfed agriculture, and grassland land-uses had a similar interception percentage, with an approximately 5% variation between their values.

The differences between the results of this research and those reported in other studies are attributed to the effect of climatological variations (Herwitz A' and Slye, 1995; Ochoa-Sánchez *et al.*, 2018), since factors such as the duration of rainfall events and the intensity, and spatial pattern of rainfall, as well as the circulation of air masses and the seasons of the year, impact the interception values. For example, convective rains impact smaller areas than orographic rains which, in turn, are smaller than frontal or cyclonic rains.

The best adjustments to the modeling of the rainfall-interception ratio occur when rainfall events are analyzed on an individual basis, rather than when they are grouped over time (Bolaños-Sánchez *et al.*, 2021). The change in land-use from forest to cropland causes the infiltration rate to decrease by up to 58% (Sun *et al.*, 2018); This proportion of water that cannot be infiltrated is added to the runoff fraction. If the infiltration rate decreases in a basin, the recharge rate of the underground aquifers decreases and, consequently, the base flow of the drainage network also decreases.

CONCLUSIONS

The WB analysis determined that rainfall is mainly divided into interception —in the case of small events— and infiltration —when the canopy and its water storage capacity are saturated. Runoff occurs as a result of high intensity or saturation of the interception. Interception in the four years ranged from 9% to 12% of the rainfall. However, most of the water transmitted to the soil was first intercepted, therefore the interception influences in the effect of water into the soil. The oyamel fir forest had the lowest interception rate, while the rest of the coverages analyzed had variations of 5% between each other. The study basin is a source of the Texcoco aquifer with great recharge potential, owing to an average annual soil infiltration of 87.3% of rainfall.

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