ISSN: 2594-0252

AGRO PRODUCTIVIDAD

AP

Potential areas for taro (*Colocasia esculenta* (L.) Schott) cultivation in Tabasco, Mexico

pág. 3

Año 15 • Volumen 15 • Número 1 • enero, 2022

Colegio de Postgraduados

Contenido

Año 15 • Volumen 15 • Número 1 • enero, 2022

Bases de datos de contenido científico

Directorios

Año 15, Volumen 15, Número 1, enero 2022, Agro productividad es una publicación mensual editada por el Colegio de Postgraduados. Carretera México-Texcoco Km. 36.5, Montecillo, Texcoco, Estado de México. CP 56230. Tel. 5959284427. www.colpos.mx. Editor responsable: Dr. Jorge Cadena Iñiguez. Reservas de Derechos al Uso Exclusivo No. 04-2017-031313492200-203. ISSN: 2594-0252, ambos otorgados por el Instituto Nacional del Derecho de Autor. Responsable de la última actualización de este número, M.C. Valeria Abigail Martínez Sias. Fecha de última modificación, 5 de febrero de 2022.

Las opiniones expresadas por los autores no necesariamente reflejan la postura del editor de la publicación.

Contacto principal
Jorge Cadena Iñiguez
Guerrero 9, esquina avenida Hidalgo, C.P. 56220, San Luis Huexotla, Texcoco, Estado de México.
agroproductividadeditor@gmail.com

Contacto de soporte
Soporte
5959284703
agroproductividadsoporte@gmail.com

Directrices para Autores/as

Naturaleza de los trabajos: Las contribuciones que se reciban para su eventual publicación deben ser resultados originales derivados de un trabajo académico de alto nivel sobre los tópicos presentados en la sección de temática y alcance de la revista.

Extensión y formato: Los artículos deberán estar escritos en procesador de textos, con una extensión de 15 cuartillas, tamaño carta con márgenes de 2.5 centímetros, Arial de 12 puntos, interlineado doble, sin espacio entre párrafos. Las páginas deberán estar foliadas desde la primera hasta la última en el margen inferior derecho. La extensión total incluye abordaje textual, bibliografía, gráficas, figuras, imágenes y todo material adicional. Debe evitarse el uso de sangría al inicio de los párrafos. Las secciones principales del artículo deberán escribirse en mayúsculas, negritas y alineadas a la izquierda. Los subtítulos de las secciones se escribirán con mayúsculas sólo la primera letra, negritas y alineadas a la izquierda.

Exclusividad: Los trabajos enviados a Agro Productividad deberán ser inéditos y sus autores se comprometen a no someterlos simultáneamente a la consideración de otras publicaciones; por lo que es necesario adjuntar este documento: Carta de originalidad.

Frecuencia de publicación: Cuando un autor ha publicado en la revista como autor principal o de correspondencia, deberá esperar tres números de ésta para publicar nuevamente como autor principal o de correspondencia.

Idiomas de publicación: Se recibirán textos en inglés con títulos, resúmenes y palabras clave en inglés.

ID Autores: El nombre de los autores se escribirán comenzando con el apellido o apellidos unidos por guion, sólo las iniciales del nombre, separados por comas, con un índice progresivo en su caso. Es indispensable que todos y cada uno de los autores proporcionen su número de identificador normalizado ORCID, para mayor información ingresar a (https://orcid.org).

Institución de adscripción: Es indispensable señalar la institución de adscripción y país de todos y cada uno de los autores, indicando exclusivamente la institución de primer nivel, sin recurrir al uso de siglas o acrónimos. Se sugiere recurrir al uso de la herramienta wyta (http://wayta.scielo.org/) de Scielo para evitar el uso incorrecto de nombres de instituciones.

Anonimato en la identidad de los autores: Los artículos no deberán incluir en ni en cuerpo del artículo, ni en las notas a pie de página ninguna información que revele su identidad, esto con el fin de asegurar una evaluación anónima por parte de los pares académicos que realizarán el dictamen. Si es preciso, dicha información podrá agregarse una vez que se acredite el proceso de revisión por pares.

Estructura de los artículos: Los artículos incluirán los siguientes elementos: Título, title, autores y adscripción, abstract, keywords, resumen, palabras clave, introducción, objetivos, materiales y métodos, resultados y discusión, conclusiones y literatura citada en formato APA.

Título: Debe ser breve y reflejar claramente el contenido, deberá estar escrito en español e inglés. Cuando se incluyan nombres científicos deben escribirse en itálicas. No deberá contener abreviaturas ni exceder de 20 palabras, se usará solo letras mayúsculas, en negritas, centrado y no llevará punto final.
Resumen y Abstract: Deberá integrarse un resumen en inglés y español (siguiendo ese orden), de máximo 250 palabras, donde se destaque obligatoriamente y en este orden: a) objetivo; b) diseño / metodología / aproximación; c) resultados; d) limitaciones / implicaciones; e) hallazgos/ conclusiones. El resumen no deberá incluir citas, referencias bibliográficas, gráficas ni figuras.
Palabras clave y Keywords: Se deberá incluir una lista de 3 a 5 palabras clave en español e inglés que permitan identificar el ámbito temático que aborda el artículo.
Introducción: Se asentará con claridad el estado actual del conocimiento sobre el tema investigado, su justificación e importancia, así como los objetivos del trabajo. No deberá ser mayor a dos cuartillas.
Materiales y Métodos: Se especificará cómo se llevó a cabo la investigación, incluyendo el tipo de investigación, diseño experimental (cuando se traten de investigaciones experimentales), equipos, substancias y materiales empleados, métodos, técnicas, procedimientos, así como el análisis estadístico de los datos obtenidos.
Resultados y Discusión: Puede presentarse en una sola sección. En caso de presentarse de forma separada, la discusión debe enfocarse a comentar los resultados (sin repetirlos), en términos de sus características mismas, su congruencia con la hipótesis planteada y sus semejanzas o diferencias con resultados de investigaciones similares previamente realizadas.
Conclusiones: Son la generalización de los resultados obtenidos; deben ser puntuales, claras y concisas, y no deben llevar discusión, haciendo hincapié en los aspectos nuevos e importantes de los resultados obtenidos y que establezcan los parámetros finales de lo observado en el estudio.
Agradecimientos: Son opcionales y tendrán un máximo de tres renglones para expresar agradecimientos a personas e instituciones que hayan contribuido a la realización del trabajo.
Cuadros: Deben ser claros, simples y concisos. Se ubicarán inmediatamente después del primer párrafo en el que se mencionen o al inicio de la siguiente cuartilla. Los cuadros deben numerarse progresivamente, indicando después de la referencia numérica el título del mismo (Cuadro 1. Título), y se colocarán en la parte superior. Al pie del cuadro se incluirán las aclaraciones a las que se hace mención mediante un índice en el texto incluido en el cuadro. Se recomienda que los cuadros y ecuaciones se preparen con el editor de tablas y ecuaciones del procesador de textos.
Uso de siglas y acrónimos: Para el uso de acrónimos y siglas en el texto, la primera vez que se mencionen, se recomienda escribir el nombre completo al que corresponde y enseguida colocar la sigla entre paréntesis. Ejemplo: Petróleos Mexicanos (Pemex), después sólo Pemex.
Elementos gráficos: Corresponden a dibujos, gráficas, diagramas y fotografías. Deben ser claros, simples y concisos. Se ubicarán inmediatamente después del primer párrafo en el que se mencionen o al inicio de la siguiente cuartilla. Las figuras deben numerarse progresivamente, indicando después de la referencia numérica el título del mismo (Figura 1. Título), y se colocarán en la parte inferior. Las fotografías deben ser de preferencia a colores y con una resolución de 300 dpi en formato JPG, TIF o RAW. El autor deberá enviar 2 fotografías adicionales para ilustrar la página inicial de su contribución. Las gráficas o diagramas serán en formato de vectores (CDR, EPS, AI, WMF o XLS).
Unidades. Las unidades de pesos y medidas usadas serán las aceptadas en el Sistema Internacional.
Citas bibliográficas: deberán insertarse en el texto abriendo un paréntesis con el apellido del autor, el año de la publicación y la página, todo separado por comas. Ejemplo (Zheng et al., 2017). El autor puede introducir dos distintos tipos de citas:

Citas directas de menos de 40 palabras: Cuando se transcriben textualmente menos de 40 palabras, la cita se coloca entre comillas y al final se añade entre paréntesis el autor, el año y la página. Ejemplo:

> Alineado al Plan Nacional de Desarrollo 2013-2018, (DOF, 2013), el Programa Sectorial de Desarrollo Agropecuario, Pesquero y Alimentario 2013-2018 establece "Construir un nuevo rostro del campo sustentado en un sector agroalimentario productivo, competitivo, rentable, sustentable y justo que garantice la seguridad alimentaria del país" (DOF, 2013).

Citas indirectas o paráfrasis: Cuando se interpretan o se comentan ideas que son tomadas de otro texto, o bien cuando se expresa el mismo contenido pero con diferente estructura sintáctica. En este caso se debe indicar el apellido del autor y el año de la referencia de donde se toman las ideas. Ejemplo:

> Los bajos rendimientos del cacao en México, de acuerdo con Avendaño et al. (2011) y Hernández-Gómez et al. (2015); se debe principalmente a la edad avanzada de las plantaciones.

Las referencias bibliográficas: al final del artículo deberán indicarse todas y cada una de las fuentes citadas en el cuerpo del texto (incluyendo notas, fuentes de los cuadros, gráficas, mapas, tablas, figuras etcétera). El autor(es) debe revisar cuidadosamente que no haya omisiones ni inconsistencias entre las obras citadas y la bibliografía. Se incluirá en la lista de referencias sólo las obras citadas en el cuerpo y notas del artículo. La bibliografía deberá presentarse estandarizada recurriendo a la norma APA, ordenarse alfabéticamente según los apellidos del autor.
De haber dos obras o más del mismo autor, éstas se listan de manera cronológica iniciando con la más antigua. Obras de un mismo autor y año de publicación se les agregará a, b, c… Por ejemplo:

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

Artículo de revista:
Wang, P., Zhang, Y., Zhao, L., Mo, B., & Luo, T. (2017). Effect of Gamma Rays on Sophora davidii and Detection of DNA Polymorphism through ISSR Marker [Research article]. https://doi.org/10.1155/2017/8576404

Libro:
Turner J. (1972). Freedom to build, dweller control of the housing process. New York: Macmillan.

Uso de gestores bibliográficos: Se dará prioridad a los artículos enviados con la bibliografía gestionada electrónicamente, y presentada con la norma APA. Los autores podrán recurrir al uso de cualquier gestor disponible en el mercado (Reference Manager, Crossref o Mendeley entre otros), o de código abierto tal como Refworks o Zotero.

AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Potential areas for taro (*Colocasia esculenta* (L.) Schott) cultivation in Tabasco, Mexico

Martínez-Herrera, Jorge[1]; Sánchez-Hernández, Rufo[2]; Ramos-Reyes, Rodimiro[3*]

[1] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experimental Huimanguillo. Carretera Huimanguillo-Cárdenas km 1, Huimanguillo, Tabasco, México. C. P. 86400.

[2] Universidad Juárez Autónoma de Tabasco. División Académica de Ciencias Agropecuarias. Carretera Villahermosa-Teapa km 25 Ramal La Huasteca, Segunda Sección, Villahermosa, Tabasco, México. C. P. 86280.

[3] El Colegio de la Frontera Sur (ECOSUR) Unidad Villahermosa. Departamento de Observación y Estudio de la Tierra, la Atmósfera y el Océano, Grupo Procesos Oceanográficos y Dinámica de Sistemas Acuáticos. Carretera a Reforma km 15.5, Ranchería Guineo 2da. Sección. Villahermosa, Tabasco, México. C. P. 86280.

* Correspondence: rramos@ecosur.mx

ABSTRACT

Objective: To analyze the productive potential of taro in the state of Tabasco, Mexico, in order to suggest the edaphoclimatic zones with the best conditions for its use.

Design/Methodology/Approach: The analysis was carried out using geographic information systems QGIS 3.16.1 and Arc GIS 10.2.2, to generate spatial interpolations of edaphic and climatic variables. The edaphoclimatic zoning for this crop in the state was generated through map algebra.

Results: The results indicate that the edaphoclimatic conditions allow the establishment of this crop in 1 608, 565 hectares, which is equivalent to 67% of the territory of Tabasco, although the optimal surface is only 655, 632 hectares (27%).

Study Limitations/Implications: The main limitations for cultivation are mainly edaphic, rather than climatic.

Findings/Conclusions: From the edaphic point of view, the most suitable soils are flat, deep, with loamy or clayey textures, with slightly acidic or neutral pH. The physiographic areas of the alluvial plain, river valley and hills are the optimal ones for the cultivation of taro. The unsuitable areas are those with permanent flooding and close to the coast, as well as in the highest areas of the Sierra de Tabasco.

Keywords: Tuber crop, taro, edaphoclimatic zoning, tropical climate, tropical soils.

Citation: Martínez-Herrera, J., Sánchez-Hernández, R., & Ramos-Reyes, R. (2022). Potential areas for taro (*Colocasia esculenta* (L.) Schott) cultivation in Tabasco, Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v15i1.1999

Editor in Chief: Dr. Jorge Cadena Iñiguez

Received: April 20, 2021.
Accepted: January 6, 2022.
Published on-line: February 5, 2022.

Agro Productividad, 15(1). January. 2022. pp: 3-10.

INTRODUCTION

Taro (*Colocasia esculenta* (L.) Schott) is a vegetable originally from Asia, which was introduced to America by black slaves; it is considered that the area of greatest variability of this genus is in the Antilles, where it is the oldest crop inherited from the indigenous Arawak people in Puerto Rico. It was introduced from Africa in 1843 and its species are cultivated in many islands of the Pacific, including New Guinea, Fiji and New Caledonia (Zapata, 2013; Mazariegos *et al.*, 2017; Barrera *et al.*, 2004). In Mexico, it has been

cultivated commercially in the states of Veracruz, Oaxaca, Puebla, Nayarit and Sinaloa, primarily for exports to the United States and Canada. Just for the farming year in Veracruz, principal national producer, 482 hectares cultivated are reported, with a production of 36,128 tons; the production value reached 158.28 million pesos (SIAP, 2019). Likewise, a variant adapted in wild form in tropical zones is produced on the edges of rivers, streams or lagoons (Rodríguez *et al.*, 2011). It is framed within exotic or non-traditional products, whose global consumption has seen an important rise, taking advantage of the interest from growing sectors of consumers that use it in human and animal diet and for different industrial uses (Eleazu, 2016; Caicedo, 2013). It is an alternative and exotic crop, also known as taro, dashen or ñame, with great potential for tropical zones (Rodríguez *et al.*, 2011).

Taro is an annual herbaceous plant, whose cycle consists of nine months. It prospers in warm-humid climates, with temperatures that range between 25 and 35 °C and at altitudes of 0-1000 masl. It prefers lose silt soils (with high content of organic matter and a pH of 5.5 to 6.5) and clayey soils, although if there is no availability of water for the harvest the activity is made difficult. In addition, it tolerates flooding, surviving up to three days under water, since it is a plant that has high water demand (López *et al.*, 2020). Currently taro is a crop that has not been exploited in Tabasco, it is produced naturally, and it is only consumed traditionally cooked, fried and in *atole*. Producing taro is a profitable business, as the market is increasingly larger, although more production is required to supply the list of clients that is increasing. Taro is a tuber full of benefits and properties, ideal within a balanced diet due to its wealth in essential nutrients and healing and preventive qualities (Figure 1).

The usable parts are tuberous underground stalks that contain between 6.87 and 10% of moisture, 1.1-7% of protein, 1.2 to 2.5% of lipids, 2.0 to 4.0% of ash, and starch content higher than 60% (Madrigal *et al.*, 2018).

The territory in Tabasco has high edaphoclimatic potential to cultivate taro, since there is favorable temperature and precipitation for the good development and growth of the

Figure 1. Commercial taro (*Colocasia esculenta* (L.) Schott) plantation in the municipality of Cunduacán, Tabasco, Mexico. Source: Dr. Rutilo López-López.

taro crop, average temperatures of 28 °C to 35°C, which is not a limitation for production of the crop. This makes it a product with high potential for its implementation in the country, participating actively in the reconversion of crops that need it (Figure 2).

The state of Tabasco is characterized by high rainfall in the summer, with warm-subhumid climates that favor the development of the taro crop; however, the producers are unaware of the agronomic management of this species (Salgado, 2003).

The crop adapts to the edaphoclimatic conditions of the state of Tabasco; specifically it prefers silty-clayey soils with pH of 5 to 7. Growing taro in Tabasco is practiced with traditional technology, in small surfaces by producers with average yields of 20 t ha^{-1}, in monocrop or interspersed with other crops at the level of family garden. The objective of this study was to analyze the productive potential for the state of Tabasco, to suggest the best edaphoclimatic zones of best conditions for its exploitation.

MATERIALS AND METHODS

Geographic Location

The territory in Tabasco has 17 municipalities and is divided into two economic regions, Grijalva and Usumacinta, of which there are five sub-regions (Centro, Chontalpa, Sierra, Pantanos and Los Ríos). The first two are located in the Grijalva zone and the last three in Usumacinta, and these names are due primarily to the hydrological representation of two large rivers that cross Tabasco. The state has a territorial extension of 24 661 km^2, with the coordinates 18° 39' 03" North, 17° 15' 03" South, 90° 59' 15" East, and 94° 07' 48" West. It borders north with the Gulf of Mexico and Campeche; east with Campeche and Guatemala; south with Chiapas; west with Veracruz de Ignacio de la Llave (INEGI, 2017). Tabasco is a state located in southeastern Mexico, which presents different uses of the soil, although more than half of the territory is used as pasture (mainly livestock producing activities). There are many other activities such as oil production, tourism, forestry and agriculture, where there is still the option of growing taro, since it is a crop with high

Figure 2. Harvest of taro (*Colocasia esculenta* (L.) Schott) corms, ready for their commercialization. Source: Dr. Rutilo López-López.

demand whose offer is not covered by the state production, which is why it has to be imported from neighboring states such as Veracruz. Edaphoclimatic factors were used that limit the cultivation of taro in the state of Tabasco (Table 1).

Interpolation and algebra of taro zoning maps in Tabasco

The geographic information systems used were QGIS 3.16.1 and Arc GIS 10.2.2, where spatial interpolations were generated of the soil and climate variables, in order to relate the entire territory in Tabasco and also to carry out the algebra of maps in order to have different zones and generate edaphoclimatic zones that are more apt for taro cultivation in the state of Tabasco.

RESULTS AND DISCUSSION

Climate zoning

The combination of elements from the climate in the state of Tabasco make practically more than 75% of the territory optimal for taro cultivation and 18% good, particularly in the municipalities of Huimanguillo, Teapa, Tenosique and Balancán; and only a small part of the sierra in Tabasco (7%), specifically in the municipalities of Huimanguillo and Tacotalpa, do not present conditions for the crop (Figure 3).

Because of the climatic requirements of taro, only a small extension of the territory in Tabasco is excluded from the cultivation of this plant. Regarding the water supply from rainfall, the entire territory of Tabasco presents a tropical climate (A), which is characterized by the record of mean annual temperature higher than 18 °C and a precipitation between 800 and 4000 mm per year. This tropical climate is subdivided into three climate regions: Af (tropical with rainfall all year); Aw (tropical with rainfall in the summer) and Am (tropical with monsoon rainfall) (Rivera-Hernández *et al.*, 2012). In every case, the water supply does not represent any limitation. Taro is a plant that grows adequately up to altitudes of 1000 masl, which is why the altitude also does not represent limitations, since the highest part of the state of Tabasco is located at 500 masl (Zavala-Cruz *et al.*, 2016). In the zone known as the Sierra, some restrictions can take place, particularly in what concerns temperatures, which in some seasons of the year can lower the critical threshold that is adequate for the crop, which is 25 °C.

Table 1. Factors and variables for the zoning of taro (*Colocasia esculenta* (L.) Schott) in Tabasco, Mexico.

Factors	Variables					
	Slope (%)		**Texture**		**pH**	
Edaphic	0-12	Optimal	Franco arenoso	Good	5.6-7.4	Optimal
	13-25	Good	Clay, clay loam, sandy clay loam; and organic	Not suitable	4.8-5.5	Good
	26-70	No suitable			<4.7	Not suitable
Climatic	**Precipitation**		**Temperature (°C)**		**Climate Type**	
	1500-2500	Optimal	22-28	Optimal	Am	Optimal
	2501-4500	No suitable			Af and Aw	Good

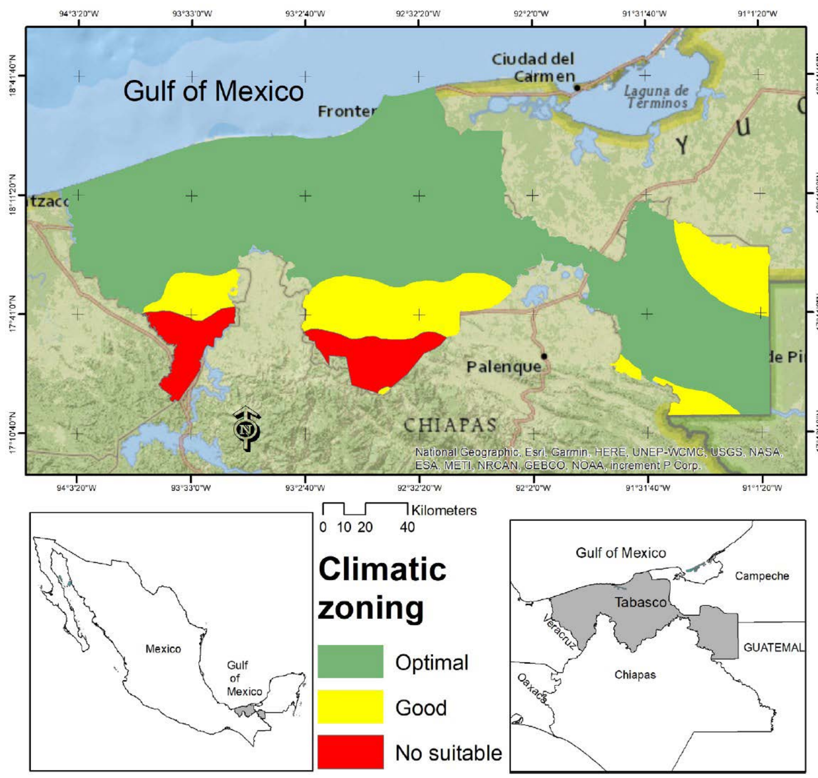

Figure 3. Climate zoning for the taro crop (*Colocasia esculenta* (L.) Schott) in Tabasco, Mexico. Source: prepared by the authors.

Soil zoning

According to the zoning of the soil aptitude for the taro crop, 34% of the surface presents an optimal aptitude; 38% is good and 28% is not apt (Figure 4), which is why it can be clearly seen that the soil is the factor of highest restriction, above the climate factor.

The soils in Tabasco are classified in 81 soil sub-units, distributed in seven physiographic zones: sierra, calcareous, hills, alluvial plain, river valley, flooding plain and coast (Palma *et al.*, 2007). From these physiographic zones, the sierra, calcareous and flooding plain zones are the ones that present most restrictions, and this is due to problems associated with the shallow depth of the soil, stoniness, water excess, permanent flooding or low fertility. Therefore, the concentrations of soil units that are optimal for taro cultivation are grouped into zones of alluvial plain, river valley and hills. Taro cultivation is adapted to low flooded lands due to its capacity to transport oxygen from the leaves to the roots, which is vital for the growth and normal functioning of the roots (Raju and Byju, 2018). However, the crop prefers silty soils that are rich in organic matter, neutral or slightly acid, and although clayey soils can offer conditions for the crop, if they present water deficit during the harvest, this can be problematic, in addition to the clayey soils presenting compacting problems, which makes corm growth difficult.

Edaphoclimatic zoning

When combining the layers of soil and the climate, the edaphoclimatic zoning is generated for the taro crop in Tabasco (Figure 5). In this image it can be seen that 67%

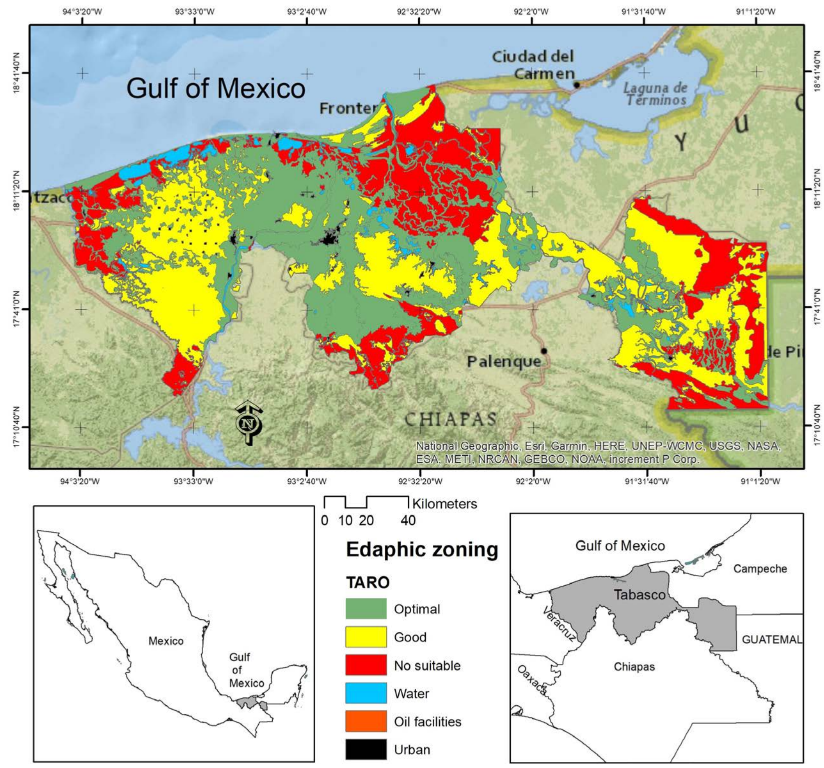

Figure 4. Soil zoning for the taro crop (*Colocasia esculenta* (L.) Schott) in Tabasco, Mexico. Source: prepared by authors.

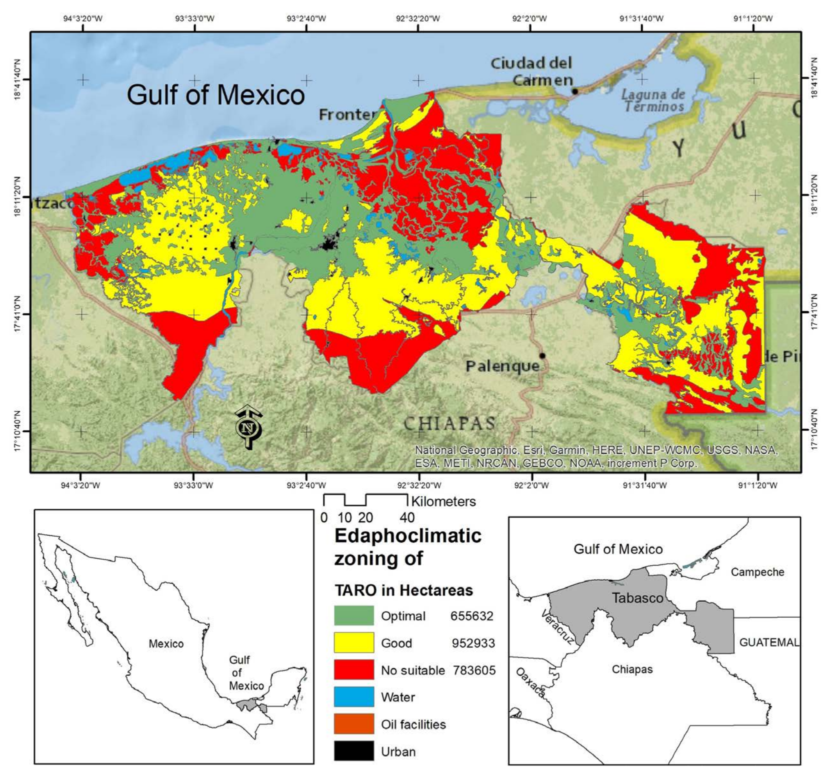

Figure 5. Edaphoclimatic zoning for the taro crop (*Colocasia esculenta* (L.) Schott) in Tabasco, Mexico. Source: prepared by authors.

of the surface of the territory in Tabasco presents conditions for the crop; however, only 27% is optimal, 40% is good, while 33% of the surface is not apt for production of the crop. These areas of greater vocation are distributed in the zones of the alluvial plain, river valley and hills, where the following soils predominate: Fluvisol, Cambisol, Regosol, Vertisol, Gleysols, Luvisol, and Acrisol (Palma *et al.*, 2007).

Taro can withstand prolonged periods of flooding, frequent in the clayey soils of Tabasco; however, the type of clay and the degree of compacting must be taken into account to select the cultivation areas (Mazariegos *et al.*, 2017).

CONCLUSIONS

The taro crop (*Colocasia esculenta* [L.] Schott) is adapted to the edaphoclimatic conditions in 67% of the territory of Tabasco (1,657,446 hectares), although with some limitations, primarily of the soil, and to a lesser degree from climate conditions, which is why the optimal areas for the crop are reduced to 677,926 hectares (27%). From the point of view of the soil, the deepest soils with good internal drainage, silt or clay textures, rich in organic matter and with slightly acidic or neutral pH are the most adequate for the crop. The climate factor does not represent an important obstacle for the development of the plant, except in a small area of the sierra, where the temperatures in some seasons of the year can decrease under the adequate threshold for the crop. The physiographic zones of the alluvial plain, river valley and hills are optimal for the growth, development and production of the taro crop.

ACKNOWLEDGEMENTS

This study was conducted thanks to the financial support from CONACyT Project No. 1153 "Generación de tecnología para la producción sustentable y uso integral de taro y yuca en Tabasco y Veracruz México" within the call to address National Problems 2015.

REFERENCES

Barrera, V., Tapia C., & Monteros, A. (2004). Caracterización Fisicoquímica, Nutricional y Funcional de Raíces y Tubérculos Andinos, In: Raíces y Tubérculos Andinos: Alternativas para la conservación y uso sostenible en el Ecuador. Instituto Nacional Autónomo de Investigaciones Agropecuarias, Quito, Ecuador & Lima, Perú. pp. 91-116.

Caicedo, W.O. (2013). Tubérculos de papa china (*Colocasia esculenta* (L.) Schott) como una fuente energética tropical para alimentar cerdos, una reseña corta sobre las características de la composición química y de los factores antinutricionales. *Revista Computarizada de Producción Porcina* 20: 278-282.

Eleazu, C. O. (2016). Characterization of the natural products in cocoyam (*Colocasia esculenta*) using GC–MS. *Pharmaceutical Biology* 54: 2880-2885,

Instituto Nacional de Estadística y Geografía (INEGI). (2017). Anuario estadístico y geográfico de Tabasco 2017. 440 p. Consultado en línea en octubre de 2021 en: https://tabasco.gob.mx/sites/default/files/users/planeacion_spf/ANUARIO%20ESTADISTICO%20WEB%2B%2B.pdf

López, J. M., Martínez-Herrera, J., Arguello, G.E. (2020). La malanga (*Colocasia esculenta* Schott) un cultivo alternativo paraTabasco. Villahermosa, Tabasco. *Revista Agroregión* 102: 16-21.

Madrigal-Ambriz, L.V., Hernández-Madrigal, J.V., Carranco-Jáuregui, M.E., Calvo-Carrillo, M.C., Casas-Rosado, R.G. (2018). Caracterización física y nutricional de harina del tubérculo de "Malanga" *Colocasia esculenta* (L.) Schott de Actopan, Veracruz, México. *Archivos Latinoamericanos de Nutrición* 68: 175-183.

Mazariegos, A., Águila, J., Milla, A., Espinoza, S., Martínez, J., López, C. (2017). Cultivo de Malanga (*Colocasia esculenta* Schott). *Agroproductividad* 10 (3): 75-80.

Palma-López, D. J.; Cisneros, D. E.; Moreno, C. E. y Rincón-Ramírez, J. A. (2007). Suelos de Tabasco: Su uso y manejo sustentable. Colegio de Postgraduados-ISPROTAB-FUPROTAB. Villahermosa, Tabasco, México. 194 p.

Raju, J., & Byju, G. (2018). Quantitative determination of NPK uptake requirements of taro (*Colocasia esculenta* (L.) Schott). *Journal of Plant Nutrition* 42 (3): 203-217.

Rivera-Hernández, B.; Aceves-Navarro, L. A.; Juárez-López, J.; Palma-López, D. J.; González Mancillas, R.; González-Jiménez, V. (2012). Zonificación agroecológica y estimación del rendimiento potencial del cultivo de la yuca (*Manihot esculenta* Crantz) en el estado de Tabasco, México. *Avances en Investigación Agropecuaria* 16: 29-47.

Rodríguez-Miranda J., J.M. Rivadeneyra-Rodríguez, E.J. Ramírez-Rivera, J.M. Juárez-Barrientos, E. Herrera-Torres, R.O. Navarro-Cortéz, B. Hernández-Santos (2011). Caracterización fisicoquímica, funcional y contenido fenólico de harina de malanga (*Colocasia esculenta*) cultivada en la región de Tuxtepec, Oaxaca, México. *Ciencia y Mar* 43: 37-47.

Salgado G., S., Núñez E., R. Bucio A., L. (2003). Determinación de la dosis óptima económica de fertilización en caña de azúcar. *Terra Latinoamericana* 21(2): 267-272.

Servicio de Información Agropecuaria y Pesquera (SIAP). (2019). Atlas Agroalimentario 2018. Consultado en línea en Octubre 2021 en: https://nube.siap.gob.mx/gobmx_publicaciones_siap/pag/2019/Atlas-Agroalimentario-2019

Zapata Martínez, J., & Velásquez Escandón, C. (2013). Estudio de la producción y la comercialización de la malanga: Estrategias de incentivos para la producción en el país y consumo en la ciudad de Guayaquil, Colombia. Tesis de Maestría en Ciencias. Universidad Politécnica Salesiana Ecuador. 12 p.

Zavala-Cruz, J., Jiménez-Ramírez, R., Palma-López D. J., Bautista Zúñiga F., Gavi-Reyes, F. (2016). Paisajes geomorfológicos: base para el levantamiento de suelos en Tabasco, México. *Ecosistemas y Recursos Agropecuarios* 3: 161-171.

AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Using discarded oyster shells to obtain biodiesel

De la Cruz-De la Cruz, Miguelina[1]; De la cruz-Burelo, Patricia[1*]; Hernández-Núñez, Emanuel[2]; Santiago-González, Ramón Eduardo[1]; Valerio-Cárdenas, Cintya[1]; Villegas-Cornelio, Víctor Manuel[1]

[1] Universidad Popular de la Chontalpa. División Académica de Ciencias Básicas e Ingenierías. CA-QVyDS. Carretera Cárdenas - Huimanguillo, Km. 2.0 Cárdenas, Tabasco, México. CP. 86500.

[2] Centro de Investigación y de Estudios Avanzados del IPN-Unidad Mérida, Departamento de Recursos del Mar, Antigua Carretera Mérida-Progreso Km 6, Col. Cordemex, Mérida, Yucatán, México. C. P. 97310.

* Correspondence: patricia.delacruz@upch.mx

ABSTRACT

Objective: To evaluate the CaO made from oyster shell (*C. virginica*) as a heterogeneous catalyst in the transesterification of edible vegetable oil used for the production of biodiesel.

Design/methodology/approach: A completely randomized experimental design was used, which grouped 3 treatments with 3 repetitions, generating a total of 9 experimental units. The response variable was the performance of the transesterification reaction that was evaluated with 2%, 3% and 4% of CaO obtained from oyster shells. The density, kinematic viscosity, acidity, and conversion efficiency to methyl esters were determined by ^{1}H NMR of the products of each treatment.

Results: The treatment with 3% catalyst showed the highest reaction yield (92.2%) compared to the treatments with 2% (86.8%) and 4% catalyst (87.13%). The ^{1}H NMR spectra confirmed the presence of methyl esters in the product of the three treatments. The treatment with 3% and 4% by weight of catalyst presented products with similar characteristics with acceptable values of density, viscosity and acid number in accordance with the ASTM D6751 and EN1421 standards.

Study limitations/implications: A concentration of 2% by weight of CaO generates a conversion percentage far from the content of methyl esters established by the ASTM D6751 and EN14214 Standards (>96.5%).

Findings/conclusions: ^{1}H NMR results indicate that the conversion efficiency to methyl esters is positively affected by the amount of catalyst. In the treatments with catalyst loading greater than 2%, the conversion to methyl esters increased significantly to values around 90%.

Keywords: oyster shells, used edible vegetable oil, transesterification, methyl esters.

Citation: De la Cruz-De la Cruz, M., De la cruz-Burelo, P., Hernández-Núñez, E., Santiago-González, R. E., Valerio-Cárdenas, C., & Villegas-Cornelio, V. M. (2022). Using discarded oyster shells to obtain biodiesel. *Agro Productividad*. https://doi.org/10.32854/agrop.v15i1.2019

Editor in Chief: Dr. Jorge Cadena Iñiguez

Received: April 30, 2021.
Accepted: December 31, 2021.
Published on-line: February 5, 2022.

Agro Productividad, 15(1). January. 2022. pp: 11-19.

INTRODUCTION

Greenhouse gas (GHG) emissions related to fossil fuel consumption are on the rise (British Petroleum, 2019). According to the Global Renewable Energy Status Report (2018), about 50% of fossil fuel consumption is due to transportation. To limit this fossil fuels dependence, the development of bioenergetics has been motivated. Biodiesel is considered a renewable, sustainable, biodegradable, non-toxic and clean form of energy (Chozhavendhan *et al.*, 2020).

Transesterification of triglycerides with short-chain alcohols (methanol and ethanol), in the presence of a catalyst, is the most widely employed method for biodiesel production (Akubude *et al.*, 2019; Borah *et al.*, 2018). On a global scale, 99% of this bioenergy is

Image by Kate Baucherel at Pixabay

obtained from edible oils (Ashraf, 2019). Homogeneous catalysts such as potassium hydroxide (KOH) and sodium hydroxide (NaOH) are widely used for obtaining high reaction yields in short times. However, they have problems in biodiesel separation, high product purification cost and zero catalyst reuse (Akubude *et al.*, 2019; Ogunkunle and Ahmed, 2019). To replace these catalysts, research has been focused on heterogeneous catalysts solids with basic properties derived from waste materials.

Due to their high calcium carbonate ($CaCO_3$) content, oyster shells represent a potential source of calcium oxide (CaO) (Ramón *et al.*, 2016). The present research evaluated CaO obtained from *Crassostrea virginica* oyster shells as a heterogeneous catalyst to produce biodiesel through the transesterification of used edible vegetable oil.

MATERIALS AND METHODS

Oyster shells (*Crassostrea virginica*) derived from human consumption were used. The edible vegetable oil used was collected from dwelling houses. The shells were washed with tap water to remove dirt particles and dried in the sun for six hours for two days. The oil was vacuum filtered to remove food residues and heated on a stirring electric grill at 105±10 °C for 30 min to remove the water content.

Catalyst Preparation

The oyster shells were crushed, passed through a manual mill, sieved through a number 60 (250 microns) mesh, and calcined in an oven at 900 °C for 2 h. They were later kept in vacuum-sealed test tubes (Figure 1).

CaO catalyst characterization

The morphology, microstructure and elemental composition of the catalyst were determined by SEM-EDX. A JEOL JSM-610 LA scanning electron microscope was used. The crystallinity was analyzed in an XRD with a Bruker model D-8 Advance diffractometer,

Figure 1. Preparation of CaO catalyst derived from oyster shells.

Cu K-alpha1 tube. The constituent substances of the catalyst were determined by FT-IR with an Agilent Cary 630 FT-IR spectrophotometer following the ATR (Attenuated Total Reflection) technique.

Characterization of the used edible vegetable oil

Based on the methods of the norms ASTM D1298, ASTM D445, NMX-F-211-SCFI-1987, NMX-F-101-SCFI-2012 and NMX-F-154-SCFI-1987, respectively, the density, viscosity, moisture % and volatile material, acid index and peroxide index of the edible vegetable oil used were determined.

Transesterification of the evaluated edible vegetable oil

An experimental design was established with nine experimental units (EU), described in Table 1. Three catalyst concentrations were evaluated, estimated by various authors as having the best reaction performance using CaO (Buasri *et al.*, 2015; Lani *et al.*, 2017; Yusuff and Popoola, 2018; Tan *et al.*, 2019).

Transesterification was carried out in a 500 mL three-way reactor (equipped with a magnetic stirrer and a thermometer), connected to a water-cooled reflux condenser (Figure 2). 50 g of the recycled edible vegetable oil was used for each EU (Yusuff and Popoola, 2018).

The type of alcohol, reaction time (2 h) and temperature (65 ^{o}C) was kept constant, following Farooq and Ramli (2015), Yusuff and Popoola (2018) and Tan *et al.* (2019). Alcohol: oil molar ratio of 36: 1 was used at a stirring speed of 700 rpm. The catalyst was separated by filtering in a vacuum. The product of each EU was passed to a separatory funnel. The denser (lower) phase was dried at 100 °C and the less dense (upper) heated to 70 °C to remove excess methanol for 30 min.

Table 1. Experimental treatments of the evaluated oil transesterification.

Treatments	Concentration	Repetition
A2	2% CaO catalyst	3
B3	3% CaO catalyst	3
C4	4% CaO catalyst	3

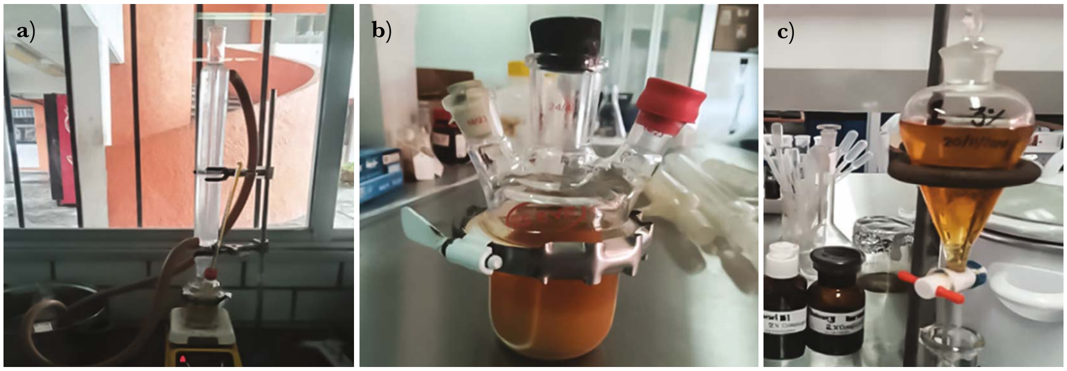

Figure 2. Transesterification. a) Reactor, b) product, c) phase separation.

Statistical analysis

The reaction yield was analyzed through a simple analysis of variance (ANOVA) to find statistically significant differences between treatments (catalyst percentage). The means comparison was assessed using Tukey's test, with a 5% significance (α=0.05). The data were then analyzed in the Statgraphics® Centurion XVIII.1.06 (Statpoint Technologies, 2010) statistical program.

Biodiesel Characterization

The methyl esters content was determined by proton nuclear magnetic resonance (^{1}H NMR), following equation 1. Kinematic viscosity, density, and acid index were analyzed following the methods established in the ASTM D445, NMX- F-075-SCFI-2012 and NMX-F-101-SCFI-2012 norms, respectively.

$$\%Conversion = \left(\frac{2A_{CH_3}}{3A_{\alpha-CH_2}}\right)*100 \qquad \text{Equation 1}$$

A_{CH_3} is the integration value of the protons of the methyl esters and $A_{\alpha-CH_2}$ is the integration value of the methylene protons (Farooq and Ramli, 2015).

RESULTS AND DISCUSSION

CaO catalyst characterization

During the calcination process, the oyster shells lost an average of 44.5% of their weight, due to CO_2 release during the gas phase (Galván and Velázquez, 2011). In the SEM images (Figure 3), the formation of particles of divergent sizes and shapes is observed, possibly as the result of a non-uniform exposure of the samples to the calcination temperature (Singh and Verma, 2019). Figure 3.1d at 200x shows a porous catalyst, which is related to abundant active reaction sites (Aderibigbe, 2020).

EDX analysis (Figure 4) exposes a high concentration of Ca and O ions. The presence of these elements as main constituents indicates that the oyster shell was converted to CaO. The Ca content is similar to that reported by Singh and Verma (2019) (54.76%), for calcined eggshells.

The XRD patterns of the catalyst (Figure 5) show peaks at $2\theta \cong 32°$, 37°, 54°, 63° and 67°, characteristic of CaO (Farooq *et al.*, 2018). Which is consistent with the high Ca and O content reported by the EDX technique. The narrow and high-intensity peaks define the crystalline structure of the catalyst (Buasri *et al.*, 2015).

The spectrum of the FT-IR analysis (Figure 6) presents similarities with those reported by Tan *et al.* (2019), for chicken bones and fish bones, with the bands for 1409 cm^{-1} and 995 cm^{-1} resulting from the Ca-O vibration.

Used edible vegetable oil characterization

The used edible vegetable oil had an acid index (0.72 mg KOH/g) less than acceptable (<2.0 mg KOH/g) (Banerjee *et al.*, 2019); and a free acids percentage (0.36%) below

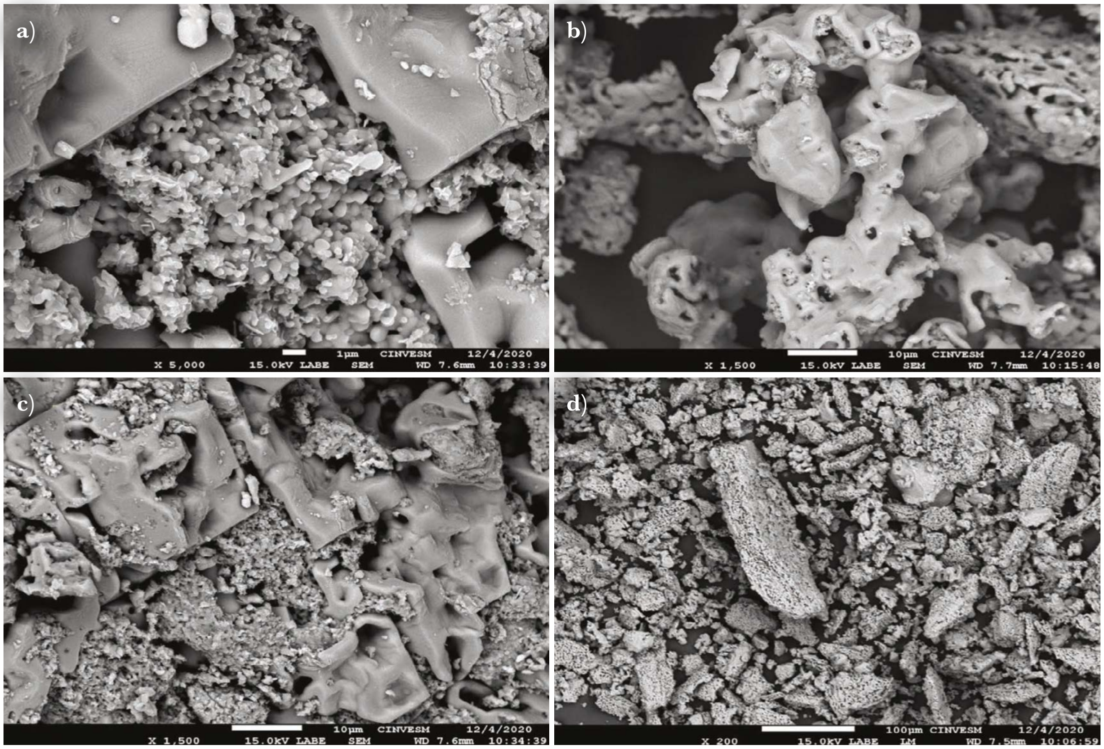

Figure 3. SEM images of oyster shell derived CaO catalyst.

Elemento	% en peso	% atómico
O K	43.17	64.70
Na K	0.80	0.83
Mg K	1.55	1.53
Si K	1.37	1.17
Ca K	53.11	31.77
Total	100 %	

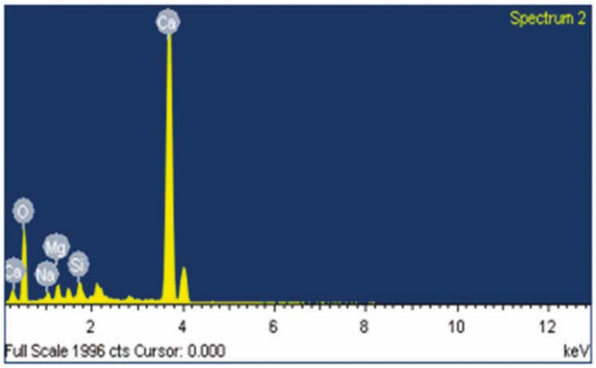

Figure 4. Elemental composition of calcined oyster shells.

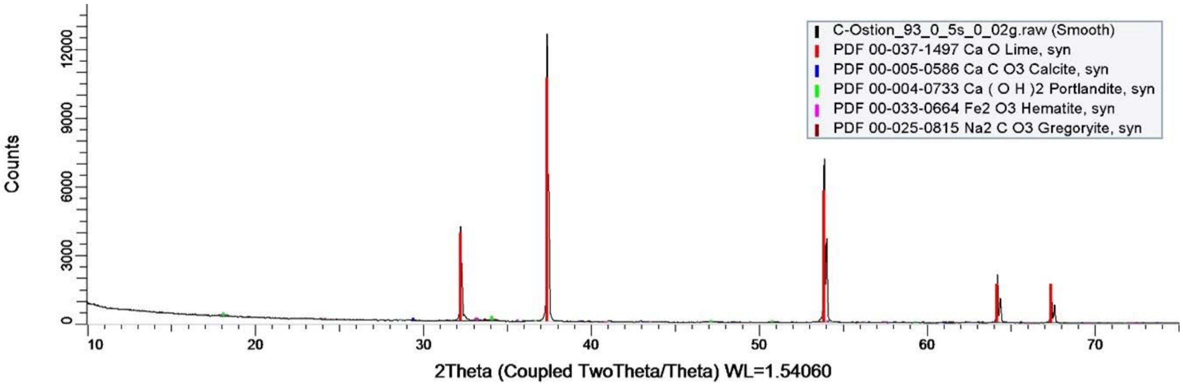

Figure 5. Diffractogram of a calcined sample of oyster shells.

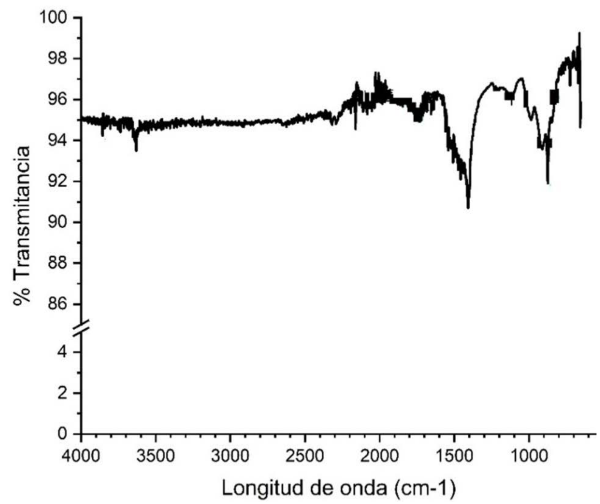

Figure 6. Spectrum of calcined oyster shell sample.

that reported by Yusuff and Popoola (2018) of 1,924%. It showed a moisture content of 0.1289%, lower than the 2.0% suggested as acceptable by Banerjee *et al.* (2019) and 0.64% documented by Tan *et al.* (2019). The density was found within the range established by the NMX-F-475-SCFI-2011 Standard (0.914-0.925) for virgin oils and was lower than the density indicated by Tan *et al.* (2019) (950 kg/m^3). The mean kinematic viscosity (39.36 mm^2/s) was located within the typical values (27.2- 53.6 mm^2/s) for fats and oils (Lanjekar and Deshmukh, 2016), and below that indicated by Farooq and Ramli (2018) (42.01 mm^2/s).

The high peroxide index (18.23 meqO_2/kg) was like those obtained by Torres *et al.* (2016), (10.5 to 19.5 meqO_2/kg) in samples of used vegetable oils from a pilot plant for biodiesel production. Although, lower than the 40 meqO_2/kg reported in the bibliography for oils in which undesirable characteristics are detected after 180 days of storage (Rivera *et al.*, 2014). The results indicate that the raw material did not show a significant deterioration that significantly affects the transesterification reaction.

Edible vegetable oil transesterification

Considering the final mass of both phases, after the heating process indicated by the methodology, treatment B3 showed a high reaction performance compared to treatments A2 and C4 (Table 3).

Table 2. Physicochemical properties of the used edible vegetable oil.

Properties	Units	Value
Density at 15 °C	kg/m^3	928.58
Viscosity at 40 °C	mm^2/s	39.36
Moisture and volatile material	%	0.1289
Acid index	mg KOH/g	0.7230
Peroxide index	meqO_2/Kg	18.2388

Table 3. Results of reaction yield by treatment.

Treatments	Yield (%)			Average
A2	86.6	87.8	86	86.8
B3	92.2	93	91.4	92.2
C4	87.8	87	86.6	87.13

With a 95% confidence level, the analysis of variance (ANOVA) of one factor and the Tukey's test, showed that there is a statistically significant difference between the highest reaction yield percentage, which was obtained in treatment B3 (92.2%), compared to the performance of treatments A2 (86.8%) and C4 (87.13%).

Biodiesel characterization

All 1H NMR spectra (Figure 7) exhibited a singlet close to 3.6 ppm, characteristic of the protons of the methoxy group (O-CH_3). As well as a triplet at approximately 2.3 ppm, typical of -CH_2 protons, adjacent to methyl (Santos, 2013; Morales, 2017), which confirms the presence of methyl esters in the three treatments. Only the product of treatment of A2 showed double doublets in the range of 4.1-4.3 ppm related to glyceride protons (Cedrón *et al*., 2014).

Treatment B3 (3% by weight of catalyst) and C4 (4% by weight of catalyst) presented products with similar characteristics with acceptable values of density, viscosity and acid number following ASTM D6751 and EN 14214 standards (Table 6). Which is related to

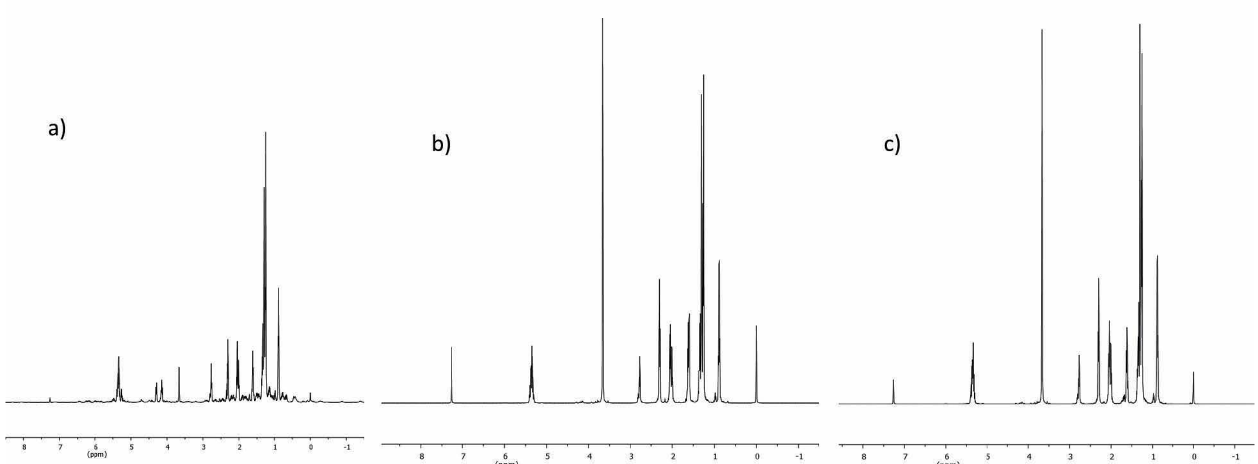

Figure 7. ^{1}H NMR spectra of treatments a) A2 (2% catalyst), b) B3 (3% catalyst) and c) C4 (4% catalyst).

Table 6. Results of the biodiesel characterization.

Treatment	Yield (%)	Density at 15 °C (kg/m^3)	Viscosity at 40 °C (mm^2/s)	Acidity (mg KOH/g)	Methyl esters (%)
A2	86.8	922.3231	18.3096	0.5075	14.63
B3	92.2	890.1291	4.4301	0.3084	90.90
C4	87.13	897.6056	4.3725	0.2985	91.14

Note: Numbers highlighted in bold meet the quality standards established in ASTM D6751 and EN 14214.

high conversion to methyl esters according to ^{1}H NMR results. Although the B3 treatment exhibited a higher and statistically significant performance.

CONCLUSIONS

The XRD, FT-IR and SEM-EDX analyzes confirmed the formation of CaO from the calcined samples of oyster shells at 900 °C. The ^{1}H NMR results indicate that the conversion efficiency to methyl esters is positively affected by the amount of catalyst. In the treatments with catalyst loading greater than 2%, the conversion to methyl esters increased significantly (≥90%). For these treatments, the physicochemical properties of the biodiesel obtained were acceptable as indicated in the ASTM D6571 and EN 1411 Standards. In this sense, local oyster shells (*Crassostrea virginica*), considered as worthless waste, represent a promising source of CaO for the successful synthesis of biodiesel, whose characteristics meet quality standards.

ACKNOWLEDGEMENTS

The authors thank the Laboratorio Nacional de Nano y Biomateriales (National Laboratory of Nano and Biomaterials) Funded by Fomix-Yucatán and Conacyt, Cinvestav-IPN, Mérida, for the characterization of the CaO catalyst and obtaining the RMN1H spectra of biodiesel.

REFERENCES

Aderibigbe, F. A., Mustapha, S. I., Adewoye, T. L., Mohammed, I. A., Gbadegesin, A. B., Niyi, F. E.,... Saka, H. B. (2020). Qualitative role of heterogeneous catalysts in biodiesel production from *Jatropha curcas* oil. *Biofuel Research Journal*, *VII*(2), 1159-1169. doi:10.18331 / BRJ2020.7.2.4

Akubude, V., Nwaigwe, K., & Dintwa, E. (2019). Production of biodiesel from microalgae via nanocatalyzed. *Materials Science for Energy Technologies, II*(2), 216-225. doi:10.1016/j.mset.2018.12.006

Ashraf, A. (2019). Review of diesel production from renewable resources: Catalysis, process kinetics and technologies. *Ain Shams Engineering Journal*. doi:10.1016/j.asej.2019.08.001

Banerjee, S., Sahani, S., & Chandra Sharma, Y. (2019). Process dynamic investigations and emission analyses of biodiesel produced using Sr–Ce mixed metal oxide heterogeneous catalyst. *Journal of Environmental Management*. doi:10.1016/j.jenvman.2019.06.119

Borah, M., Devi, A., Saikia, R., & Deka, D. (2018). Biodiesel production from waste cooking oil catalyzed by *in-situ* decorated TiO2 on reduced graphene oxide nanocomposite. *Energy*, 881-889. doi:10.1016/j.energy.2018.06.079

British Petroleum. (2019). *Statistical Review of World Energy 2019*. Obtenido de British Petroleum: https://www.bp.com/content/dam/bp/business-sites/en/global/corporate/pdfs/energy-economics/statistical-review/bp-stats-review-2019-full-report.pdf

Buasri, A., Rattanapan, T., Boonrin, C., Wechayan, C., & Loryuenyong, V. (2015). Oyster and Pyramidella Shells as Heterogeneous Catalysts for the Microwave-Assisted Biodiesel Production from *Jatropha curcas* Oil. *Journal of Chemistry*. doi:10.1155/2015/578625

Cedrón, J. C., Moncada, A., & Mendoza, P. (2014). Análisis de biodiesel preparado a partir de residuos de aceite doméstico, mediante RMN. *Revista de la Sociedad Química del Perú, LXXX*(1), 3-8.

Chozhavendha, S., Vijay Pradhap Singh, M., Fransila, B., Praveen Kumarb, R., & Karthiga Devi, G. (2020). A review on influencing parameters of biodiesel production and purification processes. *Current Research in Green and Sustainable Chemistry*, 1-6. doi:10.1016/j.crgsc.2020.04.002

Farooq, M., Ramli, A., & Naeem, A. (2015). Biodiesel production from low FFA waste cooking oil using heterogeneous catalyst derived from chicken bones. *Biodiesel production from low FFA waste cooking oil using heterogeneous catalyst derived from chicken bones*, 362-368. doi:10.1016/j.renene.2014.11.042

Farooq, M., Ramli, A., Naeem, A., Ahmad, S., Islam, U., & Mahmood, T. (2018). Biodiesel Production from Date Seed Oil (*Phoenix dactylifera* L) via Egg Shell Derived Heterogeneous Catalyst. *Chemical Engineering Research and Design, CXXXII*, 644-651. doi:10.1016/j.cherd.2018.02.002

Galván Ruiz, M., & Velázquez Castillo, R. (2011). Cal, un antiguo material como una renovada opción para la construcción. *Ingeniería. Investigación y Tecnología*, 93-102.

Global Status Report Renewable Energy. (2018). Obtenido de https://www.ren21.net/wp-content/uploads/2019/08/Full-Report-2018.pdf

Lani, N., Ngadi, N., & Taib, M. (2017). Parametric Study on the Transesterification Reaction by Using CaO/Silica Catalyst. *Chemical Engineering Transactions*, 601-606. doi:10.3303/CET1756101

Lanjekar, R., & Deshmukh, D. (2016). A review of the effecto the composition of biodiesel on NOx emission, oxidative stability and cold flow properties. *Renewable and Sustainable Energy, LIV*, 1401-1411. doi:10.1016/j.rser.2015.10.034

Morales Téllez, M. (2017). *Producción de biodiesel asistido por dispersión de alta trasquilación (Tesis de maestría). Benemérita Universidad de Puebla*. México.

Ogunkunle, O., & Ahmed, N. A. (2019). A review of global current scenario of biodiesel adoption and combustion in vehicular diesel engines. *Energy Reports*, 5, 1560-1579. doi:10.1016/j.egyr.2019.10.028

Ramón de los Santos, C., López Rodriguez, A., Sifuentes Gallardo, P., Hérnandez Rivera, M., Rivera Trejo, J. G., & Díaz Flores, L. L. (2016). Reuso de desechos de conchas de ostión «*crassostrea virginica*». para la obtención de un material como aglomerante de mortero a partir de tratamientos de molienda y calcinación. *I+D Innovación mas desarrollo, V*(12), 38-48.

Rivera, Y., Gutiérrez, C., Gómez, R., Matute, M., & César, I. (2014). Cuantificación del deterioro de aceites vegetales usados en procesos de frituras en establecimientos ubicados en el Municipio Libertador del Estado Mérida. *Revista Ciencia e Ingeniería, XXXV*(3), 157-164.

Santos Ramos, M. (2013). *Aplicación de materiales porosos tipo hidrotalcita para en la reacción de transesterificación para la obtención de biodiesel (Trabajo de grado). Instituto Politécnico Nacional*. México.

Singh, T. S., & Verma, T. N. (2019). Taguchi design approach for extraction of methyl ester from waste cooking oil using synthesized CaO as heterogeneous catalyst: Response surface methodology optimization. *Energy Conversion and Management*, 383-397. doi:10.1016/j.enconman.2018.12.077

Statpoint Tecnologies Inc. (2010). *Statgraphics Centurion ver. XVIII. 1.06: Manual de usuario*. Virginia, Estados Unidos.

Tan, H., Abdullah, M., Kansedo, J., Mubarak, N., Chan, Y., & Nolasco-Hipolito, C. (2019). Biodiesel production from used cooking oil using green solid catalyst derived from calcined fusion waste chicken and fish bones. *Renewable Energy*, 696-706.

Torres, L., Youssef , B., Ek, D., & Escalante, R. (2016). Caracterización de los parámetros físico-químicos de los aceites comestibles usados para la generación de biodiesel en la planta piloto del IT Cancún. *Revista de Investigación y Desarrollo, II*(6), 92-108.

Yusuff, A. S., & Popoola Taofeek, L. (2018). Optimization of biodiesel production from waste frying oil over alumina supported chicken eggshell catalyst using experimental design tool. *Acta Polytechnica*, 88-97. doi:10.14311/AP.2019.59.0088

AP AGRO PRODUCTIVIDAD
Colegio de Postgraduados

Use of traditional food and proposal for the dish of good eating for the Totonac region

García-Vázquez, Rafael[1], López-Santiago, Marco Andrés[2*], Valdivia Alcalá, Ramón[1], Sánchez-Toledano, Blanca Isabel[3]

[1] Universidad Autónoma Chapingo-División de Ciencias, Económico Administrativas. Carretera México-Texcoco Km. 38.5, Texcoco, Estado de México, C.P. 56230.
[2] Universidad Autónoma Chapingo-Unidad Regional, Universitaria de Zonas Áridas. Carretera Gómez Palacio-Chihuahua Km. 40, Bermejillo, Durango, C.P. 35230.
[3] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experimental Zacatecas. Calera de Víctor Rosales, Zacatecas, México. C.P. 98500.
* Correspondence: admarkos@gmail.com

ABSTRACT

Objective: To identify the use of traditional food, quantify the main foods consumed in the region, and propose a dish of good eating for the Totonac region.

Methodology: A semi-structured survey was carried out in 328 households of the municipality of Filomeno Mata, Veracruz, Mexico. The Household Dietary Diversity Score (HDDS) and a section were used to obtain information about the regional foods that the local population consumes on a daily basis.

Results: The families recognized a total of 35 regionally-produced vegetal and animal products. Each household that participated in the survey mentioned an average of 7 foods out of the whole list.

Limitations: To improve and implement a good food dish, several elements must be taken into account: age, gender, height, physical activity, or physiological condition, among others.

Conclusions: The registered foods are linked to the local culture and open a door to the generation of various resource-leveraging strategies, such as the dish of good eating for the Totonac region.

Key Words: regional food, dietary diversity, food security.

Citation: García-Vázquez, R., López-Santiago, M. A., Valdivia Alcalá, R., & Sánchez-Toledano, B. I. (2022). Use of traditional food and proposal for the dish of good eating for the Totonac region. *Agro Productividad*. https://doi.org/ 10.32854/agrop.v15i1.2057

Editor in Chief: Dr. Jorge Cadena Iñiguez

Received: June 17, 2021.
Accepted: January 11, 2021.
Published on-line: February 5, 2022.

Agro Productividad, 15(1). January. 2022. pp: 21-29.

INTRODUCTION

Food diversity has contributed very important resources to human diets through the years. The communities, over the course of their historical evolutions, have added vegetables, fruits, and roots to their diets, making them part of their development (Gispert and Álvares, 1997). Therefore, humans depend on the ecosystems; this situation is very clear in subsistence economies, where human communities take everything they need to survive directly from the ecosystems (Gómez-Baggethun and de Groot, 2007). Consequently, higher diversity creates favorable conditions for a more stable supply of food sources (Torres and Sandoval, 2015).

In this regard, accurate knowledge about the traditional foods of a particular region is decisive for the improvement of the food and nutrition security of the population (Calderón *et al.*, 2017). Therefore, the consumption of local produce establishes an important link: not only does it provide food, sustenance, and employment sources, it establishes a direct and significant relationship between human welfare, nutrition, and agrobiodiversity (Becerril, 2013).

Image by squarefrog at Pixabay

The current landscape has shown that the weight of exogenous food in rural communities is increasing. This situation led to the addition of industrialized food to the diet of rural communities, which has had negative implications in the diversity of the diet, food security, and nutrimental condition of the members of the domestic units (Otero *et al.*, 2017). This growing dependency implies a reduction in the variety of plants and animals. This situation causes a reduction in the variety of the diet and, consequently, in the agrobiodiversity of the system, resulting in a negative impact on nutrition. Therefore, there are enough proofs to clarify the importance of traditional crops and wild plants and animals for the economic benefit of small farmers and human health (Nivia and Ivette, 2009). Additionally, Hunter and Fanzo (2013) have shown their importance in rural communities. PNUMA (2008) pointed out that they are an invaluable resource because their reproduction enables the adaptation to the changing conditions. This implies a greater planification of proposals that generate strategies aimed at increasing the use of these foods.

Consequently, the objective of this study was to identify the use of traditional food, through the quantification of the main regional foods consumed by households, ultimately achieving a better use of these foods and offering an alternative in financial insolvency times. Additionally, we propose a dish of good eating for the Totonac region. The study was carried out in the municipality of Filomeno Mata, Veracruz, Mexico. This municipality is part of the Totonac culture. The study area has a wide knowledge about the use and care of natural resources.

MATERIALS AND METHODS

The study was carried out in the municipality of Filomeno Mata, Veracruz, Mexico (20° 10' and 20° 16' N, 97° 38' and 97° 45' W, between 194 and 800 masl). Its borders are the municipalities of Coahuitlán, Coyutla, and Mecatlán, Veracruz (N), the municipality of Mecatán and the State of Puebla (E), the State of Puebla (S), the State of Puebla and the municipality of Coahuitlán (W) (SEFIPLAN, 2019).

Selection and size of the sample

The target population was the private household's stratum. The study comprised 2,178 households located only in the municipal seat (INEGI, 2015). The sample was obtained using the formula for finite populations (Wayne, 2017).

$$n = \frac{N * Z_{\alpha}^{2} * p * q}{d^{2}\left(N-1\right) + Z_{\alpha}^{2} * p * q}$$

Where: N=population size; Z=1.96 trust level; p=0.5 probability of success or expected rate; q=0.5 probability of failure; d=0.05 accuracy (maximum permissible rate error). The analyzed population included 328 households. We used semi-structured interviews and their application was the result of a simple random sampling.

Tool

The questionnaire was structured in two sections. The first section included questions about the diversity or the variety of the diet classification, using the Household Dietary Diversity Score (HDDS) (CONEVAL, 2010). The objective of the questionnaire was to determine the dietary diversity of the households. However, in this case, the questionnaire was only used to establish the number of food groups that can be found in the region.

At the same time, the participants of the survey were asked about the regional foods that they consume daily and were asked to classify them according to the HDDS groups.

A pilot survey (n=10) was used to find inconsistencies in the tool. Additionally, other researches carried out in the area were used to increase the number of regional foods that are used in the area and to generate a dish of good eating for the Totonac region. Therefore, the edible natural resources mentioned by López (2019) were analyzed.

The *Plato de Bien Comer* (good food dish) is a food guide included in the Norma Oficial Mexicana (Mexican Official Standard) and is used to promote and educate the population about food security. It establishes the dietary guidance criteria in Mexico (CIAD, 2021). This categorization has three components: vegetable and fruits; cereals; pulses and food of animal origin (CIAD, 2021).

RESULTS AND DISCUSSION

Group classification of the food consumed by the Totonac households

The study area has a great food diversity: the population complements its diet with exogenous products and foods that can be obtained locally.

Local families own productive plots and backyard poultry. Usually, their production is destined for self-consumption, because their plots are small. The agricultural plots and the backyard use have mainly developed diversified food systems. Nevertheless, regional foods are losing ground on the daily diet, because they have been gradually replaced by commercial products; access to those products has become easier throughout the years. They are still present in different degrees and their diets are balanced to a certain point, thanks to this type of product.

Using the HDDS, we determined the food groups that have a higher weight in the area (Figure 1).

The families that participated in the survey reported that the groups that they more frequently consume are: beverages (323); cereals, roots, and tubers (320); lard and oils (211); meat, chicken, and sausages (178); and vegetables (151). We determined that some food groups can be replaced —at least in terms of their percentage— by local products, helping to achieve a better diet.

Beverages are the most frequently mentioned group. In this regard, the excessive consumption of sodas in households causes several diseases, including overweight. In contrast, families tend to consume coffee and beverages prepared with seasonal fruits. Therefore, we established that the use of regional foods involves a healthier diet. Additionally, they provide food to families during financial insolvency times, using products with a cultural identity, which are an alternate source to complement the diet.

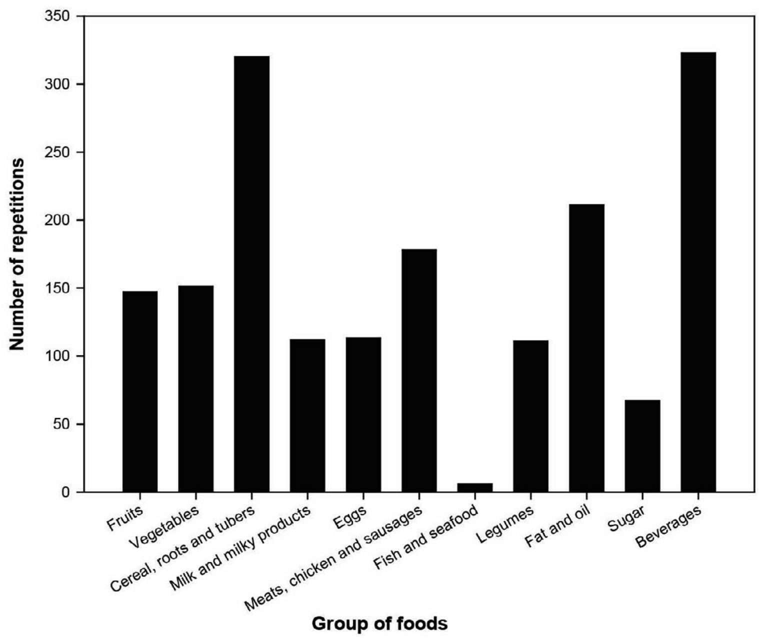

Figure 1. Group of foods consumed in Totonac households.

Local food resources used for the diet

The families who participated in the survey recognized and consumed a total of 35 regional products of vegetal and animal origin (Table 1). Each household that participated in the survey mentioned an average of 7 foods out of the whole list. For this analysis, the fruits that are consumed as seasonal aguas frescas or snacks were put in a single group. Other researches have established that there are, at least, 20 fruits grown in productive

Table 1. Indigenous foods consumed by Totonac households.

Food group	Common and scientific name
Fruits	Avocado (*Persea americana* Mill.), sweet potato (*Ipomoea potatotes* (L.) Lam.), sugar cane (*Saccharum* spp.), varied fruit trees (no scientific name), tomato (*Solanum lycopersicum*), pahua (*Persea schiedeana* Nees),
Vegetables	Chives (*Alliun choenoprasum*), squash (*Sechium edule* (Jacq.) Sw.), squash guide, (no scientific name), peppermint (*Mentha spicata*), black nightshade (*Solanum nigrum* L.), nopales (*Opuntia ficus-indica*), pichoco (*Erythrina american* Mill.), tomatillo (*Physalis gracilis* Miers), tree chili (*Capsicum annuum* L.), piquin chili (*Capsicum annuum* 'Pequin'), coriander (*Coriandrum sativum* L.).
Cereals, roots and tubers	Pumpkin (*Cucurbita argyrosperma* K. Koch), fungi (wild mushroom) (*Agaricus campestris*), corn (*Zea mays* L.), pipian (*Cucurbita argyrosperma*), manioc (*Manihot esculenta* Crantz).
Eggs	Wild eggs (no scientific name)
Meats, chicken and sausages	Armadillo (*Dasypodidae*), pork (*Sus*), hen (*Gallus gallus domesticus*).
Legumes	beans (*Phaseolus vulgaris*), asparagus (*Asparagus officinalis*), bean (*Phaseolus vulgaris* L.) guaje (*Leucaena leucocephala* (Lam.) De Wit), malvaron (*Xanthosoma robustum* Schott), papalo (*Porophyllum ruderale* (Jacq.) Cass.), quelites (*Amaranthus hybridus* L.).
Sugar	Honey (no scientific name)
Beverages	Coffee (*Coffea arabica* L.).

Source: Own elaboration based on López (2019).

plots in the region. Therefore, this study had a different classification for those fruits that are included in dishes, such as avocado, sweet potato, tomato, etc.

In this regard, based on the above-mentioned list, the sample was divided into 8 to 11 of the food groups included in the classification (Figure 2).

There are no records of local foods that belong to the following groups: milk and dairy products, seafood, lard, and oil. The reason behind this is that the study zone does not have a livestock tradition; additionally, fish and shellfish are only available in nearby cities. Therefore, the results suggest that the area lacks the necessary conditions to substitute these three groups, although it can substitute the remaining eight groups.

Meanwhile, the ten most frequently mentioned products (Figure 3) were: squash, coffee, chile de árbol, pequin peppers, beans, fruits (overall), eggs, corn, quelites (wild herbs), and tomatillos. Therefore, these produces are the most frequently consumed and have a significant presence in the studied households.

According to the classification that was used for this study, the most consumed regional foods belong to the following groups: vegetables, cereals, roots and tubers, beverages, leguminous plants and pulses, fruits, and eggs. The highest number of species were registered in the vegetables, leguminous plants, and pulses group, as well as the cereals, roots, and tubers group.

Dish of good eating for the Totonac region proposal

Based on the above-mentioned data and the Mexican Plato del Bien Comer, the food was categorized to propose a Dish of good eating for the Totonac region (Figure 4). For that purpose, the food mentioned in the research and the consulted bibliography was recorded: 59 vegetables and fruits, 1 cereal, and 8 pulses and food of animal origin. The first and the

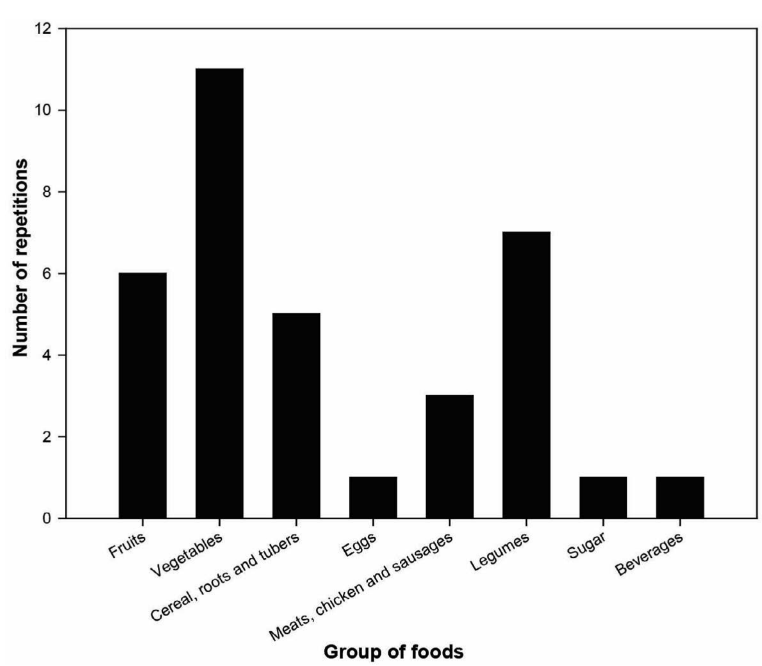

Figure 2. Native foods consumed, classified by food group.

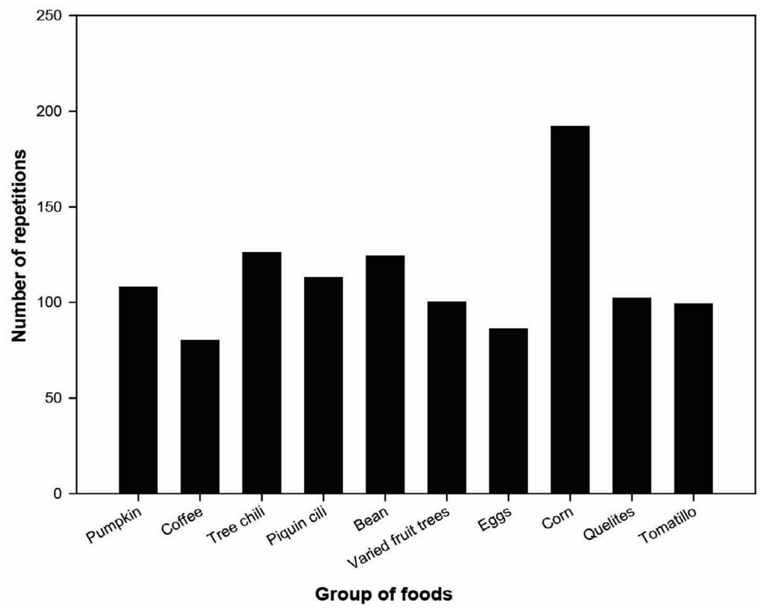

Figure 3. Local foods that are mostly consumed.

**Foods found in another research. Fruits and vegetables: anay, acamayo capulin mustache, chalahuite (2 varieties), chiltepin, custard apple, plum, foreign epazote, guava, fig tree, jobo, tangerine, mango, passion fruit, orange, nigua, papalo, pine seed, banana, banana apple tree, rose apple, white quelite, quelite, quintonil, tepetomate, shell tomato, wild tomato, purslane, sapote hair, mamey sapote, black sapote, blackberry. Cereals: no product is reported. Legumes and animal food: tree bean and fat bean.

*Foods found in the investigation. Fruits and vegetables: avocado, pumpkin, sweet potato, sugar cane, chives, squash, tree chili, piquin chilli, coriander, asparagus, varied fruit trees, tomato, guaje, squash guide, peppermint, night shade, fungi, malvaron, nopales, pahua, papalo, picocho, pipian, quelites, tomatillo, yuca, manioc. Cereals: corn, legumes and animal food: armadillo, pork, green beans, beans, hen, wild eggs.

Figure 4. Proposal of the plate of good peasant.

last categories were the most frequently used foods. There is a clear difference between the quantities reported using this classification versus the HDDS classification. A key point is that the mentioned produces are those that are currently being used in households; as times went by, many other products were no longer used. Therefore, other products that were not mentioned can be explored in a strategic use plan, widening the number of products in each category.

These results showed that a substantial set of local foods can be included in the development of this dish, revealing the local importance of products with a food identity.

Additionally, given the characteristics of the classification, some foods that were registered during this study were not taken into account for this section. Coffee fits into this context —although it is fundamental for households, it is considered a beverage and therefore it is not included in the *Plato del Bien Comer* proposed by the Mexican Government. Likewise, other products —such as garlic and pepper— are known, but used to flavor dishes and therefore do not fall into a clear category.

Generally speaking, regional foods belong to the family diet, as well as to a cultural and social context. Using them encourages an improved diet and, consequently, improves family nutrition. Additionally, government programs can provide greater visibility to local diversity, generating better food conditions.

This research established regional food as a major factor. These results have been validated by studies carried out in Llano del Higo, Jalisco, Mexico by Espinoza (2017) who points out that rural communities use and manage the vegetables, seeds, fruits, and meat in which their subsistence has taken root. In this way, natural resources have provided food culture with irreplaceable resources for the human diet (Gispert and Álvares, 1997). In this sense, some studies prove that people place major importance on the use of wild biodiversity as food (Asprilla-Perea and Díaz-Puente, 2020). Consequently, preserving those resources has become a major factor that must be taken into account, to improve food quality, given the scarcity of products in the market and the limitations put on imports (Yong *et al*., 2017).

Therefore, rural farming production is a basic food source for rural community households. Previous studies in the study area (including López (2019)) have established that it is necessary to carry out multidisciplinary studies in the indigenous communities, in order to rescue and preserve their knowledge about the use of natural capital.

Meanwhile, food diversity includes the type of food that different cultures prefer; in the case of this study, we determined that corn, *chile de árbol*, and beans are the most consumed foods in this area. These data match the findings of Cruz and Pérez (2018) who found out that the foods preferred by the local culture in deprived municipalities in Chiapas, Mexico, are those that have been handed down from generation to generation (such as corn and beans). Other researchers have determined that some foods —including the main cereals (corn, rice, and wheat)— have been an essential part of the life of certain human groups (Muñoz *et al*., 2020). Some studies have pointed out that the species with greater regional demand in the food category include: sugar cane, corn, chiltepín, wild tomato, black nightshade, chile de árbol, and tomatillo (López-Santiago *et al*., 2019). Those products are the same products mentioned in this research.

Meanwhile, the *Plato del Bien Comer* proposal made by the Mexican Federal Government is not a very viable option in rural areas, because it includes food products that are unknown to poor populations —particularly indigenous communities (Monárrez-Espino, 2009). Consequently, proposals with greater feasibility have been developed using regional food, to make this meal truly accessible. Some works have been published in this line, including a proposal for a "Mayan Dish". This *Plato del Bien Comer* for Mayan communities proposes using the resources available in the communities; this would not have a negative economic impact on the family situation (Cabrera-Araujo *et al.*, 2019). Mayan populations have consumed products that include each food group since times immemorial (Cabrera-Araujo et al., 2019). Therefore, taking into consideration the goals established in this study, a *Plato del Bien Comer* for the Totonac region is feasible.

To improve and apply this good food dish proposal, certain key elements must be taken into account: age, gender, height, physical activity or physiological condition, etc. (CIAD, 2016).

CONCLUSION

In conclusion, this study reveals the importance of taking care of the latent biodiversity, given the direct relationship that exists between lore, practices, and resources. This relationship preserves a complex dynamic that has not been properly studied yet. To improve the proposal for a rural *Plato del Bien Comer*, the nutrition advantages that these species have must be established, as well as their direct and indirect benefits vis-à-vis industrial food and whether or not they can substitute them. Another major factor that must be taken into consideration is the temporariness and quantity of the said resources' production.

REFERENCES

Torres J, Sandoval T. Seguridad Alimentaria y Aspectos Ambientales: El papel de la Diversidad. *Debate Agrario 2015*; (47).

Wayne WD. Bioestadística base para el análisis de las ciencias de la salud. 4ª ed. México LIMUSA. 2017.

Yong A, Calvez E, González Y, Permuy N, Pavón M. (2017). La conservación de alimentos, una alternativa para el fortalecimiento de la seguridad alimentaria a nivel local. *Cultivos tropicales 2017*; 38(1):102-107.

Asprilla-Perea, J., & Díaz-Puente, J. M. (2020). Uso de alimentos silvestres de origen animal en comunidades rurales asociadas con bosque húmedo tropical al noroeste de Colombia. *Interciencia*, 45(2), 76-83.

Becerril, J. (2013). Agrobiodiversidad y nutrición en Yucatán: una mirada al mundo maya rural. *Región y sociedad*, 25(58), 123-163.

Cabrera-Araujo, Z. M., Hernández-Escalante, V. M., Marín-Cárdenas, A. D., Murguía-Argüelles, R., Magaña-Be, N., Ramón-Escobar, K., ... & Juárez-Ramírez, C. (2019). Opiniones de adolescentes sobre el Plato del Bien Comer Maya como herramienta de promoción de la salud. *Salud pública de México*, 61 (1), 72-77. Doi: https://doi.org/10.21149/9362

Calderón, M. E., Taboada, O., Argumedo, A., Ortiz, E., López, A., & Jacinto, C. (2017). Cultura alimentaria: Clave para el diseño de estrategias de mejoramiento nutricional de poblaciones rurales. *Agricultura, sociedad y desarrollo*, 14(2), 303-320.

Centro de investigación en alimentación y desarrollo (CIAD). (2016). Conozca el plato del buen comer. [Accedido 2021 marzo 17]. Recuperado de https://www.ciad.mx/notas/item/1409-conozca-el-plato-del-buen-comer

Chappell, M. J., Wittman, H., Bacon, C. M., Ferguson, B. G., Barrios, L. G., Barrios, R. G., ... & Perfecto, I. (2013). Food sovereignty: an alternative paradigm for poverty reduction and biodiversity conservation in Latin America. F1000Research, 2.

Consejo Nacional de Evaluación de la Política de Desarrollo Social (CONEVAL). (2010). Dimensiones de la seguridad alimentaria: Evaluación estratégica de nutrición y abasto. [Accedido 2021 febrero 21]. Recuperado de https://www.coneval.org.mx/rw/resource/coneval/info_public/PDF_PUBLICACIONES/Dimensiones_seguridad_alimentaria_FINAL_web.pdf

Cruz, J., & Pérez, J. G. (2018). Seguridad, diversidad y alimentos culturalmente preferidos por familias rurales de municipios marginados de Chiapas, México. *Revista de Geografía Agrícola*, (61), 103-126. Doi: https://doi.org/10.5154/r.rga.2017.61.05

Espinoza, D. M. (2017). Recursos del bosque y vulnerabilidad alimentaria: El caso de Llano del higo, Jalisco, México. *Acta Sociológica*, 73, 147-169.

Gispert, M., & Álvares, A. (1997). La diversidad etnológica y alimentaria en la encrucijada de la conservación y el desarrollo. *Revista de dialectología y tradiciones populares*.

Gómez-Baggethun, E., & de Groot, R. (2007). Capital natural y funciones de los ecosistemas: explorando las bases ecológicas de la economía. *Ecosistemas*, 16(3), 4-14. Doi: 10.7818/re.2014.16-3.00

Hunter, D., & Fanzo, J. (2013). Agricultural biodiversity, diverse diets and improving nutrition. Diversifying food and diets: Using agricultural biodiversity to improve nutrition and health, 1-14.

Instituto Nacional de Estadística y Geografía (INEGI) (2015). Catálogo de claves de entidades federativas, municipios y localidades. Recuperado de http://geoweb.inegi.org.mx/mgn2k/catalogo.jsp

López, M. A. (2019). La valoración de los servicios ecosistémicos desde la cosmovisión indígena totonaca. *Madera y bosques*, 25(3). Doi: https://doi.org/10.21829/myb.2019.2531752

López-Santiago, A. A., López-Santiago, M. A., Cunill-Flores, J. M., & Medina-Cuéllar, S. E. (2019). Valor socioeconómico de las plantas para una comunidad indígena totonaca. *Interciencia*, 44(2), 94-100.

Monárrez-Espino, J. (2009). Salud y nutrición en adolescentes tarahumaras. *Revista Médica del Instituto Mexicano del Seguro Social*, 47(1), 87-S92.

Muñoz, I., Rivera, L. G., Rua, E. Y. Arango, J. S., & Larrea, L. (2020). Ruta turístico-gastronómica entre México y Colombia basada en sus productos endémicos: maíz y frijol iucma-uaem. *Revista de Turismo, Patrimonio y Desarrollo*, 12.

Nivia, E., & Ivette, P. (2009). Agriculture at a Crossroads: The International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD). Island Press, Washington.

Otero, D. E., Delfín, F., Mariaca, R. & Guízar, F. (2017). La incorporación y el aumento de oferta de alimentos industrializados en las dietas de las unidades domésticas y su relación con el abandono del sistema de subsistencia propio en las comunidades rurales mayas de Yucatán, México. *Cuadernos de Desarrollo Rural*, 14(80).

Programa de las Naciones Unidas para el Medio Ambiente (PNUMA). (2008). La Biodiversidad y la Agricultura: Salvaguardando la biodiversidad y asegurando alimentación para el mundo.

Secretaría de Finanzas y Planeación del Estado de Veracruz (SEFIPLAN). (2019). Sistema de Información Municipal, Cuadernillos municipales 2019. Filomeno Mata. Recuperado de http://ceieg.veracruz.gob.mx/wp-content/uploads/sites/21/2019/06/Filomeno-Mata_2019.pdf

Servicio de Información Agroalimentaria y Pesquera (SIAP). (2019). El Plato del Bien Comer. [Accedido 2021 febrero 17]. Recuperado de https://www.gob.mx/siap/es/articulos/el-plato-del-bien-comer#:~:text=El%20plato%20del%20bien%20comer%20es%20una%20gu%C3%ADa%20de%20alimentaci%C3%B3n,fin%20de%20mostrar%20a%20la

Torres, J., & Sandoval, T. (2015). Seguridad Alimentaria y Aspectos Ambientales: El papel de la Diversidad. Debate Agrario, (47). Recuperado de

Wayne W. D. (2017). Bioestadística base para el análisis de las ciencias de la salud. 4ª ed. México LIMUSA.

Yong, A., Calvez, E. González, Y., Permuy, N., & Pavón, M. (2017). La conservación de alimentos, una alternativa para el fortalecimiento de la seguridad alimentaria a nivel local. *Cultivos tropicales*, 38(1), 102-107.

AP AGRO PRODUCTIVIDAD

AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Phytoremediation of soils contaminated with crude and weathered oil using two rice varieties (*Oryza sativa* L.)

Morales-Guzmán, Gilberto[1*]; Rivera-Cruz, María del Carmen[2]; Ferrera-Cerrato, Ronald[3]; Zavala-Cruz, Joel[2]; Ramos-Reyes, Rodimiro[4]

[1] Universidad Popular de la Chontalpa. Carretera Cárdenas - Huimanguillo, Km. 2.0 H. Cárdenas, Tabasco, México. CP. 86500.
[2] Colegio de Postgraduados - Campus Tabasco. Periférico Carlos A. Molina s/n Km 3.5, CP 86570, H. Cárdenas, Tabasco, México.
[3] Colegio de Postgraduados, Campus Montecillos, Carretera México-Texcoco km 36.5, Montecillo 56230, Estado de México, México.
[4] El Colegio de la Frontera Sur (ECOSUR) Unidad Villahermosa. Departamento de Observación y Estudio de la Tierra, la Atmósfera y el Océano, Grupo Procesos Oceanográficos y Dinámica de Sistemas Acuáticos. Carretera a Reforma km 15.5, Ranchería Guineo 2da. Sección. Villahermosa, Tabasco, México. C. P. 86280
* Correspondence: gilberto.morales@upch.mx

ABSTRACT

Objective: To evaluate the potential for soil phytoremediation with new and weathered hydrocarbons with two rice varieties (*Oryza sativa* L.).

Materials and Methods: The assessed treatments were 150 mg kg^{-1} (control soil), 30,000; 60,000 and 90,000 mg kg^{-1} of new oil and 79,457 mg kg^{-1} of weathered oil 1 and 42,000 of mg kg^{-1} of weathered oil 2; they were established in a completely randomized design with 6×3 factorial, with four repetitions each. The evaluated variables were populations of total bacteria (colony forming units CFU per grams of dry soil), free-living nitrogen-fixing bacteria (CFU), total fungi (CFU), and total dry biomass (g). Total bacteria and fungi were quantified at the beginning of the experiment at 90 and 145 days.

Results: The highest total petroleum hydrocarbons (HTP) degradation was 73 and 72% in the 79,457 and 42,000 mg kg^{-1} concentrations of weathered HTP 1 and 2, in the rhizosphere of rice silver line 21. The total dry biomass reported significant differences ($p \leq 0.05$), evidencing a lower effect in the 60,000 mg kg^{-1} concentration in new oil, which caused a 33% reduction compared to the control.

Results/Conclusions: The rice variety line 21 has a greater potential to phytoremediate soils contaminated with crude and weathered oil in field conditions in tropical areas.

Keywords: Microorganism, Biodegradation, Petroleum hydrocarbons, Rice

Citation: Morales-Guzmán, G., Rivera-Cruz, M. del C., Ferrera-Cerrato, R., & Zavala-Cruz, J. (2022). Phytoremediation of soils contaminated with crude and weathered oil using two rice varieties (*Oryza sativa* L.). *Agro Productividad*. https://doi.org/10.32854/agrop.v15i1.2090

Editor in Chief: Dr. Jorge Cadena Iñiguez

Received: July 15, 2021.
Accepted: January 3, 2022.
Published on-line: February 5, 2022.

Agro Productividad, 15(1). January. 2022. pp: 31-41.

INTRODUCTION

Organic pollutants such as total petroleum hydrocarbons (TPHs) are currently one of the most far-reaching pollutants in terrestrial and aquatic ecosystems and for human health (Wu *et al.*, 2017; Yu *et al.*, 2018a; Haider *et al.*, 2021; Zuzolo *et al.*, 2021). Therefore, the remediation of soils contaminated with TPHs is a global challenge; consequently, in recent years various technologies have been developed to solve this problem (Ossai *et al.*, 2020). Physical and chemical methods can offer quick and fast solutions for decontamination, but require a large amount of material, heavy tools, and labor (Hussain *et al.*, 2019; Xia *et al.*, 2020). However, these methods tend to be costly and harmful to the environment (Alkani *et al.*, 2020). Biological measures have recently been preferred over chemical and physical techniques for soil remediation, due to their low cost and their ability to prevent pollutants accumulation (Bonnier *et al.*, 1980; El-Nawawy *et al.*, 1987). Research has shown that some plants are capable of remediating crude oil-contaminated soils by phytoremediation (Haider *et al.*, 2021; Ostovar *et al.*, 2021; Zuzolo *et al.*, 2021). Phytoremediation involves using plants to remove, transfer, stabilize, and / or degrade contaminants from soil, sediments, and water (Xie *et al.*, 2017; Rajaei & Seyedi 2018; Nero *et al.*, 2021). Phytoremediation is a low-cost passive method with the potential to treat toxic organic and inorganic contaminants (Jeelani *et al.*, 2017; Omara *et al.*, 2019). The objective of this research was to assess and compare the potential of two varieties of rice (*Oryza sativa* L.) to phytoremediate soils contaminated with hydrocarbons from new and weathered oil.

MATERIALS AND METHODS

Study area and soil collection

Soils were collected from the surface horizon (0-30 cm), in two sites with the same pedogenetic characteristics (Gleysols). Site one, control soil (150 mg kg^{-1} biogenic origin hydrocarbons) was collected at the Arroyo Hondo - Santa Teresa ejido, Coatzacoalcos - Cárdenas highway, Tabasco, Mexico (17° 59' 52.45" N - 93° 24' 56.58" W). Site 2, located at ejido José Narciso Rovirosa, two km southeast of the La Venta Gas Processing Complex, Huimanguillo, State of Tabasco, Mexico (18° 04' 54" N - 94° 02' 31" W). Site two is an area affected by hydrocarbon spills from 1980 to 2005, due to the pipeline's ruptures. At the time of sampling, a 79,457 mg kg^{-1} of concentration was found, dry base (weathered oil 1). In 2004 it was restored with physical-chemical processes. At the end of the restoration process, the TPHs concentrations were quantified by gravimetry, and concentrations of 42,000 mg kg^{-1} of TPHs (weathered oil 2) were recorded (Rivera-Cruz *et al.*, 2016; Arias-Trinidad *et al.*, 2017). In each one of the sites, 200 kg^{-1} of soil was collected from the surface horizon (0-30 cm) using a punctual sampling. All physical and chemical parameters were determined following the methods on the norm NOM-021-RECNAT (2002) (Table 1).

Total oil hydrocarbon analysis

The TPHs content in the collected samples was extracted with soxhlet equipment using 99.5% purity dichlomethane solvent (Sigma-Aldrich®), following the EPA 3540B method (United States Environmental Protection Agency [USEPA], 1994). The pH of the

Table 1. Physical and chemical characteristics of the three evaluated soils.

Suelo	pH %	M	N	P	K	CIC Cmol$^{(+)}$ kg^{-1}	Arcilla	Limo	Arena	Textura
		mg kg^{-1}					%			
Suelo 1	6.3	5.6	0.44	27.90	0.43	42.14	61	29	10	Arcillosa
Suelo 2	4.2	25.8	ND	3.58	0.35	43.50	48	33	19	Franco
Suelo 3	4.2	6.0	0.25	7.60	0.19	20.59	31	19	50	Migajón-arenosa

M=Organic Matter, N=inorganic nitrogen, P=phosphorus, K=potassium, CIC=Cation exchange capacity, ND=Not determined.

samples was adjusted to 2.0 with concentrated HCl and subsequently dried with $MgSO_4$. The solvent was then evaporated using a rotary evaporator, the extract was quantified by gravimetry (g kg^{-1}) in a semi-analytical balance (Sartorius, Analytic Model AC 210S, Illinois, USA) following the NMX-AA-134-SCFI-2006 method (DOF, 2006).

Experiment Establishment

The seeds of *Oryza sativa* L. (Criollo canelo variety and line 21) were collected at the municipality of Comalcalco, Tabasco, Mexico and in the INIFAP Huimanguillo Experimental Field (Jiménez, 2003), which showed 95% germination. The initial contractions of crude and weathered oil were 150 (hydrocarbons of biogenic origin), 30,000, 60,000, 90,000 mg kg^{-1} and 79,457 and 42,000 mg kg^{-1} dry base TPHs. The experimental units were set in glass containers 18 cm high and 14 cm in diameter with 1400 g of soil and one *O. sativa* plant per container (15 cm high and 18 days after emergence). The experimental units were irrigated with distilled water to maintain humidity at 80% field capacity. Sowing was carried out under similar controlled conditions for 145 days. The mean annual temperature was 26 °C and the mean annual precipitation was 2,200 mm (CONAGUA, 2014).

Microorganisms evaluation

The populations of total batteries (TB), free-living nitrogen fixers (FLNF), and total fungi (TF), were evaluated at the beginning (day 1), 90, and 145 days after the experiment was established. These bacterial groups were assessed due to their importance in the recycling of nutrients and for their contribution to the degradation of hydrocarbons. The microbiological analysis was done with the plate dilution and counting method (Madigan *et al.*, 2009), using specific culture media for total bacteria (nutritive agar, Baker®), the FLNF were quantified using the medium proposed by Rennie (1981) and for total fungus, the Potato Dextrose Agar medium (PDA, Baker®) was used.

Growth variables evaluation

After 145 days, the plants were evaluated and harvested; the height of the plant was measured (the height in cm from the base of the stem to the apex of the youngest leaf, leaf zero), the radical, aerial, and total dry biomass. The leaves, stems, and roots were dried in an oven (FELISA, Model 242-A, Mexico City) at 65 °C for 72 h until a constant weight was obtained to determine their total dry biomass.

Experimental design and statistical analysis

The experiment had a 6×3 factorial design (six concentrations and three types of hydrocarbons), resulting in 18 treatments with four repetitions each, a total of 72 experimental units distributed in a completely randomized experimental design. The considered factors were the presence of fuel oil in the soil (0, 30,000, 60,000, 90,000 mg kg^{-1} TPHs, 79,457 mg kg^{-1} TPHs 1, and 42,000 mg kg^{-1} TPHs 2) with and without established plant species (Creole canelovarietie and line 21 rice). The data were subjected to an analysis of variance and an LSD type mean comparison test (Tukey $p \leq 0.05$).

RESULTS AND DISCUSSION

Effect on the plant total dry biomass

The present concentrations of fresh and weathered hydrocarbon in the soil significantly reduced the growth and development of the two varieties of O. sativa (Criollo canelo rice and Line 21 rice). The height of the plant was statistically different ($p < 0.05$) between treatments (Figure 1A and 1B). The highest height of the two O. sativa species was obtained in the control soil (150 mg kg-1); on the contrary, the weathered soil 2 with the 90,000 mg kg-1 concentration of new TPH, showed the greatest reduction (23 and 26 %) in both varieties (Figure 1A and 1B).

The aerial, root, and total dry biomass showed significant statistical differences ($p \leq 0.05$) between treatments (Figure 2). In the case of Criollo canelo cultivar, the highest root dry biomass (12 and 10 g) was recorded in the control and the 60,000 mg kg^{-1} concentration; on the contrary, the 42,000 mg kg^{-1} concentration of weathered 2 evidenced the greatest reduction in the root biomass (50%) (Figure 2A). Regard the rice line 21 established in the

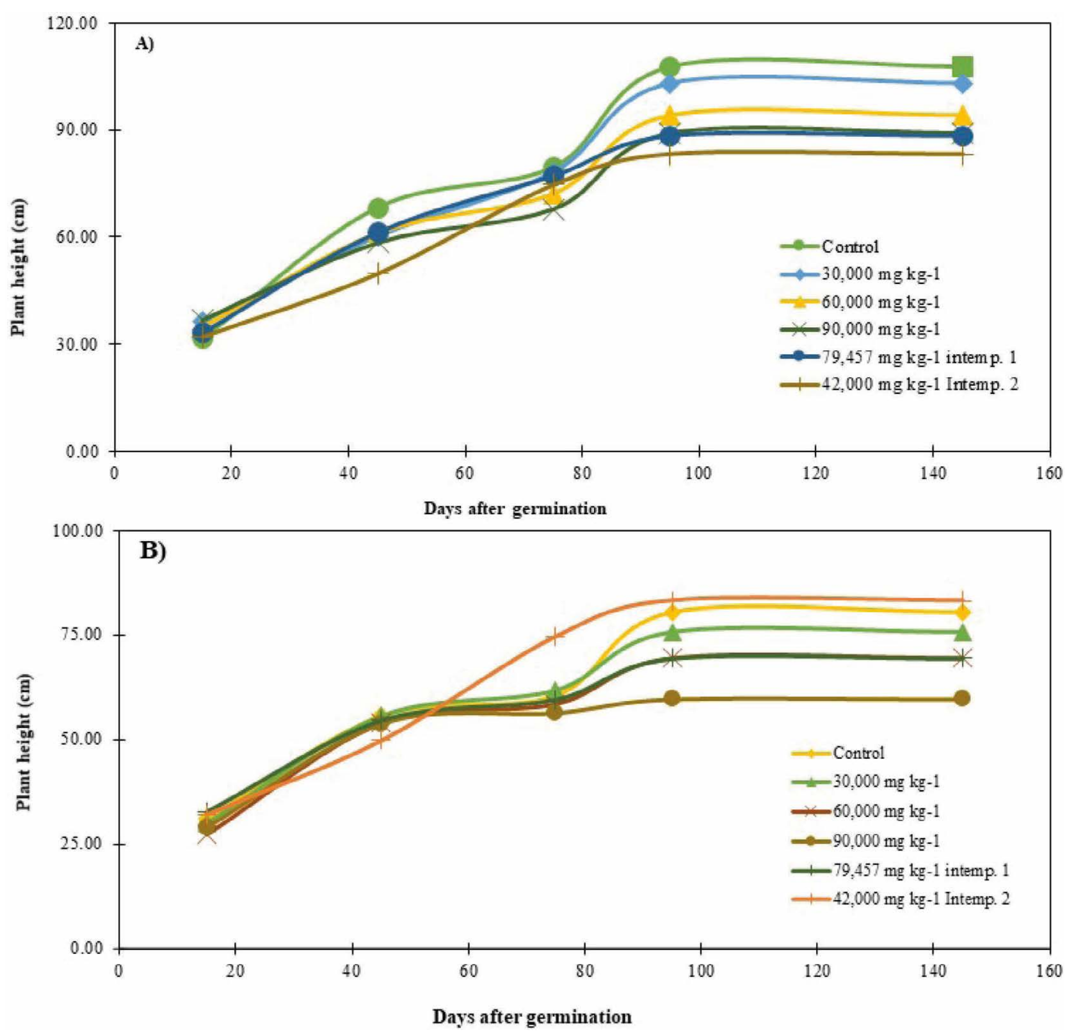

Figure 1. Height of the Criollo canelo rice plant and rice line 21 through time in different concentrations of crude and weathered oil.

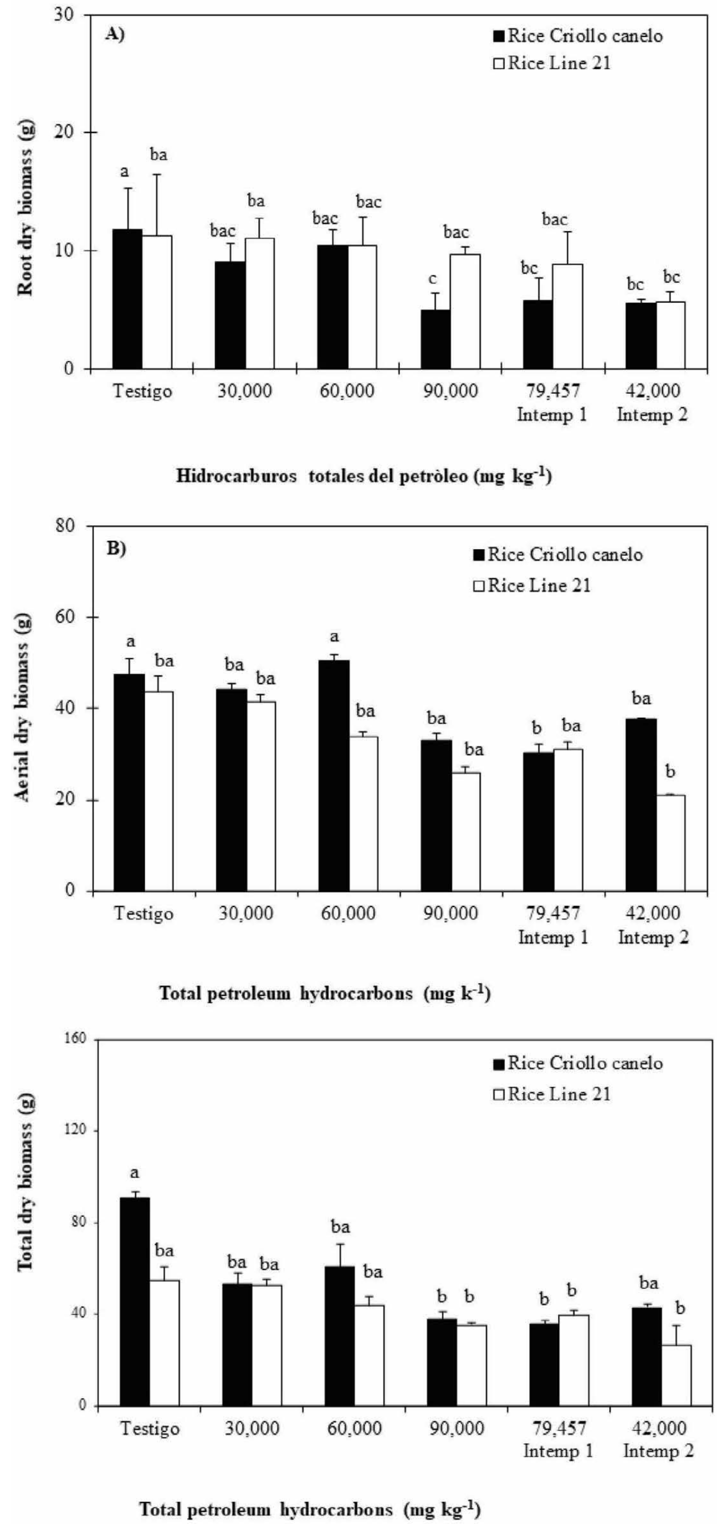

Figure 2. Effect of the concentration on the aerial dry biomass, root and total dry biomass of Criollo canelo and line 21 rice (*Oryza sativa* L.) through time in the different concentrations of crude and weathered oil. Bars with the same letters are statistically equal ($p \leq 0.05$).

control soil and the 30,000 mg kg^{-1} concentration showed the highest radical biomass (11g); on the contrary, when increasing the concentrations of petroleum hydrocarbon, the dry biomass decreased in the 2, 7, 12 and 16% compared to the control and at the lowest concentration (Figure 2B). The aerial dry biomass showed similar results to the root dry biomass which shows the tendency to increase the concentration in both rice varieties (Figure 2B). The highest total dry biomass was obtained in the Criollo canelo in the control soil and the 60,000 mg kg^{-1} concentration new TPH (91 and 63 g); compared to the rest of the treatments, which showed a maximum 33% reduction in the 30,000 mg kg^{-1} concentration of new TPH (Figure 2C). Regard line 21 rice, showed a trend like that presented by the Criollo canelo, reporting the highest total dry biomass in the control soil (55 g). On the contrary, a 42,000 mg kg^{-1} concentration of weathered TPH reduced the total dry biomass by 51% (Figure 2C).

In general, the negative effect of TPH and TPH 1, observed in the physiological variables (height, and root, aerial and total dry biomass) is attributed to the inhibition caused by the increase in the different concentrations of hydrocarbons in the soil, which suppress the nutrient's availability (Cartmill *et al.*, 2014; Ruley *et al.*, 2019; Omara *et al.*, 2020) and delay the water absorption by plants (Wang *et al.*, 2013; Ruley *et al.*, 2020; Deebika *et al.*, 2021). Likewise, oil hydrocarbons alter the physical properties of soils such as permeability, in addition to suffocating roots, especially in fine-textured (clay) or shallow soils, affecting plant growth (Akinwumi *et al.*, 2014; Grifoni *et al.*, 2020; Ostovar *et al.*, 2021). Reynoso-Cuevas *et al.* (2008) observed root damage and root length decrease in different species as the hydrocarbon's concentration increased.

Total bacterial populations in soil with new and weathered oil

The total bacterial population shows that the total hydrocarbons of crude and weathered oil (TPH) did not cause an effect at the beginning (day 1), nor at 90 days ($p \leq 0.085$, $p \leq 0.192$). On the contrary, at 145 days highly significant differences were observed ($p < 0.05$) (Figure 3). At the beginning of the experiment (time zero), the 60,0000 and 90,000 mg kg^{-1} concentrations stimulated the highest TB population in the rhizosphere of the Criollo canelo rice followed by that in line 21 rice (2.33×10^{-3} and 1.85×10^{-3} CFU), for the control without plants (Figure 3A). However, at 90 days, the maximum TB populations (1.2×10^{8} to 4.5×10^{9} CFU g^{-1}) were recorded at the 30,000 mg kg^{-1} concentration (Figure 1B) in the rhizosphere of Criollo canelo rice (Figure 3B). After 145 days, TB populations decrease two orders of magnitude (2.98×10^{6}, 1.3×10^{6} and 5.9×10^{6} CFU g^{-1}) both in the soil without plants and in the two plant species (Figure 3C).

The populations of Free-living Atmospheric N Fixing bacteria (FLNFB) showed highly significant differences between treatments ($p \leq 0.05$). At time zero, the interaction of line 21 rice and the 30,000 mg kg^{-1} and 79,457 concentrations of weathered 1 stimulated the largest population of FLNFB (9.52×10^{2}, and 9.20×10^{2} CFU), compared to the soil without plants and to the Criollo canelo rice (Figure 3 D). At 90 days after the experiment was established, the interaction in the rhizosphere of line 21 rice and the 42,000 Weathered 2 concentration stimulated the maximum population of FLNFB (4.36×10^{8} CFU) (Figure

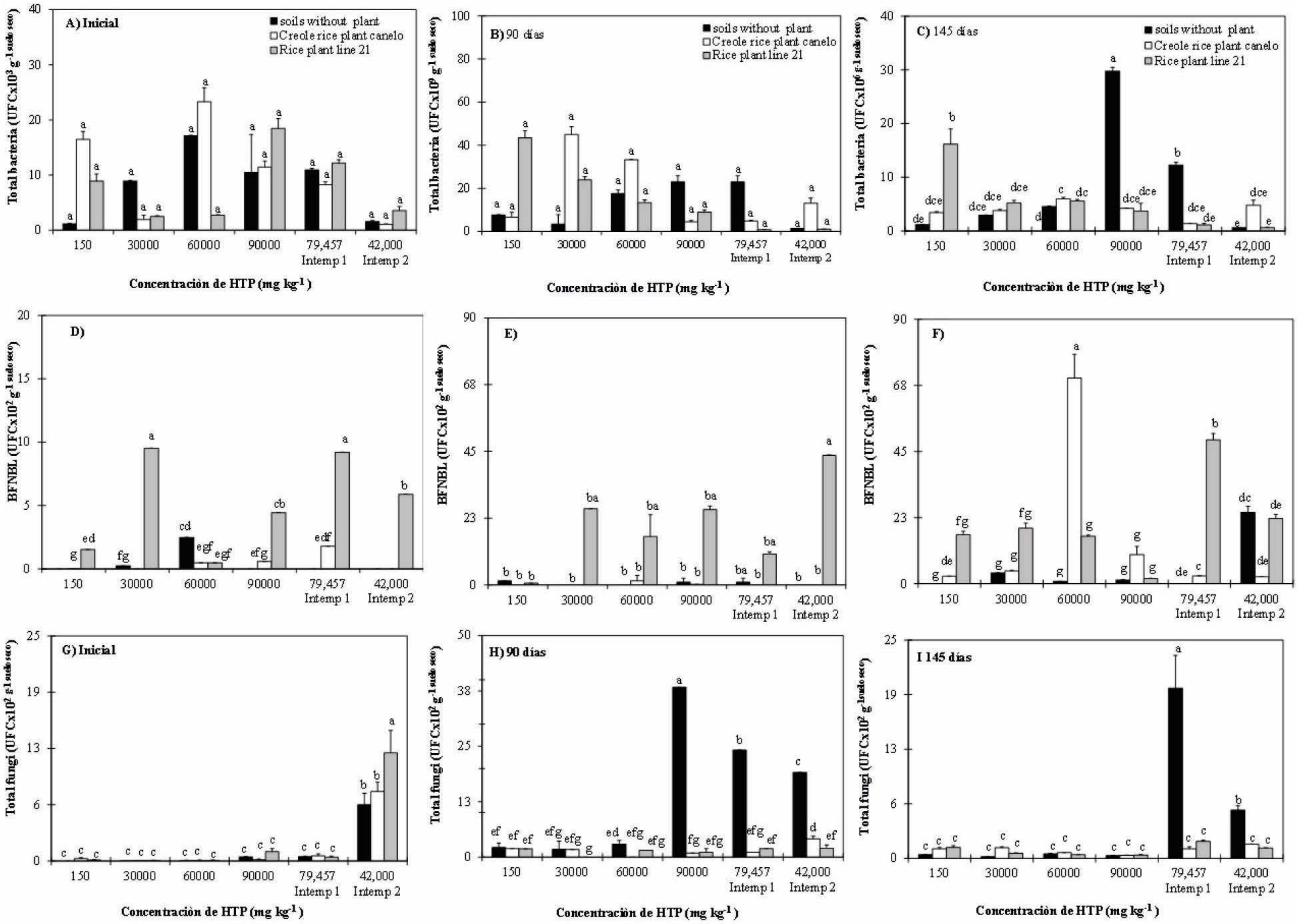

Figure 3. Kinetics of bacterial and total fungal populations in soil without plant, in soil with Criollo canelo rice plant and in soil with rice plant line 21 exposed for 145 days to soils with different concentrations of new, weathered 1 and weathered 2 crude oils (restored soil). Columns with the same letter within each time are statistically equal ($p \leq 0.05$).

3E). Population which decreased two orders of magnitude by days145 in the soil without plants as in the soil with plants (Figure 3F).

For the total fungi, significant differences ($p \leq 0.001$) between treatments were presented (Time zero, 90 and 145) (Figure G, H and I). The 42,000 weathered TPH 2 concentration presented the highest population of HT (1.2×10^3) in rhizospheric soil of line 21 rice plants on day zero (Figure 3A). On the contrary, at 90 and 145 days, the soil without plants showed the highest fungi population in the 90,000 mg kg^{-1} and 42,000 weathered 2 concentrations compared to the soil with plants (2×10^3 and 6×10^2) (Figure 3H and I).

Overall, the obtained results in the research suggest that fresh and weathered hydrocarbons presented a selective effect on soil microorganisms, favoring those with the ability to degrade or use petroleum hydrocarbons as a carbon source and energy (Freedman, 1989; Alexander, 1994; Alarcón *et al.*, 2019). In this regard, Rovina and McDougall (1967), Soleimani *et al.* (2001), and White *et al.* (2006) mention that plants, in general, can promote microbial activity through the release of organic compounds in the radical systems (amino acids, organic acids, sugars, enzymes and carbohydrates). In this sense, Bordoloi *et al.* (2012), Cartmill *et al.* (2014), and Xie *et al.* (2017) indicate that Pomacea, due to their dense root systems stimulates the bacterial populations involved in degrading petroleum hydrocarbons (González-Moscoso *et al.*, 2019). On the contrary, Rodhes and Hendricks (1990) mention that high concentrations of oil inhibit the growth of populations and the diversity of microbial communities. Chikere *et al.* (2009) indicate that the reduction of bacterial populations is an adaptive response to oil hydrocarbons due to their hydrophobic properties, which reduce the ability of plants and microorganisms to absorb water and nutrients from the soil (Alarcón *et al.*, 2019).

Effect of non-rhizospheric and rhizospheric soil on the degradation of new or weathered crude oil

The degradation of TPH in absolute terms of oil in non-rhizospheric soil (soil without plant), rhizospheric soil 1 (soil with Criollo canelo rice plants), and rhizospheric soil 2 (soil with line 21 rice plants), reported significant statistical differences ($p \leq 0.5$) (Figure 4).

Regardless of the treatments, the TPHs concentrations reduced during the 145 days, the results indicate that the highest TPHs degradation occurred in rhizospheric soils (Figure 4). The rhizospheric soil 2 presented the highest (73, 72 and 69%) degradation percentage of new and weathered crude TPH in the 30,000 mg kg^{-1} new crude TPH concentrations, 42,000 weathered TPH 2 and 79,457 from weathered TPH 1 treatment. It was followed by non-rhizospheric soil that reported degradation of 50 and 47% of the contents of 79,457 of weathered TPH 1 and 42,000 mg kg^{-1} of weathered TPH 2. On the contrary, rhizospheric soil 1 evidenced a maximum degradation of 40 and 39% equivalent to 1.9 times less than the recorded percentage in rhizospheric soil 2 and 1.2 times less than non-rhizospheric soils.

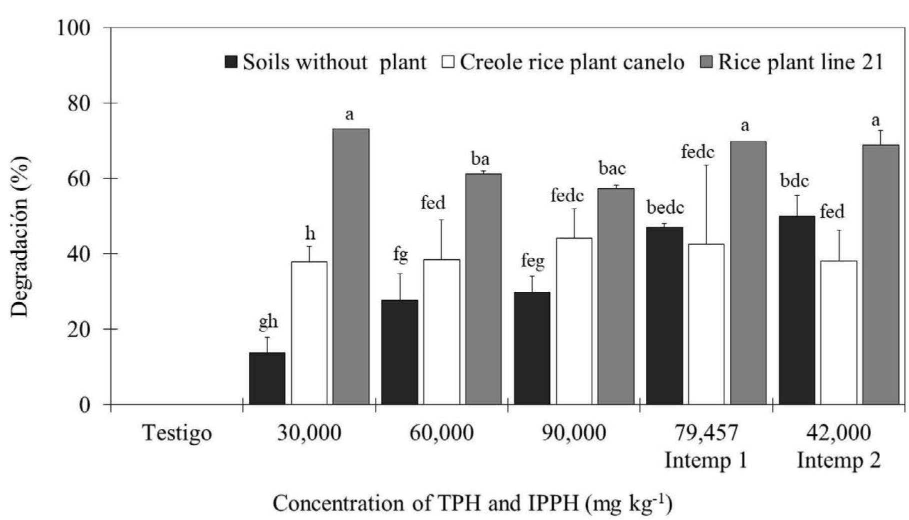

Figure 4. Degradation percentage of oil in non-rhizospheric and rhizospheric soils with *Oryza sativa* variety Criollo canelo and line 21 at 145 days. Columns with the same letter are not significantly different ($p \leq 0.05$).

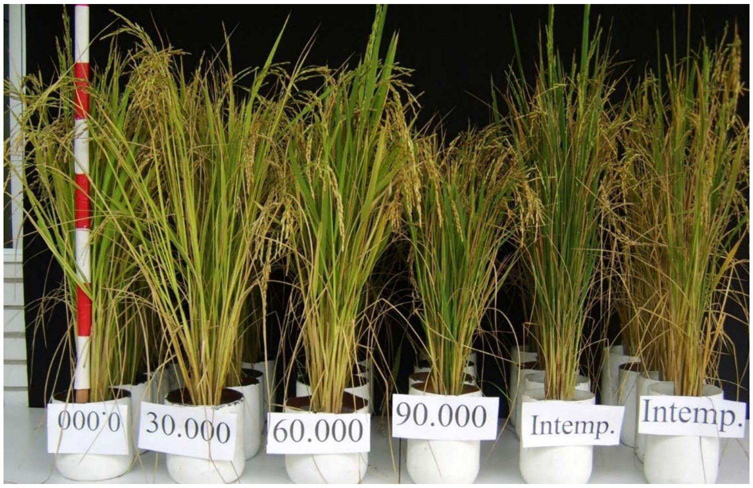

Figura 5. Rice plants (*Oryza sativa* L.) used in the experimentation.

Likewise, biodegradation of fuel oil was stimulated by line 21 rice plants (*O. stiiva* L.), which contributed by decreasing the total oil hydrocarbons proportion by 73%. Likewise, the soils without a plant (non-rhizospheric soil) showed a similar behavior until reaching a maximum 50% degradation, greater than the rhizospheric soil of the Criollo canelo rice plants, which showed a maximum degradation of 40% each.

Overall, the best results were obtained in rhizospheric soil 2 in the 79,457 concentration of weathered TPH 1. These results suggest that plant's presence increases the degradation of TPH by increasing exudates and microorganisms (bacteria and fungi) of the soil (Jeelani *et al.*, 2017; Zhang *et al.*, 2021). In this regard, Zozulo *et al.* (2020) found an 87% TPH biodegradation due to the effect of the rhizosphere in Pome plants and 89% due to the effects of Fabaceae. For their part, Kenday *et al.* (2018) found greater TPHs degradation in rhizospheric soils with respect to non-rhizospheric soils suggesting that the presence of plants in contaminated soil significantly improved TPHs elimination (Oleszczuk *et al.*, 2019: Košnář *et al.*, 2020).

CONCLUSIONS

Rice line 21 variety could be a sustainable alternative to phytoremediate soils contaminated with crude oil and weathered. The rhizospheric system of rice plants stimulated the largest population of total bacteria and fungi. The highest biodegradation of hydrocarbons occurred in the rhizospheric soil of line 21 rice plants.

REFERENCES

Akinwumi, I. I., Diwa, D., & Obianigwe, N. (2014). Effects of crude oil contamination on the index properties, strength and permeability of lateritic clay. *International Journal of Applied Sciences and Engineering Research, 3*(4): 816-824.

Alarcón, A., Garcia-Diaz, M., Hernández-Cuevas, L. V., Esquivel-Cote, R., Ferrera-Cerrato, R., Almaraz-Suarez, J. J., & Ferrera-Rodriguez, O. (2019). Impact of crude oil on functional groups of culturable bacteria and colonization of symbiotic microorganisms in the Clitoria-Brachiaria rhizosphere grown in mesocosms. *Acta biológica colombiana, 24*(2): 343-353.

Arias-Trinidad, A., Rivera-Cruz, M. D. C., Roldán-Garrigós, A., Aceves-Navarro, L. A., Quintero-Lizaola, R., & Hernández-Guzmán, J. (2017). Uso de *Leersia hexandra* (Poaceae) en la fitorremediación de suelos contaminados con petróleo fresco e intemperizado. *Revista de Biología Tropical, 65*(1): 21-30.

Bonnier, P.D., G.L. Akoun, E.C. Cadron, E.D. Edwards and W. Hockness, 1980. A technique for the disposal of oily refinery wastes. Report No. 3/10. The Hague: Concawe.

Cartmill A, Cartmill D, Alarcón A. (2014). Controlled release fertilizer increased phytoremediation of petroleum-contaminated sandy soil. *Int J Phytoremediat. 16*(3): 285–301. doi:10.1080/15226514.2013.773280.

CONAGUA. (2014). Normales Climatologicas por Estacion. Available from http://smn.cna.gob.mx/index.php?optionDcom_content&viewDarticle &idD168&tmplDcomponent Accessed 06 enero 2015. Cunningham SD, Anderson TA, Schwab AP, Hsu FC. 1996. Phytoremediation of soils contaminated with organic pollutants. Adv Agron 56: 55–114

Deebika, P., Merline Sheela, A., & Ilamathi, R. (2021). Biochar and compost-based phytoremediation of crude oil contaminated soil. *Indian Journal of Science and Technology, 14*(3): 220-228.

DOF (2006) NMX-AA-134-SCFI-2006. Norma Oficial Mexicana para suelos-hidrocarburos fracción pesada por extracción y gravimetría-método de prueba. Diario Oficial de la Federación. Martes 14 de abril de 2015. Secretaria del Medio Ambiente y Recursos Naturales. México, D. F. 27 p.

El-Nawawy, A. S., Ghobrial, F., & Elimam, A. (1987). Feasibility study of disposal of oily sludge in Kuwait. In Proceeding of the 2nd International Conference on New Frontiers for Hazardous Waste Management, Pittsburgh, PA, 27: 162-168.

González-Moscoso, M., Rivera-Cruz, M. D. C., & Trujillo-Narcía, A. (2019). Decontamination of soil containing oil by natural attenuation, phytoremediation and chemical desorption. *International journal of phytoremediation, 21*(8), 768-776.

Haider, F. U., Ejaz, M., Cheema, S. A., Khan, M. I., Zhao, B., Liqun, C., Mustafa, A. (2021). Phytotoxicity of petroleum hydrocarbons: Sources, impacts and remediation strategies. *Environmental Research*, 111031.

Hussain, I., Puschenreiter, M., Gerhard, S., Sani, S. G. A. S., Khan, W., Khan, U. D., et al. (2019). Differentiation between physical and chemical effects of oil presence in freshly spiked soil during rhizoremediation trial. Environ. Sci. Pollut. Res., 26: 18451–18464. doi: 10.1007/s11356-019-04819-6

Jeelani, N., Yang, W., Xu, L., Qiao, Y., An, S., & Leng, X. (2017). Phytoremediation potential of Acorus calamus in soils co-contaminated with cadmium and polycyclic aromatic hydrocarbons. *Scientific reports, 7*(1): 1-9.

Jiménez Ch,J.A (2003) Variedades y líneas experimentales de arroz en el estado de Tabasco. *In*: Memoria XVI Reunión Científica-Tecnológica Forestal y Agropecuaria de Tabasco. Villahermosa, Tab. Pp 89-99

Košnář, Z., Mercl, F., & Tlustoš, P. (2020). Long-term willows phytoremediation treatment of soil contaminated by fly ash polycyclic aromatic hydrocarbons from straw combustion. *Environmental Pollution*, 264: 114787.

Madigan, M. T., Martinko, J. M. y Parker, J (2009) Brock. Biología de los microorganismos. 10a. ed. rev. Pearson Educación, S. A. Madrid, Esp. 1011 p. + Ápendices

Nero, B. F. (2021). Phytoremediation of petroleum hydrocarbon-contaminated soils with two plant species: *Jatropha curcas* and *Vetiveria zizanioides* at Ghana Manganese Company Ltd. *International Journal of Phytoremediation, 23*(2): 171-180.

NOM-021- SEMARNAT. (2002). Que establece las especificaciones de fertilidad, salinidad y clasificación de suelos. Estudios, muestreo y análisis. Secretaría de Medio Ambiente y Recursos Naturales. Norma Oficial Mexicana NOM-021- SEMARNAT-2002. 75 p. Muestreo+y+Aná lisis&oq=Norma+Oficial+Mexicana%2C+Esp

Oleszczuk, P., Rakowska, M., Bucheli, T. D., Godlewska, P., & Reible, D. D. (2019). Combined effects of plant cultivation and sorbing carbon amendments on freely dissolved PAHs in contaminated soil. *Environmental science & technology, 53*(9): 4860-4868.

Omara, T., Kalukusu, R., Adupa, E., Owori, T., Kizza, D. M., & Obonge, J. (2019). Potential of Sorghum bicolor L. (Moench) and the Effect of Enhancements in Remediation of Petroleum-Vitiated Soils of an Automobile Repair Workshop in Urbanite Kampala. *Asian J Appl Chem Res. 3*(1): 1-10.

Ossai, I. C., Ahmed, A., Hassan, A., & Hamid, F. S. (2020). Remediation of soil and water contaminated with petroleum hydrocarbon: A review. *Environmental Technology & Innovation, 17*: 100526.

Ostovar, M., Ghiassi, R., Mehdizadeh, M. J., & Shariatmadari, N. (2021). Effects of crude oil on geotechnical specification of sandy soils. *Soil and Sediment Contamination: An International Journal, 30*(1): 58-73.

Reynoso-Cuevas L., Gallegos-Martínez M., Cruz-Sosa F. y Gutiérrez-Rojas M. (2008). *In vitro* evaluation of germination and growth of five plant species on medium supplemented with hydrocarbons associated with contaminated soils. *Bioresour. Technol. 99*(14): 6379-85. doi: 10.1016/j.biortech.2007.11.074.

Rivera-Cruz, M. D. C., Trujillo-Narcía, A., Trujillo-Rivera, E. A., Arias-Trinidad, A., & Mendoza-López, M. R. (2016). Natural attenuation of weathered oil using aquatic plants in a farm in southeast Mexico. *International Journal of Phytoremediation, 18*(9): 877-884.

Ruley, J. A., Tumuhairwe, J. B., Amoding, A., Opolot, E., Oryem-Origa, H., & Basamba, T. (2019). Assessment of plants for phytoremediation of hydrocarbon-contaminated soils in the Sudd Wetland of South Sudan. *Plant, Soil and Environment, 65*(9): 463-469.

SAS, (2005). Statiscal Analysis Systems "User's Guide", Versión 9.1.3. SAS Institute, Inc. Cary, NC., 664 pp

US. EPA. (1994). United States. Environmental Protection Agency Quality Assurance Management Staff. Washington, DC 20460.

Wang, Y., Feng, J., Lin, Q., Lyu, X., Wang, X., & Wang, G. (2013). Effects of crude oil contamination on soil physical and chemical properties in Momoge wetland of China. *Chinese geographical science, 23*(6): 708-715.

Wu, M., Ye, X., Chen, K., Li, W., Yuan, J., & Jiang, X. (2017). Bacterial community shift and hydrocarbon transformation during bioremediation of short-term petroleum-contaminated soil. *Environmental pollution, 223*: 657-664.

Xia, M., Chakraborty, R., Terry, N., Singh, R. P., & Fu, D. (2020). Promotion of saltgrass growth in a saline petroleum hydrocarbons contaminated soil using a plant growth promoting bacterial consortium. *International Biodeterioration & Biodegradation, 146*: 104808.

Xie, W., Zhang, Y., Li, R., Yang, H., Wu, T., Zhao, L., & Lu, Z. (2017). The responses of two native plant species to soil petroleum contamination in the Yellow River Delta, China. *Environmental Science and Pollution Research, 24*(31): 24438-24446.

Yu, L., Dong, M., Ding, B., & Yuan, Y. (2018a). Emulsification of heavy crude oil in brine and its plugging performance in porous media. *Chemical Engineering Science, 178*: 335-347.

Yu, S., Gu, X., Lu, S., Xue, Y., Zhang, X., Xu, M., & Sui, Q. (2018). Degradation of phenanthrene in aqueous solution by a persulfate/percarbonate system activated with CA chelated-Fe (II). *Chemical Engineering Journal, 333*: 122-131.

Zhang, F., Zhang, G., & Liao, X. (2021). Negative role of biochars in the dissipation and vegetable uptake of polycyclic aromatic hydrocarbons (PAHs) in an agricultural soil: Cautions for application of biochars to remediate PAHs-contaminated soil. *Ecotoxicology and Environmental Safety, 213*: 112075.

Zuzolo, D., Guarino, C., Tartaglia, M., & Sciarrillo, R. (2021). Plant-Soil-Microbiota Combination for the Removal of Total Petroleum Hydrocarbons (TPH): An In-Field Experiment. *Frontiers in microbiology, 11*: 3611.

AP AGRO PRODUCTIVIDAD

AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Analysis of the reproductive seasonality of sheep production units in Singuilucan, Hidalgo, Mexico

Barrón-González, José Eduardo[1]; Salinas-Martínez, Jesús Armando[1]; Peralta-Ortiz Jesús German; Olave-Leyva José Ignacio; González-Avalos, José[2] y Avila-Castillo, Blas Rogelio*

[1] Universidad Autónoma del Estado de Hidalgo, Área Académica de Medicina Veterinaria y Zootecnia, Instituto de Ciencias Agropecuarias, Rancho Universitario, Av. Universidad, Km 1, ExHda. De Aquetzalpa, AP 32, Tulancingo, Hidalgo, México.

[2] Universidad Autónoma del Estado de Hidalgo, Programa Educativo de Ingeniería en Manejo de Recursos Forestales, Instituto de Ciencias Agropecuarias, Instituto de Ciencias Agropecuarias, Rancho Universitario, Av. Universidad, Km 1, ExHda. De Aquetzalpa, AP 32, Tulancingo, Hidalgo, México.

* Correspondence: blas_avila8753@aueh.edu.mx

Citation: Barrón-González, J. E., Salinas-Martínez, J. A., Peralta-Ortiz, J. G., Olave-Leyva, J. I., González-Avalos, J. & Avila-Castillo, B. R. (2022). Analysis of the reproductive seasonality of sheep production units in Singuilucan, Hidalgo, Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v15i15.2033

Editor in Chief: Dr. Jorge Cadena Iñiguez

Received: May 14, 2021.
Accepted: January 2, 2021.
Published on-line: February 5, 2022.

Agro Productividad, 15(1). January. 2022. pp: 43-49.

ABSTRACT

Objective: To analyze the occurrence of the reproductive seasonality and its relation to the time of the year, feeding, race, and social environment of sheep in the municipality of Singuilucan, Hidalgo, Mexico.

Design/methodology/approach: Forty-one surveys with sheep breeders were carried out. The data obtained was subject to a logistic regression analysis, in order to analyze the degree to which the said variables intervene on the occurrence or absence of seasonal anestrus periods in ewes. The environment in which they are developed and the traditional handling of production units must be taken into account.

Results: Seasonal anestrus periods have been recorded in 95.1% of the production units; such variables as seasons of the year, feeding type, race, and social environment have a significant influence ($P<0.05$). Spring is the most influential season of the year with regard to the occurrence of anestrus periods ($P<0.05$). The reproductive season reaches its peak in late spring and in summer; ewes give birth in late autumn and in winter.

Study limitations/implications: Reproductive seasonality limits sheep production systems.

Findings/conclusions: Estrus take place in late spring and early summer. The season of the year, feeding type, race, and social environment are variables that influence the occurrence of seasonal anestrus in spring.

Key words: estrus occurrence, seasonal anestrus, semi-structured interviews.

Image by Knud Erik Vinding at Pixabay

INTRODUCTION

Reproductive handling is one of the most important aspects of sheep production systems. However, this species' reproductive seasonality poses a serious hindrance: there is a clear seasonality in the reproductive activity of sheep located in temperate regions. This seasonality is regulated by the variation in the length of days throughout the year (González *et al.*, 2014).

Reproductive seasonality is defined as a natural selection process included in the adaptation mechanisms; sheep and other species have developed endogenous rhythms that allow them to have reproductive and anestrus periods throughout the year. Yearly variations in the length of days result in an endocrine cue; the nocturnal secretion of melatonin (a hormone produced by the pineal gland) acts as a powerful seasonal synchronizer of the reproductive activity. The reproductive seasonality guarantees that births take place when food is available and the environmental temperature is appropriate for lambs (Gutiérrez, *et al.*, 2011; Henningsen *et al.*, 2016).

There is currently no full certainty about all the variables involved and to which degree they are able to explain, condition, or determine the occurrence of seasonal anestrus periods in ewes; however, several authors mention and emphasize that social signals within herds, race, and nutritional conditions are the most important variables and that they have a great impact on the herd's reproductive behavior and consequently its reproductive level (Wade and Jones, 2004; Arroyo, 2011).

Researches about seasonality in ewes in central Mexico have been carried out under controlled environments, with precise measurements, and they have been aimed only at the analysis of seasonality per photoperiod; however, they exclude nutritional and social factors (among others) which can influence reproductive behavior (Rosa and Bryant, 2003).

Therefore, the aim of this research was to analyze the reproductive seasonality and its relation with the season of the year, feeding type, race, and social environments in the sheep production systems of Singuilucan, Hidalgo, Mexico. Hidalgo stands out as second place in the national sheep meat production: 6,736 tons out of the 64,758 tons that are produced in the whole country (INFOSIAP, 2021). Production must be increased in order to satisfy the demand; currently, Mexico imports 6,734 tons of sheep meat (SIAP, 2020).

MATERIALS AND METHODS

The study was carried out in the municipality of Singuilucan, Hidalgo (20° 13' and 19° 87' N and 98° 36' and 98° 62' W). The average altitude is 2,645.88 masl. The weather is temperate and subhumid, with an average annual temperature ranging from 12 to 18 °C. During the driest month, rainfall falls below 40 mm. One-hundred ninety-one sheep producers are registered in Singuilucan (INEGI, 2010).

Data was gathered and analyzed using a combination of qualitative and quantitative research methods (Schalock *et al.*, 2000). Data was obtained from primary sources: sheep breeders, as well as their relatives and employees, were subject to semi-structured interviews, which included some specific questions.

A simple random sampling for small populations was used to obtain the interviews (Bustamante, 2011). The sample size was established through the sample size for proportion statistical method, taking into consideration a finite population, a $Z\alpha=1.96$ coefficient, and a 6% accuracy (Torres, 2006).

This research included a pilot study suggested by Daniel (2002) and Torres (2006) to provide a real value for (p). Twenty surveys were applied to sheep breeders from the municipality of Singuilucan who were randomly chosen. The purpose of this study was to determine the existence of seasonal anestrus periods in their sheep herds: 95% positive answers indicate a 5% probability of error (negative answers), which was the value allocated to (p) using the rule of three. Finally, a sample size of n=40 sheep production units was established in order to survey 191 registered units (N). However, in total, 41 surveys were carried out.

Determining and analyzing the reproductive period

The reproductive and birth period data reported by the breeders were statistically analyzed per seasons of the year, using the Kruskal-Wallis test included in the IBM SPSS Statistics software (López, 2013).

Obtaining and analyzing the seasonal anestrus

The seasonal anestrus period was obtained dividing the period without reproductive activity per type of natural anestrus, based on the months in which births and estrus periods are recorded for the herds. A 5-month gestational anestrus period (ewe gestation) and a 3-month lactational anestrus were taken into account. The latter period was obtained from the answers provided by the breeders regarding the age at which lambs are weaned (90 days), which is similar to the sheep's lactation (75-80 days) (Partida *et al.*, 2013).

Therefore, seasonal anestrus was classified as a dependent variable, while the seasons of the year, feeding type, race, and the sheep's social environment were classified as independent variables. The IBM SPSS Statistics software for research methodologies (López and Fanchelli, 2015) was used to analyze the abovementioned variables, subjecting them to bivariate and multivariate logistic regressions: the significance's percentage value was obtained by R-squared ($P<0.05$) (Paul, 2010).

RESULTS AND DISCUSSION

Occurrence of the reproductive period

Based on the 41 production units, the estrus takes place in late spring and early summer and births take place in late autumn and early winter ($P<0.05$; Table 1).

Occurrence of the seasonal anestrus

Thirty-nine production units (95.1%) reported seasonal anestrus periods, while two production units (4.8%) reported reproductive activity throughout the year (Table 2). Out of the two production units where animals were provided concentrated animal feeding, one had hair sheep without seasonal anestrus and other had wool sheep with seasonal anestrus.

In temperate regions, there is a close relationship throughout the year with the variation in the daylight hours of each season (Urviola and Riveros, 2017; Juárez *et*

Table 1. Sheep reproductive activity reported by sheep breeders from Sunguilucan, Hidalgo, México.

Months of the year	Estrus		Births	
	By month of the year	By season of the year	By month of the year	By season of the year
January	4.88	Winter[a]	63.41	Winter[a]
February	4.88		36.59	
March	7.32		19.51	
April	9.76	Spring[b]	4.88	Spring[b]
May	19.51		4.88	
June	80.49		2.44	
July	78.05	Summer[b]	2.44	Summer[b]
August	36.59		2.44	
September	17.07		2.44	
October	7.32	Fall[a]	7.32	Fall[a]
November	4.88		48.78	
December	4.88		90.24	

[ab] Different letters in the same column indicate a difference ($P<0.05$).

Table 2. Bivariate logistic regression analysis of the influence of the study variables on the occurrence of seasonal anestrus.

Variable	Production units per variable	Production units with seasonal anestrum
Season*	41	39[a]
Spring (April-June) **	37	37[a]
Summer (July-September) **	2	2
Feeding type *	41	39[a]
Grazing feed **	33	33[a]
Concentrated animal feeding **	2	1[a]
Mixed feeding **	6	5
Race type**	41	39[a]
Wool sheep	39	39[a]
Hair sheep	2	0[a]
Sheep´s social environment**	41	39[a]
Herds in the presence of males	31	31[a]
Herds in the absence of males	10	8[a]

* Independent polytomous variables; ** independent dichotomous variables.
[a] The letter above the number of production units with seasonal anestrus in relation with its number of production units per variable indicates a difference ($P<0.05$).

al., 2018). In wool sheep, the reproductive activity period takes place in autumn and early winter, without ovarian activity in spring and summer. However, anestrus periods have not been defined, since there are no reports about the influence of social factors (*e.g.*, the presence of males or lactation periods). In this sense, the results of this study show a close relationship ($P<0.05$): seasonal anestrus periods take place from April to June (spring) and most births take place from December to January (autumn-winter). Therefore, reproductive activity (estrus) occurs during the summer (July, August, and September). The difference between the occurrence of the estrus (peak reported in June) and the moment when the ewes gave birth (peak reported in December) recorded in this study might be explained by a low fertility percentage when the ovarian activity was renewed after the anestrus. Martin *et al.* (1986) mention that this behavior is mainly affected by such social factors as the incorporation of an active male. They also mention that anovulatory prepubertal, seasonal, or lactating ewes can start to leave the anestrus when they are in contact with males; physiologically, this means that LH pulses and ovulation begin to increase. Subsequently, new follicular development waves appear; a short and infertile estrus cycle usually takes place. A new normal estrus cycle then takes place, 17 to 30 days after the females have contact with the male.

Arroyo *et al.* (2007) and Macías *et al.* (2015) carried out studies in central Mexico and found out that the race of the animals has a great influence on the occurrence of seasonal anestrus. They determined that the native wool or mixed-race livestock —crossed with black-faced British races, such as Suffolk and Hampshire— has a marked seasonality, unlike hair races —such as Katahdin and Dorper which were included in two of this study's production units—, which are bred all year long (González *et al.* (2014).

Similarly to the findings of Partida *et al.* (2013) and Vázquez *et al.* (2018), three sheep production system types were identified in Singuilucan, Hidalgo: grazing feed, concentrated animal feeding, and mixed. Likewise, the feeding type is directly related to the production system; therefore, under the grazing feed system used in their production units, the ewes had a seasonal anestrus. In this sense, Arroyo (2011) mentions that this situation is closely related to food availability in certain moments of the year, which guarantees a higher birth survival rate among lambs. However, Arroyo *et al.* (2009) and González *et al.* (2014) mention that lambing periods for wool ewes take place in springtime, when the rain leads to new sprouts of the native grasses which this livestock eats under grazing feed systems. This study establishes that the lambing period took place in late autumn and winter (November, December, January, and February), when the weather is cold; the seasonal frost (heladas) diminishes food availability on dry season pasturelands (agostaderos), from December to February, and even March, until the rains begin in May (INAFED, 2010). However, in the Singuilucan region, cereals (*e.g.*, maize and barley) are usually harvested during this season; however, during the last years, some producers have taken a risk growing oats and wheat. Sheep herd graze on the residues of the harvest (both fodder and grain) that can be found in the plots. The food availability required during the sheep's lactation period could explain the births that take place during this season (Inforural, 2020).

On their part, both concentrated animal feeding production system units did not have seasonal anestrus; one had hair sheep and the other had wool sheep. Therefore, in this case, both variables can be said to have been altered by the race variable. There was no relation between variables in the mixed production system.

Another study variable was the social environment; it is closely related to the occurrence of seasonal anestrus periods, both in the presence and the absence of a male. These results match the findings of Delgadillo *et al.* (2008) who mention that male-female social communication modifies the ewe's reproductive cycle, as a result of the male's effect on the ewes' reproductive system (pheromones).

On general terms, the reproductive seasonality of sheep in Singuilucan, Hidalgo, is not only determined by the photoperiod —as has been previously reported—, but also by other closely-related variables. In this sense, photoperiods are known to be responsible for the synchronization of the ewe's reproductive period, but not for the generation of a circannual reproductive rhythm and there is even an endogenous physiological rhythm in the absence of light stimuli (Rosa and Bryant, 2003). However, further research is required in this area.

CONCLUSIONS

In the handling conditions that are typical of the sheep herds in the study region, estrus takes place in late spring and early summer. Variables such as season of the year, feeding type, race, and social environment have a significant influence on the occurrence of the seasonal anestrus during the spring.

ACKNOWLEDGEMENTS

The authors would like to thank all the students and alumni of the Área Académica de Medicina Veterinaria y Zootecnia of the Instituto de Ciencias Agropecuarias – UAEH, as well as the sheep breeders of Singuilucan, Hidalgo, for their support for this research.

REFERENCES

Arroyo, J. (2011). Reproductive seasonality of sheep in México. *Tropical and Subtropical Agroecosystems, 14*, 829-845.

Arroyo, L., Gallegos, J., Villa, A., Berruecos, J., Perera, G., & Valencia, J. (2007). Reproductive activity of Pelibuey and Suffolk ewes at 19° north latitude. *Animal Reproduction Science, 102*(1-2), 24–30. doi:10.1016/j.anireprosci.2006.09.025

Arroyo, J., Magaña, H., & Camacho, M. (2009). Regulación neuroendocrina del anestro posparto en la oveja. *Tropical and Subtropical Agroecosystems, 10*(3), 301-312.

Bustamante, C. (2011). Aproximacion al muestreo estadistico en investigaciones cientificas. *Revista de Actualización Clínica, 10*, 476-480.

Daniel, W. (2002). Bioestadística. México: LIMUSA.

Delgadillo, J., Vielma, J., Flores, J., Véliz, F., Duarte, G., & Hernández, H. (2008). La calidad del estímulo emitido por el macho determina la respuesta de las cabras sometidas al efecto macho. *Tropical and Subtropical Agroecosystems, 9*(1), 39-45.

González, A., Urrutia, J., & Gámez, H. (2014). Comportamiento reproductivo de ovejas dorper y katahdin empadradas en primavera en el norte de México. *Tropical and Subtropical Agroecosystems, 17*(1), 123-127.

Gutiérrez, C., Rangel, L., & Lassala, A. (2011). Pubertad, ciclo estral y estacionalidad. En C. Galina, & J. Valencia, Reproduccón de los animales domesticos (págs. 85-117). México: Limusa

Henningsen, J., Gauer, F., & Simonneaux, V. (2016). RFRP Neurons – The Doorway to Understanding Seasonal Reproduction in Mammals. *Frontiers In Endocrinology, 7*(36), 1-10.

INAFED. (2010). Enciclopedia de los municipios y Delegaciones de México. Recuperado el 2020, de Singuilucan: http://www.inafed.gob.mx/work/enciclopedia/EMM13hidalgo/municipios/13057a.html

INEGI. (2010). Unidad de microrregiones. Recuperado el 26 de Agosto de 2019, de SEDESOL: http://www.microrregiones.gob.mx/zap/datGenerales.aspx?entra=nacion&ent=13&mun=057

Inforural. (2020). Trigo y avena se siembran en Singuilucan. Recuperado el 2020, de Inforural: https://www.inforural.com.mx/trigo-y-avena-se-siembran-en-singuilucan/

Infosiap. 2021. Avance de la producción pecuaria por producto. [online] Available at: <http://infosiap.siap.gob.mx/repoAvance_siap_gb/pecAvanceProd.jsp> [Accessed 14 May 2021].

Juárez, A., Domínguez, A., Pinzón, L., E, A., Rivera, J., & Ramón, J. (2018). Estacionalidad reproductiva en ovejas tropicales superovuladas. *Agroproductividad, 11*(10), 133-135. doi.org/10.32854/agrop.v11i10.1257

López, P. (2013). Contraste de hipótesis. Comparación de más de dos medias independientes mediante pruebas no paramétricas: Prueba de Kruskal-Wallis. *Enfermeria del Trabajo, 3*(18), 166-171.

López, P., & Fanchelli, S. (2015). *Metodología de la investigación social cuantitativa*. Barcelona: UAB.

Macías, A., Sánchez, T., Gastelum, M., Avendaño, L., Correa, A., Álvarez, F., y otros. (2015). Actividad reproductiva estacional de ovejas pelibuey bajo condiciones áridas de México. *Archivos de Medicina Veterinaria, 47*(3), 381-386. doi:10.4067/S0301-732X2015000300016

Martin, G., Oldham, C., Cognié, Y., & Pearce, D. (1986). The physiological responses of anovulatory ewes to the introduction of rams. *Livestock Production Science, 15*(3), 219–247. doi: 10.1016/0301-6226(86)90031-x

Partida de la Peña, J., Braña, D., Jiménez, H., Ríos, F., & Buendía, G. (2013). *Produccón de carne ovina*. Ajuchitlán Querétaro: INIFAP.

Paul, J. (2010). *Academia.edu*. Recuperado el 2020, de copyleft: https://www.academia.edu/27601604/Variables_Dependientes_e_Independienteshdh

Rosa, H., & Bryant, M. (2003). Seasonality of reproduction in sheep. *Small Ruminant Research, 48*, 155–171. doi:10.1016/S0921-4488(03)00038-5

Schalock, Robert L., Gordon S. Bonhamb, Cristine B. Marchand. (2000). Consumer based quality of life assessment: a path model of perceived satisfaction. Evaluation and program planning. 23: 77-87. doi:10.1016/S0149-7189(99)00041-5

SIAP. 2020. Panorama agroalimentario 2020. [online] Available at: <https://nube.siap.gob.mx/gobmx_publicaciones_siap/pag/2020/Atlas-Agroalimentario-2020> [Accessed 14 May 2021].

Torres, M. (2006). Facultad de Ingeniería - Universidad Rafael Landívar boletín electrónico . Recuperado el 11 de Diciembre de 2019, de Tamaño de una muestra para una investigación de mercado: http://fgsalazar.net/LANDIVAR/ING-PRIMERO/boletin02/URL_02_BAS02.pdf

Urviola, A., & Riveros, J. (2017). Factores moduladores de la estacionalidad reproductiva en ungulados. *Investig. Altoandin*, 19(3), 319 – 336. doi.org/10.18271/ria.2017.297

Vázquez, I., Jaramillo, L., Bustamante, A., Vargas, S., Calderón, F., Torres, G., y otros. (2018). Structure and typology of sheep production units in central México. *Agricultura, sociedad y desarrollo, 15*(1), 85-97.

Wade, G., & Jones, J. (2004). Neuroendocrinology of nutritional infertility. *Am J Physiol Regul Integr Comp Physiol, 287*, 1277–1296. doi: 10.1152/ajpregu.00475.2004.

AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Survival of the prawn *Macrobrachium tenellum* (Smith, 1871) in confinement with the native fish *Dormitator latifrons* (Richardson, 1844)

Musin, Gabriela E.[1]; Badillo-Zapata, Daniel[2,3]; Vega-Villasante, Fernando[2]; Chong-Carrillo, Olimpia[2]; Palma-Cancino, David J.[2*]

[1] Instituto Nacional de Limnología. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) - Universidad Nacional del Litoral, Santa Fe, Argentina.
[2] Universidad de Guadalajara. Centro Universitario de la Costa, Puerto Vallarta, Jalisco, México. C.P. 48220.
[3] Programa de Cátedras CONACYT. Consejo Nacional de Ciencia y Tecnología, Ciudad de México, México.
* Correspondence: plusdpc@gmail.com

ABSTRACT

Objective: The aim of this study was to test the survival of *Macrobrachium tenellum* in different confinement conditions with *Dormitator latifrons*.

Design/methodology/approach: Using wild specimens acclimated to captive conditions of both species, three trials of different proportion were performed with treatments consisting in the presence/absence of food and shelters. Prawn/fish proportion for the assays were: 15/15 for T1, 15/3 for T2 and 10/3 for T3. In assays T1 and T2 juvenile prawns and fish were used, while in T3 post-larvae prawns and pre-juvenile fish were used. To test the survival with or without fed, it was schedule a four-day feeding *ad libitum* period, following by a two-day fed deprivation period in all the assays.

Results: In assays with juveniles of *M. tenellum* the survival rate was superior to 80% with or without shelter, when T1 showed 97-100% survival; meanwhile T3 presented a 56.67 % of survival, suggesting it's necessary to use only juvenile prawns in co-culture systems. The use of shelters and fed deprivation had no significant effects on the survival of prawns.

Limitations on study/implications: *M. tenellum* is an important aquatic resource for the central Mexican Pacific, region in which the *D. latifrons* is a representative native fish with a developing aquaculture. A polyculture or co-culture system with these species can accelerate the sustainability of both species productions.

Findings/conclusions: The results suggest this polyculture system may be feasible, and longer duration culture are recommended.

Keywords: co-culture, river shrimp, Pacific fat sleeper, shelter, feed deprivation.

Citation: Musin, G. E., Badillo-Zapata, D., Vega-Villasante, F., Chong-Carrillo, O., & Palma-Cancino, D. J. (2022). Survival of the prawn *Macrobrachium tenellum* (Smith, 1871) in confinement with the native fish *Dormitator latifrons* (Richardson, 1844). *Agro Productividad*. https://doi.org/10.32854/agrop.v15i1.2088

Editor in Chief: Dr. Jorge Cadena Iñiguez

Received: July 14, 2021.
Accepted: December 12, 2021.
Published on-line: February 5, 2022.

Agro Productividad, 15(1). January. 2022. pp: 51-58.

INTRODUCTION

Polyculture systems are becoming more relevant among producers in global aquaculture. Polyculture requires two or more cultured species in the same physical space at the same time (Stickney, 2013), preferably with different ecological niche (Wang & Lu, 2016). The

Image by kengkreingkrai at Pixabay

importance of polyculture systems in today's aquaculture lies in is capacity to be a valuable option to increase the efficiency and the sustainability of production systems (Dumont *et al.*, 2013), and in improving the system functionality by taking advantage of coexistence and interaction between species (Thomas *et al.*, 2020). Other advantages of implementing a polyculture grow-out are the mitigation of environmental impact of effluents, reduction of nitrogenous waste, and provide food security for rural population (Martínez-Porchas *et al.*, 2010).

One of the main requirements of this production systems is to utilize compatible species in environment, and that these species do not present antagonistic feeding behavior (Wang & Lu, 2016). Although is very common to use two or more fishes of high economic value (Stickney, 2013), several studies report the positive results of polyculture grow-out with prawn of the *Macrobrachium* genus and tropical fishes (García-Pérez *et al.*, 2000; Dos Santos & Valenti, 2002; Tidwell *et al.*, 2010; Cebreros *et al.*, 2013; Henry-Silva *et al.*, 2015).

Macrobrachium tenellum is a tropical species of prawn with gastronomic importance and great market value in Mexico, Guatemala and El Salvador, when is commonly referred as river shrimp (Vargas-Ceballos *et al.*, 2020). This species is found in abundance in natural populations mainly near coastal areas; has a high tolerance to temperature, salinity and dissolved oxygen fluctuation and is not an aggressive species (Espinosa-Chaurand *et al.*, 2011). Garduño-Dionate *et al.* (2017), mention that in its natural environment this prawn is over-fished, and commonly caught when individuals have not reach minimum size to secure the ecological renovation of their wild populations. The high adaptability and growing impact in their natural populations, give *M. tenellum* a high opportunity for its aquaculture development (Ponce-Palafox *et al.*, 2006; Espinosa-Chaurand *et al.*, 2011). Recent information about the grow-out of *M. tenellum* suggest is necessary to integrate methods and techniques used in other tropical shrimps to increase the cultivation effort of this resource (Peña-Herejón *et al.*, 2019). In this regard, polyculture systems represent an interesting alternative to increase the production and availability of farmed shrimps in Mexico.

The Pacific fat sleeper (*D. latifrons*), is a native fish with an increasing importance as food resource in many coastal regions of the Pacific in countries like Mexico, Ecuador and Peru (Castro-Rivera *et al.*, 2005; Basto-Rosales *et al.*, 2019). Polyculture viability of *D. latifrons* with other fishes is currently under study, and there's still a potential for the development of sustainable farm systems with other species that could benefit from the synergistic relation with the Pacific fat sleeper.

After the evaluation of the aforementioned information, we decided to test the potential compatibility of *M. tenellum* and *D. latifrons* with the implementation of a co-culture system to evaluate the survival of the prawn under confinement conditions with the fish, considering the presence and absence of food and shelters. The aim is to determine if it would be efficient to consider a polyculture system with both species.

MATERIALS AND METHODS

To evaluate our proposition, we used individuals of *M. tenellum* and *D. latifrons* collected in El Quelele Lagoon located in Nuevo Vallarta, Nayarit, Mexico, and in El Salado estuary located in Puerto Vallarta, Jalisco, Mexico. The organisms were transported under constant aeration (4 mg O_2 L^{-1}) inside a 400 L water container to the facilities of the Laboratory of Water Quality and Experimental Aquaculture (LACUIC) from the Centro Universitario de la Costa (University of Guadalajara), in Puerto Vallarta, Jalisco, Mexico. Inside the laboratory the fishes underwent an ectoparasite removal treatment in a 200 L container with a concentration of 0.3 mL of Dimilin® (diflubenzuron 22%) for 10 minutes. The fish and prawns were kept separate by species in a seven-day quarantine in 500 L water tanks with constant recirculation. Dissolved oxygen was monitored daily using an oximeter (YSI® 550 A).

After expiration of the quarantine period, we design a series of three assays using different size organisms from each species. In a first assay (T1) 15 random organisms of each species were arranged in circular tanks with 1,700 L capacity and a dimension of 2.37 m^2 (density: 13 individuals.m^{-2}), with and without shelters (eight PVC pipes in each tank), using three replicates for each treatment. Before sow, the organisms were measured in size and weight (Table 1). Then, the prawns were stocked first and later the fish. Organisms were fed *ad libitum* during a four-day period, following by a two-day feed deprivation period. To evaluate survival in this assay, organisms of each species were counted after the expiration of each period.

The second assay (T2) consisted in the confinement of three of the larger fish resulting after the first assay with 15 random prawns in 1,700 L tanks and a dimension of 2.37 m^2 (density: 8 individuals.m^{-2}) with or without shelter (eight PVC pipes in each tank) (Table 1). The fish were randomly located in the three replicates of each treatment. For this assay we proceed with feeding and feed deprivation periods similar to T1, after each period was over the surviving organisms were counted.

The third assay (T3) was realized in 40 L fish tanks, each one with 10 prawns and three fish of shorter size that the previous assays (density: 104 individuals.m^{-2}) (Table 1). As in the previous assays, we tested a treatment with shelter and without shelter (four PVC pipes in each tank) with three replicates. Feeding and feed deprivation periods were the same as the previous assays, and we counted the surviving organisms by species after every period.

All trials lasted six days and the same balanced feed was used in all of them (commercial balanced feed for tilapia "Grow fish Tilapia 2®": protein 35%, moisture 12%, fat 5.6%, crude fiber 5%, ash 8% and ELN 34.5%). During all the assays, we registered the water physicochemical parameters (temperature, dissolved oxygen and pH) on a daily basis.

Table 1. Design of the assays: initial average size (mean ± SD) and number of individuals of each species per tank (unit).

Species	Assays					
	T1		T2		T3	
	Size (cm)	Ind. $Unit^{-1}$	Size (cm)	Ind. $Unit^{-1}$	Size (cm)	Ind. $Unit^{-1}$
Macrobrachium tenellum	4.95±0.82	15	4.95±0.82	15	1.38±0.35	10
Dormitator latifrons	20.60±2.93	15	26.39±1.46	3	9.50±1.77	3

A statistical evaluation was performed with the Friedman's test taking average size, presence/absence of feeding and presence/absence of shelter as factors (explanatory variables), and the survival of the prawns as the response variable. The Friedman test was performed using the R-software (R Core Group, USA) with a statistical significance of $\alpha = 0.05$.

RESULTS AND DISCUSSION

The results in each assay suggest that the survival rate of the prawns were not affected by the feeding or fed deprivation period, the presence/absence of shelter or the average size of sow ($p<0.05$) (Table 2). The lower survival rate of *M. tenellum* obtained in T3 may be due to depredation from the fish caused by the smaller average size of the prawns (1.38 0.35), a size that is considered in the post-larval range (Jáuregui-Velázquez & Bárcenas-Gutiérrez, 2017). In this regard, New & Valenti (2017) indicate small size in prawn makes them susceptible to be eating by fishes in aquaculture systems. The prawns that not survive during T1 and T2 were found dead, dismissing the idea that they were preyed upon by fish in those assays. A better survival rate was observed in T1, suggesting that the same amount of prawns as fish not only has better biological performance but also can improve the economic revenue provided by the production system.

During our assays, many interactions between prawns and fish were observed, which may have an impact on the survival rate. In *M. tenellum* of large size is common to find some social hierarchies when some alpha males exercise dominion over the rest of the population, negatively affecting the growth and survival of said population (Vega-Villansante *et al.*, 2011). This behavior observed in several species of the *Macrobrachium* genus (Karplus & Sagi, 2010), can be countered with the use of shelter in growing areas (De Los Santos-Romero *et al.*, 2018). In our results, the use of shelter had no significant effect on the survival of the prawns, however, it's important to consider the use of shelter to prevent events of mortality or growth impairment that could affect the production system.

Another interaction observed in decapod populations is the cannibalism of the soft organisms that have just been molted by the harder ones. Cannibalism is mostly observed during molting and post-molting cycles in post-larvae and/or juvenile stages (Tidwell *et al.*, 2010). The use of different types of natural or artificial shelters has proven to be an

Table 2. Survival rate of *Macrobrachium tenellum* obtained after each period (feeding and fed deprivation) in assays with *Dormitator latifrons* (mean percentage ± SD).

	Experimental unit	Fed *ad libitum*	Fed deprivation
T1	With shelter	97.78±3.85	97.78±3.85
	Without shelter	100±0.00	100±0.00
T2	With shelter	91.11±10.18	92.44±0.84
	Without shelter	86.66±11.55	86.15±13.64
T3	With shelter	56.67±45.09	100±0.00
	Without shelter	56.67±41.63	100±0.00

The survival rate of fed deprivation period was calculated using only the survival individuals counted after the previous period. Friedman's test did not show statistical differences.

effective countermeasure for cannibalistic or other antagonist behaviors during shrimp pre-juvenile production (Karplus & Sagi, 2010). In T3 we observed a significantly lesser survival rate in the prawns; as mentioned before, this was probably caused by depredation from the fish, but it's important to not discard cannibalism. Even after not finding statistical differences in the survival for the use of shelter, the state of the art about this topic suggest it's extremely important to provide shelter when shrimps are about the average size of T3 (Ponce-Palafox *et al.*, 2006; Espinosa-Chaurand *et al.*, 2011; Vargas-Ceballos *et al.*, 2020).

This study is the first report of polyculture of *M. tenellum* with the native fish *D. latifrons*, and assays with other fishes are scarce. Vega-Villasante *et al.* (2011), tested the survival of *M. tenellum* juvenile in polyculture with different species and strains of tilapia, obtaining good survival rate for the juvenile in systems with Nile tilapia *Oreochromis niloticus* and Mozambique tilapia *O. mossambicus* (no evidence of predation by the fish), similar to our results in assays using juvenile prawns (T1, T2). However, when using more actives and growth-efficient tilapias like blue tilapia *O. aureus* and Thai-Chitralada *O. niloticus* strain (Curzon *et al.*, 2019), the mortality of *M. tenellum* was almost total (Vega-Villasante *et al.*, 2011); in this regard, observations during our assays suggest *D. latifrons* does not show antagonistic behavior when the prawns are of juvenile size (Table 2). The omnivorous nature of *D. latifrons*, and low antagonistic behavior observed toward juvenile shrimps, suggest it's a great fish option for the development of co-culture, biculture or polyculture systems with *M. tenellum*.

Other species of *Macrobrachium* are most commonly used in polyculture systems with fishes like tilapia or carp (New & Valenti, 2017), comparing the survival obtained during our research with the performance of *M. rosenbergii* in different polyculture systems, we found mixed results. Dos Santos & Valenti (2002) reported an average survival rate of 90% of *M. rosenbergii* cultivated with *O. niloticus* at different densities, and the prawn did not negatively affect the fish growth; when comparing that survival rate with the one observed during this study for T1, T2 and T3, the performance for *M. tenellum* was very similar. The similarities between survival rate of both systems may suggest that the interaction between the species can contribute to more sustainable production and more environmental friendly, also supported by the fact that in our study the survival was not affected by the fed deprivation period, suggesting that the prawn fed upon the not consumed fed and the feces of the fish. The survival of *M. tenellum* juveniles obtained in this study was similar to the survival of *M. rosenbergii* reported by Tidwell *et al.* (2010), who obtained between 87.5-96.3% with tilapia cultivated in floating cages; and higher to the survival reported by García-Pérez *et al.* (2000), who obtained 36-43% of survival for *M. rosenbergii* with tilapia, they also mentioned that high mortality of the shrimp may be related to the cannibalistic behavior observed during the grow-out and to predation by birds unrelated to the cultivation.

The survival of *M. tenellum* in our assays was more than adequate comparing with other two species of prawns. It was higher than *M. amazonicum* in a multi-trophic culture with tilapia, when the survival of the prawns fluctuated between 61.5-77.3%, without affecting the tilapia production (Henry-Silva *et al.*, 2015). Meanwhile, compared with *M. americanum* in ponds with tilapia, the prawn showed survival rate very similar to the obtained in our experiment, with low performance of post-larvae (43%), and better for juveniles with 86% (Cebreros *et al*., 2013). It's important to mention that our assays lasted less than the experiments used for comparison, however, only during our assays it was used a fed deprivation period.

Taking into account that in semi-intensive ponds, the survival of prawns and shrimps varies between 50-80%, after six to eight months, and that during storage and harvest of shrimps a minimum of 50% of survival is required to become feasible (Valenti *et al*., 2010); the results obtained in our preliminary assays are acceptable and within the expected survival range. It's pertinent to realize trials or assays with both species, at higher densities, and with longer duration.

CONCLUSIONS

All these preliminary assays are the first report of survival evaluation of *M. tenellum* in confinement conditions with *D. latifrons*. The results obtained suggest a co-culture or polyculture system with both native species can be feasible using juveniles of *M. tenellum* and fish larger than 9.50 ± 1.77 cm because predation risk is significantly lower. We do not found statistical differences between providing shelter or not, but to prevent antagonistic behavior between the species, or alpha male effect and interspecies cannibalism in the prawns during longer confinement, it is recommended the use of shelters in the tanks. Continuing research about this polyculture system will have an important impact on fisheries and aquaculture in central Mexican Pacific region, preventing exotic species introduction like tilapia may diminish the wild populations of both species and providing food security for the region with a more diverse protein source availability.

ACKNOWLEDGEMENTS

Gabriela E. Musin thanks the Government of Mexico, through the Ministry of Foreign Relations, for the scholarship awarded. Daniel Badillo-Zapata want to thanks CONACyT and the Cátedras project of CONACyT for the support provided for carrying out this study. David J. Palma-Cancino thanks the CONACyT for the grant No. 769329 provided for his postdoctoral research.

REFERENCES

Asiain-Hoyos, A., Reta-Mendiola, J.L., García-Sánchez, A.I., & Domínguez-Mora, J. (2020). The tilapia-prawn polyculture: its development in Mexico. *Agro Productividad, 13*(11): 31-36. Doi: 10.32854/agrop.v13i11.1808

Badillo-Zapata, D., Zaragoza, F.D.J., Vega-Villasante, F., López-Huerta, J.M., Herrera-Reséndiz, S., Cueto-Cortés, L., & Guerrero-Galván, S.R. (2018). Requerimiento de proteína y lípidos para el crecimiento de juveniles del pez nativo *Dormitator latifrons* (Richardson, 1844). *Ecosistemas y Recursos Agropecuarios, 5*(14): 345-351. Doi: 10.19136/era.a5n14.1554

Basto-Rosales, M.E.R., Rodríguez-Montes de Oca, G.A., Carrillo-Farnés, O., Álvarez-González, C.A., Badillo-Zapata, D., & Vega-Villasante, F. (2019). Growth of *Dormitator latifrons* under different densities in concrete tanks. *Tropical and Subtropical Agroecosystems 22*: 489-493.

Castro-Rivera, R., Aguilar-Benítez, G., & Hernández-Girón, J.P. (2005). Conversión alimenticia en engordas puras y mixtas de Popoyote (*Dormitator latifrons* Richardson) en estanques de cemento. *Revista AquaTIC, 23*: 45-52.

Cebreros, A.R., García-Guerrero, M., Molina, P.A., & Miranda, A.S. (2013). A preliminary assay on the mixed culture of red Florida tilapia and freshwater prawn *Macrobrachium americanum* stocked in outdoor tanks at different tilapia densities. *Agricultural Sciences, 4*(7): 345-352.

Curzon, A.Y., Shirak, A., Dor, L., Zak, T., Benet-Perelberg, A., Seroussi, E., & Ron, M. (2019). Hybrid origin of the Thai-Chitralada tilapia strain using DNA barcoding and microsatellite analysis. *The Israeli Journal of Aquaculture – Bamidgeh, 71*: 1-7. Doi: 10.46989/001c.20988

De los Santos-Romero, R.B., García-Guerrero, M.U., Alpuche-Osorno, J.J., & Cortes-Jacinto, E. (2018). The effect of alpha males and shelter type on growth and survival of the longarm prawn *Macrobrachium tenellum* (Smith, 1871). *Latin American Journal of Aquatic Research, 46*(3): 551-557. Doi: 10.3856/vol46-issue3-fulltext-8

Dos Santos, M.J.M., & Valenti, W.C. (2002). Production of Nile tilapia *Oreochromis niloticus* and freshwater prawn *Macrobrachium rosenbergii* stocked at different densities in polyculture systems in Brazil. *Journal of the World Aquaculture Society, 33*(3): 369-376. Doi: 10.1111/j.1749-7345.2002.tb00513.x

Dumont, B., Fortun-Lamothe, L., Jouven, M., Thomas, M., & Tichit, M. (2013). Prospects from agroecology and industrial ecology for animal production in the 21th century. *Animal, 7*(2013): 1028-1043.

Espinosa-Chaurand, L.D., Vargas-Ceballos, M.A., Guzmán-Arroyo, M., Nolasco-Soria, H., Carrillo-Farnés, O., Chong-Carrillo, O., & Vega-Villasante, F. (2011). Biología y cultivo de *Macrobrachium tenellum*: estado del arte. *Hidrobiológica, 21*(2): 99-117.

García-Pérez, A., Alston, D.E., & Cortés-Maldonado, R. (2000). Growth, survival, yield, and size distributions of freshwater prawn *Macrobrachium rosenbergii* and *Tilapia Oreochromis* niloticus in polyculture and monoculture systems in Puerto Rico. *Journal of the World Aquaculture Society, 31*(3): 446-451. Doi: 10.1111/j.1749-7345.2000.tb00894.x

Garduño-Dionate, M., Pérez-Velázquez, P.A., Lorán-Núñez, R.M., & Martínez-Isunza, F.R. (2017). Crecimiento y mortalidad del langostino moya *Macrobrachium tenellum* (Decápoda: Palaemonidae) en el río San Pedro Mezquital, Nayarit, México. *Hidrobiológica, 27*(3): 359-367.

Henry-Silva, G.G., Maia, C.S.P., Moura, R.S.T., Bessa Junior, A.P., & Valenti, W.C. (2015). Integrated multi-trophic culture of Nile tilapia (*Oreochromis niloticus*) and Amazon river prawn (*Macrobrachium amazonicum*) in brackish water. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 67*(1): 265-273. Doi: 10.1590/1678-6788

Jáuregui-Velázquez, E.E., & Bárcenas-Gutiérrez, S.Q. (2017). Estructura de tallas de *Macrobrachium tenellum* (Decapoda: Palaemonidae) en el estero El Salado, Puerto Vallarta, Jalisco, México. *Biología, Ciencia y Tecnología, 10*(37): 656-671.

Karplus, I., & Sagi, A. (2010). The biology and management of size variation. *In*: New, M.B., Valenti, W.C., Tidwell, J.H., D'Abramo, L.R. & Kutty, M.N. (Eds.) *Freshwater prawns: biology and farming*. New Jersey: Wiley-Blackwell, pp. 316-345.

Martínez-Porchas, M., Martínez-Córdova, L.R., Porchas-Cornejo, M.A., & López-Elías, J.A. (2010). Shrimp polyculture: a potentially profitable, sustainable, but uncommon aquacultural practice. *Reviews in Aquaculture, 2*: 73-85. Doi: 10.1111/j.1753-5131.2010.01023.x

New, M.B., & Valenti, W.C. (2017). Tilapia–*Macrobrachium polyculture*. *In*: Perschbacher, P.W. & Stickney, R.R. (Eds.) *Tilapia in intensive co-culture*. New Jersey: Wiley-Blackwell, pp. 156-185.

Peña-Herrejón, G.A., Sánchez-Velázquez, J., García-Trejo, J.F., Soto-Zarazúa, G.M., & Rico-García, E. (2019). Effect of stocking density on growth and survival of the prawn *Macrobrachium tenellum*, cultured in a recirculating aquaculture system. *Latin American Journal of Aquatic Research, 47*(2): 342-348. Doi: 10.3856/vol47-issue2-fulltext-14

Ponce-Palafox, J.T., García Ulloa, M.G., Arredondo Figueroa, J.L., Hernández Ocampo, D., Díaz Alvarez, J., Aldama Rojas, G., & Esparza Leal, H. (2006). El cultivo del camarón de agua dulce *Macrobrachium tenellum* en estanques rústicos. *IV Congreso Iberoamericano Virtual de Acuicultura (CIVA)*: 655-660.

Stickney, R.R. (2013). *Polyculture in aquaculture*. *In*: Christou, P., Savin, R., Costa-Pierce, B.A., Misztal, I. & Whitelaw, C.B.A (Eds.). *Sustainable food production*. New York: Springer, pp. 1366-1368.

Tidwell, J.H., Coyle, S.D., & Bright, L.A. (2010). Polyculture of Nile tilapia, *Oreochromis niloticus*, either confined in cages or unconfined in freshwater prawn, *Macrobrachium rosenbergii*, ponds. *Journal of the World Aquaculture Society, 41(*4): 616-625. Doi: 10.1111/j.1749-7345.2010.00402.x

Thomas, M., Lecocq, T., Abregal, C., Nahon, S., Aubin, J., Jaeger, C., Wilfart, A., Schaeffer, L., Ledoré, Y., Puillet, L., & Pasquet, A. (2020). The effects of polyculture on behaviour and production of pikeperch in recirculation systems. *Aquaculture Reports, 17*(2020): 100333. Doi: 10.1016/j.aqrep.2020.100333

Valenti, W.C., New, M.B., Salin, K.R. & Ye, J. 2010. *Grow-out systems: monoculture*. *In*: New, M.B., Valenti, W.C., Tidwell, J.H., D'Abramo, L.R. & Kutty, M.N. (Eds.) *Freshwater prawns: biology and farming*. New Jersey: Wiley-Blackwell, pp. 154-179.

Vargas-Ceballos, M.A., Badillo-Zapata, D., Chong-Carrillo, O., Ponce-Palafox, J.T., Hernández-Hernández, L.H., & Vega-Villasante, F. (2020). Intake of different food sources in the first zoeae stages of *Macrobrachium tenellum* (Decapoda: Palaemonidae). *Latin American Journal of Aquatic Research, 48*(1): 156-161. Doi: 10.3856/vol48-issue1-fulltext-2344

Vega-Villasante, F., Martínez-López, E.A., Espinosa-Chaurand, L.D., Cortés-Lara, M.C., & Nolasco Soria, H. (2011). Crecimiento y supervivencia del langostino (*Macrobrachium tenellum*) en cultivos experimentales de verano y otoño en la costa tropical del Pacífico mexicano. *Tropical and Subtropical Agroecosystems, 14*(2): 581-588.

Wang, M., & Lu, M. (2016). Tilapia polyculture: a global review. *Aquaculture Research, 47*: 2363-2374. Doi: 10.1111/are.12708

AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Arbuscular mycorrhizal symbiosis as sustainable alternative in the *Stevia rebaudiana* Bertoni production

Cauich-Cauich, Rodrigo Armando[1]; Tun-Suárez, José María[1]; Herrera-Parra, Elizabeth de los Ángeles[2]; Cristóbal-Alejo, Jairo[1]; Lozano-Contreras, Mónica Guadalupe[2*]

[1] Tecnológico Nacional de México/División de Estudios de Posgrado e Investigación/ Instituto Tecnológico de Conkal, Avenida Tecnológico s/n, Conkal, Yucatán, CP. 97345.

[2] Campo Experimental Mocochá, Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, Antigua carretera Mérida-Motul Km. 24.5, Mocochá, Yucatán, México, C.P. 97454.

* Correspondence: lozano.monica@inifap.gob.mx

ABSTRACT

Objective: *Stevia rebaudiana* Bertoni is a crop that can meet the demand for natural sweeteners; however, this demand requires a sustainable production, as a result of the inclusion of its steviol glycosides or active ingredients in the food and pharmaceutical industries.

Design/methodology/approach: Modern agriculture implies the integrated use of valid natural resources, such as the arbuscular mycorrhizal fungi (AMF). These microorganisms establish a symbiotic relationship with, at least, 80% of the plants, to which they provide multiple benefits. They can directly and indirectly improve crop productivity, through nutrient (particularly, phosphorus) translocation of the soil solution.

Results: As a sustainable alternative for the production of *S. rebaudiana*, they improve its nutritional state, resulting in a higher biomass production and glycoside concentration —fundamental yield parameters. Additionally, they promote resistance to biotic and abiotic stress factors and improve soil quality.

Limitations/implications: It is worth mentioning that this fungi-plant mutualism is approximately 400 million years-old; however, it has only aroused interest during the last few years.

Findings/conclusions: Although arbuscular mycorrhizal fungi (AMF) are an exploitable resource, their communities are threatened by biotic factors —such as the interaction with other microorganisms— and abiotic factors —which involve bad agricultural practices.

Keywords: mycorrhizae; sustainability, Stevia, biomass, glycosides.

Citation: Cauich-Cauich, R. A., Tun-Suárez, J. M., Herrera-Parra, E. de los Á., Cristóbal-Alejo, J., & Lozano-Contreras, M. G. (2022). Arbuscular mycorrhizal symbiosis as sustainable alternative in the *Stevia rebaudiana* Bertoni production. *Agro Productividad*. https://doi.org/10.32854/agrop.v15i1.2093

Editor in Chief: Dr. Jorge Cadena Iñiguez

Received: July 20, 2021.
Accepted: January 3, 2022.
Published on-line: February 5, 2022.

Agro Productividad, 15(1). January. 2022. pp: 59-67.

INTRODUCTION

Crop phytosanitary and nutrient management is usually based on the use of agrochemicals, with the aim of achieving a maximum exploitation of the crop's productivity; this practice requires knowledge about the agricultural needs of the plant species. However, the irrational use of these products creates problems in the agricultural sector, such as pollution, soil infertility, salinity, pest resistance, etc. (Amekawa, 2009). Sustainability is the new development trend for food production. Modern agriculture is adopting biotechnical feasible tools to achieve a sustainable production, through the application of beneficial microorganisms, both for the plant and for the soil. These

Image by Jaromír Novota at Pixabay

microorganisms have similar effects than agrochemicals and can replace them or reduce their use (Jeffries *et al.*, 2003).

Soil microorganisms are an important ingredient; some groups are more studied than others, as a result of the benefits they provide to crops. This review highlights the multiple benefits that arbuscular mycorrhizal fungi (AMF) —considered a feasible alternative for sustainable agriculture— provide to plants.

Mutualistic symbiotic relationships are 400-million-year-old (Bonfante and Genre, 2008). Currently, the AMF establish symbiosis with at least 80% of the plant species (Vierheilig, 2004). However, in an evolutionary context, plants evolved with the help of arbuscular mycorrhizae relationships (Simon *et al.*, 1993), as a result of a most interesting peculiarity of the AMF: they are forced symbionts, *i.e.*, they need a host or phytosymbiont to survive and they require a compatible relationship that provides a mutual benefit (Harrison, 1997).

During the last few years, the successful inoculation of plants of agricultural interest with AMF has been proved to promote growth and enhance the yield of the plant, as a result of an improvement in nutrient absorption.

This positive effect has also been achieved in *Stevia rebaudiana* Bertoni (Giovannini *et al.*, 2020; Tavarini *et al.*, 2018; Mandal *et al.*, 2013). This plant was introduced to Mexico mainly as a consequence of the demand for natural sweeteners, which diabetics can consume; they also replace synthetic sweeteners which cause health problems. Additionally, after its approval as food sweetener in several countries, *Stevia rebaudiana* Bertoni has acquired a high exportation potential. Its intense sweetness and medicinal properties have made it a topic of scientific and commercial interest. The importance of *S. rebaudiana* is also based on the fact that its leaves have a zero-calorie compound that is over 300 times sweeter than sucrose. This compound is made up of steviol glycosides (SG) (stevioside, steviolbioside, rebaudioside-A, B, C, D, and dulcoside-A) (Tavarini *et al.*, 2015), which are biosynthesized in a chain reaction from the pyruvate and the glyceraldehyde 3-phosphate (Brandle and Telmer, 2007; Mandal *et al.*, 2015). Among these glycosides that can be found in the leaves of the plant —which makes the leaves sweet—, stevioside and rebaudioside-A make up the highest proportion (80%).

The leaves of *S. rebaudiana* have a great proportion of its SG content; therefore, its production is mainly focused in the amount of biomass and SG content.

In this context, the main benefit of the AMF is phosphorus translocation, a key element in the SG biosynthesis. Most researches have focused on the improvement of those two important parameters. Mandal *et al.* (2013) evaluated the effect of *Rhizophagus fasciculatus* (Thaxt) on yield and SG concentration. The interaction improved the biomass and the stevioside and rebaudioside-A concentration. Tavarini *et al.* (2018) used *R. irregulare* to modify the growth habit of the plants, obtaining more branches and less height; they also reported an 88% colonization. This confirmed the susceptibility of the plants to symbiosis and AMF colonization. Vafadar *et al.* (2014) also showed that AMF inoculation efficiently increases the dry weight of the leaves of *S. rebaudiana*, using relatively low doses of chemical fertilization.

The positive effects of AMF in agriculture have been widely described; they promise an eco-friendly production. The information about the mechanisms that participate in the multiple benefits that the AMF provide to plants must increase. Most of these benefits have been attributed to the improvement of nutrient uptake by the roots. The objective of this review was to discuss the benefits and mechanisms that participate in the arbuscular mycorrhizal symbiosis for the improvement of the biomass and the metabolite of the *S. rebaudiana* crop, as well as the factors that limit the AMF use.

Stevia rebaudiana **productive potential**

Stevia has several phytochemical characteristics which makes it a crop of interest for the food industry —as sweetener, antioxidant, and dietary supplement— and for the pharmaceutical industry —for the treatment of diabetes mellitus, since it does not produce a glycaemic response. The leaves of *S. rebaudiana* can achieve a zero-calorie natural sweetness higher than sucrose (Ramesh and Megeji, 2006); they have a unique composition in the presence of labdane, phenolic acids, sterols, triterpenoid, chlorophyll, organic acids, monosaccharide, and disaccharide (Gardana *et al.*, 2010; Tavarini and Angelini, 2013).

In mid-December 2008, the Food and Drugs Administration (FDA) established that the components of *S. rebaudiana* were safe for human consumption. The same year, the JECFA/FAO (experts on food additives) agreed with the FDA's approval of *S. rebaudiana* for human consumption. In 2010, the JECFA, along with the World Health Organization, awarded the plant the safe food certification.

Since then, it has been considered a crop with high economic potential and, currently, it is considered the second sweetener worldwide. China is the main commercialization point: 50% of the production is sold in its own domestic market. China also provides at least 75% of the world production and its main exportation routes are Japan and the USA (Sarmiento-López *et al.*, 2021). Paraguay is the second producer of *S. rebaudiana* (Mogra and Dashora, 2009). Its market is focused in dry leaves commercialization, mainly supplying the EU, which has a sweetener annual consumption of ten thousand tons. Therefore, the demand of *S. rebaudiana* provides an opportunity for Mexican farmers seeking to generate a profitable crop with exportation potential.

Arbuscular mycorrhizae

Mycorrhizae imply mutualism between the arbuscular mycorrhizal fungi (AMF) and the plant. Its name comes from the Greek terms "mikos" (fungi) and "rhiza" (root). It literary means "fungi root". Specifically, it belongs to the "endomycorrhizae" type and it is the most abundant group in horticulture. This kind of fungi belong to the Glomeromycota division and are forced symbionts. The AMF have different particular structures such as hyphae, vesicles, and arbuscules. The hypahe are main structures and can be classified as "ineffective" —those that initiate the colonization and establish contact with the roots—, "absorbent" —those that explore the soil and exchange nutrients—, and "fertile" —those in charge of the asexual production of spores (Camarena-Gutiérrez, 2012).

Glomeromycota is made up of nine families (Acaulosporaceae, Archaeosporaceae, Diversisporaceae, Enthrophosporaceae, Glomeraceae, Gigasporaceae, Geosiphonaceae, Pacisporaceae, Paraglomeraceae) and at least 200 species have been described so far (Schüßler *et al.*, 2001). They are characterized by the intracellular and intercellular growth of the cortical cells of the root; they show an internal development of arbuscules (dichotomously-divided hypae, invaginated by the plasma membrane of the cortical cells, and they have short life periods) and vesicles (lipid bodies that are created in the terminal part of the hyphae) (Brundrett, 2004; Rich *et al.*, 2014).

The arbuscular mycorrhizal symbiosis takes place when an AMF spore germinates and branches in all directions, in order to find and establish contact with the roots of the plant. In this first "presymbiosis" stage, the receptive signs of the plant (flavonoids and elicitors) identify and recognize the symbiont (Harrison, 1997). However, the amount of enzymes, catalases, and peroxidases also increases; this process occurs at the same time that the appresorium (initial symbiotic structure) appears and the fungi penetrates the root. The biochemical communication between the two mutualists does not allow the plant to reject or defend itself from the fungi; this communication produces morphogenetic changes that are crucial to initiate the colonization of the roots and to guarantee mutual benefits (Akiyama, 2005).

Advantages of the arbuscular mycorrhizal symbiosis in *Stevia rebaudiana*

The symbiosis benefits for cultivated plants have been reported in survival, morphological, and productive terms. AMF are known to be capable of improving the physiological conditions of plants, even in difficult conditions, such as soil tension caused by heavy metals (Rillig, 2004), soil compaction, salinity, and drought (Miransari *et al.*, 2007). Additionally, an improvement in growth and productivity of the cultivated plants has been reported. The AMF diversity in the soil is very important, because they have a differential effect in the host (Kernaghan, 2005). Although *S. rebaudiana* is highly susceptible to AMF colonization, studies about the effects of mycorrhizal symbiosis on its quantitative and qualitative production are still limited (Giovannini *et al.*, 2020; Mandal *et al.*, 2015).

Most researches focus on the improvement of mineral nutrition: the main benefit that the AMF provides to mycorrhizae plants. However, this benefit also unchains an increase in the chlorophyl concentrations which, on their turn, modify the photosynthetic rate, resulting in a higher physiological productivity (Feng *et al.*, 2002). Although AMF cannot fix atmospheric nitrogen, their interaction with fixing microorganisms can increase the fixation of this nutrient (Rajan *et al.*, 2000).

The inoculation of *S. rebaudiana* with AMF has doubtlessly shown positive results, firstly because it leads to a greater biomass production —the commercial aspect of this crop. In this context, the inoculation of *Glomus intraradices* stimulated height, number of leaves, root length, and biomass production which are related to a higher chlorophyl content (Vafadar *et al.*, 2014; Mandal *et al.*, 2013). Parniske (2008) attributed this response to an improvement in the nutritional state of the plant, as a result of the extensive AMF hyphae network. This network covered a greater soil area, in order to absorb water and nutrients with low availability and mobility.

The AMF symbiosis can modify the quality of *S. rebaudiana*, by improving its growth habit. This situation was particularly observed when 25 mg of phosphorous were applied as P_2O_5 kg^{-1} of soil, combined with AMF symbiosis (Tavarini *et al.*, 2018), resulting in an increase of the SG yield. Nevertheless, further studies about the optimum phosphorous doses —based on the nutritional requirement of the plant— must be carried out, in order to avoid high concentrations and inhibiting AMF colonization.

On their part, Bidabadi and Masoumian (2008) proved that AMF could mitigate alterations caused by salinity stress, as a result of their positive impact on photosynthesis and the antioxidant defense system.

As essential components of soil biota, AMF also have "non-nutritional" effects: they prevent erosion, as a result of the production of a stable hydrophobic glycoprotein (glomalin), which joins the AMF hyphae, stabilizing soil aggregates and forming their long-term structure (Harrison, 1997). Arbuscular mycorrhizal symbiosis is also important for the phytoremediation of soils that have been polluted by heavy metals; the said metals are immobilized at the root and their translocation is reduced (Pawlowska *et al.*, 2000).

Mechanisms of arbuscular mycorrhizal fungi in the production of steviol glycosides

One of the main mechanisms of symbiosis is its contribution to nutrient translocation (mainly phosphorous) which is essential for the steviol glycoside pathway (Tavarini *et al.*, 2018). Although soils have very low concentrations of phosphorous, it is necessary for this process; additionally, as a result of its minimal mobility, it is not widely or easily available for the root system (Kapoor *et al.*, 2007). Under these conditions, the AMF mycelial network is the most efficient way to increase its capture (Augé, 2004). A low phosphorous level in the soil solution means that there is also a low phospholipid level in the plant membrane, resulting in a greater root exudation that stimulates an AMF colonization (Bonfante *et al.*, 2004).

Phosphorous is absorbed from the soil solution up to the plant as phosphate; the AMF's extraradical hyphae capture it quicker than diffusion; afterwards, the intraradical hyphae transport it to the cytoplasm. Alternatively, it is accumulated in vacuoles as polyphosphate granules; subsequently, it is sent through the hyphal lumen by cytoplasmic currents towards the arbusculae, where the polyphosphate is hydrolyzed and the phosphorous ion is transferred to the cell (Harrison, 1997).

On its turn, the phosphorous translocation mechanism improves the nitrogen, potassium, magnesium, and zinc assimilation (Jefferies *et al.*, 2003), incorporating an optimal nutritional state that results in the production of biomass and the concentration of steviol glycosides in the leaves.

Steviol glycosides belong to the secondary metabolites that the plant produces. During the last decade, there has been relatively little information about the AMF-induced increases in the secondary metabolite production of *S. rebaudiana*. According to Mandal *et al.* (2013), this situation is the result of the lack of in-depth studies about the effect of inoculating tropical vegetables with AMF; other studies mainly focus in the AMF-driven production of secondary metabolites in medicinal herbs (Zubek *et al.*, 2012). However, despite the growing evidence that AMF favor SG concentration, there is relatively little information about the mechanisms that are potentially involved in this increase.

Clearly, the SG biosynthesis is largely dependent on the carbon's primary metabolism and the energy supplies that the plant can process. In this regard, the AMF-plant symbiosis improves photosynthesis through morphological (*e.g.*, increase in leaf area) and physiological (*e.g.*, regulation of stomatal conductance and chlorophyl concentration) adaptations (Turgeman *et al.*, 2011) —which increases the plants carbohydrate content. SG biosynthesis takes place in the leaves and consists of three stages (Brandle *et al.*, 1998): 1) the 2-C-methyl-D-erythritol-4-phosphate pathway (MEP) (Wanke *et al.*, 2001) which leads to the conversion of pyruvate and glyceraldehyde-3-phosphate into kaurene; 2) the conversion of kaurene into steviol; and 3) the transformation of steviol into stevioside and rebaudioside-A by different glycosyltransferases (Brandle and Telmer, 2007).

A greater carbohydrate concentration increases gene transcription through the MEP pathway (Hsieh and Goodman, 2005); therefore, the AMF-plant symbiosis increases the expression of essential genes for SG biosynthesis (Mandal *et al.*, 2015). In comparison with non-inoculated plants, the transcription levels of 1-deoxy-D-xylulose-5-phosphate synthase (DXS), 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR), and 2-C-methyl-D-erythritol-2,4-cyclodiphosphate synthase (MDS) increased 1.7-1.8 times respectively in the MEP pathway. In the second stage, the transcription levels of the five genes involved (GGDPS, CPPS, KS, KO, and KAH) increased 1.4-6.5 times when mycorrhization was involved; in the third stage, glycotransferases (UGT) underwent a positive regulation, guaranteeing a greater SG concentration (Guleria *et al.*, 2014).

Limitations on the use of arbuscular mycorrhizal fungi

Biotic and abiotic factors can limit the use of AMF in the field, as well as the diversity and natural structure of the communities in the soil (Kernaghan, 2005). Plant species also determine AMF communities, based on the presymbiosis search compounds oozed by the roots to identify their preferred symbiont (Arnaut *et al.*, 1996).

Despite the scarce evidence, other soil microorganism populations can establish a synergy with the AMF, while others can inhibit spore germination and colonization (Barea *et al.*, 2002). Therefore, rhizosphere-associated microbiota has a positive or negative influence on AMF development (Gange, 2000).

Likewise, the soil's natural conditions —moisture, temperature, structure, and nutrient availability— also control AMF communities, colonization, and actions. Sometimes, these conditions are the consequence of agricultural practices (Jansa *et al.*, 2003).

Soil can contribute to AMF development at one point and hinder it later on; it can directly or indirectly limit the effects of symbiosis on AMF communities or their host plants, respectively (Augé, 2000). Neither moisture deficit (Miller, 2000), nor temperature (Matsubara and Harada, 1996) have an impact on colonization, although they can reduce spore production.

Some agricultural practices —including fertilizer doses application, inadequate crop rotation, tilling, and the use of lime— impact mycorrhizal colonization levels (Entry *et al.*, 2002). Some fertilization levels of phosphorous inhibit AMF efficiency in soy crops (Ezawa and Yoshida, 2002). Although AMF can survive in soils that have been highly polluted by heavy metals, such conditions diminish spore germination and colonization.

Arbuscular mycorrhizal symbiosis is a microbiological tool that can be used as a sustainable fertilization alternative to produce *Stevia rebaudiana*; it provides benefits that influence the improvement of biomass yield and glycoside concentration. The combination of mutual benefit mechanisms (*e.g.*, phosphorous translocation) makes the AMF and *S. rebaudiana* suitable symbionts: the plant species obtains enough phosphorous and other nutrients to improve its productivity and to concentrate its active ingredient (SG), while the AMF obtains the carbon it needs for its development.

CONCLUSIONS

Market demands require a sustainable production of *S. rebaudiana*; therefore, AMF must be taken into consideration as core elements of the cultivation and must be included in agronomy management plans. Mycorrhizae are a fundamental resource in soil structure and consequently for sustainability maintenance. Biotic or abiotic factors can threaten the communities, which must be incorporated into production plots.

ACKNOWLEDGMENTS

The authors would like to thank the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) for funding the Technology Research and Transference Project for the Use of Biofertilizers and Organic Manure in Agriculture No. 13451632421. The first author would like to thank the Consejo Nacional de Ciencia y Tecnología (CONACyT) for the postgraduate scholarship he was awarded. The authors would also like to thank the Posgrado en Agricultura Tropical Sustentable, Instituto Tecnológico de Conkal, Tecnológico Nacional de México, for their support for this research.

REFERENCES

Augé, R.M. (2000). Stomatal Behavior of Arbuscular Mycorrhizal Plants. *In*: Kapulnik Y., Douds D.D. (eds) Arbuscular Mycorrhizas: Physiology and Function. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-0776-3_10

Augé, R.M. (2004). Arbuscular mycorrhizae and soil/plant water relations. *Canadian Journal of Soil Science 84* (4): 373-381. https://doi.org/10.4141/S04-002

Amekawa, Y. (2009). Reflections on the growing influence of good agricultural practices in the global south. *Journal of Agricultural and Environmental Ethics 22* (6): 531-557. https://doi.org/bm4wg4

Arnaud, M., Hamel, C., Vimard, B., Caron, M., Fortin, J. A. (1996). Enhanced hyphal growth and spore production of the arbuscular mycorrhizal fungus *Glomus intraradices* in an *in vitro* system in the absence of host roots. *Mycological research 100* (3): 328-332. https://doi.org/cztv3w

Akiyama, K. (2005). Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* 435: 824–827. https://doi.org/10.1038/nature03608

Barea, J. M., Toro, M., Orozco, M. O., Campos, E., Azcón, R. (2002). The application of isotopic (32 P and 15 N) dilution techniques to evaluate the interactive effect of phosphate-solubilizing rhizobacteria, mycorrhizal fungi and *Rhizobium* to improve the agronomic efficiency of rock phosphate for legume crops. *Nutrient Cycling in Agroecosystems* 63 (1): 35-42. https://doi.org/bt9tbw

Bidabadi, S. S. and Masoumian, M. (2018). Arbuscular mycorrhizal symbiosis improves growth and antioxidative response of *Stevia rebaudiana* (Bert.) under salt stress. *Trends in Horticulture* 1 (3):1-8. https://doi.org/f77p

Bonfante, P., Genre, A., and Bianciotto, V. (2004). The colonisation strategies of arbuscular mycorrhizal fungi: an overview of their cellular interactions with plants and bacteria. *In*: Avance en el conocimiento de la biología de las micorrizas. J.T. Frías-Hernández, V. Olalde-Portugal, R. Ferrera-Cerrato (Eds). Primera Edición. Universidad de Guanajuato. Guanajuato, México, Pp 19-28.

Bonfante, P. and Genre, A. (2008). Plants and arbuscular mycorrhizal fungi: an evolutionary developmental perspective. *Trends in Plant Science* 13 (9): 492-498. https://doi.org/cg8hs5

Brandle, J. E., Starratt, A. N., Gijzen, M. (1998). *Stevia rebaudiana*: Its agricultural, biological, and chemical properties. *Canadian Journal of plant science* 78 (4): 527-536. https://doi.org/cn6qdn

Brandle, J. E. and Telmer, P. G. (2007). Steviol glycoside biosynthesis. *Phytochemistry* 68(14): 1855-1863. https://doi.org/d2bt6g

Brundrett, M. (2004). Diversity and classification of mycorrhizal associations. *Biological reviews* 79 (3): 473-495. https://doi.org/bt6pwk

Camarena-Gutiérrez, G. (2012). Interacción planta-hongos micorrízicos arbusculares. *Revista Chapingo serie ciencias forestales y del ambiente* 18 (3): 409-421. https://doi.org/f77d

Entry, J. A., Rygiewicz, P. T., Watrud, L. S., Donnelly, P. K. (2002). Influence of adverse soil conditions on the formation and function of arbuscular mycorrhizas. *Advances in Environmental Research* 7 (1): 123-138. https://doi.org/ds9xtf

Ezawa, T., Yamamoto, K., Yoshida, S. (2002). Enhancement of the effectiveness of indigenous arbuscular mycorrhizal fungi by inorganic soil amendments. *Soil Science and Plant Nutrition* 48 (6): 897-900. https://doi.org/bdcrwz

Feng, G., Zhang, F., Li, X., Tian, C., Tang, C., Rengel, Z. (2002). Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. *Mycorrhiza* 12 (4): 185-190. https://doi.org/bw288z

Gange, A. (2000). Arbuscular mycorrhizal fungi, Collembola and plant growth. *Trends in Ecology & Evolution* 15 (9): 369-372. https://doi.org/fh437d

Gardana, C., Scaglianti, M., Simonetti, P. (2010). Evaluation of steviol and its glycosides in *Stevia rebaudiana* leaves and commercial sweetener by ultra-high-performance liquid chromatography mass spectrometry. *Journal of Chromatography A* 1217: 1463–1470. https://doi.org/bf6f8h

Giovannini, L., Palla, M., Agnolucci, M., Avio, L., Sbrana, C., Turrini, A., Giovannetti, M. (2020). Arbuscular mycorrhizal fungi and associated microbiota as plant biostimulants: research strategies for the selection of the best performing inocula. *Agronomy* 10 (1): 106. https://doi.org/f78b

Guleria, P., Masand, S., Yadav, S. K. (2014). Overexpression of SrUGT85C2 from Stevia reduced growth and yield of transgenic Arabidopsis by influencing plastidial MEP pathway. *Gene* 539 (2): 250-257. https://doi.org/f5wfr5

Harrison, M. J. (1997). The arbuscular mycorrhizal symbiosis. In Plant-microbe interactions (pp. 1-34). Springer, Boston, MA. https://doi.org/dsz2c9

Hsieh, M. H. and Goodman, H. M. (2005). The Arabidopsis IspH homolog is involved in the plastid nonmevalonate pathway of isoprenoid biosynthesis. *Plant physiology* 138 (2): 641-653. https://doi.org/cmxcc7

Jansa, J., Mozafar, A., Kuhn, G., Anken, T., Ruh, R., Sanders, I. R., Frossard, E. (2003). Soil tillage affects the community structure of mycorrhizal fungi in maize roots. *Ecological Applications* 13 (4): 1164-1176. https://doi.org/fb9fqs

Jefferies, P., Gianinazzi, S., Perotto, S., Turnau, K., Barea, J. M. (2003). The contributionof arbuscular mycorrhizal fungi in sustainable maintenance of plant health andsoil fertility. *Biology and Fertility of Soils* 37: 1–16. https://doi.org/dhpj87

Kapoor, R., Chaudhary, V., Bhatnagar, A. K. (2007). Effects of arbuscular mycorrhiza and phosphorus application on artemisinin concentration in *Artemisia annua* L. *Mycorrhiza* 17: 581–587. https://doi.org/c72x36

Kernaghan, G. (2005). Mycorrhizal diversity: cause and effect?. *Pedobiologia* 49(6): 511-520. https://doi.org/bj75v9

Mandal, S., Evelin, H., Giri, B., Singh, V. P., Kapoor, R. (2013). Arbuscular mycorrhiza enhances the production of stevioside and rebaudioside-A in *Stevia rebaudiana* via nutritional and non-nutritional mechanisms. *Applied Soil Ecology* 72: 187-194. https://doi.org/f77g

Mandal, S., Upadhyay, S., Singh, V. P., Kapoor, R. (2015). Enhanced production of steviol glycosides in mycorrhizal plants: a concerted effect of arbuscular mycorrhizal symbiosis on transcription of biosynthetic genes. *Plant Physiology and Biochemistry* 89: 100-106. https://doi.org/f67g9v

Matsubara, Y. I., and Harada, T. (1996). Effect of constant and diurnally fluctuating temperatures on arbuscular mycorrhizal fungus infection and the growth of infected asparagus (*Asparagus officinalis* L.) seedlings. *Journal of the Japanese Society for Horticultural Science* 65 (3): 565-570. https://doi.org/bh8cv4

Miransari, M., Bahrami, H.A., Rejali, F., Malakouti, M.J., Torabi, H. (2007). Using arbuscular mycorrhiza to reduce the stressful effects of soil compaction on corn (*Zea mays* L.) growth. *Soil Biology and Biochemistry* 39: 2014–2026. https://doi.org/ffs4ww

Miller, S. P. (2000). Arbuscular mycorrhizal colonization of semiaquatic grasses along a wide hydrologic gradient. *New Phytologist* 145 (1): 145-155. https://doi.org/c59vrr

Mogra, R. and Dashora, V. (2009). Exploring the use of *Stevia rebaudiana* as a sweetener in comparison with other sweeteners. *Journal of Human Ecology* 25 (2): 117-120. https://doi.org/f77v

Pawlowska, T. E., Chaney, R. L., Chin, M., Charvat, I. (2000). Effects of metal phytoextraction practices on the indigenous community of arbuscular mycorrhizal fungi at a metal-contaminated landfill. *Applied and Environmental microbiology* 66 (6): 2526-2530. https://doi.org/cd3xdw

Parniske, M. (2008). Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews Microbiology* 6: 763-775. https://doi.org/btrpt2

Rajan, S. K., Reddy, B. J. D., Bagyaraj, D. J. (2000). Screening of arbuscular mycorrhizal fungi for their symbiotic efficiency with *Tectona grandis*. *Forest ecology and management* 126 (2): 91-95. https://doi.org/cqr3x6

Ramesh, K., Singh, V., Megeji, N. W. (2006). Cultivation of stevia [*Stevia rebaudiana* (Bert.) Bertoni]: A comprehensive review. *Advances in Agronomy* 89: 137-177. https://doi.org/dhw5v5

Rillig, M. C. (2004). Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecological Letters* 7: 740–754. https://doi.org/b8x2qd

Rich, M. K., Schorderet, M., Reinhardt, D. (2014). The role of the cell wall compartment in mutualistic symbioses of plants. *Frontiers in Plant Science* 5: 238. https://doi.org/f6bjj9

Sarmiento-López, L. G., López-Meyer, M., Sepúlveda-Jiménez, G., Cárdenas, L., Rodríguez-Monroy, M. (2021). Arbuscular mycorrhizal symbiosis in *Stevia rebaudiana* increases trichome development, flavonoid and phenolic compound accumulation. *Biocatalysis and Agricultural Biotechnology* 31: 101889. https://doi.org/f77r

Simon L., Bousquet J., Levesque R. C., Lalonde M. (1993). Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. *Nature* 363: 67–69. https://doi.org/d5p52s

Schüßler, A., Schwarzott., D, Walker, C. (2001). A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research* 105: 1413-1421. https://doi.org/c86djq

Tavarini, S., Angelini, L. G. (2013). *Stevia rebaudiana* Bertoni as a source of bioactive compounds: the effect of harvest time, experimental site and crop age on steviol glycoside content and antioxidant properties. *Journal of Science of Food and Agriculture* 93 (9): 2121–2129. https://doi.org/f4z9zq

Tavarini, S., Sgherri, C., Ranieri, A.M., Angelini, L.G. (2015). Effect of nitrogen fertilization and harvest time on steviol glycosides, flavonoid composition, and antioxidant properties in *Stevia rebaudiana* Bertoni. *Journal of Agricultural and Food Chemistry* 63 (31): 7041–7050. https://doi.org/f7nwcd

Tavarini, S., Passera, B., Martini, A., Avio, L., Sbrana, C., Giovannetti, M., Angelini, L. G. (2018). Plant growth, steviol glycosides and nutrient uptake as affected by arbuscular mycorrhizal fungi and phosphorous fertilization in *Stevia rebaudiana* Bert. *Industrial Crops and Products* 111: 899-907. https://doi.org/f7nwcd

Turgeman, T., Asher, J. B., Roth-Bejerano, N., Kagan-Zur, V., Kapulnik, Y., Sitrit, Y. (2011). Mycorrhizal association between the desert truffle *Terfezia boudieri* and *Helianthemum sessiliflorum* alters plant physiology and fitness to arid conditions. *Mycorrhiza* 21 (7): 623-630. https://doi.org/d22r45

Vafadar, F., Amooaghaie, R., Otroshy, M. (2014). Effects of plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungus on plant growth, stevioside, NPK, and chlorophyll content of *Stevia rebaudiana*. *Journal of Plant Interactions* 9 (1): 128–136. https://doi.org/f77m

Vierheilig, H. (2004). Regulatory mechanisms during the plant arbuscular mycorrhizal fungus interaction. *Canadian Journal of Botany* 82 (8): 1166-1176. https://doi.org/dkwg87

Wanke, M., Skorupinska-Tudek, K., Swiezewska, E. (2001). Isoprenoid biosynthesis via 1-deoxy D-xylulose 5-phosphate/2-C-methyl-D-erythritol 4-phosphate (DOXP/MEP) pathway. *Acta Biochimica Polonica* 48 (3): 663-672. https://doi.org/f77n

Zubek, S., Mielcarek, S., Turnau, K. (2012). Hypericin and pseudohypericin concentrations of a valuable medicinal plant *Hypericum perforatum* L. are enhanced by arbuscular mycorrhizal fungi. *Mycorrhiza* 22 (2): 149-156. https://doi.org/c537f8

Young, K.E., Zwank, P.J., Valdez, R., Dye, J.L., & Tarango, L.A. (1997). Diet of Mexican spotted owls in Chihuahua and Aguascalientes, Mexico. *Journal of Raptor Research* 31 (4): 376–380

AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Pathogens of zoonotic interest in chicken meat for sale in retail stores in Mexico

Castro-González, Numa P.

Facultad de Ciencias Agrícolas y Pecuarias, Benemerita Universidad Autónoma de Puebla, A.V. Universidad s/n San Juan Acateno Teziutlan, Puebla, México
Correspondence: numa.castro@correo.buap.mx

ABSTRACT

Objective: Determine the presence of zoonotic pathogens in chicken meat sold in retail centers in five cities of the Mexican Republic.

Design/methodology/approach: 153 samples of raw chicken meat were analyzed. All samples were analyzed using methods approved by the AOAC and the US regulatory agencies, isolation that what promised, slipped and subsequently PCR analysis was performed for *Campylobacter* spp., *Salmonella* spp., *E. coli* and *Listeria* spp.

Results: *Campylobacter* spp. it was found in 31% of the samples and *Salmonella* spp. in 1.31% of the total samples analyzed. *Campylobacter* spp. it has a higher prevalence in Tlalnepantla Estado de Mexico (74%), Puebla (33.33%) and Guadalajara Jal. (25.58%). *Salmonella* spp. it has a higher prevalence in Tlalnepantla Estado de Mexico (3,7%) and Guadalajara Jal. (4,65%).

Limitations: This study describes the prevalence of *Campylobacter* and *Salmonella* in chicken meat for sale in Mexico, however, more studies are needed to determine exactly the origin of these bacteria scale.

Findings/conclusions: According to the results obtained in this work, it can be concluded that there is contamination of the chicken meat with the bacterium *Campylobacter* spp. in a higher proportion, unlike *Salmonella* spp. This may be due to possible errors in the handling in the different areas by which the bird is handled from the farm to the commercialization.

Keywords: Chicken meat, Zoonoses, Broilers, Bacterial contamination.

Citation: Castro-González, N P. (2022). Pathogens of zoonotic interest in chicken meat for sale in retail stores in Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v15i1.2112

Editor in Chief: Dr. Jorge Cadena Iñiguez

Received: August 20, 2021.
Accepted: December 29, 2021.
Published on-line: February 5, 2022.

Agro Productividad, 15(1). January. 2022. pp: 69-76.

INTRODUCTION

Chicken meat is a food of great nutritional importance in the diet of humans, however, its consumption may represent a health risk, since in order to satisfy the demand of the population, it is intensively produced and it is processed in an industrial way and as a consequence there is greater stress for animals causing diseases that can affect human being (Castañeda-Gulla *et al.*, 2020).

It is known that there is a close relationship between animal welfare and food safety when it refers to the production of food of animal origin. Stress and the lack of animal welfare during handling on the farm or transporting animals to the slaughterhouse predispose them to suffer diseases and the increase in the elimination microorganisms such

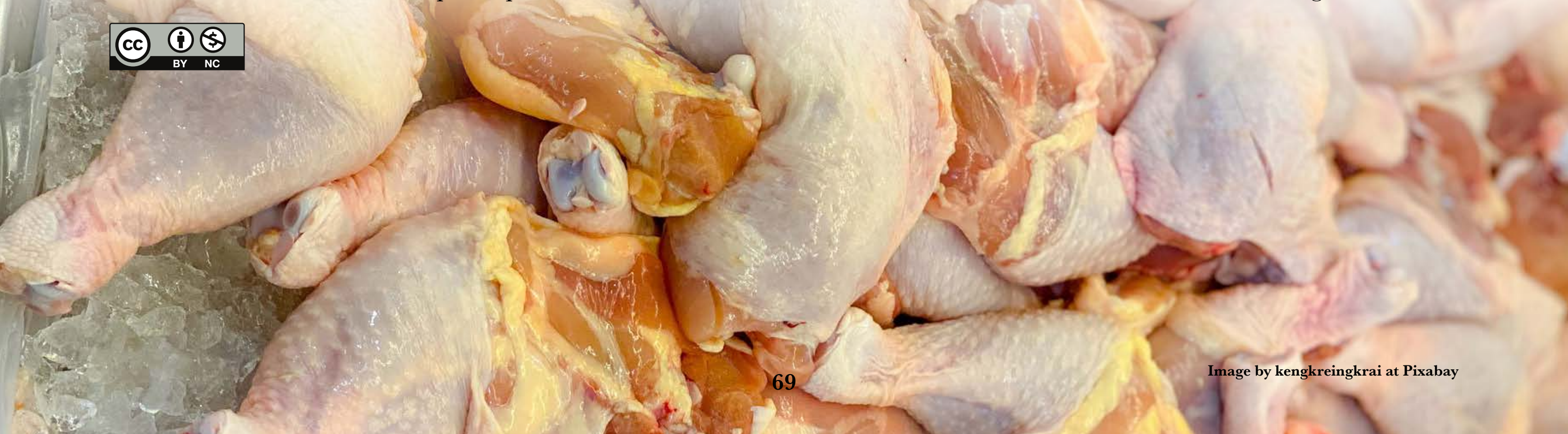

Image by kengkreingkrai at Pixabay

as *Campylobacter*, *Salmonella* and *Echerichia coli* through feces, causing the contamination of meat with these pathogens and their toxins putting the health of consumers at risk (FAO/WHO, 2009; Rostagno, 2009; López *et al*., 2016; Alpigiani *et al*., 2017; EFSA, 2019). Made additionally worrisome because of the exposure to stress conditions during the slaughter process can induce bacterial adaptation as a result of changes in the genetic expression of these pathogens (Duqué *et al*., 2021).

In the case of *Campylobacter* spp. it represents up to 70% of zoonosis cases in Europe, followed by *Salmonella* spp. both are related to products derived from the poultry sector (EFSA / ECDC, 2018) and have been isolated from live birds and in meat ready for sale (Berndtson *et al*., 1996; Willis *et al*., 1997; FAO-WHO, 2009). *Campylobacter* spp. is one of the four main global causes of diarrheal disease and is considered the most common bacterial cause of gastroenteritis in the world. Complications such as hepatitis, pancreatitis and abortions have also been observed, with varying degrees of frequency. Post-infection complications include reactive arthritis and neurological disorders such as Guillain-Barré syndrome (WHO, 2021).

Salmonella is transmitted through food and according to the European Union (Regulation (EC) No. 2160/2003) chicken meat is one of the main sources of transmission to humans causing salmonellosis and typhoid fever. It is reported that more than 1,000,000 non-typhoid *Salmonella* infections annually (EFSA, 2015; CDC, 2018), while in Mexico the number reaches around 70,000 cases of this disease each year (DGE, 2017).

Another microorganism of importance for public health is *E. coli*, which is found in 90% of the feces of birds and is recognized as an indicator of fecal contamination. In recent years it has been the cause of outbreaks with negative impact worldwide (FAO, 2021).

In Mexico, poultry production is very important, representing 0.81% of the national Gross Domestic Product (GDP), 36.65% of agricultural GDP and 63.3% of national livestock production (UNA 2019), most of the production units are of the intensive type and about 3 million 377 tons of chicken meat are produced (FAOSTAT, 2021), with a per capita consumption of 29 kg (UNA, 2019).

Mexico has a population of 126,014,024 inhabitants, of which 7% correspond to children under four years old, 8.5% to children between 5 and 9 years old, ages where children are most susceptible to contracting gastrointestinal diseases that can cause death and these are due to the consumption of contaminated food. But also, between 10 and 29 years of age it takes importance since they represent 15% of the total population (INEGI, 2021) and are the most common hosts of this type of pathogens. This being a reason to consider this as a food safety problem. Furthermore, in Mexico the impact on health is unknown and no data was found on campylobacteriosis in animals destined for human consumption.

For all the above, the objective of this study was to preliminarily detect the presence of *Campylobacter* spp., *Salmonella* spp., *E. coli* and *Listeria* spp. in chicken meat for sale in retail centers in five main cities of the Mexican Republic.

MATERIALS AND METHODS

In the months of December 2020 and January 2021, 153 samples of raw chicken meat were collected from different points of sale of the most important retail centers in

5 cities of the Mexican Republic (Tijuana BC; Guadalajara, Jal; Tlalnepantla Estado de México; Puebla and Queretaro), selected according to the importance they have for the production of broilers. The samples were collected in a period of 3 hours and transported in refrigerators with ice for transport and stored in refrigeration at 3 °C until analysis.

Sample processing and analysis

All samples were analyzed using methods approved by the AOAC and US regulatory agencies.

The analytes and methods were:

Campylobacter: first the isolation of *Campylobacter* spp. was carried out, carrying out the initial detection (presumably positive) in 48 hours, to later carry out the isolation and confirmation in a total of 4 days. Using 1625±32.5 mL of Buffer Peptone Water (BPW) which was added to 325±32.5 g of raw chicken meat. To disperse lumps, the samples were thoroughly mixed by brief manual massage using a bag (no more than 10 seconds). After mixing, 30 mL of double concentration bloodless Bolton enrichment broth was added, 30 mL of the sample was placed in a bag, mixed and shaken by hand several times. The samples were incubated for 48±2 hours at 42±1 °C under microaerobic conditions by placing 2-3 CampyGen containers (Fisher Scientific, Leicestershire, UK) inside an anaerobic flask. After 48±2 hours of incubation, 3-5 mL of enriched broth were taken for PCR analysis. The analysis was performed in a multiplex PCR for identification of *Campylobacter jejuni* and *Campylobacter coli*. This standard operating procedure is based on USDA MLG 41.05 (2021) and uses Bolton double concentration bloodless enrichment broth for enrichment and multiplex PCR for identification of *C. jejuni* and *C. coli* from broth and agar plates (isolation). Identification was carried out with multiplex PCR assays and confirmation was carried out by colony isolation on agar plates and latex agglutination test.

For the case of *E. coli* O157, STEC and *Salmonella*: PCR technology was used to amplify unique DNA sequences present in *E. coli* O157, other Shiga toxin-producing *E. coli* (STEC) and *Salmonella* that cause disease in humans. Subsequently, a 75 g sample was directly enriched with 150 mL of M1-GN broth using filter bags and incubated at 42 °C for a minimum of 12 hours (maximum 24 hours) and analyzed with the AOAC PTM 100701 "IEH methodology. *E. coli* O157, producer of Stx *E. coli* (STEC) with Intimin & Salmonella Test System", a PCR-based method that has genetic targets for *Salmonella*, *E. coli* O157 and STEC O26, O45, O103, O111, O121 and O145.

Listeria monocytogenes: the PCR method was used to amplify unique DNA sequences. present in *Listeria monocytogenes* and *Listeria* spp. that cause diseases in humans. A 75 g sample was placed in 150 mL of M1-GP medium using filter bags and incubated at 35 °C for a minimum of 21 h (maximum 48 h). After enrichment of the sample, the bacterial DNA is released from the organisms in special buffer by a lysis procedure. Two unique specific bacterial DNA fragments of *Listeria* spp. and two unique specific fragments of *Listeria monocytogenes*, not present in other bacteria, are targeted, and amplified using Taq DNA polymerase and nucleotides. After PCR amplification, the products were separated by agarose gel electrophoresis and visualized by a UV transilluminator after being stained with ethidium bromide (EtBr).

Statistical analysis

The experimental design was completely randomized, and the results obtained were analyzed by calculating the prevalence through descriptive statistics using the SPSS 18 package and the comparison between sampled cities was carried out by using a General Linear Model (GLM) and for the comparison for means, the Tukey test was used, using the statistical package SAS 9 (2002).

RESULTS AND DISCUSSION

E. coli and *Listeria* spp. were not detected in the raw chicken meat samples analyzed in this work. But the presence of *Campylobacter* spp. was detected in 31% of the total samples analyzed, showing a significant difference ($p<0.05$) (Figure 1), the percentage of positive samples for this bacterium being higher in Tlalnepantla State of Mexico 74%, Puebla with 33.33% and Guadalajara Jal. 25.58% compared to the analyzed samples from Tijuana BC 18.5% and Queretaro 10.52% (Table 1).

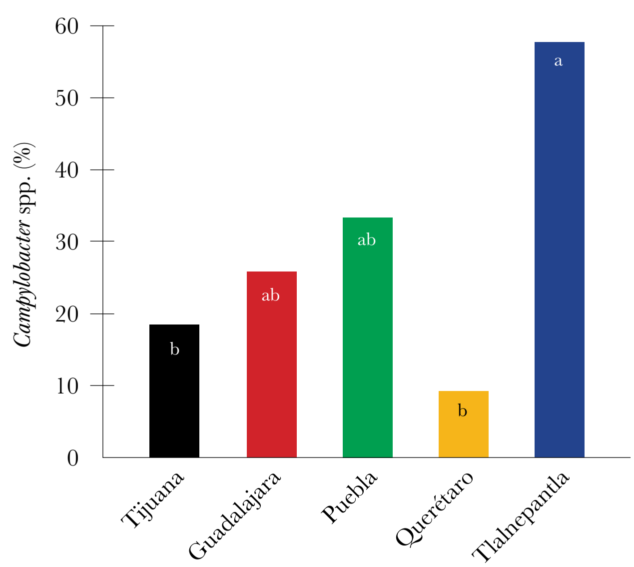

Figure 1. *Campylobacter* spp. in chicken meat for sale in retail stores in México. Literals (a, b) represents significant difference ($p<0.05$).

Table 1. Percentage of incidence of pathogens of zoonotic interest in chicken meat for sale in retail establishments in Mexico.

City	*Salmonella* spp	*E. Coli*	*Campylobacter* spp.	*Listeria* spp.
Tijuana	0/27	0/27	5/27	0/27
Tlanelpantla	1/27	0/27	20/27	0/27
Guadalajara	2/43	0/43	11/43	0/43
Puebla	0/27	0/27	9/27	0/27
Queretaro	0/19	0/19	2/19	0/19
Total	2/153	0/123	47/153	0/153
Incidence rate (%)	1.3	0	31	0

In the case of *Salmonella* spp. it was found in 1.3% of the total analized chicken meat samples, with no significant difference ($p>0.05$) between Tlalnepantla Estado de Mexico (3.7%) and Guadalajara (24.65%), but it did showed a significant difference ($p<0.05$) between these two cities and the rest of the sampled cities (Puebla, Tlalnepantla, Queretaro) in which it was not detected.

In many places worldwide, *Campylobacter* spp. has been detected in chicken meat and Mexico is no exception, and this bacterium is considered a frequent cause of gastrointestinal disease, the higher the rate of contamination of the animals, the greater the risk of contamination of the products obtained. after its process in the slaughterhouse; the main source of contamination being the content of the intestinal tract, where it can colonize in a high number of 1010 colony forming units (CFU) per gram of infected intestine, the main site of colonization being the cecum, where *Campylobacter* spp. it is found in the mucous layer of epithelial cells (Hernández-Cortez *et al.*, 2013). In addition, the bacteria can be present on the skin or feathers; thus, animals from uninfected flocks become contaminated during slaughter, especially in the plucking, evisceration and refrigeration process (Mead *et al.*, 1995; Keener *et al.*, 2004; Cervantes-García, 2020).

In this work, 31% of the raw meat samples appeared contaminated with *Campylobacter* spp. a value that is similar to that reported by EFSA (2015) for raw chicken meat samples (31.4%) in the European Union. However, the value found in this work is below that reported in raw meat by Zhao *et al.* (2001) (70%) in New York, Chrystal *et al.* (2008) (44.8%) in New Zealand, Zaidi *et al.* (2012) Mexico (58.3%), Zumbado-Gutierrez *et al.* (2014) (40%) in Costa Rica and above that found by Lucas *et al.* (2013) in Peru 16.7%, Di Giannatale *et al.* (2019) (17.38%) in Italy and by Thomas *et al.* (2020) who at carrying out a bibliographic review in Africa found that the prevalence of *Campylobacter* spp. in chicken meat was 21% of a total of 2973 samples from different African regions.

The presence of *Campylobacter* spp. in the samples of chicken meat sampled in this work, it could be due to the fact that it is a bacterium that lives in the intestine of birds and the stress is due to the lack of animal welfare during handling on the farm, transport and/or the process. of the slaughter contributes to the animals eliminating a greater amount of this micro-organism and its toxins through the feces, causing the contamination of the work equipment, the surfaces, the process water and the air, and with this there is a greater dissemination of the bacteria with the consequent contamination of the meat (Iannetti *et al.*, 2020). Therefore, greater interest should be placed on animal welfare on the farm, food safety practices and operations in processing plants (Dogan *et al.*, 2019).

According to the percentage of contaminated samples found in this work and considering that in Mexico there is a per capita consumption of 29 kg, and the annual production amounts to 3 million 377 tons of chicken meat (FAOSTAT, 2021), the data found is alarming, since it would represent a contamination of approximately 1,046,840 tons of raw chicken meat per year, the risk being high for a population between children and adults. If taking into account that a contaminated bird carcass can carry between 100 and 100,000 *Campylobacter* cells and that only 500 cells are required to cause infection (Hernández-Cortez *et al.*, 2013), then there would be a number of exposed inhabitants of approximately 36,098,000 per year, corresponding to 272,537 daily exposures nationwide.

This could be greater than the 845,024 cases per year confirmed at the laboratory level in the United States of America (Scallan *et al*., 2011; Dogan *et al*., 2020). This is relevant since the WHO (2021) reports that 1 in 10 people in the world suffer from diarrheal diseases and in the case of children under 5 years of age affected reach up to 220 million, where this disease can be fatal, being *Campylobacter* spp. one of the four most prevalent pathogens in food worldwide.

On the other hand, the presence of *Salmonella* spp. was identified in 2 of the samples of a total of 153 that were collected for the work, representing 1.3% of positive samples in raw chicken meat. The prevalence found in this work is below that reported by Van *et al*. (2007) Vietnam 53.3%, Pointon *et al*. (2008) Australia 43.3% and Adeyanju *et al*. (2014) in Nigeria 33%. The prevalence of *Salmonella* spp. can vary depending on the geographic region, the zootechnical and manufacturing practices, distribution and the biosecurity programs for the control of pathogens. In this work, during the sampling period, the prevalence is low, however, it would be important to carry out this same analysis in times of heat and high rainfall where weather conditions could influence a greater presence of this bacterium (Akil *et al*., 2014).

CONCLUSIONS

According to the results obtained in this work, it can be concluded that there is contamination of the chicken meat with the bacterium *Campylobacter* spp. in a higher proportion, unlike *Salmonella* spp. This may be due to possible errors in the handling in the different areas by which the bird is handled from the farm to the commercialization. This work serves as preliminary information and determines the need to improve the handling of the animals from the farm to the slaughterhouse in order to avoid stress and contamination with pathogens in chicken meat and thus avoid the risk to the health of the chicken's consumers. It is important that health authorities implement prevention programs for these diseases through the regulation and control of the production, distribution and marketing of poultry products and their derivatives.

ACKNOWLEDGMENT

The author is grateful to Animal Equality Mexico for the information provided and for the funding to carry out this work. He also thanks Diego Arturo Díaz Morales for his collaboration in translation.

REFERENCES

Adeyanju, G., Ishola, O., 2014. *Salmonella* and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. *Springer Plus*. 3(1):139. doi:10.1186/2193-1801-3-139 .

Akil, L., Ahmad, H.A., and Reddy, R.S., 2014. Effects of Climate Change on Salmonella infections. *Foodborne Pathogens Disease,* 11: 974-980. https://doi.org/10.1089/fpd.2014.1802.

Alpigiani, I., Abrahantes, J. C., Michel, V., Huneau-Salaün, A., Chemaly, M., *et al*., 2017. Associations between animal welfare indicators and *Campylobacter* spp. in broiler chickens under commercial settings: A case study. *Preventive Veterinary Medicine*, 147, 186–193.

Berndtson, E., Emanuelson, U., Engvall, A., Danielsson- Tham, M.L. A., 1996. 1-year epidemiological study of campylobacters in 18 Swedish chicken farms. *Preventive Veterinary Medicine*; 26:1

Castañeda-Gulla, K., Sattlegger, E., Mutukumira, A.N., 2020. Persistent contamination of *Salmonella*, *Campylobacter*, *Escherichia coli*, and *Staphylococcus aureus* at a broiler farm in New Zealand Kristine. *Can. J. Microbiol*. 66: 171–185.doi:10.1139/cjm-2019-0280

CDC, Center for Disease Control and Prevention, 2018. Salmonellosis-nontyphoidal. Aviable in:https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/salmonellosis-nontyphoidal

Cervantes-García, E., 2020. Campylobacter: emergente o reemergente Campylobacter: emergent or remergent *Rev Mex Patol Clin Med Lab* 2020; 67 (3): 142-149. doi: 10.35366/96677

Chrystal, N. D., S. J. Hargraves, A. C. Boa, and C. J. Ironside., 2008. Counts of *Campylobacter* spp. and prevalence of *Salmonella* associated with New Zealand broiler carcasses. *J. Food Prot*. 71: 2526–2532.

DGE, Dirección General de Epidemiología, 2017. Anuario de Morbilidad, 1984 -2017. Aviable in: http://www.epidemiologia.salud.gob.mx/anuario/html/anuarios.html

Di Giannatale, E., Calistri, P., Di Donato, G., Decastelli, L., Goffredo, E., *et al*., 2019. Thermotolerant *Campylobacter* spp. in chicken and bovine meat in Italy: Prevalence, level of contamination and molecular characterization of isolates. *PLOS ONE* 14(12): e0225957. https://doi.org/10.1371/journal.pone.0225957

Dogan, O.B, Clarke, J., Mattos, F., Wang, B., 2019. A Quantitative Microbial Risk Assessment Model of Campylobacter in Broiler Chickens: Evaluating Processing Interventions. *Food Control*. 100: 97–110. https://doi.org/10.1016/j.foodcont.2019.01.003.

Duqué, B., Rezé, S., Rossero, A., Membré, J-M., Guillou, S., Haddad, Nabila., 2021. Quantification of *Campylobacter jejuni* gene expression after successive stresses mimicking poultry slaughtering steps, *Food Microbiology*. 98, 103795 doi:10.1016/j.fm.2021.103795

EFSA/ECDC, European Food Safety Authority / European Centre for Disease Prevention and Control, 2015. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2013. *EFSA Journal*. 13(1): p. 3991-4153. Aviable in: https://www.efsa.europa.eu/es/efsajournal/pub/3991

EFSA, European Food Safety Authority, 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food borne outbreaks. *EFSA Journal*, 13: 3991. Doi:10.2903/j.efsa.2015.3991

EFSA, European Food Safety Authority, 2019. Animal welfare: Introduction. Aviable in: https:// www.efsa.europa.eu/en/topics/topic/animal-welfare last.

EFSA/ECDC, European Food Safety Authority and European Centre for Disease Prevention and Control, 2018. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA Journal*, 16, 5500. Aviable in: https://www.efsa.europa.eu/es/efsajournal/pub/5500

FAO, Food and Agriculture Organization of the United Nations, 2021. Gateway to poultry production and products. Products and processing. Aviable in: http://www.fao.org/poultry-production-products/products-and-processing/es/

FAOSTAT. 2021. Organización de las Naciones Unidas para la Alimentación y la Agricultura. Prevención de la *E. coli* en los Alimentos. Roma. Aviable in: http://www.fao.org/fileadmin/user_upload/fcc/news/FAO_PREVENCION.de.la.E.Coli.en.los.ALIMENTOS_FCC_ES.pdf

Fernández, H., 1992. Thermotolerant Campylobacter species associated with human diarrhea in Latin America. *J Braz Ass Adv Sc*; 44 (1): 39-4

FAO/WHO, Food and Agriculture Organization of the United Nations/World Health Organization, 2009. Risk assessment of *Campylobacter* spp. in broiler chickens: Technical Report. *Microbiological risk assessment series* No 11. Geneva .35 pp. Aviable in: https://www.who.int/foodsafety/publications/micro/MRA11_En.pdf

Hernández-Cortez, C., Aguilera, A.M.G., Castro, E.G., 2013. Campylobacter jejuni: ¿una bacteria olvidada? Situación en México. *Enf Infec Microbiol*. 2013;33(2):77-84. Aviable in: https://www.medigraphic.com/pdfs/micro/ei-2013/ei132f.pdf

Iannetti, L., Neri, D., Santarelli, G.A., Cotturone, G., Podaliri, V.M., *et al*., 2020. Animal welfare and microbiological safety of poultry meat: Impact of different at-farm animal welfare levels on at-slaughterhouse *Campylobacter* and *Salmonella* contamination. *Food Control* 109 (2020) https://doi.org/10.1016/j.foodcont.2019.106921

INIGI, Instituto Nacional de Estadistica y geografía, 2021. Comunicado de prensa número 164/20. Aviable in: https://www.inegi.org.mx/contenidos/saladeprensa/aproposito/2020/EAP_Nino.pdf

Keener, K. M., Bashor, M. P., Curtis, P. A., Sheldon, B. W., Kathariou, S., 2004. Comprehensive review of Campylobacter and poultry processing. *Comprehensive Reviews in Food Science and Food Safety*, 3(2), 105e116.

Lopez, L., Roos, R., Cressey, P., Horn, B., Lee, J., 2016. Annual foodborne disease report in New Zealand 2015. Available from https://www.foodsafety.govt.nz/science-risk/humanhealth-surveillance/foodborne-disease-annual-reports.htm.

Lucas, L. J., Vilca, L. M., Ramos, D.D., 2013. Presencia de *Campylobacter* spp en canales y ciegos de pollos de engorde en Lima, Perú. *Rev. investig. vet. Perú* [citado 2021 Jul 15] ; 24(3):346-352. Aviable in: http://www.scielo.org.pe/scielo.php?script=sci_arttext&pid=S1609-91172013000300011&lng=es.

Mead, G. C., Hudson, W. R., Hinton, M. H., 1995. Effect of changes in processing to improve hygiene control on contamination of poultry carcasses with *Campylobacter*. *Epidemiology and Infection*, 115(3), 495e500.

UE, Unión Europea, Parlamento Europeo y del Consejo. Reglamento (CE) N° 2160/2003 del Parlamento Europeo y del Consejo Sobre el Control de la Salmonella y Otros Agentes Zoonónicos Específicos transmitidos por los alimentos. Aviable in: https://www.saludcastillayleon.es/institucion/es/recopilacion-normativa/seguridad-alimentaria/normativa-general-control-oficial-alimentos/reglamento-ce-n%C2%BA-2160-2003-parlamento-europeo-consejo-17-no

Pointon, A., Sexton, M., Dowsett, P., Saputra, T., Kiermeier, A., Lorimer, M., 2008. Holds G, Arnold G, Davos D, Combs B, *et al*. A baseline survey of the microbiological quality of chicken portions and carcasses at retail in two Australian states (2005 to 2006). *J. Food Prot*. 71(6): 1123–1134. doi: 10.4315/0362-028X-71.6.1123

Rostagno, M. H., 2009. Can stress in farm animals Increase food safety risk? *Foodborne Pathogens and Disease*, 6, 767–776.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., *et al*., 2011. Foodborne illness acquired in the United States – major pathogens. *Emerg. Infect. Dis*., 17, 7–15.

Thomas, K.M., de Glanville, W.A., Barker, G.C., Benschop, J., Buza, J.J., *et al*., 2020. Prevalence of Campylobacter and Salmonella in African food animals and meat: A systematic review and meta-analysis. *Int J Food Microbiol*. 16; 315:108382. doi: 10.1016/j.ijfoodmicro.2019.108382.

UNA, Unión Nacional de Avicultores, 2019. Producción y consumo de pollo en México. Aviable in: http://www.ina.org.mx/sites/default/files/fotos/pdfs/prodyconspollo.pdf

UNA, Unión Nacional de Avicultores, 2021. Expectativas 2021. Aviable in: https://una.org.mx/industria/

USDA, U.S. Department of Agriculture, 2021. Test campylobacter Aviable in: https://www.fsis.usda.gov/sites/default/files/media_file/2020-08/1-EIOA-Micro-Sampling-Testing.pdf

Van, T.T.H., Moutafis, G., Istivan, T., Tran, L.T., Coloe, P.J., 2007. Detection of *Salmonella* spp. in Retail Raw Food Samples from Vietnam and Characterization of Their Antibiotic Resistance. *Applied and Environmental Microbiology*. 73(21): 6885–6890. doi:10.1128/AEM.00972-07

WHO, World Health Organization, 2021. Campylobacter. Aviable in: https://www.who.int/es/news-room/fact-sheets/detail/campylobacter

Willis, W.L., Murray, C., 1997. *Campylobacter jejuni* seasonal recovery observations of retail market broilers. *Poultry Science* 1997; 76:314-317.

Zaidi, M., P. McDermott, F., Campos, R., Chim, M., León, G., eta al., 2012. Antimicrobial-resistant Campylobacter in the food chain in Mexico. *Foodbourne Pathog Dis.* 9:841-847.

Zhao, C., Ge, B., De Villena, J., Sudler, R., Yeh, E., et al., 2001. Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella serovars* in retail chicken, turkey, pork, and beef from the Greater Washington, D.C., area. *Applied and environmental microbiology,* 67(12), 5431–5436. https://doi.org/10.1128/AEM.67.12.5431-5436.2001

Zumbado Gutierrez, L., Arévalo, A., Donado, M del P., Romero, Juan., 2014. Diagnóstico molecular de Campylobacter en la cadena. *Agronomía Mesoamericana* 25 (2): 357–63. https://www.mendeley.com/catalogue/556ca0cb-6fad-35ed-80c2-23a0b8ad4343/?utm_source=desktop&utm_medium=1.19.4&utm_campaign=open_catalog&userDocumentId=%7Bce1e6e0c-5e04-4005-99a6-84606dc6ef44%7D.

AGRO PRODUCTIVIDAD
Colegio de Postgraduados

Validation of a model to estimate climate effects on wheat (*Triticum aestivum* L.) production in a hydrological basin

Salazar-Solano, Vidal[1]; Moreno-Dena, Jesús M.[1*]; Rojas-Rodríguez, Isaac S.[2]; De La Torre-Valdez, Hugo C.[3]

[1] Centro de Investigación en Alimentación y Desarrollo A. C., Hermosillo, Sonora, México, C. P. 83304.
[2] Universidad de Sonora. Hermosillo, Sonora, México, C. P. 83000.
[3] Universidad Estatal de Sonora, Hermosillo, Sonora, México, C. P. 83100.
* Correspondence: jesus.moreno@ciad.mx

ABSTRACT

Objective. To validate a simulation model which enables the estimation of wheat (*Triticum aestivum* L.) production, based on climatic variables in the lower Mayo River basin in Sonora, Mexico.

Design/methodology/approach. The Soil and Water Assessment Tool (SWAT) Crop Yield simulation model was used to estimate productive wheat yields. The model was fed with climatic data for the 1979-2014 period, provided by the National Centers for Environmental Prediction. Subsequently, the results were validated using the Nash-Sutcliffe, PBIAS, and R^2 statistics. The predictive capacity of the model was validated in four of the six Hydrological Response Units with agricultural land in the study area: 26, 27, 28, and 31.

Study limitations/implications. The model does not include adaptation measures and future production scenarios based on climate data estimation must be developed.

Findings/conclusions. The influence of climate change on wheat production has been confirmed; the predictive model used is an important tool that can be adjusted and adapted to other regions and production systems.

Keywords: Climate change, farming, wheat, SWAT model.

Citation: Salazar-Solano, V., Moreno-Dena, J. M., Rojas-Rodríguez, I. S., & De La Torre-Valdez, H. C. (2022). Validation of a model to estimate climate effects on wheat (*Triticum aestivum* L.) production in a hydrological basin. *Agro Productividad*. https://doi.org/10.32854/agrop.v15i1.2136

Editor in Chief: Dr. Jorge Cadena Iñiguez

Received: September 27, 2021.
Accepted: January 10, 2022.
Published on-line: February 5, 2022.

Agro Productividad, 15(1). January. 2022. pp: 77-85.

INTRODUCTION

The countries that signed the Paris Agreement in 2016 accepted that "*the fundamental priority of safeguarding food security and ending hunger, and the particular vulnerabilities of food production systems to the adverse effects of climate change*" (Food and Agriculture Organization of the United Nations (FAO) (FAO, 2017)). This commitment endorsed previous scientific positions that warned that the climate, in addition to being an essential determinant of agricultural productivity, was also its main source of risk (Antle, 2008). It was only until the late 20th century, that scientific activity developed models of the economic impact that climatic variations have on food production (Adams, 1989; Kane *et al.*, 1992; Mendelsohn *et al.*, 1994). Subsequently, analysis based on simulation models (Rötter *et al.*, 2018) confirmed the negative impact of climate on crop productivity (Intergovernmental Panel on Climate change (IPCC), 2019). Based on the exploration of tendencies, some

Image by ykaiavu at Pixabay

researchers predict sensitive repercussions in the food sector; Field *et al*. (2014), Huong *et al*. (2019), Mendelsohn *et al*. (2010), and Nelson *et al*. (2018) warn that, in the course of the 21st century, the effects of climate change could restrict: a) economic growth, b) food security, and c) the success of poverty reduction efforts. Given this scenario, it is increasingly important to develop appropriate methodologies that support the adjustment of agricultural activity, depending on the challenges presented by the modification of environmental factors, specifically those related to climate change.

Impact of climate change on wheat production

Since the late 20th century, the argument for the negative effect of climate change on cereal production has been raised; scientists have warned that it will increase over the course of the 21st century (Rosenzweig and Parry, 1994). The most outstanding studies in this field include studies about the production of wheat —a crop that has been a staple food of humanity since the birth of civilization. Currently, 765 million tons of this grain are produced (FAO, 2021), confirming its historical relevance along with corn and rice.

The study carried out by Mereu *et al*. (2021) stands out among the recent studies about the estimation of the impact of climate change on wheat cultivation. They used a simulation model to evaluate the effects of climate change on the production of durum and common wheat in Italy; their findings suggest that yields would decline mainly in southern Italy, while the north would benefit from higher rainfall regimes. In addition to temperature and precipitation, Zhang *et al*. (2017) included the following variables in their analysis: humidity, wind, and solar radiation. They estimated the degree to which these variables influenced wheat productivity. Their results reveal that climate change could negatively impact its production in China by up to 18.26% by the end of this century.

Pequeno *et al*. (2021) and other authors simulated climate change impacts and global adaptation strategies for wheat, using new crop genetic traits —including increased heat tolerance and traits combined with additional nitrogen fertilizer applications—, as an option to maximize genetic gains. Their results predict that climate change will reduce global wheat production by 1.9% by mid-century. Hernández-Ochoa *et al*. (2018) projected wheat production in Mexico for 2050 simulating five climatic environments. All the scenarios reported yields falls, linked to the increase in temperatures; in rare cases, increases in production are explained by rainfall. Despite the vast range of studies on the subject, specific methodological strategies must be explored for each productive region to allow progress in climate change adaptation and mitigation issues. Within these lines of research, this article aims to validate a simulation model that estimates wheat production, based on climatic variables in the lower Mayo River basin in Sonora, Mexico.

MATERIALS AND METHODS

The analysis area is *the lower sub-basin of the Mayo River* (5,397 km^2), part of the Mayo River basin in Sonora, Mexico. It begins in the Adolfo Ruiz Cortines dam and its mouth flows into the Gulf of California (Figure 1). This space concentrates the substantive factors for wheat production, its main crop in terms of extension (62%) and value (46%) (Sistema de Información Agroalimentaria y Pesquera (SIAP), 2021).

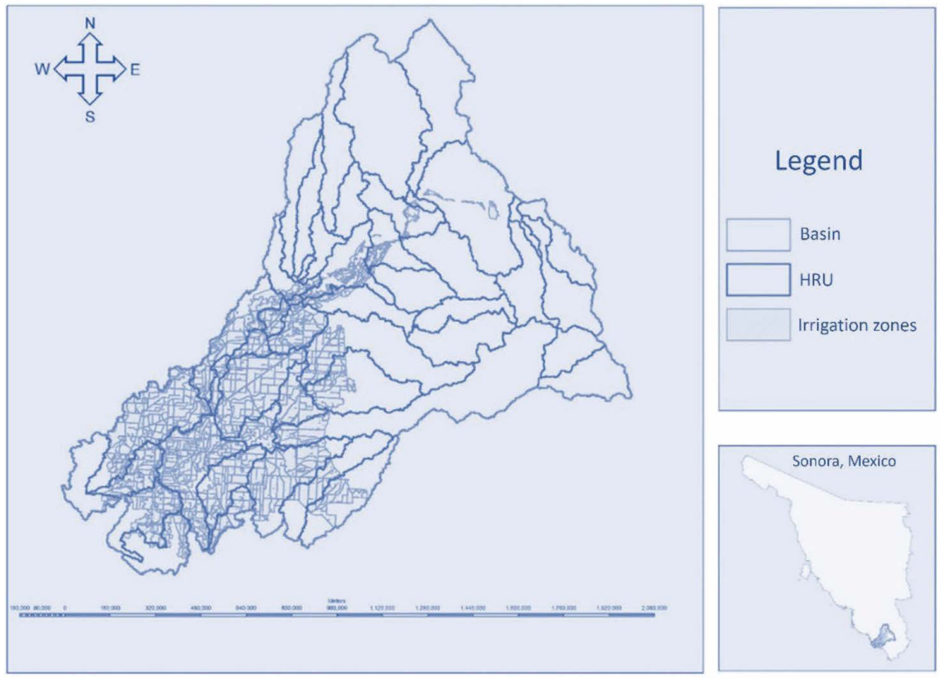

Figure 1. Mayo River Lower Basin.

Description of the applied model

The estimation of the variations in the productive yields of wheat was carried out based on an analysis structure supported by the *Soil and Water Assessment Tool (SWAT) Crop Yield* simulation model. It predicts the impact of soil and water management practices on processes such as sediment generation, erosion, and agricultural yields in a basin complex, with a variety of soils, land use, and management conditions over long periods (Neitsch *et al.*, 2011). In physical terms, SWAT operates at the basin scale in continuous time, establishing the following parameters for daily simulations: hydrology, soil, use, a digital elevation model, and climate data. The model's components include climate, hydrology, soil temperature, plant growth, nutrients, pesticides, and land management. Based on the spatially explicit parameterization, the model divides the territory into sub-basins. In their turn, the sub-basins are divided into hydrological response units (HRUs), according to specific land type and use (Akhavan *et al.*, 2010).

The HRUs represent homogeneous spaces according to the area's soil types and uses, vegetation cover, and slope. The disaggregation of the sub-basin into smaller entities allows the model to reflect differences in evapotranspiration, soil types, vegetation covers, and their generation (Akhavan *et al.*, 2010). In total, 31 HRUs make up the lower Mayo River basin; however, only six match agricultural soils; therefore, they constitute the core points of interest of this research. These are the 23, 26, 27, 28, 30, and 31 HRUs.

The SWAT model uses a digital elevation model (DEM) map to describe the watershed and its Hydrologic Response Units. The Continuo de Elevaciones Mexicano 3.0 (Instituto Nacional de Estadística y Geografía (INEGI), 2021) was used for this purpose.

The land-use maps were obtained from the Portal de Geo información (Comisión Nacional para el Conocimiento y la Biodiversidad (CONABIO) (CONABIO, 2021)). Vector land use and vegetation data sets (scale 1:250,000 - series V) were used to classify the different soil types into hydrological groups, based on infiltration characteristics (CONABIO, 2021).

Cultivation, sowing dates, irrigation, and fertilizer application

Forty *winter wheat* biophysical traits identified by SWAT were considered for wheat yield simulations. Based on the autumn-winter cycle technological package for wheat in Sonora (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), 2017), this crop is established on November 15 of each year and ends on May 2 of the following year. The volumes and dates of application of irrigation and fertilizers also come from the information contained in the above-mentioned technological package.

Weather

The Model was fed with the records of the Climate Forecast System Reanalysis (CFSR), obtained by the National Centers for Environmental Prediction (NCEP) over a 35-year period (1979-2014). In the study area, information was obtained from six weather stations; temperature records (maximum and minimum), precipitation, relative humidity, wind, and solar radiation were monitored daily. The information (including its georeferencing) was incorporated into the database entered into the model.

Model validation

To validate the model, the estimated data of the 23, 26, 27, 28, 30, and 31 HRUs were contrasted with data observed in the Distrito de Desarrollo Rural (DDR) de Navojoa, taking into account the regionalization made by Secretaría de Agricultura y Desarrollo Rural (SADER) for the 1992-2003 period. The six HRUs are located within the DDR territory; however, since there is no accurate production information for each of them, the information will be contrasted with the district's aggregate production data. Validation was performed based on three procedures or statistics:

a. Nash-Sutcliffe efficiency (NSE), which is expressed as:

$$NSE = 1.0 - \sum_{t=1}^{T} \frac{\left(y_t - f_t\right)^2}{\sum_{t=1}^{T}\left(y_t - \bar{y}\right)^2}$$

indicating how well the observed versus simulated data set fits the 1:1 line. It ranges between $-\infty$ and 1, with NSE=1 being the optimal value. Values between 0.0 and 1.0 are generally considered to be acceptable performance levels, while <0.0 values indicate that the mean observed value is a better predictor than the simulated value, indicating unacceptable efficiency (Moriasi *et al.*, 2013).

b. PBIAS, expressed as:

$$PBIAS = \left(\frac{\sum_{t=1}^{T} (f_t - y_t)}{\sum_{t=1}^{T} y_t} \right) * 100$$

where f_t is the simulated value of the model at time t; y_t is the data value observed at time t (t=1, 2,..., T). *PBIAS* measures the average tendency of the simulated data to be larger or smaller than the observed data (Gupta *et al.*, 1999). Small-magnitude *PBIAS* values are preferred. Positive values indicate an overestimation bias of the model and negative values, an underestimation bias (Gupta *et al.*, 1999).

c. R^2, represented by the following equation:

$$R^2 = \left\{ \frac{\sum_{t=1}^{T} (y_t - \bar{y})(f_t - \bar{f})}{\left[\sum_{t=1}^{T} (y_t - \bar{y})^2 \right]^{0.5} \left[\sum_{t=1}^{T} (f_t - \bar{f})^2 \right]^{0.5}} \right\}^2$$

where $\bar{y}$ is the mean of the observed data values for the entire evaluation period and $\bar{f}$ is the mean of the simulated data values for the same period. The other symbols have the same meanings defined for the above equation. The value of R^2 is equal to the square of the Pearson product-moment correlation coefficient (Legates and McCabe Jr., 1999). R^2 has a range from 0.0 to 1.0. The highest values are equivalent to better model performance.

RESULTS AND DISCUSSION

The predictive model of the wheat production behavior generated estimates for six of the 31 HRUs identified in the lower Mayo River basin: 23, 26, 27, 28, 30, and 31. Since the model overestimated the productive yields of wheat, the information was presented as production indexes, which contribute to forecast the behavior of the dependent variable (productive yield), based on the independent variables (specifically, weather) (Table 1).

Table 1. Production index (1992-2003).

Region	Average		Standard deviation	
	Observed	Estimated	observed	Estimated
HRU23	0.9545	1.1483	0.0792	0.2969
HRU26		0.9425		0.0658
HRU27		0.9423		0.0658
HRU28		0.9394		0.0823
HRU30		1.2656		0.3515
HRU31		0.9389		0.0797

Source: Own elaboration.

The first analysis identifies similar behaviors between estimated and observed production in four of the six HRUs analyzed: 26, 27, 28, and 31. In contrast, the 23 and 30 HRUs have divergent results. For validation, three statistics were calculated: Nash-Sutcliffe efficiency, PBIAS (bias), and R^2 (Table 2).

The calculation of these statistics confirmed, by inference, the analysis of the mean and standard deviation. The predictive capacity of the model is validated in the 26, 27, 28, and 31 HRUs. In the case of Nash Sutcliffe, a >0 coefficient is considered acceptable. Meanwhile a <20% BIAS probability is enough, and the closer that R^2 is to unity, the greater the resemblance between the real and observed behavior of the variable. A graph of the similarities can be seen in Figure 2. Once the predictive structure was validated, the influence of climatic variables on wheat production was analyzed. A seven-climatic variable Pearson correlation analysis was carried out for the 1980-2013 period (Table 3), confirming their influence on the behavior of the productive yields of the crop. On the one hand, precipitation and evapotranspiration were directly related to wheat yields: the

Table 2. Statistics used for model validation.

RHU	Nash-Sutcliffe	PBIAS	R^2
HRU23	−77.5	−273.00%	0.2825
HRU26	0.7	12.80%	0.7991
HRU27	0.69	13.10%	0.7995
HRU28	0.53	17.30%	0.5584
HRU30	−201.25	−434.40%	0.6453
HRU31	0.49	18.30%	0.6675

Source: Own elaboration.

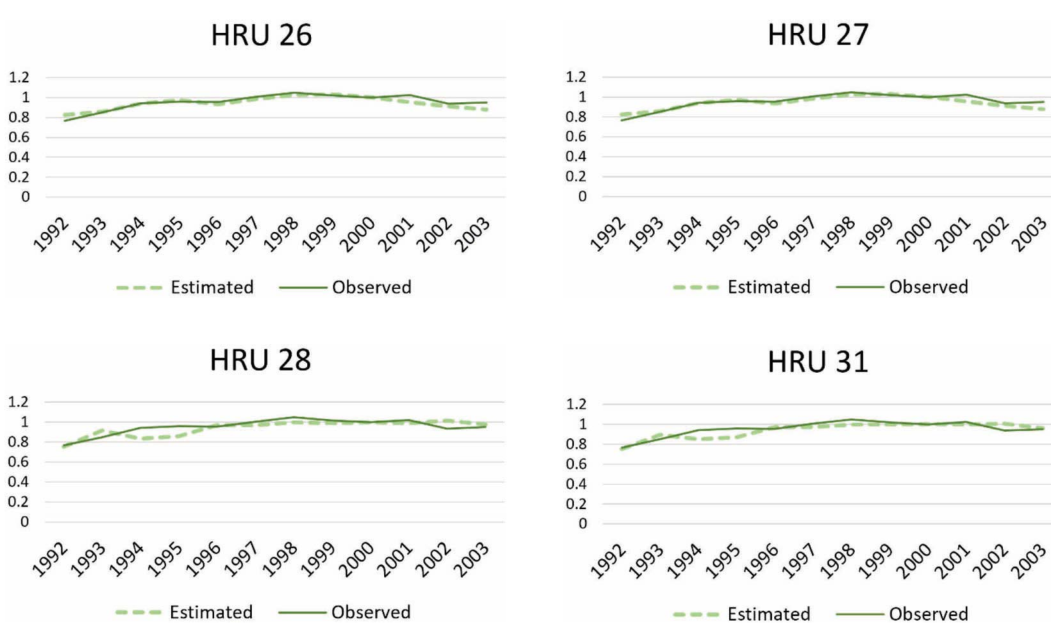

Figure 2. Wheat production index. Selected HRUs.

Table 3. Correlation coefficients between productive yields and climatic variables.

RHU	Annual precipitation	Evapotranspiration	Temperature				Solar radiation (J/m^2)
	(mm)		Annual average	Annual maximun	Annual minimum	soil average	
			(°C)				
HRU23	0.44	0.65	−0.47	−0.53	−0.33	−0.49	−0.46
HRU26	0.27	0.57	−0.31	−0.36	−0.21	−0.58	−0.33
HRU27	0.27	0.57	−0.31	−0.36	−0.21	−0.58	−0.33
HRU28	0.24	0.71	−0.28	−0.32	−0.2	−0.52	−0.28
HRU30	0.39	0.63	−0.48	−0.54	−0.35	−0.5	−0.43
HRU31	0.24	0.71	−0.28	−0.32	−0.2	−0.52	−0.28

Source: This table was developed by the authors.

higher the precipitation and the evapotranspiration level, the higher the yields. On the other hand, the temperature and solar radiation variables show an inverse relationship.

These results are consistent with those obtained by Hernandez-Ochoa *et al.* (2018) and Mereu *et al.* (2021), who highlighted the importance of temperature for the productive yields of wheat. However, the highest correlations in temperature of the 23 and 30 HRUs were not statistically significant; this could be explained by the annual precipitation, which was higher in both cases. These results match the findings of Zhang *et al.* (2017), who determined that other climatic variables (such as evapotranspiration and solar radiation) impact wheat yields. One of the advantages of this model is the ease with which various climatic variables can be incorporated. In contrast, most academic literature mainly focuses on the effects of temperature and precipitation. This work does not consider technological change or adaptation measures; the introduction of this type of strategy helps to mitigate the effects of climate change (Pequeno *et al.*, 2021), hence the importance of its incorporation in the modeling assumptions. However, this does not lessen the depth of our results.

The use of environmental simulation models is a tool to estimate changes in wheat yields, using a structure that —unlike models emanating from conventional economics— takes the environment into consideration, as one of the factors that determines the production of agricultural systems. The influence of the climate on the development of the wheat crop ultimately impacts its yields and profitability. In this sense, an intense generation of scientific knowledge has been motivated by the search for substantive solutions to prevent and remedy the effects of its vulnerability to climate change.

CONCLUSIONS

The influence of climate change on wheat production has been corroborated. As part of these academic efforts, this work validated a procedure that confirms the influence of climate change on the production system. In this process, areas of opportunity were identified to be covered in later stages of the overall research process, involving, among other aspects: a) developing productive yield scenarios, based on the estimation of climatic data; b) strengthening the accuracy of the predictive capacities of the model, in terms of productive yields; c) incorporating the effects of technological change; and d) replicating the use of the model in other agricultural production systems and water spaces.

REFERENCES

Adams, R. M. (1989). Global Climate change and Agriculture: An Economic Perspective. *American Journal of Agricultural Economics, 71*(5), 1272-1279. https://doi.org/10.2307/1243120

Akhavan, S., Abedi-Koupai, J., Mousavi, S.-F., Afyuni, M., Eslamian, S.-S., & Abbaspour, K. C. (2010). Application of SWAT model to investigate nitrate leaching in Hamadan–Bahar Watershed, Iran. *Agriculture, Ecosystems & Environment, 139*(4), 675-688. https://doi.org/10.1016/j.agee.2010.10.015

Antle, J. M. (2008). *Climate change and Agriculture: Economic Impacts*. 3.

Comisión Nacional para el Conocimiento y la Biodiversidad (CONABIO). (2021, marzo 24). *Portal de Información Geográfica*. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO). http://www.conabio.gob.mx/informacion/gis/

Field, C. B., Barros, V. R., & Intergovernmental Panel on Climate change (Eds.). (2014). *Climate change 2014: Impacts, adaptation, and vulnerability: Working Group II contribution to the fifth assessment report of the Intergovernmental Panel on Climate change*. Cambridge University Press.

Gupta, H. V., Sorooshian, S., & Yapo, P. O. (1999). Status of Automatic Calibration for Hydrologic Models: Comparison with Multilevel Expert Calibration. *Journal of Hydrologic Engineering, 4*(2), 135-143. https://doi.org/10.1061/(ASCE)1084-0699(1999)4:2(135)

Hernandez-Ochoa, I. M., Asseng, S., Kassie, B. T., Xiong, W., Robertson, R., Luz Pequeno, D. N., Sonder, K., Reynolds, M., Babar, M. A., Molero Milan, A., & Hoogenboom, G. (2018). Climate change impact on Mexico wheat production. *Agricultural and Forest Meteorology, 263*, 373-387. https://doi.org/10.1016/j.agrformet.2018.09.008

Huong, N. T. L., Bo, Y. S., & Fahad, S. (2019). Economic impact of climate change on agriculture using Ricardian approach: A case of northwest Vietnam. *Journal of the Saudi Society of Agricultural Sciences, 18*(4), 449-457. https://doi.org/10.1016/j.jssas.2018.02.006

Instituto Nacional de Estadística y Geografía (INEGI). (2021). *Datos de relieve*. https://www.inegi.org.mx/app/geo2/elevacionesmex/

Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). (2017). *Paquete tecnológico del cultivo de trigo O.I. 2017-2018*. INIFAP.

Intergovernmental Panel on Climate change (IPCC). (2019). *Special Report on Climate change and Land–IPCC site*. https://www.ipcc.ch/srccl/

Kane, S., Reilly, J., & Tobey, J. (1992). An empirical study of the economic effects of climate change on world agriculture. *Climatic Change, 21*(1), 17-35. https://doi.org/10.1007/BF00143251

Legates, D. R., & McCabe Jr., G. J. (1999). Evaluating the use of "goodness-of-fit" Measures in hydrologic and hydroclimatic model validation. *Water Resources Research, 35*(1), 233-241. https://doi.org/10.1029/1998WR900018

Mendelsohn, R., Arellano-Gonzalez, J., & Christensen, P. (2010). A Ricardian analysis of Mexican farms. *Environment and Development Economics, 15*(2), 153-171. https://doi.org/10.1017/S1355770X09990143

Mendelsohn, R., Nordhaus, W. D., & Shaw, D. (1994). The Impact of Global Warming on Agriculture: A Ricardian Analysis. *The American Economic Review, 84*(4), 753-771.

Mereu, V., Gallo, A., Trabucco, A., Carboni, G., & Spano, D. (2021). Modeling high-resolution climate change impacts on wheat and maize in Italy. *Climate Risk Management, 33*, 100339. https://doi.org/10.1016/j.crm.2021.100339

Moriasi, D. N., Gowda, P. H., Arnold, J. G., Mulla, D. J., Ale, S., & Steiner, J. L. (2013). Modeling the impact of nitrogen fertilizer application and tile drain configuration on nitrate leaching using SWAT. *Agricultural Water Management, 130*, 36-43. https://doi.org/10.1016/j.agwat.2013.08.003

Neitsch, S., Arnold, J., Kinry, J. R., & Williams, J. R. (2011). Soil and water assessment tool theoretical documentation. *Version*.

Nelson, G., Bogard, J., Lividini, K., Arsenault, J., Riley, M., Sulser, T. B., Mason-D'Croz, D., Power, B., Gustafson, D., Herrero, M., Wiebe, K., Cooper, K., Remans, R., & Rosegrant, M. (2018). Income growth and climate change effects on global nutrition security to mid-century. *Nature Sustainability, 1*(12), 773-781. https://doi.org/10.1038/s41893-018-0192-z

Organización de las Naciones Unidad para la Alimentación y la Agricultura (FAO). (2021). FAOSTAT. *Datos sobre alimentación y agricultura*. http://www.fao.org/faostat/es/#home

Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO). (2017). *La estrategia de la FAO sobre el cambio climático* (p. 52). http://www.fao.org/3/i7175s/i7175s.pdf

Pequeno, D. N. L., Hernández-Ochoa, I. M., Reynolds, M., Sonder, K., MoleroMilan, A., Robertson, R. D., Lopes, M. S., Xiong, W., Kropff, M., & Asseng, S. (2021). Climate impact and adaptation to heat and drought stress of regional and global wheat production. *Environmental Research Letters, 16*(5), 054070. https://doi.org/10.1088/1748-9326/abd970

Rosenzweig, C., & Parry, M. L. (1994). Potential impact of climate change on world food supply. *Nature, 367*(6459), 133-138. https://doi.org/10.1038/367133a0

Rötter, R., Hoffmann, M., Koch, M., & Müller, C. (2018). Progress in modelling agricultural impacts of and adaptations to climate change. *Current Opinion in Plant Biology, 45*, 255-261. https://doi.org/10.1016/j.pbi.2018.05.009

Sistema de Información Agroalimentaria y Pesquera (SIAP). (2021). *Producción anual agrícola*. https://nube.siap.gob.mx/cierreagricola/

Zhang, P., Zhang, J., & Chen, M. (2017). Economic impacts of climate change on agriculture: The importance of additional climatic variables other than temperature and precipitation. *Journal of Environmental Economics and Management, 83*, 8-31. https://doi.org/10.1016/j.jeem.2016.12.001

AGRO PRODUCTIVIDAD

Estimation of water erosion in the Necaxa system, Puebla, Mexico

Roblero-Hidalgo, Rodrigo[1]**; Chávez-Morales, Jesús**[2*]**; Ibáñez-Castillo Laura A.**[3]
Palacios-Vélez, Oscar L.[4]

[1] Instituto Mexicano de Tecnología del Agua, Progreso, Jiutepec, Morelos, México, C.P. 62550.
[2] Colegio de Postgraduados, Campus Montecillo, Montecillo, Texcoco, Estado de México, México, C.P. 56230.
[3] Universidad Autónoma Chapingo, Chapingo, Estado de México, México, C.P. 56230.
[4] Colegio de Postgraduados, Campus Montecillo, Montecillo, Texcoco, Estado de México, México, C. P. 56230.
* Correspondence: chavezje@colpos.mx

ABSTRACT

Objective: To estimate the potential and current water erosion in the Necaxa system and to propose alternatives aimed to reduce the siltation problem in the hydraulic infrastructure.

Design/methodology/approach: The Universal Soil Loss Equation (USLE) was applied, using a Geographic Information System to process the map algebra.

Results: The current estimated water erosion was 159 t ha^{-1} per year; this factor impacts the loss of farmland and soil nutrients, the accumulation of matter towards urban areas, the siltation of riverbeds and dams, and the loss of the system's hydraulic works capacity.

Study limitations/implications: No data about runoff plots in the field is available; therefore, it is not possible to compare current water erosion values with those obtained by the USLE applied, using the map algebra technique.

Findings/conclusions: The reforestation and conservation agriculture proposal would help to reduce erosion to 16 t ha^{-1} per year. However, if control actions are not carried out and the current vegetation cover is not preserved, the problem can increase until it reaches potential erosion values >200 t ha^{-1} per year.

Keywords: erosion, basin management, map algebra.

Citation: Roblero-Hidalgo, R., Chávez-Morales, J., Ibáñez-Castillo L. A. & Palacios-Vélez, O. L. (2022). Estimation of water erosion in the Necaxa system, Puebla, Mexico. Agro Productividad. https://doi.org/10.32854/agrop.v15i1.2003

Editor in Chief: Dr. Jorge Cadena Iñiguez

Received: April 23, 2021.
Accepted: January 12, 2022.
Published on-line: February 5, 2022.

Agro Productividad, 15(1). January. 2022. pp: 87-102.

INTRODUCTION

The United States Department of Agriculture (USDA) has been carrying out researches about water erosion and applying soil loss quantification models since the middle of the 19th century; the USDA analyzed the effects of different vegetable covers and crop rotation on the surface runoff and the erosion in experimental runoff plots (Oropeza Mota, 2007).

In 1935, with the creation of the Soil Conservation Services, the USDA started their studies about the mechanics of the erosion processes. These studies included the research about the raindrop impact on bare soil (splash erosion), which enabled the establishment of the first models used to estimate the soil loss caused by water erosion. In Mexico, similar studies started in 1970 by the Área de Física de Suelos of the Colegio de Postgraduados (currently, the postgraduate department of Edaphology). In the basin of the Texcoco River, 50-m^2 experimental plots with different soil uses were established for this purpose (Oropeza Mota, 2007).

In 1958, Wischmeier and Smith (1978) developed the USLE using erosion production mathematical models. The USLE was aimed to predict the average soil loss in agricultural plots, under different farming systems, handling practices, soil types, precipitation patterns, and topography. The USLE application allowed to estimate crop soil loss, soil nutrients loss, drag and accumulation of matter towards urban areas, siltation of riverbeds and dams, and the loss of hydraulic works capacity (Montes-León *et al.*, 2011).

Studies about erosion have been carried out in other countries and regions, including: Argentina (Rodríguez-Vagaría and Gaspari, 2015; Olmos *et al.*, 2008); Spain (Lozano-García and Parras-Alcántara, 2011); Africa (Dumas, 2012); Guatemala (Luna Lemus, 2016); Morocco (Saldaña Días *et al.*, 2014); Venezuela (Pacheco Gil, 2012); Chile (Bonilla *et al.*, 2010; Muñoz-Marcillo *et al.*, 2014; Laval Molkenbuhr, 2009; and Pizarro *et al.*, 2009); Central Greece (Stefanidis *et al.*, 2017); Haiti (Morales Ascarrunz, 2014);and Peru (Jiménez Callejo *et al.*, 2008; Díaz R., 2015; and Portuguez M., 2015).

In Mexico, the main erosion studies have been carried out in the following states: Chihuahua (Alatorre *et al.*, 2014); Veracruz (Melchor-Marroquín and Chagoya-Fuentes, 2016); Tabasco (Río Grijalva basin) (Zavala-Cruz *et al.*, 2011); Durango (López-Santos *et al.*, 2012); Durango, Zacatecas, Sonora, Chiapas, and Tabasco (Flores Islas, 2016); San Luis Potosí (Durán Trejo, 2012); Jalisco (Flores-López *et al.*, 2002; and Torres Benites *et al.*, 2003); Querétaro (Alejandrina *et al.*, 2013); and Estado de México (Pedraza Villafaña, 2015). Montes-León *et al.* (2011) developed a national map of potential erosion in Mexico. The objective of this study is to calculate the potential and current erosion and control alternatives, based on land use change and soil conservation practices in the basins that make up the Necaxa system, in northern Puebla, Mexico.

MATERIALS AND METHODS

The study was carried out in the X hydrological-administrative region (Golfo Centro), 27th Hydrological Region (Tuxpan-Nautla). The following sub-basins were analyzed: the San Marcos River (1,635 km^2) in the Cazones River basin and the Necaxa River (900 km^2) and the Laxaxalpan River (1,608 km^2) in the Tecolutla River basin (Figures 1 and 2).

The following dams can be found in the hydrological system of the study sub-basins: Los Reyes, Laguna, Nexapa, Tenango, and Necaxa (Figure 3).

Collected data

- Digital Elevation Model (DEM), with a 15-m pixel resolution, 1:50,000 scale (INEGI, 2013).
- Edaphological, series II, 1:250,000 scale (INEGI, 2007).
- Land use and vegetation, VI, 1:250,000 scale (INEGI, 2003).
- Average annual precipitation of the Extractor Rápido de Información Climatológica, ERIC III (SMN, 2017).

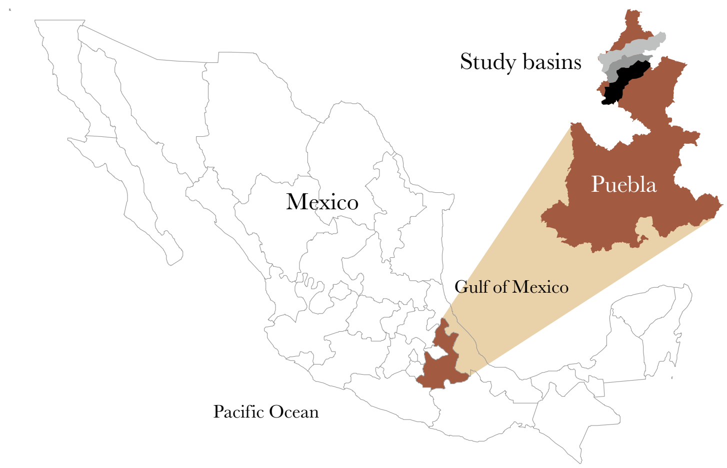

Figure 1. Location of the sub-basins of the San Marcos, Necaxa, and Laxaxalpan rivers.

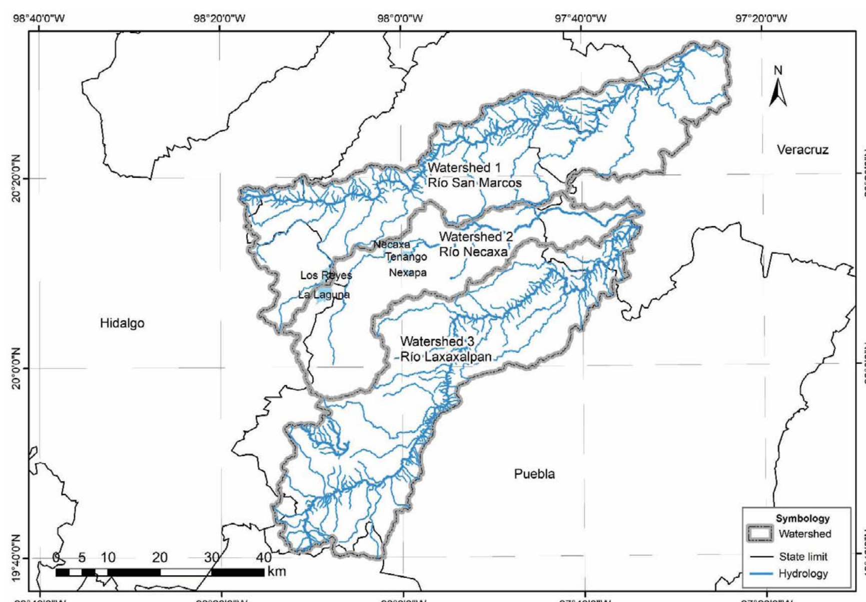

Figure 2. Hydrology of the sub-basins of the San Marcos, Necaxa, and Laxaxalpan rivers.

The USLE proposed by Wischmeier & Smith (1978) was used to determine hydric erosion:

$$A=R*K*L*S*C*P \tag{1}$$

Where: A=Average annual soil loss (ton/ha*year); R=Rainfall erosivity (MJ*mm/ha*h); K=Soil erodibility factor (ton*ha/MJ*mm); L=Slope length gradient factor (Dimensionless); S=Slope inclination gradient factor (Dimensionless); C=Cover Management Factor (Dimensionless); P=Support Practice Factor (Dimensionless).

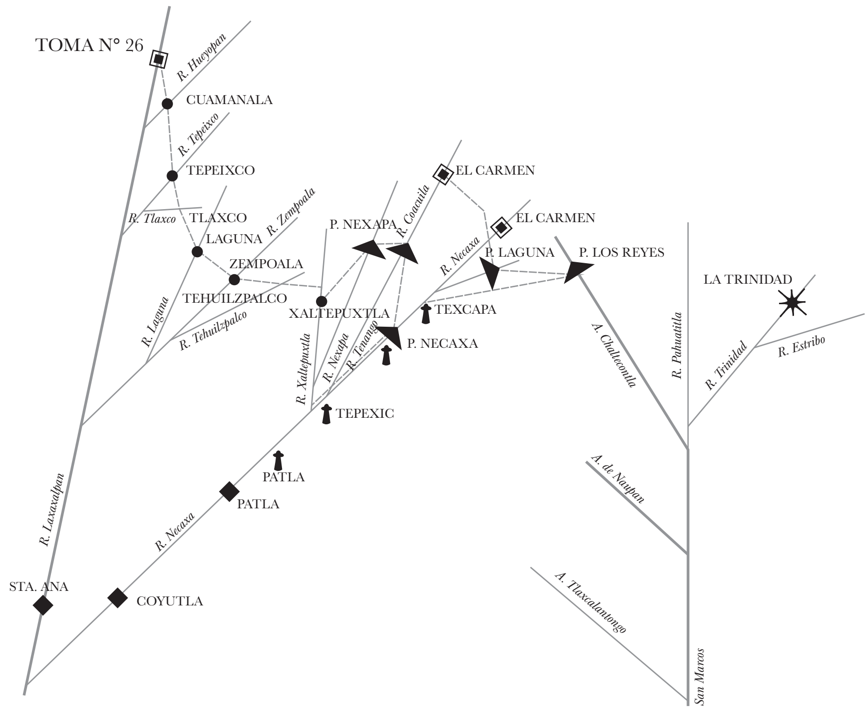

Figure 3. Linear diagram of the dams of the Necaxa system (SRH, 1969).

Rainfall runoff erosivity factor (R)

The R-factor is related to the weather factor and represents the potential energy of the rainfall produced by a storm when it reaches its maximum intensity in 30 m. The method proposed for Mexico by Becerra (2017, quoted by Montes-León *et al.* (2011)) was used, selecting Region 9.

$$R = 7.0458 * P - 0.002096 * P^2 \quad (2)$$

Where: P=is the average annual precipitation (mm).

The average annual precipitation (P) data was obtained from the ERIC III data base, which includes 97 stations within and nearby the study area (SMN, 2017). Forty-four stations met the criteria: 20+ years and 80% of available data.

Using the geographical coordinates (Long. X and Lat. Y) and the P values, a raster model was created for each station, using R-factor interpolated data, from the vectorial factor (*.shp), ArcGIS (ESRI, 2016), Figure 4.

Soil Erodibility Factor (K)

The term "soil erodibility" indicates the susceptibility of a given soil to erosion. The K-factor indicates the said susceptibility degree and is defined based on the characteristics of the soil: texture, organic matter content, structure, and permeability.

The edaphological data of the series II maps (INEGI, 2007) includes the type of soils and textures. Based on this information, the method proposed by the Food and Agriculture

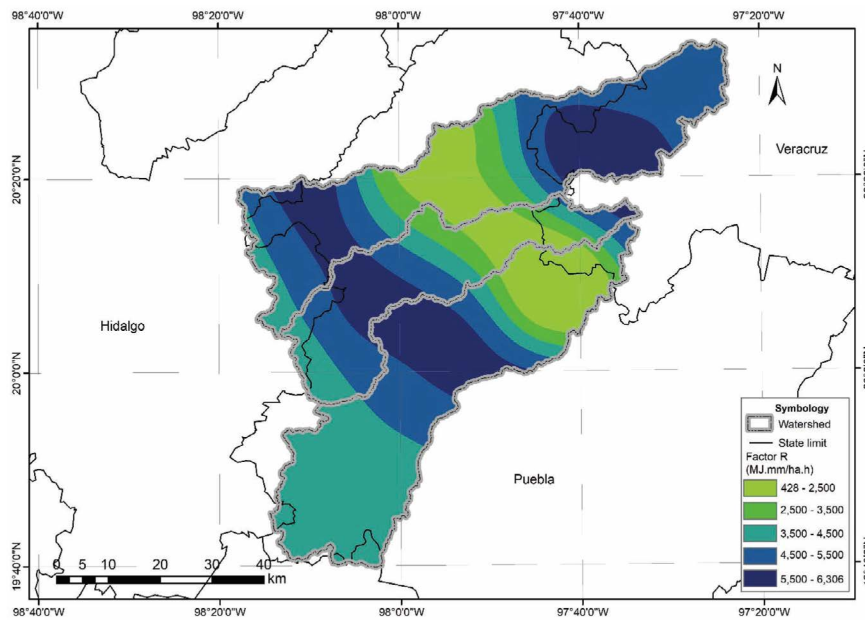

Figure 4. Spatial distribution of the R-factor. The Erosivity values (R) range from 428 to 6,306 MJ*mm/ $ha^{-1}h^{-1}$; the lower values of the analyzed basins can be found in the middle strip.

Organization (FAO) (FAO, 2015) was used to determine the K-factor. The K values (t h^{-1}/ MJ^{-1}mm) were assigned based on this data (presented in a vectorial format (*.shp)). This information can be found in Table 1.

The minimum K values (0.007) match Nitisol and Phaeozem which are fine-textured soil types. The maximum K-factor value (0.079) matches the medium-textured Durisol. Based on this vectorial data (*.shp), a raster model (*.tif) was generated, using the interpolated K values, Figure 5.

Table 1. K-factor values for each type of soil in the study area.

Type of soil	Order	Texture		
		Heavy	Medium	Fine
Acrisol	AC		0.04	0.013
Andosol	AN		0.04	
Cambisol	CM		0.04	
Durisol	DU		0.079	
Fluvisol	FL	0.026		
Kastanozem	KS			0.013
Leptosol	LP		0.02	
Luvisol	LV		0.04	0.013
Nitisol	NT			0.007
Phaeozem	PH		0.02	0.007
Planosol	PL			0.026
Regosol	RG		0.04	
Umbrisol	UM		0.04	
Vertisol	VR			0.026

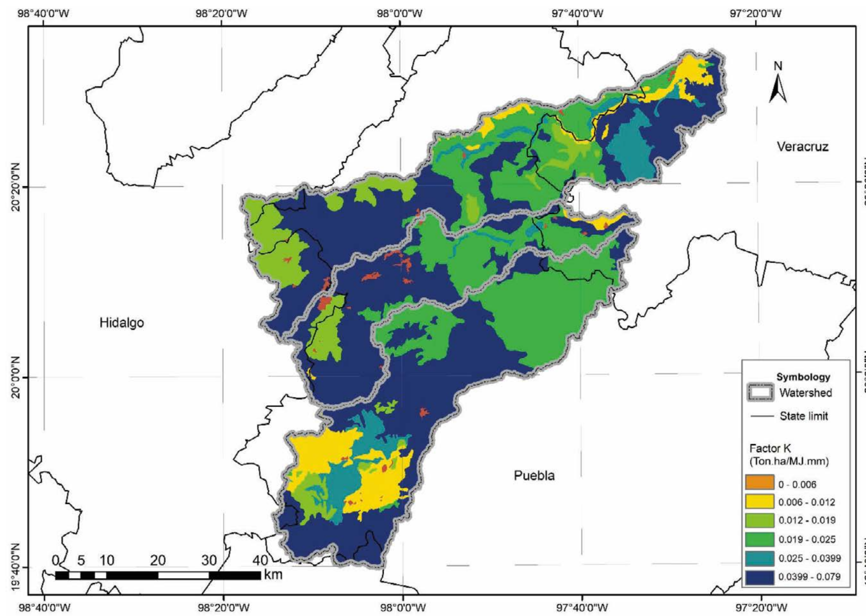

Figure 5. K-factor distribution.

The higher values of K can be found in the high parts of the basins, while the lower values can be found in the low parts (Figure 5).

Slope length gradient factor (L)

This factor is defined as the distance between the point where the runoff originates and any other point where the slope diminishes, promoting sediment deposition; or between the origin point and the point where the runoff water enters in a well-defined channel, which can be part of a stream network or a built interception channel (*e.g.*, a diversion channel). The L-factor is calculated using the Geographic Information System proposed by Desmet and Gover (Luna Lemus, 2016):

$$L_{(i,j)} = \frac{\left[A_{(i,j)} + D^2\right]^{(m+1)} - A_{(i,j)}^{(m+1)}}{x^m * D^{m+2} * \left(22.13^m\right)} \quad (3)$$

Where: L=Slope length gradient factor, dimensionless. i=x-direction index, from i=1, 2, …, limit, number of pixels in x. j=y-direction index, from j=1, 2, …, limit number of pixels in y. A=Unit Contributing Area of each pixel, m^2. D=Side of each pixel, m. x=Form coefficient (x=1 for pixel systems). m=Coefficient dependent on the slope-length interactions, calculated as follows:

$$m = \frac{F}{1+F} \quad (4)$$

F is calculated according to the following formula:

$$F = \frac{\sin\beta / 0.0896}{3*(\sin\beta)^{0.8} + 0.56} \qquad (5)$$

Where: β=Slope at each pixel level (expressed in radians; radians to degrees conversion factor: 0.01745), radians.

The SIG ArcGis software (ESRI, 2016) was used to calculate equations (3), (4), and (5), based on the digital elevation model (DEM).

Based on the visualization of the properties of the raster model of the DEM, the value obtained is D=15.203 m, which matches the length of each pixel.

The value factor $L_{(i,j)}$ was calculated using all these elements.

Slope length gradient factor (S)

The S-factor was calculated using the methodology proposed by Renard (Montes-León *et al.*, 2011), where two conditions on the slope are analyzed. It is expressed as:

$$\tan\beta_{(i,j)} < 0.09 \qquad S_{(i,j)} = 10.8 * \sin\beta_{(i,j)} + 0.03 \qquad (6)$$

$$\tan\beta_{(i,j)} \geq 0.09 \qquad S_{(i,j)} = 16.8 * \sin\beta_{(i,j)} - 0.50 \qquad (7)$$

The DEM and the SIG ArcGis software (ESRI, 2016) were used to obtain the S-factor for the abovementioned conditions; when all the above mentioned conditions are introduced, the layer-factor $S_{(i,j)}$ is obtained.

Subsequently, using the $L_{(i,j)}$ and $S_{(i,j)}$ rasters, the following multiplication was carried out, $L_{(i,j)} * S_{(i,j)}$, Figure 6.

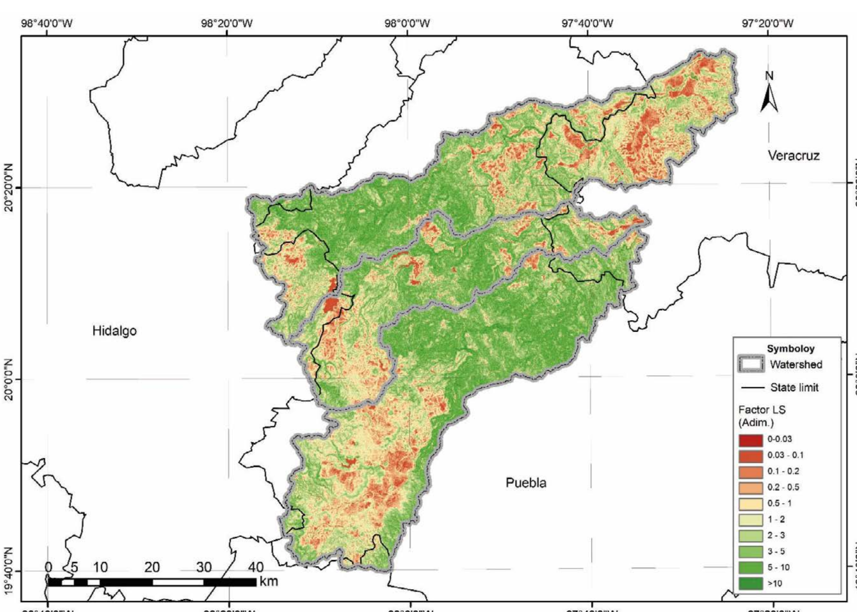

Figure 6. Distribution of the $L_{(i,j)} * S_{(i,j)}$ factors.

The highest LS values were obtained in the central part of the basins, while the lowest values were found at the basin exit, mainly in the sub-basin of the San Marcos River.

Cover Management Factor (C)

The C-factor measures the combined effect of vegetal cover and handling practices on the erosion rates. This factor is the main element to plan soil conservation and to calculate how these covers can eventually impact soil recovery. This factor ranges from 0 to 1 (1: plots without cover; 0: completely covered plots). In order to assign a value to C, the table developed by Montes-León (2011) was used, along with the land use and vegetation vectorial layer, series VI; the vectorial, polygon-shaped file was transformed into a raster format (Figure 7).

The main land use and vegetation that prevails in the sub-basins belong to the annual rainfed agriculture type; vegetation covers 22.3% of the study area, with a C value of C=0.75.

Support Practices Factor (P)

Mechanical practices are frequently used in farmlands that have >2% slopes, to control water erosion and surface runoff, as well as to preserve the soil. The most important mechanical practices are contour furrows, strip cropping, and terraces systems parallel to the contours.

The USLE's P-factor is defined as the ratio of the soil loss under a conservation practice to the soil loss without such conservation practice when the crops are established in the same direction of the slope. The superficial roughness created by tilling, sowing, and farming, as well as other mechanical treatments directed to the level curves seek to reduce erosion. The assessment of the P-factor includes the following support practices: contours

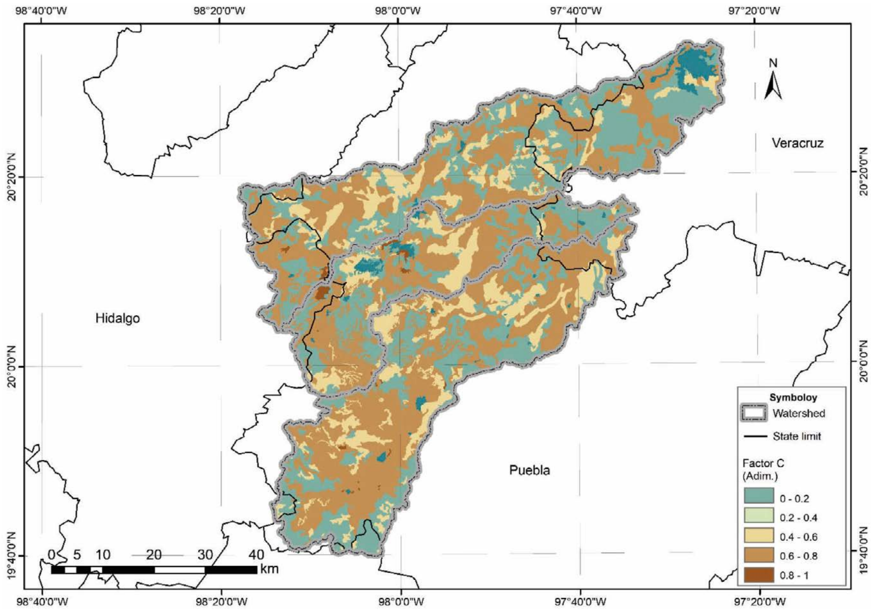

Figure 7. C-factor distribution.

furrows (tilling, sowing, farming), strip cropping, terraces, and superficial drainage. Factor 1 means that no conservation practices were carried out in the area; therefore, mechanical practices do not reduce erosion.

RESULTS AND DISCUSSION

Potential erosion, Ap. The ArcGIS (ESRI, 2016) software was used to process the $Ap=R * K * L * S$ factors data of equation (1) and the product of the Figures 4*5*6, obtaining the potential t ha-1 erosion per year (Figure 8). The prevailing erosion values in the study area exceeded 200 t ha^{-1} per year, distributed in a central strip of the basins.

Current erosion, Aa. Equation 1, $Ap=R * K * L * S * C * P$ (the product of Figures 4*5*6*7) was applied to obtain the current erosion; P=1 was taken into consideration, given that there are no support conservation practices (Figure 9).

When estimating the average potential and current erosion for each sub-basin, we observed that the average erosion would range from 187 to >200 t ha^{-1} per year, if there were no cover in the sub-basins surface (Table 2). According to the classification of Table 2 and compared with the national map developed by Montes-León *et al.* (2011), we can observe that the sub-basins erosion is intense or very high, just like the values reported by UNESCO - PHI (2017). Additionally, Colín-García *et al.* (2013) obtained similar values for a basin influenced by the Gulf area, with losses between 10 and 200 t ha^{-1} per year. We also took into consideration the values obtained by Montes-León *et al.* (2011), which were higher than 200 t ha^{-1}. The highest potential erosion is located in the sub-basins of the middle part of the Necaxa system (Figure 9).

In order to analyze the current erosion of the San Marcos sub-basin, we observed an intense erosion with a soil loss higher than 200 t ha^{-1} per year in an area of 423.08 km^2

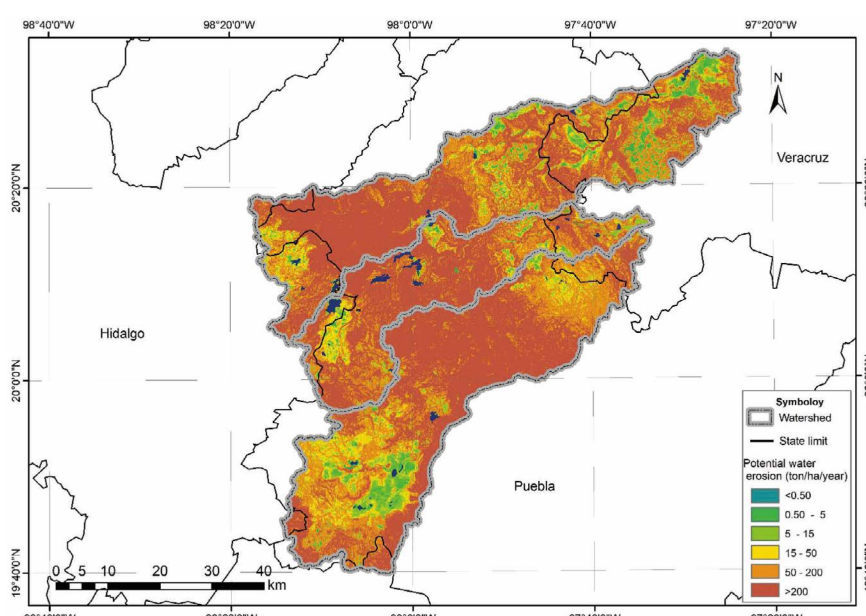

Figure 8. Potential water erosion (ton/ha/year).

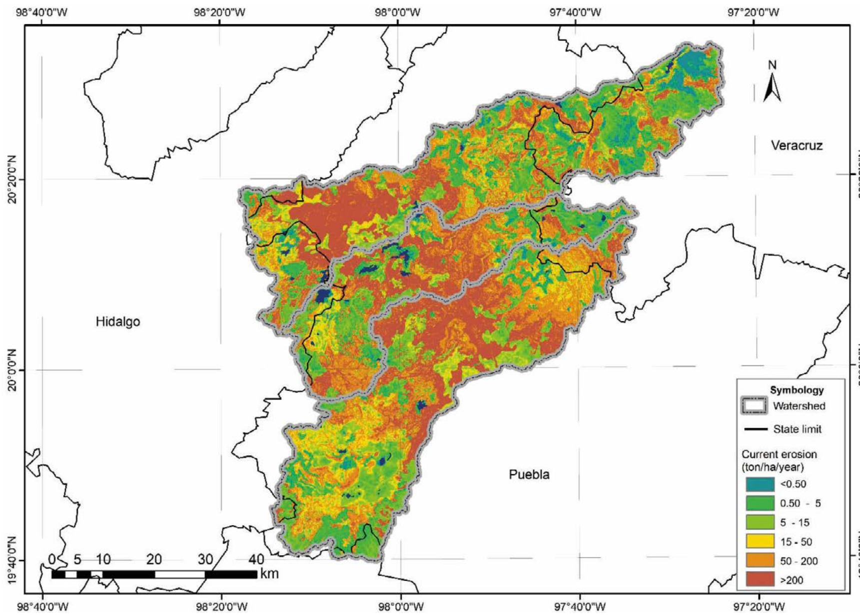

Figure 9. Current erosion (ton/ha/year).

(25.89%). If the current handling remains unmodified, the potential erosion will increase, reaching an area of 852.71 km^2 (52.17%): a surface increase of 26.28% (Table 2).

Table 2. Potential and current erosion of the sub-basins.

Sub-basins	Erosion range t ha^{-1} year	Class	Potential erosion area		Current erosion area	
			km^2	%	km^2	%
Río San Marcos	<0.5	Normal	11.48	0.70	119.39	7.31
	0.5-5	Light	65.07	3.98	343.54	21.02
	5-15	Moderate	64.46	3.94	219.45	13.43
	15-50	Severe	177.91	10.88	211.48	12.94
	50-200	Very severe	462.91	28.32	317.12	19.41
	>200	Intense	852.71	52.17	423.08	25.89
Río Necaxa	<0.5	Normal	20.22	2.25	47.64	5.29
	0.5-5	Light	16.32	1.81	160.01	17.77
	5-15	Moderate	23.72	2.63	113.46	12.60
	15-50	Severe	78.26	8.69	95.59	10.62
	50-200	Very severe	237.37	26.36	217.00	24.10
	>200	Intense	524.45	58.25	266.55	29.61
Río Laxaxalpan	<0.5	Normal	6.99	0.43	32.90	2.05
	0.5-5	Light	36.26	2.26	243.01	15.12
	5-15	Moderate	68.87	4.28	268.19	16.68
	15-50	Severe	183.85	11.43	258.34	16.07
	50-200	Very severe	458.52	28.52	385.74	24.00
	>200	Intense	853.41	53.08	419.36	26.09

In order to analyze the current erosion of the Necaxa River sub-basin, we observed an intense erosion with a soil loss higher than 200 t ha^{-1} per year in an area of 266.55 km^2 (29.61%). If the current handling remains unmodified, the potential erosion will increase, reaching an area of 524.45 km^2 (58.25%): a surface increase of 28.64% (Table 2).

Finally, in order to analyze the current erosion of the Laxaxalpan River sub-basin, we observed an intense erosion with a soil loss higher than 200 t ha^{-1} per year in an area of 419.36 km^2 (26.09%). If the current handling remains unmodified, the potential erosion will increase, reaching an area of 853.41 km^2 (53.08%): a surface increase of 26.99% (Table 2).

The Necaxa system does not include all the sub-basins (Figure 8 and 9); therefore, the solution proposed only apply to those sub-basins located in the north side, not to the whole system (Figures 10 and 11). Figure 11 shows that the greatest surface of current erosion is located in the low parts of the basin, as a result of the topographic slopes and the rainfed agriculture areas.

After calculating the current erosion of the San Marcos sub-basin of the Necaxa system, we observed an intense erosion with a soil loss higher than 200 t ha^{-1} per year in an area of 18.93 km^2 (33.16%). If the current handling remains unmodified, the potential erosion will increase, reaching an area of 43.69 km^2 (76.48%): a surface increase of 43.32% (Table 3).

Regarding the current erosion of the Necaxa River sub-basin of the Necaxa system, we observed an intense erosion with a soil loss higher than 200 t ha^{-1} per year in an area of 120.75 km^2 (27.07%). If the current handling remains unmodified, the potential erosion will increase, reaching an area of 274.00 km^2 (61.42%): a surface increase of 34.35% (Table 3).

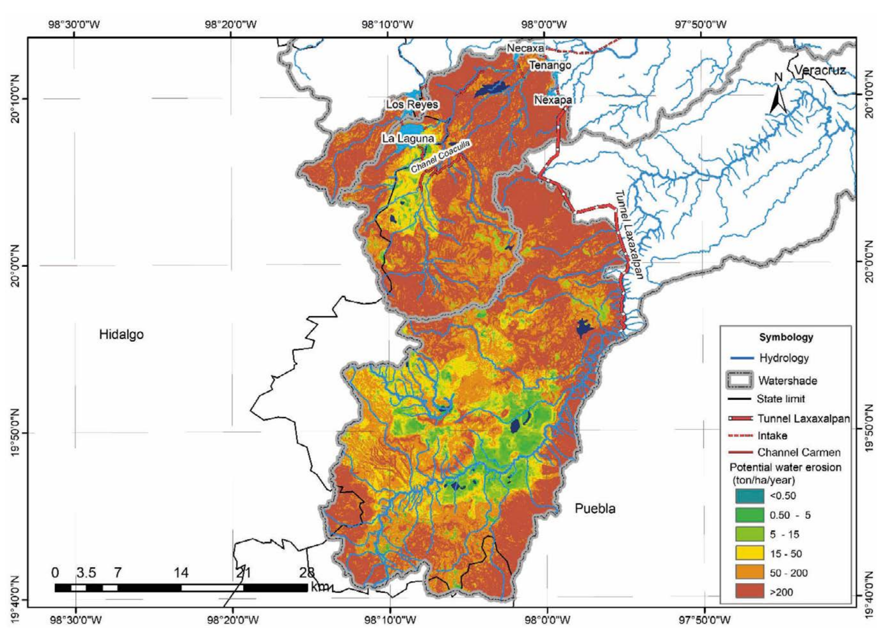

Figure 10. Potential erosion in the Necaxa system.

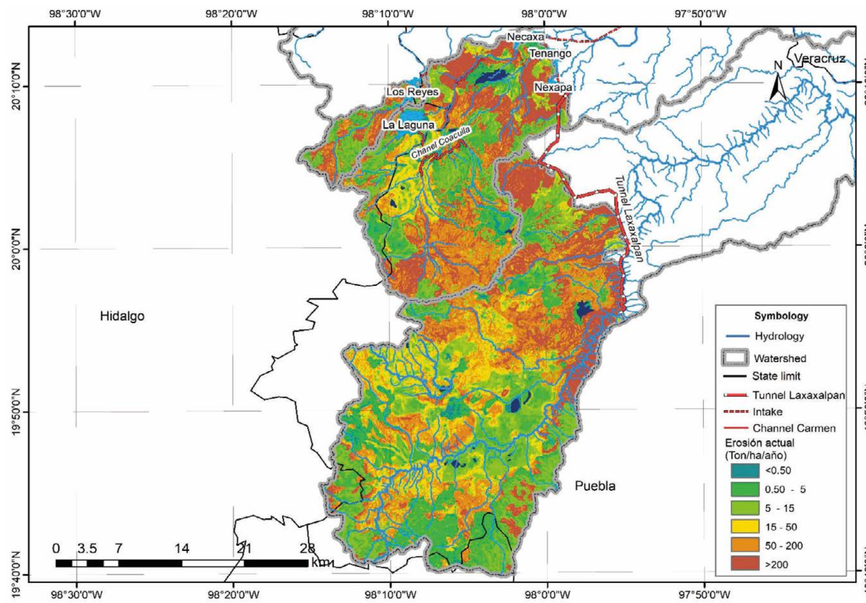

Figure 11. Current erosion in the Necaxa system.

Finally, regarding the current erosion of the Laxaxalpan River sub-basin of the Necaxa system, we observed an intense erosion with a soil loss ranging from 50 to 200 t ha^{-1} per year in an area of 190.91 km^2 (21.40%). If the current handling remains unmodified, the

Table 3. Potential and current erosion in the sub-basins of the Necaxa system.

Sub-basins	Erosion range t ha^{-1} year	Class	Potential erosion area		Current erosion area	
			km^2	%	km^2	%
Río San Marcos	<0.5	Normal	0.25	0.43	0.56	0.98
	0.5-5	Light	0.01	0.02	9.92	17.37
	5-15	Moderate	0.56	0.98	13.86	24.28
	15-50	Severe	0.63	1.09	1.40	2.46
	50-200	Very severe	12.00	21.00	12.42	21.76
	>200	Intense	43.69	76.48	18.93	33.16
Río Necaxa	<0.5	Normal	11.07	2.48	19.43	4.36
	0.5-5	Light	5.69	1.28	81.67	18.31
	5-15	Moderate	10.33	2.32	76.27	17.10
	15-50	Severe	36.78	8.24	52.19	11.70
	50-200	Very severe	108.24	24.26	95.74	21.46
	>200	Intense	274.00	61.42	120.75	27.07
Río Laxaxalpan	<0.5	Normal	5.95	0.67	21.52	2.41
	0.5-5	Light	31.92	3.58	180.05	20.18
	5-15	Moderate	59.67	6.69	181.91	20.39
	15-50	Severe	135.42	15.18	160.34	17.97
	50-200	Very severe	277.14	31.06	190.91	21.40
	>200	Intense	382.16	42.83	157.34	17.64

potential erosion will increase, reaching an area of 382.16 km^2 (21.43%): a surface increase of 26.99% (Table 3).

Overall, the Necaxa system reports a current average erosion of 159 t ha^{-1} per year, which indicates an erosion increase of 183%, as a result of the lack of appropriate soil surface and vegetation handling. Unless appropriate handling activities are carried out in the basin, the siltation of the dams will continue.

As an alternative solution to the siltation problems of the water bodies and the hydraulic infrastructure, actions that strengthen the protection of natural resources must be proposed. These measures must be based on modifications to the C- and P- parameters, through reforestation measures and mechanical agricultural practices (Figure 12).

A conservation proposal for the edaphic resources of the Necaxa River system includes reforesting 214 km^2 of pine-oak forests in zones that currently hold secondary vegetation. Additionally, agricultural management practices based on conservation agriculture have been proposed: leaving approximately 30% of the harvest waste on the soil surface, planting crops on the contour of the plot, and implementing terraces. All these measures should be carried out on of the three sub-basins (704 km^2) of the Necaxa system under study, consequently allowing a decrease in the erosion problem (Table 4).

A reduction of the moderate erosion has been reported as a result of the measures proposed for the San Marcos sub-basin of the Necaxa System: 5-15 ha^{-1} per year in a 22.34 km^2 area (39.15%). Meanwhile, a reduction of the light erosion has been reported as a result of the measures proposed for the Necaxa sub-basin of the Necaxa system: 0.5-5 ha^{-1} per year in a 163.74 km^2 area (36.72%).

Finally, reduction of the light erosion has been reported as a result of the measures proposed for the Laxaxalpan sub-basin of the Necaxa system: 0.5-5 ha^{-1} per year in a

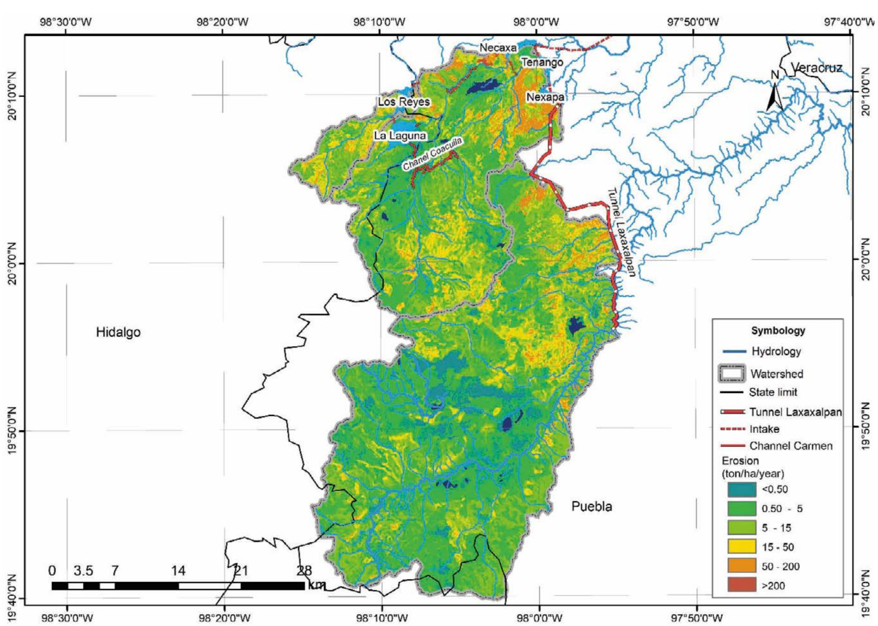

Figure 12. Solution option based on basin management practices.

Table 4. Solution options to avoid an increase in the siltation of the Necaxa system.

Sub-basins	Erosion range t ha^{-1} por año	Class	Area	
			km^2	%
Río San Marcos	<0.5	Normal	0.90	1.57
	0.5-5	Light	10.88	19.07
	5-15	Moderate	22.34	39.15
	15-50	Severe	18.42	32.28
	50-200	Very severe	4.36	7.64
	>200	Intense	0.17	0.29
Río Necaxa	<0.5	Normal	30.50	6.84
	0.5-5	Light	163.74	36.72
	5-15	Moderate	137.94	30.93
	15-50	Severe	73.99	16.59
	50-200	Very severe	36.48	8.18
	>200	Intense	3.32	0.74
Río Laxaxalpan	<0.5	Normal	98.50	11.05
	0.5-5	Light	413.72	46.39
	5-15	Moderate	254.47	28.53
	15-50	Severe	94.71	10.62
	50-200	Very severe	28.98	3.25
	>200	Intense	1.40	0.16

413.72 km^2 area (46.39%). The proposed measures aim to achieve a tenfold reduction of erosion with regard to the current situation: *i.e.*, reducing the current 159 ton/ha*year erosion to an average of 0.5 (light) to 15 (moderate) t ha^{-1} per year. This measure would guarantee a longer useful life for the Necaxa system.

CONCLUSIONS

This study about the current and potential erosion shows that reforestation measures and conservation agricultural practices would cut down erosion in the Necaxa system from the current annual average of 159 t ha^{-1} to 0.5-15 t ha^{-1} per year. This study also proposes possible solutions based on the basin management, applying the USLE through the Geographical Information Systems. Otherwise, if no action is carried out, erosion could exceed 200 t ha^{-1} per year, which would worsen siltation problems in water bodies and infrastructure; additionally, soils will lose their productivity and the ecosystem will face adverse changes.

REFERENCES

África Dumas, S. (2012). Riesgo de erosión hídrica en la cuenca hidrográfica del río Mundo. La Universidad Complutense de Madrid.

Alatorre, L. C., García, A. K., Rodríguez, A. J., Erives, V., & González, E. (2014). Estimación de la erosión potencial en la cuenca de la Laguna Bustillos, Chihuahua, México. *In*: Garcia Ruiz, J.M., Geoecología, cambio ambiental y paisaje, 249–258. Consejo Superior de Investigaciones Científicas, Ernestina M. del Puerto, Santa Cecilia, Tláhuac, 13010 Ciudad de México, CDMX.

Alejandrina, M., León, M., Domínguez, M. A., & Ventura, E. (2013). Metodología para la estimación del riesgo de erosión hídrica en cuencas hidrográficas utilizando un SIG. Maestría en Hidráulica. Facultad de Ingeniería-División del Posgrado, UniversidadAutonoma de Queretaro, C.U: Cerro de las Campanas, Queretaro, Qro. 76010

Bonilla, C. A., Reyes, J. L., & Magri, A. (2010). Water erosion prediction using the revised universal soil loss equation (RUSLE) in a gis framework , central Chile. *Chilean journal of agricultural research, 70*(1), 159–169. Doi: 10.4067/S0718-58392010000100017.

Colín-García, G., Ibáñez-Castillo, L. A., Reyes-Sánchez, J., & Arteaga-Ramírez, R. (2013). Diagnóstico de La Erosión Hídrica de la Cuenca del Río Pichucalco. *Ingeniería Agrícola y Biosistemas, 5*(1), 23–31. Doi: 10.5154/r.inagbi.2013.04.002

Díaz R, J. (2015). Diagnóstico del potencial de erosión hídrica mediante técnicas de geoprocesamiento en la sub-cuenca del río Angasmarca, La Libertad, Perú. *Anales Científicos, 76*(2), 283–293. Doi: 10.21704/ac.v76i2.793

Durán Trejo, S. (2012). Riesgo a la erosión hídrica y práctica de manejo de suelos en la microcuenca La Concodia, Armadillo de los Infantes, S.L.P. Universidad Autónoma de San Luis Potosí.

ESRI. (2016). ArcGIS Desktop: Release 10.4. En Redlands CA. Environmental Systems Resource Institute.

FAO, O. de las N. U. para la A. y la A. (2015). Base referencial mundial del recurso suelo 2014. Sistema internacional de clasificación de suelos para la nomenclatura de suelos y la creación de leyendas de mapas de suelos (FAO, Organ). publications-sales@fao.org.

Flores-López, H. E., Martínez-Menes, M., Oropeza-Mota, J. L., Mejía-Saenz, E., & Carrillo-González, R. (2002). Integración de la EUPS a un SIG para estimar la erosión hídrica del suelo en una cuenca hidrográfica de Tepatitlán, Jalisco, México. *Terra Latinoamericana E-ISSN:21*, 233–244.

Flores Islas, E. (2016). Estimación de la pérdida de suelo por erosión hídrica en tres subcuencas de la República Mexicana, utilizando la ecuación universal de pérdida de suelo. Universidad Nacional Autónoma de México.

INEGI. (2013). Continuo de Elevaciones Mexicano 3.0 (CEM 3.0). Instituto Nacional de Estadística y Geografía. Disponible en: http://www.inegi.org.mx/geo/contenidos/datosrelieve/continental/continuoelevaciones.aspx

INEGI, I. N. de E. y G. (2003). Conjunto de datos vectoriales de la carta de Uso de Suelo y Vegetación. Escala 1:250,000. Serie III. Instituto Nacional de Estadística y Geografía.

INEGI, I. N. de E. y G. (2007). Conjunto de datos vectoriales Edafológicos. Escala 1:250,000. Serie II (Continuo Nacional). Instituto Nacional de Estadística y Geografía.

Jiménez Callejo, L., Martínez De Anguita, P., Ángeles Ruiz, M., Dunin-borkowski, S. M., & Guerrero, D. (2008). Metodología para la zonificación del riesgo de erosión en cuencas Andinas. Estudio de caso en el río Chalaco, Piura. Uned. *Espacio, Tiempo y Forma, VI* (1), 11–32. Doi: 10.5944/etfvi.1.2008.1456

Laval Molkenbuhr, E. (2009). Determinación de erosión actual y fragilidad de suelos en la V Región utilizando datos satelitales y SIG.

López-Santos, A., Sánchez Cohen, I., Esquivel Arriaga, G., & González Barrios, J. L. (2012). Evaluación de la vulnerabilidad de los suelos del estado de Durango, México en el contexto del cambio climático. *Agrociencia, 16*(3), 117–127.

Lozano-García, B., & Parras-Alcántara, L. (2011). Erosión actual y potencial en suelos ácidos del sur de España. *Terra Latinoamericana, 29*(1), 35–46.

Luna Lemus, M. F. (2016). Determinación de la erosión hídrica y la producción de sedimentos en la cuenca del río Pixcayá aplicando herramientas SIG, Tesis de maestria. Universidad del Valle de Guatemala. 18 Av. 11-95 Guatemala, 01015, Guatemala. 105–117.

Melchor-Marroquín, J. I., & Chagoya-Fuentes, J. L. (2016). Diagnóstico de la erosión hídrica en la cuenca del río Tuxpan, Veracruz, México. Aqua-LAC, 8(2), 25–35. Doi: 10.29104/phi-aqualac/2016-v8-2-03

Montes-león, M. A. L., Uribe-Alcántara, E. M., & García-Celis, E. (2011). Mapa Nacional de Erosión Potencial. *Tecnología y Ciencias del Agua, 2*(1), 5–17.

Muñoz-Marcillo, J. L., Morante-Carriel, J., & Miranda-Ullón, P. (2014). Erosión potencial por reconversión productiva en subcuenca Llay-Llay, Chile. Aplicación de unidades de respuesta a la erosión. *Ciencia y Tecnología, 7*(2), 35–47.

Olmos, L. A., Spalleti, P., Farias, H. D., & Pilán, M. T. (2008). Verificación de modelos de producción de sedimentos en cuencas del N.O. Argentino. *Ingeniería del Agua, 15*(2), 1134–2196. Doi: 10.4995/ia.2008.2926

Pacheco Gil, H. A. (2012). El índice de erosión potencial en la vertiente norte del Waraira Repano, estado Vargas, Venezuela. *Revista colombiana de geografía. 21*(2), 85–97.

Pedraza Villafaña, A. (2015). Estimación de la erosión hídrica mediante dos métodos de la Ecuación Universal de Pérdida de Suelo (EUPS) en la cuenca del río Chapingo, Texcoco. Universidad Autónoma del Estado de México.

Pizarro, R., Moralez, C., Vega, L., Olivares, C., Rodrigo, V., & Balocchi, F. (2009). Propuesta de un modelo de estimación de la erosión hídrica para la región de Coquimbo, Chile (Documentos Técnicos del PHI-LAC, No. 18).

Portuguez M., D. M. (2015). Estimación de la pérdida de suelos por erosión hídrica en la cuenca del río Siguas utilizando geoinformática. *Anales Científicos, 76*(2), 324–329. Doi: 10.21704/ac.v76i2.797

Rodríguez-Vagaría, A., & Gaspari, F. (2015). Estimación de la admisibilidad de pérdidas de suelo por erosión hídrica en la cuenca del arroyo Napaleofú , provincia de Buenos Aires-Argentina. *Revista Geográfica Venezolana, 56*(1), 105–119.

Saldaña Días, M. M., Nemmaoui, A., Cantón Castilla, M. Y., Aguilar Torres, M. A., & Aguilar Torres, F. A. (2014). Estimación de la erosión potencial en la cuenca del río Moulouya aguas arriba de la presa Mohamed V. *Mapping, 23*(168), 4–16.

SMN. (2017). Estaciones Climatológicas 2016. Servicio Meteorológico Nacional. Disponible en: http://smn.cna.gob.mx/tools/RESOURCES/estacion/EstacionesClimatologicas.kmz

SRH. (1969). Boletín hidrológico 42. Región hidrológica 27 (Tuxpan-Nautla). Disponible en: http://hidrosuperf.imta.mx/pronacch/Boletines/42/tomo 1/Boletín 42 Tomo 1.pdf

Stefanidis, S., Chatzichristaki, C., & Stefanidis, P. (2017). Development of a GIS toolbox for automated estimation of soil erosion. *Environmental Impact Assessment and Risk Analysis*, June, 665–673.

Torres Benites, E., Cortes Becerra, J., Mejía Sáenz, E., Exebio García, A., Santos Hernández, A. L., & Delgadillo Piñón, M. E. (2003). Evaluación de la degradación de los suelos en la cuenca “El Josefino”, Jesús María, Jalisco. *Terra Latinoamericana, 21*(1), 117–126.

UNESCO - PHI. (2017). Evaluación de la degradación de suelos en el estado de puebla, México. En Procesos de erosión. Sedimentación en cauces y cuencas. (Número III, pp. 80–96). United Nations Educational, Scientific and Cultural Organization. Disponible en: http://eias.utalca.cl/isi/publicaciones/erosion_y_sedimentacion_vol1.pdf

Wischmeier, W. H., & Smith, D. D. (1978). Predicting rainfall erosion losses a guide to conservation planning (Número 537). *U.S. Department of Agriculture, Agriculture Handbook* no. 537.

Zavala-Cruz, J., Palma-López, D. J., Fernández Cabrera, C. R., López Castañeda, A., & Shirma Tórres, E. (2011). Degradación y conservación de suelos en la cuenca del río Grijalva, Tabasco. Colegio de Postgraduados, Secretaría de Recursos Naturales y Protección Ambiental y PEMEX.

AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Genetic improvement and its effect on the genetic diversity of habanero chili (*Capsicum chinense* Jacq.)

Castillo-Aguilar, Crescencio C.[1]**; Chiquini-Medina Ramón A.**[2]**; Chiquini-Medina Ricardo A.**[3*]

[1] Colegio de Postgraduados, Campus Campeche, Sihochac, Champotón, Mexico, C.P.24050.
[2] Centro de Bachillerato Tecnológico Agropecuario Núm. 169, Hecelchakán, Campeche, México, C.P. 24800.
[3] Tecnológico Nacional de México/Instituto Tecnológico de Chiná, Campeche, Campeche, México, C.P. 24520.
* Correspondence: ricardochiquini@yahoo.com.mx

ABSTRACT

Objective: To determine the impact of genetic improvement on the maintenance and preservation of the genetic diversity of habanero chili (*Capsicum chinense* Jacq.).

Methodology: We reviewed topics related to the genetic improvement and diversity of habanero chili. We included historic reviews, as well as interviews with plant breeders and nursery growers of the Yucatán Peninsula. The information thus obtained was classified, analyzed, and discussed.

Results: The introduction and use of hybrids and varieties is affecting the genetic diversity of habanero chili, restricting the cultivation of local varieties and, therefore, the use of the Designation of Origin.

Conclusions: Regional genetic improvement is needed to preserve genetic diversity.

Key words: Improvement, Diversity, Varieties, Native.

Citation: Castillo-Aguilar, C. C., Chiquini-Medina R. A. & Chiquini-Medina R. A. (2022). Genetic improvement and its effect on the genetic diversity of habanero chili (*Capsicum chinense* Jacq.). *Agro Productividad*. https://doi.org/10.32854/agrop.v15i1.2022

Editor in Chief: Dr. Jorge Cadena Iñiguez

Received: March 3, 2021.
Accepted: December 28, 2021.
Published on-line: February 5, 2022.

Agro Productividad, 15(1). January. 2022. pp: 103-109.

INTRODUCTION

Considered a genetic pool center for habanero chili (*Capsicum chinense* Jacq.), the Yucatán peninsula in Mexico produces around 3,500 tons of fruit per year, 85% of which is sold fresh and 15% for industrial use (López-Espinoza *et al.*, 2018). The Yucatán peninsula is characterized by the genetic diversity of habanero chili, which is expressed in the different forms, sizes, colors, tastes, and pungency of native varieties. There are several ways to characterize the variability of chili: phenotypically (Latournerie *et al.*, 2002; Trujillo-Aguirre and Pérez-Yanes, 2005; Canto-Flick, 2007); isoenzymatically (Corona-Torres *et al.*, 2000); agronomically (Latournerie-Moreno *et al.*, 2015); biochemically (Coutinho *et al.*, 2015; Guzman *et al.*, 2010); and molecularly (López-Espinoza *et al.*, 2018).

Despite various efforts, the genetic diversity of chili is endangered, among other things, as a consequence of the introduction of uncertified seeds, which in many cases are hybrids and may scatter in the peninsular region and reproduce as local genetic materials. In

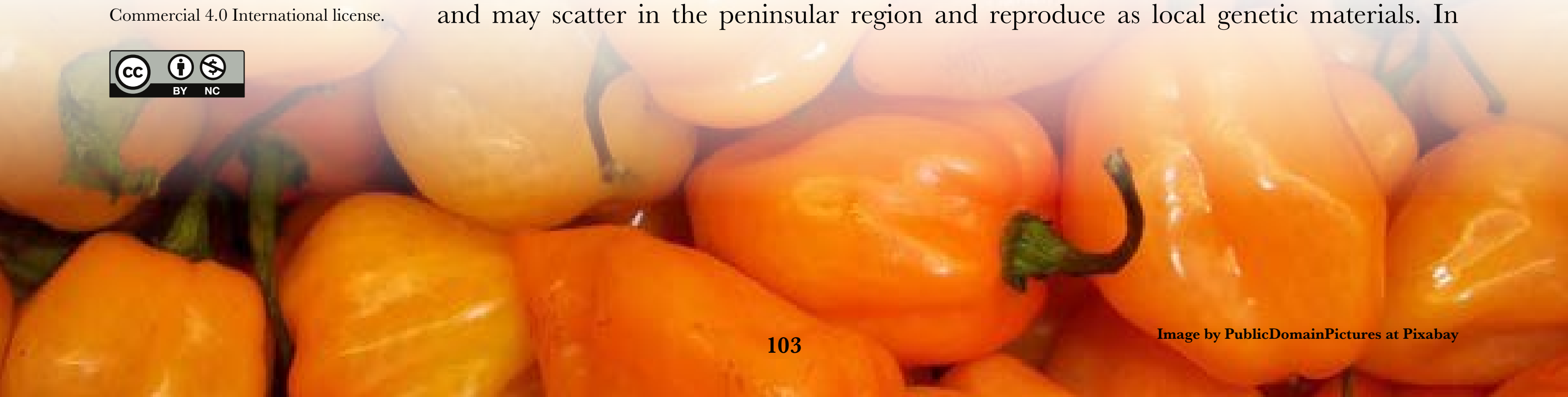

Image by PublicDomainPictures at Pixabay addition to this problem, there are no genetic improvement programs that contribute to preserve the genetic resources of habanero chili. Therefore, the objective of this article was to document the impact of genetic improvement and the cultivation of improved habanero chili hybrids and varieties on the maintenance and preservation of its genetic diversity.

MATERIALS AND METHODS

The information contained in this work is based on the review of bibliographic information regarding the genetic improvement and diversity of habanero chili (*Capsicum chinense* Jacq). We took into account articles, books, and phone interviews with people involved in the cultivation of this species. We interviewed the main habanero chili plant growers of the peninsula, who supply approximately 70% of the demand of plants for cultivation: Carlos Burgos of the Invernaderos Ma'Alob Pakal nursery (Tenabo, Campeche); Luis Domínguez Pacheco of the La Nueva Era nursery (Hopelchén, Campeche); Ricardo Julián Cutz of the Viveros Cutz nursery (Suma, Yucatán); and Miguel Ángel Barbosa López of the Vivero Barbosa nursery (Muna, Yucatán). The information for the State of Quintana Roo was provided by the ABC *Capsicum* company via phone and e-mail. Additionally, plant breeder José Jorge Gerardo Trujillo Aguirre (MSc) provided a historic account on the genetic improvement of habanero chili.

RESULTS AND DISCUSSION

The production of habanero chili in the Yucatán peninsula started with the exploitation of small areas called *mecates* (20 m×20 m), whose produce was sold locally. The improvement of communication routes and the market's demand for the chili fruit resulted in its intensive exploitation. The fruit of the habanero chili plant is trilocular, top-shaped, and orange-colored when ripe, and has a characteristic smell and burning taste (Trujillo-Aguirre, 2021). Between 1975 and 1982, the Instituto Nacional de Investigaciones Agrícolas (INIA) —now Instituto Nacional de Investigaciones Agrícolas Pecuarias y Forestales (INIFAP)— worked to obtain habanero chili varieties, an activity it resumed in 1992. On a regional level, the Instituto Tecnológico Agropecuario (ITA) of Conkal and the ITA of Chiná, in Campeche, only had research papers and dissertations. In 2002, the Centro de Investigación Científica de Yucatán (CICY) brought a group of researchers together to conduct different types of studies about habanero chili.

The INIA, founded in 1960, started its habanero chili genetic improvement program with a series of harvests of native habanero, according to its taste, color, smell, and pungency in Campeche, Yucatán, Quintana Roo, and Belize (1965-1975). As an output, the INIA edited and published the technical brochure No. 1 SARH-INIA, 1981, under the title "Habanero INIA y Habanero Uxmal, Nuevas Variedades de Chile para la Península de Yucatán" (INIA Habanero and Uxmal Habanero, New Varieties of Chili for the Yucatán Peninsula). This brochure mentions the origin of the improvement material and the selection process used to obtain improved cultivars, as well as its characteristics (Trujillo-Aguirre, 2018; Trujillo-Aguirre *et al.*, 1994). These varieties were created at the experimental field of Uxmal, Yucatán, with materials obtained from individual harvests carried out in commercial plantations of habanero chili native cultivars established in Yucatán (lines: Yuc-75-01 and Cam 75-24). On the one hand, the INIA variety was characterized by 54-cm-high plants with three fruits per axil, orange-colored when ripe, 5.1 cm long, 3.2 cm wide, and a yield of 19,442 kg ha^{-1}. On the other hand, the Uxmal variety was characterized by 62-cm-high plants with four fruits per axil, orange-colored (deep yellow) when ripe, 5.4 cm long, 3.4 cm wide, and a yield of 19,578 kg ha^{-1}. The said varieties were registered in 1981 before the Servicio Nacional de Inspección y Certificación de Semillas (SNICS) (Trujillo-Aguirre, personal communication, 2021). On the same year, technical brochure No. 17 SARH-INIA was edited by Octavio Pozo Campodónico (MSc), National Coordinator of Chilis, who worked at the Huastecas experimental field of INIFAP. Pozo Campodónico mentioned the importance of habanero chili in the Yucatán peninsula and its popularity in other domestic markets (Trujillo-Aguirre, 2018). Years later, in 1984, "Presente y pasado del Chile en México" (Present and Past of Chili in Mexico) was published; this document included the first references to the cultivated area, the yield, and the production of habanero chili.

In late 1989, the presence of a Gemini virus was discovered in the horticultural area of Yucatán; consequently, the INIFAP launched in late 1995 a program to harvest native habaneros, in order to recover and preserve their seed (Gaceta SIIDATEY, 2014). Through a program carried out with the support of the State of Yucatán's government and the involvement of the Fundación Produce Yucatán, the obtained germplasm was used to distribute native seed among those producers who had to establish new plantations. The INIFAP launched a series of efforts —of its sole and exclusive authorship— to obtain improved varieties with the fruit characteristics required by the market, such as resistance and tolerance to virosis (Trujillo-Aguirre, 1994; Gaceta SIIDATEY, 2014).

Towards late 1998, the INIFAP's habanero chili improvement program resulted in the registration of three new varieties: Jaguar, Mayapán, and Calakmul. The Jaguar variety was developed in Tamaulipas and adapted to the conditions of the Yucatán peninsula, is harvested at 120 to 125 days, and has a triangular, orange-colored fruit when ripe. The Mayapán variety, harvested at 120 to 125 days, has a triangular fruit, brilliant green before ripening and orange when ripe; its characteristics are adequate for the fresh market and the industry. This variety was obtained through the mass selection method during the first stages and the single seed descent method during the final process (Trujillo *et al.*, 2006b). The Calakmul variety, harvested at 120 to 125 days, has a triangular fruit, green before ripening and red when ripe; its characteristics are adequate for fresh consumption and potentially industrial use (Figure 1) (Gaceta SIIDATEY, 2014). The Jaguar and Calakmul varieties were obtained by the mass selection method at the beginning and the pedigree selection method at the end (Berny, 2011; Ramírez *et al.*, 2012).

The Jaguar variety was obtained from the habanero chili collection of the Banco de Germoplasma de Chile del Campo Experimental las Huastecas (CEHUAS)-INIFAP, which contains materials native to the Yucatán, Quintana Roo, Campeche, and Veracruz production areas, collected during the 1980's and early 1990's (Ramírez-Meraz *et al.*, 2018). The characterization and morphological description efforts, plus the increasing global demand for habanero chili, were the base for the declaration of designation of origin for this product, published in the Diario Oficial de la Federación (DOF) on June 4, 2010, comprising the states of Campeche, Quintana Roo, and Yucatán. This designation was backed by the Norma Oficial Mexicana (NOM-189) (Secretaría de Economía, 2012), the official Mexican standard for the habanero chili in the Yucatán peninsula, which includes specifications and testing methods. It was modified and published in the Diario Oficial de la Federación on February 21, 2018.

The boost to the production of habanero chili in the Yucatán peninsula entailed a genetic erosion problem, caused by the introduction of variety and hybrid seeds, together with the practice of harvesting ripe fruits and scattering them by means of multiplication, a practice resulting from ignorance and novelty.

In a personal communication, Trujillo (2021) mentions that, in 2006, a batch of 76 introduced varieties of habanero chili with different colors and tastes were planted in the community of Muna, Yucatán. The aim was to elaborate a production scheme based on the idea of obtaining seeds out of the best harvested fruits and saving them for the next cycle (Figure 2). The adoption of this practice is feasible only for self-sufficiency purposes, not for production schemes. Around the same time, seedlings in germination trays were offered to producers, but without any reference on the material they would plant in their fields. Another institution involved in genetic improvement in Yucatán is the Centro de Investigación Científica de Yucatán (CICY) which, based on the genetic diversity of habanero chili in the peninsula, in 2002 launched a genetic improvement program based on harvests carried out in the region.

Peninsular: it started with 58 initial characterized accessions which were subsequently reduced to 38. The genetic improvement method chosen was phenotypic mass selection,

Figure 1. From left to right, Mayapán, Jaguar, and Calakmul INIFAP varieties.

Figure 2. Morphological variability of habanero chili, expressed as fruit color and form, starting from 1994.

Figure 3. Characterized varieties of the CICY at the SNICS. From top to bottom and from left to right: mayan Ixchel, mayan ba'alche, mayan kauil, mayan ek, mayan chac, mayan chan, mayan kisin, and mayan k'iin.

which consists in selecting a number of plants with the desired characteristics (morphotype) among a certain population, applying a specific selection pressure in each cycle. In total, five selection cycles were used, and the resulting varieties were: mayan Ixchel, mayan ba'alche, mayan kauil, mayan ek, mayan chac, mayan chan, mayan kisin, and mayan k'iin (Santana-Buzzy *et al.*, 2018).

In spite of the genetic improvement programs in the Yucatán peninsula, according to data provided by the habanero chili plant producers in 2021, production is based on the cultivation of introduced varieties and hybrids, such as PX 11459057, which represents between 70% and 80% of production in the states of Yucatán and Campeche. Rey Pakal and Palenque, as well as Chichenitza and Helios (5%) are also among the most used materials.

Production in the State of Quintana Roo is based on the use of the Jaguar, Mayapán, and Mayan Chan varieties. In Yucatán, only the CICY produces small quantities of locally-adapted seeds. The INIFAP Yucatán does not report an improvement program in the peninsula and the seed for its varieties comes from the INIFAP Tamaulipas.

To date, the CICY has a habanero chili germplasm bank with 250 accessions. It is important to preserve, characterize, and protect the habanero chili genetic diversity in germplasm banks, considering samples of populations grown by several generations of regional producers. Genetic improvement programs must not include materials that have been introduced and reproduced in the Yucatán peninsula.

The chosen selection method results in a high genetic uniformity among the new varieties. In fact, it is considered the best method for autogamous species, such as habanero chili, for in spite of being a very long improvement process, it is very efficient to obtain varieties with a high genetic value. The mass, individual, crossbreeding, and pedigree selection methods have the least impact on regional genetic diversity, and use diversity within and among populations.

In this respect, as part of their assessment of different populations of habanero chili, López- Espinosa *et al.* (2018) found an intermediate diversity among the studied populations. These authors add that genetic differentiation among habanero chili populations in the Yucatán peninsula is low, which means that most genetic diversity (95.5%) is represented among the populations. Improved varieties are mostly hybrids and are not allowed in the DO, since they do not meet the NOM-189 criteria for the habanero chili in the Yucatán peninsula. Hence the need of a genetic improvement program that focuses on generating improved native varieties that are able to compete with introduced genotypes.

The use of NOM-189 certified and protected varieties is suitable to monitor traceability in production and certification, as well as compliance by Mexican certification bodies such as ANCE (Asociación de Normalización y Certificación A.C.), EMA (Entidad Mexicana de Acreditación), and IMPI (Instituto Mexicano de Protección Intelectual), regarding the designation of origin or collective brand (Marca Colectiva), as proposed by the Área de Normalización de la Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentos (SAGARPA).

CONCLUSION

The Yucatán peninsula needs a genetic improvement program to produce habanero chili native varieties whose characteristic form, scent, color, and taste allow them to achieve competitive yields and preserve genetic diversity.

ACKNOWLEDGEMENTS

We are indebted to Jorge Gerardo Trujillo (MSc) for the invaluable information he contributed to this article.

REFERENCES

Aguilar- Rincón. V. H., Corona-Torres, López-López P., Latournerie- Moreno L., Ramírez -Meraz M., Villalón- Mendoza H., Aguilar -Castillo J. A. (2010) Los chiles de México y su Distribución. SINAREFI, Colegio de Postgraduados, INIFAP, IT-Conkal, UANL, UAN. Montecillo, Texcoco, Estado de México. 114 p

Canto- Flick A. (2007). Caracterización morfológica y determinación de los niveles de capsaicina en 18 accesiones de chile habanero (*Capsicum chinense* Jacq.) Tesis Instituto Tecnológico de Mérida. Maestro en Ciencias en Ingeniería Bioquímica. Mérida, Yucatán, México.

Corona-Torres T., García-Velázquez A., Castillo-González F., Montero-Tavera V., Azpiroz-Rivero H. S. (2000) Caracterización isoenzimática de la diversidad genética de colectas de chile (*Capsicum annuum* L. y *Capsicum chinense* Jacq.). *Revista Chapingo Serie Horticultura 6*:5-17,

Coutinho, J. P., Barbero, G. F., Avellán, O. F., Garcés-Claver, A., Godoy, H. T., Palma, M., & Barroso, C. G. (2015). Use of multivariate statistical techniques to optimize the separation of 17 capsinoids by ultra performance liquid chromatography using different columns. *Talanta, 134*, 256-263.

Gaceta SIIDETEY. 2014. Investigación del INIFAP en chile habanero. Organo oficial de la ciencia y tecnología del estado en Yucatán Año 6 numero 14

Godínez-Hernández, Y., Anaya-López, J. L., Dıaz-Plaza, R., González-Chavira, M., Torres-Pacheco, I., Rivera-Bustamante, R. F., Guevara-González, R. G. (2001). Characterization of resistance to Pepper Huasteco Geminivirus in chili peppers from Yucatan, Mexico. *HortScience, 36*, 139-142.

Guzman, I., Hamby, S., Romero, J., Bosland, P. W., & O'Connell, M. A. (2010). Variability of carotenoid biosynthesis in orange colored *Capsicum* spp. *Plant Science, 179*(1-2), 49-59.

González-Estrada. T. Gutiérrez, L., Contreras, F., Peraza, S., Xool. E., Trujillo-Aguirre. J. J. G. en II Reunión Estatal de Investigación Agropecuaria, Forestal y Pesca. Fundación Produce Yucatán A.C. 20, 21 Y 22 de enero 2005. pag 279, 283.

Latournerie-Moreno. L., Chávez-Servia J. L.., Pérez-Pérez M.., Castañón- Nájera.G., Rodríguez Herrera S. A.., Arias Reyes L. M.,Ramírez-Vallejo P. (2002). Valoración *in situ* de la diversidad morfológica de chiles (*Capsicum annuum* L. y *Capsicum chinense* Jacq.) en Yaxcabá, Yucatán. *Revista Fitotecnia Mexicana 25*:25-33.

Latournerie-Moreno L., López-Vázquez J. S., Castañón-Nájera G., Mijangos-Cortéz J. O., Espadas-Villamil G., Pérez-Gutiérrez A. Ruiz-Sánchez E. (2015) Evaluación agronómica de chile habanero (*Capsicum Chinense* Jacq.). *Agroproductividad 8*:24-29.

López-Espinosa1 S. T, Latournerie -Moreno L., Castañón-Nájera G., Ruiz-Sánchez E., Gómez-Leyva J. F., Andueza-Noh R. H., Mijangos-Cortés J. O.. 2018. Diversidad genética de chile habanero (*Capsicum chinense* Jacq.) MEDIANTE ISSR. *Rev. Fitotec. Mex. Vol. 41* (3): 227 – 236

Santana-Buzzy N., Canto-Flick A., Balam-Uc E., Avilés-Viñas S., Zetina-Rosado G., Montalvo-peniche M.C-, Valle-Gough R., Bello-Bello J., Lecona-Guzmán C., Solís-Marroquín ., Gómez-Uc E., Muñoz-Ramírez L., Peña-Yam L., Pérez-Pastrana J., Guzmán-Antonio A. 2018. Mejoramiento genético del chile habanero: selección y registro de variedades mejoradas. En: Mejoramiento genético del chile habanero de la península de Yucatán. González-REstrada T., Zúñiga-Ahuilar J.J. Vázquez-Flota T. Compiladores. CONACYT-CICY. Mérida-México

Secretaría de Economía (2012) Norma Oficial Mexicana NOM-189- SCFI-2012, Chile Habanero de la Península de Yucatán (*Capsicum Chinense* Jacq.) Especificaciones y Métodos de Prueba. Diario Oficial de la Federación, Primera Sección. México, D.F. 30 de diciembre de 2012

Trujillo-Aguirre J.J.G., Pérez-Lianes, C.R. (2005) Caracterización de nueve materiales de Chile Habanero en Muna, Yucatán. México. 43 pp.

Trujillo-Aguirre J.J.G., Díaz-Plaza R. 1994. Society for Tropical Horticulture Interamerican. "Obtencion de cultivares de chile habanero con buenas características horticolas y con tolerancia a la virosis transmitida por mosquita blanca". Campeche, Camp. 13 al 19 Nov. de 1994.

Trujillo –Aguirre J J G. 2018. Caracterización de los recursos genéticos de *Capsicum chinense* Jacq. en la Península de Yucatán en: Mejoramiento genético de chile habanero de la península de Yucatán Tomas González-Estrada, Jose Juan Zuniga Aguilar, Felipe Vazquez Flota editores CICY.pag. 45-51.

Ruiz-Sánchez E., Gómez-Leyva J F., Andueza-Noh R H., Mijangos-Cortés J. O. 2018. Diversidad Genética de Chile Habanero (*Capsicum chinense* Jacq.) Mediante ISSR *Rev. Fitotec. Mex. Vol. 41* (3): 227 – 236.

Vera-Sánchez K. S., Cadena-Iñiguez J, Latournerie-Moreno L., Santiaguillo-Hernández J. F., Rodríguez-Contreras A., Basurto-Peña F. A., Castro-Lara D., Rodríguez-Guzmán E., López-López P., Ríos-Santos E. (2016). Conservación y Utilización Sostenible de las Hortalizas Nativas de México. Servicio Nacional de Inspección y Certificación de Semillas. México, D. F. 132 p.

AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Physiological response of three wild castor bean (*Ricinus communis* L.) ecotypes exposed to different substrate moisture levels

Peña-Uribe, Gabriel de J.[1*]**; Vázquez-Chun, Carolina**[1]**; Valdez-Cepeda, Ricardo D.**[2]**; Armendáriz-Erives, Sigifredo**[1]**; López-Santos, A.**[1*]

[1] Universidad Autónoma Chapingo, Unidad Regional Universitaria de Zonas Áridas, Bermejillo, Durango, México, C. P. 35230.

[2] Universidad Autónoma Chapingo, Centro Regional Universitario Centro Norte, Morelos, Zacatecas, C. P. 98053.

* Correspondence: biol.mar.gabriel@gmail.com; alopez@chapingo.uruza.edu.mx

ABSTRACT

Objective: To analyze the morphological and physiological responses of three wild castor bean (*Ricinus communis* L.) ecotypes to four different gravimetric moisture levels.

Design/methodology/approach: The wild castor bean ecotype seeds were collected in the arid region of the State of Durango, Mexico. Three potential ecotypes were selected according to seed size and shape. A completely random greenhouse culture was established with three wild castor bean ecotypes; they were planted in substrate with four gravimetric moisture levels ($T1_{\Theta}=24\pm2\%$; $T2_{\Theta}=20\pm2\%$; $T3_{\Theta}=16\pm2\%$; $T4_{\Theta}=14\pm2\%$). The physiological measurements were carried out with LICOR's LI-6400XT portable photosynthesis system. A two-way ANOVA was conducted to obtain differences between the factors and their interactions.

Results: Ecotypes 1 and 2 had larger stems and leaves than ecotype 3. The differences in plant growth due to the effects of a 24% and 20% gravimetric moisture content were not significant ($p=0.05$). Ecotype 3 presented the highest photosynthetic rate (14.77 ± 6.14 μmol CO_2 $m^{-2}s^{-1}$); however, the differences between ecotypes were not significant. The differences were determined based mainly on substrate moisture.

Study limitations/implications: Determining the water requirements of castor bean crops allows for the optimization of water use in regions where this resource is scarce.

Findings/conclusions: Ecotype 1 seeds —which were very large, very round, and had low eccentricity— are associated with plants that have larger and wider stems and leaves. This genotype could be domesticated considering a substrate moisture content of 24% and 20%.

Key words: Intracellular carbon dioxide, Leaf vapor pressure deficit, Morphometry, Photosynthetic rate, Stomatal conductance.

Citation: Peña-Uribe, G. de J., Vázquez-Chun, C., Valdez-Cepeda, R. D., Armendáriz-Erives, S., & López-Santos, A. (2022). Physiological response of three wild castor bean (*Ricinus communis* L.)ecotypes exposed to different substrate moisture levels. *Agro Productividad*. https://doi.org/10.32854/agrop.v15i1.2045

Editor in Chief: Dr. Jorge Cadena Iñiguez

Received: May 23, 2021.
Accepted: January 3, 2022.
Published on-line: February 5, 2022.

Agro Productividad, 15(1). January. 2022. pp: 111-119.

INTRODUCTION

The seeds of castor bean (*Ricinus communis* L.) (Euphorbiaceae) are used to produce castor oil, which is widely used in the chemical industry (Severino *et al*., 2012; Lakhani *et al*., 2015). The characteristics of its oil have made castor bean a non-food crop of great

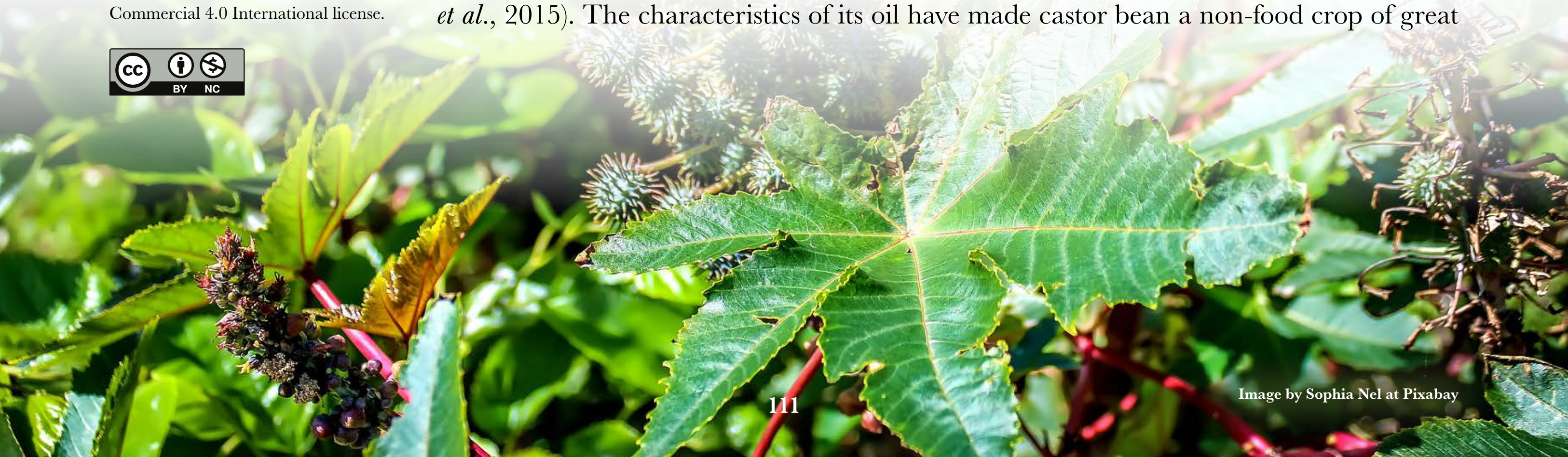

Image by Sophia Nel at Pixabay

economic importance in the last few years (Severino *et al.*, 2012). In addition, various authors (Da Silva *et al.*, 2006; Dos Santos *et al.*, 2017; Buendía-Tamariz *et al.*, 2018) emphasize the fact that castor oil is a high-quality raw material to produce biofuel. Small-scale producers, mainly from India, China, Brazil, and Mozambique, account for 96% of the worldwide castor bean seed production (Severino and Alud, 2013). Only in 2008 did Mexico figure as the eighth castor bean producer worldwide; afterwards, production data have decreased (FAOSTAT, 2021). Although the demand for castor oil is constantly increasing, the supply is limited by the current production of castor bean, which cannot satisfy the global market's demand (Severino *et al.*, 2012).

Limited water availability is a recurring phenomenon and a main restricting factor in crop productivity in the arid tropics (Vijaya-Kumar *et al.*, 2005; Sausen and Rosa, 2010). Castor bean's tolerance to water deficit has been studied from diverse viewpoints. Different authors (Dai *et al.*, 1992; Lacerda *et al.*, 2009; Sausen and Rosa, 2010; Brito-Pinto *et al.*, 2014; Dos Santos *et al.*, 2017) agree that castor bean has high tolerance levels to water deficit and can even have a low seed yield with little available water, a situation where other species could not be cultivated (Severino *et al.*, 2012). Nevertheless, castor bean cultivation is still unknown in different regions of Mexico, which reduces its promotion and the development of crop systems (Buendía-Tamariz *et al.*, 2018).

Establishing the water requirements of castor bean cultivation allows for the optimization of water use in regions where this resource is scarce. Although castor bean is considered a species with low genetic diversity (Allan *et al.*, 2008; Foster *et al.*, 2010), it has a high phenotypic polymorphism, from very tall to very small plants (Lakhani *et al.*, 2015). Therefore, there might be significant differences in morphometric and physiological responses among castor bean ecotypes at different gravimetric moisture levels. The aim was to analyze the morphologic and physiological responses of three castor bean ecotypes collected in the arid regions of the State of Durango, Mexico, based on four soil moisture content levels. The underlying hypothesis was that ecotypes have different morphometric and physiological responses to different substrate gravimetric moisture levels.

MATERIALS AND METHODS

This research was conducted between June 2017 and May 2018 under greenhouse conditions at the Unidad Regional Universitaria de Zonas Áridas de la Universidad Autónoma Chapingo (25.893° N, 103.600° W). The climate in the region is very arid and semi-hot, with an annual average temperature between 18 °C and 22°C (BWhw). Temperatures inside the greenhouse fluctuated between 3.9 and 36.09 °C, with an overall average of 25.07±6.2 °C (Table 1).

Preparation for the experiment

The wild castor bean seeds were collected in the arid region of the State of Durango, Mexico (Table 2). Three potential seed ecotypes were selected according to size and shape (data are not shown). The first group comprised large seeds with lower eccentricity and higher roundness (Ecotype 1). The second group included medium-sized seeds, with high eccentricity, and intermediate roundness (Ecotype 2). The third group consisted of small

Table 1. Mean environmental conditions in the greenhouse during the experiment.

Year	Month	Min.T.	Max.T.	Mean T.	Hum. (%)
		°C			
2017	July	24.31	40.63	31.36	39.81
	August	23.22	43.67	32.13	40.59
	September	20.89	39.77	28.9	43.97
	October	16.7	41.1	26.51	42.73
	November	11.33	41.18	23.16	33.07
	December	8.34	29.08	16.21	54.59
2018	January	5.89	32.86	16.53	38.76
	February	13.84	38.2	23.58	44.11
	March	15.92	43.72	27.76	36.06
	April	18.36	43.1	28.65	36.02
	May	20.8	43.56	30.15	37.9

Min.T., minimum temperature; Max.T., maximum temperature; MeanT., mean temperature; and Hum., Air humidity.

seeds with highly dispersed eccentricity and roundness values (Ecotype 3). Out of each group, 36 seeds were selected. They were planted in 72-cell seedbeds with peat moss substrate. The seedbeds were irrigated daily with purified water until transplant day. The seedlings were transplanted 21 days after planting, when they had already developed two leaves.

The substrate that was used in the experiment came from soil collected in an experimental plot. According to the *World Reference Base for Soil Resources* (IUSS Working Group WRB, 2015), the soil was identified as aridic Calcisol (CLad). In addition, the soil was characterized in the laboratory following the Norma Oficial Mexicana NOM-021-RECNAT-2000 guidelines, which establish specifications for soil fertility, salinity, and classification. Bulk density was determined with the paraffin method (AS-03); soil texture

Table 2. Collection sites of castor ecotypes and their environmental characteristics.

Eco	Site	Lat.	Long.	WRB Key	RSG	Climate
E3	Gómez Palacio	25.613726	−103.4932	SNszw+LVsow/3	SOLONETZ	BWhw
E1	Guatimape	24.807201	−104.9197	SNaxszn+VRmzszp/2	SOLONETZ	BS1kw
E3	Leandro Valle	25.086666	−105.065	FLeu+KSlvcc/1	FLUVISOL	BSohw
E1	Nazareno	25.397986	−103.4201	RGsowca+CLad+VRcrca/2	REGOSOL	BWhw
E2	Nazas	25.228944	−104.113	CLlv+KSlvcc/2	CALCISOL	BWhw
E2	Nazas	25.232055	−104.1168	CLlv+KSlvcc/2	CALCISOL	BWhw
E2	Nazas	25.2306944	−104.1371	FLeu+KSlvcc/1	FLUVISOL	BWhw
E1	San Luis del Cordero	26.196666	−105.2017	CLskptp+RGskca/2R	CALCISOL	BWhw
E3	Villa Unión	23.967611	−104.0496	Flca+KSvpcn/2	FLUVISOL	BS1kw
E3	Villa Unión	23.9595	−104.0539	Flca+KSvpcn/2	FLUVISOL	BS1kw

Eco, ecotype; WRB Key, World Reference Base for Soil Resources key; and RSG, reference soil group.

was established using the Bouyoucos hydrometer method (AS-09); the AS-05 method was used to determine soil moisture; organic matter was established with the Walkley and Black method (AS-07); and electric conductivity was measured using the AS-16 method. Soil texture was identified as sandy clay loam (Table 3).

Experimental design

Twelve treatments were established based on the three castor bean ecotypes and four gravimetric moisture levels ($T1_{\Theta}=24\pm2\%$; $T2_{\Theta}=20\pm2\%$; $T3_{\Theta}=16\pm2\%$; $T4_{\Theta}=14\pm2\%$). Four repetitions were prepared, giving a total of 48 pots or experimental units. Six kilograms of dry soil were placed in each of the forty-eight 7.5-liter pots. One liter of tap water was added to each pot in order to dampen the substrate. Then a castor bean seedling was placed in each pot. All pots were labelled with the relevant ecotype, the corresponding gravimetric moisture percentage, and the repetition number. The treatments were distributed according to a completely random experimental design.

Irrigation

Water for irrigation was extracted from a deep well. Water electric conductivity was 2.85 dS m^{-1} according to the Orion Star A222 portable conductivity meter (Thermo Scientific, USA). During a 30-day acclimatization period, plant irrigation was homogenous: approximately 900 g of water were added per pot. Subsequently, pots were weighed every two days before and after irrigation, in order to estimate the quantity of water to be restored.

Morphological variables

Plants were measured every week throughout the experiment. A measuring tape was used to measure the stem height (SH) —from the substrate base to the last internode. To measure the stem width (SW), a permanent marker was first used to make a mark on the stem 1 cm above the soil surface. This mark served as a reference to measure the stem width using a Vernier caliper. Leaf length (LL) and leaf width (LW) were measured and the results were used to calculate the leaf area (LA).

Physiological variables

A LI-6400XT portable photosynthesis system (LICOR Inc., Lincoln, Nebraska, USA) was used to measure the physiological response of the three castor bean ecotype plants to the different substrate moisture levels. Once all the plants had at least one leaf with a length of over 5 cm, measurements were done every 15 days. The LI-6400XT was calibrated with

Table 3. Biophysical characteristics of aridic Calcisol collected from the experimental plot.

Sample	BD	FC	PWP	USM	OC	OM	pH	EC	N	CO_3
	g cm^{-3}	%						dS m^{-1}	%	
Depth (cm) 0 a 15	1.28	27.24	14.63	12.6	0.23	0.4	8	7.72	0.1	7.3

BD, bulk density; FC, field capacity; PWP, permanent wilting point; USM, usable soil moisture; OC, organic carbon; OM, organic material; pH, hydrogen potential; EC, electric conductivity; N, nitrogen; and CO_3, carbonates.

a photon flux density within the photosynthetically active radiation spectrum of 1000 μmol $m^{-2}s^{-1}$. This device was used to estimate photosynthetic rate (*A* in μmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (*gs* in mol H_2O $m^{-2}s^{-1}$), intracellular carbon dioxide concentration (*Ci* in μmol CO_2 mol^{-1}), leaf water vapor deficit (VpdL in kPa), and leaf temperature (Tleaf in °C).

Statistical analyses

Morphological and physiological variables were subjected to normality tests. Variables that presented a non-normal distribution were standardized. Two-way ANOVAs were conducted to identify the effects of the different factors on the response variables. When the results allowed the identification of significant effects of the factors, Tukey's test was applied to conduct the corresponding comparisons. Statistical analyses were conducted with the MiniTab 17 software (MiniTab Inc., USA).

RESULTS AND DISCUSSION

Morphometry

The most striking results are described in the rest of this section. Ecotype 1 and 2 plants had the tallest stems, which were especially similar when cultivated in substrates with 24% and 20% gravimetric moisture levels (Table 4). In addition, ecotype 1 plants had the widest stems when the substrate had a gravimetric moisture level of 24%. Ecotype 1 and 2 plants also had the longest and widest leaves when grown in substrates with 24% and 20% gravimetric moisture levels. Significantly, ecotype 1 plants had the largest leaf area, especially when cultivated in substrates with a gravimetric moisture content of 24%.

In general, these results are consistent with those reported by Brum *et al.* (2011), who found that castor bean seedlings grown from heavier seeds emerge faster and are taller than seedlings grown from lighter seeds. Other authors (*e.g.*, Lacerda *et al.*, 2009; Silva *et al.*, 2009; Brito-Pinto *et al.*, 2014) emphasized that, if water availability increases, the height, leaf area, and yield of castor bean plants also increases. It is worth mentioning that plants were smaller than the "Al Guarany 2002" variety (59.3 cm), according to Brito-Pinto

Table 4. Means and standard deviations of castor bean morphometric variables grouped by factors.

Factors \ Variables	Stem height (cm)	Stem width (cm)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)
Ecotype					
E1	12.50 ± 3.88^{A}	0.71 ± 0.20^{A}	7.20 ± 2.07^{A}	9.24 ± 3.13^{A}	71.28 ± 37.51^{A}
E2	8.51 ± 2.57^{B}	0.58 ± 0.19^{B}	6.75 ± 2.13^{A}	8.31 ± 2.39^{AB}	60.89 ± 34.46^{A}
E3	7.46 ± 2.34^{C}	0.53 ± 0.18^{B}	5.45 ± 1.84^{B}	7.23 ± 1.99^{B}	44.08 ± 23.97^{B}
Substrate moisture					
$T1_{\theta}$ 24±2%	10.91 ± 4.56^{A}	0.74 ± 0.22^{A}	7.52 ± 2.44^{A}	9.52 ± 2.48^{A}	76.63 ± 42.25^{A}
$T2_{\theta\alpha}$20±2%	9.93 ± 3.36^{A}	0.62 ± 0.17^{B}	6.88 ± 1.59^{A}	8.59 ± 1.75^{A}	60.87 ± 23.98^{B}
$T3_{\theta\alpha}$16±2%	8.74 ± 2.51^{B}	0.51 ± 0.12^{C}	5.34 ± 1.61^{B}	6.84 ± 3.33^{B}	39.30 ± 23.65^{C}
$T4_{\theta\alpha}$14±2%	6.63 ± 2.31^{C}	0.36 ± 0.09^{D}	4.89 ± 1.52^{B}	5.78 ± 1.17^{B}	34.99 ± 12.87^{C}

Results of Tukey's means tests are shown, capital letters represent similar groups.

et al. (2014). This could be explained by pot size: Brito-Pinto *et al.* (2014) used 30-L pots (four times larger than the ones we used in our work). However, the leaf area of our three ecotypes is larger than the leaf area of the "Al Guarany 2002" variety (35.37 cm^2). Leaf area is an important factor, because it is directly related to the leaf's capacity to intercept light and carry out photosynthesis (Severino and Auld, 2013).

Physiology

The results for the physiological variables were as follows: only the leaves of ecotype 2 and 3 plants had a higher gs than genotype 1 (Table 5). Likewise, the lower gs is associated to the leaves of plants that were cultivated in the substrate with the lowest gravimetric moisture level (16%); their temperature and photosynthetic rate were also considerably lower than those of the leaves of plants in substrates with higher moisture content levels. However, the differences between *A*, *Ci*, *VpdL*, and *Tleaf* variable values for leaves of the three ecotypes were statistically similar. Likewise, the leaves of plants cultivated in the two lower substrate moisture levels presented higher water vapor deficits than those associated to a substrate gravimetric moisture level of 25%.

Overall, plants cultivated in pots with a higher substrate moisture level presented higher physiological activity values. This is consistent with reports by other authors (Lacerda *et al.*, 2009; Silva *et al.*, 2009; Brito-Pinto *et al.*, 2014), who mention that castor bean yields are closely related to the available moisture. According to Da Matta *et al.* (2001), values for A in plants with the highest available moisture levels are similar to those of bean (16.3 μmol CO_2 $m^{-2}s^{-1}$) and eucalyptus (14.4 μmol CO_2 $m^{-2}s^{-1}$). Moreover, Sausen and Rosa (2010) obtained maximum photosynthesis values of 15 μmol CO_2 $m^{-2}s^{-1}$ with 18% of soil moisture, while Dos Santos *et al.* (2017) recorded maximum photosynthesis values of 16.2 μmol CO_2 $m^{-2}s^{-1}$.

On the one hand, different authors (Lawlor and Tezara, 2009; Broeckx *et al.*, 2014; Dos Santos *et al.*, 2017) maintain that the decrease of *gs* is a result of stomatal closure, which is a key phenomenon and the first defense against dehydration; this might have been the

Table 5. Means and standard deviations of the physiological variables of castor bean grouped by factors.

Variables / Factors	*A*	*gs*	*Ci*	*VpdL*	*Tleaf*
Ecotype					
1	11.11±5.32	0.10 ± 0.07^{B}	147.33±53.88	2.66±0.72	31.14±3.42
2	12.93±5.66	0.12 ± 0.11^{AB}	127.70±149.40	2.85±0.86	32.43±3.36
3	14.77±6.14	0.16 ± 0.12^{A}	154.90±73.00	2.84±0.97	32.81±3.32
Substrate Moisture					
$T1_{\theta\alpha}24\pm2\%$	14.95 ± 5.61^{A}	0.14 ± 0.11^{A}	127.60±132.00	3.00 ± 0.96^{A}	33.36 ± 3.17^{A}
$T2_{\theta\alpha}20\pm2\%$	13.22 ± 5.21^{A}	0.14 ± 0.11^{A}	149.38±73.50	2.68 ± 0.81^{AB}	31.81 ± 3.30^{B}
$T3_{\theta\alpha}16\pm2\%$	7.62 ± 4.10^{B}	0.06 ± 0.04^{B}	161.11±57.03	2.51 ± 0.52^{B}	29.90 ± 3.04^{C}

A, photosynthetic rate (μmol CO_2 $m^{-2}s^{-1}$); gs, stomatal conductance (mol H_2O $m^{-2}s^{-1}$); Ci, intracelular carbon dioxide concentration (μmol CO_2 mol^{-1}); VdpL, leaf water vapor deficit (kPa); and Tleaf temperatura foliar (°C). Results of Tukey's means tests are shown, capital letters represent similar groups.

case of the plants subjected to a substrate gravimetric moisture level of 16%. Furthermore, according to Heckenberger *et al*. (1998) and de Freitas *et al*. (2011), this decrease in stomatal conductance might also be the result of a decrease in the stomatal conductance values of castor bean plants subjected to water deficit, as a consequence of the low stomata density during leaf growth in water stress conditions.

On the other hand, the tendency of *A* to decrease and *Ci* to increase as the soil moisture availability decreases is noticeable (Table 4). The increase in *Ci* is inconsistent with reports by Ocheltree *et al*. (2014), who maintain that the reduction in the plants' stomatal conductance also entails a reduction in the CO_2 diffusion rate and the internal CO_2 concentration. This situation can reduce the efficiency of carbon fixation in plants. In light of this, the responses of plants to water deficit seem to be complex and to involve adaptive changes and genotype influence (Chaves *et al*., 2002).

Barbour and Buckley (2007) and other authors mention that an increase in leaf water vapor deficit directly affects stomatal closure. Nevertheless, this was not observed on castor bean leaves. Oddly enough, the highest *VpdL* was observed in plants that were grown in pots with higher substrate moisture levels, which were also those with higher *gs*. This is consistent with Davies and Zang (1991), who maintain that stomatal response is frequently associated with soil water content, rather than with leaf water status. Therefore, this could mean that the plant can transpire continually without problems, which allows water to move constantly through the plant. According to several authors (McDonald *et al*., 2002; Snyder *et al*., 2003), this can provide more benefits for the plant, such as a better nutrient absorption from the soil.

CONCLUSIONS

Castor bean ecotype 1 has big, very round seeds with low eccentricity, which yielded plants with wide stems, with a 24% substrate gravimetric moisture level. This ecotype's plants are also associated with very long and wide leaves, when they are grown in substrates with 24% and 20% gravimetric moisture levels. Notably, castor bean ecotype 1 plants had the larger leaf area, especially when cultivated in substrates with a 24% gravimetric moisture content. In general, results indicate that this genotype could be introduced in an improvement program, considering these two substrates gravimetric moisture content conditions for its cultivation.

ACKNOWLEDGEMENTS

G.J.P.-U. is extremely grateful for the PhD grant (CVU 514128) awarded to him by the Consejo Nacional de Ciencia y Tecnología (CoNaCyT). The authors are indebted to the Universidad Autónoma Chapingo for the funding of the project "Potencialidades de la higuerilla como un cultivo alternativo en el norte de México para la adaptación y mitigación al cambio climático" (Castor bean's potential as an alternative crop in northern Mexico for climate change adaptation and mitigation) (Clave E-07-2016).

REFERENCES

Allan, G., Williams, A., Rabinowicz, P. D., Chan, A. P., Ravel, J., & Keim, P. (2008). Worldwide genotyping of castor bean germplasm (*Ricinus communis* L.) using AFLPs and SSRs. *Genet. Resour. Crop Evol. 55*: 365–378. doi:10.1007/s10722-007-9244-3

Barbour, M.M., & Buckley, T.N. (2007). The stomatal response to evaporative demand persists at night in *Ricinus communis* plants with high nocturnal conductance. *Plant, Cell and Environment. 30*: 711–721. doi: 10.1111/j.1365-3040.2007.01658.x

Brito-Pinto, M.A., Reisser-Júnior, C., Carlos-Timm, L., Franke-Brixner, G., dos Anjos e Silva, S.D., & Helwing-Penning, L. (2014). Development and initial growth of castor bean (*Ricinus communis*) crop submitted to different soil water tensions. *Agrociencia 48*: 501-510.

Broeckx, L.S., Fichot, R., Verlinden, M.S., & Ceulemans, R. (2014). Seasonal variations in photosynthesis, intrinsic water-use efficiency and stable isotope composition of poplar leaves in a short-rotation plantation. *Tree Physiol. 34*: 1-14. doi:10.1093/treephys/tpu057

Brum, B., Lopes, S.J., Storck, L., Lúcio, A.D., Oliveira, P.H., Milani, M., (2011). Canonical correlation between variables seed, seedling, plant and grain yield in castor bean. *Ciênc. Rural. 41*: 404–411. doi: 10.1590/S0103-84782011000300007

Buendía-Tamariz, M.N., Trejo-Calzada, R., Sánchez-Cohen, I., Flores-Hernández, A., López-Santiago, M.A., & Pedroza-Sandoval, A. (2018). Growth analysis of four varieties of *Ricinus communis* L. in an arid region of México. *Interciencia Journal. 43*: 457-479.

Chaves, M.M., Pereira, J.S., Maroco, J, Rodríguez, M.L., Ricardo, C.P.P., Osorio, M.L., Carvalho, I., Faria, T., & Pinheiro C. (2002). How plants cope with water stress in the field. Photosynthesis and growth. *Annals of Botany. 89*: 907-916. doi: 10.1093/aob/mcf105

Da Silva, N.D.L., Maciel, M.R.W., Batistella, C.B., & Maciel-Filho, R. (2006). Optimization of biodiesel production from castor oil. *Appl. Biochem. Biotechnol. 130*: 405-414. doi: 10.1385/ABAB:130:1:405

Da Matta, F., Loos, R.A., Rodrigues, R., & Barros, E. (2001). Actual and Potential Photosynthetic Rates of Tropical Crop Species. *Bras. Fisiol. Veg. 13*: 24-32. doi:10.1590/s0103-31312001000100003

Dai, z., Edwards, G.E., & Ku, M.S.B. (1992). Control of Photosynthesis and Stomatal Conductance in *Ricinus communis* L. (Castor Bean) by Leaf to Air Vapor Pressure Deficit. *Plant Physiol. 99*: 1426-1434. doi: 10.1104/pp.99.4.1426

Davies, W.J. & Zhang, J. (1991) Root signals and the regulation of growth and development of plants in drying soil. *Annual Review of Plant Physiology and Plant Molecular Biology 42*: 55-76. doi: 10.1146/annurev.pp.42.060191.000415

de Freitas C.A.S., de Silva, A.R.A., da Bezerra, F.M.L., Lacerda, C.F., de Pereira-Filho, J.V., & de Sousa, G.G. (2011). Produção de matéria seca e trocas gasosas em cultivares de mamoneira sob níveis de irrigação. *Revista Brasileira de Engenharia Agrícola e Ambiental, 15:* 1168-1174. doi: 10.1590/S1415-43662011001100009

Dos Santos, C.M., Endres, L., Ferreira, V.M., Silva, J.V., Rolim, E.V., & Wanderley-Filho, H.C.L., (2017). Photosynthetic Capacity and Water use Efficiency in *Ricinus communis* (L.) under Drought Stress in Semi-Humid and Semi-Arid Areas. *Annals of the Brazilian Academy of Sciences 89:* 3015-3029. doi:10.1590/0001-3765201720160729

Foster, J. T., Allan, G. J., Chan, A. P., Rabinowicz, P. D., Ravel, J., Jackson, P. J., & Keim, P. (2010). Single nucleotide polymorphisms for assessing genetic diversity in castor bean (*Ricinus communis*). *BMC Plant Biol. 10*:13. doi: 10.1186/1471-2229-10-13

Hecknenberger, U., Roggatz, U., Schurr, U. (1998) Effect of drought stress on the cytological status in *Ricinus communis*. *Journal of Experimental Botany 49:* 181-189.

Lacerda, R. D., Guerra, H.O.C., & Júnior, G.B. (2009). Influência do déficit hídrico e da matéria orgânica do solo no crescimento e desenvolvimento da mamoneira BRS 188-Paraguaçu. *Rev. Bras. Ciencia Agrar. 4:* 440-447.

Lakhani, H.N., Patel, S.V., Bodar N.P., & Golakiya B.A. (2015). RAPD analysis of genetic diversity of castor bean (*Ricinus communis* L.). *Int.J.Curr.Microbiol.App.Sci. 4:* 696-703.

Lawlor, D.W., & Tezara, W. (2009). Causes of decreased photosynthetic rate and metabolic capacity in waterdeficient leaf cells: a critical evaluation of mechanisms and integration of processes. *Ann Bot. 103:* 561-579. doi:10.1093/aob/mcn244

McDonald, E.P., Erickson, J.E., & Kruger, E.L. (2002). Can decreased transpiration limit plant nutrient acquisition in elevated CO_2? *Functional Plant Biology. 29:* 1115–1120. doi: 10.1071/FP02007

Ocheltree, T., Nippert, J., & Prasad, P. (2014). Stomatal responses to changes in vapor pressure deficit reflect tissue-specific differences in hydraulic conductance. *Plant Cell Environ. 37:* 132-139. doi: 10.1111/pce.12137

Sausen, T.L., & Rosa, L.M.G. (2010). Growth and carbon assimilation limitations in *Ricinus communis* (Euphorbiaceae) under soil water stress conditions. *Acta Botanica Brasilica. 24:* 648-654. doi: 10.1590/S0102-33062010000300008

Severino, L.S., Auld, D.L., Baldanzi, M., Cândido, M.J., Chen, G., Crosby, W., & Lavanya, C. (2012). A review on the challenges for increased production of castor. *Agronomy journal 104:* 853-880. doi: 10.2134/agronj2011.0210

Severino, L.S., & Auld, D.L. (2013). A framework for the study of the growth and development of castor plant. *Industrial Crops and Products 46*: 25-38. doi: 10.1016/j.indcrop.2013.01.006

Silva, S.M.S., Gheyi, H.R., Beltrão, N.E.M., dos Santos, J.W., & Soares, F.A.L. (2009). Dotações hídricas em densidades de plantas na cultura da mamoneira cv. BRS Energia. *Rev. Bras. Ciencia Agrar. 4*: 338-348.

Snyder, K.A., Richards, J.H., & Donovan, L.A. (2003). Night-time conductance in C3 and C4 species: do plants lose water at night? *Journal of Experimental Botany. 54:* 861-865. doi: 10.1093/jxb/erg082

Vijaya-Kumar, P., Ramakrishna, Y., Bhaskara-Rao, D., Sridhar, G., Srinivasa-Rao, G., & Rao, G. (2005). Use of remote sensing for drought stress monitoring, yield prediction and varietal evaluation in castor beans (*Ricinus communis* L.). *International Journal of Remote Sensing, 26:* 5525-5534. doi: 10.1080/01431160500285258

Identification of *Pseudomonas viridiflava*, causal agent of onion (*Allium cepa* L.) bulb rot

Ramírez-Razo, Karina[1]; Cristóbal-Martínez, Ana L.[1]; Alvarado-Rosales, Dionicio[1]; Serret-López, María[1]; Aranda-Ocampo, Sergio[1*]

[1] Colegio de Postgraduados, Km. 36.5 Carretera Federal México-Texcoco, Montecillo, Texcoco, Estado de México, México, C.P. 56230.

* Correspondence: saranda@colpos.mx

Citation: Ramírez-Razo, K., Cristóbal-Martínez, A. L, Alvarado-Rosales, D., Serret-López, M., & Aranda-Ocampo, S. (2022). Identification of Pseudomonas viridiflava, causal agent of onion (Allium cepa L.) bulb rot *Agro Productividad*. https://doi.org/10.32854/agrop.v15i1.2110

Editor in Chief: Dr. Jorge Cadena Iñiguez

Received: August 10, 2021.
Accepted: December 14, 2022.
Published on-line: February 5, 2022.

Agro Productividad, 15(1). January. 2022. pp: 121-128.

ABSTRACT

Objective: To phenotypically and molecularly characterize and identify the causal agent of onion (*Allium cepa* L.) bulb rot in Morelos, Mexico.

Methodology: Fluorescent bacteria from onion bulb tissue with symptoms of rot were isolated; the LOPAT test was used to describe them and subsequently they were identified by partial 16S rRNA gene amplification. Pathogenicity in vegetables and plants was evaluated injecting a suspension with 108 CFU mL^{-1} of the pathogen.

Results: Phenotypic characterization and 16S rRNA nucleotide sequencing showed 100% identity with *Pseudomonas viridiflava* as the causal agent of onion bulb rot. The pathogen caused infection in broccoli (*Brassica oleracea* L.), spring onion (*Allium fistulosum* L.), purple onion (*Allium cepa* L.), cauliflower (*Brassica oleracea* var. *botrytis* L.), leek (*Allium porrum* L.), and carrot (*Daucus carota* L.), as well as in plant species such as jalapeño pepper (*Capsicum annuum* var. *annuum* L.), bean (*Phaseolus vulgaris* L.), and tomato (*Solanum lycopersicum* L.).

Implications: This information is important for agriculture in Mexico. *Pseudomonas viridiflava* is a bacterial pathogen with high potential to infect new hosts. This is the first report of *P. viridiflava* causing onion rot in Mexico.

Conclusions: *Pseudomonas viridiflava* is the causal agent of onion bulb rot in Morelos, Mexico. Other vegetables (such as spring onions and leek) can be potential new hosts in Mexico.

Keywords: pectinolytic bacteria, 16S rRNA, pathogenicity, vegetables.

INTRODUCTION

Onion (*Allium cepa* L.) is the second most important vegetable crop in Mexico. Its export —mainly to the United States— is valued at 3.5 million dollars per year (TecnoAgro, 2017). *Pseudomonas viridiflava* is a bacterial pathogen with a wide range of hosts; it is genetically included within the *Pseudomonas syringae* species complex which is the most important phytopathogenic bacteria worldwide (Mansfield *et al.*, 2010; Bartoli *et al.*, 2015). *P. viridiflava* populations are characterized by their pectinolytic activity and the lack of oxidase and arginine dihydrolase. This sets it apart from the *P. syringae* species complex, according to the LOPAT determinative test (levana - oxidase - potato rot - arginine dihydrolase - hypersensitivity in tobacco [*Nicotiana tabacum* L.]) (Lelliot and Stead, 1966).

In other countries, *P. viridiflava* can cause rot in bulbs, stems, and fruits in a wide range of hosts. Currently, many of these hosts are crops of economic

Image by Bruno /Germany at Pixabay

importance in Mexico, for example, vegetables and fruits such as: tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annum* L.), melon (*Cucumis melo* L.) (Al-Karablieh *et al*., 2017), watermelon (*Citrullus lanatus* L.), carrot (*Daucus carota* L.), lettuce (*Lactuca sativa* L.) (Nuebling *et al*., 2016), bean (*Phaseolus vulgaris* L.) (González *et al*., 2003), grapes (*Vitis vinifera* L.) (Goumans and Chatzaki, 1998), and citrus (Beiki *et al*., 2016).

It also affects ornamental flowering plants, such as chrysanthemums (*Chrysanthemum* sp.) (Goumans and Chatzaki, 1998) and the family Rosaceae (Choi *et al*., 2020). Although *P. viridiflava* is considered an opportunistic pathogen that can survive as a saprophyte and epiphyte, records indicate that severe epidemics have caused significant economic losses in crops such as melon (*Cucumis melo* L.), tomato (*Solanum lycopersicum* L.), chrysanthemum (*Chrysanthemum* sp.) (Goumans and Chatzaki, 1998), onion (*Allium* sp.) (Gitaitis *et al*., 1998), celery (*Apium graveolens* L.) (Hunter and Cigna, 1981), carrot (*Daucus carota* L.) (Godfrey and Marshall, 2002), and potato (*Solanum tuberosum* L.) (Macagnan *et al*., 2007).

There are not many studies about onion bulb rot in Mexico. Bulbs with symptoms of rot —probably caused by a bacterial infection— were observed in field onion crops of the State of Morelos, Mexico. Therefore, the objective was to phenotypically and molecularly characterize and identify the causal agent of onion bulb rot, evaluating the *in vitro* pathogenicity in different vegetables and plants species under greenhouse conditions, as well as the *in vitro* sensitivity of the agent to the bactericide.

MATERIALS AND METHODS

Pathogen isolation

In 2019, Blanca Morelos variety onion bulbs with symptoms of rot were collected in field in the town of Cuautla, Morelos (18° 48' 45" N; 98° 57' 17" W). The tissue samples were disinfected with 1% sodium hypochlorite for 1.0 min and washed three times with sterile distilled water. Tissue pieces (0.5 g) were stirred in 20 mL of saline solution (0.85% NaCl) for 1 h; 20 μL of this suspension were placed in plates with King's B (KB) culture medium and were incubated at 28 °C for 72 h. Five fluorescent colonies from that suspension were isolated in ultraviolet light (25W Transilluminator TFL-40, California, USA); an isolate was selected for the subsequent study from one these colonies which had the same morphological characteristics.

Physiological and biochemical characterization

The fluorescent isolate was characterized for the LOPAT determinative test (Lelliot and Stead, 1966) and as per the protocols described by Schaad *et al.* (2001).

In vitro sensitivity to bactericides

Fifteen commercial bactericides (including four biological products) were evaluated using the dose recommended on the label and a modification of the procedure described by Klančnik *et al.* (2010): 100 μL of a bacterial suspension were inoculated with 10^8 CFU. mL^{-1} in KB medium and evenly distributed on the culture medium surface; then, 0.5-cm wide filter paper discs, previously embedded in the bactericide solution, were placed.

Plates were incubated for 72 h at 28 °C. Sensitivity was determined by the formation of a bacterial growth inhibition halo around the filter paper embedded with the bactericide.

Molecular identification

DNA was obtained from pure colonies using the CTAB method (William and Copeland, 2012). The partial 16S rRNA gene amplification was performed with the 8F:5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R:5'-GGTTACCTTGTTACGACTT-3 primers and under the PCR conditions described by Weisburg (1991). Amplified fragments were sequenced in Macrogene Inc. (Korea); sequences were assembled and edited using the BioEdit Sequence Aligment Editor Software v.7.2.6 (Hall, 2005); the consensus sequence generated was compared with those contained in the National Center for Biotecnology Information (NCBI), with the BLAST nucleotide (dvV5) 2.10.0 option.

***Pseudomonas viridiflava* pathogenicity**

Pathogenicity was evaluated *in vitro* in 15 vegetables and in 8 plants species, under greenhouse conditions. Vegetables were disinfected with soapy water, alcohol (70%), and three washings with sterile distilled water. Inoculation was carried out by 100-μL injection of suspension with 10^8 CFU.mL^{-1}. The treatments were kept in a humidity chamber and incubated at 28 °C for 72 h. Each vegetable was inoculated with three repetitions. The control was inoculated with sterile distilled water. A completely randomized design was used. The plants species were sown in pots with a sterile substrate of agrolite, peat moss, and soil (2:2:1). They were kept in a greenhouse with >60% relative humidity and a 25-30 °C temperature. A BD Plastipak hypodermic syringe was used to inoculate 0.5-mL infiltration of a suspension with 10^8 CFU.mL^{-1} in the abaxial surface of three leaves, which were then kept in a greenhouse for 35 d. Each plant species was inoculated with five repetitions. The control plants were inoculated with sterile distilled water.

RESULTS AND DISCUSSION

Biochemical characterization

The physiological and biochemical characterization of the onion bulb isolated strain showed high similarity to the metabolic profile described in other studies about *P. viridiflava* (Heydari *et al.*, 2012; Sarris *et al.*, 2012). Such characterization is 91% identical to the characteristics of the *P. viridiflava* ATCC 13223 (American Type Culture Collection) reference strain and nine isolates identified as *P. viridiflava* that cause rot in melon (Al-Karabieth *et al.*, 2017) (Table 1).

Using the LOPAT test, the isolated strain from onion produced a fluorescent pigment in KB medium and induced a hypersensitivity reaction in tobacco leaves; it also had negative results for oxidase and arginine dihydrolase; it did not produce levana and caused rot on potato slices.

According to Lelliot and Stead (1966), it was identified as *P. viridiflava* group II of Pseudomonas and had 100% similarity with *P. viridiflava* strain identified in tomato (*Solanum lycopersicum* L.), eggplant (*Solanum melongena* L.), celery (*Apium graveolens* L.), and amaranth (*Amaranthus blitum* L.) (Al-Karablieh *et al.*, 2017).

Table 1. Physiological and biochemical characterization, pathogenicity, and in vitro sensitivity to bactericides of *Pseudomonas viridiflava* isolated from onion.

Test	***P. viridiflava*** **onion**	***P. viridiflava*** **ATCC 13223**[1]	**Pathogenicity**		***In vitro*** **sensitivity to bactericides**	
			In vitro **vegetables**		**Antibiotics and coppers**	
Gram stain	–	ND	Garlic (*Allium sativum* L.)	–	Agricultural Cuprimycin (Oxytetracycline hydrochloride)	–
Fluorescence	+	ND	Broccoli (*Brassica oleracea* var. *Italica* Plenck)	+	Bactrol 2X (Streptomycin and Oxytetracycline	–
Levana	–	–	Cambray onion (*Allium fistulosum* L.)	+	Cuprimycin 17 (Streptomycin sulfate)	–
Oxidase	–	–	Purple onion (*Allium cepa* L.)	+	Agricultural Bactrimicin (Oxytetracycline hydrochloride)	–
Potato rot	+	+	Mushroom (*Agaricus campestris* Fr.)	–	Cuprimycin 500 (Streptomycin sulfate, oxytetracycline, and tribasic copper sulfate monohydrate)	–
Arginine dihydrolase	–	–	Cauliflower (*Brassica oleracea* var. *botrytis* L.)	+	Kasumin (Kasugamycin)	–
Tobacco hypersensitivity	+	+	Ginger (*Zingiber officinale* L.)	–	Final Bacter (Gentamicin sulfate and Oxytetracycline hydrochloride)	+
Catalase	+	ND	Prickly pear cactus (*Opuntia ficus indica* L. Miller)	-	Phyton (Copper sulfate pentahydrate)	–
Gelatin hydrolysis	+	+	Pore (*Allium porrum* L.)	+	Copper oxychloride	–
Nitrate reduction	–	+	Carrot (*Daucus carota* L.)	+	**Biological**	
Starch hydrolysis	–	ND	**Greenhouse plant**		Quatz IV (Quaternary ammonium)	+
Oxidative/fermentative	O	O	Amaranth (*Amaranthus hypochondriacus* L.)	–	Bacter Best (Organic compounds)	–
Use of:			Oats (*Avena sativa* L.)	–	Bioxtermin (*Bacillus thuringiensis* var. *kurstaki*)	–
Glucose	+	+	Jalapeño pepper (*Capsicum annuum* var. *annuum* L.)	+	Serenade powder (*Bacillus amyloliquefaciens*)	+
Lactose	+	ND	Bean (*Phaseolus vulgaris* L.)	+	Fungifree (*Bacillus velezensis*)	–
Maltose	+	+	Tomato (*Solanum lycopersicum* L.)	+		
Cellobiose	+	ND	Purple corn (*Zea mays* L.)	–		
Trehalose	+	ND	Cucumber (*Cucumis sativus* L.)	–		
Dulcitol	–	ND	Weath (*Triticum* sp.)	–		
Inositol	+	+				
Sorbitol	+	+				

[1] ATCC (American Type Culture Collection); Source: Al-Karabieth *et al.*, 2017; ND=Test not determined; O=Oxidative metabolism.

Molecular identification

The BLAST analysis of the 16S rRNA nucleotide sequencing of the strain isolated from onion had 100% identity with the RM207.1a strain (16S rRNA gene) of *Pseudomonas viridiflava* (accession AY604845.1 4) which infected Arabidopsis thaliana (Figure 1).

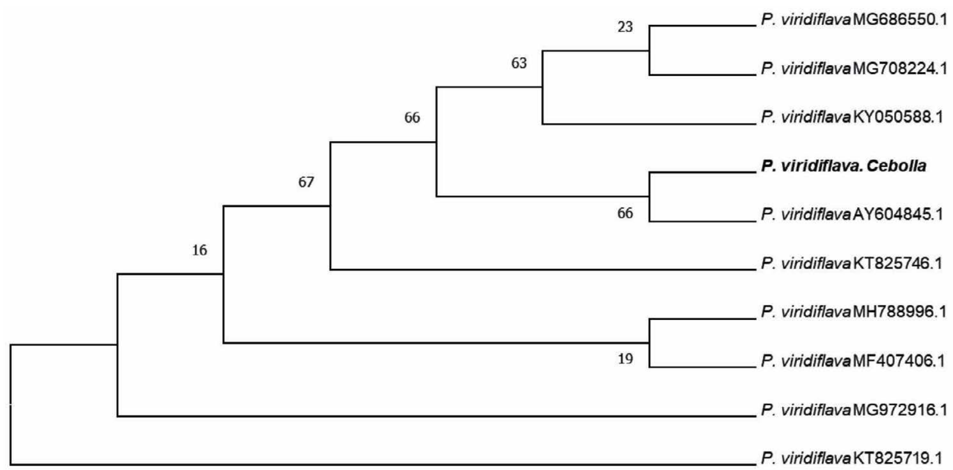

Figure 1. Consensus tree of *Pseudomonas viridiflava* isolated from onion based on partial 16S rRNA gene sequencing, using the Neighbor-Joining (NJ) method. Jukes-Cantor model 500 bootstrap replicates with nine sequences of the gene bank, aligned with the highest similarity index and coverage with Genbank sequences of related bacterial strains.

Other countries have reported significant economic losses as a consequence of the severe onion bulb rot caused by *P. viridiflava* (Gitaitis *et al.*, 1998; Tsuji *et al.*, 2021). In Mexico, *Erwinia chrysanthemi* (de Jesús *et al.*, 2003) and recently *Burkholderia gladioli* (Serret-López *et al.*, 2020) were found to cause rot in onion bulbs. Consequently, this study is the first report of *P. viridiflava* as a causal agent of onion bulb rot in Mexico. P. viridiflava pathogenicity is mainly based on the production of the pectate lyase enzyme —which causes tissue maceration— and the expression of the type III secretion system for the virulence effector production within the host cell (Araki *et al.*, 2007). *P. viridiflava* can survive as an epiphyte on onion leaves; therefore, it is considered as an inoculum source infecting under certain environmental and management conditions. The highest rot severity in onion was associated with epidemics occurring during long rain periods, under excessive fertilization conditions, and with high nitrogen content in leaves (Gitaitis *et al.*, 2003). In the same way, different weed species surrounding onion crops have been identified as the main inoculum source for this pathogen (Gitaitis *et al.*, 1998). *P. viridiflava* infection in citrus leaves was influenced by temperature, humidity, low oxygen concentrations, varietal susceptibility, and pathogen virulence (Beiki *et al.*, 2016).

Pseudomonas viridiflava pathogenicity

The *in vitro* inoculation of *P. viridfilava* isolated from onion in Mexico caused rot in such vegetables as broccoli, spring onions, red onion, cauliflower, leek, and carrot, but not in ginger, mushrooms, garlic, and prickly pear (Table 1). These results are in line with previous reports (Goumans and Chatzaki, 1998); however, based on the bibliography and to our knowledge, this study results provide the first record of experimental rot in spring onions and leek by *P. viridiflava*. *P. viridiflava* caused symptoms of necrosis and foliar chlorosis in jalapeño pepper, tomato, and bean plants, as well as symptoms of plant stunting in beans (Table 1) (Figure 2).

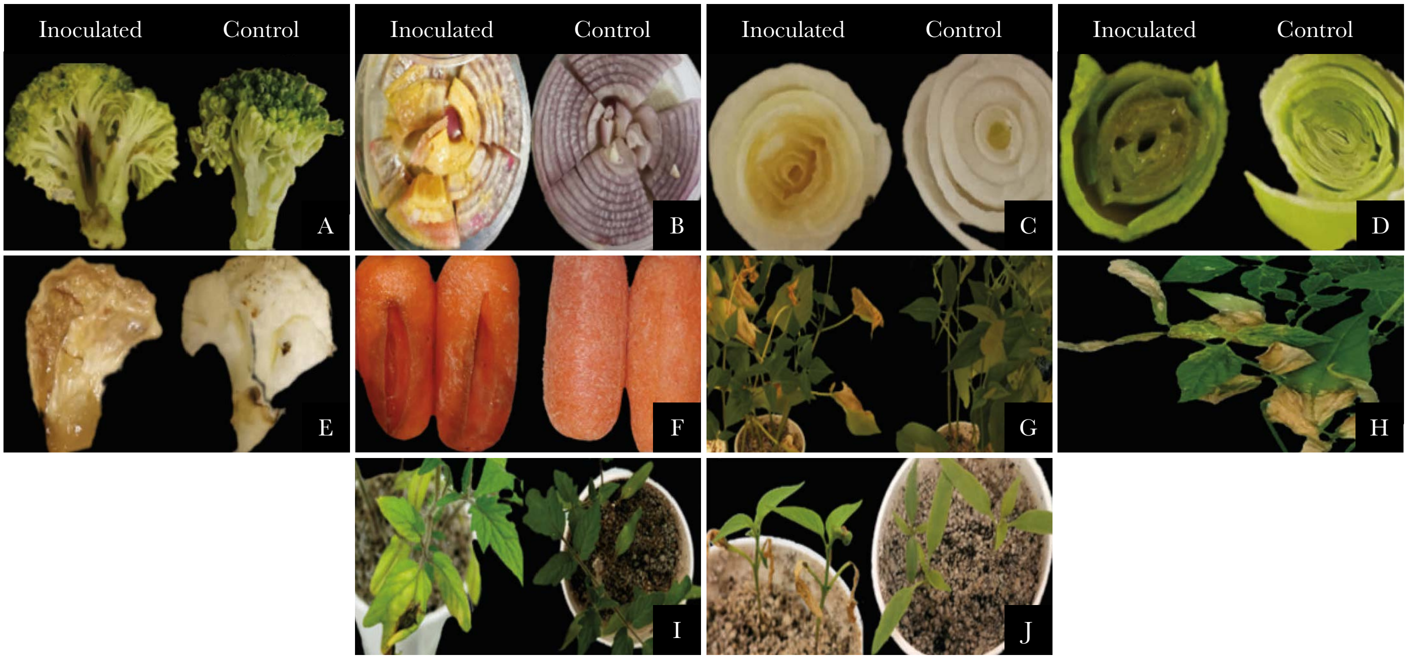

Figure 2. Symptoms caused by *Pseudomonas viridiflava*. *In vitro* rot in vegetables: A) broccoli, B) red onion, C) spring onion, D) leek, E) cauliflower, and F) carrot. Greenhouse plants: G) and H) growth reduction and necrosis in bean leaves, I) necrosis and chlorosis in tomato, and J) necrosis in jalapeño pepper leaves.

The observed symptoms are in line with those reported for these plant species (EPPO, 2019). Growth reduction was observed in infected bean plants; *P. viridiflava* was originally isolated from bean plants with stunting symptoms in Switzerland (Goumans and Chatzaki, 1998). Likewise, stunting symptoms in alfalfa plants (*Medicago sativa* L.) infected with *P. viridiflava* were observed by Heydari *et al.* (2012). *P. viridiflava* pathogenicity and host range varies with the geographic location, plant susceptibility, and pathogen virulence; genetic populations of this pathogen include variants that differ in their proteolytic activity and exopolysaccharide production (Bartoli *et al.*, 2014). No symptoms were developed by vegetables and control plants inoculated with sterile distilled water. From the tissue of infected vegetables and plants, colonies with the same morphological characteristics of *P. viridiflava* were re-isolated in pure culture, fulfilling Koch's postulates. The identity of the inoculated and re-isolated strain was confirmed by PCR, with the amplification and partial 16S rRNA gene sequencing, as well as the above mentioned protocol.

In vitro sensitivity to bactericides

Out of the 14 evaluated bactericides, *P. viridiflava* isolated from onion was sensitive under *in vitro* conditions to Final Bacter (gentamicin + oxytetracycline), Serenade (*Bacillus amyloliquefaciens*), and Quatz IV (quaternary ammonium) (Table 1). The above suggests that this strain has a high resistance capacity to commercial bactericides. Bartoli *et al.* (2014; 2015) identified phenotypes with variable bactericide resistance among *P. viridiflava* strains from different geographic origin and host. However, unlike our results, most strains were susceptible to copper, streptomycin, and oxytetracycline. *B. amyloliquefaciens* has proved to be effective in the control of *P. viridiflava* in Persian buttercups (*Ranunculus asiaticus*) (Fascella *et al.*, 2012); meanwhile, quaternary ammonium is used to disinfect greenhouse facilities and tools.

CONCLUSIONS

Pseudomonas viridiflava is the causal agent of onion rot in Morelos, Mexico. Such vegetables as spring onions and leek may be potential new hosts. *P. viridiflava* isolated from onion in Morelos is resistant under *in vitro* conditions to different antibiotics and coppers. Currently, there is an important legal restriction for the antibiotic application in agriculture; therefore, the use of biological products like Serenade (*B. amyloliquefaciens*) and efficient disinfectants (*e.g.*, quaternary ammonium) should be further evaluated in the field, in order to establish the optimal management of this pathogen.

REFERENCES

Al-Karablieh, N., Mutlak, I., & Al-Dokh, A. (2017). Isolation and identification of *Pseudomonas viridiflava*, the causal agent of fruit rotting of *Cucumis sativus*. *Jordan Journal of Agricultural Sciences, 13*, 79-91. https://doi.org/10.12816/0039717

Araki, H., Innan, H., Kreitman, M., & Bergelson, J. (2007). Molecular evolution of pathogenicity-island genes in *Pseudomonas viridiflava*. *Genetics, 177*, 1031-1041. https://doi.org/10.1534/genetics.107.077925

Bartoli, C., Berge, O., Monteil, C. L., Guilbaud, C., Balestra, G. M., Varvaro, L., ... & Morris, C. E. (2014). The *Pseudomonas viridiflava* phylogroups in the *P. syringae* species complex are characterized by genetic variability and phenotypic plasticity of pathogenicity‐related traits. *Environmental Microbiology, 16*, 2301-2315. https://doi.org/10.1111/1462-2920.12433

Bartoli, C., Lamichhane, J. R., Berge, O., Varvaro, L., & Morris, C. E. (2015). Mutability in *Pseudomonas viridiflava* as a programmed balance between antibiotic resistance and pathogenicity. *Molecular Plant Pathology, 16*, 860-869. https://doi.org/10.1111/mpp.12243

Beiki, F., Busquets, A., Gomila, M., Rahimian, H., Lalucat, J., & García-Valdés, E. (2016). New *Pseudomonas* spp. are pathogenic to citrus. *PLoS One, 11*, e0148796. https://doi.org/10.1371/journal.pone.0148796

Choi, O., Lee, Y., Kang, B., Kim, S., Bae, J., & Kim, J. (2020). Bacterial shoot blight of sweet crab apple caused by *Pseudomonas viridiflava*. *Forest Pathology, 50*, e12603. https://doi.org/10.1111/efp.12603

de Jesús Yáñez, M., Fucikovsky, L., Lorbeer, J. W., González, A., & Aranda, S. (2003). *Erwinia chrysanthemi* Burkholder, McFadden and Dimock and other phytobacteria causal agents of onion (*Allium cepa* L.) bulb decay, and their detection. *Revista Mexicana de Fitopatología, 21*, 189-198.

Fascella, S., Boeri, G. L., Cangelosi, B., Pasini, C., Benuzzi, M., & Curir, P. (2012). Experiences of Biological Control of *Pseudomonas viridiflava* on Cut Flowers of *Ranunculus asiaticus*. In II International Symposium on Horticulture in Europe 1099 (pp. 291-295). https://doi.org/10.17660/actahortic.2015.1099.33

EPPO (European and Mediterranean Plant Protection Organization). (2019). *Pseudomonas viridiflava*. EPPO Global Database. Fecha de consulta: 9 de diciembre de 2019. Disponible en https://gd.eppo.int/taxon/PSDMVF

Gitaitis, R. D., Sanders, F. H., Diaz-Perez, J. C., & Walcott, R. R. (2003). Integrated management of bacterial streak and bulb rot of onion. In *Pseudomonas syringae* and related pathogens (pp. 443-449). Springer, Dordrecht. https://doi.org/10.1007/978-94-017-0133-4_48

Gitaitis, R., MacDonald, G., Torrance, R., Hartley, R., Sumner, D. R., Gay, J. D., & Johnson III, W. C. (1998). Bacterial streak and bulb rot of sweet onion: II. Epiphytic survival of *Pseudomonas viridiflava* in association with multiple weed hosts. *Plant Disease, 82*, 935-938. https://doi.org/10.1094/pdis.1998.82.8.935

Godfrey, S. A. C., & Marshall, J. W. (2002). Identification of cold-tolerant *Pseudomonas viridiflava* and *P. marginalis* causing severe carrot postharvest bacterial soft rot during refrigerated export from New Zealand. *Plant Pathology, 51*, 155–162. https://doi.org/10.1046/j.1365-3059.2002.00679.x

Goumans, D. E., & Chatzaki, A. K. (1998). Characterization and host range evaluation of *Pseudomonas viridiflava* from melon, blite, tomato, chrysanthemum and eggplant. *European Journal of Plant Pathology, 104*, 181-188.

González, A. J., Rodicio, M. R., & Mendoza, M. C. (2003). Identification of an emergent and atypical *Pseudomonas viridiflava* lineage causing bacteriosis in plants of agronomic importance in a Spanish region. *Applied and Environmental Microbiology, 69*, 2936-2941. https://doi.org/10.1128/aem.69.5.2936-2941.2003

Hall, T. (2005). BioEdit: biological sequence alignment editor for Win95/98/NT/2K/XP. URL http://www.mbio. ncsu. edu/bioedit/bioedit. html.

Heydari, A., Khodakaramian, G., & Zafari, D. (2012). Characterization of *Pseudomonas viridiflava* causing alfalfa root rot disease in Hamedan province of Iran. *Journal of Plant Pathology and Microbiology, 3*, 135. https://doi.org/10.4172/2157-7471.1000135

Hunter, J. E., & Cigna, J. A. (1981). Bacterial Blight Incited in Parsnip by *Pseudomonas marginalis* and *Pseudomonas viridiflava*. *Phytopathology, 71*, 1238-1241.

Klančnik, A., Piskernik, S., Jeršek, B., & Možina, S. S. (2010). Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. *Journal of Microbiological Methods, 81*, 121-126. https://doi.org/10.1016/j.mimet.2010.02.004

Lelliott, R. A., & Stead, D. E. (1987). Methods for the Diagnosis of Bacterial Diseases of Plants. Blackwell Scientific Publications,Oxford.

Macagnan, D., Romeiro, R. D. S., Macedo, D. M. D., & Schurt, D. A. (2007). Podridão-mole em pós-colheita de batata (*Solanum tuberosum*) incitada por *Pseudomonas viridiflava*. *Summa Phytopathologica, 33*, 307-308. https://doi.org/10.1590/s0100-54052007000300019

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., ... & Foster, G. D. (2012). Top 10 plant pathogenic bacteria in molecular plant pathology. *Molecular Plant Pathology, 13*, 614-629. https://doi.org/10.1111/j.1364-3703.2012.00804.x

Nuebling, S., Schmidt, H., & Weiss, A. (2016). Variation of the Pseudomonas community structure on oak leaf lettuce during storage detected by culture-dependent and-independent methods. *International Journal of Food Microbiology, 216*, 95-103. https://doi.org/10.1016/j.ijfoodmicro.2015.09.007

Sarris, P. F., Trantas, E. A., Mpalantinaki, E., Ververidis, F., & Goumas, D. E. (2012). *Pseudomonas viridiflava*, a multi host plant pathogen with significant genetic variation at the molecular level. *PloS one, 7*, e36090. https://doi.org/10.1371/journal.pone.0036090

Schaad, N. W., Jones, J. B., & Chun, W. (2001). Laboratory guide for the identification of plant pathogenic bacteria (No. Ed. 3). *American Phytopathological Society* (APS Press).

Serret-López, M., Aranda-Ocampo, S., Espinosa-Victoria, D., Ortiz-Martínez, L. E., & Ramírez-Razo, K. (2021). Polyphasic characterization of *Burkholderia gladioli* isolated from onion and evaluation of its potential pathogenicity for other crops. *Revista Mexicana de Fitopatología, 39*, 21-40. https://doi.org/10.18781/r.mex.fit.2007-2

TecnoAgro. (2017). La producción de cebolla en México. Revista en línea 119. Disponible en 14 https://tecnoagro.com.mx/no.-119/la-produccion-de-cebolla-en-mexico

Tsuji, M., & Shinichi, F. (2021). The first report of bacterial streak and rot of onion caused by *Pseudomonas viridiflava* in Japan. *Plant Disease*, (ja). https://doi.org/10.1094/pdis-02-21-0296-pdn

William, S., Feil, H., & Copeland, A. (2012). Bacterial genomic DNA isolation using CTAB. Sigma, 50 (6876).

Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology, 173*, 697-703. https://doi.org/10.1128/jb.173.2.697-703.1991

AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Water quality in the central zone of the Texcoco aquifer, Mexico

Pascual-Ramírez, F.[1]**; Martínez-Ruiz, A.**[2]**; Prado Hernández, Jorge V.**[3*]**; Cristóbal-Acevedo, D.**[3]

[1] Universidad Nacional Autónoma de México, Instituto de Investigaciones en Ecosistemas y Sustentabilidad Campus Morelia, Morelia, Michoacán, México, C. P. 58190.
[2] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experimental San Martinito, Tlahuapan, Puebla, México, C.P. 74100.
[3] Universidad Autónoma Chapingo, Departamento de Suelos, Chapingo, Texcoco, México, México, C. P. 56230.
* Correspondence: jpradoh@chapingo.mx

ABSTRACT
Objective: To determine water quality in the central zone of the Texcoco aquifer, Mexico, for human use and consumption and agricultural use.
Methodology: The physical, chemical, and biological indicators of the water from 16 wells in urban areas of the central zone of the aquifer were determined. The sampling was carried out, based on the parameters and definition of water quality per use established in the current official Mexican standards.
Results: According to the physical indicators and concentrations of CO_3^{2-}, HCO_3^-, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, PO_4^{3-}, Na^+, K^+, Fe, Cu, and Zn, the water in the sample was suitable for human use and consumption and agricultural use. Based on the cadmium (Cd) concentration, the water was unsuitable for human use and consumption and agricultural use, in 12 and 6 wells, respectively. According to the lead (Pb) concentration, water was suitable for agricultural use in the 16 wells studied; however, it was unsuitable for human use and consumption in any of the wells. In eight wells analyzed, the presence of fecal coliforms was lower than the permissible limit for agricultural use.
Implications: This study complements researches done in other areas of the aquifer. The causes of water pollution are unknown and researches about the vulnerability of the aquifer and the possible polluting sources should be carried out.
Conclusions: The water from the aquifer in the central zone has limitations for human use and consumption and agricultural use, as a consequence of the high Cd and Pb concentrations and its microbiological quality.

Keywords: Aquifer contamination, groundwater, wells.

Citation: Pascual-Ramírez, F., Martínez-Ruiz, A., Prado Hernández, Jorge V., & Cristóbal-Acevedo, D. (2022). Water quality in the central zone of the Texcoco aquifer, Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v15i1.2118

Editor in Chief: Dr. Jorge Cadena Iñiguez

Received: September 4, 2021.
Accepted: January 13, 2022.
Published on-line: February 5, 2022.

Agro Productividad, *15*(1). January. 2022. pp: 129-136.

INTRODUCTION

Mexico has 653 aquifers that supply 38.9% of the water for various uses. In 2020, 157 overexploited aquifers were reported (CONAGUA, 2021). Several aquifers present inadequate water quality —in physical, chemical or biological terms, or in all three of them—, as a result of various factors that may involve human activities or of the mineral constitution of their rocks (Foster and Hirata, 1988). In the Mezquital Valley, Hidalgo, Mexico, inadequate biological quality of shallow groundwater was recorded; although it was used for domestic purposes and irrigation, it had been polluted by wastewater from Mexico City (Downs, Cifuentes-Garcia, and Suffet, 1999). Cardona, Carrillo, and Armienta (1993) found that the high content of heavy elements in the water of the aquifer of the City of San Luis Potosí, Mexico, is largely caused by the composition of its rocks; human activities have a minor effect. A similar situation was recorded in the Meoqui-Delicias aquifer in the state of Chihuahua, Mexico, where there are high arsenic concentrations (Espino-Valdez, Barrera-Prieto, and Herrera-Peraza, 2009). In other aquifers, groundwater may be polluted by landfill leachate (Pérez-López, Vicencio-de La Rosa, Alarcón-Herrera, & Vaca-Mier, 2002) or by the transit of wastewater in unlined canals (Ramírez, Robles, Sainz, Ayala, and Campoy, 2009; Ramírez-Flores, Robles-Valderrama, Ayala-Patiño, and Martínez-Rodríguez, 2012; Robles, Ramírez, Durán, Martínez, and González, 2013).

A study carried out in the metropolitan area of Mexico City by Soto-Galera, Marai-Hiriart, and Bojórquez-Tapia (1990) showed that the Texcoco aquifer had the largest number of wells in the area. However, groundwater pollution was not a risk, because there were few pollution sources —such as landfills or fuel deposits, gas stations, and industries. Nevertheless, that situation drastically changed in recent years. For example, Guzmán-Quintero, Palacios-Vélez, Carrillo-González, Chávez-Morales, and Nikolskii-Gavrilov (2007) observed that biological quality of wastewater from the Texcoco River was unsuitable for agricultural, public, and urban uses.

The Texcoco aquifer covers an area of 934 km^2, and it has a predominantly urban public use. This aquifer is very important, because it supplies a large population: in 2015, 3,105,559 people lived within its limits (DOF, 2019). In addition to water for human consumption, the aquifer supplies different productive activities, through 921 wells distributed in fourteen municipalities (DOF, 2015).

It faces severe overexploitation problems, with a deficit of 111.02 hm^3 (DOF, 2020). Studies about the aquifer's water quality are scarce and isolated, despite the fact that most of the surface runoff —sewage transported through unlined canals from towns, farms, and other activities—could be a source of groundwater pollution. One of the most recent studies was carried out by Martínez-Luna (2014), who focused solely on the wells of the municipality of Texcoco, finding some indications of inadequate water quality.

The continuous fall of the Static Water Level in the Texcoco aquifer (an average of 1.21 m per year) (Carrillo, Gómez, Valle, and Prado, 2016), the rise in water demand, and the increase of sewage discharges and wastewater from other activities in unlined canals pose the imperative need to find out the annual evolution of the water quality in the said aquifer. The information obtained from the central zone of the aquifer will expand the information about the water quality in the Peñón-Texcoco and Lago Nabor Carrillo areas (DOF, 2019). The information could be useful for the authorities in charge of implementing a sustainable management plan for the aquifer, which would guarantee adequate quality water. Water samples from 16 wells of the Texcoco aquifer were analyzed; those wells were located in places with a high population density and close to unlined surface runoffs. Their quality for human use and agricultural consumption was determined according to current official Mexican standards; physical, chemical, biological, and heavy metals indicators of negative impact on human health were taken into account.

MATERIALS AND METHODS

Location of the Texcoco aquifer

The Texcoco aquifer (key 1507) is located in the central-eastern portion of the Estado de México, within the Valley of Mexico hydrological basin (19° 18' and 19° 38' N and 98° 39' and 99° 03' W), with an area of 934 km^2. It comprises the municipalities of Chicoloapan, Chimalhuacán, Chiconcuac, Papalotla, and Texcoco; it covers almost all of Atenco, Chiautla, Ixtapaluca, Nezahualcóyotl, La Paz, and Tepetlaoxtoc, and part of Acolman, Ecatepec de Morelos, and Tezoyuca. There are ten main surface rivers on the aquifer, the vast majority of which are unlined and carry stormwater runoff and wastewater to the federal zone of Ex-Lago de Texcoco. Its final destination is the collector drain of the Valley of Mexico (DOF, 2019).

Water sampling and determinations

Water samples were taken from 16 wells, located in areas of high population density in the municipalities of Atenco, Chicoloapan, Chiautla, Chimalhuacán, Ixtapaluca, La Paz, Tepetlaoxtoc, and Texcoco (Figure 1). Physical, chemical, and heavy metal indicators were determined for the 16 samples. Biological determinations were made in eight of them, whose water comes from wells located near unlined surface runoffs that carry sewage or waste from other activities. The samples were collected according to the guidelines established in the NOM-014-SSA1-1993 standard (DOF, 1994), in the months of June and July 2016.

Three repetitions were used to obtain the potential of hydrogen (pH), electrical conductivity (CE), and total dissolved solids (STD) —which were considered as physical indicators. Chemical determinations were also made with three repetitions and the concentrations of calcium $\left(Ca^{2+}\right)$, magnesium $\left(Mg^{2+}\right)$, sodium $\left(Na^{+}\right)$, potassium $\left(K^{+}\right)$, carbonate $\left(CO_3^{2-}\right)$, bicarbonate $\left(HCO_3^{-}\right)$, chlorides $\left(CL^{-}\right)$, sulfates $\left(SO_4^{2-}\right)$, and phosphates $\left(PO_4^{3-}\right)$ were obtained. Five repetitions were made to obtain the concentrations of the following heavy metals: cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), and zinc (Zn). With regard to the biological characterization, the most likely number of coliform bacteria per 100 mL (NMP 100 mL^{-1}) was determined based on three repetitions.

The determinations were made in specialized laboratories in Mexico, applying the guidelines established in current Mexican regulations, issued by the Secretaría de Comercio y Fomento Industrial and by the Secretaría de Economía in the Diario Oficial de la Federación. Heavy metals were obtained by atomic absorption spectrophotometry, pH and CE by potentiometry, SDT by a CE ratio, Ca^{2+}, Mg^{2+}, CO_3^{2-}, HCO_3^{-}, and Cl^{-} by volumetry, Na^{+} and K^{+} by flame spectrometry, and SO_4^{2-} and PO_4^{3-} by visible spectrometry (standards: NMX-AA-042-SCFI-2015, NMX-AA-072-SCFI-2001, NMX-AA-004-SCFI-2013, NMX-AA-006-SCFI-2010, NMX-AA-008-SCFI-2011, PROY-NMX-AA-029/1-SCFI-2008, PROY-NMX-AA-034/1-SCFI-2008, PROY-NMX-AA-051/1-SCFI-2008, PROY-NMX-AA-051/1-SCFI-2008, PROY-NMX-AA-051/2-SCFI-2008, PROY-NMX-AA-051/4-SCFI-2008, NMX- AA-115-SCFI-2001).

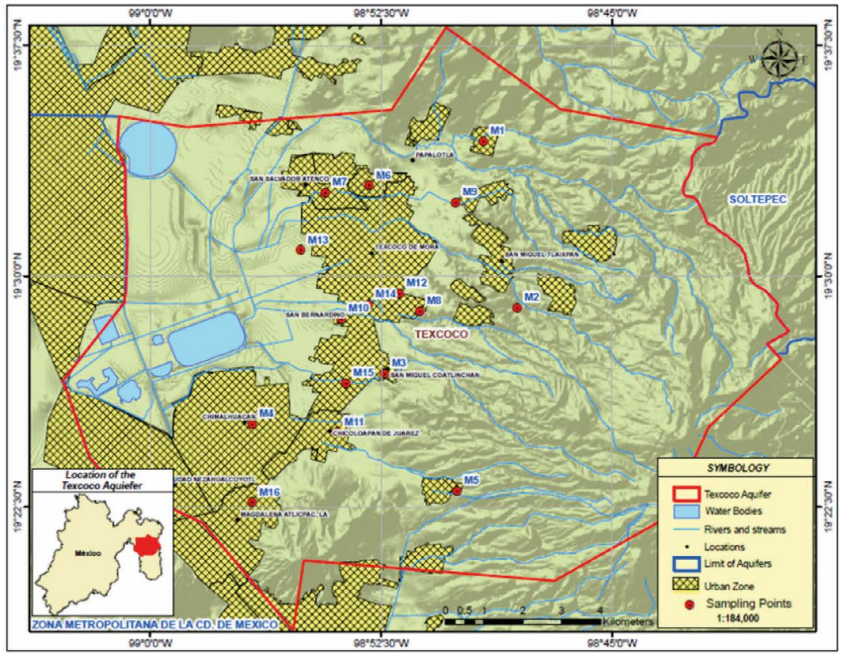

Figure 1. Limit of the Texcoco aquifer and location of the sampling wells.

Water quality analysis

The suitability of the water for human use and consumption was defined based on the permissible limits established in the modifications to the NOM-127-SSA1-1994 standard (DOF, 2000) and, for agricultural irrigation, based on the values established by the Ley Federal de Derechos de Agua 2016 (CONAGUA, 2016) and by the Criterios Ecológicos de Calidad del Agua of the CE-CCA-001/89 standard (DOF, 1989).

RESULTS AND DISCUSSION

According to the pH, CE, and STD results (Table 1), the water from the 16 wells analyzed was suitable for human use and consumption. Only in two of them (Chimalhuacán and La Pastoría), the CE value makes it unsuitable for agricultural irrigation.

In the 16 wells, the Ca^{2+}, Mg^{2+}, CO_3^{2-}, HCO_3^-, Cl^-, Na^+, K^+, SO_4^{2-} and PO_4^{3-} concentrations (Table 2) indicated that water was unsuitable for human use and consumption and agricultural use. These results are reliable, because the error of the ionic balance between anions and cations was less than the $\pm$8.0% allowed (Custodio and Llamas, 1976).

The chlorides and sulfates concentrations in the El Cooperativo well increased by 10.0 and 1.9 mg L^{-1}, respectively, with regard to the findings of Martínez (2004); meanwhile, in the San Luis Huexotla well, the said concentrations increased by 7.0 and 2.3 mg L^{-1}, respectively. In the remaining wells of the municipality of Texcoco, the pH, CE, Na^+, and coliforms did not present an appreciable difference.

The determinations of heavy elements resulted in minimum and maximum standard deviations of 0.0009 and 0.00128 (Zn), 0.00025 and 0.00096 (Fe), 0.00019 and 0.00043 (Cu), 0.00042 and 0.00103 (Cd), 0.0009 and 0.0021 (Pb) mg L^{-1}; therefore, the reliability of the results has been established.

Table 1. Results of the physical indicators.

Sample	Location	Municipality	pH	CE (μS cm^{-1})	STD (mg L^{-1})
M1	Tepetlaoxtoc	Tepetlaoxtoc	7.6	207.0	84.6
M2	San Miguel Tlaixpan	Texcoco	7.6	171.0	71.1
M3	San Miguel Coatlinchán	Texcoco	7.2	182.0	75.7
M4	Chimalhuacán	Chimalhuacán	7.6	503.0	210.0
M5	Coatepec	Ixtapaluca	7.1	102.0	42.6
M6	San Andrés	Chiautla	7.6	267.0	103.0
M7	La Pastoría	Atenco	7.1	644.0	267.0
M8	San Luis Huexotla	Texcoco	6.9	774.0	32.3
M9	San Joaquín Coapango	Texcoco	7.1	412.0	171.0
M10	San Bernardino	Texcoco	7.0	456.0	193.0
M11	Santiago Cuautlalpan	Texcoco	7.2	319.0	133.0
M12	Tecamachalco	La Paz	7.5	297.0	125.0
M13	San Vicente	Chicoloapan	7.0	329.0	135.0
M14	U. H. Emiliano Zapata ISSSTE	Texcoco	7.1	182.0	75.5
M15	Colonia Lázaro Cárdenas	Texcoco	7.5	306.0	125.0
M16	El Cooperativo	Texcoco	7.2	152.0	63.5

Table 2. Results of the chemical indicators.

Sample	Concentration (mg L^{-1})							
	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	HCO_3^-	Cl^-	SO_4^{2-}	PO_4^{3-}
M1	39.01	17.01	24.2	9.10	103.70	11.09	0.012	0.002
M2	13.93	4.88	25.8	8.40	70.15	11.09	0.007	0.012
M3	20.23	8.11	26.6	7.80	84.18	11.09	0.014	0.006
M4	53.62	89.63	37.7	11.50	210.45	28.84	0.073	0.018
M5	21.89	7.34	15.4	5.40	32.33	19.97	0.031	0.009
M6	43.17	28.79	27.8	11.60	134.20	15.53	0.011	0.020
M7	63.49	164.61	35.6	14.90	289.75	28.84	0.103	0.000
M8	21.39	7.49	14.9	5.90	45.75	11.09	0.007	N/D
M9	72.14	60.41	31.1	11.10	227.53	11.09	0.014	0.029
M10	66.69	67.98	31.4	9.20	195.20	24.41	0.050	0.013
M11	48.38	31.07	30.6	9.70	127.49	19.97	0.010	0.023
M12	20.61	30.29	30.4	8.40	106.75	24.41	0.012	0.005
M13	33.94	29.77	30.6	9.70	127.49	15.53	0.016	0.014
M14	20.66	5.56	26.9	8.30	62.83	15.53	0.018	0.015
M15	50.98	33.95	27.4	9.60	137.25	11.09	0.016	0.018
M16	16.14	6.46	23.5	6.50	68.32	11.09	0.011	0.004

The water from the 16 wells was suitable for human use and consumption and agricultural use, since their Zn, Fe and Cu concentrations were lower than the 2.0, 0.3 and 0.2 mg L^{-1} limits established for human use and consumption (Figure 2). In 12 of the

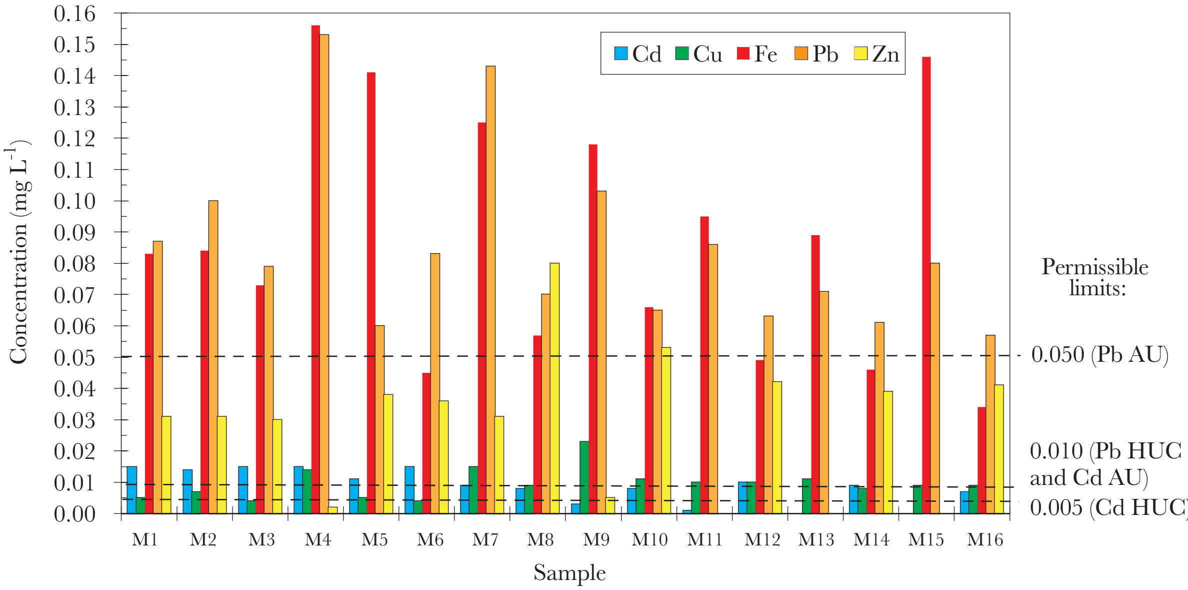

Figure 2. Cadmium (Cd) and lead (Pb) maximum concentrations and permissible limits for human use and consumption (HUC) and agricultural use (AU).

wells (1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, and 16), water was unsuitable for human use and consumption, because Cd exceeded the maximum permissible value of 0.005 mg L^{-1}. Likewise, it was unsuitable for agricultural use in six of them (1, 2, 3, 4, 5, and 6), because it exceeded the permitted Cd concentration of 0.01 mg L^{-1}. The water from three of the wells was acceptable by a narrow margin (7, 12, and 14). With regard to maximum permissible Pb levels, water was unsuitable for human use and consumption (0.01 mg L^{-1}), but it was suitable for agricultural use (0.50 mg L^{-1}) in all the wells.

The predominant subsoil material of the first 40 m of the Texcoco aquifer is made up of clays with high porosity and low permeability (DOF, 2019) that can protect it from surface pollution (Ramos-Leal, Medrano-Noyola, Tapia-Silva, Silva-Garcia, and Reyes-Garcia, 2012). These characteristics of the aquifer should protect it from pollution by Cd and Pb, and other pollutants. However, other characteristics of the aquifer —such as a poor design and installation of the sanitary seal in extraction wells and the fractures generated by the differential subsidence, as a result of the overexploitation of the aquifer (Vargas and Ortega-Guerrero, 2004)— must be taken into consideration, because they can increase the probability of pollution by surface sources (Hernández-Espriú *et al.*, 2014; Hizar-Álvarez, Carrillo-Rivera, Ángeles-Serrano, Hergt and Cardona, 2004). In some aquifers where subsidence has not been considered, a contradiction between the vulnerability and quality of groundwater has been reported (Hernández-Espriú *et al.*, 2014; Ramos-Leal *et al.*, 2014).

The water from the eight wells that were subject to a biological analysis is unsuitable for human use and consumption, as a result of the presence of total coliforms; however, it can be used for agricultural irrigation —whose maximum permissible limit is 1,000 NMP 100 mL^{-1} (Table 3). The presence of coliforms is caused by anthropogenic pollution; they have also been detected in other aquifers in Mexico (Pérez *et al.*, 2002; Ramírez *et al.*,

Table 3. Result of the microbiological indicator.

Sample	M1	M2	M3	M4	M5	M6	M7	M8
Total coliforms (NMP 100 mL^{-1})	2.0	<2.0	9.0	<2.0	5.0	<2.0	49.0	8.0

2009; Ramírez *et al*., 2012; Robles *et al*., 2013), where sewage is discharged into the surface runoff without lining. Indeed, in the unlined stream of the Texcoco River, the presence of fecal coliforms far exceeds the permissible limits for irrigation and for public and urban use (Guzmán *et al*., 2007).

The results of this research —along with the report about the poor water quality for human use and consumption in the Peñón-Texcoco and Lago Nabor Carrillo areas, as a consequence of its chlorides, STD, Fe, and Mn contents (DOF, 2019)— suggest an inadequate groundwater quality in a large extent of the Texcoco aquifer.

CONCLUSIONS

The water quality from the 16 wells analyzed was suitable for human use and consumption and agricultural use, based on the physical-chemical parameters analyzed and certain heavy metals (Zn, Fe, and Cu) it contains. Regarding the Cd content, it was unsuitable for human use and consumption in 75% of the wells studied and unsuitable for agricultural use in 38% of them. In all the wells studied, water was unsuitable for human use and consumption, but it was suitable for agricultural use, when its Pb content and presence of total coliforms were taken into account. The sustained depletion and poor water quality of the aquifer suggest the urgent implementation of responsible water extraction policies and proper wastewater management.

REFERENCES

Cardona, B.A., Carrillo, R.J.J., & Armienta, H.M. A. (1993). Elemento traza: contaminación y valores de fondo en aguas subterráneas de San Luis Potosí, SLP, México. *Geofísica Internacional, 32*(2), 277-286.

Carrillo, G.M., Gómez, P.Y.A., Valle, G.S. & Prado, H.J.V. (2016). Behavior of groundwater levels in Texcoco Aquifer (1507) when they are lowered by excessive pumping from 1968 through 2014. En 2016 ASABE Annual International Meeting, 17 a 20 de julio de 2016 (Paper No. 2462745). Orlando, Fl., USA: American Society of Agricultural and Biological Engineers. doi:10.13031/aim.20162462745

CONAGUA (Comisión Nacional del Agua). (2016). Ley Federal de Derechos: disposiciones aplicables en material de aguas nacionales 2016.

CONAGUA (Comisión Nacional del Agua). (2021). Detalle de los acuíferos en México 2020: Aguas del Valle de México. Sistema Nacional de Información del Agua.

Custodio, E., & Llamas, M.R. (1976). Hidrología Subterránea. Madrid, España: Omega.

Downs, T.J., Cifuentes-García, E., & Suffet, I.M. (1999). Risk Screening for Exposure to Groundwater Pollution in a Wastewater Irrigation District of the Mexico City Region. *Environmental Health Perspectives, 107*(7), 553-561. doi: 10.1289/ehp.99107553

DOF (Diario Oficial de la Federación). (1989, 13 diciembre). Acuerdo por el que se establecen los Criterios Ecológicos de la Calidad del Agua CE-CCA-001/89. Secretaría de Desarrollo Urbano y Ecología.

DOF (Diario Oficial de la Federación). (1994, 12 noviembre). PROYECTO de Norma Oficial Mexicana NOM-014-SSA1-1993, Procedimientos Sanitarios para el muestreo de agua para uso y consumo humano en sistemas de abastecimiento de agua públicos y privados. Secretaría de Salud.

DOF (Diario Oficial de la Federación). (2000, 22 noviembre). Modificación a la Norma Oficial Mexicana NOM-127-SSA-1994, Salud Ambiental. Agua para uso y consumo humano. Límites permisibles de calidad y tratamiento a que debe someterse el agua para su potabilización. Secretaría de Salud.

DOF (Diario Oficial de la Federación). (2019, 13 septiembre). ACUERDO por el que se dan a conocer los resultados del estudio técnico de las aguas nacionales subterráneas del acuífero Texcoco, clave 1507, en el Estado de México, Región Hidrológico-Administrativa XIII, Aguas del Valle de México. Secretaría de Medio Ambiente y Recursos Naturales.

DOF (Diario Oficial de la Federación). (2020, 17 septiembre). ACUERDO por el que se actualiza la disponibilidad media anual de agua subterránea de los 653 acuíferos de los Estados Unidos Mexicanos, mismos que forman parte de las regiones hidrológico-administrativas que se indican. Secretaría de Medio Ambiente y Recursos Naturales.

Espino-Valdez, M. S., Barrera-Prieto, Y., & Herrera-Peraza, E. (2009). Presencia de arsénico en la sección norte delacuífero Meoqui-Delicias del estado de Chihuahua, México. *TECNOCIENCIA Chichuahua, 3*(1), 8-18.

Foster, E., & Hirata, R. (1988). Groundwater pollution risk assessment, a methodology using available data. Lima, Perú: Panamerican Center for Sanitary Engineering and Environmental Sciences, World Health Organization, Panamerican Health Organization, Environmental Health Program.

Guzmán-Quintero, A., Palacios-Vélez, O. L., Carrillo-González, R., Chávez-Morales, J., & Nikolskii-Gavrilov, I. (2007). La contaminación del agua superficial en la Cuenca del Río Texcoco, México. *Agrociencia, 41*, 385-393.

Hernández-Espriú, A., Reyna-Gutiérrez, J.A., Sánchez-León, Cabral-Cano, E., Carrera-Hernández, J., Martínez-Santos, P., …Colombo, D. (2014). The DRASTIC-Sg model: an extension to the DRASTIC approach for mapping groundwater vulnerability in aquifers subject to differential land subsidence, with application to Mexico City. *Hydrogeology Journal, 22*,1469–1485. doi:10.1007/s10040-014-1130-4

Huizar-Alvarez, R., Carrillo-Rivera, J.J., Ángeles-Serrano, Hergt, T., & Cardona, A. (2004). Chemical response to groundwater extraction southeast of Mexico City. *Hydrogeology Journal, 12*, 436–450. doi:10.1007/s10040-004-0343-3

Martínez-Luna, D. (2014). Análisis físico-químico y bacteriológico de los pozos de agua potable de Texcoco, Estado de México. Tesis de Licenciatura, Universidad Autónoma Chapingo, Chapingo, Texcoco, Estado de México, México.

Pérez-López, M., Vicencio-de La Rosa, M., Alarcón-Herrera, M., & Vaca-Mier, M. (2002). Influencia del basurero municipal en la calidad del agua del acuífero de la ciudad de Durango, México. *Revista Internacional de Contaminación Ambiental,18*(3),111-116.

Ramírez, E., Robles, E., Sainz, M. G., Ayala, R., & Campoy E. (2009). Calidad microbiológica del acuífero de zacatepec, morelos, méxico. *Revista Internacional de Contaminación Ambiental, 25*(4), 247-255.

Ramírez-Flores, E., Robles-Valderrama, E., Ayala-Patiño., R., & Martínez-Rodríguez, B. (2012). Calidad amebológica del agua de pozos utilizados para suministro de agua potable en el Estado de Hidalgo. *Ingeniería,16*(3), 219-228.

Ramos-Leal, J.A., Medrano-Noyola, C., Tapia-Silva, F.O., Silva-García, J.T., & Reyes-García, L.R. (2012). Assessing the inconsistency between groundwater vulnerability and groundwater quality: the case of Chapala Marsh, Mexico. *Hydrogeology Journal, 20*, 591–603. doi:10.1007/s10040-011-0823-1

Robles, E., Ramírez, E., Durán, A., Martínez, M., & González, M. (2013). Calidad bacteriológica y fisicoquímica del agua del acuífero Tepalcingo-Axochiapan, Morelos, México. *Avances en Ciencias e Ingeniería, 4*(1), 19-28.

Soto-Galera, E., Marai-Hiriart, M., & Bojórquez-Tapia, L. A. (2000). Entidades de la Zona Metropolitana de la Ciudad de México propensas a la contaminación de agua subterránea. *Boletín del instituto de Geografía de la UNAM, 43*, 60-75.

Vargas, C., & Ortega-Guerrero, A. (2004). Fracture hydraulic conductivity in the Mexico City clayey aquitard: Field piezometer rising-head tests. *Hydrogeology Journal, 12*, 336–344. doi:10.1007/s10040-003-0302-4

AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Morphology and forage quality in buffel, rhodes, and blue grama grasses in Valle del Mezquital

Velázquez-Martínez Mauricio[1]; **Rodríguez-Ortega Leodan T.**[2]; **Rojas-García Adelaido R.**[3]; **Enríquez-Quiroz Javier F.**[4]; **Santiago-Hernández Francisco**[1]; **Ramírez-Rojas Sergio G.**[5]; **Hernández-Guzmán Filogonio J.**[2*]

[1] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuaria (INIFAP), Campo Experimental San Luis, Carretera San Luis Potosí-Matehuala km 14.5, Soledad de Graciano Sánchez, San Luis Potosí, México, C.P. 78431.

[2] Ingeniería en Producción Animal de la Universidad Politécnica de Francisco I. Madero, Domicilio Conocido SN, Tepatepec, Francisco I. Madero, Hidalgo, México, C.P. 42660.

[3] Universidad Autónoma de Guerrero, Carretera Acapulco-Pinotepa Nacional km 197, Cuajinicuilapa, Guerrero, México, C.P. 41940.

[4] INIFAP Campo Experimental La Posta, Carretera Federal Veracruz-Córdoba km 22.5, Paso del Toro, Municipio de Medellín, Veracruz, México, C.P. 94277.

[5] INIFAP Campo Experimental Zacatepec, Km. 0.5 Carretera Zacatepec, Galeana - Cuautla S/N, Centro, Zacatepec, Morelos, México, C.P. 62780.

* Correspondence: fjesushg@hotmail.com

ABSTRACT

This study aims to evaluate the production and quality of buffel (*Pennisetum ciliare*), rhodes (*Chloris gayana*), and blue grama (*Bouteloua gracilis*) forage with three defoliation dates —at 50, 80, and 110 days after regrowth (dar)— in Valle del Mezquital. Crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), total digestible nutrients (TDN), relative feed value (RFV), and metabolizable energy (ME) were determined. A completely randomized design with three repetitions, a Tukey test for mean separation (α=0.05), and SAS Proc GLM for data analysis were used. The highest forage production occurs in the rhodes forage at 110 dar (12,936 kg DM ha^{-1}). The highest CP (10.6%) was found in the buffel forage at 50 dar; in the rhodes forage, both values (80 and 110) were lower than 7%. The highest RFV was obtained by exotic grasses; however, higher TDN was recorded for the blue grama grass. Rhodes grass obtained the lowest ME at 80 dar (1.76), while the highest ME was obtained by buffel at 50 dar (1.91). The three varieties can be defoliated when the plants show intermediate yield and nutritional value, *i.e.*, 80 days after the regrowth begins; nevertheless, buffel and rhodes should be defoliated at 50 dar.

Keywords: forage grasses, *Bouteloua*, grasslands.

Citation: Velázquez-Martínez M., Rodríguez-Ortega, L. T., Rojas-García, A. R., Enríquez-Quiroz, J. F., Santiago-Hernández, F., Ramírez-Rojas S. G., & Hernández-Guzmán F. J. (2022). Morphology and forage quality in buffel, rhodes, and blue grama grasses in Valle del Mezquital. *Agro Productividad*. https://doi.org/10.32854/agrop.v15i1.2158

Editor in Chief: Dr. Jorge Cadena Iñiguez

Received: September 17, 2021.
Accepted: January 3, 2022.
Published on-line: February 5, 2022.

Agro Productividad, 15(1). January. 2022. pp: 137-143.

INTRODUCTION

Natural grasslands cover less than 15% of the Chihuahuan Desert, approximately 5% of which is occupied by blue grama grass (*Bouteloua gracilis*) (Granados-Sánchez *et al*., 2011). Buffel grass has spread throughout the whole Mexican Northeast; according to Gómez *et al*. (2007), 104,783 ha, 27,167 ha, and 600,000 ha were planted in Coahuila, Nuevo León,

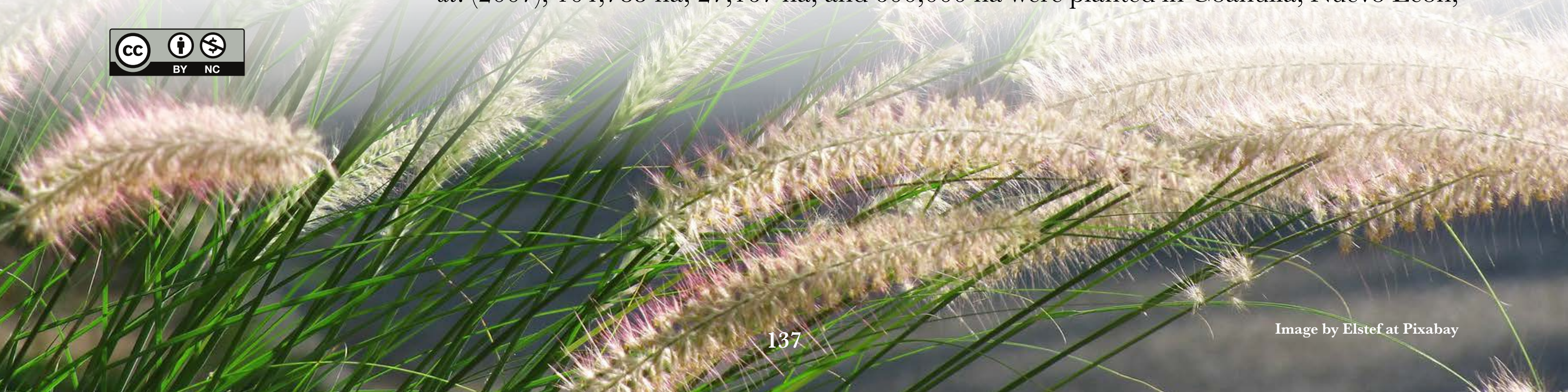

Image by Elstef at Pixabay

and Tamaulipas, respectively. Cattle are fond of buffel and other grasses from semi-arid areas; however, the cells of each plant of older regrowth grass have a limited access to nitrogen, cellulose, hemicellulose, and cell content, because lignin thickens the wall and, therefore, makes it less accessible to microbial enzymes (Abas *et al.*, 2005; Castillo-López and Domínguez-Ordoñez, 2019). The main determining tissue in the digestibility of the C_3 and C_4 grasses (*Poaceae*) is the abundance of mesophyll (Buxton and Redfearn, 1997). Meanwhile, according to Bernal-Flores *et al.* (2017), the greater aggregation of lignin inside oats gramaoccurs mainly in xylem, bundle sheath, and bundle sheath extension. This determines digestibility and, together with ammonium, limits the formation of volatile fatty acids and metabolic protein (Okoruwa and Igene, 2014). The relative feed value (RFV) is an index for the prediction and classification of the nutritional quality of forage, based on the combined analysis of expected animal consumption, quantity of forage, and dry matter digestibility (Amiri *et al.* 2012; Núñez *et al.*, 2014). Blue grama grass is one of the most widely distributed native mexican grasses in semi-arid of Mexico; buffel grass is the most widely spread species that has been introduced in northeastern Mexico; finally, rhodes grass is able to adapt to temperate semi-arid highlands environments. However, there is no nutritional and morphological composition information about their phenological stages. The objective of this research was to evaluate the morphological, chemical, and nutritional composition of defoliated blue grama, buffel, and rhodes grass at 50, 80, and 110 days after regrowth.

MATERIALS AND METHODS

The experiment was carried out in facilities of the Universidad Politécnica de Francisco I. Madero, Hidalgo, Municipality of Francisco I. Madero, Hidalgo, which is located at 2,017 masl, has a dry temperate climate, with an average annual temperature of 16 °C, and 550 mm of precipitation. The site has a Vertisol type soil with clayey texture (Granados-Sánchez *et al.*, 2001). The work was carried out from April 1 to November 9, 2019.

Plant materials and pasture management

The experimental seeds of buffel grass (var. Titan) and blue grama (var. Cecilia) —provided by INIFAP-San Luis— were cultivated in the spring-summer of 2016 productive cycle, while the seeds of rhodes grass (var. Bell) —purchased from a commercial establishment— were cultivated in the 2017 agricultural cycle. The three grass species were established in July 2017 with a density of 15 kg pure live seed (PLS) ha^{-1} in three areas of 3.5 × 1.5 m per grass. There were four strip irrigations with an irrigation capacity of 1 L h^{-1}, to which 40 mm m^{-2} were applied on April 1 and 20, as well as on May 3 and 20, 2019. No fertilizer was applied during the whole study period. The uniformization cut was made on July 17, 2019, 10 cm above the ground. The experimental cuts were made at 50, 80, and 110 days after the standardization cut (dar) in three random sites per variety. On each sampling date, grass was harvested with scissors at 10 cm above the ground and then divided into leaves, stems, inflorescences, and dead material. Later, each component was placed in previously labeled paper bags, which were placed in a forced air stove at 60 °C until they reached constant weight. A H-5851 Ohaus Scout scale was used to weigh the samples. Total forage production was the sum of all morphological components. Afterwards, the components were mixed and ground using a 400 Pulvex pulverizer mill (<1.0 mm) and placed in paper bags for subsequent bromatological analysis. The analysis was performed in triplicate by a certified laboratory (AgroLab de México SA de CV, Gómez Palacio, Durango), in order to obtain the following values: crude protein (CP, %), neutral detergent fiber (NDF; %), acid detergent fiber (ADF; %), total digestible nutrients (TDN; %), relative feed value (RFV), metabolizable energy (ME; Mcal kg feed^{-1}), net energy for maintenance (NE_m), net energy for lactation (NE_l) and net energy gain (NE_g).

The records of monthly temperature and precipitation (Figure 1) were obtained from a meteorological station located 3 km away from the experimental site. The experimental design was completely randomized with three repetitions. The results were analyzed with SAS Proc GLM (2009) and subject to the Tukey test (α=0.05).

RESULTS AND DISCUSSION

During the study, the average annual temperature was 18.5 °C, the annual precipitation was 395 mm, and no frost was recorded.

Forage production

The highest forage production was observed in rhodes at 110 dar (12,936 kg ha^{-1}; Table 1), followed by buffel and blue grama (P<0.001) at 50 and 80 dar. These results confirm that buffel and rhodes (grasses of African origin) outnumber native American grasses by 1.7 and 2.7 times, respectively, as reported by Ibarra *et al.* (2005).

Leaf:stem ratio

At 50 dar, the leaf:stem ratio (Table 1) was higher for Buffel (1.68; P<0.01), while it was similar between rhodes and blue grama (P>0.05). At 110 dar, blue grama grass had a better ratio (0.94; P<0.01) than the introduced grasses, and rhodes and buffel had a similar ratio (P>0.05). The leaf:stem ratio of African grasses drastically decreases in older regrowths, unlike blue grama; this characteristic of blue grama may also be related to its better nutritional value as the grass gets older. The forage pastures quality depends on the frequency and intensity of defoliation; therefore, the higher the leaves ratio, the higher the

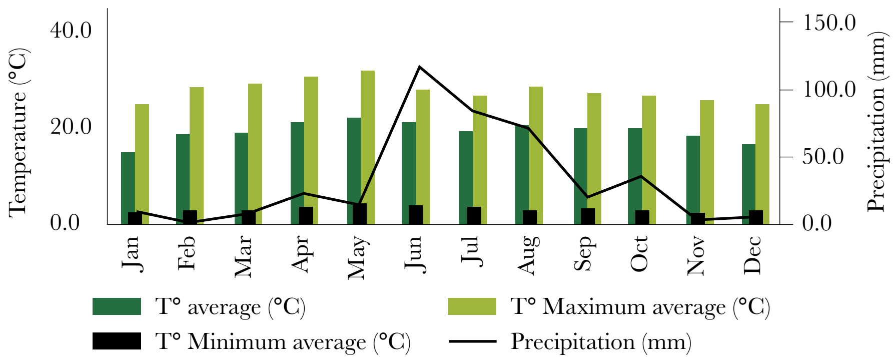

Figure 1. Annual temperature and precipitation 2019, in Francisco I. Madero, Hidalgo, Mexico.

Table 1. Morphological composition (g DM m^2) and forage production (kg DM ha^{-1}) established in Francisco I. Madero, Valle del Mezquital, Hidalgo, Mexico.

Grass name	dar	leaves	stems	infl	DM	sum	total (ha)	L:S
Rhodes	50	$170^{a\dagger}$	146^a	20^a	5^a	357^a	$8\,923^a$	1.16^b
Buffel		120^b	72^b	11^b	2^b	204^b	$5\,108^b$	1.68^a
Blue grama		57^c	53^b	4^b	2^b	120^b	$3\,012^b$	1.07^b
Average		116	90	12	3	224	225	1.3
Statistical significance		**	*	*	*	**	**	**
SEM		6.51	10.47	1.42	0.501	17.42	435	0.067
Rhodes	80	185^a	199^a	16^a	19^a	419^a	$10\,468^a$	0.93^b
Buffel		148^b	104^b	17^a	5^b	273^b	$6\,833^b$	1.42^a
Blue grama		72^c	76^b	9^a	3^b	160^c	$3\,999^c$	0.95^b
Average		135	126	14	9	275	7 100	1.1
Statistical significance		**	**	NS	*	**	**	*
SEM		3.27	7.38	4.83	2.04	16.37	409	0.065
Rhodes	110	200^a	264^a	19^a	33^a	507^a	$12\,936^a$	0.76^b
Buffel		154^b	207^b	27^a	15^b	300^b	$10\,263^b$	0.74^b
Blue grama		84^c	90^c	12^b	4^c	189^c	$4\,719^c$	0.94^a
Average		146	187	19	17	355	9 306	0.81
Statistical significance		**	**	**	**	**	**	**
SEM		3.16	5.53	1.36	1.44	7.25	182	0.02

† The same lowercase letters per column are statistically similar averages (P>0.05). dar=days after regrowth. DM=Dead Material. SEM=Standard error of the mean. *P<0.05, **P<0.01. NS=not significant differences (P>0.05).

forage quality (Lemaire *et al.*, 2009). Likewise, the higher the nutritional quality of forage, the higher the voluntary consumption by ruminants, as a result of the higher speed of passage in their gastrointestinal tract (Núñez *et al.*, 2014). Finally, a higher lignin ratio is observed at a higher rebound age: that is, ferulic acid is deposited in juvenile stages and p-coumaric acid is deposited throughout development (Casler and Hatfield, 2006).

Nutritional quality

For the three grass species studied, the older the plant, the lower the CP, TDN, RFV, ME, NE_l, NE_m, and NE_g (Table 2). In addition, NDF and ADF increased as the plants grew older, as reported by Bernal-Flores *et al.* (2017). The CP content in buffel grass at each cutting date was higher than in rhodes and blue grama (P<0.001). This is a key element in the management of grasslands, since it implies that more than 50% of the amino acids absorbed by ruminants ranges from 70 to 100% N. Likewise, it is important for the synthesis of microbial protein in the rumen and is used for growth, maintenance, and animal production (Rodríguez *et al.*, 2007; Das *et al.*, 2014). In the case of rhodes grass, CP decreased by 7% at 80 and 110 dar; this percentage is critical for the multiplication of microorganisms and therefore protein and metabolizable energy.

The forage pastures quality is given by the constitution of the epidermal tissues; therefore, in C_4 species such as the grasses included in this study, the mesophyll, phloem, epidermis,

Table 2. Chemical composition of rhodes, buffel, and blue grama grasses at three dates after regrowth in Francisco I. Madero, Valle del Mezquital, Hidalgo, Mexico.

Grass name	dar	CP (%)	NDF (%)	ADF (%)	TDN (%)	RFV (%)	Mcal kg^{-1}			
							ME	NE_l	NE_m	NE_g
Rhodes	50	7.49$^{c\,\dagger}$	74.42^{b}	45.17^{c}	53.09^{b}	66.20^{b}	1.87^{ab}	1.14	1.02^{a}	0.49
Buffel		10.7^{a}	69.5^{c}	46.55^{b}	52.9^{c}	70.4^{a}	1.91^{a}	1.17	1.13^{b}	0.52
Blue grama		8.03^{b}	78.59^{a}	49.0^{a}	56.50^{a}	60.10^{c}	1.85^{b}	1.13	1.05^{ab}	0.49
Average		8.73	74.18	46.91	54.17	65.57	1.88	1.15	1.07	0.50
Statistical significance		***	**	***	***	**	*	NS	*	NS
SEM		0.045	0.605	0.024	0.032	0.754	0.011	0.032	0.022	0.026
Rhodes	80	6.60^{c}	75.61^{b}	46.72^{c}	52.69^{b}	63.50^{b}	1.76	1.12	1.01	0.48
Buffel		9.30^{a}	70.46^{c}	47.98^{b}	52.89^{a}	67.60^{a}	1.86	1.15	1.04	0.51
Blue grama		7.85^{b}	79.45^{a}	51.15^{a}	52.90^{a}	56.50^{c}	1.84	1.11	1.01	0.46
Average		7.92	75.17	48.62	52.83	62.53	1.82	1.12	1.02	0.48
Statistical significance		***	***	***	**	***	NS	NS	NS	NS
SEM		0.031	0.311	0.033	0.026	0.613	0.023	0.023	0.024	0.016
Rhodes	110	6.38^{c}	75.30^{b}	48.33^{c}	51.02^{c}	64.40^{a}	1.88	1.07	0.96^{b}	0.42
Buffel		9.10^{a}	71.87^{c}	48.90^{b}	51.59^{b}	51.47^{c}	1.88	1.11	1.06^{a}	0.46
Blue grama		7.09^{b}	80.15^{a}	52.21^{a}	52.60^{a}	56.87^{b}	1.87	1.13	1.04^{ab}	0.48
Average		7.52	75.77	49.81	51.74	57.58	1.87	1.1	1.02	0.45
Statistical significance		***	***	***	***	***	NS	NS	*	NS
SEM		0.041	0.289	0.041	0.027	0.403	0.016	0.024	0.02	0.026

† The same lowercase letters per column are statistically similar averages (P>0.05). CP=Crude Protein. NDF=Neutral Detergent Fiber. ADF=Acid Detergent Fiber. TDN=Total Digestible Nutrients. RFV=Relative Feed Value. ME=Metabolizable Energy (Mcal kg^{-1}). NE_l=Net Energy of Lactation. NE_m=Net Energy of Maintenance. NE_g=Net Energy Gain. TDN=Total Digestible Nutrients. dar=days after regrowth. ***P<0.001, **P<0.01. SEM=Standard error of the mean, NS=no statistical significance (P>0.05).

parenchyma, and bundle sheath degrade slowly or partially (Guevara and Ramia, 2007). Therefore, in the case of the side oats grama (*B. curtipendula*), according to Bernal-Flores *et al.* (2017), the digestibility of the phloem and mesophyll is high and 70% of the chloroplast protein is concentrated in the latter. Meanwhile, xylem and sclerenchyma are high in lignin, especially in the primary veins, which consequently entails partial degradation. Blue grama grass had the highest TDN values in the three regrowth ages (P<0.001). This is a very positive output, because cattle cannot consume all the forage of the plots of a single ranch at one time. In the best of cases, ranchers organize certain sections for the dry season with physiologically mature plants. The grasses lose quality from pollination, as a consequence of the removal of soluble nutrients from leaves and especially from stems. These nutrients fill caryopses in inflorescences and therefore the lignin ratio increases (Li *et al.*, 2014). Buffel grass, like blue grama, showed a higher amount of TDN than rhodes (P<0.01) at 80 dar.

The influence of the nutrient digestibility percentage in the rumen results in a higher production of the following volatile fatty acids: acetate, propionate, and butyrate (Okoruwa and Igene, 2014). This increased production is reflected in a higher cattle productivity.

Therefore, the native blue grama grass will have a more positive effect on the ruminal microbiota: a greater production of metabolizable energy at a higher regrowth age. This can help to organize grazing in ranches in the Mexican deserts. That is, first defoliate introduced grasses and then native grasses. Therefore, to provide better pasture quality to ruminants in the natural grasslands of the Mexican deserts, ranchers will have to use grasses at 80 dar (2.5 months of regrowth), when forage production, CP, ME, and RFV are the most adequate. In the case of grasslands with rhodes and buffel grasses, grazing between 50 and 80 dar is recommended. The NDF and ADF percentages in the three grass species increased along with the age of the plants, as reported by Agnusdei *et al.* (2011). Meanwhile, blue grama grass showed higher NDF and ADF than rhodes and buffel grass ($P<0.001$); however, it also had a higher digestibility (TDN; $P<0.001$).

Overall, the highest RFV were recorded with younger plants: buffel grass stood out at 50 ($P<0.01$) and 80 ($P<0.001$) dar, while rhodes grass at 110 dar obtained better results ($P<0.001$). However, the TDN of blue grama grass was greater at 110 dar, although this is not reflected in the RFV, as a result of the higher DM production in rhodes and buffel. This production is related with the matrix that obtains the relative value of forage. Differences ($P<0.01$) in metabolizable energy were observed only at 50 dar. The metabolizable energy is given by the digestibility and concentration of protein, fat, non-fibrous, and fibrous carbohydrates in the forage (Weiss, 1996). Meanwhile, the energy content of forages is given mainly by the content, type, and digestibility of carbohydrates (Núñez, 2014); which affects the dry matter intake of cattle.

CONCLUSIONS

The introduced buffel and rhodes grasses surpassed blue grama grass by 1.7 and 2.7 times in forage production, respectively; however, blue grama grass had a consistent leaf:stem ratio. Buffel grass had a higher crude protein and relative value of forage, while rhodes grass produced a greater amount. Out of the three grass species, buffel and rhoes had the best pasture quality at 80 dar and 50 dar, respectively. On its turn, the total digestible nutrients in blue grama grass were higher in the most senescent stage.

REFERENCES

Abas, I., Ozpnar, H., Kutay, H.C., Kahraman, R. and Eseceli, H. (2005). Determination of the metabolizable energy (ME) and net energy lactation (NEL) contents of some feeds in the Marmara Region by *in vitro* gas technique. *Turk J Vet Anim Sci. 29* (3). 751-757.

Amiri, F., Rashid, A. and Shariff M. (2012). Comparison of nutritive values of grasses and legume species using forage quality index. *Songklanakarin J. Sci. Technol. 34* (5). 577-586.

Bernal-Flores, A., Quero-Carrillo, A.R., Zabaleta-Mancera, H.A., Valdez-Carrasco, J. y Ortega-Cerrilla, M.E. (2017). Atributos histológicos relacionados con digestibilidad en *Bouteloua curtipendula* (Michx.) Torr. de México. *Revista Fitotecnia Mexicana. 40* (3). 299-308.

Buxton, D.R. and Redfearn, D.D. (1997). Plant Limitations to Fiber Digestion and Utilization. *The Journal of Nutrition. 127* (5). 814–818. Doi: 10.1093/jn/127.5.814S

Casler, M.D. and Hatfield, R.D. (2006). Cell wall composition of smooth bromegrass plants selected for divergent fiber concentration. *J. Agric. Food Chem. 54* (21). 8206-8211. Doi: 10.1021/jf060319o

Castillo-López, E. y Domínguez-Ordóñez, M.G. (2019). Factores que afectan la composición microbiana ruminal y métodos para determinar el rendimiento de la proteína microbiana. Revisión. *Revista Mexicana de Ciencias Pecuarias. 10* (1). 120-148. Doi: 10.22319/rmcp.v10i1.4547

Das, L.K., Kundu, S.S., Kumar, D., and Datt, C. (2014). Metabolizable protein systems in ruminant nutrition: A review. *Veterinary World 7*(8). 622-629.

Gómez de la, F.E., Díaz, S.H., Saldívar, F.A., Briones, E.F., Vargas, T.V. and Grant, W.E. 2007. Patrón de crecimiento de pasto buffel [*Pennisetum ciliare* L. (Link.) Sin. *Cenchrus ciliaris* L.] en Tamaulipas, México. *Técnica Pecuaria en México. 45* (1). 1-17.

Granados-Sánchez, D., López-Ríos, G.F. y Hernández-Hernández, J. (2003). Agricultura nhanñhu-otomí del Valle del Mezquital, Hidalgo. *Terra Latinoamericana 22* (1). 117-126.

Guevara, O.L.I. y Ramia, M. (2007). Anatomía foliar de *Panicum* L., sección Parvifolia (Poaceae, Paniceae) en Venezuela. *Rodriguesia 58*(1). 73-83. DOI:10.1590/2175-7860200758108

Ibarra, F.F., Martín, R.M.H., Moreno, M.S., Denogean, B.F.G. y Gerlach, B.L.E. (2005). El zacate buffel como una alternativa para incrementar la rentabilidad de los ranchos en la zona serrana de Sonora. *Revista Mexicana de Agronegocios. 9* (16). 521-529.

Li G., Pan J., Cui K., Yuan M., Hu Q., Wang W. and Peng S. (2017). Limitation of Unloading in the Developing Grains Is a Possible Cause Responsible for Low Stem Non-structural Carbohydrate Translocation and Poor Grain Yield Formation in Rice through Verification of Recombinant Inbred Lines. Front. *Plant Sci. 8*. 1-16. doi:10.3389/fpls.2017.01369

Lemaire, G., Da Silva, S.C., Agnusdei, M., Wade, M. and Hodgson, J. (2009). Interactions between leaf lifespan and defoliation frequency in temperate and tropical pastures: A review. *Grass and Forage Science. 64* (4). 341–353. DOI:10.1111/j.1365-2494.2009.00707.x

Núñez H.G., Rodríguez H.K., Granados N.J.A., Anaya S.A. y Figueroa V.U. (2014). Calidad nutricional y utilización de forrajes en explotaciones lecheras en la región lagunera. *AGROFAZ. 14* (1). 33-41.

Okoruwa, M.I. and Igene, F.U. (2014). Comparison of Fermentation Kinetics (*in vitro*) of Napier Grass and Fruit Peels for Ruminants: The Pattern of Organic Matter Degradability, Volatile Fatty Acids Concentration, stimated Methane and Microbial Biomass Production. *J. Agriculture and Veterinary Science. 7* (1). 21-28. DOI:10.9790/2380-07132128

Rodríguez, R., Areadne, S. and Rodríguez, Y. (2007). La síntesis de proteína microbiana en el rumen y su importancia para los rumiantes. *Revista Cubana de Ciencia Agrícola. 41* (4). 303-311.

SAS (SAS/STAT User's Guide Version 9.2). 2009. SAS Institute Inc. Cary, North Carolina, USA. 5136 p.

Weiss, W.P., Conrad, H.R. and St. Pierre, N.R. (1992). A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. *Animal Feed Science and Technology. 39* (1). 95–110. doi:10.1016/0377-8401(92)90034-4