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AGRO PRODUCTIVIDAD

AP

Evaluation of the productivity and rooting of cuttings of ten *Eucalyptus* clones

pág. 57

Año 13 • Volumen 13 • Número 9 • septiembre, 2020

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AGRO PRODUCTIVIDAD

Año 13 • Volumen 13 • Número 9 • septiembre, 2020

CONTENIDO

AP AGRO PRODUCTIVIDAD

SADER SECRETARÍA DE AGRICULTURA Y DESARROLLO RURAL · COLEGIO DE POSTGRADUADOS · CONACYT Consejo Nacional de Ciencia y Tecnología · Master Journal List Clarivate Analytics

ZOOLOGICAL RECORD® · latindex · CABI

PERIÓDICA Índice de Revistas Latinoamericanas en Ciencias · biblat Bibliografía Latinoamericana en revistas de investigación científica y social · CAB ABSTRACTS

CENGAGE Learning · Dialnet · EBSCO INFORMATION SERVICES

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> Ogata N. (2003a).
> Ogata N. (2003b).

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Thysanoptera diversity associated with Mexican lemon (*Citrus aurantifolia* Christm.)

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ABSTRACT

Objective: To calculate the monthly relative abundance of *Thysanoptera* species, according to the Margalef, Simpson and Shannon-Wiener diversity indices.

Design/Methodology/Approach: The work was carried out in three geographic units with conventional management, during January-December, 2019 in the Reserva de la Biosfera Zicuirán-Infiernillo (Biosphere Reserve), Michoacán, Mexico. In each unit, 10 trees were selected through simple random sampling. Thrips counts were performed on ten shoots per tree every 15 d, for a total of 7200 shoots in the three geographic units. *Thysanoptera* individuals were placed in entomological jars. The variables were: number of thrips collected per shoot in sampled tree and geographic unit (orchard). To estimate the specific richness and structure of species, the program "calculation of diversity indices DIVERS" was used.

Results: In the three geographical units studied, the recorded presence of *Thysanoptera* accounted for 12 to 17 species. For Nueva Italia 12 recorded species, two were permanent (16.66%), five abundant (41.66%), one scarce (8.3%) and four rare (33.33%). In Zicuirán, three species were permanent (17.64%), six abundant (35.29%), two scarce (11.76%) and six rare (35.29%). In Los Hoyos, four species were permanent (26.66%), four abundant (26.66%) and seven rare (46.66%). The abundance of species was represented by the genus *Frankliniella* and the species *Scolothrips sexmaculatus* and *Scirtothrips citri*. The highest species richness and abundance was found from January to May. In October and November, the value of the calculated indices was zero, which shows less richness and abundance of individuals. The best species uniformity was recorded during January and December, which meant a more stable and homogeneous relation.

Study limitations/Implications. Pest resurgence, presence of *Candidatus liberibacter* spp. and its vector *Diaphorina citri*.

Findings/Conclusions: in Nueva Italia, 12 species were taxonomically determined; in Los Hoyos 15, and in Zicuirán 17 species, which are reported for the first time in the state of Michoacán, Mexico. At the geographic unit "Los Hoyos" diversity was higher, uniform and stable.

Keywords: *Citrus*, *Frankliniella bispinosa*, *Scolothrips sexmaculatus*, *Scirtothrips citri*, thrips.

INTRODUCTION

The concept of diversity has been intensively discussed by ecologists for years, deriving some semantic, conceptual, and technical problems (Hurlbert, 1971). Diversity indices then, provide utility and scientific relevance, despite some debates and precautions

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which may arise when applying them. High levels of biodiversity suggest good functions in the ecosystems, a high capacity to react to external pressures and optimal adaptation to a changing environment (Odum and Warrett, 2008). Diversity studies make it possible to relate changes in the composition of species with environmental and edaphic variables (Durán and Zambrano, 2002), and also suggest obtaining a more detailed scenario of the interrelations among environmental factors and the distribution of species (Palmer *et al*., 2000).

For the diversity-alpha represents the species in a specific habitat, its importance relies on the application of the Simpson (Simpson, 1949), and Shannon-Wiener (Shannon, 1948) indices that express the values of richness and relative abundance in an agroecosystem. Low equity (or high dominance by few species) or equity in the representation of individuals of each species detected in the samplings (Carmona and Carmona, 2013). The advantage of the indices is that they summarize diverse information in a single value and allow comparisons, via statistical verification, between the diversity at the same or different habitats over time (Moreno, 2001). Mexico along with Brazil, Colombia and Indonesia rank among the first places of species richness on the planet (Ceballos *et al*., 2005).

In Mexico, the Zicuirán-Infiernillo Biosphere Reserve, in Michoacán, is the Protected Natural Area that preserves one of the most fragile and riskiest ecosystems, such as the dry tropical forest, which is also a provider of environmental goods and services such as the water catchment, soil retention, climate regulation, carbon reduction in the atmosphere, hydrological services; and it is an important site which concentrates a great diversity of species and endemism (SEMARNAT, 2006).

From an agricultural point of view, citrus (*Citrus* sp.) is the main crop in this region. Trees that have multiple shoots throughout the year, thanks to the warm climate, with the absence of a defined winter, which favors the presence of diverse biological organisms (Medina *et al*., 2001). Thrips are among them, playing an important role, by reducing the yield and quality of the fruits (Curti *et al*., 2000). Given the great importance that this group of insects represents in the cultivation of Mexican lemon (*Citrus aurantifolia*), it was considered to carry out this work in the Zicuirán-Infiernillo Biosphere Reserve, with the objective of calculating the monthly relative abundance of the *Thysanoptera* species, according to the Margalef, Simpson and Shannon-Wiener diversity indices.

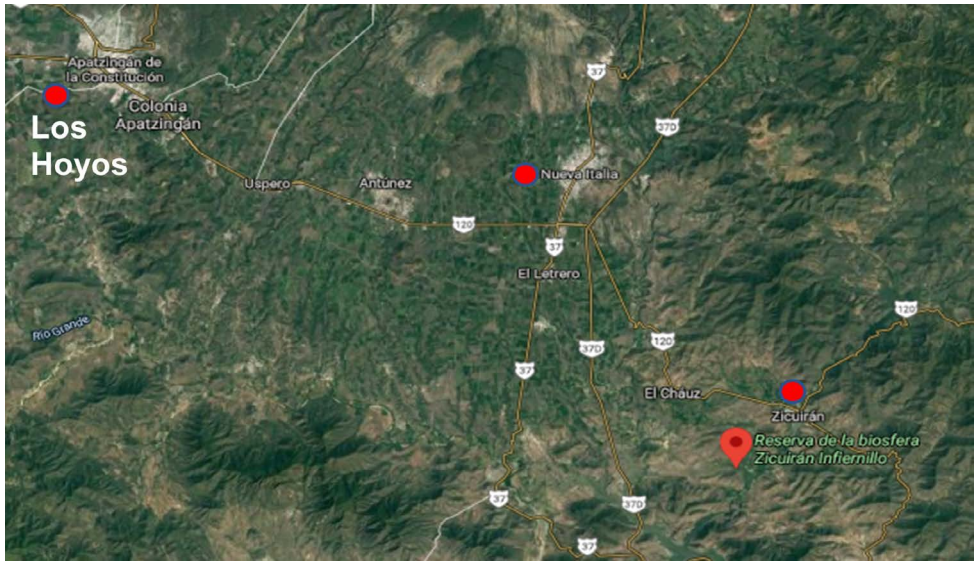

Figure 1. Location of the three geographic units, in the Zicuirán-Infiernillo Biosphere Reserve (Rzedowski 1978; Google Earth, 2020).

MATERIALS AND METHODS

The work was carried out in three geographic units or Mexican lemon orchards with conventional management, from January to December 2019. These units were ejidos 1) Nueva Italia, municipality of Fco. J. Mújica; 2) Zicuirán, municipality of Huacana; and 3) Los Hoyos municipality of Apatzingán, Michoacán, Mexico. The three areas are located in the Zicuirán-Infiernillo Biosphere Reserve, considered a great valley in which altitudes above sea level fluctuate from 200 m in the lower parts to 1600 m in the highland areas that delimit it (Rzedowski, 1978; INEGI 1985) (Figure 1).

In each unit, ten trees were selected by simple random sampling (Castillo, 2002). Thrips counts were made on ten shoots per tree every 15 d, to make a total of 7200 shoots in the three geographic units. The methodology consisted of washing the vegetative parts with a spray bottle containing soapy solution (5 mL of cationic softener® in 95 mL of water),

in order to collect the thrips (Johansen, 1987). A plastic container 25 cm long by 20 cm wide and 8 cm deep was placed at the bottom of the atomizer and the bud. Stored contents were then filtered on an analytical sieve with tight permanent tissue with a measuring range of 45 μm so that the insects would remain on the mesh and we can easily isolate them with a brush. *Thysanoptera* were deposited in entomological flasks containing 70% alcohol, with their respective passport data. The variables studied were the number of thrips collected per shoot in the sampled tree and geographic unit (orchard). To know the specific richness and structure of species, the program "calculation of diversity indices DIVERS" was used (Pérez and Sola, 1993), and in this study, they were the following:

Margalef Diversity Index

$$D_{Mg} = \frac{S-1}{\ln N}$$

Table 1. Number of *Thysanoptera* individuals per species and geographical unit, in the Zicuirán-Infiernillo Biosphere Reserve, Michoacán, Mexico. 2019.

Especie	Unidades geográficas		
	Nueva Italia	Los Hoyos	Zicuirán
Scirtothrips citri	369	372	465
Leptothrips mcconnelli	97	92	83
Scolothrips sexmaculatus	395	444	466
Frankliniella cephalica	250	204	94
Liothrips mexicanus	1	0	0
Karnyothrips sp.	1	3	3
Frankliniella bispinosa	185	252	238
Lacandonithrips elegantis	1	2	0
Microcephalothrips sp. 1	1	0	0
Frankliniella curticornis	205	126	124
Frankliniella minuta	187	69	63
Stomatothrips brunneus	20	40	30
Scolothrips pallidus	0	0	51
Liothrips sp.	0	1	1
Microcephalothrips sp. 2	0	0	2
Leptothrips macro-ocellatus.	0	2	2
Frankliniella insularis	0	0	10
Scirtothrips totonacus	0	0	1
Leucothrips theobromae	0	0	1
Caliothrips phaseoli	0	0	10
Frankliniellia cubensis	0	3	0
Leucothrips furcatus	0	1	0
Neurothrips punanus	0	1	0
Número total de individuos (N)	1712	1612	1644
Número total de especies (S)	12	15	17

where: S=number of species. N=total number of individuals.

Its result, generally, is different from "zero" (Mallet, 1996). To know the dominance of species, the Simpsom Index was calculated

$$\lambda = \sum p_i^2$$

where: p_i^2 =proportional abundance of species "*i*", that is, the number of individuals of species "*i*" divided by the total number of individuals in the sample. This value shows the probability that two individuals, taken at random from a sample, are of the same species. To know the average uncertainty, the Equity Index (Shannon-Wiener) was calculated (Moreno, 2001).

$$H' = -\sum(p_i \ln p_i)$$

RESULTS AND DISCUSSION

This work exemplifies the use of effective numbers of species that give confidence and consistency to the biological diversity of thrips. Species numbers also comply with a series of mathematical properties, consistent with the intuitive interpretation of the biological concept of diversity (Jost, 2006), and that avoid plausibly inaccurate conclusions in conservation biology (Jost *et al.*, 2010). In the three geographic units studied, the recorded presence of *Thysanoptera* in *Citrus aurantifolia* trees was 12 to 17 species (Table 1, Figure 2).

Table 1 shows that the number of species of thrips shows heterogeneity within an ecosystem, since the minimum value was 12 and the maximum 17, respectively. Case and Cody (1987) point out that the appreciation of biota in a given place is influenced not simply as a relic of past historical events, but as an entity in constant change. Halffter and Escurra (1992) attribute the simplification of ecosystems to the application of toxic products, which result in biomass and diversity losses. In Nueva Italia, of 12 registered species, two were permanent (16.66%), five abundant (41.66%), one scarce (8.3%) and four rare (33.33%). In Zicuirán, three species were permanent (17.64%), six abundant (35.29%), two scarce (11.76%) and six rare

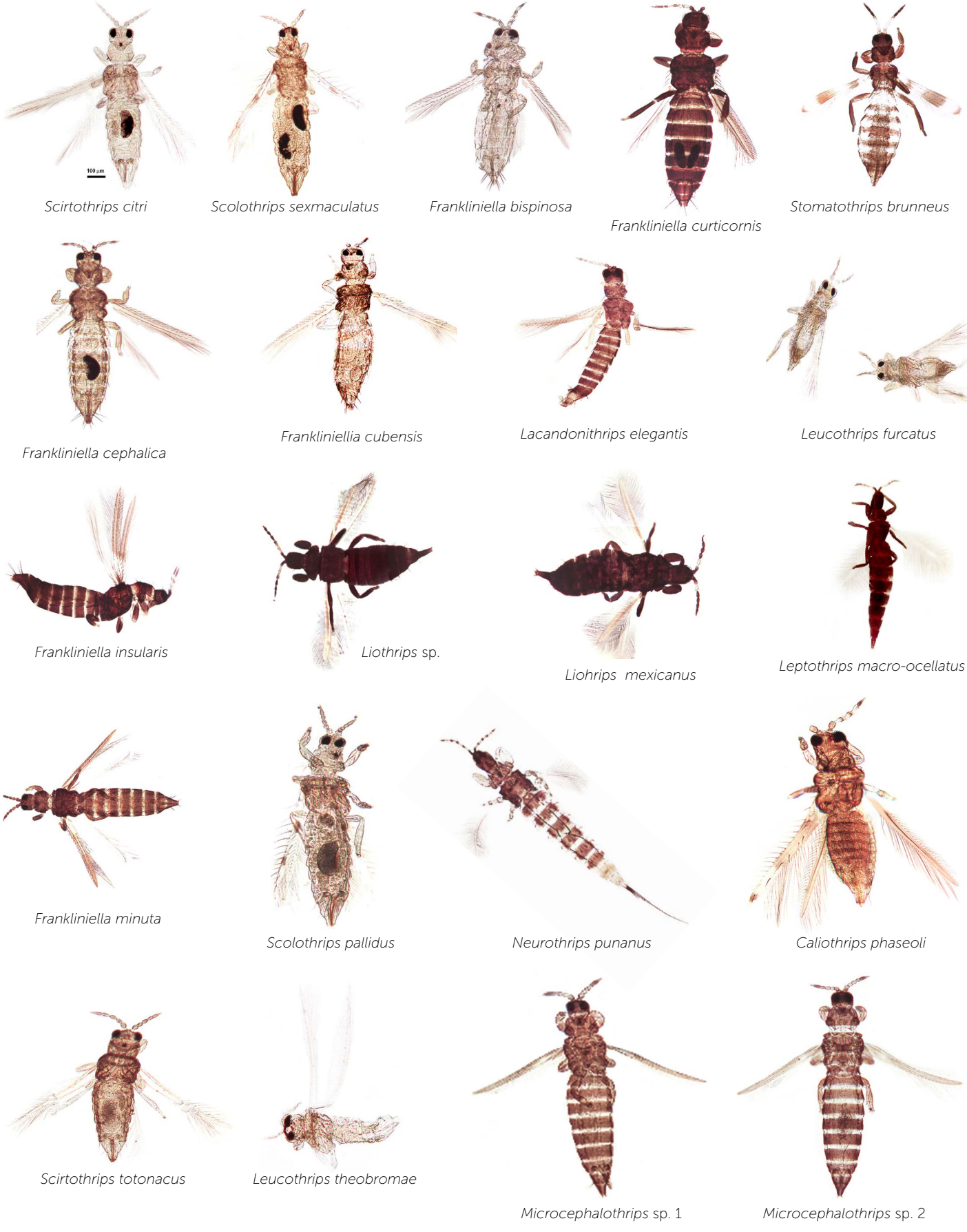

Figure 2. *Thysanoptera* genera and species collected in vegetative shoots of Mexican lemon, in three geographic units of the Zicuirán-Infiernillo Biosphere Reserve, Michoacán, Mexico; 2019.

(35.29%). In Los Hoyos, four species were permanent (26.66%), four abundant (26.66%) and seven rare (46.66%) (Table 2).

The information in Table 2 coincides with Segnini (1995), when mentioning that biodiversity depends not only on species richness, but on the relative dominance and abundance of each of them. As well as on the physical structure of the environment, since some species of thrips can just stay on, others feed of, and some others reproduce on plants (Mound, 1997). According to the number of individuals collected monthly per species (Table 3, 4 and 5), the diversity indices were calculated for each geographic unit (Table 6, 7 and 8).

Regarding Tables 3, 4, and 5, it can be seen that the species distribution pattern is only explained by the physical conditions of the environment. At the same time, it is observed that the abundance of species in the three localities was represented mainly by the genus Frankliniella and the species *Scolothrips sexmaculatus* and *Scirtothrips citri*. The percentage of *Thysanoptera* previously mentioned at Nueva Italia was 92.92, at Los Hoyos, 91.19; and at Zicuirán 88.80. The above coincides with classic works by Moulton (1948), Priesner (1933) and Wilson and Schmida (1984), cited by Johansen (1987), who points out the importance of thrips in Mexico, where the *Frankliniella* genus stands out. Pertaining to the absence or reduced number of collected thrips, perhaps because the specific microhabitat where they live is unknown; because their reproduction is scarce, due to the phytosanitary management that is carried out in the crops; or, in other cases, because there have not been recent collections in the localities. However, the results obtained are important for Entomology since, according to the Thysanopterii-fauna inventory of the year 1993, from the state of Michoacán only 9.3% of Suborder *Terebrantia* and 22.85% of Tubulifera within the total species described in the country (Johansen 1987) are known. Halffter (1998) and Zuccaro and Bulla

Table 2. *Thysanoptera* species in *C. aurantifolia*, grouped into abundance classes, in three geographic units in the Zicuirán-Infiernillo Biosphere Reserve, Michoacán, Mexico. 2019.

Locality	Species			
	Permanent [z]	Abundant [y]	Scarce [x]	Rare [w]
Nueva Italia	*S. sexmaculatus*	*S. citri*	*S. brunneus*	*Karnyothrips* sp.
	F. bispinosa	*L. mcconnelli*		*Liothrips mexicanus*
		F. cephalica		*L. elegantis*
		F. curticornis		*Microcephalothrips* sp. 1
		F. minuta		
Zicuirán	*S. sexmaculatus*	*F. cephalica*	*F. insularis*	*Liothrips* sp.
	S. citri	*F. bispinosa*	*C. phaseoli*	*Karnyothrips* sp.
	L. mcconnelli	*S. pallidus*		*Microcephalothrips*. sp. 2
		F. curticornis		*Leptothrips* sp.
		F. minuta		*S. totonacus*
		S. brunneus		*L. theobromae*
Los Hoyos	*S. sexmaculatus*	*L. mcconnelli*		*Liothrips* sp.
	S. citri	*F. cephalica*		*Karnyothrips* sp.
	F. bispinosa	*F. minuta*		*L. elegantis*
	F. curticornis	*S. brunneus*		*F. cubensis*
				L. furcatus
				Leptothrips sp.
				N. punanus

[z] Present for 11 and 12 months of the year; [y] present for eight to ten months; [x] present from three to seven months; [w] present in one and two months of the year.

S. sexmaculatus=Scolothrips sexmaculatus, S. citri=Scirtothrips citri, S. brunneus=Stomatothrips brunneus, F. bispinosa=Frankliniella bispinosa, L. mcconnelli=Leptothrips mcconnelli, F. cephalica=Frankliniella cephalica, F. curticornis= Frankliniella curticornis, F. minuta=Frankliniella minuta, L. elegantis=Lacandonithrips elegantis, S. palidus=Scolothrips pallidus, F. insularis=Frankliniella insularis, C. phaseoli=Caliothrips phaseoli, S. totonacus=Scirtothrips totonacus, L. theobromae=Leucothrips theobromae, F. cubensis=Frankliniellia cubensis, L. furcatus=Leucothrips furcatus, N. punanus=Neurothrips punanus.

Table 3. Number of recorded thrips of each species of *Thysanoptera* per month, in the *C. aurantifolia* cultivation at the geographic unit ejido Nueva Italia, Michoacán. 2019.

Species	Number of thrips collected in *C. aurantifolia*												Total
	jan	feb	mar	apr	may	jun	jul	aug	sep	oct	nov	dec	
S. citri	67	54	79	51	53	41	12	8	2	0	0	2	369
L. mcconnelli	11	12	21	17	13	9	8	5	0	0	0	1	97
S. sexmaculatus	61	56	68	62	65	39	20	10	3	2	3	6	395
F. cephalica	54	27	60	32	34	21	10	6	2	1	0	3	250
L. mexicanus	0	1	0	0	0	0	0	0	0	0	0	0	1
Karnyothrips sp.	0	0	0	1	0	0	0	0	0	0	0	0	1
F. bispinosa	26	23	38	29	26	21	8	7	3	1	1	2	185
L. elegantis	0	0	0	0	0	0	1	0	0	0	0	0	1
Microcephalothrips sp. 1	0	0	0	0	0	1	0	0	0	0	0	0	1
F. curticornis	39	31	37	27	24	22	17	7	0	0	0	1	205
F. minuta	24	20	38	35	31	22	14	3	0	0	0	0	187
S. brunneus	2	4	1	3	5	3	1	0	1	0	0	0	20
Accumulated species	**8**	**9**	**9**	**10**	**10**	**11**	**12**	**12**	**12**	**12**	**12**	**12**	**12**

Table 4. Number of recorded thrips of each species of *Thysanoptera* per month, in the *C. aurantifolia* cultivation at at the geographic unit ejido Zicuirán, Michoacán. 2019.

Species	Number of thrips collected in *C. aurantifolia*												Total
	jan	feb	mar	apr	may	jun	jul	aug	sep	oct	nov	dec	
S. citri	71	68	67	69	65	53	49	14	4	0	0	5	465
L. mcconnelli	19	21	12	9	11	5	3	2	0	0	0	1	83
S. sexmaculatus	82	74	69	61	57	53	46	21	0	0	0	3	466
S. pallidus	18	11	7	3	9	0	2	0	0	0	0	1	51
F. cephalica	21	13	11	16	10	12	7	4	0	0	0	0	94
Liothrips sp.	0	0	0	0	1	0	0	0	0	0	0	0	1
Karnyothrips sp.	1	2	0	0	0	0	0	0	0	0	0	0	3
F. bispinosa	42	47	39	42	36	21	9	0	0	0	0	2	238
F. curticornis	27	22	25	13	21	13	3	0	0	0	0	0	124
F. minuta	13	17	11	9	7	2	4	0	0	0	0	0	63
S. brunneus	8	3	5	7	2	0	1	2	0	0	2	0	30
Microcephalothrips sp. 2	0	0	1	0	0	0	1	0	0	0	0	0	2
L. macro-ocellatus	0	0	0	0	0	0	0	1	1	0	0	0	2
F. insulares	4	0	2	1	0	0	1	2	0	0	0	0	10
S. totonacus	0	0	0	1	0	0	0	0	0	0	0	0	1
L. theobromae	0	0	0	1	0	0	0	0	0	0	0	0	1
C. phaseoli	6	0	0	2	1	0	0	0	0	0	0	0	9
Accumulated species	**12**	**12**	**13**	**15**	**16**	**16**	**17**	**17**	**17**	**17**	**17**	**17**	**17**

(1985), point out that it is difficult to imagine a social development like the current one, without affecting the natural environment, and the most fragile element is biodiversity, which is why abundance of species was contrasting.

The values in Table 6 show that, during the months of March, May, August, September and October, the uniformity value was higher, which means the relation that exist between abundance and species richness, since collected individuals presented roughly the same

Table 5. Number of recorded thrips of each species of *Thysanoptera* per month, in the cultivation of *C. aurantifolia* at the geographic unit ejido Los Hoyos, Michoacán, Mexico. 2019.

Species	Number of thrips collected in *C. aurantifolia*												Total
	jan	feb	mar	apr	may	jun	jul	aug	sep	oct	nov	dec	
S. citri	62	56	58	60	48	35	24	13	6	2	3	5	372
L. mcconnelli	16	18	21	13	7	9	6	2	0	0	0	0	92
S. sexmaculatus	66	71	73	72	53	42	31	15	6	4	2	9	444
F. cephalica	38	32	41	34	28	19	9	2	0	0	0	1	204
Liothrips sp.	0	0	0	0	0	0	0	0	0	0	0	1	1
Karnyothrips sp.	0	2	1	0	0	0	0	0	0	0	0	0	3
F. bispinosa	31	39	46	41	37	29	11	9	2	0	1	6	252
L. elegantis	0	0	1	0	0	1	0	0	0	0	0	0	2
F. cubensis	0	0	0	0	1	0	2	0	0	0	0	0	3
L. furcatus	1	0	0	0	0	0	0	0	0	0	0	0	1
L. macro-ocellatus	1	0	0	0	0	0	0	0	0	0	0	1	2
F. curticornis	14	17	23	24	18	13	9	3	1	0	1	3	126
F. minuta	12	9	11	13	10	7	5	2	0	0	0	0	69
S. brunneus	7	9	3	6	9	2	0	0	0	0	1	3	40
N. punanus	1	0	0	0	0	0	0	0	0	0	0	0	1
Accumulated species	**11**	**12**	**13**	**13**	**14**	**14**	**14**	**14**	**14**	**14**	**4**	**15**	**15**

Table 6. Result of the DIVERS program that shows the diversity indices corresponding to the geographical unit of ejido Nueva Italia, Michoacán 2019 (Pérez and Sola, 1993).

Month	No. of individuals (abundance)	Species richness (S)	Uniformity (E)	Index			ln "S"[z]
				Margalef	Simpson	Shannon	
February	228	9	0.86670	1.47342	0.160	1.90	2.197
March	342	8	0.90518	1.19974	0.161	1.88	2.079
April	257	9	0.87884	1.44168	0.156	1.93	2.197
May	251	8	0.91275	1.26685	0.164	1.89	2.079
June	179	9	0.88093	1.54220	0.155	1.93	2.197
July	91	9	0.89697	1.77350	0.142	1.97	2.197
August	46	7	0.97328	1.56714	0.138	1.89	1.945
September	11	5	0.96096	1.66813	0.145	1.54	1.605
October	4	3	0.94639	1.44270	0.166	1.03	1.098
November	4	2	0.81128	0.72135	0.500	0.56	0.693
December	15	6	0.88560	1.84635	0.190	1.58	1.79

[z] Natural logarithm of species richness.

amount in their respective species. The value of the Margalef index (0.72135) indicates that in the month of November there was no species richness, since only three individuals of *S. sexmaculatus* and one of *F. bispinosa* were collected. The main characteristic of this index is that the higher its value (greater than one), the greater diversity exists.

In the Simpson index, the maximum value was obtained in the month of November (0.500) close to one, which indicates that in this month diversity was lower. But, at the same time, this value represents a 50% probability that, of the four individuals collected, two taken at random, would be of the same species. However, in the months from December to October, as the species richness (S) and number of individuals increased, the value of the Simpson index decreased (0.1). Referring to the Shannon index, when its value is equal to zero, most of the individuals in a population correspond to the same species. Thus, the higher it is or it approaches

Table 7. Result of the DIVERS program that shows the values of the diversity indices corresponding to the geographical unit of ejido Zicuirán, Michoacán, 2019 (Pérez and Sola, 1993).

Month	No. of individuals (abundance)	Species richness (S)	Uniformity (E)	Index			ln "S"[z]
				Margalef	Simpson	Shannon	
February	278	10	0.83358	1.59925	0.174	1.91938	2.302
March	249	11	0.78884	1.81234	0.188	1.89157	2.397
April	234	13	0.73490	2.19969	0.195	1.88499	2.564
May	220	11	0.77280	1.85404	0.194	1.85310	2.397
June	159	7	0.80340	1.18369	0.248	1.56335	1.945
July	127	12	0.63842	2.27076	0.285	1.58642	2.484
August	46	7	0.73210	1.56714	0.299	1.42460	1.945
September	5	2	0.72193	0.62133	0.600	0.50040	0.693
October	0	0	0.0	0.0	0.0	0.0	0.0
November	2	0	0.0	0.0	0.0	0.0	0.0
December	12	5	0.88486	1.60972	0.212	1.42413	1.609

[z] Natural logarithm of species richness.

Table 8. Result of the DIVERS program that shows the diversity indices corresponding to the geographical unit of ejido Los Hoyos, Michoacán, 2019 (Pérez and Sola, 1993).

Month	No. of individuals (abundance)	Species richness (S)	Uniformity (E)	Index			ln "S"[z]
				Margalef	Simpson	Shannon	
January	249	11	0.79055	1.81243	0.17820	1.89565	2.397
February	253	9	0.85801	1.44577	0.17642	1.88525	2.197
March	278	10	0.81496	1.59925	0.17290	1.87652	2.302
April	263	8	0.88871	1.25625	0.17862	1.84803	2.079
May	211	9	0.85770	1.49481	0.17174	1.88457	2.197
June	157	9	0.84266	1.58220	0.17712	1.85152	2.197
July	97	8	0.86715	1.53015	0.19201	1.80319	2.079
August	46	7	0.83701	1.56714	0.21739	1.62875	1.945
September	15	4	0.85279	1.10781	0.29524	1.18222	1.386
October	6	2	0.91830	0.55811	0.46667	0.63651	0.693
November	8	5	0.92838	1.92359	0.14286	1.49418	1.609
December	29	8	0.87038	2.07882	0.16502	1.80999	2.079

[z] Natural logarithm of species richness.

to the ln (natural logarithm) of "S" (species richness), the greater the diversity and, according to the discriminatory criterion of the statistical program proposed by Pérez and Sola (1993), it is inferred that in most months of the year there was a general population rich in species.

Table 7 shows that the highest species richness and abundance was from January to May. In October and November, the value of the calculated indices was zero, which means less richness and abundance of individuals. The values corresponding to uniformity in the months of January and December were the highest, which meant a more stable and homogeneous relation among species. When analyzing the Margalef index, it can be seen that in the months of October and November its result was zero and, according to Moreno *et al*. (2001), when this result is obtained there is no richness and the population studied is represented by a single species. However, in the months of April and July, their values were 2.19969 and 2.27076, respectively, which means that the higher the richness, the higher the abundance of species. In the Simpson index, the highest value corresponded to the month of September (0.600). During this month only two species were collected: *Scirtothrips citri*, with four individuals, and *Leptothrips macro-ocellatus*, with one. This means that if two individuals are chosen at random, there is a high probability that both would correspond to *S. citri*,

suggesting strong influence by the dominant species. In relation to the Shannon-Wiener index, the highest value (2.06303) was recorded in January and represented high uncertainty that two individuals taken at random were of the same species, since the abundance was more uniform when collecting 12 out of 17 species reported in our study, with an average collection of 26 individuals per month in each one. These data coincide with that reported by González and Torruco (2001), by pointing out that when this index increases, the diversity in the study area is greater. On the contrary, in the month of September, the value was 0.50040, which represents low uncertainty that two individuals taken at random would correspond to the same species.

The values in Table 8 indicate that the uniformity was stable, so that the equity among the different species was more homogeneous. As at the geographical unit ejido Nueva Italia, at least one species was present throughout the year. According to Magurran (1988), in an ecological context, the components of an ecosystem, such as "variety and relative abundance of species", are well correlated. In addition, they indicate the way in which resources and energy are shared in the biological systems and populations (Sheldon, 1969). The Margalef index, in the month of October, presented the lowest value (0.55811) when collecting four individuals of *S. sexmaculatus* and two of *S. citri*. These values are closely related to the Simpson index, whose highest value was 0.46667. The value of the Shannon-Wiener index, in the month of October, represented low uncertainty, by indicating to which species the next collected individual will belong, since two species were recorded that month, of which *S. sexmaculatus* was dominant. The values corresponding to the other months represented high uncertainty, since their value was close to the natural logarithm of "S" (species richness).

The information obtained in the three locations shows that the species were distributed, according to their hierarchy of abundance, from very abundant to some very rare. According to what Halffter and Ezcurra (1992) cited, the greater the degree of dominance of some species and rarity of the others, the lower the biodiversity in the community. However, Peet (1974) points out that it is normal to find these conditions, since the earth is nothing more than a fine mosaic of conditions and resources that define different niches that are habitable or not by different species.

CONCLUSIONS

In Nueva Italia, 12 species were taxonomically determined; in Los Hoyos, 15; and in Zicuirán, 17, the latter are reported for the first time for Michoacán, Mexico. In the three units, the abundance of species was represented by five species: *Scirtothrips citri*, *Frankliniella bispinosa*, *F. cephacila*, *F. curticornis* and *Scolothrips sexmaculatus*. While *Liothrips* sp., *Stomatothrips totonacus*, *Leucothrips theobromae, Leucothrips furcatus*, *Neurothrips punanus*, *Leptothrips elegantis*, *Leptothrips* sp., *Karnyothrips* sp., and *Microcephalothrips* sp.1 were registered as rare species, since, on average, just one to three individuals of each species were collected during the year. The species *Stomatothips brunneus*, *Frankliniella insularis* and *Caliothrips phaseoli* were also considered scarce species. In the geographical unit Los Hoyos, diversity was higher, homogeneous and stable, compared to that registered in Nueva Italia and Zicuirán.

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Study of the quality and antioxidant properties of tomatoes (*Solanum lycopersicum* L.) under different postharvest and dehydration conditions

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ABSTRACT

Objective: To study the effect of storage temperatures and dehydration conditions (solar and convective drying; SD, CD), on the quality, physicochemical parameters and antioxidant properties of tomato fruits.

Methodology: The physicochemical characteristics pH, titratable acidity, soluble solids (°Bx) and color parameters (*L**, *a** and *b**), were evaluated. The lycopene, carotenoids and antioxidant activity percentages retention of tomatoes fruits stored at 7 and 22 °C for 5 days and subjected to SD (Temperature (T) of 67 °C and luminescence of 685 lum/sqf) and CD (T 70 °C, flow rates 0.5, 1.0 and 1.5 m/s), were analyzed.

Results: The fruits reached humidities of 17 and 15% for SD and CD. The parameters pH, °Bx, *L**, *a**, *b** were highest with 22 °C and CD (1.5 m/s). The value of the carotenoids was higher in fruits stored at 7 °C and subjected to CD (1.0 and 1.5 m/s) and SD with values of 83.85, 85.98 and 99.43%, respectively. The CD (0.5 m/s) and SD improved lycopene (94.37 and 95.14%) and the antioxidant activity with values of 73.06 and 97.21%.

Implications: The application of solar dehydration depends on luminescence condition; however, it is inexpensive and environmentally friendly alternative.

Conclusions: The results derived in a viable alternative for the conservation and commercialization of tomato fruits in rural communities.

Keywords: dried tomato, solar dehydration, lycopene, carotenoids, antioxidant activity.

INTRODUCTION

The tomato (*Solanum lycopersicum* L.) of the Solanaceae family, is one of the essential vegetables due to its consumption and economic relevance, with a cultivation area of 4.6 million hectares worldwide (FAO, 2017). In 2017, fresh tomato production in Mexico was 4,243,058 tons (Servicio de Información Agroalimentaria y Pesquera,

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2016), being Sinaloa, San Luis Potosí, Michoacán, Jalisco and Puebla, states with the highest production. However, the increase of the production and sometimes the low demand of the market propitiate alterations in the commercialization affecting the fresh tomato conservation, due to its high moisture content (92%), which result in the physical-chemical and microbiological changes. Dehydration is one of the most used processes in the conservation of fruits and vegetables (Ibarz and Ribas, 2005), highlighting drying as one common operation in the processing of food products, to increase their shelf life. The demand for dehydrated tomatoes has increased internationally, due to its use for the preparation of different dishes (Catalano *et al*., 2013). Additionally, the application of solar drying is being promoted as a conservation alternative, inexpensive, and friendly to the environment (Ojike *et al*., 2010). Moreover, several studies highlight the nutritional composition of tomatoes and the presence of lycopene, β-carotene, vitamins C and E, and phenolic compounds (Leonardi *et al*., 2000; Luna-Guevara and Delgado-Alvarado, 2014). The antioxidant properties of these compounds are associated with the prevention of carcinogenic and cardiovascular diseases (Juroszek *et al*., 2009; Luna-Guevara *et al*., 2019). Similarly, some physicochemical properties of tomato are essential because they are related with the selection criteria by the consumer, they are also crucial as quality factors during processing (Ghavidel and Davoodi, 2010).

This research aimed to study the effect of storage temperatures, dehydration conditions and the influence of dehydrator types (solar and convective), on the quality, physicochemical parameters and antioxidant properties of tomato fruits.

MATERIALS AND METHODS

Vegetal material

In a greenhouse with a fertirrigation system, 100 fruits of tomato cv Reserva-vilmorin were harvested in the Aquixtla region, Puebla, Mexico. The second and third bunch of five flowering plants located in the center of the greenhouse were marked to guarantee the degree of physiological maturity (PM). The PM degree is considered by the local producers for commercialization and corresponds to the slightly red coloration (color chart No.5) (USDA, 2019).

Pretreatments

The fruits were selected with similar size, washed, and disinfected with a sodium hypochlorite solution 5% (v/v). Subsequently, the tomatoes were divided into four lots, two of them were stored at 7 °C ± 2 °C and the other two lots at 22 °C ± 2 °C, both remained for 5 days.

Dehydration processes

Preparation of the sample

The stored fruits were cut into four portions of approximately 20 g and submitted to the drying conditions. For solar dehydration (SD) treatments was used an SSB-2008 dryer (1.4 m × 0.8 m × 0.4 m dimensions), which consists of a collector, air channel and tray sample, hot air extractor and thermoelectric cell for electric power generation. The samples were exposed to sunlight during the month of June, and the environmental conditions (average temperature of 67 °C and luminescence of 685 lum/sqf) were monitored with a data logger (HOBO Mod. H08-004-00). While a cabinet dryer was considered for convection drying (CD) treatments, which operated at 70 °C and air flows of 0.5, 1.0 and 1.5 m/s conditions, the samples were weighed every 30 min for 5 h until a constant weight was reached (Montiel-Ventura *et al*., 2018).

Physicochemical and composition characterization of dehydrated fruits

Color

The parameters of Hunter scale color L^* (luminosity, white-black), a^* (red-green) and b^* (yellow-blue) were analyzed with a colorimeter (Minolta Mod CR-300) and were used to calculate total color difference (ΔE) with equation:

$$\Delta E = \left(\left(L_1^* - L_2^* \right)^2 + \left(a_1^* - a_2^* \right)^2 + \left(b_1^* - b_2^* \right)^2 \right)^{(1/2)} \quad (1)$$

Other physicochemical properties

The dried tomatoes were pulverized in a coffee grinder (KRUPS, Mod GX410011V73). The physicochemical properties such as pH, total soluble solids (°Brix) and titratable acidity (% citric acid) were evaluated in dehydrated fruits according to the methods of the AOAC 918.12, 932.012 and 942.15, (AOAC, 2010).

Lycopene content

The content was analyzed according to Sadler *et al*. (1990), in 0.1 g of the dried product previously homogenized in 1 mL of water. To each sample were added 19 mL of the mixture hexane, acetone and ethanol (2:1:1) (v/v), vigorously shaken for 15 min and the

non-polar phase was collected. Finally, the absorbance was evaluated at 503 nm, and the concentrations were calculated according to the following expression:

$$L = \frac{Abs \times EC}{TS} \quad (2)$$

Where: L is the lycopene content (mg of lycopene/kg), Abs absorbance, and EC is the extinction coefficient (31.2), TS is the dried sample (g).

Total carotenoids

These compounds were evaluated according to the Lichtenthaler and Wellburn (1983) methodology. One sample was macerated with 5 mL of acetone (80% v/v), 2 g of calcium carbonate and 2 g of sea sand, and the mixture was centrifuged at 3500 rpm for 10 minutes. The absorbance was evaluated at 470, 645, and 662 nm, the acetone was used as blank, and the carotenoids contents were calculated with the following equations:

$$C_c = \frac{1000\ Abs_{470} - 2.27C_a - 81.4C_b}{227} \quad (3)$$

$$C_a = 11.75\ Abs_{662} - 2.35\ Abs_{645} \quad (4)$$

$$C_b = 18.61\ Abs_{645} - 3.96\ Abs_{662} \quad (5)$$

Where: Abs is the absorbance, C_a is chlorophyll a, C_b is chlorophyll b, and C_c is the content of carotenoids (μg of carotenoids / 100 g of sample).

Antioxidant activity (AA)

The AA was evaluated according to the methodology proposed by Mongkolsilp *et al.* (2004), by neutralizing the DPPH radical (1,1-diphenyl-2-picrylhydrazyl). The extracts were obtained with 0.5 mL of sample mixed with 3 mL of methanol (80% v/v), which were stirred at 125 rpm by 12 h at 40 °C. 2 mL of DPPH (0.1 mM) (Sigma-Aldrich, MO, USA) were added to 500 μL of extract and the mixture was stored by 30 minutes in dark conditions. The absorbance was measured at 517 nm using methanol as the blank. The AA was expressed in percentages of inhibition and was calculated using the following equation:

$$I = \frac{(Abs_b - Abs_s)}{Abs_s} \times 100 \quad (6)$$

Where: I is the% inhibition, Abs_b is absorbance of the blank, and Abs_s is the absorbance of the methanolic extract.

Percentages of retention

The results of the antioxidant compounds were expressed as percentages of retention of lycopene, carotenoids and antioxidant activity, which were calculated in the ratio of the concentrations of dehydrated fruits on the concentrations of fresh fruits * 100.

Statistical analysis

The analysis were performed by triplicate, the averages and ± SD were calculated. Likewise, the experimental data were analyzed through a randomized block experimental design, with the Minitab Statistical Software version 18.1 (Inc. All Reserved) program using ANOVA analysis and Tukey test means with a significance level of 0.05.

RESULTS AND DISCUSSION

Dehydration conditions

The fresh fruits with an approximate initial moisture content of 90 ± 0.30% were subjected to the dehydration processes. From the CD (70 °C with flow rates of 0.5, 1.0 and 1.5 m/s) conditions and according to Mariem *et al.*, 2014, the final moisture content of 15% was determined. While the fruits exposed to SD showed a moisture percentage of 17% with 67 °C and drying time of 7 h. These moisture contents were higher than those reported by Ghavidel and Davoodi (2010) with values of 5.9-6.9% (T 65 ± 2 °C and flow rate of 1 m/s) and 4.5-6.0% with CD and SD, respectively. The differences in moisture content with SD can be related to variations in drying temperature or the season to guarantee solar radiation.

Effect of the dehydration treatments on physicochemical properties

The highest pH values were obtained in the fruits exposed to CD with 70 °C and flow rate 1 m/s. While the tomatoes with SD were more acids with pH 3.91 and 4.02 in fruits previously stored at 7 and 22 °C, respectively (Table 1).

The quality of dehydrated fruits depends on many factors such as the tomato variety, the content of soluble solids (°Brix), the sizes and shapes of the fresh fruit segments subjected to dehydration treatments (Gallo *et al.*, 2010). While Coste *et al.* (2010) mention that T close to 50 °C during CD allows the preservation of product and increase the production of certain enzymes related to the sensory profile and level of acceptance of dried tomato. Specifically, the SD treatments increased the acidity of

the dehydrated products due to the partial fermentation that occurred in samples and the activity of the pectic enzymes in the first hours of the drying process (Okanlawon *et al.*, 2002). A similar trend was observed with the °Bx; this parameter was higher in the fruits submitted to CD. The drying conditions can produce a more significant loss of water, resulting in major levels of soluble solids in dehydrated tomato fruits (De Abreu *et al.*, 2014). While the lowest values of titratable acidity (TA) observed in stored fruits at 22 °C with CD at 70 °C are related to the decrease in the content of organic acids in tomatoes, which is due to the fact that these compounds are used as substrate in the processes of respiration of the fruit (Sanchez-Moreno *et al.*, 2006).

Concerning the color parameters of dehydrated fruits; the values of *L** reflected higher luminosity with tomatoes preserved at 22 °C and CD (flow rate of 1.5 m/s) and with SD, while an increase in darkness (decrease in the *L** value) was observed with CD and flow rate of 0.5 m/s (21.07 and 21.31).

The values of the parameters *a** and *b** increased in conditions of 7 °C and CD (1.5 m/s), the increase in the values of *b** indicate yellow colorations in the fruits, while the parameter *a** is an indicator of the red color (Brandt *et al.*, 2006).

Additionally, the values of ΔE (calculated in relation to the color parameters of the fresh fruit) were lower in the fruits with CD and with higher flow velocities (1.0 and 1.5 m/s) with both storage, while the results of the fruits subjected to solar dehydration (SD) did not differ from each other (P≥0.05).

Table 1. Physicochemical parameters of tomato fruit subjected to different storage temperature and treatments to dehydration by convection and solar.

Parameter	Convection drying			Solar drying
	70 °C/0.5 m/s	70 °C/1.0 m/s	70 °C/1.5 m/s	T 65 °C
Temperature of storage: 7 °C				
pH	4.29±0.09^{abc}	4.35±0.11^{a}	4.25±0.05^{abc}	3.91±0.20^{d}
°Brix	4.96±0.35^{ab}	5.36±0.92^{ab}	6.00±0.55^{a}	5.53±0.11^{ab}
AT%	0.48±0.01^{a}	0.50±0.02^{a}	0.47±0.03^{a}	0.49±0.01^{a}
*L**	21.31±1.24^{d}	28.81±0.22^{abc}	27.27±0.79^{c}	29.14±0.23^{ab}
*a**	13.56±0.16^{f}	23.52±0.49^{b}	25.80±0.65^{a}	21.61±0.14^{c}
*b**	7.91±0.79^{d}	12.57±0.49^{ab}	13.12±0.57^{a}	12.47±0.18^{ab}
ΔE	11.76±0.90^{a}	2.00±0.06^{c}	4.50±0.63^{b}	1.15±0.29^{c}
Temperature of storage: 22 °C				
pH	4.29±0.16^{ab}	3.99±0.06^{bcd}	3.97±0.03^{cd}	4.02±0.05^{bcd}
°Brix	4.23±0.32^{b}	4.90±0.34^{ab}	5.66±0.25^{a}	4.13±0.66^{b}
AT%	0.49±0.01^{a}	0.48±0.01^{a}	0.44±0.01^{a}	0.48±0.02^{a}
*L**	21.07±0.88^{d}	27.73±0.19^{bc}	29.77±0.18^{a}	27.45±0.11^{bc}
*a**	13.66±0.60^{f}	20.01±0.82^{d}	18.45±0.41^{e}	20.22±0.62^{cd}
*b**	7.26±0.40^{d}	10.27±0.86^{c}	11.44±0.14^{bc}	10.68±0.46^{c}
ΔE	10.16±0.98^{a}	1.79±0.67^{c}	1.29±0.11^{c}	1.88±0.29^{c}

Values reported as the average ± Standard Deviation (n=3). Equal letters do not present significant differences (P≥0.05).

Some processing conditions of tomato affect the color due to the formation of brown pigments; according to Okanlawon *et al.* (2002) the color change (red to dark red) might be due to Maillard reactions, which are caused by dehydration conditions. Even though for De Abreu *et al.* (2014), the darkening is a chemical process that relates the T, the dehydration time and the structure of the dehydrated material, generating changes in the sensory and nutritional quality of the dehydrated products. Another report mentions that the increases in the values of the *b** parameter indicate yellow colorations in the fruits, which can be favored in conditions of refrigeration and may be due to the synthesis of flavonoids such as quercetin (Luna-Guevara and Delgado-Alvarado, 2014). While, the parameter *a** is an indicator of the red color in fresh and processed tomatoes, and a determining factor in the quality and commercialization of the fruit (Juroszek *et al.*, 2009).

Effect of treatments on antioxidant properties

In this study, the Figure 1 shows the percentages of retention for carotenoids, lycopene, and AA of the fruits subjected to the different conservation treatments. Retention of carotenoids was higher in fruits stored at 7 °C and subjected to CD (air flow rates of 1.0 and 1.5 m/s) and SD with values of 83.85, 85.98 and 99.43%, respectively (Figure 1A).

Concerning the values of lycopene, these were higher in tomatoes stored at 22 °C, flow rate 0.5 m/s and SD conditions with results of 94.37 and 95.14% (Figure 1B). The results obtained with the retention percentages of the AA, were constant with the different storage

conditions and drying treatments, with values of 73.06 to 97.21% (Figure 1C).

According to Andritsos *et al*. (2003) and Azeez *et al*. (2019), the conditions of T 45-55 °C are recommended for the dehydration of tomato due to the better protection of antioxidant compounds and quality aspects such as color. Another study carried out by Montiel-Ventura *et al*. (2018), the fruits subjected to CD maintained the highest contents of lycopene under conditions of 40 °C and 50 °C with 540, 390 min.

The highest antioxidant potential of tomato is due to the content of phenolic compounds, ascorbic acid, and lycopene. However, several dehydration techniques used during the conservation of this fruit can significantly influence these compounds (Yahia *et al*., 2007). While, Veillet *et al*. (2009) considered that the AA of dehydrated tomatoes depends on some carotenoids that are released due to the degradation of cellular components during thermal processing. De Abreu *et al*. (2014) mentioned that the differences of the antioxidant properties vary due to the cultivar, process variables such as T, the incidence of light, and drying time.

Concerning the values of lycopene, these were higher in tomatoes stored at 22 °C, this temperature promotes the maturation of the fruit and increases the contents of this antioxidant compound (Giovanelli *et al*., 1999). In this study, the low flow rate of CD and SD conditions increased lycopene retention, according to Periago *et al*. (2007), the lycopene contents rise during CD, because of the rupture of tomato cells throughout dehydration inducing the availability of this antioxidant. However, Demiray et al. (2013) suggested that conditions of 70 to 80 °C can significantly affect the loss of lycopene, which can be related to the lower retention values obtained in this study with higher flow velocities (1.0 and 1.5 m/s). While Bechoff et al. (2010) consider that the stability of the carotenoids during the SD compared with the CD, is due to the UV radiation only affects the surface and does not penetrate the inner part of the tissue, preventing its degradation.

CONCLUSION

The results showed that storage and drying conditions influenced the physicochemical and functional properties. The processes of convection dehydration were more effective in preserving color specifically

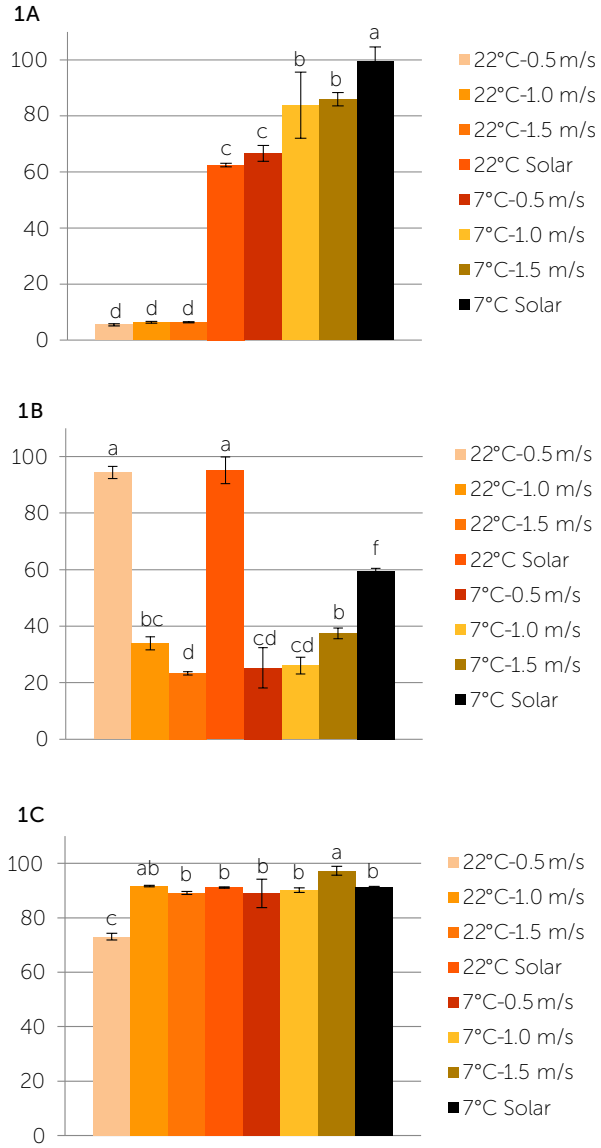

Figure 1. Percentages of retention of antioxidant compounds in tomato fruits subjected to dehydration by convection and solar dehydration. 1A) Total Carotenoids, 1B) Lycopene and 1C) Antioxidant Activity. Values reported as the mean ± standard deviation (n=3). Same letters do not present significant differences (P≥0.05).

with parameter a*, which is related to the red tones of dehydrated fruit. However, fruits stored at room temperature (22 °C) and subjected to solar dehydration presented the highest percentages of lycopene, this being the main antioxidant of tomato fruit. Finally, the proposed storage conditions and solar dehydration treatments can be a viable alternative for conservation and commercialization for tomato farmers in rural communities.

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Unconventional plants as a source of phytochemicals for broiler chicken

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ABSTRACT

Objective: To carry out a review to know the modes of action and the commercial application of unconventional plants as growth promoters in broiler chicken production.

Design/Methodology/Approach: A bibliographic review of unconventional and commonly used plants was carried out, which have shown efficacy as growth promoters on broiler chickens.

Results: Numerous reports have demonstrated the efficacy of phytochemicals present in plants as antioxidants, antimicrobials, and immune-stimulants.

Limitations of the study/Implications: Alternative use of unconventional plants can help to develop sustainable production systems and the production of innocuous meat products.

Findings/Conclusions: The use of additives of natural origin in poultry feed represents a viable option to replace or reduce the use of antibiotics and growth promoters on broiler chickens.

Keywords: Phytochemicals, secondary metabolites.

INTRODUCTION

Bacterial resistance by the use of antibiotics as growth promoters has caused the search for alternatives to develop sustainable production systems for meat products that consider the welfare of the consumers of those products and the animals from which products are obtained (Lillehoj and Lee, 2012). The use of herbal products is an antimicrobial alternative and promoter of animal growth due to their contents of secondary metabolites (Dhama *et al*., 2015).

The herbs begin to be used in the poultry industry as promoters of growth in the fight against various infections for their potential as alternatives to antibiotics and their content of bioactive substances that could improve production parameters attributed to some phytochemicals which may favorably modify the metabolism of the animal (Dhama

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et al., 2015; Vinus *et al*., 2018). However, additional complications arise because herbal additives for animal feed can vary widely by agro-climatic origin, processing and composition, when the additive is an extract or an essential oil (Hashemi and Davoodi, 2010). The objective of this review was to provide an overview of recent knowledge about the use of some unconventional plants as a source of phytochemicals for feeding broiler chickens, excluding the use of extracts and essential oils.

Unconventional plants as sources of phytochemicals

Sweet wormwood (*Artemisia annual*). Active compounds: artemisinin, flavonoids, phenols, purines and lipids (Brisibe *et al*., 2009). Effect: 2 to 4% of the diet as anti-parasite, antioxidant (Cherian *et al*., 2013), coccidiostats (Brisibe *et al*., 2009), reduces the count of enterobacteria and increases the content of lactobacilli in the intestine (Panaite *et al*., 2018; 2%).

Garlic (*Allium sativum*). Active compounds: ajoene, allyl cysteine, diallyl sulfide and allicin. Effect: the inclusion of up to 3% garlic improves weight gain, feed conversion, decreases the mortality rate (Makwana *et al*., 2015; Karangiya *et al*., 2016; Patel *et al*., 2017; Belal *et al*., 2018). It has antimicrobial properties, increases the quantity of antibodies, decreases the concentration of triglycerides, cholesterol, low-density lipoproteins and increases the levels of high-density lipoproteins (Toghyani *et al*., 2011; Faghani *et al*., 2014).

Anise (*Pimpinella anisum*). Active components: The seed contains trans-anethole eugenol, methyl-chavicol, anisaldehyde, estragole, coumarins, scopoletin, umbelliferone, estroles, terpene hydrocarbons, polyenes and polyacetylenes (Barakat *et al*., 2016). Effect: the inclusion of anise seed in the feed improves weight gain, feed consumption and feed conversion (Yazdi *et al*., 2014; Mahmood *et al*., 2014a; Barakat *et al*., 2016), increases the quantity of antibodies against avian influenza virus (10 g kg^{-1} of feed; Yazdi *et al*., 2014), Gumboro and Newcastle (1 g kg^{-1} of feed; Mahmood *et al*., 2014b) and increases the amount of immunoglobulins [(5-15 g kg^{-1} of feed; Barakat *et al*., 2016); (0.5-1 g L^{-1} of water; Al-Shammari *et al*., 2017)].

Cinnamon (*Cinnamomum zeylanicum*). Active compounds: cinnamaldehyde, 2-hydroxycinnamaldehyde, cinnamyl acetate, coumarin, eugenol and caryophyllin (Saeed *et al*., 2018). Effects: improves weight gain, feeding efficiency, digestion, intestinal microflora activity and immune response (Toghyani *et al*., 2011; Singh *et al*., 2014; Faghani *et al*., 2014; Saeed *et al*., 2018). It decreases the plasma concentration of cholesterol, triglycerides, low-density lipoproteins and increases high-density lipoproteins (Faghani *et al*., 2014). Antioxidant and anti-inflammatory activity (Bansode, 2012). Dose: 0.02 to 7%.

Coriander (*Coriandrum sativum*). Active components: essential oil, tannins, terpenoids, reducing sugars, alkaloids, phenolic compounds, flavonoids, fatty acids, sterols and glycosides (Hosseinzadeh *et al*., 2014). Effect: coriander seeds (1 to 3% in the diet) improve weight gain, feed conversion (Al-Jaff, 2011; Saeid and Al-Nasry, 2010; Abou-Elkhair *et al*., 2014), stimulate the immune system (Hosseinzadeh *et al*., 2014), decreases cholesterol, glucose, low-density lipoproteins and high-density lipoproteins (Al-Jaff, 2011).

Chile (*Capsicum annuum*). Active compounds: capsaicin, capsisin and capsanthin. Effects: antioxidant capacity (Thiamhirunsopit *et al*., 2014), increases weight gain, feed consumption and reduces feed conversion, information obtained by evaluating doses between 0.25-1.7 % (Al-Kassie *et al*., 2011a; Thiamhirunsopit *et al*., 2014; Puvaca *et al*., 2015). It strengthens the immune system of poultry (Al-Kassie *et al*., 2011a; 0.25-1.00%) and is effective against Salmonella infection (Alaa, 2010; 1-2%). Chili pepper decreases the blood concentration of cholesterol (Alaa, 2010; Al-Kassie *et al*., 2011a; Puvaca *et al*., 2015), triglycerides, low-density lipoproteins, increases the concentration of high-density lipoproteins (Puvaca *et al*., 2015), and total serum protein (Alaa, 2010).

Black cumin (*Nigella sativa*). Active compounds: alkaloids, essential oils, imoquinone, dithymoquinone, thymol, carvacrol, nigelycine, nigelidin and hedrin (Azeem *et al*., 2014). Effects: viable alternative to replace the use of antibiotics, growth promoter, antioxidant and immune-regulator (Azeem *et al*., 2014; Kumar and Patra, 2017). Black cumin increases the quantity of antibodies against Newcastle, Gumboro and Bronchitis (Durrani *et al*., 2007; Kumar and Patra, 2017). The addition of cumin seeds to the diet causes greater weight gain, feed consumption and better feed conversion [Durrani *et al*., 2007 (4%); Kumar and Patra, 2017 (0.5-2.0%)]. In addition, adding 0.4% to the diet reduces the concentration of triglycerides and cholesterol (Kumar and Patra, 2017).

Turmeric (*Curcuma longa*). Active compounds: curcumin, cinnamic acid, curlone and niacin (Guil-Guerrero *et al.*, 2017). Effects: antioxidant (Guil-Guerrero *et al.*, 2017), improves the immune response (Nayaka *et al.*, 2012) of chickens infected with Newcastle (Guil-Guerrero *et al.*, 2017) and Eimeria (Lee *et al.*, 2010). Turmeric powder supplementation improves weight gain and feed conversion (0.5%) (Al-Sultan, 2003; Abou-Elkhair *et al.*, 2014), and decreases blood triglycerides (Nouzarian *et al.*, 2011).

Eucalyptus (*Eucalyptus globulus*). Active component: terpene-eucalyptol and tannins (Farhadi *et al.*, 2017). Effects: *Eucalyptus* leaves improved productive behavior, it is also associated with the manipulation of the intestinal microbiota and better immunity (Hassan *et al.*, 2011; Farhadi *et al.*, 2017).

Ginger (*Zingiber officinale*). Active compounds: gingerdiol, gingerol, gingerdione and phenolic compounds. Effects: antioxidant properties (Zhang *et al.*, 2009). The addition of 0.02 to 1.5% to the feed improves weight gain, feed conversion, reduces mortality in chickens (Oleforuh-Okoleh *et al.*, 2014; Youssef *et al.*, 2016; Belal *et al.*, 2018) and stimulates the immune system (Valiollahi *et al.*, 2014).

Laurel (*Laurus nobilis*). Active compounds: cineol, eugenol, acetyl and methyl eugenol, α- and β-pinene, felandrene, linalool, geraniol and terpineol (Zekovic *et al.*, 2009). Effect: by using 2 to 6 g kg^{-1} of bay leaves in feed, the total of bacteria and aerobic bacteria in the colon is inhibited (Nafea *et al.*, 2018).

Moringa (*Moringa oleifera*). Active compounds: polyphenols, vanillic-, ferulic-, melilotic- acids, vitamins A, E, C and complex B. Effects: alternative source of protein in diets at levels of 50 to 140 g kg^{-1} of leaves in the diet replacing the soybean meal (*Glycine max* L.), finding as a result greater weight gain and feed conversion (Melesse *et al.*, 2013). The inclusion of moringa leaves (1, 2, 5, 10 and 15%) in the diet reduced abdominal fat, increased omega 3 and 6 fatty acids, improving meat color, attributed to greater oxidative stability (Cui *et al.*, 2018).

Neem (*Azadirachta indica*). Active compounds: triterpenoids (azadirachtin, nimbine, salanine, meliacin). Effects: by supplementing 0.25% of Neem leaves, weight gain increase, and it improves feed conversion; it also has antimicrobial, antiviral and antifungal action (Ansari *et al.*, 2012).

Black pepper (*Piper nigrum*). Active compounds: glutathione peroxidase, glucose-6-phosphate dehydrogenase, vitamin C, curcurmin and piperine (Khalaf *et al.*, 2008). Effects: the addition in the diet (0.02 to 1.0%) improves weight gain, feed intake and feed conversion (Al-Kassie *et al.*, 2011b; Shahverdi *et al.*, 2013; Valiollahi *et al.*, 2014; Abou–Elkhair *et al.*, 2014). Black pepper improves the immune system through increasing the concentration of immunoglobulins in serum (Al-Kassie *et al.*, 2011b; Abou-Elkhair *et al.*, 2014; Valiollahi *et al.*, 2014); also, it decreases the blood concentration of cholesterol, triglycerides, low-density lipoproteins and increases the concentration of high-density lipoproteins (Al-Kassie *et al.*, 2011b; Shahverdi *et al.*, 2013; Puvaca *et al.*, 2015; Singh *et al.*, 2018).

Green tea (*Camellia sinensis*). Active compounds: catechins, flavonoids with antioxidant activity (Farahat *et al.*, 2016). Effect: the addition of green tea leaves (0.5 to 2% in the diet) decreases the plasma cholesterol level (Yang *et al.*, 2003), improves the immune response (Wang *et al.*, 2018) to coccidiosis (Jang *et al.*, 2007) and to H9N2 influenza (Lee *et al.*, 2012) and Newcastle (Farahat *et al.*, 2016) viruses.

CONCLUSION

There are several products of herbal origin that can be used in the production of broiler chickens as growth promoters, natural antibiotics and antivirals, as well as alternative sources of antioxidants, all of which offers a viable alternative to partially or totally replace antibiotics in the diet.

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Arbuscular mycorrhizal inoculation in *Citrus volkameriana* Tan & Pasq

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ABSTRACT

Objective. We evaluated the effect of arbuscular mycorrhizal inoculation in *Citrus volkameriana* Tan & Pasq plants with the application of *Glomus* spp. Zac- 19 (*G. claroides*, *G. diaphanum* and *G. albidum*) to assess growth under greenhouse conditions without fertilizer application.

Methodology. The evaluated treatments were inoculation with 2,4,6,8, and 10 g of inoculum, plus a control without inoculation in an experimental design of complete randomized blocks with three replications. Variables were plant height, stem diameter, number of leaves, foliar area, dry weight, and mycorrhizal colonization.

Results. A statistically significant response ($p \leq 0.01$) to inoculation was observed, registering higher growth of mycorrhizal plants. The inoculation was considered appropriate for all the levels evaluated. The outstanding treatment was 10 g of inoculum that produced plants with 110.16 cm in height for the last sampling; 38.56 leaves per plant; 1.13 cm stem diameter; 35.95 g dry weight of root; 76.88 g dry weight of the aerial part of the plant; 225.03 cm^2 of leaf area per plant and 88.87% of mycorrhizal colonization.

Conclusions. The application of 10 g per plant to the roots of *Citrus volkameriana* from the *Glomus* Zac-19 arbuscular mycorrhizal consortium promoted the production of more vigorous plants for grafting, without the application of chemical fertilizer.

Keywords: Microorganisms, Glomus spp Zac- 19 consortium

INTRODUCTION

In the state of Campeche, Mexico, it is estimated that there are 7220 ha of commercial orchards and 2101 ha of backyard orchards in citrus reconversion with the use of patterns resistant to the citrus quick decline disease virus (Citrus Tristeza virus, in Spanish VTC). The sour orange tree (*Citrus aurantium*) had been the most used grafting rootstock until the appearance of the Tristeza virus, which has led to the search for other rootstocks, such as the lemon tree *Citrus volkameriana* Tan & Tasq, which is tolerant to *Phytophtora parasitica* and VTC, tolerant to calcareous soils such as those existing in the Yucatán peninsula, Mexico (Cruz and de la Garza, 2003). Given the need to have quality rootstocks produced in the nursery, the possibility of

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using arbuscular mycorrhizal fungi (in Spanish HMA) arises because they establish a symbiotic relationship with plants (Smith and Read, 2008). Their main effect is the increased efficiency in the absorption of nutrients by the plant due to the extra radical hyphae. Many researchers have recorded an increase in the absorption of N, P, by plants and increasing in their growth (Zaidi *et al*., 2003). In addition, there is evidence that mycorrhizae-enhanced citrus plants have better growth compared to non-mycorrhizae plants (Srivastava *et al*., 2002). Thus, it becomes important the mycorrhizal effect on the production of citrus plants in nurseries; as the initial part of the citrus production process, when they will be grafted and afterwards, taken to the field. The effectiveness of HMA in the production of citrus seedlings has been demonstrated in several studies (Alarcon and Ferrera-Cerrato, 1999). Effects of mycorrhizal inoculation on *Citrus volkameriana* rootstocks were found by Haghighatnia *et al*., (2011) when registering higher growth in plants, expressed in height, stem diameter, number of leaves, dry weight and nutrient content. Authors such as González-Chávez and Ferrera-Cerrato (2000) with the use of phosphoric rock as fertilizer in sour orange tree and *Glomus* spp Zac-19 (*G. claroides*, *G. diaphanum* and *G. albidum*), registered increases in seedlings growth from 135 days after transplantation (in Spanish, ddt); the height of the inoculated plants was three times greater than the height of uninoculate plants. In a more recent study in sour orange plants (*C. aurantifolia*) inoculated with different species of mycorrhiza, Orton and Ustuner (2014) observed that after ten months the mycorrhizal plants showed increments in height, number of leaves and dry weight. Others like Navarro *et al*. (2015) obtained effects by mycorrhizal inoculation, in plant height and fresh weight of *C. macrophyla*. Similar results were obtained by Qiang-Sheng and Ying-Ning (2015) in *Citrus tangerine* with *Glomus mosseae* in plant height, stem diameter, plant dry weight and photosynthetic index. The HMA inoculation in *Citrus volkameriana* Tan & Pasq seedlings showed plants 60 cm in height and 5 mm in diameter at the base of the stem at 120 days without the addition of fertilizer or fungicides. This size was considered sufficient to perform the graft (Quisehuatl *et al*., 2015). Based on the above, this study aimed to evaluate the effect of mycorrhizal inoculation with the *Glomus* spp Zac-19 consortium on the growth of the *Citrus volkameriana* Tan & Pasq. rootstock.

MATERIALS AND METHODS

The experiment was established in the nursery of the Secretaría de Desarrollo Rural del gobierno del estado de Campeche, Mexico, using clay soil, which was previously sterilized. Certified seed of *Citrus volkameriana* Tan & Pasq from Cuba, which was pretreated by applying an immersion bath in neutral water for 48 h for pre-germination and avoiding the use of vain seed. Before sowing the seeds were disinfected with 5% sodium hypochlorite for 7 min, and then rinsed with neutral water. The seeds were sown in 200-cavity plastic trays, which were given the same seed cleaning treatment. Peat moss was used as substrate and sowing was carried out on January 10, depositing a seed in each cavity of the trays at a depth of 2 cm. Subsequently, the trays were covered with black plastic in order to homogenize and accelerate germination, placing them in a cool and dry place. On the fifth day after sowing (in Spanish, dds), the black plastic was removed and the trays were arranged in terraces, waiting for the total emergence of the seedlings, an event that began at 15 dds and lasted eight days.

The transplant substrate was a mixture of pure forest soil, which was sterilized with a 456 g (1 lb) can of methyl bromide. After 7 d of fumigation and an aeration period of 3 d, the substrate was used to fill polyethylene nursery bags with a volume of 2.1 kg. On March 14 (63 dds) plants were transplanted to black polyethylene bags, also inoculating the roots of the plants with the *Glomus* spp Zac-19 consortium, with approximately 100 spores in the root of alfalfa (*Medicago sativa* L.) colonized in 72%. The plants were extracted from the trays, and a small blow was given to extract the root ball, contacting the root zone of each plant with the inoculum in the dose corresponding to each treatment.

The inoculum that did not adhere to the root was applied directly to the bottom of the bag. Then, each bag was filled with the forest soil used for transplanting the plants. Once the inoculation and transplantation were finished, the plants were irrigated. The treatments were defined by the use of five doses of mycorrhizal inoculation with the *Glomus* spp Zac-19 consortium: 2, 4, 6 8 and 10 g, plus an uninoculated control, in total six treatments, arranged in an experimental design of complete randomized blocks, with three replications. The experimental unit consisted of a group of 25 plants, with a separation of 1 m between units. The treatments were

randomly assigned to each experimental unit within each block, with a separation between them of 1 m. Within each experimental unit, 16 plants were sampled throughout the term of the research. Evaluated variables were: plant height, stem diameter, number of leaves, foliar area (integrator of foliar area LI-COR model li-3100), dry weight of root, dry weight of the aerial part of the plant, and percentage of mycorrhizal colonization (Philips and Hayman, 1970). The statistical analysis of the results was carried out by means of an analysis of variance and Tukey's mean comparison test (α=0.05), with the use of the statistical program Statistical Analysis System (SAS).

RESULTS AND DISCUSSION

Plant height and stem diameter

Growth in plants of *Citrus volkameriana* was attributed to the effect of the arbuscular mycorrhizal inoculation with *Glomus* spp Zac-19, observed effect from 60 d after transplantation (ddt) in plant height, stem diameter, number of leaves per plant and leaf area (data not shown). The height of the *Citrus volkameriana* plants inoculated with 10 g of the inoculum showed larger plants from 60 ddt and up to 240 ddt, which was attributed to better plant nutrition, because these fungi improve the availability of nutrients for the plant, specially nitrogen and phosphorus (Table 1). Mycorrhizal plants showed more vigorous plants and uniform green coloration. The magnitude of the difference in the plant height for the best treatment (10 g), compared to the control treatment was 78.66 cm, with a final growth per day for the treatment of 10 g of inoculum in the order of 2.62 cm.

These results coincide with those found by Alarcón and Ferrera-Cerrato (2003) who found significant effects in this variable due to the effect of mycorrhizal inoculation in *Citrus volkameriana* Tan & Pasq, considering that HMA are nutritional enhancers (nitrogen and phosphorus), since they favor the concentration and synthesis of amino acids. The results found also coincide with what was observed by Dixon *et al*. (1989) because in their work with *Glomus fasciculatum* and *G. etuunicatum* in *Citrus janbhiri*, they observed that the use of mycorrhization caused greater growth in inoculated plants. The effect of mycorrhization in nursery plants, on plant height has been shown in various species; thus Ruiz *et al*. (2016) registered taller plants of papaya (*Carica papaya* L.), guava (*Psidium juagava* L.) and avocado (*Persea* sp.), when inoculated with different strains of arbuscular mycorrhizal fungi in two types of soil.

Results similar to those found for plant height were found for stem diameter, where the best treatment was 10 g, finding that eight months after transplantation (in November), the average diameter in the inoculated plants was 1.03 cm, while in the control plants the average diameter per plant was 0.45 cm, a difference that resulted in vigorous *C. volkameriana* plants (Table 1).

Obtained results agree with that established by Antunes and Cardoso (1991), who report that the diameter of the stem required in plants for grafting is 5 mm after six months after transplantation, a diameter exceeded in *Citrus volkameriana* plants with any of treatments with inoculation. The results found match those observed by Alarcon and Ferrera-Cerrato (2003) in *C. volkameriana* plus the inoculation with *Glomus* spp, Zac-19 to obtain at 210 ddt plants with 95% more diameter than the non-inoculated ones (45 mm). The above suggests that mycorrhizal symbiosis has a favorable

Table 1. Average height (cm) and diameter (cm) of *Citrus volkameriana* Tan & Pasq. Plants, due to the effect of mycorrhizal inoculation with *Glomus* spp. Zac-19.

Inoculum (g)	Days after transplant							
	150		180		210		240	
	AP	DT	AP	DT	AP	DT	AP	DT
0	13.743d	0.18b	21.85d	0.27c	27.81c	0.40c	29.78c	0.45c
2	21.35c	0.57a	33.86c	0.74b	60.93b	0.92ab	77.79b	1.02b
4	22.62c	0.57a	34.67c	0.74b	60.89b	0.92ab	78.84b	1.02b
6	30.99ab	0.57a	60.93a	0.73b	76.76a	0.99ab	94.10a	1.04ab
8	27.38b	0.60a	49.47b	0.69b	69.54ab	0.87b	100.34a	0.98b
10	32.02a	0.62a	61.76a	0.82a	77.75a	1.03a	102.34a	1.13a
DMSH	4.50	0.09	10.42	0.07	11.39	0.13	10.44	0.10

Treatments with the same letter are statistically equal (Tukey, α=0.05).

effect on obtaining healthy and vigorous plants in less time to graft them. Additionally, obtaining transplant plants with a larger stem diameter will offer advantages in their management and transfer from the nursery to the orchard: structurally stronger plants, capable of withstanding adverse environmental conditions, such as the action of winds and competition with other plant species for water and light.

Number of leaves and dry weight

Vegetative growth of *C. volkameriana* was favored by inoculation with *Glomus* spp. Zac-19 (Table 2). All the treatments with inoculation exceeded the average number of leaves to the treatment without inoculation in all the samplings. At 240 d, it was observed that the plants inoculated with 10 g obtained up to 77.45% higher in number of leaves compared to the control. In evaluations with different types of mycorrhizae and soil types, it was found that the inoculated plants presented 56.25% more leaves, compared to the treatment without inoculation (Ortas and Ustumer, 2014), a value lower than that found in the conditions of our research. This implies a production of citrus plants in the nursery with greater growth and vigor due to the effect of mycorrhizal inoculation, a result of the benefits attributed to a better absorption of nutrients by the plant. Pimienta-Barrios *et al*. (2009) complement this by pointing out an increase in photosynthesis, which is expressed as a better establishment in the field, thus representing an adaptive advantage.

The mycorrhizal plants had higher dry and fresh leaves weight compared to plants not inoculated, and a similar tendency was observed for the total leaf area (Table 2). On average, the inoculated plants presented 13.88 g of root dry weight and 49.12 g of dry weight of the aerial part of the plant, higher values compared to not inoculated plants of 12.26 and 10.80 g, respectively. The outstanding treatment for its effect on the dry weight of the plant and the foliar area was the mycorrhizal inoculation with 10 g of inoculant per plant, which was related to presenting plants with greater height, number of leaves and foliar area compared to the control treatment.

The higher value of fresh and dry weight of the plants is attributed to a greater assimilation of nutrients by the plants, an indirect measure of their photosynthetic activity. At evaluations with the mycorrhizal consortium *Glomus* spp. Zac-19 on *Citrus volkameriana*, Alarcon and Ferrera-Cerrato (2003) found a significant effect for dry weight on inoculated plants. Similar effects were also found by Ruiz *et al*. (2016) when evaluating six strains of HMA, in avocado, guava and papaya, where the inoculated plants showed higher fresh and dry weight compared with plants without inoculation. The use of mycorrhizal fungi to produce nursery plants should be considered as a practice to use by the plant producer, since these microorganisms deserve special attention for their positive effect on plant growth by increasing the radical absorption efficiency of nutrients (Viera *et al*., 2017). The root dry weight/dry weight ratio of the aerial part of the plants, for all the amounts of inoculum used, was on average lower in the inoculated plants (0.43) than in the uninoculated plants (1.13). These are similar results to those found in *Capsicum* by Aguilera and Gomez *et al*. (1999). These results indicate a greater distribution of carbon from the root towards the areal portion of the plant, meaning a greater foliar expression. Regarding leaf area, its increased expression in plants *C. volkameriana* was attributed to the effect of mycorrhizal inoculation, due to the fact that all the treatments inoculated presented higher average values of leaf area (190.89), compared to the control (92.51 cm^2). The increase in biomass from the expanded leaf area should be considered as a

Table 2. Average number of leaves per plant, dry weight of root and dry weight of stem and leaves per plant of inoculated plants of *Citrus volkameriana* Tan & Pasq. due to the effect of mycorrhizal inoculation with *Glomus* spp. Zac-19.

Inoculum (g)	Number of sheets (dat)				Dry weight (g)		
	150	180	210	240	raíz	Tallo y hojas	Área foliar
0	16.42d	18.17e	19.56d	21.73c	12.26c	10.80d	92.51 e
2	26.63b	24.96d	32.33bc	34.95ab	22.54b	43.81c	171.28d
4	26.48b	29.85b	34.96ab	36.97a	22.60b	56.06 b	176.43cd
6	26.43b	33.58a	35.36ab	37.31a	24.20b	61.07b	185.22bc
8	21.56c	27.41c	29.52c	32.52b	25.45b	61.78b	196.50b
10	31.76a	33.69a	36.48a	38.56a	35.97a	76.88a	225.03a
DMSH	4.42	2.24	3.23	4.07	6.16	9.87	11.30

source of photo assimilate production (Cookson *et al*., 2005). Therefore, it is a critical variable for productivity.

Total mycorrhizal colonization, colonization per vesicle, number of colonized segments and number of segments per vesicle

The fundamental aspect in all mycorrhizal inoculation study consists in determining whether the inoculated fungi establish the symbiotic association with the roots of the plants. According to the results obtained, it can be established that there are highly significant statistical differences for the variables Total colonization, Colonization per vesicle (Figure 1). It is possible to say that *C. volcameriana* is satisfactorily colonized by the *Glomus* spp Zac-19 consortium. Our percentages of mycorrhization were very good, if the report by González-Chávez and Ferrera-Cerrato (2000) is taken as a reference, from their research with sour orange, where they obtained maximum values close to 60%. These results indicate that the roots inoculation of *C. volkameriana* with 10 g of inoculum of the mycorrhizal consortium Zac-19, gave as result the root-fungus symbiosis, as it is shown from the values of total colonization and colonization per vesicle, in the order of 88.87% and 88.79%, respectively. There are higher values compared to those reported for other horticultural, fruit and forestry species such as: Tree tomato (*Solanum betaceum* Cav) 22.8% (Viera *et al*., 2017); grasses *Brachiaria brizantha* cv. Toledo, *B. dictyoneura* cv. Llanero, *Desmodium ovalifolium* cv. Maquenque, *Panicum maximum* (CIAT 36000) and *Paspalum notatum*, 47 A 94% (Monroy *et al*., 2013); blueberry 9.8-15% (Bautista *et al*., (2017). In *Citrus aureantium* (Ortas and Ustuner (2014) when working with different types of soil and mycorrhiza species, they found colonization percentages from 17.33 to 50.34%, and that are lower values than 86.27% as the total colonization found in our research. Results agree with those obtained by Gonzalez-Chavez and Ferrera-Cerrato (2000), who in their research with two citrus species, they found colonization percentages from 37 to 64%. The results obtained allow establishing affinity between the *Glomus* spp Zac-19 consortium and the species evaluated in our study, suggesting that this species is susceptible to colonization by this type of endophytes, which induces a response in the growth of the inoculated lemon plants (Figure 1).

The high percentages of total colonization in the roots of the inoculated plants are expressed as a better response in variables such as plant height, number of leaves per plant, stem diameter, dry weight and foliar area, which allows us to assume that the symbiotic association with mycorrhizal fungi resulted in favorable changes on growth rates and produced biomass.

CONCLUSIONS

The management practice of mycorrhizal inoculation to the root system of *Citrus volkameriana* Tan & Pasq. plants at the time of transplantation is important because the plants presented advantages in growth compared to non-mycorrhizal ones. This gave advantages to those plants for their establishment in the orchard, in addition to a reduction in cultivation costs due to the effect of natural fertilization.

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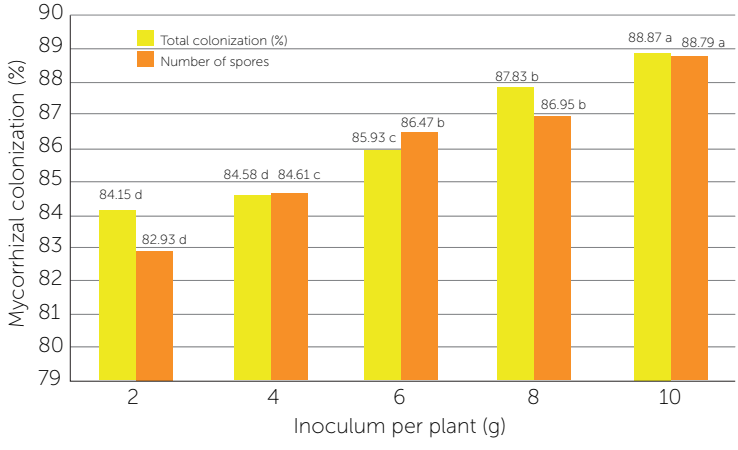

Figure 1. DMSH: 0.94 and 1.32 for total colonization and vesicle colonization, respectively. Treatments with the same letter are statistically equivalent. (Tukey, α=0.05)

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Phenology and quality of habanero pepper fruits (*Capsicum chinense* Jacq.) due to nutrient solution in hydroponics

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ABSTRACT

Objective: To evaluate the phenological development and fruit quality of habanero pepper (*Capsicum chinense* Jacq.) with three nutrient solutions.

Design/Methodology/Approach: In a hydroponic system the nutrient solutions of Steiner, Hoagland and Soria were used to evaluate the phenology, morphology and pungency (spicy-hotness) of the habanero pepper fruits.

Results: It was observed that the nutrient solution of Soria brought flowering 4 d ahead, but it delayed fruiting by 2 d compared to the other treatments. Steiner solution incremented length (4 cm), diameter (2.9 cm), weight (8 g) and moisture content (89%) of the fruit compared to the other nutrient solutions. Capsaicin and dihydrocapsaicin increased in plants watered with Steiner nutrient solutions (24 and 12.5 mg g^{-1}, respectively) and Soria (23 and 12.7 mg g^{-1}, respectively), as well as Scoville units (>538,000).

Study limitations/Implications: Although favorable results were found in some treatments, more studies are required to evaluate the nutrient composition of the different organs of habanero pepper plants, by phenological stages.

Findings/Conclusions: Considering phenology, fruit quality and capsaicinoids content, the Steiner nutrient solution could be a suitable option for cultivating habanero peppers in hydroponic systems.

Keywords: capsaicinoids, flowering, pungency (spicy-hotness).

INTRODUCTION

Applying the appropriate amount of nutrients to plants allows them to perform their basic metabolic functions (Nieves-González *et al*., 2015). In many cases the fertilization of a crop is based on the empirical knowledge of farming producers (Sonneveld and Voogt, 2009) and not on the requirement of the cultivated species. An alternative for the production of vegetables are hydroponic systems, which use balanced nutrient solutions that allow water and nutrients to be efficiently absorbed by the plants (Juárez *et al*., 2006). However, in vegetables such as the habanero pepper (*Capsicum chinense* Jacq.), studies on the use of nutrient solutions in hydroponic systems are scarce (López-Gómez *et al*., 2017).

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The habanero pepper is a crop of great economic importance due to its spicy fruits (Ruíz-Lau *et al*., 2011). Flowering and fruiting depend on strong amounts of macronutrients (N, P, Ca, K and Mg) to produce flowers and fruits (Prado, 2006). Competition for nutrient elements and the production of photoassimilates can modify the phenology of the habanero pepper (Meneses-Lazo *et al*., 2018). Likewise, nutrition influences the size and quality of pepper fruits in hydroponic systems (López-Gómez *et al*., 2017), as well as the capsaicinoids content (Medina-Lara *et al*., 2008). Therefore, the objective of this work was to evaluate the phenological development and quality of the habanero pepper fruits (*Capsicum chinense* Jacq.) responding to different nutrient solutions in a hydroponic system.

MATERIALS AND METHODS

This research was carried out in a greenhouse of the Technological Institute of Conkal. Seeds of habanero pepper (*Capsicum chinense* Jacq.) Orange variety (Geneseeds, Jalisco, Mexico) were sown in 200-cavity polystyrene trays, Canadian moss (SunGro Horticulture, Massachusetts, USA) was used as substrate. At 20 days after sowing (in Spanish, dds) foliar fertilization began (19:19:19 of N:P:K, 1.0 g L^{-1}, twice a week). At 50 dds, the transplant was carried out in 15 L pots and a mixture of tezontle (inert volcanic gravel) and coconut fiber (*Cocos nucifera* L.) was used as the substrate, in a 3:2 v/v ratio, previously steam sterilized. The agronomic management of the crop was that recommended by Soria *et al*. (2002).

Nutrient solutions

The nutrient solutions of Steiner (1984), Hoagland and Arnon (1950), and Soria *et al*. (2002) were used, and each solution was a treatment. The Steiner (T1) and Hoagland (T2) nutrient solutions were chosen because they are balanced universal solutions that include all the essential macronutrients (N, P, K, Ca, Mg, S), while the solution of Soria *et al*. (2002) was chosen as the control (T3) because it is of the customary use in Yucatán, México by pepper and habanero producers. However, the sources of N and P for this solution were modified. In the place of urea (46% N), ammonium nitrate (33% N) was used to avoid microorganisms which convert urea to ammonium; and, instead of phosphoric acid, monopotassium phosphate was used (to avoid a very acidic pH).

Thus, Steiner solution was formulated with: 9, 3, 4, 3 and 1 meq L^{-1} of $Ca(NO_3)_2$, KNO_3, $MgSO_4$, K_2SO_4, KH_2PO_4, respectively. Hoagland & Amon's with 1, 8, 6, 4, and 1 meq L^{-1} of NH_4NO_3, $Ca(NO_3)_2$, KNO_3, $MgSO_4$, KH_2PO_4, respectively. And that of Soria was formulated according to the requirements of four pepper phenological stages: transplant (7.9, 4.9 and 2. 6 meq L^{-1} de NH_4NO_3, KH_2PO_4 y KNO_3, respectively); growth and flowering (2.7, 1.2 and 2.5 meq L^{-1} de NH_4NO_3, KH_2PO_4 y KNO_3, respectively); fruiting (0.7 and 1.2 meq L^{-1} de NH_4NO_3 y KH_2PO_4, respectively); and production (0.3 and 1.4 meq L^{-1} de KH_2PO_4 y KNO_3, respectively). Micronutrients (30 mg L^{-1}) were added to the three nutrient solutions (treatments).

Reproductive phenology and fruit morphology

The time to flowering and fruiting was calculated, a stage was considered as flowering or fruiting when 50% + 1 of the evaluated plants presented flowers or fruits (Garruña-Hernández *et al*., 2012). The fruits were weighed with an analytical balance and the pericarp thickness, length and diameter of the fruit were determined with a Vernier caliper.

Ash content and moisture percentage in fruits

To determine the ash content, the fruits were weighed fresh, dried in an oven at 60 °C for 5 d, and ground until obtaining fine powder. In addition, crucibles were placed in an oven at 150 °C for 4 h, then they were placed in a drying hood to lower the temperature and the weight of the empty crucible was determined, in each crucible 1 g of dried fruit powder was placed and they were put in a muffle furnace at 600 °C for 5 h to obtain the weight of crucible with ash. To determine the weight of ash, to the weight of the crucible with ash was subtracted the weight of the empty crucible. To determine the percentage of ash, the ash weight was divided by the sample weight in grams and multiplied by one hundred.

Capsaicinoid content

For determination of capsaicinoids the fruits were placed in an oven at 60 °C for 5 d, ground, and 1 g of dried and powdered fruit was weighed and placed in a 250-mL flask. Three replicates were generated by treatment. To each flask, 25 mL of acetonitrile were added and placed in a shaker at 60 °C for 4 h in the dark, with stirring at intervals of 20 min. Subsequently, the samples were filtered and the filtrate obtained was placed in flasks that were made up to 25 mL with acetonitrile. The samples were homogenized and 2 mL were placed in dark vials for reading on high resolution liquid chromatography. The data were expressed in content (mg g^{-1}-of PS, Spanish for DW) and pungency (Scoville heat units, SHU). One

SHU is equivalent to 0.015 mg g^{-1} of capsaicinoids dry weight.

A randomized complete block experimental design with five replications was used. Three treatments were evaluated (T1=Steiner nutrient solution; T2=Hoagland & Amon nutrient solution; T3=Soria *et al.*, modified nutrient solution). Twenty-five plants were established per treatment. An analysis of variance (ANOVA, $p \leq 0.05$) and a Tukey test ($\alpha=0.05$) were performed on the data. The data in percentages were transformed by the square root of the arc sine.

RESULTS AND DISCUSSION

Times to flowering and fruiting

The habanero pepper plants advanced flowering 4 d ahead with the Soria solution (72 dds), followed by the plants with Hoagland's, and Steiner solutions (76 dds) (Figure 1A). According to Medina-Lara *et al.* (2008), flower production depends more on N concentrations than those of K. The Soria solution has high doses of N in growing and flowering stages; thus, it is probable that the increase in the concentrations of N and K in the Soria solution favored the precocity of flower buds in the plants. Similarly, the increase in P concentrations in the nutrient solution can favor flower production (Coutinho *et al.*, 2014). Plants with Soria solution had high doses of P during the transplant stage (4.9 meq L^{-1}), it is probable that this also contributed to early flowering.

Plants irrigated with Steiner and Hoagland solutions brought the fruiting stage 2 d ahead (90 DAP) compared to plants watered with Soria solution (92 dap). But, at 90 dds, the plants with Hoagland solution (52%) had a lower percentage of plants with fruits than the plants watered with Steiner (68%) (Figure 1B).

In this regard, Medina-Lara *et al.* (2008) reported that the application of N with 15 meq L^{-1} promotes fruit production in habanero pepper. Similarly, Nieves-González *et al.* (2015) mentioned that the application of P with 1.5 meq L^{-1} increased the production of habanero pepper fruits. Steiner and Hoagland solutions supply similar concentrations of NO_3^- and $H_2PO_4^-$ constantly in the crop, which probably avoided competition for nutrients during fruit formation. In addition, the formulation of both solutions included other elements such as Ca, Mg and S, which are also important for plant and fruit growth (Barker and Pilbeam, 2014).

Fruit morphology

The habanero pepper fruits watered with Steiner nutrient solution had greater length and diameter (4.0 and 2.9 cm, respectively) compared to fruits watered with Hoagland (3.2 and 2.4 cm, respectively) and Soria (3.8 and 2.4 cm,) solutions (Figure 2A and 2B). The fruits of the Steiner treatment were visually more robust (Figure 3A-3C) and with better appearance (Figure 3D-3F).

Similarly, the fruits of the plants watered with Steiner solution presented greater weight (8 g) compared to those of Hoagland (5.5 g) and Soria (6.5 g) solutions (Figure 2D). However, the fruits of the treatment with the Soria nutrient solution had greater pericarp thickness (2.2 mm) than the other treatments (Steiner: 2.04 mm, and Hoagland: 2.02 mm) (Figure 2C and 3G-I). Some authors mention that in hydroponic cultivation the habanero

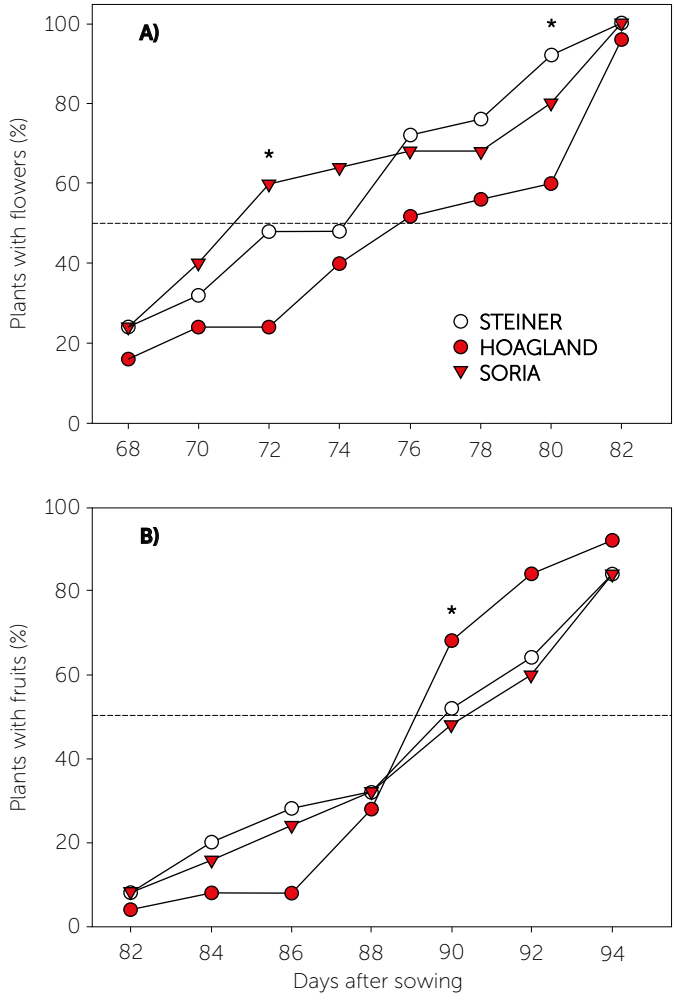

Figure 1. Time to flowering (A) and fruiting (B) of habanero pepper plants (*Capsicum chinense* Jacq.), fertilized with different nutrient solutions (Steiner, Hoagland and Soria) in a hydroponic system. The dotted line indicates 50% of individuals with flowers or fruits. The values represent the means ± standard error, *=statistical differences (ANOVA, $p \leq 0.05$); n=25.

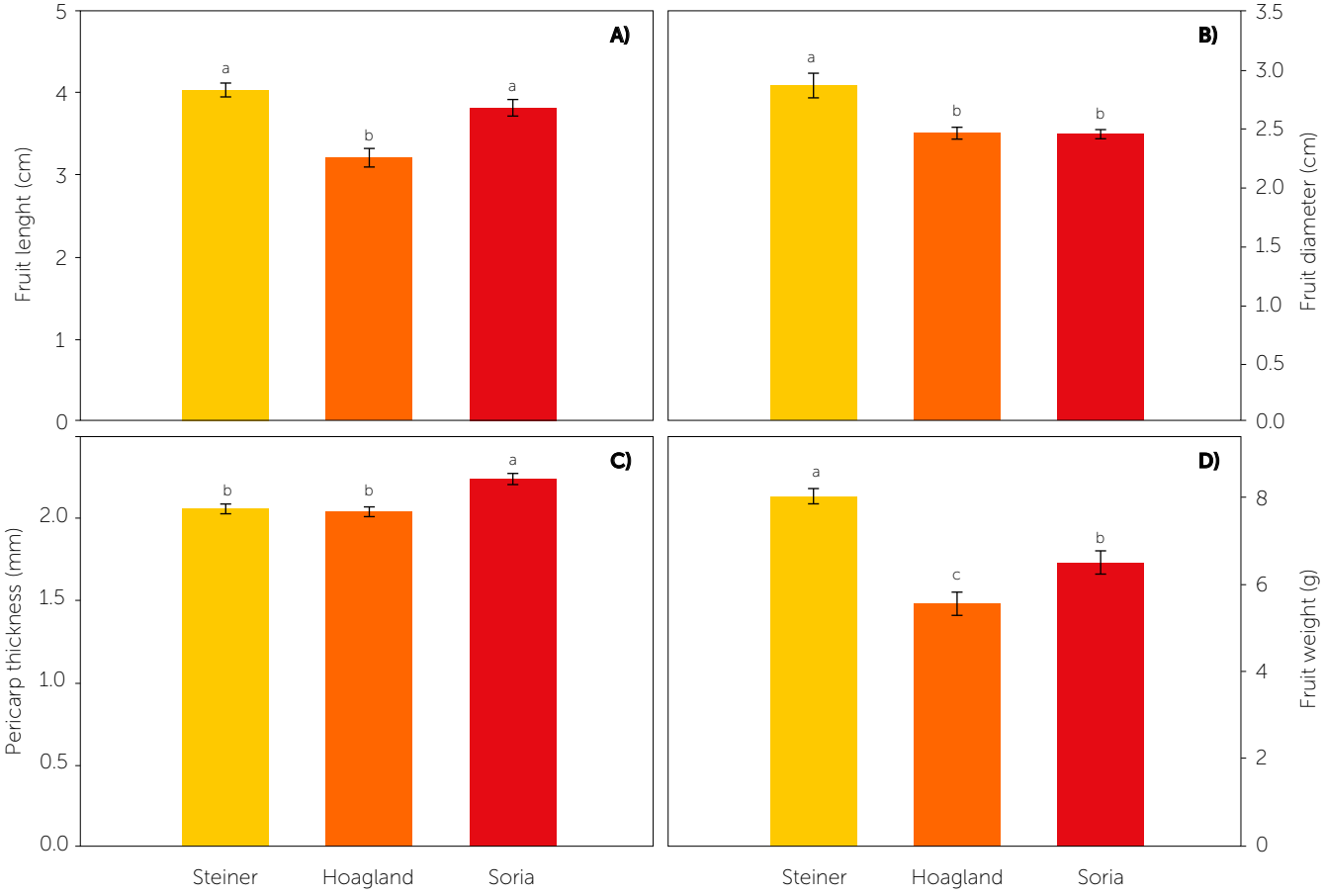

Figure 2. Fruit length (A), fruit diameter (B), pericarp thickness (C) and fruit weight (D) of habanero pepper plants (*Capsicum chinense* Jacq.), fertilized with different nutrient solutions (Steiner, Hoagland and Soria) in a hydroponic system. Data are means ± standard error; different letters indicate statistical differences between treatments (Tukey, α=0.05); n=100.

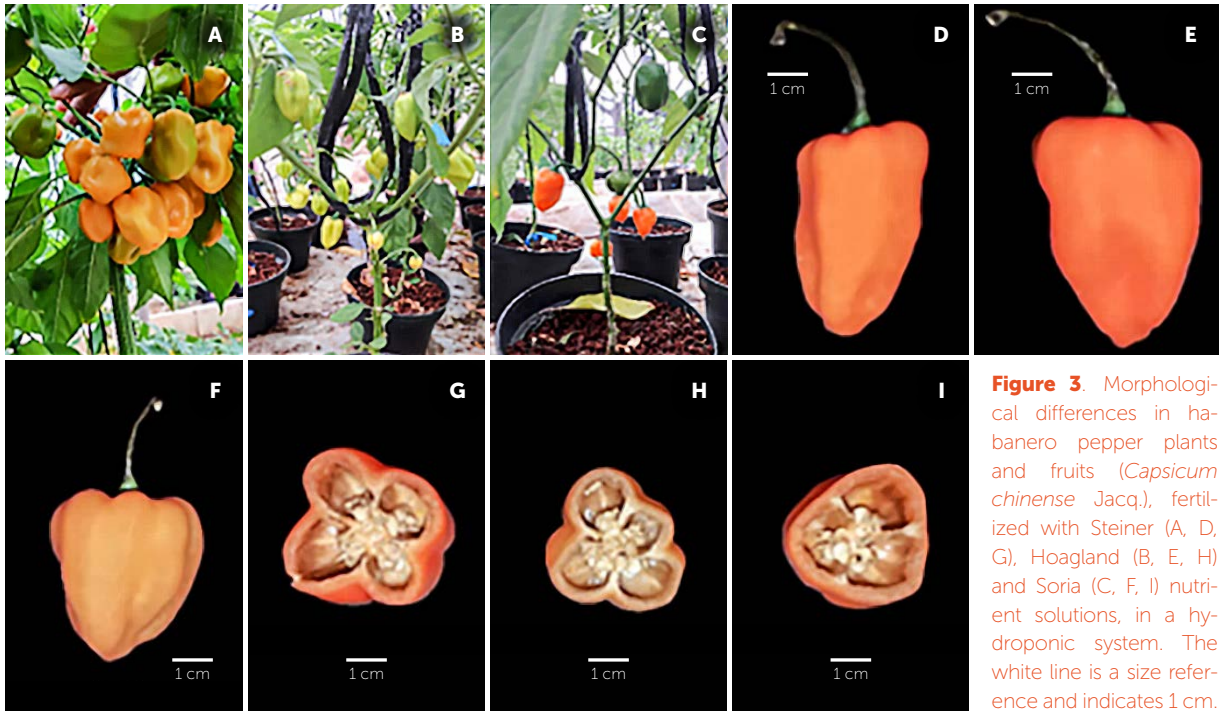

Figure 3. Morphological differences in habanero pepper plants and fruits (*Capsicum chinense* Jacq.), fertilized with Steiner (A, D, G), Hoagland (B, E, H) and Soria (C, F, I) nutrient solutions, in a hydroponic system. The white line is a size reference and indicates 1 cm.

pepper can reach a length between 3.5 and 3.6 cm, with diameters between 2.3 and 2.5 cm (Tucuch-Haas *et al.*, 2012; López-Gómez *et al.*, 2017).

Fruit weight was statistically different among treatments, and no statistical differences were found in the weight of ash. However, the Steiner and Soria solutions presented a higher percentage of ash in the fruit (8.76 and 8.89%, respectively), respect to the Hoagland solution (8.26%). On the other hand, the fruits with Steiner solutions had a higher percentage of humidity (89%) compared to the fruits of the plants watered with Hoagland and Soria solutions (84 and 86%, respectively) (Table 1). The percentage of ash is an indicator of the accumulation of biomass in plants, a product of their photosynthetic activity (Azcón-Bieto and Talón, 2013). It is probable that the plants, watered with Steiner and Soria solutions, allocated more photo-assimilates to the fruits, which favored their size and increased the percentage of ash and moisture.

Table 1. Weight and percentage of ashes and moisture content of habanero fruits (*Capsicum chinense* Jacq.) grown with three nutrient solutions.

Treatments	Ash weight (g)	Ash (%)	Moisture content (%)
Steiner	0.921±0.002	8.76±0.04 a	89±0.2 a
Hoagland	0.923±0.002	8.26±0.09 b	84±0.4 c
Soria	0.920±0.002	8.89±0.03 a	86±0.1 b

* Data are means±standard error; different letters in columns indicate statistical differences among treatments (Tukey, α=0.05); n=9.

Physicochemical characteristics of the fruit

Capsaicin (CAP) in the fruits of the plants watered with Steiner and Soria solutions (23.94 and 23.27 mg g^{-1} respectively) was statistically higher compared to the fruits of the treatment with Hoagland (19.26 mg g^{-1}) (Table 2). In dihydrocapsaicin (DHCAP), the fruits with the Soria solution (12.65 mg g^{-1}) were statistically superior to the fruits with the Hoagland solution (10.31 mg g^{-1}) (Table 2). Likewise, it was observed that the fruits with the Steiner and Soria solutions had a higher content of total capsaicinoids (total CAP's) (36.42 and 35.92 mg g^{-1}, respectively) compared to the Hoagland treatment (29.58 mg g^{-1}). According to Aldana-luit *et al.* (2014) there is a positive correlation between the concentration of NO_3^- and the accumulation of capsaicinoids in the placentas of habanero pepper. However, Medina-Lara *et al.* (2008) mention that the supply of N in high concentrations can decrease the capsaicin content in the fruits. In this case, the application of N, throughout the entire cultivation, led to contrasting effects on the accumulation of capsaicinoids between the Steiner and Hoagland solutions, probably as a result of the difference in the concentration of N (Steiner: 12 meq L^{-1} of NO_3^-; Hoagland: 14 meq L^{-1} of NO_3^- y 1 meq L^{-1} of NH_4^+). The treatment with more N (Hoagland) decreased the spicy-hotness (pungency) of the fruits. It is probable that the increase in the pungency of the fruits with the Soria solution is due to a greater distribution of photo-assimilates in the fruits than in the rest of the plant, caused by the decrease in nutrients in the production stage (nutritional deficit), which would cause stress in the plant. Then the plant, in response, would channel the photo-assimilates to sites where they are most required (Azcón-Bieto and Talón, 2013). In the Scoville units (SHU) the fruits of the treatments with the Steiner and Soria solutions (546,000 and 538,800 SHU, respectively) statistically surpassed the fruits watered with the Hoagland solution (443,550 SHU) (Table 2).

According to Canto-Flick *et al.* (2008), the degree of pungency in habanero pepper can vary considerably due to genetic or environmental factors. In this regard, Garruña-Hernández *et al.* (2013) report 15.9 mg g^{-1} of capsaicinoids with 238,500 SHU in ripe habanero pepper fruits, in a high CO_2 environment (760 mg kg^{-1}). In this study, it is probable that the difference in the degree of pungency of the peppers is due both to environmental conditions and to the nutrition applied to the plants; favoring the obtention of fruits with differences in the content of capsaicinoids and in the SHU values.

Table 2. Contents of capsaicin (CAP), dihydrocapsaicin (DHCAP), total capsaicinoids (total CAP's) and Scoville units (SHU) in habanero pepper fruits (*Capsicum chinense* Jacq.), evaluated with three nutritional solutions.

Treatments	CAP	DHCAP	Total CAP's	SHU
	mg g^{-1}			
Steiner	23.94±0.72 a	12.48±0.23 ab	36.42±0.94 a	546,300±14,000 a
Hoagland	19.26±0.21 b	10.31±0.48 b	29.58±0.66 b	443,550±9,900 b
Soria	23.27±0.27 a	12.65±0.13 a	35.92±0.36 a	538,800±5,400 a

Data are means±standard error; different letters in each column indicate statistical differences among treatments (Tukey, α=0.05); n=3.

CONCLUSIONS

Steiner nutrient solution advanced the fruiting stage and improved fruit morphology, by producing larger and heavier fruits. The most spicy-hot (pungent) habanero pepper fruits were obtained with the nutrient solutions of Steiner and Soria. Considering the phenology and the quality of the fruits, Steiner nutrient solution may be an option for cultivating habanero peppers in hydroponic systems.

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Risk factors associated with mastitis and raw milk quality in small farms of Texcoco, México

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ABSTRACT

Objective: to determine the nutritional and physicochemical quality, and the presence of aflatoxins in raw milk, as well as risk factors for developing mastitis in 20 family farms in the region of Texcoco, Mexico.

Methods: MilkoSCan FT1 was used for nutritional and physicochemical analysis of milk. Somatic cells were quantified and the cow's health status was tested using Somaticell; furthermore, the presence of Aflatoxin M1 was determined using lateral flow immunochromatography.

Results: the milk evaluated in this study reported normal nutritional values according to NMX-F-700-COFOCALEC-2012, which guarantees its quality for human consumption. The pH ranged from 5.0 to 8.4, which indicates deficient temperature control in some farms, leading to problems with acidity. The logistical analysis showed that adequate udder cleaning during milking is important to avoid it being a risk factor for an increase in somatic cells and degree of mastitis, although not the milking technique or teat sealing. The presence of aflatoxin AFM1 was not reported in raw milk.

Study implications: the Somaticell® technique renders a qualitative and efficient diagnosis of clinical mastitis.

Conclusions: raw milk quality from this region guarantees consumers with a safe and apt product for human consumption or transformation into dairy byproducts.

Key words: raw milk, Somaticell® test, risk factors.

INTRODUCTION

Small-scale dairy farms often have technological limitations to obtain raw milk or other dairy products, such as cheese, cream, or yoghurt, which meet hygiene and safety standards in order to avoid public health problems (Zumbado and Romero 2015; Villagómez and Pérez, 2017). These products can be affected by the cows' overall health, contamination with toxins or pathogenic organisms, which

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are consequence of deficient management practices and inadequate milking techniques, manipulation of milk in tanks and storage containers which can damage the milk's color, taste, physicochemical and sanitary composition (Moreno *et al*., 2007). Therefore, a periodic diagnosis of mastitis, sterilization of milking equipment, physicochemical and nutritional milk analysis, as well as the detection of toxins in cow feed and raw milk, should be standardized protocol in these farms. Among the methods used to evaluate udder health and quantify the degree of mastitis, the Somaticell test is very sensitive and allows for the classification of milk obtained from affected udders (Ruíz and Sandoval, 2018; Remón *et al*., 2019), in addition to quantifying somatic cells in storage tanks (Pereira *et al*., 2014). The presence of aflatoxins in cow feed and in milk has been reported by some studies conducted in farms in the Estado de México, reporting the presence of aflatoxin AFM1 in raw milk above permissible limits (Péres *et al*., 2008), putting at risk the health of consumers (Urban *et al*., 2009). This has been attributed to the cows consuming feed contaminated with AFB1, which is why continuous monitoring is recommended to avoid a public health problem. The nutritional and physicochemical quality of raw milk was evaluated, in order to determine the presence of aflatoxin M1, determining the risk factors during milking for developing mastitis in family owned bovine production units in the micro-region of Texcoco, Estado de México.

MATERIALS AND METHODS

A cross sectional sampling of 20 family owned small-scale production units was carried out, with a total of 565 Holstein cows (4 to 35 cows herd^{-1}) located in the municipalities of Texcoco and San Andrés Chiautla, Estado de México (19° 24' and 19° 33' N; 98° 38' and 99° 02' W) (INEGI, 2009), during the months of March to July, 2019, based on a direct poll and sampling of raw milk in storage containers and tanks.

The interview form consisted of 71 questions related to general aspects of the farm, milk production, hygiene practices, and cattle management. Milk quality was based on the Mexican norm NOM-155-SCFI-2012, which classifies degree of udder infection according to somatic cell count (Figure 1) into four degrees (healthy: 0-200,000; subclinical: 200,001-400,000; clinical: 400,001-1,000,000; acute: >1,000,000). Physicochemical and nutritional analyses were carried out using infrared spectrometry in MilkoSCan FT1, measuring: proteins (gL^{-1}), casein (%), fat (gL^{-1}) and lactose (gL^{-1}). In order to count somatic cells and overall cow health, the Somaticell test was used taking 2260 samples from udder quarters of 565 cows. In order to determine the association between somatic cell count and related risk factors (milking technique, udder cleanliness, and teat sealing), logistic regression was used analyzing the significance of regression coefficients with the Wald test (Chi squared), using PRO LOGISTIC from SAS (SAS 1992, ver. 2). In addition, the presence of aflatoxin M1 was determined in 50 milk samples, utilizing lateral flow immunochromatography, with a sensitivity of 350 ppt.

RESULTS AND DISCUSSION

General description of the farms. The dairy herds of the family milk farms (FMF) in the region of study included a total of 565 cows, 58% of which are in production and 19% of which are dry. This indicates fertility planning throughout the year, in order to have a continuous offer for the market and a useful life of 5 to 6 births. Artificial insemination and replacement production are common practice. The milk is sold without any processing on the farm to consumers, intermediaries, or artisanal collection centers where it is transformed into fresh cheese, yoghurt and creams, with results similar to those reported by de Espinosa *et al*. (2010). Average production is 6 kg animal-1d^{-1} (Sánchez *et al*., 2015; Álvarez *et al*., 2012).

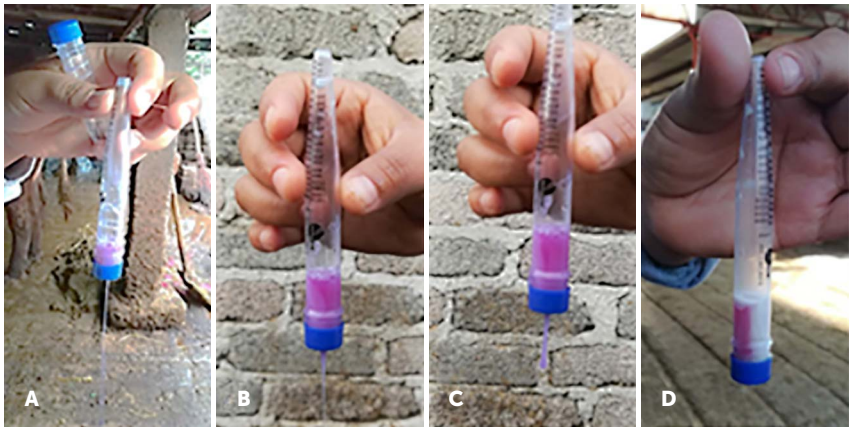

Figure 1. Aspect of the milk samples tested with Somaticell in order to determine presence of mastitis. A: Healthy, B: Subclinical, C: Clinical, D: Acute.

Milk production. On average each FMF produced 16±1.9 L animal^{-1} day^{-1}, which is sold without any processing in the region directly on the farm to consumers,

collection centers, and artisanal companies who make fresh cheese, yoghurt, and creams. It is common for small-scale producers to sell raw milk door to door and get a higher price per liter compared to warehouse prices. Since these small-scale farms are family run, the production cost is lower, and they are able to establish a labor chain that includes local buyers, intermediaries, processing plants (cheese factories) and final distributor, as reported by Ruiz *et al*. (2017). This payment system based on volume offered is not adequate, since there is no additional compensation for milk quality, such as fat percentage and amount of total solids.

Mastitis diagnosis. In this region hand milking is more common than mechanical milking, and it is associated with a higher percentage of mastitis when compared to the mechanical process ($p<0.05$), due to presenting higher degree of clinical mastitis (29.2% and 26.6%, respectively), compared to other degrees of mastitis (Table 1). These results demonstrate that the absence of good milking practices, and deficient sanitary conditions at the time of cleaning and udder sterilization, can be underlying causes of a higher incidence of mastitis in these herds. This is inconsistent with findings by Ruiz *et al*. (2011), who reported a higher incidence of clinical mastitis associated with mechanical milking. There is also a higher frequency with afternoon milking (55%), with clinical type showing the highest degree of mastitis.

Mastitis diagnosis according to udder quarter. There was no difference reported when evaluating each quarter individually ($P>0.05$). The average prevalence of each quarter was 14% (Table 2). It was found that the highest presence of mastitis in all of the quarters was clinical mastitis (55.8%), and 23.7% reported acute mastitis. These percentages represent the most severe degrees of mastitis. This demonstrates that the lack of a cleaning routine and adequate udder hygiene are associated with an increase in mastitis.

The quantitative diagnostic method Somaticell allowed for a more accurate somatic cell count (SCC). Figure 2 shows the distribution of the variation of mastitis in all of the udder quarters evaluated, where 57% of the variation corresponds to values between 400 and 1000 SCC (clinical mastitis), while 24% ranged between 1001 and 1970 SCC (acute mastitis). The sum of these two figures represented 81% of the cases reported, these being the most severe degrees of mastitis (Gómez *et al*., 2015).

Calculating possible risk factors according to possible causes of mastitis

In this region hand milking is more common than mechanical milking, with 56% of done manually and the rest mechanically. Hand milking has been associated with higher percentage of mastitis when compared to mechanical milking.

The whole set of data about milking variables, udder cleanliness, and teat sealing were not statistically significant (X^2, $p>0.0.5$), therefore they could not be taken into account for a model of risk factors for degrees of mastitis in small-scale dairy herds in this region. On its own, the variable of udder cleanliness is very important ($Pr>ChiSq$ 0.05); that is, the risk of presenting mastitis is higher if appropriate udder cleanliness is not carried out, which indicates that when the hygiene is more complete in the milking routine, the risk of presenting mastitis and udder contamination is 3.66 times lower (Table 3), with a 1 to 15 confidence interval. These findings are consistent with Ramírez (2015) who also reports that udder cleanliness is the most important factor associated with mastitis. The milking technique and teat sealing did not increase or decrease mastitis development.

Table 1. Frequency of mastitis (%) in Holstein cows depending on time of milking.

	Mastitis Classification				
	Healthy	Subclinical	Acute	Clinic	Total
Milking type					
By hand	3.0	8.5	29.2	13.4	54.2[a]
Mechanics	1.8	7.2	26.6	10.4	45.8[b]
Milking time					
Morning	3.0	7.2	22.8	12.2	45.1[b]
Afternoon	1.8	8.5	33.0	11.6	55.0[a]

($P<0.001$, Chisq test).

Table 2. Frequency of the degree of mastitis (%) in each of the four udder quarters of dairy cows in family owned production units.

Degree of mastitis	Udder quarter				
	CDD	CDI	CTD	CTI	Total
Healthy	1.0	0.9	1.4	1.5	4.8[d]
Subclinical	3.7	4.4	4.0	3.7	15.7[c]
Clinic	14.2	13.9	14.0	13.8	55.8[a]
Acute	6.5	5.7	5.6	5.9	23.7[b]

Columns with different letters are different ($P<0.01$, Chisq test); CDD: Right front quarter; CDI: Left front quarter; CTD: Right rear quarter; CTI: Left rear quarter.

Figure 2. Manual and mechanical milking system.

Nutritional quality of raw milk. It is fundamental for milk to conserve its nutritional and hygienic quality, even if this is related to a higher cost to the consumers. The estimated values for fat content varied between 3.6 and 4.42, due to the amount of fiber included in the cow's diet; with higher fiber content values, there is higher fat percentage in the milk. In these small herds, the fodder/concentrate ratio is high, with the proportion most likely increasing due to elevated concentrate costs and the fact that many produce their own fodder. The average lactose content was 4.21 and protein was 3.01, while the highest values were 4.63 and 3.87, respectively. These values are very similar to those reported in other studies, and they comply with milk quality norms and are not modified by the presence of mastitis.

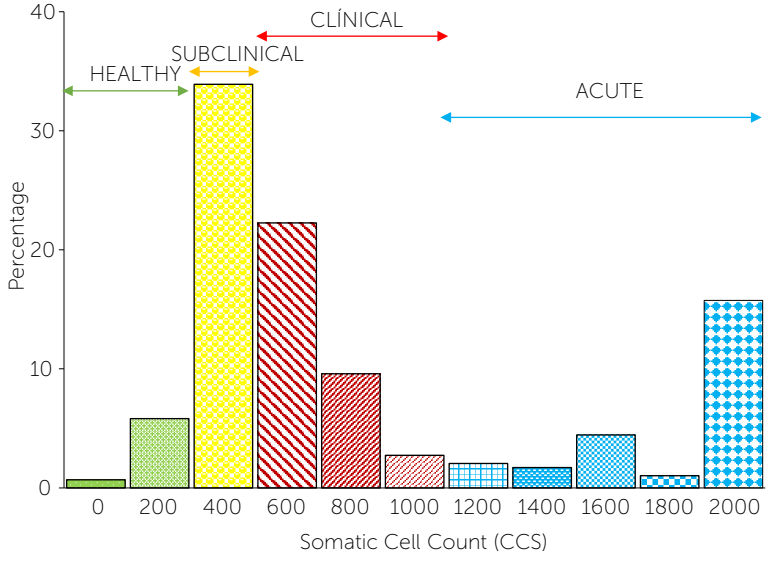

Figure 3. Distribution of mastitis variation in udder quarters of Holstein dairy cows in the Texcoco, Estado de México.

Table 3. Logistic regression analysis associating degree of mastitis with milking technique, udder cleanliness and teat sealing.

Variable	GL	Est*	E.E.	ChiSq. Wald	Pr>ChiSq	Parameter Estimater	ODD RATIO	
							Confident	Límites
							95%	
Intercept	1	1.15	0.30	15.07	0.01			
Milking Type	1	0.33	0.50	0.45	0.50	1.39	0.53	3.73
Udder cleaning	1	1.30	0.66	3.82	0.05	3.66	1.07	14.93
Sealing nipples	1	−0.25	0.52	0.23	0.63	0.78	0.277	2.18

*Est=estimator.

Other studies have reported that mastitis decreases the percentage content of fat and milk solid nonfat (MSNF), as well as reduces lactose levels (Bramley, 1996). The average MSNF value was reported at 7.96, with the highest value reported at 8.72 (Table 4).

A good indicator of milk adulterated with water is the cryoscopic point, which corresponds to milk freezing temperature, which normally ranges between −0.553 and −0.551 °C (Table 4), due to the presence of water soluble dairy components, mainly minerals and lactose.

The value reported in this study is within this interval, and this adulterating practice should be avoided because it could cause a public health problem from contamination with water microorganisms.

Table 4. Nutritional and physicochemical composition of raw milk in dairy farms in Texcoco.

Variable	Mínimum	Mean	Máximum	Standard error
Nutritional composition				
Fat	2.38	3.61	4.42	0.046
Lactose	3.29	4.21	4.63	0.035
Protein	2.51	3.01	3.87	0.034
NFS	6.4	7.96	8.72	0.064
TS	9.94	12.49	13.345	0.08
Density	1030.31	1037.56	1044.65	0.392
Casein	1.805	2.29	2.65	0.029
Urea	−0.1	0.01	0.02	0.003
Physicochemical composition				
Cryoscopic Point	0.39	0.5	0.55	0.004
pH	5.0	7.06	8.4	0.134

The pH values reported ranged between 5.0 and 8.4 (Table 4), with a higher tendency towards more acidic levels; however higher, more neutral pH levels, were also found with a trend toward alkalinity values, similar to what is reported by Negri (2005), who mentioned that higher alkalinity pH could be an indicator of high incidence of mastitis. Therefore, it is important to be aware of the pH value, considering that alkaline levels are linked to high permeability of membranes in cows' mammary glands, which leads to higher Na^+ and Cl^- ion concentrations, as well as a reduction in lactose and inorganic P (Negri, 2005; Asif and Sumaira, 2010).

Aflatoxin M1 presence. Analysis of n=50 samples of raw milk to determine presence of aflatoxin M1 did not result in positive readings, which coincides with results reported by Ortiz (2009), in production units in Arequipa, Peru. However, they are not similar to reports by Pérez *et al*. (2008) in the Texcoco region, who reported levels above those established by the European Union (0.05 μg kg^{-1}), and above the daily recommended intake in Mexico for raw and pasteurized milk (0.5 μg L^{-1}). The results obtained could be attributed to the absence or very low levels of AFLAB1 present in the fodder and grains consumed by the animals.

CONCLUSIONS

In the study area, milk producers are characterized by planning the reproductive activities of herds during the entire year, which has the benefit of maintaining a constant milk offer in the market, which is sold without processing and directly to the consumer. The sanitary and physicochemical quality of milk complies with established norms. No aflatoxin AFM1 levels were found, which guarantees the consumer with a product suitable for human consumption.

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Characterization of stakeholders in the value chain and commercialization channels of string cheese in Vega de Alatorre, Veracruz, México

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ABSTRACT

Purpose: Characterize Stakeholders in the Value Chain and Commercialization Channels of String Cheese in Vega de Alatorre, Veracruz, México.

Methodology: The study was performed in the period June-August 2019, including 61 surveys responded by milk producers and 22 dairy product processers (cheesemakers). Local Agri-food Systems (LAFS) and value chain approaches were applied.

Results: The value chain of string cheese in this municipality consists of providers of supplies, dairy cattle breeders, gatherers, cheesemakers, sellers, and final consumers. String cheese is commercialized through 5 channels: (a) products are distributed by cheesemakers to wholesalers and retailers, (b) products are sold in food stores, establishments or restaurants within the region, (c) products are sold to distributors who buy the product directly in the factory, d) direct sale in a grocery or traditional dairy store in the municipality and (e) products are sold at small-scale to final consumers in houses.

Study Limitations: Distrust of some stakeholders to provide information for the study, due to the insecurity in the state of Veracruz.

Conclusion: Limitations were found in the development and integration of the value chain. The sale of cheese is carried out without sales contracts or cooperation agreements. Prices are set subjectively by the dominant stakeholder, and in order to influence the market price, producers and processors arbitrarily make conditional on the supply or demand for milk and cheese, which creates market failures and inefficiencies.

Key words: Local Agri-food Systems, market failures, stakeholders.

INTRODUCTION

Bovine livestock is one of main sectors in Veracruz, Mexico, with 3.6 million hectares dedicated to this activity and approximately four million heads of livestock, which ranked first in inventory and sixth in milk production with 702 million liters in 2016 (LAFS, 2016). This industry in the state is considered a source of income by most producers (68%), as family enterprise (24%) and to a lesser extent (8%) as a business with corporate vision (Díaz *et al*., 2011). In the municipality of Vega de Alatorre, dual purpose

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system (DP) is aimed at producing weaned calves and milk; the latter is sold both as it is, and also processed by cheesemaking factories that are distributed in the villages of the municipality. To assess DP stockbreeding under an agroecosystemic model, the social component of the activity should be considered, as well as ecological, technological, and productive aspects (Vilaboa *et al*., 2009). In this municipality, livestock breeding is one of the most important activities of the primary sector, this has allowed the development of milk transformation into cheese. In the 50's, cheesemaking activity began to be carried out in the Emilio Carranza village for self-consumption, and afterwards it scaled up to be sold locally. Cheese is produced both in a traditional manner and semi-industrialized process. Cheesemaking process has evolved in this region. Currently, there are different types of production and cheese is produced in more locations. Production differentiation was registered by production scale, diversity of processed dairy products, type of labor, use and type of supplies, technology used and level of organization (Martínez *et al*., 2009). Thus, they can be characterized according to the technological level from traditional to semi-industrial. Finished products are string cheese, queso fresco, panela cheese, white cheese, double cream cheese, grated white cheese, and Cotija cheese, in addition to cream, ricotta and buttermilk subproducts. The string cheese is one of the most demanded in the market, and perhaps it is the most consumed cheese in Veracruz (Ramírez y Vélez, 2012). This is an enzymatic coagulation cheese, usually made from whole and raw milk; and it is widely produced at traditional and industrialized level in different regions of the country. Based on the above, the purpose of the study was to characterize different stakeholders of the value chain and commercialization channels of string cheese in Vega de Alatorre, Veracruz, Mexico.

MATERIALS AND METHODS

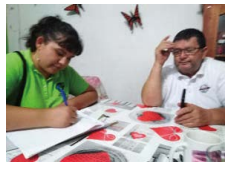
The study was carried out in the municipality of Vega de Alatorre (20° 02' N and 96° 57' W) at 10 meters above mean sea level. It belongs to Nautla region and has an area of 340.17 km^2, representing 0.47% of the state's total area. The study consisted in the assessment of the structure and characterization of stakeholders of the value chain of string cheese in accordance with LAFS model. A survey was performed, and data was collected from primary stakeholders and dairy product processors from June to August 2019. They were interviewed in one-to-one meetings. 61 surveys were responded by dairy cattle farmers and 22 by dairy product processers (cheesemakers). Surveys were intended to assess the value chain of string cheese, as well as to characterize above stakeholders (Figure 1).

RESULTS AND DISCUSSION

The value chain under study consists of raw material suppliers, dairy cattle breeders, gatherers, cheesemakers, distributors, and final consumers (Figure 2). Cheese production arises from the creation of small traditional and semi-industrialized cheese factories in the municipality that are part of the value generation from the milk-cheese production chain. Cheese is in great demand by local consumers and from nearby municipalities, as well as far places. Among this variety of products sold in the municipality, string cheese is the most demanded; therefore, the one with the highest production in cheese factories; this agrees with Dominguez-López *et al*. (2011), and string cheese is one of the best-selling cheeses in Mexico.

Raw Material Suppliers

Supply procurement is vital for dairy cattle breeders of the municipality, it is carried out with different providers: 1) Local dairy cattle breeders Association, where medicines and commercial balanced food they need are bought, 2) vet offices located in municipal seat or in Emilio Carranza or Misantla localities, where medicines are bought, 3) transportation of a private company sells balanced feed for cattle and pigs, and corn through the communities of the municipality and 4) some companies with established routes that sell food or corn to small producers, and the total amount is deducted from the payment of the milk delivered.



Interviews with key informants to collect data on the number of producers and cheesemakers in the municipality.

A pilot test of the surveys to dairy cattle breeders and cheesemakers was performed.

Surveys responded by dairy cattle breeders and cheesemakers.

Figure 1. Stages of the survey responded by stakeholders of the value chain of string cheese in Vega de Alatorre, Veracruz, México.

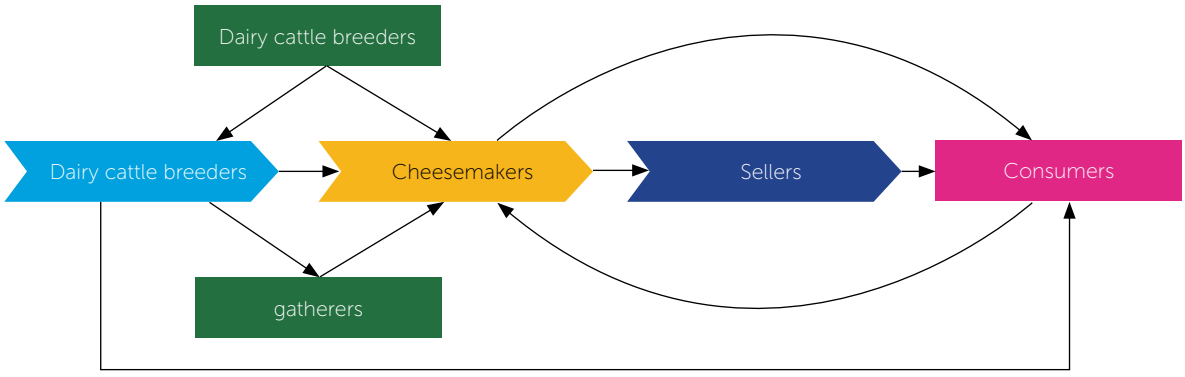


Figure 2. Structure of value chain of string cheese in Vega de Alatorre, Veracruz, México.

There are also different providers of raw materials that are necessary to make cheese: (1) the managers or owners of semi-industrialized cheese factories buy and warehouse large volumes of their stock in another state and, in turn, they resell it to small cheese factories, (2) they buy from providers that bring products to the region from other places. For example, every 20 days, one provider of supplies from the city of Xalapa and other from the state of Puebla offer their products to cheese factories. Another example is salt, which is mainly purchased from a provider in Martínez de la Torre who is a wholesaler for cheese stores. The bags for packaging are purchased from a provider in Martínez de la Torre, who delivers bags every 8 days to cheese shops; (3) There is an establishment in the municipal seat that distributes products for cheese industry, which provide services to some cheesemakers in the region.

Characterization of dairy cattle breeders

90% of the primary producers (dairy cattle breeders) interviewed are men. This number agrees with the results observed in previous studies carried out in the Papaloapan region and central Veracruz state (Vilaboa *et al*., 2009; Juárez-Barrientos *et al*., 2015). Their average age is 54.6±13.4 years, and range between 22 and 80 years, and when they were grouped into six ranges, it was found that 32.79% are between 51 and 60 years. These results observed exceed 50 years, which was reported by Orantes-Zebadúa *et al*. (2014) in DP bovine producers in the Center of Chiapas, and similar to the 56 years reported by Juárez-Barrientos *et al*. (2015) in PD producers from the Rural Development District 008 of Veracruz (DDR 0008). Education level of 37.70% of the producers is elementary school. On the other hand, some producers any degree of study and others have a postgraduate degree.

The years of experience in the activity was from 3 to 65 years, with an average of 31.4±16.3 years. This number is below the 38 years observed in DDR 008 (Juárez-Barrientos *et al*., 2015). 23% of people interviewed have another source of income (salaried employee, business, pension or income from real estate) and 77% are engaged in other activities of the primary sector (corn, beans, lime, banana, etc.) 79% dedicate their ranch exclusively to livestock, 18% to livestock and agriculture, and 3% to livestock and forestry. This lack of diversification of activities makes them vulnerable to market changes (Vilaboa *et al*., 2009).

The size of area of production units is between 5 and 250 ha, with an average of 39.8±48.2 ha, a number similar to that reported for breeders in DDR 008 and higher than 20 ha in José Azueta, Veracruz (Martínez *et al*., 2012). 57% of the producers have small properties, 30% ejido lands, 10% rented land, and 3% in gratuitous loan agreement. 87% directly manage the farm, 8% a member of his/her family, and 5% a cowboy or manager. 48% of the workforce is family, similar to the 48.4% found in DDR 008 (Juárez-Barrientos *et al*., 2015). 57% of the producers have the DP production system, 25% milk, 15% breeding stock and 3% meat. 98% have grazing cattle and 2% semi-stabled cattle; 72% of the grazing system is rotational, 15% continuous and 13% alternate. 70% do not keep productive or economic records. 59% of the farmers belong to a Local Livestock Association, mainly due to the benefits of belonging to the association, distributed as follows: 56% Emilio Carranza, 25% Vega de Alatorre, 6% Misantla, 6% Nautla, 6 % Colipa and 3% Nautla. There are two livestock associations in the municipality, one is from Emilio Carranza, which has the largest number of members (358 members), and the other from Vega de Alatorre, with 112 members. 41% of the farmers who are not members of one of the above associations does not

have many benefits but prefer to pay for the bills without a discount offered to members of associations. Most of the people who are not members of any association are small producers.

The cattle are mainly Swiss × Cebu cross breed (84%), which is similar with the results observed in studies carried out in the region (Martínez *et al*., 2012; Orantes-Zebadúa *et al*., 2014; Juárez-Barrientos *et al*., 2015). The number of animals per producer ranges from 9 to 292 heads of livestock, with an average of 59±60.2 bovines per producer; Of the total number of bovines, 26% are cows being milked both manually (95%) and 5% with a machine. 98% of the producers do only one milking per day. These results are higher (48.4%) than those registered by DDR 008 (Juárez-Barrientos *et al*., 2015). The type of breeding used by the majority (93%) of producers is natural breeding, and only 7% use artificial insemination with natural breeding. 74% of the cattle breeders do not receive technical assistance, 25% receive it from independent professionals and only 2% from personnel of government agencies. Most of them (98%) do not have support from government agency programs, and only 3% have loans for their farm. Regarding infrastructure, 85% have rustic milking areas built with only a sheet of metal and wood roof, without walls or floors, which is similar to that described by Martínez *et al*. (2012); the remaining 15% have cement buildings in their milking parlor.

The average production per cow was 5.3 L of milk, a production higher than the 4.4 L observed in DDR 008 (Juárez-Barrientos *et al*., 2015) and lower than the 6.2 L observed by Martínez *et al*. (2012), with an average of 82 L per day per producer. Martínez *et al*. (2012) reported that the months with the highest milk production are from July to September, which is the rainy season, and decreases in the dry season, from December to May. In turn, during high-production periods, the price of milk drops, and low-production periods, it rises.

90% of the milk obtained is for cheesemakers, 5% for self-consumption and 5% for cheese for sale at home. In 2018, the average milk price was MXN $5.00, and it varied from MXN MXN $6.50 in low-production periods to MXN $4.80 during high-production periods. One of the nonconformities or disadvantages of the farmers is that the price is determined by the cheesemaker at his/her discretion. Most of the cattle breeders sell milk to cheesemakers who pay the best price and if they trust or are friend of the processor. 59% of the farmers deliver the milk to the ranch; truck drivers hired by the cheese factories pick it up, and the remaining 41% deliver milk directly to the cheese factory because they obtain a better price. Milk is paid in cash and on a weekly basis; the owner of the cheese factory prepares the packs with the resulting quantity of the liters of milk and according to the price set, and delivers them in two ways: (1) to the truck drivers who deliver them to the producers, and (2) directly to the producers who go to the cheese shop to receive their pay. Producers sometimes request a loan or advance on their pay that is either granted or not at a discretion of the cheesemaker. 39% of the farmers deliver milk to the same cheesemaker, and 41% have changed for a better price, due to delays in the payment of milk or due to unjustified discounts. 56% of cattle breeders buy cheese from the cheesemaker who delivers their milk, and 44% buy it elsewhere. As they are family businesses, both in the management of PU and in the transformation of milk, 85% of farmers and processors involve their children in activities, such as going to pay for the milk or administrative issues in the case of the cheese shops.

Dairy Product Processors

32 traditional and semi-industrialized cheese factories were located. These factories have different sizes to process milk, from 180 to 9500 L per day. 69% of these were surveyed, ant the information below was below.

Socioeconomic Profile of Dairy Product Processors (Cheesemakers)

The participation of women in this phase of the process was 32%, which differs from that observed in Aculco state of Mexico, where 100% are men, and their wife and children are involved in activities related to the cheese factory (Martínez *et al*., 2009). Regarding age, people from 20 to 70 years old were registered. 32% were between 41 and 50, with an average age of 48 years, which is older than 43 years reported in the municipality of Aculco, State of Mexico (Martínez *et al*., 2009). The education level of processors was 36% with elementary, 32% with middle, 18% with high school, 9% with a degree and 5% without formal schooling.

Gatherers play an important role within the chain, since they collect milk production from producers who are generally far from cheese factories (Martínez *et al*., 2009; Espinosa-Ayala *et al*., 2013). It was observed that the cheesemakers in Vega de Alatorre obtain their milk directly from the producer or through the gatherers, who are classified into: 1) gatherers-producers, who

deliver their own milk and the milk of other producers according to the agreed route with the cheesemaker, (2) independent gatherers, who collect milk from producers with their own means and deliver it to the cheese factory according to the route assigned by the cheesemaker, (3) gatherer-worker, who is in charge of collecting milk with a vehicle of their own cheese factory in the course of the morning, and later they work making cheese, and (4) gatherer-cheese maker: The owner of the cheese factory gathers milk from producers and then, makes cheese.

Transformation Volume

90% of the cheese factories only gather milk, and the remaining 10% their raw material is mixed, as they have their own gathering and production from their dairy herds. Mixed production is lower in the municipality of Vega de Alatorre compared to that observed in Aculco, where 41.2% of the producers have milking herds and their production is combined with external collection to supplement the daily volume required of the company (Martínez *et al*., 2009). The varieties of products made in the cheese factories are one to ten different products. 54% of cheesemakers make string cheese, a product preferred by buyers, which agrees with that reported by Martínez *et al*. (2009), who found in Aculco, state of Mexico, that of the 37 existing cheese factories, 33 made string cheese, which represents 66% of the total cheese production. The cheese conversion is 9.2 L per kilo of string cheese, 8.1 L for fresh cheese, and 8.9 L for other cheeses such as panela and white cheese. Regarding workforce, 59% is hired, 18% a family member and 23% mixed (hired and family members), which depends on the size and volume of the cheese factory. The number of workers ranges from 1 to 15 people.

Commercialization

String cheese commercialization is performed via 3 different channels: (1) the cheesemaker carries production to Poza Rica, Veracruz, where products are sold both to wholesalers and retailers, depending on the purchased volume; the price for wholesalers is MXN $75.00, and MXN $80.00 per kilogram of cheese. Since it is a good place for selling cheese products, some of them have bought stands in the local market or in the city, which allows a direct sale to the consumer, (2) products are sold in food stores, establishments or restaurants within the region. This is similar to the selling model of manchego in Cuenca, Spain (Trejo *et al*., 2011), who reported 4 ways of cheese commercialization (traditional own stores, other traditional stores, retailers, and exporting wholesalers); (3) products sold to intermediaries who come to the factory to buy the product to resell it in other cities of the state and the country such as Reynosa, Monterrey, Mexico City, Tuxpan, Veracruz, Tamaulipas, etc., (4) direct sale in a grocery or traditional dairy store in the municipality. These are managed by themselves. They also sell products beside highways or other places within the village (5) products are sold at small-scale to final consumers in houses. When products are sold to intermediaries, the transaction is made only informally, since no purchase agreements are entered into, which agrees with the findings reported by *et al*. (2009) regarding that purchase is based on agreements secured by good faith and honor of the parties and no formal agreements are entered into (Figure 3).

CONCLUSIONS

String cheese is the product with the highest level of production and commercialization in Vega de Alatorre. The structure of stakeholders of the value chain is semi-integrated, since there is alliance in some stakeholders horizontally, vertically but not in all, since mistrust was detected in several of the links that interact with each other. The value chain of string cheese consisting of providers of supplies, consisting of providers of supplies, dairy cattle breeders, gatherers, cheesemakers, sellers, and final consumers. It has five commercializing channels. There are market failures that limit the integration of the

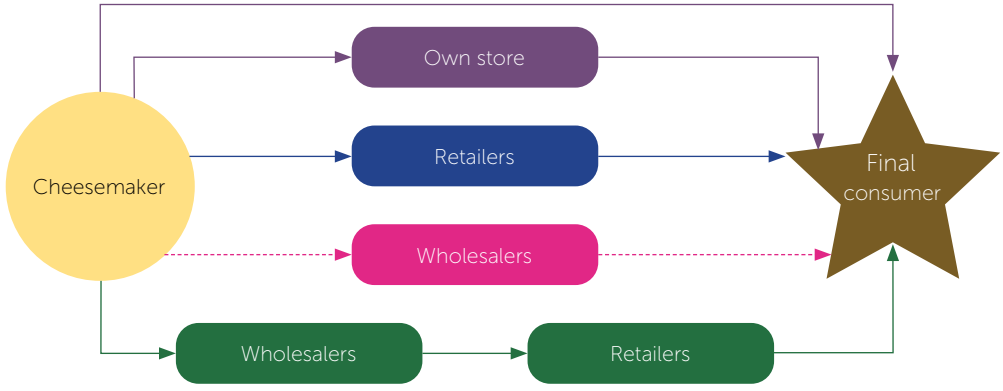


Figure 3. Commercialization channels of value channel of string cheese in Vega de Alatorre, Veracruz, Mexico.

value chain, such as the determination of the price of milk by cheese makers and the price of cheese by traders. This has an impact on other links in the chain.

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The importance of environmental education in students: analyzing the transition from middle to high school in Nuevo Laredo, Tamaulipas, Mexico

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ABSTRACT

Objective: A poll on first-semester high school students (n=246) took place at Nuevo Laredo, Tamaulipas, Mexico, to find out if they received environmental education as an extracurricular class during middle school and how this training could influence their perceptions and positions on environmental issues.

Design/methodology/approach: A questionnaire that contained aspects regard water culture, recycling, species care and transportation, as well as general knowledge of the environment and its regulations, was applied to students. The SPSS statistical software was used to categorize two groups: students who received extracurricular environmental education and those who did not. Subsequently, the non-parametric Mann Whitney tests were performed on the data.

Results: Out of the total number of participants, 167 said they had received extracurricular environmental education in middle school. The other 79 participants reported not to have done so. Students who received more environmental education in middle school exhibit a better attitude regard water management.

Limitations of the study/implications: To interpret the results, it is necessary to consider that the study took place only in the city of Nuevo Laredo, Tamaulipas and that a convenience sampling was used.

Findings/Conclusions: There are still students that do not receive enough environmental education. We conclude that it is important to intensify efforts to change this situation, as environmental training has shown to positively influence the attitudes of students who received it.

Keywords: Environmental awareness, environment, protection of non-renewable resources.

INTRODUCTION

The presence of environmental problems in a country may reflect a lack of awareness of a society that daily demands the consumption and extraction of natural resources from its ecosystems and other global sites. For this reason, it is important to develop programs whose main purpose is a social construction, focused on generating environmental awareness and empathy. Training and information dissemination strategies must be developed to mitigate the presence of activities and actions that do not lead to good environmental practices. For example, the "Earth Charter", since 2000 establishes a series of items where the incorporation of formal environmental education (EE) is requested worldwide as an element of social construction for a sustainable lifestyle (Estrella Suárez & González Vázquez, 2014). In addition to the above, EE represents a set

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of efforts that requires the participation of diverse social actors, government, and business sectors. Formal environmental education is described as a process incorporating the subject into the education programs at the propaedeutic level. Likewise, the Earth Charter in its heading 14 indicates the necessity to add this knowledge and values to construct a sustainable lifestyle (SEMARNAT, 2006). Formal EE in Mexico is set in the curricular profile as part of the obligations established by the environmental legal framework in the country at the federal, state, and municipal levels. In the General Law for Ecological Balance and Environmental Protection (LGEEPA, 2018), article 3, fraction 38, EE is defined as an educational process directed to society formally and informally to build a society with environmental values and respect for nature to guarantee life preservation.

At the federal level, the LGEEPA has a section entitled Ecological Research and Education. It is made up of articles 39, 40 and 41. This section only establishes that the competent authorities must promote the incorporation of formal environmental education content (SEMARNAT, 2006) at the basic level (Primary-Secondary) and Higher Education. Article 39 requests the Ministry of Public Education (SEP) to build this content under the supervision of the Ministry of the Environment and Natural Resources (SEMARNAT) (LGEEPA, 2018). Also, the Code for the Sustainable Development of Tamaulipas, Mexico, (CODE, 2019) in article 4 fraction 19, indicates the legal definition for the environmental education term in the same way as it is presented for the LGEEPA. In the state, EE incorporation is contained in Chapter III "public utility and social interest in sustainable development"; therefore, it must be part of the citizen's actions and state educational institutions as indicated in article eight section four, where it indicates that the state education system must incorporate formal EE (CODE, 2019). Following what is established in the Tamaulipas state code, the Secretary for the Environment has, as part of its competences, monitoring the incorporation of environmental contents into state educational programs. Placing this research in the context of Nuevo Laredo, Tamaulipas, the regulation for environmental protection and sustainable development (Regulation, 2017), in its article four describes the concept of environmental education in the same way as the Code and the LGEEPA, which generates concordance at the three government levels and ensures understanding in the usage of the concept. Based on the above, the objective was to determine if the first-semester high school students had participated in any extracurricular modality for environmental education during middle school and if this training relates to their perceptions of environmental aspects and their present life.

MATERIALS AND METHODS

In this study n=246 first semester students from public high schools at Nuevo Laredo city, Tamaulipas, Mexico, participated, from which 136 were women, 102 men, and eight provided no information (Table 1).

Table 1. Characterization of this study participants.

Age (years)	Female	Male	Total
16	76	46	122
17	53	49	102
18	7	7	14
Total	136	102	238

The study was conducted in public high schools in the city of Nuevo Laredo, Tamaulipas, Mexico. Each one of them was visited, and permission was requested to access the first-semester class groups. There, students gave their consent to be part of the poll and explained what their participation would consist of. The students received the questionnaires printed on white paper sheets, answered the questions, and returned their questionnaire. The anonymity of their information and responses was guaranteed.

Table 2. Categories and topics addressed in this research.

Category	Topics
Environmental knowledge	Biological diversity. Ecosystem. Invasive species. Exotic species. Protected Natural Areas.
Environmental culture for daily life.	Management of polluting waste at home. Transportation and care of living organisms. Saving water. Recycling at home. Existing regulations.

Instrument

A 20-question questionnaire was applied, the contents of which dealt with the categories and topics shown in Table 2. The questions presented to the students are shown in Table 3.

The questionnaire was prepared by the research team and was reviewed by two experts, who provided feedback on the content and approach of the instrument. Fifteen students

also participated in a pilot test that allowed adjusting the wording of the statements. For all questions, a four-point Likert scale was used as follows: totally disagree, disagree, agree and totally agree. The type of study carried out was quantitative, observational, cross-sectional and relationally oriented to the comparison of two independent groups.

The data analysis was carried out using the SPSS version 24 statistical software and consisted of four phases below detailed.

Phase 1. Data cleaning. This phase consisted of identifying missing values in each set of responses, as well as looking for outliers and poorly captured values. A visual inspection and contrasting of the values captured in SPSS with the answers directly obtained from the students on paper of the questionnaires was conducted. No outliers or wrong values were identified.

Phase 2. Data preparation and characterization. A contingency table was obtained to visualize the age and gender of the participants. Subsequently, through frequency tables, the characterization of the responses obtained by the two groups approached in this research was obtained, for which, the responses "Totally disagree" and "Disagree" were unified, as well as the "Totally agree" and "agree " responses in all questions.

Phase 3. Mann-Whitney tests Development. In this phase, a Mann Whitney test was performed for each of the questions in the questionnaire. Interest groups were defined based on the question that indicates whether students received extracurricular environmental education in middle school or not. The test, which was conducted with the original responses that the students provided, recorded the Mann-Whitney U value and the PValue to indicate the significance of the differences found.

Phase 4. Search for statistically significant results. Values of less than 0.05 were sought to consider them significant since a 95% confidence level was taken as a reference. For statistically significant values, the mean ranks were inspected to identify which of the groups answered with a greater tendency towards "totally agree" and which with a "totally disagree" tendency.

RESULTS AND DISCUSSION

The obtained responses characterization is shown in Table 4, the results of the Mann-Whitney tests in Table 5 and their mean ranks are presented in Table 6.

Table 3. Questionnaire applied to participants.

Id	Statements presented to the participants
1	I know the concept of biological diversity
2	I know the concept of ecosystem
3	I know the meaning of invasive species
4	I know the meaning of exotic species
5	It is good to transport insects or plants from one city to another
6	It is good to transport fish from one city to another
7	I turn off the faucet when I brush my teeth
8	I take a shower in less than five minutes
9	It is good to take care of water
10	Water in Nuevo Laredo is scarce
11	It is good to throw away plastic bottles
12	Aluminum cans should be thrown away
13	It is good to throw oils in the sink
14	It is good to throw oils in the W.C.
15	I recycle at home
16	There are laws that fine those who pollute
17	I know the meaning of Protected Natural Area

The results indicate that students who received more environmental training had more knowledge and a good attitude towards most of the investigated aspects. However, it could only be shown that in-favor-of-water care positions were statistically significant ($p<0.05$). According to the collected responses, it is also evident that there are still students who do not receive sufficient environmental education. These results reveal the importance of environmental education in students and are consistent with the recommendations issued at the global, federal, state and municipal levels. For the interpretation of these results, it is necessary to consider that the study was carried out only in the city of Nuevo Laredo, Tamaulipas and that a convenience sampling was used.

These results suggest that the environmental training received during middle school positively influence the knowledge, attitudes and perceptions of students in high school. Specifically, this could be statistically demonstrated with the culture of water care.

CONCLUSIONS

Environmental education for sustainability is a strategy incorporated into the different educational levels of the country. Middle school and high school are no

Table 4. Characterization of the obtained responses.

Id	Statement	Students who did not take extracurricular environmental education in middle school		Students who took extracurricular environmental education in middle school	
		Disagree	Agree	Disagree	Agree
1	I know the concept of biological diversity	13(16.5%)	66(83.5%)	27(16.2%)	139 (83.2%)
2	I know the concept of ecosystem	9(11.4%)	69(87.3%)	14(8.4%)	150(89.8%)
3	I know the meaning of invasive species	38(48.1%)	40(50.6%)	79(47.3%)	88(52.7%)
4	I know the meaning of exotic species	20(25.3%)	59(74.7%)	29(17.4%)	138(82.6%)
5	It is good to transport insects or plants from one city to another	60(75.9%)	19(24.1%)	129(77.2%)	36(21.6%)
6	It is good to transport fish from one city to another	55(69.6%)	24(30.4%)	129(77.2%)	37(22.2%)
7	I turn off the faucet when I brush my teeth	12(15.2%)	67(84.8%)	17(10.2%)	150(89.8%)
8	I take a shower in less than five minutes	58(73.2%)	21(26.6%)	123(73.7%)	39(23.4%)
9	It is good to take care of water	8(10.1%)	70(88.6%)	6(3.6%)	153(91.6%)
10	Water in Nuevo Laredo is scarce	43(54.4%)	34(43.0%)	69(41.3%)	88(52.7%)
11	It is good to throw away plastic bottles	37(46.8%)	41(51.9%)	65(38.9%)	94(56.3%)
12	Aluminum cans should be thrown away	54(68.4%)	23(29.1%)	105(62.9%)	53(31.7%)
13	It is good to throw oils in the sink	76(96.2%)	2(2.5%)	153(91.6%)	8(4.8%)
14	It is good to throw oils in the W.C.	74(93.7%)	4(5.1%)	145(86.8%)	17(10.2%)
15	I recycle at home	34(43.0%)	44(55.7%)	52(31.1%)	107(64.1%)
16	There are laws that fine those who pollute	27(34.2%)	51(64.6%)	48(28.7%)	112(67.1%)
17	I know the meaning of Protected Natural Area	21(26.6%)	57(72.2%)	26(15.6%)	136(81.4%)

Table 5. Mann-Whitney Tests Results.

Id	Statement	Mann-Whitney's U	PValue
1	I know the concept of biological diversity	5945.00	0.18
2	I know the concept of ecosystem	6346.00	0.91
3	I know the meaning of invasive species	6510.50	0.99
4	I know the meaning of exotic species	6215.50	0.42
5	It is good to transport insects or plants from one city to another	6357.00	0.73
6	It is good to transport fish from one city to another	6068.50	0.31
7	I turn off the faucet when I brush my teeth	5962.50	0.15
8	I take a shower in less than five minutes	6312.50	0.85
9	It is good to take care of water	5306.50	0.01 *
10	Water in Nuevo Laredo is scarce	5012.50	0.02 *
11	It is good to throw away plastic bottles	5747.50	0.34
12	Aluminum cans should be thrown away	5645.50	0.34
13	It is good to throw oils in the sink	5955.50	0.44
14	It is good to throw oils in the W.C.	5772.50	0.19
15	I recycle at home	5671.00	0.24
16	There are laws that fine those who pollute	5692.50	0.24
17	I know the meaning of Protected Natural Area	5657.50	0.15

Table 6. Mean ranks obtained for both groups in the Mann-Whitney test.

Id	Statement	Students who did not take extracurricular environmental education in middle school		Students who took extracurricular environmental education in middle school	
		N	Mean Rank	N	Mean Rank
1	I know the concept of biological diversity	79	115.25	166	126.69
2	I know the concept of ecosystem	78	122.14	164	121.20
3	I know the meaning of invasive species	78	123.03	167	122.99
4	I know the meaning of exotic species	79	118.68	167	125.78
5	It is good to transport insects or plants from one city to another	79	124.53	165	121.53
6	It is good to transport fish from one city to another	79	129.18	166	120.06
7	I turn off the faucet when I brush my teeth	79	115.47	167	127.30
8	I take a shower in less than five minutes	79	122.09	162	120.47
9	It is good to take care of water	78	107.53	159	124.63
10	Water in Nuevo Laredo is scarce	77	104.10	157	124.07
11	It is good to throw away plastic bottles	78	113.19	159	121.85
12	Aluminum cans should be thrown away	77	112.32	158	120.77
13	It is good to throw oils in the sink	78	115.85	161	122.01
14	It is good to throw oils in the W.C.	78	113.51	162	123.87
15	I recycle at home	78	112.21	159	122.33
16	There are laws that fine those who pollute	78	112.48	160	122.92
17	I know the meaning of Protected Natural Area	78	112.03	162	124.58

exceptions. However, few subjects address this content; therefore, it is important to strengthen the issues of environmental awareness and valuing natural resources with extracurricular activities. In this way, we consider that it is also important to incorporate regional elements that promote the student's location in the environmental context with information that is useful in their daily life.

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Evaluation of the productivity and rooting of cuttings of ten *Eucalyptus* clones

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ABSTRACT

Objective: evaluating the productivity of mother plants and the rooting percentage of cuttings of *Eucalyptus grandis*, *Eucalyptus urophylla* and *Eucalyptus urograndis*.

Design: Ten clones of each species were evaluated in a clonal miniature garden in a gutter system fitted with drip irrigation.

Results: The assessed number of sprouts and viable cuttings variables registered high significant differences (P<0.0001) between clones: UP1 (17,947 shoots and 34.05 viable cuttings), UG2 (12,120 shoots and 22.96 viable cuttings) and G2 (10,254 shoots and 23.96 viable cuttings). Regard the measured parameters in the irrigation water, the average values of EC 0.5 mS/cm and pH 5 to 7 were obtained. The stem cuttings established in the module had high significant differences (P<0.0001) in terms of the rooting percentage. Three clones were superior, the best of which, clone G2, reports 78.58% rooting, which developed in environmental conditions of relative humidity greater than 80% and temperature between 25-30 °C.

Findings/conclusions: With the obtained values from the measured variables, a rooting productivity projection was carried out considering a 54 mother plants/m^2 density, resulting in a minimum average annual production of viable cuttings from 10,000 to 27,000 depending on the clone, with 2,000 to 18,000 cuttings with rooting possibilities per square meter.

Keywords: Asexual propagation, Clonal miniature garden, plant growth regulators.

INTRODUCTION

The *Eucalyptus* genus has a diversity of approximately 700 species. It is native to Australia and nearby islands and has industrial importance for cellulose production, lumber milling and chipboards. Their first forest plantations in Mexico date back to 1950, with less than 7,000 hectares planted with broadleaf species, mainly eucalyptus. The "Pulsar" company had a eucalyptus plantation with a 300,000 hectares area at Emiliano Zapata, Tabasco, Mexico (Martínez *et al*., 2006). The objective of this work was to evaluate the productivity of mother plants and rooting percentage of cuttings of *Eucalyptus grandis*, *Eucalyptus urophylla* and *Eucalyptus urograndis*.

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MATERIALS AND METHODS

Study Area Location

The study was developed in a nursery of the Proplanse S.A. de CV company, located at km 12.5 of the Balánca-El Triunfo highway, Balancán, Tabasco, Mexico (UTM coordinates, zone 15 Q 659601 m E and 1976492 m N) where a clonal miniature garden with mother plants of the *Eucalyptus* genus was established.

Background of the evaluated clones

In this research, ten different clones of *Eucalyptus grandis* (1), *Eucalyptus urophylla* (8) and *Eucalyptus urograndis* (1) obtained from selected trees in operational plantations and trials because of their outstanding productivity and economic interest, the characteristics of diameter and height of these trees are summarized in Table 1.

The clone capture process consisted of knocking down selected trees to promote sprouts emission, from these, vegetative material was collected. The cuttings were rooted and multiplied to obtain mother plants of the clonal miniature garden. A total sample population of n=130 individuals as an experimental unit (Table 2) was evaluated.

An analysis of variance (ANOVA) was performed on the assessed variables, the number of regrowths, number of viable cuttings and survival percentage, the value of the F statistics and its significance are shown. With mean comparison analysis with 5% TUKEY test to determine homogeneous or heterogeneous groups. For this, the STATISTIX 10 statistical software was used.

Mother plant productivity

The mother plants productivity data recollection consisted of counting the sprouts obtained per vegetative unit sampled from each clone, later the viable cuttings made from these sprouts were counted.

Table 1. Summary of the characteristics of the mother trees of *Eucalyptus* Labill.

Number	Material	Species	Source	Diameter (cm)	Height (m)
1	UP1	*E. grandis*	Arboretum, Balancán	47	31
2	G2	*E. urophylla*	Arboretum, Balancán	57	35
3	UG2	*E. urograndis*	Arboretum, Balancán	53	36
4	PC11	*E. urophylla*	V. Carranza, Huimanguillo	53	41
5	PC12	*E. urophylla*	V. Carranza, Huimanguillo	60	39
6	PC21	*E. urophylla*	V. Carranza, Huimanguillo	60	42
7	PC16	*E. urophylla*	V. Carranza, Huimanguillo	59	38
8	PC30	*E. urophylla*	V. Carranza, Huimanguillo	54	41
9	PC37	*E. urophylla*	V. Carranza, Huimanguillo	53	52
10	PC31	*E. urophylla*	V. Carranza, Huimanguillo	53	39

Table 2. Sample size of the experimental unit per evaluated clone.

Number	Clone	Species	Source	N	(n)
1	UP1	*E. grandis*	Arboretum, Balancán	38	15
2	G2	*E. urophylla*	Arboretum, Balancán	36	15
3	UG2	*E. urograndis*	Arboretum, Balancán	66	15
4	PC11	*E. urophylla*	V. Carranza, Huimanguillo	180	15
5	PC12	*E. urophylla*	V. Carranza, Huimanguillo	44	15
6	PC21	*E. urophylla*	V. Carranza, Huimanguillo	48	15
7	PC16	*E. urophylla*	V. Carranza, Huimanguillo	78	15
8	PC30	*E. urophylla*	V. Carranza, Huimanguillo	5	5
9	PC37	*E. urophylla*	V. Carranza, Huimanguillo	5	5
10	PC31	*E. urophylla*	V. Carranza, Huimanguillo	341	15
Total				841	130

Table 3. Days elapsed from cutting to next harvest.

Clone	Evaluation					
	I	II	III	IV	V	Average
UP1	19	21	21	20	21	20
G2	20	17	22	20	20	20
UG2	21	20	22	17	42	24
PC11	31	21	19	16	40	25
PC12	21	21	23	20	36	24
PC21	22	21	22	20	37	24
PC16	18	21	24	24	28	23
PC30	17	22	24	24	29	23
PC37	17	22	24	24	26	23
PC31	19	21	26	22	24	22
General average						23

Table 3 shows the number of days elapsed between each harvest per clone, obtaining a 23 d general average.

Growth regulator application and establishment of stakes

For the stimulation of adventitious roots in the cuttings made from the clonal miniature garden, a growth regulator treatment was carried out using exogenous auxins, in this case, AIB (Indole-3-butyric acid) in its RADIX™ 1500 commercial presentation, formulated as an impregnable powder with 0.15% as the active ingredient.

Irrigation Water Characterization

The management area for cuttings production has evolved in such a way that the spacing of the mother plants has been reduced. Higashi *et al*. (2004) mention the types of production systems and the distances between plants: field clonal garden (0.5 × 0.5 m), clonal miniature garden in gutters on a sand substrate (0.1 × 0.1 m) with drip irrigation system, clonal garden in 8 L plastic containers and mini clonal fiberglass garden (0.1 × 0.1 m). Higashi *et al*. (2002) point out that there is no nutrient solution for all plant species under growing conditions, the nutrients necessary for development are the same, but the amounts extracted differ between and within species. One of the factors determining the success of a clonal propagation operational program employing cuttings is the used nutrient solution in the miniature garden; the chemical properties of irrigation water (pH and Electrical Conductivity) influence the usage of nutritive elements. Silveira *et al*. (1999) cited by Higashi *et al*. (2002) recommend that the electrical conductivity should be in the range of 1.25 to 2.3 mS/cm for a hydroponic clonal miniature garden system and the pH between 5.8 and 6.0. However, positive results have been obtained with EC and pH outside these recommended ranges.

Table 4. Average values of the water quality evaluation obtained from the irrigation of a clonal miniature garden.

Evaluation	Productivity		pH water		C.E. (mS/cm)	
	Sprouts	Viable cuttings	average	Deviation standard	average	Deviation standard
I	8	18	5.48	0.709	0.009	0.008
II	7	15	4.61	0.942	1.092	0.927
III	11	21	4.26	0.849	0.636	0.420
IV	12	23	3.81	0.641	0.55	0.393
V	14	20	3.71	0.362	0.63	0.147

RESULTS AND DISCUSSION

During the evaluation period of the clonal miniature garden, the irrigation water parameters were taken, the values behaving differently in each evaluation (Table 4.).

Table 5 shows the number of viable shoots and cuttings produced by each mother plant.

Number of sprouts

During the trial period, with respect to the variable number of regrowth, an increasing trend in the amount of regrowth per individual of each clone was noted according to the age of the mother plant: in the evaluation I, the UP1 clone produced an average of 11 regrowths per individual, 21 regrowths produced on average in the intermediate evaluation (III) and the V evaluation with 28 regrowths on average per individual (Figure 1).

A possible response to this effect of increasing the number of regrowth was the nutrition applied in the clonal miniature garden and the effect of the type of pruning carried out in the regrowth crop. From the analysis of variance summarized in Table 5, three homogeneous groups were formed where the best group was constituted by the UP1 clone

Table 5. Analysis of variance of the measured variables in a miniature garden and means comparison per clone via the Tukey statistical test. Degree of significance: highly significant (<0.0001), averages with a common letter are not significantly different (p>0.05).

ClonE	Sprouts number	Viable cuttings number
UP1	17.947 ^{A}	34.05 ^{A}
G2	10.254 ^{BC}	23.29 ^{B}
UG2	12.120 ^{B}	22.96 ^{B}
PC11	7.929 ^{CD}	12.69 ^{D}
PC12	9.296 ^{BCD}	15.80 ^{CD}
PC21	9.581 ^{BCD}	16.59 ^{CD}
PC16	10.173 ^{BC}	18.83 ^{BC}
PC30	8.095 ^{BCD}	16.43 ^{BCD}
PC37	9.760 ^{BCD}	18.72 ^{BCD}
PC31	6.625 ^{D}	15.29 ^{CD}
F	19.78	22.00
P-VALUE	<0.0001	<0.0001

(A) with an average of 17.95 sprouts, followed by the UG2 clone with 12.12 sprouts (B) and group D constituted For clone PC31 with only 6.36 sprouts, the other clones were heterogeneous between B, C and D. Figure 2 shows the measured variables, observing clones such as G2 (median 10 sprouts), PC12 (median 9), PC21 (median 9) and PC31 (median 8), the lower whisker (0) indicate that during the evaluation period there were biases in some individuals and no regrowth was obtained.

Viable stem cuttings

Overall, obtaining viable stem cutting during the productivity evaluation behaved as follows: In the first evaluation, 18 viable stem cutting were obtained on average, 15 stem cutting in the second evaluation, and 21 viable stem cutting in the third evaluation and 23 viable stem cutting on average in the fourth evaluation which ended in December with 20 viable stem cutting. Figure 3 shows the behavior and trend of the number of regrowth variable of each clone according to the progress of the evaluation.

Three homogeneous groups formed in which the best group was the UP1 clone with an average of 34.05 viable cuttings (A), followed by the UG2 clone with average 22.96 viable cuttings (B) and group C by the PC12 clone (14.96 viable cuttings), PC31 (14.68 viable cuttings), PC30 (13.80 viable cuttings) and PC11 (11.84 viable cuttings); clones PC16, PC37, G2 and PC21 were heterogeneous (Table 6). Figure 4 summarizes the analyzed data by clone, the central tendency, and its means.

Wendling *et al*. (2003) obtained 5 to 7 cuttings per mini stain for each collection in higher production, and 1.2 to 3 in a lower production using four *Eucalyptus grandis* clones. Analysis of variance percentage of rooting

The results of the analysis of variance for the rooting percentage of the cuttings made of the mini garden are shown in Table 6, the values of F and their significance degree, with P<0.0001 showing highly significant differences. For verification, a Tukey's multiple comparison test was carried out (Table 6. Analysis of variance). Two homogeneous groups superior to the others were formed, where clone G2 reported 78.58%

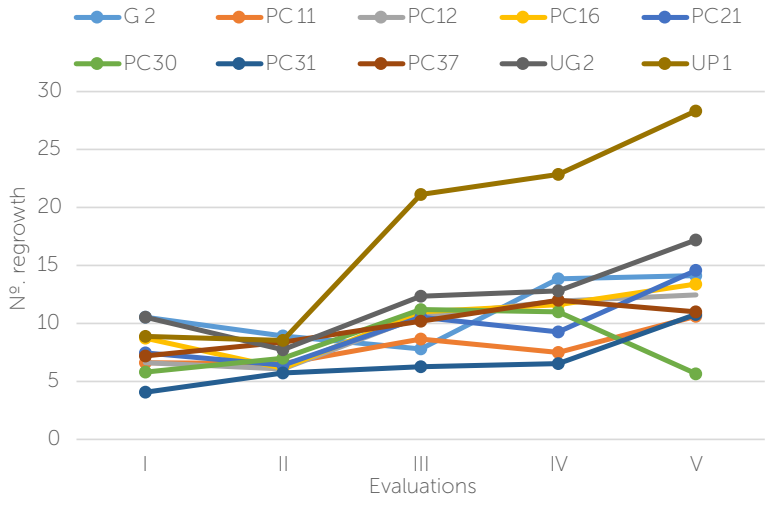

Figure 1. Average behavior of sprouts per clone according to the data collected during the evaluation period.

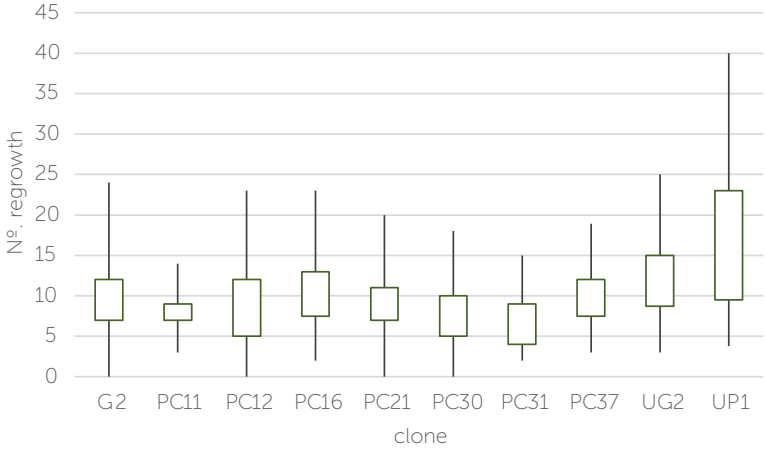

Figure 2. Number of sprouts per clone. Outliers represented by asterisks (*)

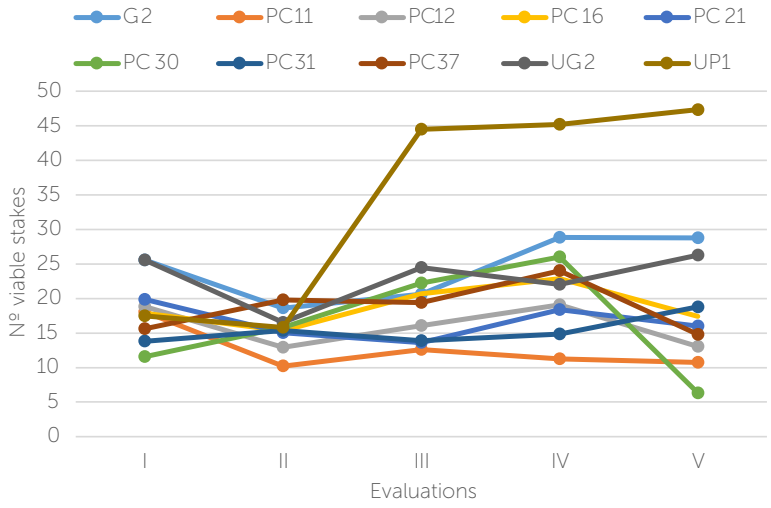

Figure 3. Behavior of the variable per clone for each evaluation.

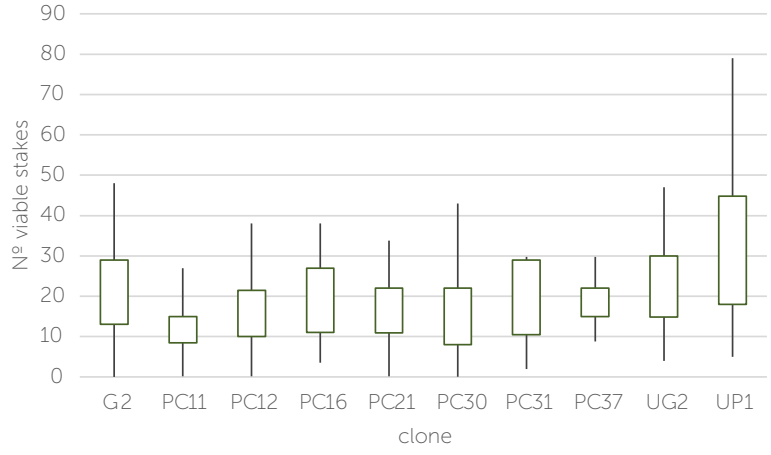

Figure 4. Box plot number of viable cuttings per clone

Table 6. Analysis of variance and Tukey's multiple comparison test (p≤0.05) of the rooting percentage.

Clone	Module	
	% rooting	CV (%)
UP1	68.75 ^{AB}	22.16
G2	78.58 ^{A}	13.44
UG2	68.00 ^{AB}	29.70
PC11	46.44 ^{ABC}	43.57
PC12	34.39 ^{BC}	41.85
PC21	33.32 ^{BC}	56.67
PC16	19.34 ^{C}	70.15
PC30	15.67 ^{C}	78.53
PC37	30.36 ^{C}	44.05
PC31	42.76 ^{ABC}	59.59
F	7.96	
p-value	<0.0001	

rooting (A), followed by clone PC37 (30.36%), PC30 (19.58%) and PC16 (19.34), in the heterogeneous group (A, B and C) constituted by clones UP1, UG2, PC11, PC31, PC12, PC21 with only 68.75, 68.00, 46.44, 42.76, 34.39 and 33.32 percent rooting.

According to Arnold *et al*. (1991) and Leakey (1985) the second preselection level occurs in clonal propagation, using only those clones with greater than 70% rooting capacities and according to the data obtained, the clones G2, UG2 and UP1 would pass (Figure 5).

Monitoring of pH and the irrigation water C.E. allows to assess their values and, where appropriate, correct them to adapt them to 0.5 mS/cm EC and pH 5 to 7, due to the relation with the mother plants intake, reflected in the number of sprouts and rooted cuttings. According to the obtained results and the criteria of a clonal operative program, clone G2 reported the adequate rooting percentage; However, UP1 and UG2 clones present an average close to the adequate, therefore, these three clones are suitable to start the clonal operating program of the Proplanse company; the values of the variables in the rooting phase are within the recommended range for clonal propagation utilizing cuttings.

CONCLUSIONS

The implementation of a clonal *Eucalyptus* miniature garden system with high cuttings productivity and

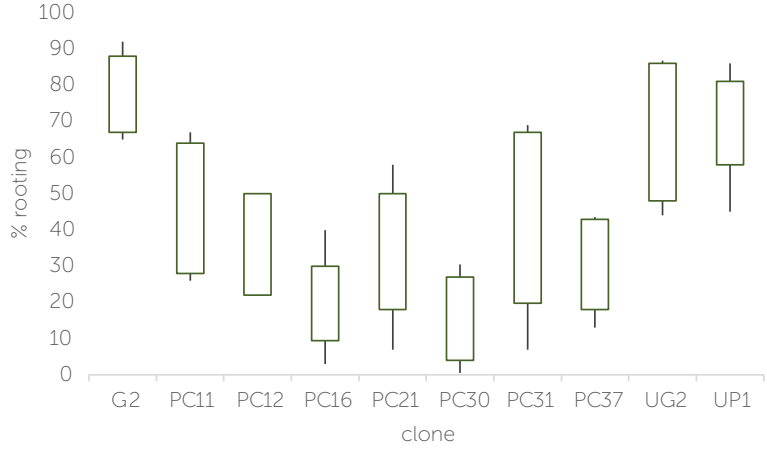

Figure 5. Rooting Percentage per clone, obtaining three highly significant clones; UG, UG2 and UP1.

good rooting capacity for seedling production is feasible. According to the analysis of the parameters measured in the clonal miniature garden, there is a relationship between viable cuttings and the number of shoots according to the clone, in such a way that three superior clones were identified (UP1 (17,947 shoots and 34.05 viable cuttings), UG2 (12,120 shoots and 22.96 viable cuttings) and G2 (10,254 shoots and 23.96 viable cuttings).

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Growth and nutritional value of the silage of two cultivars of *Pennisetum purpureum* at different ages

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ABSTRACT

Objetive: To evaluate the growth, chemical composition and quality of the silage of 45 d and 60 d cuts of *Pennisetum purpureum* sp. Gigante and *Pennisetum purpureum* cv. Taiwan grasses.

Design/methodology/approximation: The study took place at the Germplasm Bank of the Rosario Izapa Experimental Field in Tuxtla Chico, Chiapas, Mexico. Chemical analysis of the silage determined crude protein (CP), crude fat (CF), crude fiber (CF), ash (A), neutral detergent fiber (NDF), acid detergent fiber (ADF), and pH. Plant height, leaf length, leaf width and stem diameter were also determined. A completely randomized design was used with three repetitions per treatment as per PROC GLM and means comparison using the Tukey procedure.

Results: The CP content of both grasses decreased in the 45 d to 60 d period; from 7.18 % to 5.36 % in Taiwan grass and from 7.53 % to 6.05 % in *Pennisetum* sp. Gigante. Plant height, length and width of leaf, and stem diameter were greater in the Taiwan variety ($P<0.05$) at 30 d, 45 d and 60 d; the pH of the silage was similar in both grasses evaluated ($P>0.05$) at 45 d and 60 d.

Study limitations/implications: A review during low water periods is required (December-April).

Findings/conclusions: Taiwan grass registered a greater growth rate in all periods; while Gigante grass showed higher nutritional value at 45 and 60 days of cutting.

Key words: fodders, humid tropics, feed, bovines.

INTRODUCTION

Bovine production in tropical regions depends on the production of forage since it is the most affordable form of feed available (Animasaun *et al*., 2018). The prevailing way of raising cattle as livestock in Mexico occurs in tropical regions, where calves are raised for meat production, or for dual-purpose meat and dairy production. The cattle population for this

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purpose is 34 820 271 heads, and their diet is based on extensive grazing on a surface area of 77 842 256 ha which represents 23 % of the national territory (INEGI, 2018), comprised of 50 % native pastures and seasonal grazing sites, 25 % induced pastures, and the rest of introduced pastures.

One of the limiting factors that lead to low productivity is the deficient diet caused by the seasonality of forage production which is dependent on precipitation (Reyes *et al*., 2019). In most of the national territory rainfall is seasonal (June through October) (García, 2004), and the dry season extends from November through May during which the growth of forage species is limited and they have a lower nutrient content (May *et al*., 2011).

The use of cut fodders with higher productivity is a growing practice in livestock production systems in the tropics, but there is no consistent evidence showing this is the most appropriate or productive practice for the different agroecosystems (López *et al*., 2020). *Pennisetum purpureum* sp. is a South African species that was introduced into Mexico and which after a process of natural adaptation has a distribution from 0 m to 2,200 m altitude in various land types with the exception of flood lands and saline soils. This perennial grass grows erect, and is robust and vigorous. It has been used as cut grass fodder because of its greater growth, nutritional value and production of biomass per surface area as a result of its photosynthetic capacity, efficient assignation of carbohydrates to the leaves, and greater specific foliar area (Quero and Miranda, 2013), with varieties such as Taiwan, Gigante or Elephant, Kin grass, Merkeron and Napier, in addition to the cultivars recently introduced into the country like OM-22, CT-115 and Maralfalfa (López *et al*., 2011). Knowing the effect of environmental factors on adaptability and productivity of these grasses is important, and to this end an agronomic evaluation of morphological composition and physiological behavior throughout its biological cycle is necessary. Because of the growth characteristics of *P. purpureum*, it has been used as cut fodder, and the characteristics of growth, yield, and chemical composition have been evaluated (González *et al*., 2011; Nava *et al*., 2013; Hinojosa *et al*., 2014).

Ensilage is a technique used to take advantage of good quality forage during the productive season and to store it for later use (Gutiérrez *et al*., 2015). The nutritional value of the silage depends upon the nutritional value of the original forage material, and to preserve the initial quality as much as possible is the main objective of ensilage (Garcés *et al*., 2004). Success depends on the amount of fermentable sugars, the water content of the forage, and the carbohydrate:protein ratio; via the fermentation activity of lactic bacteria, the pH drops to approximately 4.0, which inhibits the growth of microorganisms that could cause silage decay (Vargas *et al*., 2014). Due to the above, the objective was to determine the growth characteristics and nutritional value of *Pennisetum purpureum* sp. Gigante and *Pennisetum purpureum* cv. Taiwan grass silage, at different cutting ages.

MATERIALS AND METHODS

Evaluation of grass growth took place at the Tropical Forage Germplasm Bank in the Rosario Izapa Experimental Field, run by INIFAP (14° 40' 16.1'' N, 92° 42' 59.1'' W) at 435 m altitude, with warm humid climate, summer rains, an average annual temperature of 25.2 °C and rainfall of over 4500 mm (Figure 1). The proximal chemical analysis of the silage of the grasses was carried out at the Animal Nutrition laboratory at the Southern University Center at Universidad de Guadalajara in Ciudad Guzmán, Jalisco, Mexico.

Growth evaluation. The study was performed during the months of July to November of 2018, on a plot of land with grasses established for approximately two years. A uniform cut of 30 cm was made, no fertilizer was applied, and weed control was performed manually at the time of uniform cutting. To carry out the evaluation, five cultivars (clusters) were chosen randomly on three 4 × 15 m plots of each grass and three plants were measured from each, making a total of 45 plants per variety of grass at 15, 30, 45 and 60 d from the uniform cutting.

Ensilage process. For the ensilage process, grasses harvested at 45 d and 60 d (five clusters per repetition) were crushed with a gasoline engine chipper, to a particle size of 1.5 cm. to 2.5 cm and deposited in 60 cm lengths of 4" diameter PVC tubing. The material was compacted with a mallet to eliminate oxygen and the tubes were hermetically sealed on both ends with plastic bags, and then placed horizontally on a dry surface in the shade. The silage material was retrieved at 30 d to make the corresponding observations. The samples were dried in a forced air furnace at 65 °C for 48 h, they were ground in a mill through a 2 mm sieve, placed in labeled plastic bags and sent to the laboratory for proximal chemical analysis.

Figure 1. Elephant grass (*Pennisetum purpureum* sp. Gigante) and taiwan grass (*Pennisetum purpureum* cv. Taiwan grasses).

Chemical analysis of the grass silage. Total dry matter (DM), crude protein (CP) via the Kjeldahl method, ether extract (EE) via the Soxhlet method, crude fiber (CF) via the Weende method, ash (A), and organic matter (OM) by difference – all through techniques described by the AOAC (2012) – were determined on the silage of Taiwan and Gigante varieties of grass harvested at 45 and 60 days of growth. The fiber fraction determinations (NDF and ADF) were made with alpha amylase without correcting for ash according to the specifications outlined by Van Soest *et al*. (1991).

Evaluated variables. The determinations of plant height (PH), leaf length (LL), leaf width (LW) and stem diameter (SD) were made 15, 30 and 60 days after uniform cutting. The plant height was measured from the base to the fold of the third leaf with a 3.0 m long measuring tape with 1.0 mm precision. The leaf length was determined from the insertion of the leaf blade with the sheath to the terminal apex. The leaf width was measured in the middle part of the leaf with a digital Vernier, and the diameter of the stem in the middle part of the third internode from the base using a digital Vernier.

Silage pH. To determine the pH, 10.0 g of a fresh sample were taken at 30 d of ensilage, placed in a 250 mL beaker; 100 mL of distilled water was added and it was agitated for 10 min on a magnetic plate at 300 rpm: After this time a pH reading was taken using an ORION® brand potentiometer calibrated to two pH values (4.0 and 7.0).

Experimental design and statistical analysis. A completely random design was used, two treatments (grass varieties) with three repetitions, each repetition with five clusters and three plants per cluster. The total height (cm), leaf length (cm), leaf width (mm), stem diameter (mm) and pH of silage were analyzed. The data obtained were analyzed via PROC GLM and the means comparison via the Tukey procedure (SAS, 2011).

RESULTS AND DISCUSSION

The crude protein content in the Gigante grass variety was 7.53 % at 45 d and decreased to 6.03 % at 60 d of age (Table 1). In the Taiwan grass the value decreased from 7.18 % to 5.36 % in the same period. The NDF content in the silage of the Gigante grass at 45 d of cutting was 36.92 %, and in the Taiwan grass 55.34 %, with a difference of 18.42 % more in this variety, while at 60 d of cutting the difference was 28 % more NDF in the Taiwan grass silage.

One of the principal factors that affect the nutritional quality of the fodders is the age of the plant, so that generally speaking the CP content decreases and the NDF content increases as the plant ages.

In a study performed by González *et al*. (2011), it was observed that the crude protein content decreased as the age of the plant increased in two cultivars of *P. purpureum* (Elephant), increasing the NDF content. As a result, harvesting between 35 d and 56 d of age is recommended, which is when the grass has the highest nutritional content and quality (López *et al*., 2020).

The CP content of the Taiwan grass reported by various authors is variable; it has been observed that it decreases as the cutting age increases. In regard to this, Araya and Boschini (2005) report 12.52 % at 70 d and 10.07 % at 98 d; 15.05 % and 12.27 % at six and eight weeks of age, respectively (Barrón *et al*., 2009); 14.9 % and 13.0 % at 42 d and 56 d respectively (González *et al*., 2011). The results obtained in this study are lower, but the same

tendency was observed, since the CP content decreased as the age of the plant increased. Inversely, the NDF content increases as the cutting age increases. In regard to this, Barrón *et al*. (2009) report 56.0 % and 66.83 % at six and eight weeks, respectively.

The pH results of the silage of Gigante and Taiwan grasses at 45 d y 60 d of cutting show that there was no difference between treatments (Table 1). The pH of the silage of the grasses evaluated at 45 d is found to be within the recommended values because there were pH levels below 4.5. Vargas *et al*. (2014) mention that pH is an indicator of silage quality, and the value should be between 3.8 and 4.5. The quality of silage obtained in this study was adequate, if pH and crude protein content are considered. In that regard, Duran (2007) mentions that the minimal analytical determinations necessary to evaluate the nutritional quality of the silage are pH and nutrient content; among these, crude protein levels should be higher than 7.0.

Table 1. Chemical composition (g 100 g^{-1}) and pH of *Pennisetum purpureum* sp. Gigante and *Pennisetum purpureum* cv. Taiwan silage at 45 and 60 day cuttings.

Component	45 days of cutting		60 days of cutting	
	Elephant grass	Taiwan grass	Elephant grass	Taiwan grass
Crude protein	7.53	7.18	6.03	5.36
Ethereal extract	2.75	2.72	2.26	2.13
Crude fiber	34.88	32.61	37.41	35.46
NDF	36.92	55.34	38.24	66.24
ADF	53.67	31.37	56.22	37.47
Ash	13.45	13.44	13.30	13.24
Silage pH	3.94a	4.13a	4.64a	4.10a
SEM	0.10		0.26	

FDN: neutral detergent fiber; FDA: acid detergent fiber. Same letters in the row are not significantly different; EEM: standard error of the mean. Initials in table are based on Spanish terms.

Table 2. Means comparison of total height, leaf length, leaf width, and stem diameter in *Pennisetum purpureum* sp. Gigante and *Pennisetum purpureum* cv. Taiwan grasses at four cutting ages.

Cut ages (days)	Elephant grass	Taiwan grass	SEM
Total height (cm)			
15	29.22a	45.40a	13.65
30	37.94b	85.56a	25.10
45	52.52b	143.54a	28.56
60	80.56b	199.02a	27.69
Leaf length (cm)			
15	27.12b	62.56a	16.07
30	42.02b	93.26a	12.50
45	50.86b	111.32a	11.36
60	55.82b	115.04a	10.91
Leaf width (mm)			
15	13.52b	23.10a	5.63
30	12.38b	24.30a	4.11
45	13.90b	29.36a	4.34
60	16.78b	33.88a	3.42
Stem diameter (mm)			
15	6.24b	12.72a	3.66
30	6.98b	18.67a	2.98
45	7.34b	19.08a	2.22
60	7.66b	19.28a	2.55

Same letters in the same row are not statistically different, according to the Tukey test; EEM: standard error of the mean.

The total height of the *Pennisetum purpureum* sp. Gigante and *Pennisetum purpureum* cv. Taiwan grasses at 15 days of age was similar (P>0.05); while at 30 d, 45 d and 60 d of cutting, it was greater in the Taiwan variety (Table 2). The values for the Taiwan grass obtained in this study are greater than those reported by Madera *et al*. (2013), who registered a height of 74.5 cm at 45 d regrowth from *Pennisetum purpureum* cv Morado, as well as those obtained by Vivas *et al*. (2019) at 1.50 cm plant height at 50 d of age; on the other hand, what was obtained from the Gigante grass was lower than that reported by the same authors, indicating that the Gigante grass has lower growth than the Taiwan grass at 30 d, 45 d and 60 d in the same growth conditions.

The leaf length at 15, 30, 45 and 60 d of cutting, was greater in the Taiwan grass (P<0.05); at 45 d the difference was 35.44 cm more in Taiwan, and 59.22 cm at 60 d, more than 100% greater in the Taiwan variety (Table 2). The length and width of the leaf are related to the leaf area index, and they are an indicator of light interception and photosynthetic capacity, since as the leaf length and width increase there is more light interception and greater photosynthetic capacity; these characteristics affect the plant's growth rate and the fodder material's storage capacity (Pérez *et al*., 2004). The leaf width of the Taiwan grass was greater than in the Gigante grass at 15, 30, 45 and 60 d (P<0.05). At 60 d in the Taiwan grass,

the value was higher in 101.9% (17.1 mm) with respect to the leaf width of the Gigante grass (Table 2).

Authors like Madera *et al*. (2013) observed that the leaf length of the purple Taiwan grass at 45 and 60 d was 60.0 cm and 62.5 cm, respectively; while the leaf width was 2.63 cm and 2.80 cm in the same time periods, respectively. Reyes *et al*. (2019) report the leaf length at 45 d of regrowth of *P. purpureum* cv. Morado grass was 85.6 cm, while in *Pennisetum* sp. Maralfalfa it was 78.7 cm and 96.4 cm, at 45 and 60 d respectively; in the same study, they report the leaf width at 45 and 60 d was 2.92 cm and 2.13 cm in the Morado grass, while in the Maralfalfa grass it was 2.03 cm and 2.46 cm in the same periods. The results of this study show that the leaf length and width of the Gigante grass were lower than those reported by the above-mentioned authors, while the values observed in the Taiwan grass were greater. The stem diameter was greater ($P<0.05$) in the Taiwan grass at 15, 30, 45 and 60 d (Table 2). The results obtained in this study are similar to those observed by Reyes *et al*. (2019) in *Pennisetum purpureum* cv. Morado grass, whose values were 16.0 and 16.3 mm at 45 and 60 d, respectively, while in values of 10.7 and 12.4 mm were found in *Pennisetum* sp. Maralfalfa grass in the same period.

CONCLUSIONS

The protein content depends upon the type of grass and age of cutting; as the age of the grass increased, the protein content decreased. The Taiwan grass showed a better adaptability to humid tropic conditions as was reflected in greater growth, while the Gigante grass showed greater nutritional value.

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Inventories in the sugar market in Mexico

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ABSTRACT

Objective: To determine the minimum inventory which guarantees the domestic consumption supply and sugar exports in Mexico. The hypothesis was that an optimal inventory would lower storage costs and increase the sugar producers' income.

Methodology: To achieve the objective a spatial and temporal equilibrium model applied to the Mexican sugar market for the 2015 sugar cycle was formulated.

Results: The sugar industry in Mexico maintains an average monthly inventory of 831 thousand tons of sugar, a high inventory for most of the year. The inventory level could decrease to 416 thousand tons, given that this level guarantees the supply of the domestic demand and exports in the assessed year.

Implications: A management policy that keeps sugar inventories at their minimum level allows for a reduction of storage costs by 594 million pesos (MXN) and increases the income of the sugar producers by 635 million pesos (MXN).

Conclusions: Due to the positive effects on the producer's income, it is recommended that the sugar sector promotes a minimum inventory policy.

Keywords: supply, demand, exports, temporal, spatial equilibrium model.

INTRODUCTION

Sugar is used as a raw material for certain products, this determines that it is one of the most important energy foods in the agri-food sector in Mexico. During the sugar cycle from October 2014 to September 2015, the apparent national consumption of sugar in Mexico was 4.41 million t (CONADESUCA, 2016a). Between 2008 to 2013, the annual per capita consumption of sugar fluctuated between 40 and 50 kg, higher than that of other basic products such as beans, rice and wheat, and was only surpassed by corn (FAO, 2017). With an average factory yield of 11.16%, sugar production during the 2015 sugar cycle was 5.98 million t. 73.6% of the total production supplied domestic consumption, and 26.4% was exported to the United States (CONADESUCA, 2016b). Sugar comes from the industrialization of sugarcane (*Saccharum* spp.) from 54 sugar mills distributed at the

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Gulf, South, West, Northeast and Central Mexico (1 in Campeche, 1 in Colima, 2 in Chiapas, 6 in Jalisco, 3 in Michoacán, 2 in Nayarit, 3 in Oaxaca, 2 in Puebla, 1 in Quintana Roo, 4 in San Luis Potosí, 3 in Tabasco, 2 in Tamaulipas, 2 in Sinaloa, 2 in Morelos and 20 in Veracruz). During the 2015 sugar cycle, the harvested sugar cane area was 785 thousand ha, from which production of 53.68 million t was obtained (CEMA, 2016). As it depends on the biological and climatic conditions, the production of sugarcane and sugar is seasonal. The sugar cycle begins in October of one year and ends in September of the next, establishing months where sugar availability is reduced, and others such as January, February, March and April, where it is common that production exceeds a million t (CONADESUCA, 2016b).

The seasonality of sugar production determines temporary excess supply that generates price volatility. Part of the sugar production is sold during March, April and May, at prices that reflect temporary excess supply. In addition to these excesses, Mexican sugar prices are volatile due to the inelastic demand that characterizes this good. For Mexico, the price elasticity of sugar demand is −0.12 (FAPRI, 2016), which indicates that it is highly inelastic respect to the changes in its price. Data from the SNIIM (2016) indicate that between 2011 and 2016, wholesale prices at the Iztapalapa Central de Market, in Mexico City, had volatility with maximum prices of 718 pesos per 50 kg bag, more than double the minimum price, which was 310 pesos. These low prices are due to the sugar production seasonality, determined by the biological and climatic conditions that affect the production of sugarcane in the field. Apparently, the sugar industrial sector in Mexico has had no interest in influencing prices, suggesting a perfect competition market.

Due to the seasonality of the sugarcane production and the consumption uniform distribution, it is necessary to store and manage sugar inventories. Data from CONADESUCA (2016b) indicate that inventories at the beginning (October) of the 2013, 2014 and 2015 harvests were 966, 1,460 and 831 thousand t, and their final inventories (September) were 1,460, 831 and 810 thousand tons, respectively. If the initial inventory is compared with the average monthly domestic consumption of sugar (367 thousand t), the high difference raises the question of whether the management of sugar inventories is optimal.

Two approaches to inventory management can be distinguished: the "push" and the "pull" (Ballou, 2004). The push approach estimates the demand, and based on this forecast the required inventory to satisfy the demand is calculated; a company must forecast the quantity of product that will be required to produce and be sold. A disadvantage of the push approach is that sales and demand forecasts are often not accurate and may lead to unwanted inventory build-up and high storage costs; however, high inventories have the advantage of meeting any unexpected increases in demand.

The pull approach maintains a minimum inventory because a company only produces what is demanded. The advantage of this approach is the low storage cost due to minimal inventory. The disadvantage been the risk of not being able to supply an unexpected increase in demand. The Mexican sugar sector appears to practice a "Push" inventory control, which generates high storage costs. The high level of inventories seems to relate to the high sugar production, which in turn depends on the production of sugarcane, to the consumption decrease due to the substitution of this good for high fructose corn syrup, and to the restrictions to increase exports. Considering the importance of sugar, this research formulates a model that replicates the functioning of the sweetener market, determining the minimum inventory that allows the supply of consumption and exports for the 2015 sugar cycle. The research hypothesis assumes that a policy that guarantees a minimum inventory would reduce storage costs and increase the income of the sugar producers.

MATERIALS AND METHODS

A spatial and temporal equilibrium model was used to the sugar market for the 2015 sugar cycle that considers the production, consumption and exports of standard and refined sugar, the distribution of production, the supply of consumption and warehouse. The model formulation was based following García-Salazar and Skaggs (2015) and Borja-Bravo *et al.* (2013). Assuming i (i=1,2.. H=54) standard sugar mills, s (s=1,2.. S=54) refined sugar mills, j (j=1,2.. J=32) standard sugar markets, d (d=1,2..D=32) refined sugar markets, e(e=1,2...E =12) ports of exit for exports, and t (t=1,2..T=12) time periods, the model is:

$$MaxNSP = \sum_{t=1}^{T} \pi^{t-1} \sum_{j=1}^{J} \left[\lambda_{jt} y_{jt} + \frac{1}{2} \omega_{jt} y_{jt}^2 \right] + \sum_{t=1}^{T} \pi^{t-1} \sum_{d=1}^{D} \left[\lambda_{dt} y_{dt} + \frac{1}{2} \omega_{dt} y_{dt}^2 \right] + \sum_{t=1}^{T} \pi^{t-1} \sum_{e=1}^{E} \left[p_{et}^{e} x_{et}^{e} \right]$$
$$+ \sum_{t=1}^{T} \pi^{t-1} \sum_{e=1}^{E} \left[p_{et}^{r} x_{rt}^{r} \right] - \sum_{t=1}^{T} \pi^{t-1} \sum_{i=1}^{I} \left[v_{it} x_{it} + \frac{1}{2} \eta_{it} x_{it}^2 \right] - \sum_{t=1}^{T} \pi^{t-1} \sum_{s=1}^{S} \left[v_{st} x_{st} + \frac{1}{2} \eta_{st} x_{it}^2 \right]$$
$$- \sum_{t=1}^{T} \pi^{t-1} \sum_{i=1}^{I} \sum_{i=1}^{J} \left[p_{ijt}^{c} x_{ijt}^{c} \right] - \sum_{t=1}^{T} \pi^{t-1} \sum_{i=1}^{I} \sum_{i=1}^{J} \left[p_{ijt}^{f} x_{ijt}^{f} \right] - \sum_{t=1}^{T} \pi^{t-1} \sum_{i=1}^{I} \sum_{e=1}^{E} \left[p_{iet}^{c} x_{iet}^{c} \right]$$
$$- \sum_{t=1}^{T} \pi^{t-1} \sum_{i=1}^{I} \sum_{e=1}^{E} \left[p_{iet}^{f} x_{iet}^{f} \right] - \sum_{t=1}^{T} \pi^{t-1} \sum_{s=1}^{S} \sum_{d=1}^{D} \left[p_{sdt}^{c} x_{sdt}^{c} \right] - \sum_{t=1}^{T} \pi^{t-1} \sum_{s=1}^{S} \sum_{d=1}^{D} \left[p_{sdt}^{f} x_{sdt}^{f} \right]$$
$$- \sum_{t=1}^{T} \pi^{t-1} \sum_{s=1}^{S} \sum_{e=1}^{E} \left[p_{set}^{c} x_{set}^{c} \right] - \sum_{t=1}^{T} \pi^{t-1} \sum_{s=1}^{S} \sum_{e=1}^{E} \left[p_{set}^{c} x_{set}^{c} \right]$$
$$- \sum_{t=1}^{T} \pi^{t-1} \sum_{i=1}^{I} \left[p_{it,t+1} x_{it,t+1} \right] - \sum_{t=1}^{T} \pi^{t-1} \sum_{s=1}^{S} \left[p_{st,t+1} x_{st,t+1} \right] \quad 1)$$

The target function is subjected to the following restrictions:

$$x_{it} + x_{it-1,t} - x_{it,t+1} \geq \sum_{j=1}^{J} \left[x_{ijt}^{c} \right] + \sum_{j=1}^{J} \left[x_{ijt}^{f} \right] + \sum_{e=1}^{E} \left[x_{iet}^{c} \right] + \sum_{e=1}^{E} \left[x_{iet}^{f} \right] \quad 2)$$

$$x_{st} + x_{st-1,t} - x_{st,t+1} \geq \sum_{d=1}^{D} \left[x_{sdt}^{c} \right] + \sum_{d=1}^{D} \left[x_{sdt}^{f} \right] + \sum_{e=1}^{E} \left[x_{set}^{c} \right] + \sum_{e=1}^{E} \left[x_{set}^{f} \right] \quad 3)$$

$$\sum_{i=1}^{I} \left[x_{ijt}^{c} \right] + \sum_{i=1}^{I} \left[x_{ijt}^{f} \right] \geq y_{it} \quad 4)$$

$$\sum_{i=1}^{I} \left[x_{iet}^{c} \right] + \sum_{i=1}^{I} \left[x_{iet}^{f} \right] \geq x_{et}^{e} \quad 5)$$

$$\sum_{s=1}^{S} \left[x_{sdt}^{c} \right] + \sum_{s=1}^{S} \left[x_{sdt}^{f} \right] \geq y_{dt} \quad 6)$$

$$\sum_{s=1}^{S} \left[x_{set}^{c} \right] + \sum_{s=1}^{S} \left[x_{set}^{f} \right] \geq x_{et}^{r} \quad 7)$$

$$\sum_{e=1}^{E} \left[x_{et}^{e} \right] = xa_t \quad 8)$$

$$\sum_{e=1}^{E} \left[x_{et}^{r} \right] = xb_t \quad 9)$$

$$\sum_{i=1}^{I} \sum_{j=1}^{J} \sum_{t=1}^{T} \left[x_{ijt}^{f} \right] + \sum_{i=1}^{I} \sum_{e=1}^{E} \sum_{t=1}^{T} \left[x_{iet}^{f} \right] + \sum_{s=1}^{S} \sum_{d=1}^{D} \sum_{t=1}^{T} \left[x_{sdt}^{f} \right] + \sum_{s=1}^{S} \sum_{r=1}^{R} \sum_{t=1}^{T} \left[x_{srt}^{f} \right] = w \quad 10)$$

$$\left[\sum_{t=1}^{T} \left[x_{et}^{e} \right] + \sum_{t=1}^{T} \left[x_{et}^{r} \right] \right] = q_e \quad 11)$$

$$x_{i12,13} = x_{i0,1} \quad 12)$$

$$x_{s12,13} = x_{s0,1} \quad 13)$$

$$y_{jt}, y_{jt}, x_{it}, x_{st}, \ldots\ldots x_{st,t+1} \geq 0 \quad 14)$$

Where for month t: $\pi^{t-1} = (1/1+i_t)^{t-1}$ is the discount factor with i_t equal to the inflation rate; λ_{jt} and λ_{dt}, is the intercept of the standard and refined sugar demand function in j and d; y_{jt} and y_{dt} is the amount of sugar consumed in j and d; ω_{jt} and ω_{dt} is the slope of the demand function in j and d; p^e_{et}, x^e_{et}, p^r_{et} and x^r_{et} is the international price and the exported quantity of the sugar per e; v_{it} and v_{st} is the intercept of the sugar supply function in i and s; x_{it} and x_{st} is the amount of produced sugar in i and s; η_{it} and η_{st} is the slope of the supply function for sugar at i and s; p^c_{ijt}, x^c_{ijt}, p^f_{ijt} and x^f_{ijt} are the costs of transportation by truck and rail and the quantity of sugar shipped from i to j; p^c_{iet}, x^c_{iet}, p^f_{iet} and x^f_{ijt} are the costs of transportation by truck and rail and the quantity of sugar shipped from i to e; p^c_{sdt}, x^c_{sdt}, p^f_{sdt} and x^f_{sdt} are trucking and rail transportation costs and quantity of sugar shipped from s to d; p^c_{set}, x^c_{set}, p^f_{set} and x^f_{set} are trucking and rail transportation costs and quantity of sugar shipped from s to e; $p_{it,t+1}$, $p_{st,t+1}$, $x_{it,t+1}$ and $x_{st,t+1}$ is the warehouse cost and the amount of sugar stored in i and s from t to $t+1$; xa_t and xb_t is the national quantity of exported sugar at t; w is the annual national shipment of sugar by rail; q_e is the exported quantity of sugar sent by e.

The target function maximizes the Net Social Payoff (NSP) and is equal to the area under the sugar demand curve, plus the value of exports, minus the area under the supply curves, minus the costs of transportation and storage. Constraints 2 and 3 indicate how sugar production is distributed. Constraints 4, 5, 6 and 7 indicate how consumption is supplied. Restrictions 8 and 9 indicate that the sum of sugar exports made by all ports of departure is equal to the observed monthly exports. Restriction 10 establishes a limit to mobilizations carried out by rail, and restriction 11 a limit to total sugar exports by port. Constraints 12 and 13 indicate that the final inventories are equal to the initial ones, and restriction 14 establishes the non-negativity conditions. The model considered all sugar consuming regions (31 states and Mexico City), 54 sugar mills and 12 exit points for exports. The minimum inventory scenario was defined as follows: a) First, the base model was set for the 2015 sugar cycle and; b) The assessed scenario consisted of reducing the initial inventory of standard and refined sugar, until a minimum that allowed the supply of the internal consumption and exports was determined for the cycle.

The model used monthly information for the 2015 sugar cycle. The supply and demand functions were calculated using the price elasticity, producer and consumer prices, and the quantities of sugar produced and consumed. The elasticities were taken from FAPRI (2016), sugar production by mill came from CONADESUCA (2016a), and the consumer price of sugar from SNIIM (2016). The producer price per mill was estimated by subtracting the cost of transportation from the consumer's price from each production area to the potential consuming regions. Sugar consumption was estimated as follows: a) The states monthly consumption of standard sugar was obtained by multiplying the monthly national consumption of standard sugar by the participation of each state in the population; b) The monthly state consumption of refined sugar was obtained by multiplying the monthly national consumption of refined sugar by the state's share of the value of the production of soft drinks and their biscuit industry. The assessed information came from CONADESUCA (2016a), INEGI (2014) and INEGI (2010).

The international price of sugar corresponds to the monthly average price of futures contracts, 11 listed in New York (CONADESUCA, 2016a). The information on the monthly exported quantity of sugar came from CONADESUCA (2016a). Exports distributed by port were obtained from SIAP (2016). To calculate the international price in pesos (MXN), the exchange rate was used (CONADESUCA, 2016a). The wholesale prices of sugar from SNIIM (2016) in the 32 cities of the country were used to derive the producer prices.

The transportation costs were calculated with a fixed factor and a variable one that depends on the distance (García-Salazar *et al.*, 2005). Distance matrices were constructed from the mills to the markets and export ports. The fixed and variable factors for the railway came from the SCT (2016), for trucks were estimated. A function was used where transport costs and distance are the dependent and independent variables, the necessary information came from transport companies that trade sugar. The cost of storage considered the cost of entry and exit maneuvers and monthly insurance.

RESULTS AND DISCUSSION

The 2015 sugar cycle data indicate that production, consumption and exports were 5.985, 4.408 and 1.581

million tons, respectively. Out of the total production, 70.4% was standard sugar and the remaining 29.6% refined sugar. 75.3% of consumption corresponded to standard sugar and 24.7% to refined sugar. For exports, 38.9% was refined and 61.1% standard sugar (Table 1).

Sugar production was seasonal, 13.6% was obtained during December, 16.2% during January, 17.3% in February, 16.7% in March and 16.7% in April; in August and September there was no production. The sugar production seasonality relates to the sugar cycle, which depends on the sugarcane seasonality production. With a monthly average of 367 thousand tons, sugar consumption showed a slightly seasonal behavior, since the demand registered in December, February, March and April was greater than 10% of the total consumption; during each of these months, sugar production was greater than 800 thousand t. The average monthly exports were 132 thousand t, and the distribution throughout the year was not uniform, since 13.6, 14.1, and 11.0% of external sales were made during July, August and September, months which have low production.

Temporary oversupply is observed at the peak production months such as December, January, February, March and April. With a monthly average of 1.497 million tons, the inventory variation was variable throughout the year; November had the lowest level of production with 406 thousand t, and May the highest with 2.590 million t (Table 1).

To validate the base model, the observed inventories were compared with the estimated ones; small differences are observed between the two, barely 2.3% in November, hence, the base model can be used to carry out scenarios. The results of the model (Table 2) indicate that during the 2015 sugar cycle the economic surplus of the sugar market was 283,741 million pesos. Exports were valued at 12,502 million pesos, transportation costs from the production areas to the domestic markets and ports were 1,967 and 839 million pesos, while storage costs amounted to 2,128 million pesos. The NSP in the baseline scenario was 291,309 million pesos, and the consumption expenditure (for domestic sales) and producers' income were 40,735 and 48,303 million pesos.

Table 1. Sugar production, consumption, exports, and inventories during 2015. Thousands of tons.

Variable	ININ	oct	nov	dec	jan	feb	mar	apr	may	jun	jul	aug	sept	Annual
Observed values from October 2014 to September 2015														
PSS		2	168	608	721	741	701	681	453	128	8	0	0	4,211
PRS		1	59	205	249	292	301	316	267	78	7	0	0	1,774
SSC		259	189	337	282	315	326	304	287	323	241	224	232	3,320
RSC		68	72	116	98	128	118	141	112	102	13	65	55	1,089
SSE		16	9	13	69	124	86	121	144	82	114	123	65	967
RSE		23	10	10	12	28	60	49	58	53	102	100	110	615
Estimated values with the shipments and reception of sugar obtained from the base model														
PSS		1	168	608	721	741	701	681	453	128	9	0	0	4,211
PRS		1	59	205	249	292	301	316	267	78	7	0	0	1,774
SSC		259	189	337	282	315	326	304	287	323	241	224	232	3,320
RSC		68	72	116	98	128	118	141	112	102	13	65	55	1,089
SSE		16	9	13	69	124	86	121	144	82	114	123	65	967
RSE		23	10	10	12	28	60	49	58	53	102	100	110	615
Observed inventory and estimated inventory with the base model														
Obs. Inv.	831	462	406	741	1,246	1,677	2,090	2,471	2,590	2,236	1,783	1,271	811	17,784
Est. Inv.	831	468	416	751	1,260	1,696	2,108	2,490	2,608	2,254	1,800	1,287	827	17,965
Dif. (%)	0	1.5	2.3	1.4	1.1	1.2	0.9	0.7	0.7	0.8	0.9	1.3	2.0	1.0
Inventories in the minimum inventory scenario														
Scenario	416	53	0	336	844	1,281	1,693	2,075	2,193	1,838	1,384	872	411	12,982

ININ=Initial inventory; PSS and PRS=Production of standard sugar and production of refined sugar; SSC and RSC=Standard sugar consumption and refined sugar consumption; SSE and RSE=Standard sugar exports and refined sugar exports.

Table 1 shows that initial inventories could decrease from 831 to 416 thousand t, guaranteeing the consumption and export supply. If the above happens, annual inventories (sum of monthly inventories) may decrease from 17.97 to 12.98 million t; the monthly average inventory would be 1.08 million t and would be zero during November. Its maximum value would occur during May with 2.19 million t.

The reduction of the inventories to the minimum would not affect the economic surplus, nor on the exportation value. Transportation costs to move the sugar production would decrease by 15 million pesos due to lower inventories which determine a lower availability of the product and, therefore, an adjustment in trade flows. Transportation costs to move sugar from the mills to ports would also decrease by 26 million pesos due to the changes that would take place in logistics. The cost of storage would decrease by 594 million pesos, increasing the producer's income by 635 million pesos. The net result of the scenario would be positive, since the inventories decrease to a minimum would improve the social welfare, due to the generation of more SNV, which would increase by 635 million pesos; an increase of 0.2%, compared to the base model.

Based on the above results, a policy that promotes the location of inventories at their minimum level is recommended. In the analyzed year, the level of observed inventories is high, reflecting unnecessary storage costs. Sugar producers will surely take better profits if they manage to reduce part of the unnecessary inventories to achieve a satisfactory supply of domestic consumption and exports.

CONCLUSIONS

The formulation of a spatial and temporal equilibrium model of the sugar market made it possible to determine a minimum inventory that would be required to supply both, domestic consumption, and exports of the sweetener for the 2015 sugar cycle. The inventory could decrease to 400 thousand tons and guarantee the supply; this would make it possible to considerably reduce the storage costs and improve the sugar producers' income.

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Table 2. Effects of a minimum inventory on the Mexican sugar market. Millions of pesos (MXN) and thousands of tons.

Scenario	ES	VE	IRTC	CTER	SC	NSP	CE	PI	INV
	Millions of pesos (MXN)								Thousands of tons
Base sit.	282,833	12,504	1,967	839	2,117	290,414	40,817	48,398	17,965
Base sit.†	283,741	12,502	1,967	839	2,128	291,309	40,735	48,303	17,965
Dif. (%)	0.3	0.0	0.0	0.0	0.5	0.3	-0.2	-0.2	0.0
Scenario	283,741	12,502	1,952	813	1,534	291,944	40,735	48,938	12,982
Change	0	0	−15	−26	−594	635	0	635	−4,983
Change in %	0.0	0.0	−0.8	−3.1	−27.9	0.2	0.0	1.3	−27.7

† Using estimated price. ES=Economic surplus; VE=Value of exports; IRTC=Internal route transportation costs; CTER=Cost of transportation of external routes; SC=Storage costs; NSV=Net Social Value; CE=Consumption expenses; PI=Producer's income; INV=Inventory.

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Tourism awareness as a tourism development strategy: status of the issue

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ABSTRACT

Objective: Tourism has become the main engine of economic, social and environmental development in several countries, so promoting tourism awareness among tourists and the local population should be a priority. The present study aims to suggest a status of the research carried out on the topic of tourism awareness.

Design/methodology/approach: The type of analysis is through a retrospective and exploratory bibliometric study. The analysis materials were scientific articles and a training manual published between 2000 and 2020, registered by Scopus, Emerald insight and Dialnet, using "tourism awareness" as the keyword.

Results: When considering the three senses in which tourism awareness ought to operate, it is concluded that studies are more focused on the relationship and contact of the host community with the tourist. It is observed that four out of six articles in this sense consider that education, training, and government policies around tourism awareness should be developed in a better way in the destinations, in order to be an element that contributes to the development of communities and reduces poverty in developing countries.

Study limitations/implications: It is considered a limitation not to include thesis dissertations.

Findings/conclusions: It is necessary to make visible the importance of tourism awareness as a local development strategy for communities, in addition to including tourism awareness on the part of tourists.

Keywords: tourism, local development, sustainability, identity.

INTRODUCTION

Mexico, as a country, has stood out for having exceptional natural and cultural resources in its territory, and for this reason it has become an international benchmark when it comes to tourism. Much of this wealth is also constituted by its inhabitants, who become the factor that gives quality and warmth to all tourism activities that take place in different destinations. The arrival of international tourists worldwide has increased, from the 25 million registered in 1950 to 1,500 million in 2019, which represents an increase of 4% in tourist activity compared to 2018. In the case of Mexico, the country ranks seventh within the top ten destinations that receive 40% of global arrivals, with France, Spain and the United States being the countries that register the first places, respectively (Organización Mundial del Turismo, 2019).

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Taking into account the above figures, the importance of tourism will continue to grow since it is considered a factor of economic, environmental and sociocultural development. In this sense, faced with the challenges of improving the tourist experience for visitors, it becomes more important to develop the social aspect, strengthening tourism awareness in the host communities and in the tourists themselves, since on these depends, to a large extent, the return and recommendation of visitors. According to the Real Academia Española de la Lengua (2019), among the definitions of awareness there are: "Knowledge of good and evil that allows the person to morally judge reality and acts, especially their own" and "Moral or ethical sense that is characteristic of a person". Therefore, it can be established that awareness is that internal voice that allows each person to decide on their actions, good or bad, taking into account their previously learned morals and ethics; likewise, the collective awareness becomes more important by representing a factor of identity and sense of belonging among the population. Now, this awareness can be applied in many ways; however, if it applied in the field of tourism, it is defined as the way of acting in a positive way for the benefit of the tourist and the local population. In addition, it is identified as a viable option to preserve and rescue the natural and cultural heritage that they shape; on the one hand, the identity of the local community and on the other, the main motivation for the shift of tourism flows toward a destination. Based on the above, the objective of this study was to analyze the research carried out on the topic of tourism awareness, in order to show the importance it has for the development of tourism activities at various levels.

MATERIALS AND METHODS

The type of analysis is by means of a retrospective bibliometric study and exploratory in nature. The design was non-experimental, cross-sectional. The analysis materials were 12 scientific articles and a training manual published between 2000 and 2020, since they were the only ones found in the Scopus, Emerald insight and Dialnet search engines, using "tourism awareness" as the keyword.

RESULTS AND DISCUSSION

Speaking about tourism is very complex and defining it is even more so; however, tourism scholars and some national and international institutions have worked to identify tourism as a field of academic and scientific study based on its recognition as a complex phenomenon in its economic, environmental and sociocultural dimensions (Monterrubio, 2015). For instance, the World Tourism Organization is taken up as an international body in charge of promoting tourism, which it defines as "the activities that people carry out during their trips and stays in places other than their usual environment, for a consecutive period of time of less than a year, for leisure purposes, and other reasons not related to the exercise of a paid activity in the place visited" (OMT, 1998). In the same context, but from a National Institution, the Tourism Ministry in Mexico mentions that tourism "is the momentary movement that people make including the actions they carry out during their trips and stay outside their usual environment" (SECTUR, s/f). Although it is true that these definitions are part of two important institutions at the international and national level, in addition to being references when talking about tourism, they do not show the social side of it, focusing only on economic aspects from the activities carried out by the tourists, forgetting about the human part of tourism. From this perspective, the definition of Goeldner and Ritchie (2003) in Monterrubio (2005) mentions that: "tourism can be defined as the processes, activities and results generated from the relationships and interactions between tourists, tourism providers, host governments, host communities and environments that are involved in serving and receiving visitors". This definition focuses on two important elements that make up the tourism phenomenon: tourist and host community, and therefore, the reason for there to be "tourism awareness", since this can be the mediating element for the interaction between these two elements to be carried out successfully.

Authors such as Ramírez (1994) mention that tourism awareness is the mental attitude that should regulate our individual and collective acts, operating in three senses: the first addresses the relationship and contact of the receiving community with the tourist; the second, facing service providers; and finally, with regard to the conservation of resources susceptible to be exploited by tourism. Therefore, this tourism awareness focuses on the positive attitude that a person must have in the humane and kind treatment of the tourist, in the services offered, and in relation to the conservation and enhancement of the tourism heritage (Mireles, 2015).

This tourism awareness occurs to the extent that the host community understands the benefits that it will obtain from the economic use of tourism (Ibánez and Cabrera, 2011). Likewise, SECTUR (2019) mentions that tourism awareness refers to attitudes and behaviors of the inhabitants of a touristic place, which humanize the reception of tourists through hospitality and understanding, a definition that allows assessing the importance of humanizing tourism activities so that there is real understanding between the local community and tourists.

Based on the previous concepts, it can be identified that tourism awareness is focused on the host community, service providers, and more commonly, on nature conservation; however, it is important to highlight the importance of mentioning the "tourist" in these definitions as another element of tourism awareness, since a good relationship between the local community and the tourist depends largely on the tourism awareness they have, thus managing to identify whether he or she is a "responsible tourist", considering it as a traveler who understands his or her responsibility to the planet and seeks to create a positive impact on the destination, bring benefits to the local population, and not deplete resources (Instituto de Turismo responsible, 2018).

Relationship and contact of the host community with the tourist:

The study by Timothy (2000) titled "Building Community Awareness of Tourism in a Developing Country Destination" highlights the importance of creating public awareness about tourism in the various sectors the population, as it represents a development strategy for their country, whether they are part of the decision-making process or only benefit from the tourism spillover. In both senses, the population is required to know about tourism, its sociocultural and environmental impacts, but also how to mitigate them. Thus, there will be a population with more power and capable of deciding the future of the local tourism industry.

Under the same section, but written ten years later, is the study by Saarinen (2010) "Local tourism awareness: Community views in Katutura and King Nehale Conservancy, Namibia", where the author mentions Namibia's policy of sensitizing local communities about tourism so that they become agents of tourism development and not just mere objects, so it is considered that tourism awareness is beneficial for processes of empowerment and poverty reduction. However, the author recognizes that this issue has not been widely studied and highlights that this is one of the great challenges in the development of tourism.

In this sense, it would be necessary to promote the empowerment of communities, which implies the expansion of the goods and capacities of the population to participate in, negotiate with, influence, control and hold accountable the institutions that affect people's lives (World Bank, 2002). Therefore, it is necessary for the local population to have ***access to information*** to know the tourism potential of their resources, as well as ***inclusion and participation*** of excluded groups for decision-making, ***responsibility*** of federal, state and local governments in order to be accountable for the decisions made which affect the wellbeing of the population, and to build the ***local organizational capacity***. Van Niekerk and Saayman (2013) with the study, "The influences of tourism awareness on the travel patterns and career choices of high school students in South Africa" mention that there should be tourism awareness tools to develop tourism awareness in the community and make society become more involved in the tourism product. One of those used was to introduce tourism as a secondary school subject in 2001, in such a way as to sensitize the younger generations, and indirectly, also parents. Through this strategy, it is possible to better understand the dynamics of tourism activities, change attitudes and behaviors toward tourists, have a reflective knowledge of tourism, and the importance of taking advantage and disseminating its natural and cultural resources in a sustained way.

The study by Kouassi and Maroto (2016), on Tourism Education, Training and Awareness in Ivory Coast, mentions that without education it is difficult to promote a tourism awareness that allows the growth of tourism equitably. Therefore, they mention a model of tourism which they aspire to reach (responsible tourism).

This type of tourism implies a commitment to the local community, its cultural heritage, environment and natural heritage, so responsibilities must be assumed and demanded from all the actors involved in tourism. Emphasis is placed on the role played by the State, since it is in charge of defining the tourism development policy, as well as the prior training of human resources so that true tourism awareness can be contemplated.

A study carried out in Mexico by Sánchez and Cruz (2017), titled "International hiking and kidnapping on the border with Mexico" (***Excursionismo internacional y secuestros en la frontera de México***), 1997-2016, analyzes the relationship between kidnappings and hikers, concluding that kidnappings cause a decrease in their arrival. This research takes up the theme of tourism awareness to point out that it is a factor closely linked to the concept of tourism ambiance or environment, which is conceived as a set of attitudes that the local community shows to visitors and which is directly influenced by the socioeconomic benefits spilled toward the host population. Thus, an appropriate tourism climate, fostered by tourism awareness in the population can directly contribute to the image of tranquility and security that the community should offer to the visitor. The work carried out by Ruíz and Pozo (2020), who developed the theme of tourism awareness of inhabitants in the district of Rímac-Peru, determined the level of tourism awareness of the inhabitants taking into account three aspects: the attitude of the inhabitant toward the tourist, the cultural identity of the inhabitant, and the participation of the inhabitants in tourism. It is the most recent study that takes up the theme of tourism awareness; however, their results are not very encouraging, since they mention that the population of the Rimac district has a medium level of tourism awareness (attitude), which indicates that the population recognizes the importance of tourism. In terms of identity, the level is high given the magnitude of natural and cultural resources it has. Finally, with respect to the participation of the population, a low level was found as they do not assume responsibilities in the management of tourism activities, which is a great challenge if what you want is to include the community in tourism development, even if it is not directly benefited from tourism (Ruíz and Pozo, 2020), and according to the results, the importance of empowering communities is once again shown, so that they are informed, included, and take responsibility for their own development.

Facing tourism service providers

According to the Training Manual: Tourism Awareness Program in the country of Chile, whose objective is for the population to identify the role to be assumed regarding tourism (tourists, hosts, tourism industry) (SERNATUR, 2018), is part of an initiative that could be replicated in other countries and be part of an investigation. It is presented as a reference text for the training carried out within the framework of the Program. It mentions what tourism is, the importance of tourism in the country, as well as assessing the different areas of tourism and its inhabitants. It can be recovered that this manual instructs tourism service providers and the general population (if they have access to the document) to be "conscious tourists" when they have to play that role, since it mentions that when traveling, one becomes an ambassador of the place of origin. At the end of this document, there is a section called "Know your country", which allows you to learn about the natural and cultural attractions of the country, highlighting that in order to know other countries you must first know your own. It should be noted that this document is the only one that briefly includes tourism awareness by the tourist, a situation that is important to highlight since the General Assembly of the United Nations declared 2017 the International Year of Sustainable Tourism to emphasize how the tourist and in general the tourism sector can contribute to the fulfillment of the Sustainable Development Goals.

The World Tourism Organization (2017) launched a campaign called "Travel Enjoy Respect" by developing the manual of "Practical Tips for the Responsible Traveler" as a strategy to raise awareness about the value and contribution of tourism in host communities, based on ***La Carta del Turismo y El Código del Turista*** (OMT, 1985) and the ***Código Ético Mundial para el Turismo*** (OMT, 1999). Likewise, the Institute for Responsible Tourism (*Instituto de Turismo Responsable*, 2018), published the Responsible Traveler Manifesto so that tourists know, consume, enjoy and value their travel experiences in a sustainable way.

The study carried out on the assessment of the rural lodging service in Moctezuma, Sonora, Mexico, by Alcaraz *et al*. (2018), does not focus entirely on the issue of tourism awareness since its main objective is to evaluate the quality of the service through the "quality service" method. However, it is taken up again briefly, since in the research results it is mentioned that one of the weaknesses of this establishment is the lack of training, education and tourism awareness in employees (Alcaraz *et al*., 2018). Therefore, it is established again that tourism awareness as a strategy must be included, in this case, in the service providers, since they are the ones who have most contact with tourists, and in addition, the tourism development policy of the communities must implement constant training for this sector.

Conservation of resources susceptible to tourist use

Concerning this point, the study carried out by Ralitsa Antimova, Jeroen Nawijin and Paul Peeters (2012) titled "The awareness/attitude-gap in sustainable tourism: A theoretical perspective", mentioned that a person with awareness is not necessarily prone to change their attitude/behavior (Antimova *et al*., 2012). This is explained by three theories: at the individual level that mainly describes individual behavior (values, beliefs, attitudes, social norms and intentions), the interpersonal level (relationship between individuals and social networks), and the community level (communication, media and social norms and culture). It concludes by mentioning that intention does not lead to action in the case of theories at the individual level, so it is likely that from the theories at the community level it can be argued that the dominant social norms are those that determine common action or inaction, so that the application of sustainable behaviors for the protection of resources are aimed at what the bulk of the population has determined as the right thing, and recommends further research on the awareness/attitude/behavior gap from the game theory and the approach of social practices (Antimova *et al*., 2012).

A valuable contribution of this approach is developed by Vecsilir and Tommei (2013) called: "Toward a territorial project for a cultural landscape. Humahuaca Ravine, Jujuy, Argentina." is the authors mentioned that the Ravine was declared a World Heritage Site by UNESCO in the category of "cultural landscape", so the results of the survey of the main plans, programs and proposals for the new territory are shown. Under this objective, the result is that the 2006 Sustainable Tourism Development Plan proposes, among some other strategies, the creation of Resource Survey programs, as well as tourist awareness, demand segmentation and implementation of a Tourism Observatory (Vecslir & Tommei, 2013). In this research, only the recommendation to create a tourism awareness program is mentioned, which highlights the importance of this strategy for the conservation of the natural site and mitigation of possible negative impacts.

One of the studies carried out in Mexico under this theme is that by Palafox *et al*. (2015), titled: "Cozumel and the transformation of its landscape by cruise ship tourism", which analyzes the transformation of the island landscape from tourism development, specifically cruise ship tourism, which is promoted by the state. However, it mentions that the tourism demand related to cruise ships generates a more negative impact compared to the economic spill that it generates, leaving aside the other segment of tourism (diving) which seeks to preserve the natural wealth of the community by showing coral reefs. In this sense, if there was tourism awareness related to nature conservation, it decreases due to the arrival of tourists via cruise ship, an issue that must be taken up again if the destination is to recover (Palafox *et al*., 2015). A second case study found in Mexico is titled "Towards a Model of Sustainable Tourism in Protected Natural Areas: Case Study of the Chankanaab Natural Park in Cozumel, Mexico", whose main objective was to determine the tourist load capacity of the destination that is considered massive for cruise ship hikers. Briefly, it is mentioned in the results section that one of the functions of the Park is environmental education, for which cultural events, tourism awareness programs, as well as preparation of educational material are carried out; however, it refers to the fact that one of the main deficiencies of the park is precisely tourism awareness, as it is not promoted adequately (Segrado *et al*., 2015). Finally, there is the study by Limpho Lekaota (2017) titled "Awareness and education about sustainable tourism in Katse and Mohale tourism development areas in Lesotho", whose objective was to analyze the perceptions of stakeholders on awareness and knowledge of the community about tourism, and points out that it is through tourism awareness and community knowledge on tourism issues that environmental sustainability can be achieved (Lekaota, 2017). Unlike previous studies, this is research focused directly on tourism awareness, and therefore it offers a broader vision of how to work on this issue from the National Policy.

Community awareness about tourism and tourists has not been well documented (Timothy, 2000; and Saarinen, 2010). It is necessary to develop more research on the topic of tourism awareness worldwide, but especially in Mexico, since there are scarce published scientific studies. According to national policies (Mexico), tourism with a social focus should be implemented for it to generate economic benefits in local communities, so carrying out research on this issue can make visible the impacts caused by a lack of tourist awareness and provide the necessary elements for the creation of programs and projects that allow a successful understanding and participation among the different tourism actors, such as tourists, the local community, service providers, and others involved in tourism.

CONCLUSIONS

It was identified that the first sense, "relationship and contact of the host community with the tourist", is the theme that has been most developed, emphasizing the need for education and training of the local community in order to develop greater tourism awareness, even to the point of suggesting it as a subject in schools.

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Agronomic evaluation of corn (*Zea mays* L.) genotypes in the warm dry region of Chiapas, Mexico

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ABSTRACT

Objective. To evaluate the agronomic behavior of corn (*Zea mays* L.) experimental genotypes in three contrasting environments in the Central region of Chiapas, Mexico.

Design/methodology/approach. The experiments took place during the 2016 spring-summer agricultural cycle at Francisco Villa, Villaflores (730 m); San Luis, Suchiapa (600 m) and Ocozocoautla (800 m), at the Central region in the state of Chiapas, Mexico. At the three assessed sites, the climate is warm subhumid with rains in summer and intra-stival drought during the second half of July and the first half of August. The genotypes XT-5614, XT-3402, XT-5610, XT-5612, XT-5627, and BG7415W from the Biogene Company were evaluated, which are used in commercial crops at the Center of Chiapas. All genotypes showed viability greater than 90%. Three experimental sites were evaluated, in a randomized complete block design with four replications. The experimental unit consisted of four 5 m long rows 0.8 m apart. The useful plot was formed by two central furrows. The evaluated variables were: days to male flowering (DMF), days to female flowering (DFF), plant height (PH), cob height (CH), cob length (CL), cob diameter (CD), rows per cob (RC), grains per row and grain yield (YLD) at 14% moisture. These were analyzed with an analysis of variance (ANOVA) and for the genotype × environment interaction (GEI) the additive main effects and multiplicative interaction model (AMMI) were used, with the SAS statistical software and the GEA-R software.

Results: The combined analysis of variance detected differences between genotypes (G) for most of the variables except in grains per row. and days to male and female flowering; there were significant differences between environments (A) for all variables, while for the GEI, there were significant differences for the number of rows per cob. The CV was 1.26 (DFF) at 10% (YLD), which indicates an acceptable control (<20%) of the experimental variability. The results indicated genetic variation between evaluated genotypes, which allows the selection of the most outstanding ones. The evaluation environments showed differential effects and this condition is necessary for the evaluation of germplasm for a genetic improvement process.

Study limitations/implications: Cob height registered acceptable values given that short plants favor rotting in hot climates when weeds are present before harvest. The flowering of the genotypes was considered acceptable and was earlier (55 d) at the Suchiapa site.

Findings/conclusions: Among the assessed genotypes there were significant differences for grain yield, plant height, cob height, cob length and the number of rows; the genotype-by-environment interaction was not significant. The XT 5627 and XT 5610 genotypes showed higher stability, and the former showed higher grain yield. The highest yields were recorded in the environment from Francisco Villa, Villaflores, at the Frailesca region, Chiapas.

Keywords: Hybrids, genotype × environment interaction, yield.

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INTRODUCTION

In Mexico, corn (*Zea mays* L.) cultivation is important from a socioeconomic point of view because it is a basic component of the Mexican diet: 7.5 million hectares are cultivated in the country; however, only 22.1 million tons of grain are produced each year to cover food demand (Tadeo *et al*., 2015; SIAP, 2016). The Central Depression of the state of Chiapas, Mexico, had an altitude ranging between 600 to 800 m, with a warm dry climate and an average annual rainfall of 1000 mm, which includes RDD 01 Tuxtla and 04 Villaflores of the Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA). Corn is the most important annual crop by planted area while generating an important source of employment. According to SIAP (2016), 415,000 ha are sown in this region with an average yield of 3.2 t ha^{-1}. The genetic improvement programs of different companies and Public Research Centers (PRC) have their own corn germplasm, and can therefore generate genotypes of different genetic structure, such as free pollination varieties (FPV), synthetic varieties (SV), single cross hybrids (SCH), double-cross hybrids (DCH), trilinear hybrids (TLH) and intervarietal hybrids (IVH), to achieve greater adaptation to the environment (Ramírez *et al*., 2015; González *et al*., 2016). Therefore, before recommending a genotype for planting, it is necessary to evaluate its phenotypic response to different agroecological environments. The study of the genotype-by-environment interaction (GEI) in plant genetic improvement is important since it is the result of the response of each genotype to environmental variations (Crossa and Vargas, 2000). To achieve greater progress, it is necessary to adequately establish methodologies to be used for GEI evaluation and to accurately estimate the differential response of the genotypes through environmental testing (Canales *et al*., 2016). Several studies have demonstrated the effectiveness of the AMMI model and the biplot graph to describe the GEI of experimental maize genotypes in multiple environments (Lozano-Ramírez *et al*., 2015; Sánchez-Ramírez *et al*., 2016); therefore, the objective was to evaluate the agronomic behavior of experimental genotypes of corn (*Zea mays* L.) in three contrasting environments at Chiapas Central region, Mexico.

MATERIALS AND METHODS

The experiments were established during the 2016 spring-summer agricultural cycle at the towns of Francisco Villa, Villaflores (730 m); San Luis, Suchiapa (600 m) and Ocozocoautla (800 m), located in the Central region of the state of Chiapas. In all three sites, the climate is warm subhumid with rains in summer and the presence of intra-estival drought during the second half of July and the first half of August.

Genetic Materials. The evaluated experimental genotypes were: XT-5614, XT-3402, XT-5610, XT-5612, XT-5627 and the commercial BG7415W genotype from the Biogene company, which is used in commercial plantings at Chiapas central region. All genotypes showed viability greater than 90%. The genotypes were distributed in the three experimental sites, in a randomized complete block experimental design with four replications. The experimental unit consisted of four rows of 5 × 0.8 m, with a useful plot formed by the two central rows.

Evaluated variables. Days to male flowering (DFM), days to female flowering (DFF), plant height (AP), cob height (CH), cob length (CL), cob diameter (CD), rows per cob (RC), grains per row and grain yield (YLD) at 14% moisture. A combined analysis of variance (ANOVA) was performed and the additive main effects and multiplicative interaction (AMMI) model was used to evaluate the genotype-by-environment interaction (IGA). The analysis of variance was assessed with the SAS software system (SAS, 2000) while the GEA-R program was used for the AMMI model (Pacheco *et al*., 2015).

RESULTS AND DISCUSSION

The combined analysis of variance detected differences between genotypes (G) for most of the variables except in grains per row and days to male and female flowering; Between environments (E) there were significant differences for all variables, for the environment × genotype interaction (GEI) there were significant differences only for the number of rows per cob (Table 1). The values of the coefficient of variation (CV) ranged from 1.26 (DFF) to 10% (YLD), which indicates an acceptable control (<20%) of the experimental variability. The results show a genetic variation between the evaluated genotypes, which allows selecting the outstanding ones. The evaluation environments represent the agroecological conditions, where maize is grown in the Central region of Chiapas, and because they are contrasting, differential effects were shown. This condition is necessary for the germplasm evaluation process for genetic improvement (Córdoba, 1991). Table 2 presents the Gollob analysis, which allows visualizing the statistical significance of the components of the AMMI

model. Component 1 (CP1) was statistically significant and explained 80% of the sum of squares of the genotype by environment interaction.

Table 1. Statistical significance of the combined analysis for various phenotypic characteristics of maize genotypes (*Zea mays* L.) in three environments. Spring-summer 2016 cycle.

F.V.	Grain yield	AP	AM	DFM	DFF	LM	DM	NH	GH
Genotipe (G)	*	**	**	NS	NS	**	NS	**	NS
Environment (A)	**	**	**	**	**	**	**	**	**
G × A	NS	NS	NS	NS	NS	NS	NS	*	NS
C.V. (%)	10.1	3.9	9.7	1.4	1.26	3.9	3.9	5.3	4.6

The * and ** significant level at P<0.05 and P<0.01, respectively; N.S.=Not Significant; C.V.=Coefficient of variation. YLD=Grain yield (kg ha^{-1}); PH=plant height (m); CH=cop height (m); DMF=days to male flowering (DDS); DFF=days to female flowering (DDS); CL=Length of cob (cm); CM=Cob diameter (cm); NR=number of rows; GR=grains per row.

Table 2. Mean squares and statistical significance of the Gollob test for the components of the AMMI multiplicative model for six evaluated genotypes of *Zea mays* L. in three environments. 2016 spring-summer agricultural cycle.

Component	Sum of squares	% Explained±	%Accumulated	Mean Square
CP1	7679624	80.01	80.01	1279937 **
CP2	1919561	19.99	100	479890 NS

± Percentages with respect to the sum of squares of the genotype × environment interaction.

Table 3 presents the obtained yields in each evaluation environment. At Francisco Villa, Villaflores, Chiapas site, an average of 8,207 kg ha^{-1} were obtained, exceeding by 39% that recorded at the Ocozocoautla and San Luis, Suchiapa sites. These results are explained because at Francisco Villa precipitation was higher and without intra-estival drought. However, at San Luis and Ocozocoautla, there was a drought period (without rain) of 20 and 30 days, respectively, the drought occurred during the flowering period, at this stage, the corn plants are highly susceptible to water deficit (Márquez, 2009).

The XT 5627, XT 3402 and XT 5610 genotypes stood out from the rest with yields of 6662, 6594 and 6359 kg ha^{-1} (Table 3), respectively, which exceeded the control (BG7415W) by 384 kg and more. Due to the length of the cob, the XT 3202, XT 5612 and XT 5627 genotypes stood out, while the BG7415W genotype stood out due to the number of rows. These yields exceed the average of 3,200 kg ha^{-1} reported by producers (SIAP, 2016).

Table 4 presents plant height, cob, days to male flowering and female to flowering. The evaluated genotypes presented an average plant height of 2.4 to 2.7 m, which was not a problem since the genotypes did not present lodging; also, these values are favorable for forage production as an added value from grain yield. Regard to the cob height, the values are acceptable as short plants favor cob rotting in hot climates, especially when excess weeds are present before harvest. The flowering of the genotypes was considered acceptable and they were earlier (55 days) at the Suchiapa site, probably due to high temperatures in this locality and, also, there occurrence of intra-estival drought, compared to the other two

Table 3. Test of means of main effects for grain yield and yield components of six genotypes of corn (*Zea mays* L.) evaluated in three environments. Spring-summer 2016 agricultural cycle.

Factor	Grain yield	LM	DM	NH	GH
Genotipo					
XT 5614	6017 b+	13.9 b	4.4 a	14.5 bc	34.9 a
XT 3402	6594 a	15.5 a	4.3 a	15.4 b	35.4 a
XT 5610	6359 ab	14.1 b	4.2 a	13.8 c	34.4 a
XT 5612	5888 b	15.4 a	4.2 a	14.4 c	34.4 a
XT 5627	6662 a	15.8 a	4.3 a	13.7 c	34.4 a
BG7415W	5976 b	14.7 b	4.4 a	16.5 a	36.3 a
DSH	584	0.73	0.21	0.99	2.0
Environment					
Ocozocoautla	5063 b	15.2 a	4.3 a	15.1 a	36.5 a
Francisco Villa, Villa Flores	8207 a	14.9 ab	4.4 a	14.3 b	35.1 b
San Luis, Suchiapa	5222 b	14.6 b	4.1 b	14.7 ab	33.1 c
DSH	467.7	0.4	0.13	0.6	1.2

YLD = Grain yield (kg ha-1); CL = Length of cob (cm); DC = Diameter of cob (cm); NR = Number of rows; GR = Grains per row. + Means with the same letter in a column are not statistically different.

experimental sites. Asynchrony between male and female flowering in all genotypes was not significant.

Figure 1 shows the biplot graph that facilitates visualizing the stability of the genotypes through the environments. Yields are recorded on the "X" axis and the effects of genotype-by-environment interaction (GEI) are recorded on the ordinate axis "Y". The segments represent the environments and the numbers the genotypes. The AMMI1 model was used because the first principal component explained 80% of the GEI. The biplot shows that the XT 5627 (5) and XT 5610 (3) hybrids were the closest to the origin, which indicates greater stability (Lozano-Ramírez *et al.*, 2015; Sánchez-Ramírez *et al.*, 2016). The rest of the hybrids registered an acceptable performance behavior, but it considerably changes from one environment to another.

Table 4. Test of means of main effects for plant height, cob height and phenology of six genotypes of corn (*Zea mays* L.) evaluated in three environments. 2016 spring-summer agricultural cycle.

Factor	AP (m)	AM (m)	DFM (dds)	DFF (dds)
Genotipe				
XT 5614	2.5 bc^{+}	1.06 b	58.5 a	59.4 a
XT 3402	2.6 ab	1.10 ab	58.8 a	59.8 a
XT 5610	2.6 ab	1.20 a	59.0 a	60.0 a
XT 5612	2.7 a	1.21 a	58.9 a	59.8 a
XT 5627	2.4 c	1.00 b	58.6 a	59.6 a
BG7415W	2.7 a	1.12 ab	58.7 a	59.7 a
DSH	0.14	0.13	1.0	0.9
Environment				
Ocozocoautla	2.6 a	1.2 a	60.3 a	61.3 a
Francisco Villa, Villa Flores	2.6 a	1.1 b	59.9 a	60.9 a
San Luis, Suchiapa	2.5 b	1.1 b	55.2 b	56.1 b
DSH	0.08	0.07	0.6	0.6

PH=Plant height (m); CH=Height of cob (m); DMF=Days to male flowering; DFF=Days to female flowering. das=Days after sowing. $^{+}$Means with the same letter in the column are not statistically different.

CONCLUSIONS

Among the assessed genotypes, there were significant differences for grain yield, plant height, cob, cob length and the number of rows; the genotype-by-environment interaction was not significant. The XT 5627 and XT 5610 genotypes showed higher stability and the former showed higher grain yield. The highest yields were recorded in the environment of Francisco Villa, Villaflores, in the Frailesca region, Chiapas.

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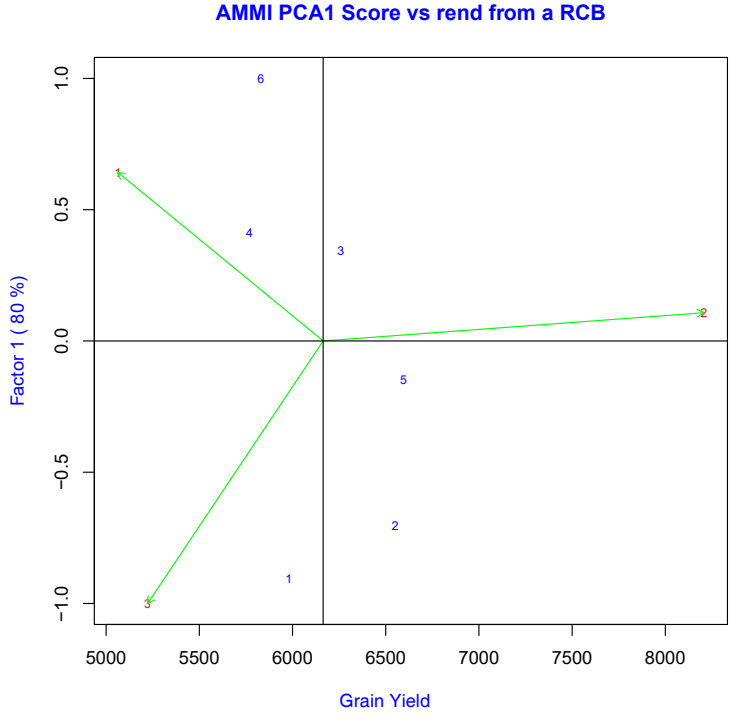

Figure 1. Biplot shows the CP1 (G×A) pattern as a function of the average yield of six maize hybrids (*Zea mays* L.) evaluated in three environments. 2016 spring-summer agricultural cycle.

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Quality and Yield of basil (*Ocimum basilicum* L.) essential oil under hydroponic cultivation

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ABSTRACT

Objective: to assess the production of basil (*Ocimum basilicum* L.) grown in a greenhouse under open hydroponic system, using tezontle (volcanic gravel) as substrate.

Methodology: three planting densities were evaluated: D1, D2 and D3 (14, 28 and 71 plants m^{-2}, respectively) and two concentrations of Steiner nutrient solution (S1: 100%, and S2: 50%).

Results: the highest values for fresh and dry weight of the aerial part, were the treatments S1 D1, S1 D2 and S2 D1. With S1 D1 the largest leaf area was obtained. Dry matter obtained was 14.03 mg g−1 of essential oil.

Findings: the use of hydroponics with an increase in sowing density can generate up to 70.21 t ha^{-1}.

Keywords: aromatic plant, sowing density, essential oil.

INTRODUCTION

Basil (*Ocimum basilicum* L.) is an aromatic plant native to South Asia of the Lamiaceae family, characterized by containing essential oils in all the aerial parts of the plant that give the species a particular smell and flavor. Those are product of its chemical components, which include estragol, eugenol, linalool, linalyl acetate, camphor, and o-cymene (Muñoz, 2002; Sam *et al*., 2002). Although in the past the chemical composition and properties of its oil were unknown, since ancient times it has been appreciated in traditional medicine. Nowadays, with the advancement in the knowledge and action of its components, basil essential oil has an immense value for perfumery, cosmetic, pharmaceutical and food industries (Muñoz, 2002; Kalita and Khan, 2013). In addition, antioxidant properties, insecticidal, nematicidal, fungicidal and microbicidal activities are attributed to it (Wannisorn *et al*., 2005; Bozin *et al*., 2006; Kelen and Tepe, 2007; Politeo *et al*., 2007; Abdullah *et al*., 2008).

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This species is cultivated in many countries, but the European Union and the United States are the largest importers and exporters where basil is marketed as fresh, dry, frozen, as essential oil, or in any other available form. Cost depends on the origin and cultivation type (Guerrero and Ruiz, 2012). In Mexico, basil cultivation covers 363 ha, mainly in Baja California Sur, Morelos, Nayarit and Baja California; the main producer is Baja California Sur, 273 ha (SIAP, 2015). *O. basilicum* cultivation is developed mainly in the field, without defined programs of irrigation, nutrition and planting densities. However, in order to increase yields and extending the growing season, the producers venture into greenhouse cultivation; even tough adequate nutrition has not been established to increase performance of the essential oil from the species. Considering the species potential is great, it seems possible that protected cultivation, complemented with the use of a balanced nutrient solution, in open hydroponic production system, can be an intensive production alternative, which facilitates supply throughout the year. Based on the above, the yield of basil (*Ocimum basilicum*) cultivated in hydroponics was evaluated at different planting densities and concentrations of nutrient solution.

MATERIALS AND METHODS

Basil seeds from Hortaflor™ were sown in 200-cavities germinating trays filled with a commercial organic substrate (Peat Moss™). Two daily irrigations with running water were applied until the appearance of the first true leaves; and from that stage on, it was watered once a day with a 50% Steiner nutrient solution until the moment of transplantation, which was carried out when the seedlings had 4 or 5 true leaves, at 45 d after sowing (in Spanish, dds).

The transplant was carried out in tezontle (inert volcanic gravel), granulometrics (3 to 5 mm in diameter) disinfected with 5% sodium hypochlorite, placed in beds 2.10 m long × 0.60 m wide × 0.20 m high, divided with Styrofoam plates in three equal parts. Each of these parts constituted a sowing unit (0.42 m^2), where basil seedlings were placed according to the studied planting densities D1, D2 or D3 (14, 28 and 71 plants m^{-2}, respectively). In general, 14 plants m^{-2} is the sowing density used by growers in conventional field cultivation. The Steiner universal nutrient solution (Steiner, 1984) was used at 100 and 50% (S1 and S2, respectively); the pH of the solutions was kept at 5.5. Watering was applied by dripping, 5 min four times a day, a daily flow of 1.33 L of nutrient solution, for each sowing density (0.42 m^2). The experimental design was completely randomized with four replicates, and a 3×2 factorial arrangement. The experimental unit consisted of three plants per density.

The harvest was carried out at the beginning of flowering (60 days after transplantation). Plants were cut from the base of the stem. The height of the main stem was measured in millimeters. Aerial parts were immediately weighed on a digital scale (ACCULAB VI-3 mg, USA). Total leaf area was determined with a LI-COR™-MODLI-3100 leaf area integrator. The leaves and stems of each harvested plant were placed in brown paper bags to be dried in an LC-Oven LAB-Line oven for 4 to 5 days, at a maximum temperature of 25 °C until constant weight was obtained.

The oil extraction was carried out by steam distillation of 35 g of dry matter, obtained by drying in the shade of three plants for each planting density. The chemical composition of basil oil was obtained by analyzing the samples in a gas chromatograph (HP 6890) coupled to a mass spectrometer (HP-5973). The components of the oil were identified by comparing their mass spectrum with the spectrometer database. The analysis was done following the methodology of Aligiannis *et al*. (2001) using linalool as a standard. With the data obtained, analysis of variance and Tukey means comparison test were performed, analyzing the data with the SAS statistical software, version 6.1 (SAS, 1994).

RESULTS AND DISCUSSION

In the final height of the plants, little significant difference was observed among treatments. However, the treatments S1 D1, S1 D2, and S1 D3 showed the highest values and S2 D3 registered the lowest plant growth (Figure 1). Although the highest value obtained was 30.17 cm, at S1 D3, great heterogeneity of sizes was also observed in this density, attributed to the competition for nutrients and light. Similar observations were made by Velásquez (2019), who reported that a high planting density caused a decrease in yield, due to competition and proliferation of pathogens. Considering size variation under S1 D3, the treatments S1 D1 and S1 D2 were considered as the best, if the objective of the producer was to obtain taller plants.

The obtained results suggest that by providing a 100% nutrient solution, basil reaches its optimum growth,

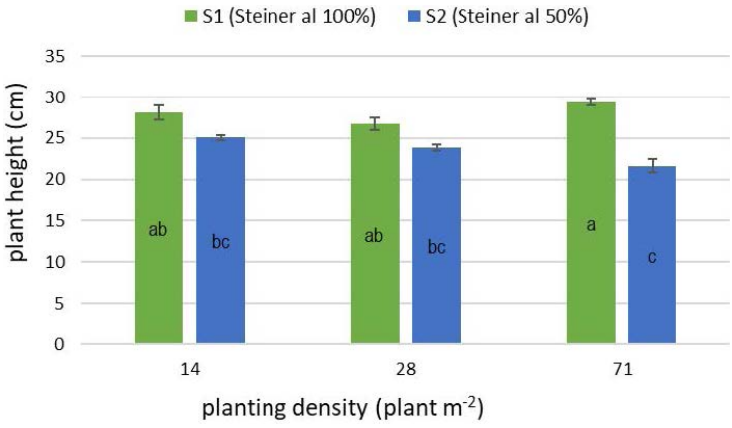

Figure 1. Basil (*Ocimum basilicum* L.) plant height, cultivated with two nutrient solutions (S1: 100% and S2: 50%) and three planting densities (D1: 14, D2: 28 and D3: 71 plants m^{-2}) ± standard error. Means with different letters show significance (Tukey α=0.05).

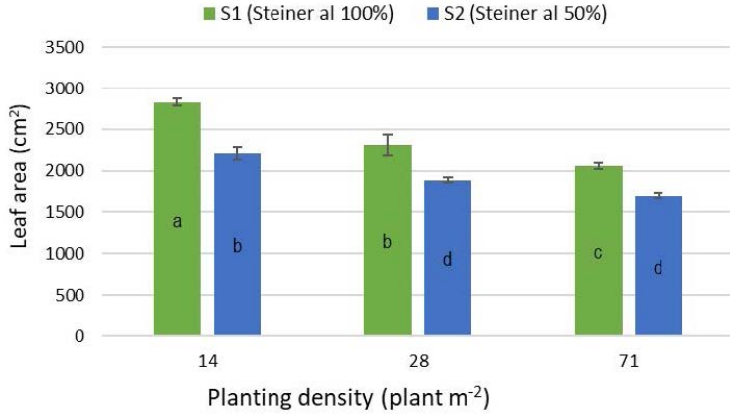

Figure 2. Total leaf area of basil plants (*Ocimum basilicum* L.), grown with two nutrient solutions and three planting densities, (plants m^{-2}) ± standard error. Means with different letters show significance (Tukey α=0.05).

obtaining those 13 nutrients which are necessary for its different physiological functions. Such a circumstance agrees with Santos *et al.* (2005), who conclude that nutrient availability affects basil height growth. Their results showed a positive response in height by providing 100% of nutrients and an inverse effect by reducing nutrients to 50%.

An increase in fresh and dry weight of the aerial part could be observed, with the treatments S1 D1, S1 D2 and S2 D1, while S1 D3, S2 D2 and S2 D3 showed lower values (Table 1). Although these results show that any of the three aforementioned treatments can be used, it is considered that with S1 D2 higher yields of fresh and dry weight per unit area can be obtained; since it is the one with the largest number of plants compared to S1 D1 and S2 D1. Open field crops in Mexico reach a yield of 8.89 t ha^{-1} of fresh weight (SIAP; 2015), while with the use of this system with S1 D2, up to 70.21 t ha^{-1} can be obtained, increasing production by 600 %.

Regarding leaf area, the statistical difference of S1 D1 (2828.44 cm^2) was clear, over the rest of the treatments (Figure 2) S1 D2 was statistically lower (2308.89 cm^2). However, if the 100% increase in plants per unit area is considered, then the recommended treatment for this variable is S1 D2, as S1 D3 treatment was the one that presented the lowest value, possibly due to the competition for light and nutrients.

It has been shown by Sifola and Barbieri (2006), that basil is a species that responds positively to the increase of nitrogen and phosphorous fertilizers, causing an increase in the foliar area, which generates an increase in fresh- and dry weight of the plant. This also causes an accumulation of secondary metabolites; a circumstance that was observed in this research when the tallest plants of S1 D1 and S1 D2 were the ones that also presented the highest values of fresh and dry weight. The yield of essential oils obtained ranged between 10.47 and 14.03 mg g^{-1} of dry matter. However, the comparison of means did not show significant differences among the evaluated treatments (Figure 3). With S1 D2, the percentage obtained was the one with the lowest value, but even so, it was 1.047%, higher than the 0.13% obtained by Teixeira *et al.* (2002). Furthermore, although statistical

Table 1. Means comparison of fresh- and dry weight of the aerial part of basil (*Ocimum basilicum* L.) plants cultivated with two nutrient solutions and three planting densities.

Nutrient solution Steiner	Planting density (plant m^{-2})	Fresh weight aerial part (g)	Standard error	Dry weight aerial part (g)	Standard error
100%	14	354.5a	±16.05	39.0a	±1.76
	28	250.7ab	±22.80	27.5ab	±2.51
	71	194.2b	±30.41	23.3bc	±3.65
50%	14	280.3ab	±19.40	33.6ab	±2.33
	28	205.3b	±26.43	24.6bc	±3.17
	71	172.3b	±38.54	18.9c	±4.24

* Means with different letters show significance (Tukey α=0.05); SE=Standard error. Each value is the mean of 3 replicates ± standard error.

differences were not evident among treatments, the choice S2 D2 allowed obtaining the highest yield of essential oil (14.03 mg g^{-1} DM).

From the sample analyzed in the gas chromatograph, a total of six components were obtained, the most abundant observed was linalool (Table 2). No differences were observed among treatments, in terms of essential oil components. Despite the fact that the concentration and composition of the essential oil from basil has been reported as irregular due to the chemotypes of the species, several authors agree that the majority component of this species is linalool (Smith *et al*., 1997; Sánchez *et al*., 2000; Teixeira *et al*., 2002; Fernández *et al*., 2004), a circumstance that was observed in our research. In the present study, it was demonstrated without any doubt that greenhouse cultivation with a hydroponic system, at the planting density D2, allows the production of basil with high yields and commercial quality, both for the fresh market and for the essential oils industry.

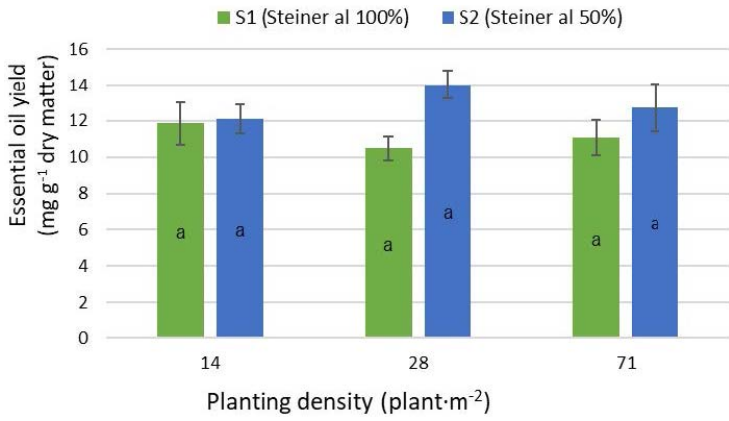

Figure 3. Yield of the essential oil from basil (*Ocimum basilicum* L.) grown with two nutrient solutions and three planting densities. Values are means ± standard error. Means with different letters show significance (Tukey α=0.05).

CONCLUSIONS

The planting density D2 (28 plants per m^2) is suggested. When the cultivation is required for leaf production, the use of the 100% nutrient solution (SI) is recommended, whilst, for the production of essential oil, we suggest the 50% nutrient solution (S2).

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Table 2. Major compounds in the essential oil of basil (*Ocimum basilicum* L.), grown with two nutrient solutions and three planting densities.

Compound	Retention time (min)	Area (%)
Linalol	12.889	32.633
Terpin-4-ol	15.768	0.850
Borneol	19.685	1.148
Cyclohexane-1-ethenyl-1-methyl-2,4-bis(1-methyleth)	23.246	1.377
Methyl eugenol	23.660	1.527
1,6-cyclodecadiene, 1-methyl-5-methylene-8-(1-methyl)	26.044	0.630

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Design of a HACCP Plan for Vacuum-Packed Beef Cuts

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ABSTRACT

Objective: To elaborate a Hazard Analysis and Critical Control Points (HACCP) plan for the process of vacuum-packed beef cuts, in a Federal Inspection Type (FIT) meat processor located in eastern Estado de México, with the aim of improving its conditions of hygiene and innocuousness.

Design/Methodology/Approximation: The activities performed in the business were revised applying the HACCP methodology and the dangers identified were analyzed, to determine whether the danger found was significant for the innocuousness of the food, finally concluding if it represented, or not, a critical control point (CCP).

Results: A CCP was identified in the phase of metal detection, the dangers identified were the passing of metal shards from the transport rails, pieces of knives or saws, and pieces of needles from prior steps in the process.

Study limitations/implications: Documentation about case studies of beef cuts, as well as about management of their CCP is limited.

Findings/Conclusions: The CCP1 Metal Detection is a control measure that the business performs; however, under analysis, there was evidence of an opportunity to improve the current operation, which is why the adaptation of registry formats that could allow monitoring and controlling the CCP was proposed.

Keywords: CCP, FIT, meat cuts, innocuousness, packaging.

INTRODUCTION

The Hazard Analysis and Critical Control Points (HACCP) system, which has scientific foundations and systematic nature, allows identifying specific dangers and control measures in order to guarantee innocuousness of foods. It is an instrument to evaluate the dangers and establish control systems that are focused on prevention instead of being based primarily on testing the final product (CODEX ALIMENTARIUS, 2005). The HACCP system can be applied all along the entire food chain, from the primary producer to the final consumer, and its application must be based on scientific trials of dangers for human health. In addition to improving the safety of foods, applying the HACCP system can offer other significant advantages, also facilitating the inspection by the regulatory authorities, and promoting international trade by increasing the confidence in the innocuousness of the foods (CODEX ALIMENTARIUS, 2005).

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Currently, the importance of food safety is reflected not only in the need to avoid health problems, but also in the repercussions from trading contaminated foods. All of these circumstances have led to methodologies being created, designed or adapted, or systems that allow guaranteeing or ensuring the innocuousness and quality of foods (Nápan & Pérez, 2017).

Meat is a food rich in nutrients that provides the ideal conditions for microbes to grow and this defines its perishable nature. Some organisms simply ruin it, while others make it a threat for health (Saucier, 2016). Deterioration is a complex process where a combination of chemical and biological mechanisms occurs that makes the product unacceptable (García *et al*., 2015). The foods most frequently involved in epidemics and cases of foodborne diseases (FD) are those of animal origin. In 48 % of the epidemics that took place between 1973 and 1987 in the USA, where the causal factor was identified, the products involved were beef, eggs, pork meat, poultry meat, fish, crustaceans, mollusks, or dairy products (OMS, 2016).

The Hazard Analysis and Critical Control Points (HACCP), which strongly depends on prerequisite programs that include Best Hygiene Practices (BHP) and Standard Sanitation Operating Procedures (SSOP) provides better hygiene conditions of the process that are necessary for meat production and safe meat products in the meat chain (Tomasevic *et al*., 2016).

A FIT (Federal Inspection Type) establishment is an animal slaughter facility for supply, refrigerating and industrialization of meat products and byproducts that is object of permanent sanitary inspections to ensure that they comply with the regulations that the Ministry of Agriculture and Rural Development (Secretaría de Agricultura y Desarrollo Rural, SADER) marks for Mexico considering food safety (SENASICA, 2015). Currently the clients are increasingly more demanding in terms of the conditions of the products they enjoy, which is why they seek those that satisfy their expectations and offer confidence and safety. Beef consumption in Mexico is important. Data from 2014 indicate that the consumption of this type of food in Mexico was 1.85 million tons. However, only 48 % of it came from Federal Inspection Type (FIT) meat processors, while the other 52 % came from municipal meat processors (Ventura *et al*., 2017). This study aims to design a proposal for a HACCP plan in processing of vacuum-packed secondary beef cuts, for a meat cut packer that recently obtained its FIT certification, located in eastern Estado de México, to ensure innocuousness.

MATERIALS AND METHODS

The study is centered on a business that is a Federal Inspection Type (FIT) meat processor, located east of Estado de México and west of Mexico City, which has a chamber for slaughter, deboning, carving, packing, packaging and storing meat cuts. The research had a field phase where the HACCP methodology was applied in terms of the revision of activities carried out in the business, with the aim of identifying all the physical, chemical and biological potential dangers that it is essential to anticipate in the production process, and another analysis phase of the dangers identified during the first, with the purpose of defining whether the danger found is significant for food innocuousness, and thus, through the use of a decision tree to conclude if the stage evaluated was a critical control point (CCP) or not. The agroindustry selected is a medium-sized business that has 36 workers distributed in the different areas; it is focused on slaughter and obtaining meat byproducts. The process that was used for the development of the HACCP plan is obtaining vacuum-packed secondary beef cuts. With the visits carried out to the packaging plant, through observation and collection of documental information, the preliminary tasks and the HACCP principles were developed.

Sequence for the implementation of the HACCP system

- Training the HACCP team.
- Describing the product.
- Defining the use and destination of the product.
- Elaborating the flow diagram of the process.
- *In situ* confirmation of the flow diagram.
- Analyzing dangers (principle 1).
- Determining critical control points (CCP) (principle 2).
- Establishing the critical limits (principle 3).
- Designing a security system (principle 4).
- Designing corrective measures (principle 5).
- Designing verification and validation procedures (principle 6).
- Designing a registry and documentation system (principle 7).

RESULTS AND DISCUSSION

Training of the HACCP Team

In the first meeting the study's objectives were made

public; in the second meeting an organigram was proposed to guarantee the reliability of the system, which was made by the advisory team and operating manager, validated by the general director, and the other members of the HACCP team were selected, the positions and responsibilities of each member of the team are shown next.

- Quality chief (leader) with the responsibility of coordinating the team to achieve the objectives established.
- Operating manager (facilitator) with the responsibility of providing tools, data and information of importance to the product.
- Systems manager (secretary) with the responsibility of performing the registry of meetings and agreements, in addition to filing, consulting and receiving documentation.
- Advisory team with the responsibility of guiding and maintaining communication with all the areas during product development.

Product Description

The product is obtained from the secondary beef cuts, vacuum packed, refrigerated or frozen, according to the client's request. They come from deboning of front and back quarters of the cattle carcass, destined for human food and wrapped in polyethylene sheets and vacuum bags, packed in cardboard boxes or plastic baskets, and sent out at 0 to 4 °C when refrigerated or at −18 °C when frozen. The product should be free of clots, filth and should have a bright red color, with white or slightly yellow fat, and characteristic odor (NOM 251-SSA1; 2009). It was found that the business carries out these activities completely and maintains the criteria of the cold chain, as well as the organoleptic characteristics established by the norm for production of this product.

Determination of the Use and Destination of the Product

The product is destined to self-service stores for its sale, distribution and consumption; it is recommended to follow the next instructions for safe management of the meat cuts obtained:

- Keep refrigerated or frozen at a temperature of −18 to 4 °C.
- Thaw in refrigerator at a maximum temperature of 4 °C, to keep the cold chain of the product and preserve its shelf life.
- Keep the raw meat separate from other types of foods in the store.
- Cook completely, making sure to have reached total cooking.
- Once the package is open, consume the whole product.
- It is recommended to cook for 10 to 12 min or until the internal temperature of the meat reaches 68 °C minimum.
- Do not consume the product raw.

Process Flow Diagram and *in situ* Verification

Figures 1, 2 show the phases of the process, from the moment of the reception of the half carcasses until the vacuum-packed meat cuts are obtained, which was verified and corrected according to the changes found in the in situ verification. A path diagram was also developed that helped to identify the phases of the process, thus fulfilling the last Pre-HACCP activity.

HACCP Principles

Analysis of dangers (Principle 1)

Derived from the analysis of dangers, probability and severity were identified for each of them, and their significance was evaluated with the support from the severity framework of the HACCP methodology. The most important biological dangers of the production process of vacuum-packed meat cuts are: *E. coli O157:H7*, *Salmonella* spp. and *Listeria monocytogenes*, which can be introduced from the slaughter until the moment of eviscerating or from the incorrect manipulation of the carcass on the product by the staff. *Salmonella* spp. is a primarily intestinal parasite bacterium of animals including humans, liberated to the environment from feces, where certain survival ability is shown in the materials that it contacts; under favorable conditions, it multiplies (Caballero, 2008). This can be prevented with a staff Hygiene Program, or in later steps of the process, such as vacuum packing to reduce microbial growth. Maldonado (2013) recommends improving animal slaughter, as well as the sanitary conditions in meat processors, since contamination of the meat on the carcass is the direct result of the transfer of pathogens present in cattle and its later meat contamination. This is what various studies suggest, arguing that beef consumption is associated with infections produced by *Salmonella* spp. and *E. coli 0157:H7*.

Within the beef cutting process and according to the bibliography, clenbuterol is identified as the most

important chemical danger; it is an anabolic agent that is used illegally in livestock production to increase the weight in bovines and other species. High doses are required for clenbuterol to express its anabolizing function, which is why residues in meat represent a danger for consumers, who can present severe intoxication (COFEPRIS, 2018). Therefore, it is recommended to implement a reliable supplier program (FIT Meat Processor) to prevent purchasing meat contaminated with clenbuterol, and to request a certification of Reliable Producer from SAGARPA along with the implementation of a traceability program.

Jiménez (2011) found between the years 2002 to 2008, more than two thousand cases of intoxication from clenbuterol in Jalisco, Distrito Federal, Guanajuato and Michoacán, which are the states with most cases. The use of clenbuterol has become a problem not only of public health but also of economic and ethical type, since it represents profits for those who distribute it and use it regardless of the consequences; therefore, coordinated actions, timely information, and strict observance of the law could contribute to the compliance of the demand for healthy and innocuous meat products. Based on the Federal Law of Animal Safety, the foundations are provided to SAGARPA for this ministry to carry out the activities that lead to legal action against those who use clenbuterol illegally.

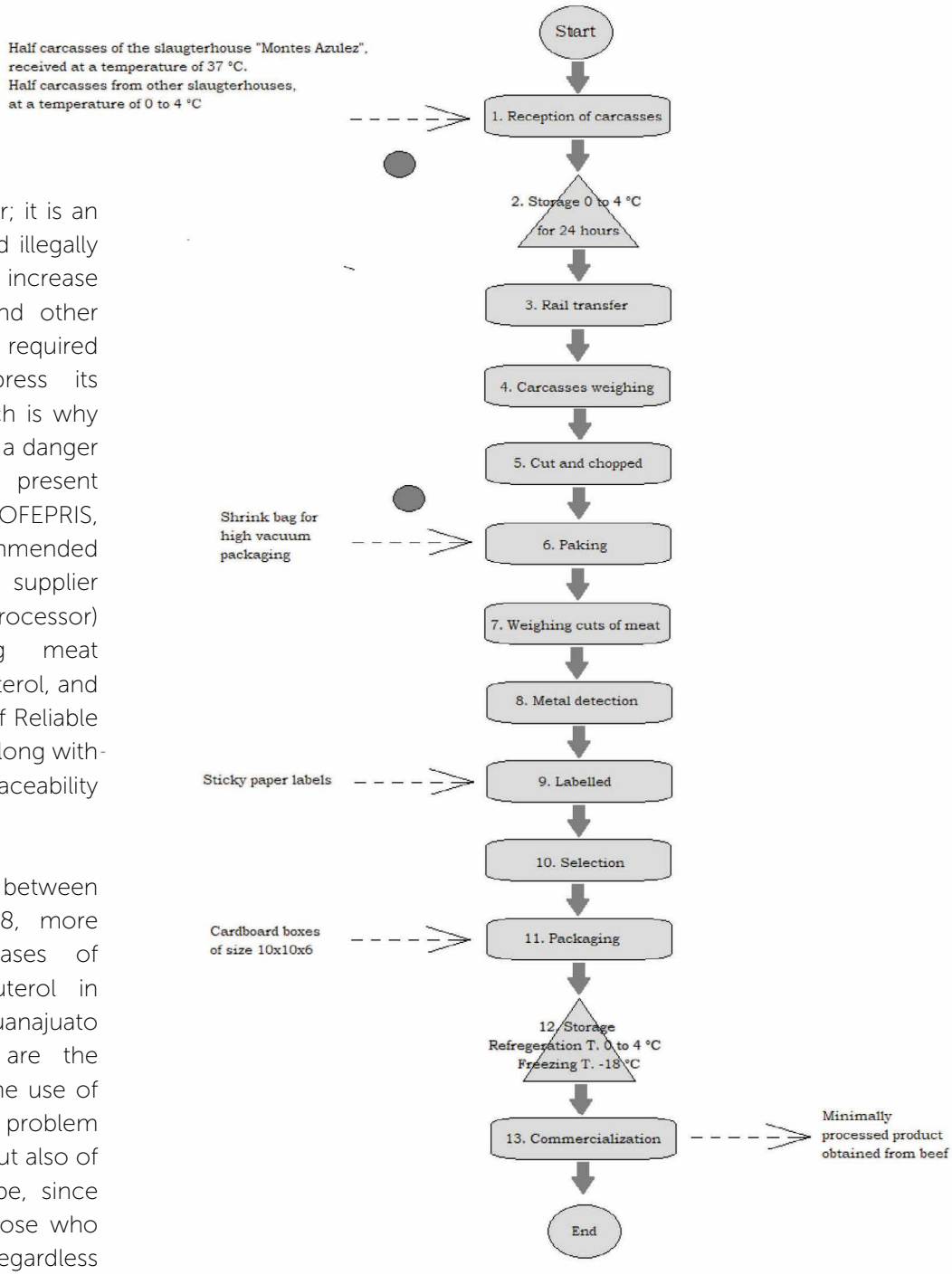

Figure 1. Flow diagram of the meat cuts processing. Source: Prepared by authors.

Evidences of meat contaminated with this chemical were not found in this study; the documents that certified the supplier and endorsed the livestock as clenbuterol-free animals were reviewed. The most important physical dangers of the production process were wires that can come from the slaughter process and presence of metal pieces from utensils used in the cutting and deboning phase (knives, saws, metallic mesh gloves), although this can be prevented with the revision of equipment and work tools and with a metal detector. The chemical and biological dangers mentioned before were not significant; however, the physical dangers mentioned were

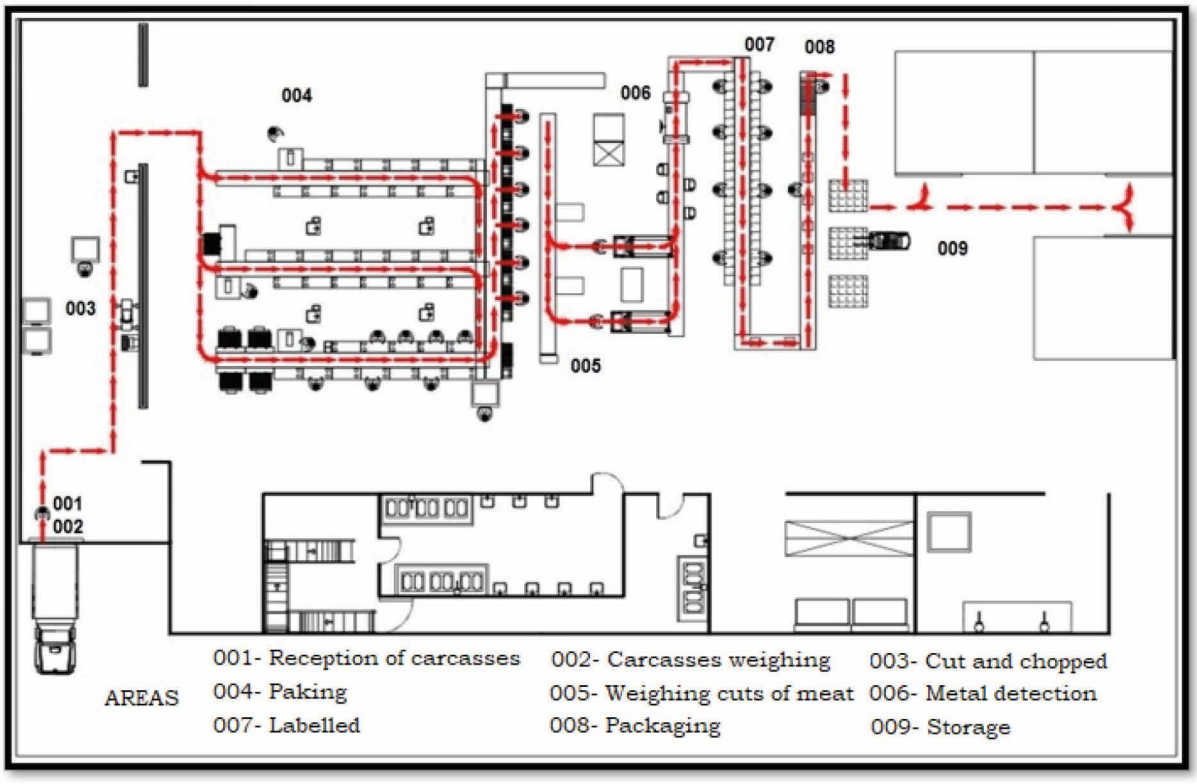

Figure 2. Path diagram of the vacuum-packed meat cuts process. Source: Prepared by authors.

significant and it is necessary to take adequate control measures to manage the risk that was identified in the phase of metal detection.

Defining Critical Control Points (Principle 2)

In the metal detection phase dangers were identified that come from steps prior to the vacuum packaging of beef, which could be metal shards from rails, pieces of knives or saws from the cutting area, and pieces of needles from applying injections on the live animal. With the decision tree diagram, it was determined that the phase of metal detection ***is a critical control point (CCP)***. To control the physical danger, such as metal and wire fragments in the meat, a metal detector should be used as indicated by the National Institute of Normalization (2012).

The United States Department of Agriculture (USDA, 1999), in its general HACCP publication for raw ground products of beef and poultry, established the metal detector as a CCP since it is a control measure for contamination with metal shards, regardless of whether these are introduced by means of the packaging process or in some other point prior to this; it specifies that no metal particle can be bigger than 0.079 cm (0.0313 inches) in size.

Establishing Critical Limits (Principle 3)

The critical control point detected was called the critical limit, according to the characteristics of the equipment used in this phase, which is the case of the sensitivity of the metal detector, with a range of no more than 2.5 mm for iron and non-iron metals and for stainless steel no more than 0.5 mm, a maximum band speed of 4 m s^{-1}. According to the United States Department of Agriculture (USDA, 1999) in its general HACCP publication for raw meat and poultry products, the critical limit in metal detectors is established where no metal particle could be larger than 0.079 cm (0.0313 inch) in size.

Establishing a Security System (Principle 4)

In order to guarantee the fulfillment of the critical limits in the size of metallic residues established, a security method is proposed, by assigning one person in the plant to carry out this activity, who will be in charge of controlling the calibration of the metal detectors, which must pass "pattern samples" with different sizes of metallic residues under the detection arch every hour, respecting the sensitivity parameters of the metal detector and the band speed. There are very modern metal detectors that have an auto-control system, and therefore, they are free of maintenance while others do require verification

after long periods of operation to confirm that they still function correctly (Monreal, 2011).

Establishing Corrective Actions (Principle 5)

Two corrective actions were proposed that are mentioned next: the immediate, which is when the pattern samples could not be identified by the metal detector, and the process must be stopped at that moment to pass the total product of the lot in the most recent hour through the metal detector; and the preventive, which is to verify that the metal detector works with the corresponding parameters, monitoring and calibrating each hour with a pattern sample and every 4 h, with band speed at 4 m s^{-1}.

Establishing Verification Procedures (Principle 6)

Verification of the frequency established proposed by the team should be carried out by trained staff, through monitoring in one-hour lapses, confirming that the operation is being performed correctly and making the corresponding record.

Establishing Documentation for All the Procedures and Records (Principle 7)

To carry out the evidence of verifications, preventive and corrective actions, the elaboration of registry and vigilance formats for the CCP was proposed. For example: in metal detection, the times, calibration, maintenance and person in charge of doing the operation should be recorded.

When identifying the phase of metal detection as Critical Control Point, the critical limits were established (minimum and maximum size of iron, non-iron and stainless steel residues) according to the characteristics of the equipment used by the business. The most important functioning parameter of the metal detector is the sensitivity for the detection of particles: with a range not larger than 2.5 mm for iron and non-iron metals, and not larger than 0.5 mm for stainless steel; the other parameter was the maximum band speed of 4 m s^{-1}. Once the critical limits were established, vigilance procedures were proposed assigned to a person in the plant to carry out such an activity, through good control in the calibration of metal detectors, making pattern samples pass under the detection arch, with frequency of 1 h, respecting the sensitivity parameters of the metal detector and band speed. In case of there being a deviation in the critical control point identified, immediate and preventive measures are established, as well as monitoring and supervision to confirm that the operation is performed correctly, and making the corresponding registry in the formats proposed.

CONCLUSIONS

The CCP1 metal detection is a measure that is carried out in the business; however, when defining its critical limits and monitoring activities, an opportunity to improve the current operation was evidenced. This confirms the importance of the HACCP methodology as an essential system for the detection of the CCP which allows guaranteeing businesses the innocuousness of their products.

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Bioethanol production from agro-industrial *Saccharum* spp. residues: using *Trametes versicolor* in simple fermentation and saccharification processes

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ABSTRACT

Objective: To quantify the production of total and reducing sugars, as well as bioethanol from sugarcane bagasse (*Saccharum* spp.) pretreated with *Trametes versicolor* and 20 %(W/V) NaOH solution.

Design/methodology/approach: An experimental 2^K design was used to determine the effect of the volume of inoculant solution added to wild sugarcane bagasse samples (Factor A; 8 and 10 mL), its incubation period (Factor B; 18 and 24 d), and the bagasse weight/volume of 20 %(W/V) NaOH solution (Factor C; 5 and 20 %(W/V), on the production of total and reducing sugars during simple saccharification. ANOVA analysis was used to evaluate the significance of these factors. The production of bioethanol was achieved via simple fermentation using a commercial strain of *Saccharomyces cerevisiae*.

Results: It was found that the highest yields of reducing and total sugars were obtained with the use of 10 mL of inoculant solution and with an 18 d incubation period during biological pretreatments. The ANOVA analysis suggests that Factors A and B influence the release of sugars, while Factor C was irrelevant. The bioethanol production registered concentrations of up to 7.3 mg mL^{-1} for every 5 g of sugarcane bagasse.

Limitations/implications: This study focused on how treatments using *T. versicolor* and NaOH affected the release of sugars. Optimization of bioethanol production needs to be considered subsequently in another study.

Findings/conclusions: The results have implications for the use of pretreatments for the production of bioethanol from agro-industrial residues.

Keywords: biofuel, cellulose, residues, fermentation.

INTRODUCTION

Biomass is the fundamental resource for the production of biofuels. Biofuels are considered an alternative energy source to petroleum, with the characteristic of being renewable and low environmental pollution indices. Similar to other biofuels such as biodiesel and biogas, bioethanol is easily produced, although it can compete with food production for human consumption when it is processed directly from crops. Using agro-industrial residues as a source of bioethanol aims to make its production more appealing by not competing with arable lands, contributing to decrease production costs since it is a low cost material, and by generating a lower carbon footprint. In this sense, current bioethanol production from agro-industrial residues does not solve the problem of providing energetic security, since there are still many challenges such as excessive water consumption

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during pretreatment of agro-industrial residues, fostering the production high-added value products within secondary biorefining of agro-industrial residues, and finding strategies to strengthen the energetic density in biofuels which is naturally low.

Lignocellulosic residues, such as sugarcane bagasse (*Saccharum* spp.) aside from cellulose, primarily contain the biopolymers hemicellulose and lignin in their matrix. The presence of these components depends on the species, age, and growth phase of the plant (Cardona and Sanchez, 2007). In order to produce bioethanol, it is necessary to remove the complex matrix of hemicellulose and lignin which surrounds cellulose, by applying pretreatments. Any pretreatment applied to agro-industrial residue should take into account increasing to the maximum the enzymatic conversion of polymeric scaffolds, minimizing the loss of sugars, and avoiding the production of inhibitors which could affect subsequent fermentation processes. There are a great number of physicochemical pretreatments (based on trituration, vapor, acids, strong bases, or a combination of these) and biological pretreatments reported, with well-established advantages and disadvantages (Alvira *et al*., 2010; Cardona *et al*., 2010). The purpose of pretreatments is the removal of lignin and hemicellulose, or rather to break the crystalline structure of cellulose in order to make subsequent saccharification and fermentation processes more efficient. In other words, pretreatments increase the accessibility of enzymes during the processes of hydrolysis, allowing the transformation of cellulose and hemicellulose into fermentable sugars (Sun and Cheng, 2002). Biological and physico-biological pretreatments are based on microorganisms, such as fungi, bacteria or algae, and they are generally characterized as being environmentally friendly, and not generating enzymatic inhibitors of any kind; however, they are slow. Nevertheless, the manipulation of a variety of microorganisms, including those genetically modified, can be attractive in order to make subsequent saccharification and fermentation pretreatment processes of lignocellulosic material more efficient (Alvira *et al*., 2010). Some examples of these systems include strains of the yeasts *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* (thermotolerant strain), which have been used for simple saccharification and fermentation (SHF), simultaneous saccharification and fermentation (SSF), or pre-saccharification followed by SSF (Kádár *et al*., 2010; Mesa *et al*., 2011; Cardona *et al*., 2010). These types of systems have achieved satisfactory results in accordance to those expected from glucose production from residues. *Trametes versicolor* is a white-rot fungus which degrades lignin. These characteristics permit easier saccharification of exposed cellulose in order to transform it to glucose, which in turn can be transformed into bioethanol via fermentation. The release of reducing and total sugars from pretreated fibers is achieved by using a mixture of commercial cellulases during simple saccharification. The production of bioethanol was developed in conditions of simple fermentation using *Saccharomices cerevisiae*. Taking this into consideration, this study evaluated the effects of biological pretreatments using *Trametes versicolor* on the production of total and reducing sugars from sugarcane bagasse.

MATERIALS AND METHODS

Sugarcane bagasse (*Saccharum* spp.) was collected from the sugar refinery "Emiliano Zapata", in Zacatepec, Morelos, Mexico. For its use, we proceeded to dry the bagasse by solar radiation for 72 hours, and subsequently sieved until 0.5 × 0.5 mm fibers were obtained.

The bagasse cane fiber was pretreated in two stages. First pretreatments were applied using *Trametes versicolor*, while in the second stage, a 20 %(W/V) NaOH solution was added under thermal conditions. The experimental design consisted in alkaline-biological pretreatments applied to sugarcane bagasse for the removal of lignin and to decrystallize the resulting cellulose structure, taking into account eight different conditions with two replicas, thus resulting in a 2^k experimental design with two levels of response. This allowed the data analysis through an ANOVA approximation.

A. Obtaining *Trametes versicolor*. *Trametes versicolor* was cultivated in a PDA medium for 6 d at 25 °C (Felisa incubator). An inoculum from this microorganism was prepared by placing a sample (1 cm^2 of the fungus) in another liquid medium through puncturing, forcing it to grow in spores. The composition of the second liquid medium was 30 g L^{-1} malt extract, 10 g L^{-1} yeast extract, and 10 g L^{-1} glucose anhydride. Afterwards the spores were incubated for another 96 hours at 25 °C at a speed of 100 rpm (LabTech incubator, Model LSI-3016A), then subsequently washed, liquefied, and finally centrifuged (Hermle Centrifuge, Model Z323K) with Czapek Dox solution.

B. *Trametes versicolor* inoculation. 5 g of sugarcane bagasse were placed in glass bottles (413 mL) with a cotton plug, to which a certain amount of inoculum solution was added (Factor A: Inoculum volume, 4 and 10 mL). See Table 1. Before the inoculation, 30 mL of Czapek Dox solution was added and the bagasse was sterilized for 15 min at 121 °C. The bagasse was incubated for a certain time (Factor B: Incubation time; 18 and 24 d), and was subsequently washed with ~2L of distilled water in order to eliminate any microorganism residues. The samples were dried and stored for their later use.

C. Alkaline pretreatments. The biologically pretreated fibers had a second conditioning when alkaline treatments were applied. For this process, a 20 %(W/V) NaOH solution was incorporated in accordance with the proportion of weight of the bagasse treated biologically vs volume of alkaline solution (Factor C: 5 and 20 %(W/V), as indicated in Table 1. These samples were again thermally treated at 121 °C for 30 minutes, and subsequently washed until a neutral pH was obtained, and then dried. Figure 1 depicts the sequence of processes carried out during alkaline pretreatments.

Table 1. Description of factors used during pretreatment based on solutions with *Trametes versicolor* and 20 %(W/V) NaOH.

Pretreatment	Factor A	Factor B	Factor C
	Inoculum volume (mL)	Incubation time (Days)	Weight of pretreated bagasse/ Volume of NaOH solution (%W/V)
1	4	18	5
2	10	18	5
3	4	24	5
4	10	24	5
5	4	18	20
6	10	18	20
7	4	24	20
8	10	24	20

Simple Saccharification

In order to saccharify pretreated bagasse, 1.5 g of sample was initially put in contact with an enrichment medium and 0.2 mL of Tween 80, at pH=4.8 (Mesa *et al.*, 2010). The enrichment medium was composed of 0.2 g L^{-1} $CaCl_2$, 1 g L^{-1} NaCl, 1.7 g L^{-1} $MgSO_4$, 2 g L^{-1} K_2HPO_4 and 3 g L^{-1} of yeast extract. The pH was conditioned with a 50 mM citric acid solution. Once these conditions were obtained, the mixture was supplemented with 1.8 mL of cellulases 1,4-(1,3:1,4)-β-D-Glucan, 4-glucano-hydrolase (Celluclast 1.5 L, ≥700 units/g, *Trichoderma reesei* ATCC 26921, Sigma Aldrich) and incubated (180 rpm, 5 °C) for 72 hours (Giese *et al.*, 2012). Once the saccharification process was concluded, the supernatant was separated from the bagasse by centrifugation at 3500 rpm for 20 minutes.

Simple Fermentation

An inoculum of *Saccharomices cerevisiae* was prepared for simple fermentation of the saccharified samples. For this, it was necessary to seed fresh commercial yeast in an agar medium of yeast extract (10 g L^{-1} yeast extract,

Figure 1. Pretreatment process applied to sugarcane bagasse (*Saccharum* spp.).

15 g L^{-1} bacteriological agar, 20 g L^{-1} dextrose and 20 g L^{-1} peptone), during an incubation period of 3 days at 25 °C. After incubation, the mixture was stored at 4 °C. At the same time, a nutritive solution (20 g L^{-1} dextrose, 20 g L^{-1} peptone and 10 g L^{-1} yeast extract) was prepared and added for the inoculum to grow, which required another incubation period at 30 °C and 100 rpm for 24 h. Our simple fermentation was carried out in batches. In order to achieve this, ~24 mL of the centrifuged liquid fraction of the saccharified samples was transferred to 50 mL test tubes and conditioned to a pH=6.5. A 0.1 M NaOH solution was added by dosage. The inoculum produced from the yeast was re-suspended and incorporated to this same saccharified portion, and subsequently incubated at 30 °C for 8 h. The fermented sample was centrifuged for 20 min at 3500 rpm in order to analyze the supernatant and discard the precipitate (de Souza *et al.*, 2012). Figure 2 illustrates the sequence of procedures applied during simple saccharification and simple fermentation.

Quantification of total sugars, reducing sugars and ethanol concentration

The quantification of total sugars was achieved with the phenol-sulfuric acid method with glucose calibration (Dubois *et al.*, 1956), in both saccharification and fermentation stages under each of the proposed conditions. Similarly, the DNS colorimetric technique was used to determine reducing sugar concentrations (Harisha, 2007), which is based on the capacity of glucose to reduce 3, 5-dinitrosalicylic acid. All of the colorimetric determinations were conducted at 540 nm in a UV-Visible Jenway spectrophotometer, Genova Model. Finally, the quantification of bioethanol was also performed through colorimetry using the enzyme assay equipment Megazyme (Megazyme International Ireland, Wicklow, Ireland) to detect ethanol. In this stage, the assay consisted of two subsequent enzymatic reactions. During the first reaction (Equation (1)), the bioethanol is catalyzed by alcohol dehydrogenase (ADH) to acetaldehyde, in the presence of nicotinamide adenine dinucleotide (NAD^+),

$$CH_3CH_2OH + NAD^+ (ADH) \rightarrow CH_3CHO + NADH + H^+ \quad (1)$$

Afterwards, the same acetaldehyde is oxidized to acetic acid in the presence of aldehyde dehydrogenase (Al-DH) and NAD^+ (Equation (2)).

$$CH_3CHO + NAD^+ + H_2O(Al-DH) \rightarrow CH_3COOH + NADH + H^+ \quad (2)$$

The quantification of bioethanol was achieved by determining the amount of NADH produced, which presents a significant absorption at 340 nm.

RESULTS AND DISCUSSION

Table 2 shows the quantification of total sugars after saccharification and fermentation processes, applied to samples pretreated sequentially with *T. versicolor* and NaOH. At a glance, these results reveal two tendencies when 10 mL of the inoculum was added to the wild samples (Factor A): higher production of total sugars after saccharification, and lower concentration

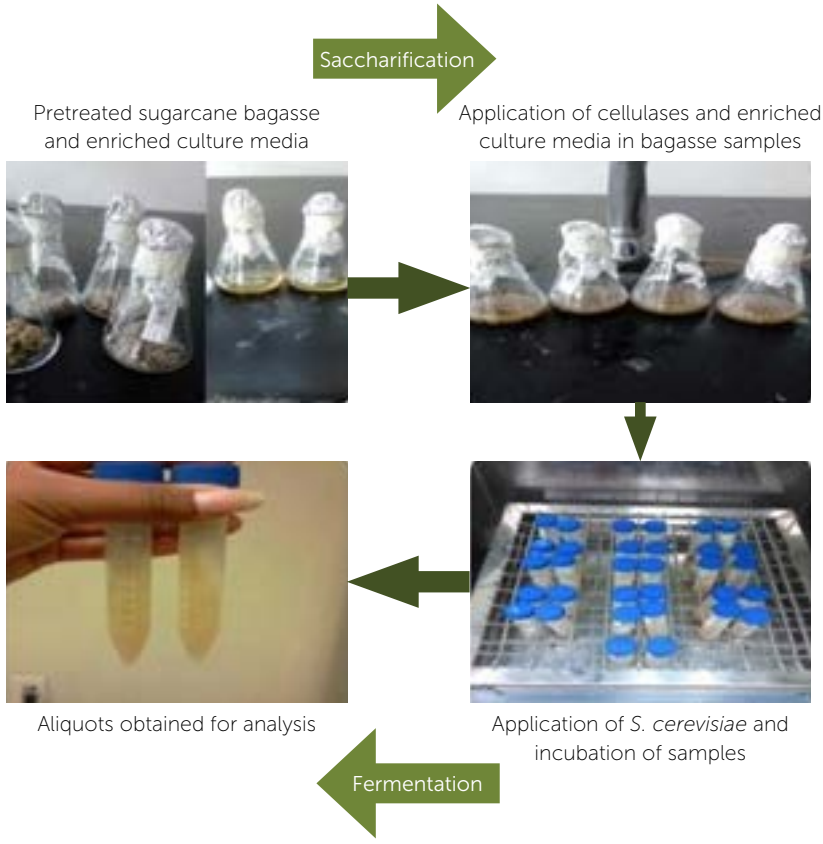

Figure 2. Sequence of processes for saccharification of pretreated sugarcane bagasse samples (*Saccharum* spp.), and of fermentation in their respective liquid extracts, both in simple conditions.

Table 2. Quantification of total sugars after simple saccharification and fermentation processes of samples pretreated with *Trametes versicolor*/NaOH.

No.	Factor A	Factor B	Factor C	Total sugars (mg/mL)			
	Inoculum volume (mL)	Incubation Time (days)	% W/V Bagasse/ NaOH Solution	After Saccharification		After Fermentation	
				Rep. 1	Rep. 2	Rep. 1	Rep. 2
1	4	18	5	13,35	25,28	13,52	16,25
2	10	18	5	30,61	35,98	10,6	9,4
3	4	24	5	37,52	32,32	16,62	13,85
4	10	24	5	25,8	39,05	14,79	12,21
5	4	18	20	16,46	17,5	8,39	8,35
6	10	18	20	36,19	34,38	14,75	12,62
7	4	24	20	24,62	22,21	17,78	18,68
8	10	24	20	46,69	25,35	16,25	15,91

of these sugars after fermentation. These tendencies occur independently of the bagasse/NaOH %(W/V) ratio (Factor C), which allows one to assume that this factor was irrelevant during saccharification and fermentation.

A simple analysis can also be applied to the results of reducing sugars, as shown in Table 3, highlighting that analogous trends are present in the concentration of reducing sugars as with total sugars; namely, that the addition of 10 mL of *Trametes versicolor* inoculum influences the production of sugars, and that Factor C is irrelevant. This should be taken into account given that the bioethanol yield directly depends on the amount of reducing sugars present. The results shown in Table 3 also suggest that in both saccharification and fermentation processes, a big portion of the total sugars actually corresponds to reducing sugars, within the margins of experimental error.

Elevated sugar concentrations are to be expected in our saccharified samples due to the high cellulose content in the sugarcane bagasse (~50%) (Sun and Cheng, 2002). In order to determine which factor or source had the highest attributable effect on production of total and reduced sugars, an ANOVA analysis was conducted considering the factorial design described above. Table 4 shows the ANOVA analysis with a significance of α=0.05 for both types of sugars. The value of α indicates from which probability ANOVA will not detect a significant difference. Thus, at lower probability there will be higher certainty that there is a significant difference.

The ANOVA analysis confirms the initial observations. Factor C % (bagasse weight/volume of 20 %(W/V) NaOH solution) is not significant in the release of reducing or total sugars during the saccharification process. On the contrary, the volume of solution incorporated containing *Trametes versicolor* (Factor A) has an important effect,

Table 3. Quantification of reducing sugars and ethanol after simple saccharification and fermentation processes of samples pretreated with *Trametes versicolor*/NaOH.

No.	Factor A	Factor B	Factor C	Total sugars (mg/mL)				Ethanol (mg/mL)	
	Inoculum volume (mL)	Incubation Time (days)	% W/V Bagasse/ NaOH Solution	After Saccharification		After Fermentation			
				Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
1	4	18	5	8.29	18.64	8.67	15.15	4.03	5.42
2	10	18	5	32.68	24.99		4.02	4.63	5.69
3	4	24	5	25.3	25.53	9.41	10.82	6.13	6.33
4	10	24	5	34.31	24.85	9.22	7.34	5.52	5.49
5	4	18	20	14.54	16.93	1.83	2.34	5.05	5.47
6	10	18	20	36.65	38.58	11.64	6.95	7.31	6.81
7	4	24	20	18.56	17.33	14.37	12.38	5.08	4.81
8	10	24	20	28.41	25.17	12.3	12.77	5.64	5.64

as well as its interference on the number of incubation days (Factor AB). In other words, statistically speaking the volume of inoculum that was added to the wild samples affects the production of both types of sugars. On the other hand, the ANOVA analysis also suggests that the days of incubation in this study (18 and 24 d) are not relevant for the release of reducing sugars, but is important for total sugars. It is important to point this out, since one of the disadvantages of biological pretreatments is the time required for the biomass to be exposed to microorganisms. We suggest a separate study to explore this finding further.

Table 4. ANOVA analysis for the quantification of total and reducing sugars after saccharification of wild samples.

Origin of variation	Total sugars		Reducing sugars	
	F	F (0.05)	F	F (0.05)
A	8,57	0,019	36,44	0
B	2,29	0,169	0,24	0,637
AB	2,34	0,164	8,48	0,02
C	0,32	0,584	0,01	0,927
AC	1,8	0,217	1,81	0,216
BC	0,27	0,616	6,55	0,034
ABC	0,55	0,478	0,05	0,832

The impact of fermentation on sugars is also taken into account. This is analyzed in Table 5. Regardless of the type of pretreatment applied to the sugarcane bagasse, it stands out that simple fermentation induced a significant decrease in reducing sugars. Once more the effect of adding 10 mL of inoculum solution was noted, since the increased concentration of reducing sugars permitted higher consumption and better yield when producing bioethanol, compared to adding 4 mL.

It is difficult to correlate the direct effect of the of the biological-alkaline pretreatment conditions with bioethanol efficiency, excluding the intuitive idea of having an elevated concentration of reducing sugars. The fermentation process has its own variables that need to be controlled, including fermentation temperature, yeast concentration, time of exposure and presence of inhibitors, among others. Therefore, a separate study is needed to analyze optimization of fermentation using samples pretreated with *Trametes versicolor*. The importance of this study is valued when we consider that sugarcane is one of the principal crops in the country, and especially in the state of Morelos. Thus, finding adequate methods for producing bioethanol from its residues is of great importance.

CONCLUSIONS

This study established conditions for production of reducing and total sugars, as well as bioethanol, from sugarcane bagasse that was pretreated with *Trametes versicolor*. A volume of 10 mL of this inoculum had a significant influence on the liberation of reducing and total sugars, and in the subsequent production of bioethanol. Under these same conditions, the incubation time intervals for the fungus acting on the fiber (18 and 24 d) allowed obtaining a concentration of reducing sugars in a range of 25.17 to 38.58 mg mL^{-1}. Slightly higher concentrations were found in the samples incubated for 18 d. Our ANOVA analysis sugests that during the alkaline pretreatment, the proportion of pretreated sugarcane bagasse mass *vs* the volume of 20 %(W/V NaOH solution used were not significant enough to influence the production of sugar.

Table 5. Concentration of reducing sugars consumed and bioethanol production.

Pretreatment	Factor A	Factor B	Factor C	Reducing Sugars				Ethanol (mg/mL)	
	Inoculum volume (mL)	Incubation time (days)	% W/V Bagasse/NaOH Solution	Consumption (m/mL)		% Consumption			
				Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
1	4	18	5	−0,38	3,49	−4,58	18,72	4,03	5,42
2	10	18	5	22,8	20,97	69,76	83,91	4,63	5,69
3	4	24	5	15,89	14,71	62,81	57,62	6,13	6,33
4	10	24	5	25,09	17,51	73,13	70,46	5,52	5,49
5	4	18	20	12,71	14,59	87,41	86,18	5,05	5,47
6	10	18	20	25,01	31,63	68,24	81,99	7,31	6,81
7	4	24	20	4,19	4,95	22,58	28,56	5,08	4,81
8	10	24	20	16,11	12,4	56,71	49,27	5,64	5,64

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