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EDITOR'S LETTER

Dear collaborators, readers, and members of the Editorial Committee:

We put at your disposal the 34th Issue of the Journal of Scientific Dissemination of the Universidad Autónoma de Chiapas: *Espacio I+D, Innovación más Desarrollo*. This issue is very special since we closed Volume 12 and began a new era, without changing its editorial nature. We refer to the fact that, due to an institutional restructuring, as of this edition, the editorial team that makes this publication, composed of professional editors, designers, and communicators, joins the Secretariat of University Identity and Social Responsibility (SIRESU) of the UNACH, from where we will continue to be a window for the dissemination of science, culture, and knowledge in the south-southeast region. In this way, internally we will support the *Academic Project 2022- 2026: For the consolidation of excellence*, from the Science, Technology and Innovation Programme with the same quality and punctuality that we have done throughout these 11 years and 34 issues.

This issue brings together materials from various institutions, carried out in coordination with the Escuela de Ciencias Químicas based on the call published within the framework of the 1st. International Congress of Applied Biotechnology (March 2023). In this issue, we will publish the first three works entitled: "Study of the effect of drought induced by polyethylene glycol in Capsicum frutescens in a hydroponic system"; "Relaxant activity of Hyptis suaveolens on isolated guinea pig tracheal rings"; and "Antifungal Effect Of Encapsulated Metarhizium anisopliae spores on the development of bean plants (Phaseolus vulgaris L) infected with Fusarium moniliforme".

In addition to these, this issue includes the following articles: "Remittances and Financial Inclusion in Municipalities of Northeast Mexico: A Bayesian spatiotemporal Analysis" and "Evaluation of graduated students from the School of Medicine through the EGEL results and ENARM score.".

On the other hand, we add the book *University Memories. Images of the past and voices of the present (UNACH)* in the "*Letters without paper*" collection, which from this moment is in digital format and open access, that is, free download and consultation, in order to bring the youngest university community closer, above all, to the reflection of the creation process and development of one of the most important universities in the south-southeast region. With this title, we have already added 19 books to this collection.

The Espacio I+D magazine glimpses a new panorama, in which new challenges in editorial matters will undoubtedly arise, in this increasingly changing reality, as always, we will prepare ourselves to face them in the best way.

Finally, we thank the members of the Editorial Committee who have completed their term with the publication and we welcome the new members and our new directors who represent the cultural, academic, and administrative support necessary for the permanence of this project.

Thank you for being a part of this media outreach!

"*Por la conciencia de la necesidad de servir*"
Universidad Autonoma de Chiapas

Espacio I+D, Innovación más Desarrollo

ARTICLES

Reception: **April 1, 2023** | Acceptance: **July 7, 2023**| Publication: **October 1, 2023**

Antifungal Effect Of Encapsulated *Metarhizium anisopliae* Spores on the Development of Bean Plants (Phaseolus vulgaris L) Infected with *Fusarium moniliforme*

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To quote this article:

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— Abstract—

The annual bean (*Phaseolus vulgaris* L.) crop production in Mexico is reduced mainly by diseases and pests. An alternative for biological control is the use of *Metarhizium anisopliae* spores due to their ability to colonize the rhizosphere, but their effectiveness decreases due to sensitivity to environmental factors. Therefore, the objective of this study was to analyze the biocontrol potential of an alginate formulation with spores of *M. anisopliae* in the bean crop with Fusarium infected. A completely randomized experimental design was proposed using healthy plants as control; plants were also inoculated with a suspension of Fusarium oxysporum in the substrate, and additionally to a part of these plants, 1 g of capsules of alginate with M. anisopliae spores per plant was deposited at the stem base, the morphometric parameters were measured every eight days for eight weeks, as well as the appearance of Fusarium wilt symptoms. After analyzing the data obtained, it was shown that there were significant differences 64 days after inoculation (ddi), with greater leaf and root dry weight, as well as root length in plants where there were interactions between Fusarium and encapsulated spores of Metarhizium, which suggests that the presence of the microorganisms positively affected the treatment of the plants, surpassing the benefits of the application of Metarhizium reported as a growth promoter. In addition, the incidence of the root disease will occur during the first 32 days of cultivation. It was concluded that the application of encapsulated spores of M. anisopliae is a viable option as a biocontrol to reduce the effect of Fusarium wilt in the bean crop.

Keywords:

Encapsulated; Metarhizium anisopliae; alginate; disease incidence.

Due to their high protein content, beans are considered one of the main foods of gastronomy in Mexico for both the rural and urban populations, so it is vital to maintain sufficient production to meet demand since various factors influence the quality of the grain, such as agronomic management and cultivation and storage conditions, as well as being threatened by pests and diseases during its development in the field. The Fusarium species are the most aggressive, which limits the productivity of the common bean, mainly *Fusarium solani* (Toghueo *et al*., 2016). The main symptom of fusariosis is root rot, which begins with the reduction of the root system in length and weight, thus decreasing the ability of the plant to absorb nutrients and water, leading to its death. Another genus that affects this crop is Rhizoctonia, which can cause serious damage in susceptible varieties, up to 94% of the incidence of the disease and 39% in resistant varieties (Ketta & Hewedy, 2021). The conventional management to control these pathogens is the application of chemical fungicides, and the abuse of these compounds has affected other components of the ecosystem, such as the inhibition of pollinators and the reduction of beneficial microbial communities in the soil, even causing the appearance of resistant pathogens. In addition, the accumulation of these inorganic compounds affects the health of the user and the environment. In recent decades, alternatives have been sought to reduce these effects, the most promising being the use of broad-spectrum antagonist biocontrol agents (bacteria and fungi) (Uzman *et al*., 2019; Sánchez-Rodriguez *et al*., 2016).

It has been shown that non-pathogenic microorganisms associated with the plant generally protect it through rapid colonization and, therefore, use the available substrates, being limited for pathogenic organisms hindering the growth of the latter (Mayerhofer *et al*., 2019). Entomopathogenic fungal species such as *Beauveria bassiana* and some belonging to the genus Metarhizium have been used to control insects in food-important crops, they can colonize their roots (Ahmad *et al*., 2020; Razinger *et al*., 2020; Krell *et al*., 2017), promote growth and even increase their resistance to viral and fungal pathogens (Shaalan *et al*., 2021; Shaalan & Ibrahim, 2018) due to their ability to produce bioactive molecules by their secondary metabolism such as hormones and antibiotics, (Shah *et al*., 2022). The entomopathogen *M. anisopliae* naturally attacks insects of various orders (Yousef *et al*., 2018) by combining hydrolytic enzymes such as glucanases, lipases, amylases, chitinases and toxic metabolites such as destruction and cytochalasins. Unfortunately, its prevalence in the environment is limited because it is sensitive to environmental factors such as temperature, humidity, and radiation (Putnoky-Csicsó *et al*., 2020), reducing its effectiveness in crops; therefore, to prolong the benefits provided by the interaction of *M. anisopliae* with its host plant, it requires coverage that allows it to increase

its permanence and environmental resistance. In this regard, research has been carried out that proposes the inclusion of spores of the microorganism in a non-toxic polymeric matrix (Manzanarez-Jiménez *et al*., 2023) and is biocompatible. Sodium alginate is a polymer that has been used for the encapsulation of cells, which has allowed the release of cells under specific conditions and has achieved 80 to 95% survival (Sarma *et al*., 2023; De Oliveira Lopes *et al*., 2020), being a good alternative for the protection of fungal biocontrol agents, including entomopathogens (Lei *et al*., 2022). Therefore, the objective of this work was to analyze the biocontrol potential of an alginate formulation with encapsulated *M anisopliae* spores in the cultivation of beans (*Phaseolus vulgaris* L.). infected with Fusarium

2. MATERIALS AND METHODS

2.1 Microorganisms

Metarhizium anisopliae was obtained from the National Institute of Agricultural and Livestock Forestry Research (INIFAP), and *Fusarium moniliforme* was molecularly identified (no.GU982311.1).

2.2 Encapsulation of Metarhizium anisopliae spores in sodium alginate

The alginate capsules were prepared by ionic gelation, as reported by Meirelles *et al*., (2023) with some modifications. The 4% p/v sodium alginate solution (Sigma-Aldrich-Merck brand) was prepared and sterilized at 15 Lb for 15 minutes. Subsequently, in a laminar flow hood, that solution was mixed with a spore suspension ($1x10^7$ spores/mL.) of *M. anisopliae*, which was prepared from a 7-day Czapek broth culture at 4% v/v. It was kept under agitation until the mixture was uniform and deposited into a 20 mL needleless syringe, from which it was dropped dropwise onto a solution of 10% calcium chloride ($CaCl_2$) (Meyer Brand) previously sterile and at room temperature that was kept under continuous stirring, the mixture (Na-spore alginate) upon contact with the CaCl2 solution instantly forms the beads, encapsulating the spores. The beads or capsules were kept in the $CaCl_2$ solution for 30 min, after which time they were washed with sterile water and placed in a dry and sterile container, finally dried in an oven at 45 °C for 48 h.

2.3 Plant material

The bean seeds were obtained from the greenhouse of the Tecnológico Nacional de México, Tuxtla campus from preservation crops.

2.4 Establishment of the experiment at the greenhouse level

From a previous culture in Petri dishes with potato dextrose agar (PDA) for seven days at 28 ± 2°C, the pathogen inoculum suspension was prepared at a suspension concentration of $1x10^6$ spores/mL *F. moniliform*, for which 10 mL of sterile distilled water and glass beads that were slowly stirred were added to each plate to detach the spores from the mycelium. Each bean seed was sown in 100 g of Peat moss substrate in black nursery bags at a 4 cm depth; 12 days after germination, the plants were transplanted into pots containing 500 g of the same substrate, and four treatments were evaluated: 1) bean - neutral control -(Fr), 2) bean + *F. moniliform* (Fr + Fm), 3) beans + capsules of *M. anisopliae* (Fr + CMa), 4) beans + capsules of *M. anisopliae* + *F. moniliform* (Fr + CMa + Fm). At the time of transplanting for treatments 3 and 4, 1 g of M. anisopliae spore capsules were deposited at a distance of no more than 5 cm from the base of the stem. 7 days after the bean plants had contact with the M. anisopliae capsules, plants from treatments 2 and 4 were inoculated with 20 mL of a suspension of *F. moniliform*. The development of symptoms of fusariosis was analyzed using six plants per treatment; observations were made at 15-day intervals for two months. The variables evaluated were fresh weight and leaf and root dry weight (g), root length (cm), plant height (cm), chlorophyll (SPAD), stem diameter (mm), and root incidence (%).

For each experimental unit, the variables stem diameter, height, and root length were measured with a vernier and/or flexometer, to obtain the fresh and dry weights, the plants were removed from the substrate, washed, and dried with absorbent paper and separated into two sections: root and leaf, then weighed on an analytical balance and deposited in paper bags and dried in an oven at 45ºC until constant dry weight values were obtained.

The percent disease incidence (IR) in roots was determined by the following formula: IR (%) =n/N x 100. Where n is the number of plants showing disease symptoms with at least one diseased root, and N is the total number of samples used (Cruz-Rodriguez *et al*., 2020).

2.5. Experimental design and statistical analysis

A randomized complete block design was used, four blocks with six repetitions monitored four times; with a total of 96 experimental units. For statistical analyses, one-way ANOVA at a 5% significance level was used using the Statgraphics program.

3. RESULTS AND DISCUSSION

Effect of M. anisopliae encapsulates on the growth parameters of bean plants

The application of capsules with *M. anisopliae* spores had a positive effect on leaf and root system development in bean plants at 64 days after inoculation (ddi) (Table 1). The parameters focused on root growth such as fresh and dry weight, in addition to length, are those that mainly show variations between treatments 1 and 2, the latter being the one in which the lowest values of the entire experiment are observed because it is the tissue where the Fusarium infection process begins. In the case of treatment 4, which was also inoculated with *F. moniliform*, this presents not only significant differences in root growth with the rest of the treatments but also in leaf development, such is the case of the number of leaves with this treatment (66 leaves), a 55% increase was observed concerning the plants of treatment 1, that developed under normal conditions with an average value of 42.5 leaves.

Some of the processes that result from the interaction of the cells of the entomopathogenic fungus with the plant cell tissue, initiate through the production of hydrolytic enzymes by the fungus, which allow the degradation of the components of the plant cell wall and, this way, the hyphae can penetrate the surface of the cells thus colonizing the roots, facilitating the uptake of nutrients and water (Liao *et al*., 2013). Another of the reported mechanisms of the genus Metarhizium is its ability to activate defense signaling pathways in the plant cell that results in the production of molecules such as phytohormones that accelerate growth. This was observed in *Arabidopsis thaliana* plants, due to the presence of *Metarhizium robertsii*, through an increase in the production of Indole Acetic Acid (IAA), which promoted the development of root hair (Liao *et al*., 2017). Finally, the cells of the entomopathogenic fungus can act as an elicitor, to accelerate the production of phytochemicals that protect the cell from F. moniliform *attack*, and reduce symptoms in plants due to the presence of the pathogen.

The presence of *F. moniliform* significantly affected chlorophyll production in treatments 2 and 4 with values of 29.84 + 4.72 and 31.7 + 0.12 respectively, however, it did not present significant differences with treatment 1, while the interaction of the entomopathogen with the plant in treatment 3, showed the highest relative index of chlorophyll with 40.41 + 4.24, this index is expressed in SPAD units and has been positively correlated with the state or nitrogen absorption capacity of the plants, Casierra *et al*., (2012) point out that the content of photosynthetic pigments can increase or decrease due to stress factors (light, temperature, humidity, nutrients, etc.), to the physiological age or photosynthetic capacity of the plant, in this case, the variation is due to biotic stress.

Analysis of the incidence of disease caused by Fusarium moniliform

Fusarium species are known as causative agents of root and stem wilt and rot, one of the first symptoms is browning of the root tissue due to the infection mechanism of these pathogens and at an advanced level chlorosis, vascular discoloration and decay are observed, for this reason the monitoring of the incidence in the root (IR) was carried out during the 64 days that the experiment lasted, as seen in Figure 1, the percentage of IR was presented in both treatments where the pathogen was inoculated, at the 8 days after inoculation (16 ddi), the percentage of incidence increased as the crop cycle progressed, with the incidence being higher in all samples of treatment 3, reaching an IR of 25.8 + 0.56 % and after 48 days a decrease in the presence of Fusarium necrosis was observed up to 14.6 + 4.6 %. In the case of treatment 4 plants, which were pre-treated with *M anisopliae* capsules showed slight resistance in the first days of infection, 3.2 + 0.4 % at 16 days and reaching a maximum incidence of 8.6 % at 64 days of the entomopathogen, which is not statistically different from the IR of treatment 3 at 16 ddi. This shows that the presence of *M anisopliae* markedly decreased the normal development of the disease; making the entomopathogenic plant-fungus symbiosis effective against Fusarium. It is suggested that growth inhibition mechanisms against *F. moniliform* include the combination of the activity of hydrolytic enzymes produced by the entomopathogen, which act on the cell wall of Fusarium together with the induction of phytoalexins by bean plants (García-Enciso *et al*., 2018).

CONCLUSION

The formulation of alginate to encapsulate the spores of *Metarhizium anisopliae* spores allowed their release into the soil, being indirectly evident by the growth-promoting effect on bean plants, it was also shown that the interaction of the spores of the entomopathogen with the plant tissue reduces the incidence of *F moniliform* in the root. Therefore, the addition as a pretreatment of capsules with spores of *Metarhizium anisopliae* is an option to control fusariosis in bean cultivation, and thereby reduce in the future the application of chemical substances in the field for the control of diseases in crops caused by fungi.

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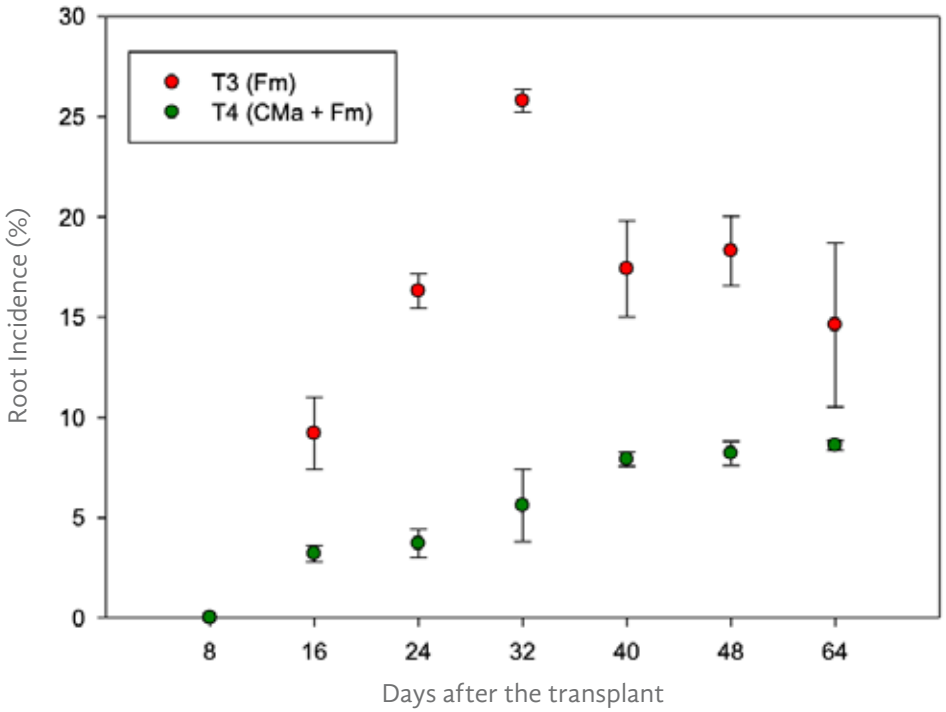

Figure 1. Percentage incidence of fusarium root blight in Phaseolus vulgaris L. Fm: F. moniliforme. CMa: M. anisopliae spore capsules

Table 1

Morphometric variables of bean (Phaseolus vulgaris L). plants infected with Fusarium 64 days after inoculation

		Variables							
No.	**Treatment**	Fresh leaf weight (g)	Dry leaf weight (g)	Fresh root weight (g)	Dry root weight (g)	Root length (cm)	Number of leaves	Plant height (cm)	Relative Chlorophyll Index (SPAD units)
1	Fr	12.97 ± 4.63 c	2.48 ± 0.50 c	2.47 ± 0.21 c	0.54 ± 0.02 c	27.1 ± 0.22 c	42.5 + 0.71 b	60.4 + 0.71 ab	34.94 + 1.18 b
2	Fr + Fm	16.35 ± 4.75 c	3.11 ± 0.63 c	2.08 ± 0.12 d	0.48 ± 0.03 d	26.4 ± 0.0 d	45.0 + 8.49 b	45.0 + 8.49 c	29.84 + 4.72 bc
3	Fr + CMa	25.81 ± 0.0 b	4.68 ± 0.93 b	3.38 ± 0.42 b	0.66 ± 0.05 b	29.04 ± 1.07 b	48.5 + 9.31 b	75.75 + 17.04 b	40.41 + 4.24 a
4	Fr + CMa + Fm	48.05 ± 0.0 a	9.46 ± 0.0 a	6.49 ± 0.0 a	1.13 ± 0.0 a	34.29 ± 0.77 a	66.0 + 1.21 a	110..0 + 2.5 a	31.7 + 0.12 c

Mean values of three replicates followed by at least one letter, which are not significantly different at P < 0.05 (Tukey's test). **Fr: bean; CMa: M. anisopliae spore capsules; Fm: F. moniliforme.**

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Relaxant activity of *Hyptis suaveolens* on isolated guinea pig tracheal rings

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— Abstract—

Asthma is a chronic disease that leads to difficulty in breathing due to the restriction of airflow. This is attributed to the contraction of bronchial smooth muscle tissue by diverse inflammatory mediators. Since existing therapies have not achieved adequate control of the symptomology of most patients, new options are needed. *Hyptis suaveolens* is used in the State of Chiapas, Mexico, to treat asthma, but its activity has not been corroborated scientifically. The aim of the current contribution was to determine the muscle relaxant activity of *Hyptis suaveolens* in the model of rings isolated from guinea pig trachea. The hexane, dichloromethane, and methanol extracts of the plant were prepared by maceration. The extract with the greatest activity was separated by column chromatography to find the most active fraction. The participation of β adrenergic and muscarinic receptors was determined by constructing concentrationresponse curves from the following treatments: the active subfraction of *Hyptis suaveolens* (56177 µg/mL), isoproterenol (3170 µM) in the absence and presence of propranolol (0.3 µM), and carbachol ($1x10^{-16}$-0.01 M) in the absence and presence of the active subfraction (100 y 177 µg/mL) or of atropine (0.3 y 3 µM). The dichloromethane extract was the most active ($p<0.05$), and its most active subfraction (F4') showed an EC_{50} of 91.19 ± 1.83 µg/mL. The relaxant effect of F4' was not inhibited by propranolol. F4' behaved as a competitive antagonist of muscarinic receptors ($p<0.01$). In conclusion, *Hyptis suaveolens* exerts a muscle relaxant activity on rings isolated from guinea pig trachea. An active subfraction of the dichloromethane extract acted as a competitive antagonist on muscarinic receptors, but β_2 adrenergic receptors did not participate in its mechanism of action.

Keywords:

Hyptis suaveolens; asthma; relaxant activity; medicinal plants; guinea pig tracheal rings.

The number of people affected by asthma worldwide is approximately 300 million, which is estimated to rise to 400 million by 2025 (Taur and Patil, 2011). In Mexico, about 7.0% of the population (~8.5 million inhabitants) suffers from this chronic respiratory disease (Arteaga-Badillo *et al*., 2020). The most common symptoms of asthma are coughing, wheezing, chest tightness, and shortness of breath (dyspnea) (WHO, 2023).

The difficulty of asthma patients to breathe stems from the capacity of various inflammatory mediators to contract airway smooth muscle, thus restricting airflow and preventing an adequate gaseous exchange of O_2 and CO_2 in the blood (Arteaga-Badillo *et al*., 2020). Excessive narrowing of the airways is considered to be the main cause of the morbidity and mortality that results from this disease. The exacerbation of symptoms may lead to disability, a loss of workdays, and hospitalization (Dowell *et al*., 2014).

The drugs administered to manage asthma are anti-inflammatory agents (e.g., corticosteroids, antileukotrienes, and chromones) and bronchodilators (e.g., β_2 adrenergic agonists, anticholinergics, and methylxanthines) (Arteaga-Badillo *et al*., 2020). Bronchodilators achieve symptomatic improvement by relaxing airway smooth muscle tone. The different pharmacological regimens available to reduce symptoms are unsatisfactory for many patients because of producing adverse effects. As a consequence, patients often suspend treatment, leading to the reappearance of symptoms (Taur and Patil, 2011). Some studies indicate that over 50% of asthma patients are unable to control their symptoms (Arteaga-Badillo *et al*., 2020). Hence, many asthma patients seek complementary or alternative medicine (Taur and Patil, 2011), such as the use of immunomodulatory agents and herbal medicine (Arteaga-Badillo *et al*., 2020). Certain medicinal plants can improve airflow by relaxing airway smooth muscle tone (Águila *et al*., 2015), thus relieving the symptoms of patients.

In the state of Chiapas, Mexico, the *Hyptis suaveolens* (Lamiaceae) Poit. plant is used in traditional medicine to treat different diseases, including asthma. The plant is known locally as “chía”, “confitura”, and “hierba de burro”. Although experimental evidence exists for the anti-inflammatory, antinociceptive, anti-cancer, anti-hyperglycemic, and anti-ulcer activity of *Hyptis suaveolens* (Santos *et al*., 2007; Vera-Arzave *et al*., 2012; Bayala *et al*., 2020; Mishra *et al*., 2021), no scientific study has yet been published on the relaxant effects of this plant on bronchial smooth muscle for the treatment of asthma. Therefore, the aim of the current contribution was to determine the relaxant effect of *Hyptis suaveolens* on the smooth muscle of isolated guinea pig tracheal rings.

METHODOLOGY

Vegetable material

Hyptis suaveolens was collected in the municipality of Copainalá (Chiapas, Mexico) in August, 2018. A specimen of the collection was identified as *Hyptis suaveolens* by the CHIP herbarium (registration number 27939), which belongs to the Ministry of Environment, Housing, and Natural History of Tuxtla Gutiérrez, Chiapas.

Preparation of extracts and chromatographic fractionation

The aerial parts (stems and leaves) of *Hyptis suaveolens* were dried in the shade at room temperature and then pulverized. Subsequently, the steps that characterize a bioassay-guided study were followed (Cornejo-Báez *et al*., 2020), beginning with the maceration of 20 kilos of plant material in different solvents: hexane, dichloromethane, and methanol (in increasing order of polarity) (Sharma & Gupta, 2015). The plant material was in contact with each solvent for three days, after which time the solution was filtered and the plant material concentrated under reduced pressure in a rotary evaporator (Sharma & Gupta, 2015). The process was carried out three times with each solvent, yielding 2.5% of the hexane extract, 2.3% of the dichloromethane extract, and 5.6% of the methanol extract. The most active extract was dichloromethane (440 g), and therefore it was fractionated by column chromatography (Cornejo-Báez *et al*., 2020) in a column packed with silica gel. Mixtures of hexane and ethyl acetate in ratios of 9:1, 7:3, and 5:5 generated fractions F1, F2, and F3, respectively. Ethyl acetate and methanol were used for the mobile phase to provide fractions F4 and F5, respectively. The yields of these fractions were 22% (F1), 21% (F2), 17% (F3), 21% (F4), and 18% (F5). Subsequently, 80.5 g of the most active fraction (F4) was separated by silica gel column chromatography. The subfractions were obtained with mixtures of hexane and ethyl acetate (Sharma & Gupta, 2015) in increasing polarity as eluents: 9:1 (F1´), 8:2 (F2´), 7:3 (F3´), and 5:5 (F4´). The yields were 26% (F1´), 29% (F2´), 33% (F3´), and 1.7% (F4´).

Pharmacological experiments

Animals

Male guinea pigs of the Hartley strain (300-450 g) were used. All procedures complied with the national regulation for the care and experimentation with laboratory animals (SAGARPA.NOM-062-ZOO-1999). The protocol

was approved by the internal ethics committee (CICUAL) of the School of Medicine of the Instituto Politécnico Nacional (registration number: CICUAL-08/4-12-2017).

Drugs

Acetylcholine chloride, carbachol, isoproterenol, propranolol, and atropine were purchased from Sigma-Aldrich (Mexico). All solutions were prepared with distilled water on the same day that the biological tests were performed.

Extracts, fractions, and subfractions

The extracts, fractions, and subfractions were suspended in a solution of Tween 80 (0.05%) and distilled water, prepared on the same day they were evaluated (Arrieta *et al*., 2018).

Preparation of the tracheal rings

The guinea pigs were sacrificed by administering an overdose of pentobarbital (95 mg/kg) intraperitoneally. The trachea was immediately dissected, and the attached tissue was cleaned. The trachea was always maintained in a Krebs solution, which consisted of the following compounds (measured in mM): NaCl (118), $NaHCO_3$ (25.0), glucose (11), KCl (4.7), $CaCl_2{\cdot}2H_2O$ (2.2), KH_2PO_4 (1.2), and $MgSO_4{\cdot}7H_2O$ (1.2). The Krebs solution was kept at a pH of 7.4 and a temperature of 37 °C, and was constantly ventilated with a mixture of 95% O_2 and 5% CO_2. Eight rings were obtained from each guinea pig trachea, and these were placed in isolated organ chambers with a volume of 10 mL. The rings were clamped between two hooks inserted into the lumen. One hook was fixed to the chamber and the other to a Biopac TSD 125c force transducer that recorded isometric tension on a Biopac MP150 polygraph. Each ring was adjusted to a tension of 2 g and allowed to rest for 30 min. Upon completion of this time, the viability of the tissue was determined by adding a solution of 30 µM acetylcholine. After washing the tissue with Krebs solution every 15 min for 1 h, the experiments were performed (Arrieta *et al*., 2018). The values were digitized and analyzed with data acquisition software (Acknowledge 4.0).

Evaluation of the relaxant activity of *Hyptis suaveolens*

The tissue was contracted with carbachol (3 µM) and when a plateau was reached, the vehicle or one of the treatments was applied. The concentrations

of the active agents were applied in increasing order, one every 7 min. The concentrations were 17.7, 31.6, 56.2, 100, 177, and 316 µg/mL for the extracts, 75, 100, 133, 177, 237, and 316 µg/mL for the fractions, and 56-177 µg/mL for the subfractions (Arrieta *et al*., 2018). In all cases, the maximum contraction induced by carbachol alone was considered as 100%.

Assessment of the participation of β2 adrenergic receptors

The tracheal rings were incubated with a propranolol solution (0.3 µM) or the vehicle for 15 min. Subsequently, they were contracted with carbachol (3 µM) until reaching a plateau (15 min), and then increasing concentrations of the active subfraction F4' (56, 75, 100, 133, and 177 µg/mL) or isoproterenol (3-170 µM) were added, one concentration every 7 min. Isoproterenol served as a reference drug in an independent experiment (Arrieta *et al*., 2018). The maximum contraction induced by carbachol alone in the presence of the vehicle was considered as 100%.

Evaluation of the participation of muscarinic receptors

The rings were incubated with atropine (0.3 and 3 µM), F4' (the most active subfraction, at 100 and 177 µg/mL), or the vehicle for 15 min in independent experiments. Upon completion of this time, increasing concentrations of carbachol (1x10-16 to 0.01 M) were applied every 7 min. The maximum contraction achieved by carbachol in the presence of the vehicle was considered as 100% (Sánchez-Mendoza *et al*., 2008).

Statistical analysis

Data are expressed as the mean ± the standard error of the mean (SEM) of at least six assays. EC_{30} and EC_{50} values (the concentration that produces 30% and 50% of the maximum effect, respectively) were calculated by linear regression (Talladira, 2000). The Student's *t*-test was used to compare the difference between two groups, and Dunnett's test to compare the differences between several groups. Statistical significance was set at $p \leq 0.05$.

RESULTS

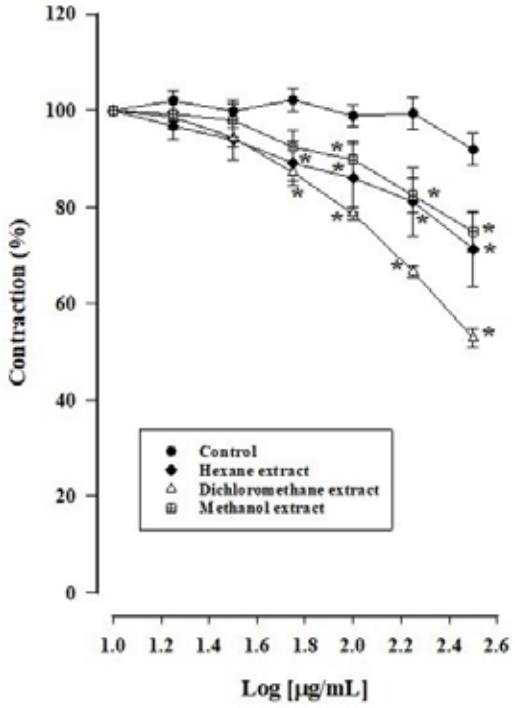
The relaxant effect generated by Hyptis suaveolens

The hexane, dichloromethane, and methanol extracts relaxed the smooth muscle of tracheal rings in a concentration-dependent manner (Figure 1), with the greatest effect produced by the dichloromethane extract. The latter

extract reached a maximum relaxation value of 47.16 ±1.93% at a concentration of 316 µg/mL and had an EC30 value of 138.07 µg/mL.

The fractionation of the dichloromethane extract by column chromatography provided five fractions. Whereas F1 was inactive, fractions F2-F5 showed a concentration-dependent relaxant effect, and F4 was clearly the most effective and potent (Table 1).

Fraction F4 (ethyl acetate) was then separated into four subfractions. F4´, obtained with a mixture of hexane/AcOEt (5:5), afforded the greatest relaxant effect. Both F4 and F4´ exhibited equal efficiency, furnishing an almost 100% relaxant effect (Figure 2), though at different concentrations.



Data are expressed as the mean ± SEM of six assays. * p≤0.05 versus the control, ANOVA followed by Dunnett's test.

Figure 1. The relaxant effect of *Hyptis suaveolens* extracts on the smooth muscle of guinea pig tracheal rings contracted with carbachol (3 µM).

F4´ was more potent than F4, as can be appreciated by the EC_{50} values (Table 2). To determine the complexity of the F4´ subfraction, thin layer chromatography was performed. Three compounds were detected, suggesting the participation of more than one compound in the relaxant activity of this subfraction.

Table 1

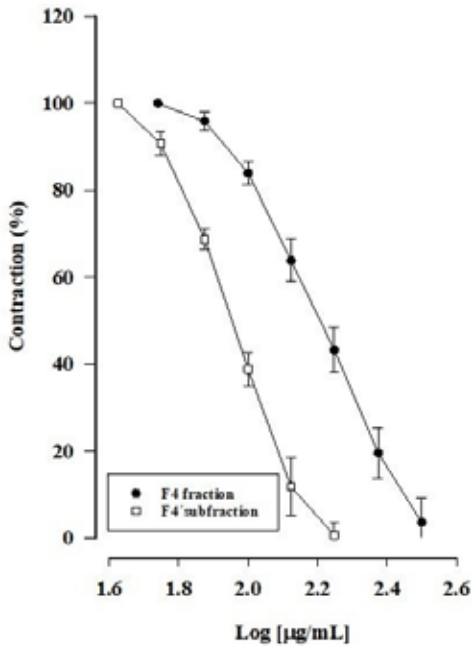
The maximum relaxant effect (Emax) and EC_{30} values of the fractions of the dichloromethane extract of Hyptis suaveolens when applied to the smooth muscle of guinea pig tracheal rings contracted with carbachol (3 µM)

Treatment	Emax (%) ± EEM	CE30 (µg/mL) ± EEM
Control	3.07 ± 3.98	-
F1	14.41 ± 5.13	-
F2	*64.17 ± 4.92	**191.66 ± 10.53
F3	*61.9 ± 4.44	**156.78 ± 4.93
F4	*96.37 ± 5.66	114.08 ± 3.44
F5	*50.49 ± 4.73	**225.01 ± 16.73

* p≤0.05 versus the control, ANOVA followed by Dunnett's test; ** p≤0.05 versus F4, ANOVA followed by Dunnett's test.

Evaluation of the participation of β_2 adrenergic receptors

The relaxant effect of F4´ was not inhibited by the presence of propranolol, indicating that the activation of β2 adrenergic receptors was not involved (Figure 3A). In contrast, the relaxant effect of the reference drug isoproterenol (a β-adrenergic receptor agonist) was entirely inhibited by the presence of propranolol (Figure 3B).



Data are expressed as the mean ± SEM of six assays. * p≤0.05 between groups, Student's *t*-test.

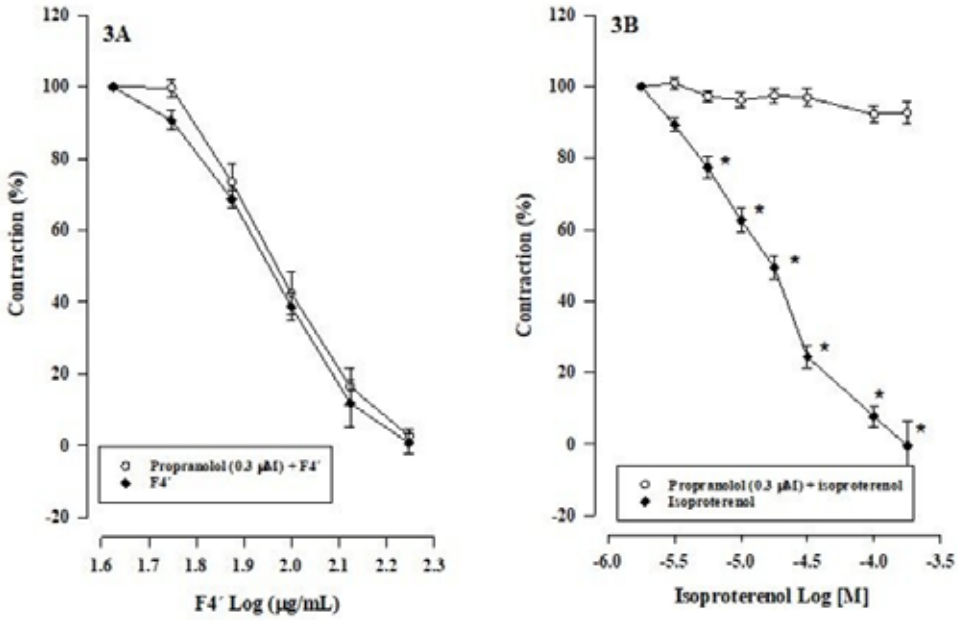
Figure 2. The relaxant effect of fraction F4 and subfraction F4´ on the smooth muscle of guinea pig tracheal rings contracted with carbachol (3 µM)

Table 2

The maximum relaxant effect (Emax) and EC50 values of fraction F4 and subfraction F4', tested on the smooth muscle of guinea pig tracheal rings contracted with carbachol (3 µM)

Fractions	Emax (%) ± EEM	CE_{50} (µg/mL) ± EEM
F4 (Ethyl acetate)	96.37 ± 5.66	151.47 ± 3.91
F4´ (Hexane/AcOEt 5:5)	99.33 ± 2.90	*91.19 ± 1.83

* p ≤ 0.001 between the two groups, Student's *t*-test.



Data are expressed as the mean ± SEM of six assays. * p≤0.05 between the two groups, Student's t-test.

Figure 3. The relaxant effect produced by subfraction F4´ (A) and isoproterenol (B) on the smooth muscle of guinea pig tracheal rings contracted with carbachol (3 µM), both in the absence and presence of propranolol pretreatment

Participation of muscarinic receptors

The concentration-response curves of carbachol were shifted to the right in the presence of F4´ (at 100 and 177µg/mL) (Figure 4). This decline in potency is reflected in the EC_{50} values (Table 3). On the other hand, the presence of F4´ did not modify the maximum contractile effect of carbachol (Figure 4). Thus, F4´ behaves like a competitive antagonist of muscarinic receptors. The reference drug atropine (a competitive antagonist of muscarinic receptors)

also caused a shift to the right in the concentration-contractile response curves of carbachol (Figure 5). This decreased potency is reflected in the EC_{50} values (Table 4).

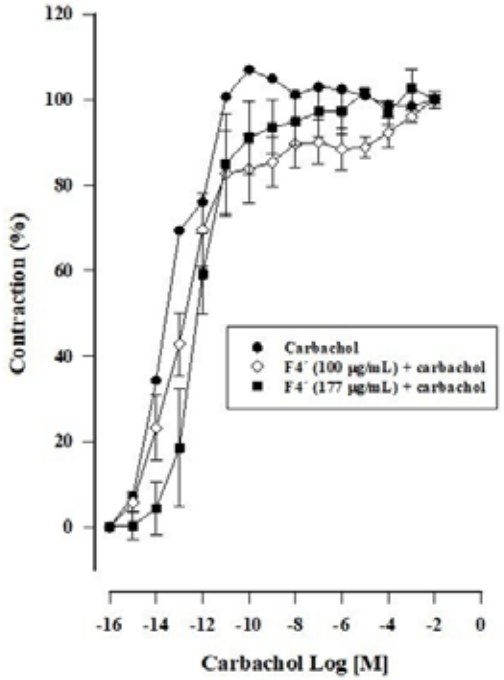


Figure 4. The effect of the F4´ subfraction on the smooth muscle of guinea pig tracheal rings subjected to carbachol-induced contractions. Data are expressed as the mean ± SEM of six assays

Table 3

The EC_{50} values of carbachol tested on the smooth muscle of guinea pig tracheal rings in the absence and presence of subfraction F4´ (100 and 177 µg/mL)

Treatment	CE_{50} (M) ± EEM
Carbachol	$2.27 \times 10^{-15} \pm 2.86 \times 10^{-15}$
F4´ (100 µg/mL) + Carbachol	$*2.29 \times 10^{-13} \pm 3.53 \times 10^{-13}$
F4´ (177 µg/mL) + Carbachol	$*1.92 \times 10^{-12} \pm 2.61 \times 10^{-12}$

* p≤0.01 compared to the carbachol-only group, ANOVA followed by Dunnett's test.

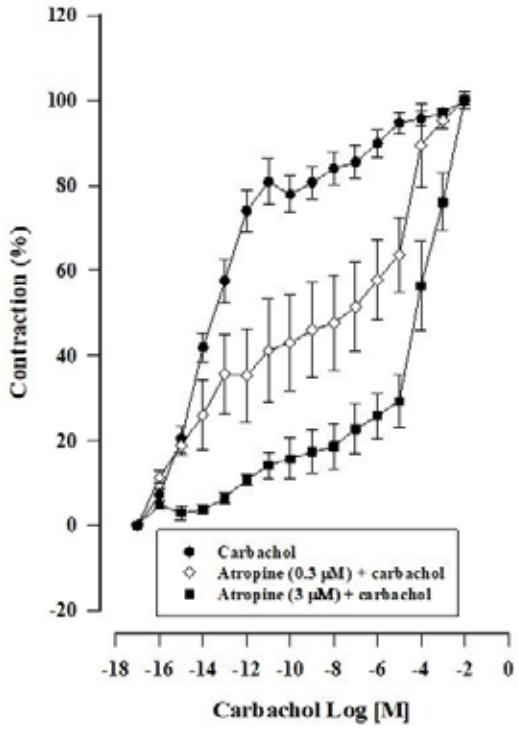


Figure 5. The effect of atropine on the smooth muscle of guinea pig tracheal rings subjected to carbachol-induced contractions.

Table 4

The EC_{50} values of carbachol when tested on the smooth muscle of guinea pig tracheal rings in the absence and presence of atropine

Treatment	CE_{50} (M) ± EEM
Carbachol	$2.79 \times 10^{-13} \pm 1.56 \times 10^{-13}$
Atropine 0.3 µM + Carbachol	$*1.050 \times 10^{-08} \pm 1.1 \times 10^{-08}$
Atropine 3 µM + Carbachol	*0.0078 ± 0.0136

* p≤0.01 compared to the carbachol-only group, ANOVA followed by Dunnett's test.

DISCUSSION

Scientific support for relaxant effect of *Hyptis suaveolens* on bronchial smooth muscle is herein provided for the first time. This plant has been reported to produce an anti-inflammatory effect in an *in vivo* model (Misrha, 2021), which is relevant because asthma patients have elevated levels of IgE, which trigger an inflammatory reaction through the release of mediators such as histamine, prostaglandins, and leukotrienes. These in turn lead to the contraction of airway smooth muscle (Taur & Patil, 2011). Hence,

Hyptis suaveolens could possibly contribute to the treatment of asthma through dual activity: the inhibition of inflammation and relaxation of bronchial smooth muscle.

In the bioassay-guided study of *Hyptis suaveolens*, the greatest relaxant activity on tracheal rings was found for the dichloromethane extract, which was separated into five fractions. Since four fractions were active (F2-F5), there is more than one compound with a relaxant activity in this extract. The most active fraction was F4.

From the fractionation of F4, four subfractions were obtained, with F4´ showing the greatest relaxant activity. Thin layer chromatography of F4´ suggests that it consists of at least three compounds. In future research, these compounds should be isolated and evaluated in order to determine the one or the combination capable of producing the best relaxant effect.

Regarding the possible mechanism of action of F4 and F4´, airway smooth muscle undergoes relaxation by directly activating relaxant mechanisms or inhibiting the effect of contractile agonists (e.g., acetylcholine and leukotrienes) (Thirstrup, 2000). Relaxant mechanisms are directly activated through the stimulation of AMPc and/or GMPc, the inhibition of cyclic nucleotide degradation, and the modulation of the activity of cell membrane ion channels. In the current study, an evaluation was made of the participation of β_2-adrenergic receptors and muscarinic receptors in the relaxant activity of subfraction F4´.

β_2-adrenergic receptors are coupled to guanine nucleotide-binding proteins (G proteins). When activated, they stimulate the enzyme adenylyl cyclase, which catalyzes the formation of the second messenger AMPc. The latter leads to the activation of protein kinase A (PKA), and this in turn phosphorylates myosin light chain kinases and several other proteins. The phosphorylation of myosin light chain kinases decreases their affinity for Ca^{2+}/calmodulin, which results in smooth muscle relaxation (Barisione *et al*., 2010; Alkawadri *et al*., 2022).

To assess the contribution of β_2-adrenergic receptors to the relaxant activity of F4´, relaxation curves were constructed for F4´ in the presence and absence of propranolol (a β-adrenergic receptor antagonist). Given that the relaxation curves were not modified, the participation of these receptors in the mechanism of action of F4´ was ruled out. The reference drug isoproterenol (a β-adrenergic agonist) was also examined in the presence and absence of propranolol. Its relaxant effect was inhibited by propranolol.

In regard to muscarinic receptors, they are involved in the control of airway smooth muscle tone and the diameter of the trachea. M_2 and M_3 receptors are located in smooth muscle. M_2 receptors have been reported to prevent the relaxation normally caused by β-adrenergic receptors by inhibiting the activity of the enzyme adenylyl cyclase. There is also evidence of the

M_2 receptor-induced potentiation of the contractile role of M_3 muscarinic receptors (Soukup *et al*., 2017; Alkawadri *et al*., 2022). M_3 receptors are the main subtypes involved in the contractile response in smooth muscle (Soukup *et al*., 2017). When an agonist binds to these receptors, they activate a Gq protein that binds to the enzyme phospholipase C (PLCβ), which generates the second messengers IP3 and DAG, known to contribute to smooth muscle contraction (Soukup *et al*., 2017).

According to the current results, carbachol and F4´ compete for the same binding site on M_3 muscarinic receptors (Blumenthal, 2019). In the presence and absence of F4´, carbachol was equally effective in contracting smooth muscle. In the presence of F4´, however, a greater concentration of carbachol was required to achieve the same contractile effect, indicating reduced potency. Hence, F4´ behaves like a competitive antagonist of M_3 receptors. In confirmation of this conclusion, the reference drug atropine (a competitive antagonist of muscarinic receptors) had the same effect on carbachol, causing a lower potency without affecting its efficiency.

Short-acting anticholinergics (ipratropium and oxitropium) and one long-acting anticholinergic (tiotropium) is currently used in the treatment of asthma. One approach now employed in the development of new anticholinergic drugs for asthma is to design compounds with prolonged action on muscarinic M_3 receptors and with less effect on M_2 receptors (Soukup *et al*., 2017). In this sense, future research is necessary on the compounds responsible for the activity of F4' in order to evaluate the relaxant effect and antagonistic behavior individually and in combinations. Additionally, it is important to continue to explore other mechanisms of action of *Hyptis suaveolens*.

CONCLUSIONS

The present bioassay-guided study of *Hyptis suaveolens* demonstrated that produces a relaxant effect on the smooth muscle of guinea pig tracheal rings, which could possibly be useful for the improvement of the symptoms of asthmatic patients. The most active was subfraction (F4´). According to the results of exploring the mechanism of action of F4´, muscarinic receptors but not β_2-adrenergic receptors are involved. F4´ acted as a competitive antagonist of muscarinic receptors. .

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Study of the effect of drought-induced by polyethylene glycol in *Capsicum frutescens* in a hydroponic system

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— Abstract—

Drought is the most common factor limiting crop development and productivity, severely affecting agriculture. In Mexico, one of the crops of economic and gastronomically important sensitive to water stress is the *Capsicum* genus. In the present study, the application of proline on *Capsicum frutescens* plants exposed to drought induced by polyethylene glycol (PEG) in a hydroponic system was evaluated. Using a 2^2-factorial design, a total of 60 seedlings were evaluated for 120 hours divided into four treatments: PEG (0 and 10 %) and Pro (0 and 10 mM) as study variables. The results showed that exposure to 10 mM of Pro induced a significant increase in the chlorophyll concentration and endogenous proline (leaf and root) in seedlings in the absence of stress. In addition, 80 % survived of seedlings, and an increase in proline content was observed in those exposed to 10 mM Pro + 10 % PEG. Similarly, there was an increase in chlorophyll content (13 $\mu g \cdot mL^{-1}$), relative water content (RWC) in the root (77.6 %), and percentage of electrolytes in the leaf and root (~44 and ~52 % respectively) compared to the treatment of 0 mM Pro + 10 % PEG. These results suggest that the pre-application of proline has a positive effect on seedlings' survival under PEG-induced drought conditions.

Keywords:

Drought; Capsicum frutescens; tolerance; survival.

Drought is considered the main stress that affects the growth and productivity of agricultural crops, and economic losses are estimated to exceed those caused by other types of abiotic stress. Unfortunately, due to climate change, more extreme conditions are expected in the coming years (dos Santos *et al*., 2022). One of the crops affected of great importance due to the wide variety of culinary and economic uses is chili. The genus *Capsicum* belongs to the family Solanaceae and is native to tropical and subtropical America. As of 2019, 40 species have been identified, of which five are of great economic relevance (*C. annum, C. chinense, C. baccatum, C. frutescens, and C. pubescens*) and have been domesticated or semi-domesticated depending on the region in which they are grown. However, it experiences a significant impact on its production due to water stress during the different phenological stages of the crop, which limits its development and productivity (Tripodi & Kumar, 2019; Toppino *et al*., 2021). The effects of water deficit on plants depend on their stage of development, the time of exposure, the species, and the degree of severity, which leads to metabolic alterations such as the reduction in the synthesis of photosynthetic pigments, loss of turgidity, increase in reactive oxygen species, among others (Jalil & Ansari, 2020; Taiwo *et al*., 2020). Currently, Mexico is the fourth largest producer of fresh chili in the world and the five main species of economic importance are cultivated (Food and Agriculture Organization [FAOSTAT], 2023). In addition, around 25 wild or semi-domesticated species are present in the territory, one of which is *C. frutescens*, distributed in the southeast of the country (La Cruz-Lázaro *et al*., 2017), the gene pool of semi-domesticated species offers the opportunity to use it as unique study models for crop breeding. In recent years, various investigations have been carried out to mitigate the effects of water stress and improve plant tolerance. The exogenous application of biostimulants to increase performance and minimize the effects of environmental stress is one of the most promising strategies (Sahoo *et al*., 2019). The amino acid proline (Pro) is considered one of the most important signaling molecules, in addition to participating as an osmoprotectant by presenting a positive correlation between proline accumulation and stress tolerance (Elewa *et al*., 2017; Suekawa *et al*., 2019). However, the application of exogenous proline in various agricultural crops under drought conditions reduced the effects and increased their tolerance (Elewa *et al*., 2017; Farooq *et al*., 2017; Alkahtani *et al*., 2021). However, plant response varies based on genotypes, severity, and extent of drought. Therefore, the effect of proline application and polyethylene glycol (peg) -induced drought on semi-domesticated chili (C. frutescens) seedlings in a hydroponic system was evaluated in this study.

2. MATERIALS AND METHODS

2.1 Plant material

The chili seeds were germinated in polystyrene seedbeds with a mixture of peat (Peat moss®) and agrolite (Termolita® Hortiperl) in a ratio of 3:1 v/v. After 30 days of germination, the seedlings were transplanted to a hydroponic system with Hoagland solution (Hoagland & Arnon, 1950) for 3 days under constant aeration for adaptation (de Freitas *et al*., 2019), with a photoperiod of 16/8 h light/dark, at an average temperature of 25 ± 2°C. Hoagland's solution contains 50 *µM* de $CaCl_2$ (Sigma Aldrich®), 12.5 *µM* de H_3BO_3 (Sigma Aldrich®), 1 *Mm* $MnSO_4$ (Sigma Aldrich®), 1 *µM* de $ZnSO_4$ (Sigma Aldrich®), 0.5 *µM* de $CuSO_4$ (Sigma Aldrich®), 0.1 µM $(NH_4)_6Mo_3O_{24}$ (Sigma Aldrich®), 0.1 *µM* de NiCl (Sigma Aldrich®), 10 *µM* Fe-EDTA (Sigma Aldrich®), 1.2 *mM* de KNO_3 (Sigma Aldrich®), 0.8 *mM* de $Ca(NO_3)_2$ (Sigma Aldrich®), 0.2 *mM* de KH_2PO_4 (Sigma Aldrich®), and 0.2 *mM* de $MgSO_4$ (Sigma Aldrich®).

2.2 Water stress induced by polyethylene glycol

The experiment was performed using a 2^2-factorial design to analyze the effect of proline and polyethylene glycol (PEG-8000 Sigma Aldrich®) -induced drought on chili seedlings. A total of 60 seedlings distributed in four treatments were evaluated. After acclimatization of the chili plants, they were treated with proline for 48 h, which was done by supplementing Hoagland's solution in the hydroponic culture, using concentrations of 0 and 10 mM proline (Sigma Aldrich®).

Subsequently, the seedlings were exposed to 0 and 10 peg for 120 h.

At the end of the exposure to drought stress, the percentage of survival, relative water content (RWC), electrolyte quantification, chlorophyll content, and endogenous proline were evaluated for each of the treatments.

2.3. Survival rate

The survival rate of chili seedlings was determined by calculating the percentage of live plants at the end of the experimental phase concerning the number of live plants at the beginning, using equation (2), proposed by Linares (as cited in Peñalba, 2022).

$$Survival\ \% = \frac{P_V}{P_v + P_m} * 100 \qquad (2)$$

Where:
P_v = live plants
P_m = dead plants

2.4. Relative Water Content (RWC)

To evaluate the CRA of leaves and roots, the fresh weight (P_F) of the seedlings was determined after collection. Subsequently, they were dried at 65 °C in a Hamilton Beach® hot air desiccator for 48 h to obtain the constant dry weight (P_S). Equation (1) described by Jothimani & Arulbalachandran, (2020) was used.

$$\% CRA = \frac{P_F - P_S}{P_F} * 100 \quad (1)$$

Where:
% CRA = Relative water content
P_F = Fresh weight of plant tissue
P_s = Dry weight of plant tissue

2.5. Electrolyte Quantification

To evaluate the percentage of electrolyte leakage, the methodology described by Restrepo *et al.* (2013) was followed with some modifications. Discs of the fresh plant material were placed in test tubes with tri-distilled water. Initial conductivity (CE_1) was measured, using a CON-BTA (Vernier®) conductivity probe, after 2 hours of incubation at room temperature (30±2 °C). Subsequently, the samples were incubated at 120°C for 20 min, and the final conductivity (CE_2) was measured. The percentage of electrolytes released was calculated using equation (3).

$$Electrolytes\ \% = \frac{CE_1}{CE_2} * 100 \quad (3)$$

2.6. Chlorophyll content

The total chlorophyll content was determined, using the method of Inskeep & Bloom (1985) with modifications. 50 mg of fresh leaf was macerated with 80 % acetone (MEYER®) and incubated at 4°C for 60 min, then centrifuged at 10,000 rpm for 5 min. The quantification of total chlorophyll was per-

formed by the UV-visible spectrophotometry technique, using a HACH® brand spectrophotometer, DR model 5000, at wavelengths (λ) of 664 and 647 nm, using equation (4).

$$Total\ chlorophyll\ (\mu g \cdot mL^{-1}) = 17.95A_{647} + 7.90A_{664} \quad (4)$$

2.7. Determination of endogenous proline content in leaf and root

The Escalante-Magaña protocol (2020) was used to extract and quantify endogenous proline in leaf and root samples. The reaction was carried out with acid ninhydrin (Sigma Aldrich®) and glacial acetic acid (MEYER®), and the samples were incubated at 96°C for 60 min. Subsequently, the organic phase was extracted with toluene (MEYER®) and the amount of proline was quantified by spectrophotometry at λ of 520 nm, using equation (5).

$$\mu mol\ proline\ * g^{-1} = \left(\frac{\frac{(\ \mu g\ proline\ * mL^{-1})(\ mL\ toluene\ )}{115.5\ \mu g * \mu mol^{-1}}}{\frac{g\ \ sample}{5}} \right) \quad (5)$$

2.8. Statistical Analysis

The data obtained were evaluated using one-way analysis of variance (ANOVA) and the comparison of means was performed by LSD test ($P \leq 0.05$), through Statgraphics Centurion XIX® software (Statgraphics Technologies, Inc., Madrid, Spain).

3. RESULTS

To determine the effect of proline application on chili seedlings (*C. frutescens*) 35 days after germination were subjected to water stress induced by -8000 PEG 10% for 120 h. The results indicate that exposure to 10 mM Pro significantly improved survivability reaching a value of 80% in seedlings pre-treated with proline (Table 1), compared to 40% in untreated seedlings.

3.1. Chlorophyll content

The chili seedlings previously treated with proline had a higher chlorophyll content, compared to the control treatment in the absence of stress (Table 2). On the other hand, when exposing seedlings to PEG-induced drought stress, it was observed that treatment with 10 mM Pro + 10% PEG had a

higher total chlorophyll content than treatment with 0 mM Pro + 10% PEG, which showed a significant decrease due to the effects of drought.

3.2. Relative Water Content (RWC)

It was observed that the RWC, both in the aerial part and the root system of the chili seedlings significantly decreased under drought conditions (Table 3), compared to the control (no PEG). However, seedlings previously treated with proline and exposed to PEG had a significantly higher RWC in the root system, compared to seedlings without prior proline treatment. These results suggest that the application of proline could have helped maintain hydration in the roots during drought.

3.3. Electrolyte leakage percentage

PEG-induced drought exposure significantly increased electrolyte leakage into leaf and root tissues in chili (*C. frutescens*) seedlings (Table 4), relative to control. However, pre-treatment with proline reduced electrolyte loss in both tissues, compared to 0 mM Pro + 10% PEG treatment.

3.4. Endogenous proline content

The values obtained indicate that the endogenous proline content in the leaves and roots of *C. frutescens* increased significantly in response to treatment with 10% PEG, compared to treatments in the absence of stress (Table 5). This suggests that plants exposed to PEG-induced drought increased proline synthesis as a defense response to water stress. In addition, a significant difference in endogenous proline concentration was observed in the leaves and roots of *C. frutescens* treated with exogenous proline. It is important to note that the seedlings previously treated with exogenous proline and subsequently exposed to stress, had the highest content of endogenous proline.

4. DISCUSSION

Drought stress reduced the survival rate of chili seedlings, the relative water content in leaf and root tissues, and total chlorophyll, as well as an increase in the percentage of electrolyte leakage and endogenous proline content in both tissues. This is because plant water potential and turgidity are significantly reduced, which can interfere with plant metabolic functions (Pandey *et al*., 2019; Abobatta, 2020).

Jothimani and Arulbalachandran (2020) reported that the water deficit induced by 20% PEG in tomato cultivation critically affects photosynthesis, due to the increase in reactive oxygen species (ROS), altering the total chlorophyll content, and damage to cell membranes. While Restrepo *et al*. (2013) demonstrated that electrolyte loss can indicate the integrity of cell membranes and that the impairments are mainly due to lipid peroxidation under stress conditions, due to ROS acting on the free radicals of the lipids that make up cell membranes in corn plants exposed to abiotic stress.

Even though the effects of drought-induced by 10% PEG in *C. frutescens* were negative, in the seedlings that were pre-treated with proline at the concentration of 10 mM, a decrease in the negative impact of stress was observed. This led to a significant increase in the survival rate, the RWC of the root system, and a decrease in the percentage of electrolyte leakage, suggesting that the integrity of the cell membrane was maintained, and the efficiency of photosynthesis by presenting an increase in the total chlorophyll content, compared to untreated plants.

Proline is considered a multifunctional amino acid under stressful conditions. Its accumulation in high concentrations is related to the ability to provide protection and participate in various cellular processes, such as osmotic regulation, energy, nutrient availability, and changes in redox balance. The accumulation of this amino acid under dehydration conditions, such as in seedlings exposed to PEG, is because the anabolism of Pro, mainly in the leaves, is activated and catabolism is repressed (Abobatta, 2020; Alvarez *et al*., 2022; Hosseinifard *et al*., 2022).

Previous application of exogenous proline to drought-exposed chili seedlings may likely have activated physiological and biochemical mechanisms that enhanced their ability to tolerate PEG-induced stress, which enabled an increase in chlorophyll content, reduced electrolyte leakage, and increased survival rate. Semida *et al*. (2020) report an increase in chlorophyll in onion plants treated with proline (1 – 2 mM) in a foliar manner, indicating that one of the mechanisms that may be related to the increase is due to the protection of the structure and function of photosystems. In addition, proline can also act as an antioxidant, protecting cells from reactive oxygen species by reducing chloroplast cell membrane damage and chlorophyll degradation (Ashraf *et al*., 2018; Merwad *et al*., 2018; Cha-um *et al*., 2019).

Regarding the decrease in the percentage of electrolyte leakage and the increase in the concentration of endogenous proline, Farooq *et al*. (2017) reported that the foliar application of osmoprotectants (proline, gamma-aminobutyric acid) in wheat reduced lipid peroxidation of cell membranes, allowing greater stability in the integrity of cell membranes; and that proline, applied exogenously, improved the endogenous proline content, under

conditions of drought stress could be related to an increase in the content of proline precursors (ornithine, glutamic acid, and arginine).

CONCLUSION

Previous application of exogenous proline at a concentration of 10 *mM* has a positive effect on percent survival, electrolytes, relative water content, chlorophyll, and proline in chili plants *C. frutescens* exposed to 10% PEG under *in vitro* conditions. These findings could lay the groundwork for investigating different concentrations of proline and establishing the optimal concentration to improve percent survival in hydroponic cultivation under drought-stress conditions.

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Table 1

Effect of proline application on Capsicum frutescens under PEG drought

Treatments		
		Survival %
% PEG	[mM Pro]	
0	0	100^{Aa}
	10	100^{Aa}
10	0	40^{Bc}
	10	80^{Ab}

Note. Different capital letters indicate a statistically significant within-treatment difference (% PEG); whereas, different lowercase letters indicate a statistically significant difference between LSD treatments ($P \leq 0.05$).

Table 2

Total chlorophyll content in drought-exposed Capsicum frutescens

Treatments		
		Chlorophyll (μg/mL)
% PEG	[mM Pro]	
0	0	19.03^{Bb}±1.4
	10	28.96^{Aa}±2.1
10	0	6.05^{Bd}±1.7
	10	13.14^{Ac}±2.9

Note. Different capital letters indicate a statistically significant within-treatment difference (% PEG); whereas different lowercase letters indicate a statistically significant difference between LSD treatments ($P \leq 0.05$).

Table 3

Relative water content (RWC) in Capsicum frutescens seedlings under drought

Treatments			
		Aerial part %	Root system %
% PEG	[mM Pro]		
0	0	86.80^{Aa}±1.3	85.32^{Aa}±1.2
	10	85.95^{Aa}±0.9	85.34^{Aa}±1.6
10	0	80.46^{Ab}±5.2	55.90^{Bc}±2.6
	10	82.44^{Ab}±1.4	77.63^{Ab}±2.6

Note. Different capital letters indicate a statistically significant within-treatment difference (% PEG); whereas different lowercase letters indicate a statistically significant difference between LSD treatments ($P \leq 0.05$).

Table 4

Percentage of electrolyte leakage in drought-exposed Capsicum frutescens seedlings

Treatments		Aerial part %	Root system %
% PEG	[mM Pro]		
0	0	14.87^{Ac}±2.6	31.16^{Ac}±2.4
	10	12.31^{Ac}±1.1	30.88^{Ac}±1.1
10	0	58.47^{Aa}±0.9	72.96^{Aa}±3.9
	10	43.91^{Bb}±3.0	51.81^{Bb}±3.6

Note. Different capital letters indicate a statistically significant within-treatment difference (% PEG); whereas different lowercase letters indicate a statistically significant difference between LSD treatments (P ≤ 0.05).

Table 5

Endogenous proline content in drought-exposed Capsicum frutescens seedlings

Treatments		Leaf (µmoles Pro·gPF/mL)	Root (µmoles Pro·gPF/mL)
% PEG	[mM Pro]		
0	0	33.52^{Bd}±1.4	29.77^{Bd}±4.9
	10	57.06^{Ac}±3.2	37.16^{Ac}±2.6
10	0	168.87^{Bb}±2.3	99.17^{Bb}±3.1
	10	212.38^{Aa}±11.8	129.03^{Aa}±4.5

Note. Different capital letters indicate a statistically significant within-treatment difference (% PEG); whereas different lowercase letters indicate a statistically significant difference between LSD treatments (P ≤ 0.05).

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Evaluation of graduated students from the School of Medicine through the EGEL results and ENARM score

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— Abstract—

The EGEL-Medicine and the ENARM allow us to infer the performance of the graduates and the educational quality of the institutions that train them. The purpose of this article is to describe the performance of university graduates of the Universidad Autónoma de Chiapas in the Human Medicine degree in the EGEL evaluations in the years 2018-2019 and ENARM in the years 2016-2020. This is a cross-sectional study with a convenience sample of 997, of which 70.11% corresponded to graduates of the 1993 curriculum and the rest (29.89%) to the 2013 plan. We looked over the National reports issued by CENEVAL (2018-2019) and CIFHRS (2016-2020), and the institutional records for graduating. In the EGEL-Medicine test, 95.6% of the graduates obtained satisfactory performance, and the rest were outstanding, obtaining averages greater than 8.5 in the degree (56.9%). The area of medical diagnosis was the best performing (90.4% with a satisfactory opinion). Concerning the ENARM, the percentage of those accepted increased by 10% in the last registration. Conclusions: The graduates with higher averages in the bachelor's degree are the ones who manage to obtain outstanding opinions in the EGEL-medicine test. UNACH has fallen in the national ranking in the ENARM, as well as its scores. The ENARM average obtained by UNACH graduates in the years 2016 to 2020 is slightly higher than the national one (61.3 vs 60.7).

Keywords:

Educational measurement; clinical competencies; medical education.

Mexico is one of the countries with the highest number of schools of human medicine; as of 2018, 160 universities were counted (Heinze *et al*, 2018), of which only 80 have been accredited (COMAEM, 2021). In recent years, the number of graduates with this degree has increased considerably, despite this, Mexico is still below the average of the countries belonging to the Organization for Economic Cooperation and Development (OECD), with 2.5 doctors per 1000 inhabitants (or fewer, according to geographical area). The number of specialists is also lower than in other countries (119 per 100,000 inhabitants). The medical specialization process is regulated by the Inter-institutional Commission for the Training of Human Resources (CIFRHS), through the National Evaluation for Medical Residency Applicants (ENARM), which selects doctors so they can study a medical specialty (approximately 17,000 in 2020 and 2021).

Every year, a number of doctors stop providing their services for various reasons, which justifies the need to train new doctors to occupy these places. Despite this, there are few mechanisms established for the regulation of the training of health personnel, which should range from the curriculum of universities, the number of graduates, and the number of places available to specialize. Among the instruments that allow us to compare the performance of medical graduates at the national level are the

General Graduation Exam to obtain the Degree in Medicine (EGEL-Medicine) and the ENARM. The Mexican Council for the Accreditation of Medical Education (COMAEM) has proposed the unification of criteria in the operation and evaluation of medical schools and colleges in our country, however, unifying the programs of national universities is a distant goal.

Both exams have been considered useful for measuring the knowledge and/or aptitude of a doctor, the EGEL is proposed as a means to determine if the graduate has the basic knowledge and skills for his/her performance as a general practitioner (López *et al*, 2015) and the ENARM, as a selection tool for those who wish to pursue a medical specialty (Guerrero *et al*, 2016). There is no exact methodology described in the Mexican literature to determine the quality of knowledge and aptitude in medical graduates. It has been used by other institutions in the medical training of the country to the professional examination or the Objective Structured Clinical Examination (ECOE) as tools of greater objectivity, however, there is no information available in the national literature on which institutions apply these exams or the results of their graduates in them, being the EGEL and the ENARM the predominant tests according to what has been observed, prepared by external, standardized, thus allowing the comparative analysis between institutions (Flores *et al*, 2012).

The EGEL-Medicine and ENARM exams allow us to analyze the performance of graduates, although there are competencies that cannot be

evaluated in depth in this way, such as skills or abilities, they can help establish areas of improvement for the quality of the institutions that teach the degree in medicine (Flores *et al*, 2001). Determining the educational institution's training quality and the skills of its graduates is important to guarantee the adequate training of health professionals, allowing graduates better future job opportunities and higher quality health services for users. The instruments to be analyzed only evaluate the knowledge of the graduates (the exams to be analyzed present the performance of the graduates only in terms of knowledge), but allow us to infer weaknesses or strengths in the institutional study plans and programs. Following this, this analysis summarizes the findings related to the general average of the degree, performance in the EGEL-Medicine during the years 2018-2019 and the ENARM from the years 2016 to 2020 of the graduates of the Faculty of Human Medicine C-II of the UNACH.

EGEL-MEDICINE AND ENARM

The Ministry of Public Education (SEP), according to the Official Gazette of the Federation in its agreement 286, from 2000, established the General Graduation Exam to obtain a bachelor's degree (EGEL) by the National Center for the Evaluation of Higher Education (CENEVAL) as a graduation process in Mexico. This evaluation is applied at the national level and seeks to evaluate the essential knowledge in the bachelor's degree for the exercise of the profession. The exam consists of 193 multiple-choice agents and comprises the following areas:

- Health promotion/prevention
- Medical Care: Medical Diagnosis
- Healthcare: Treatment and Evaluation
- Sociomedicine

The results are classified into three levels of performance: unsatisfactory (ANS), satisfactory (DS), and outstanding (DSS). To pass the exam, the sustainer must obtain at least three areas with satisfactory or outstanding performance (satisfactory performance). It may also be that of the four areas, he or she gets at least two with outstanding performance and the remaining with satisfactory performance (Outstanding Performance). At the national level, the areas of best (outstanding) performance are medical diagnosis and health promotion.

In the case of UNACH, the Internal Regulations for Professional Evaluation for graduates of the Faculty of Human Medicine of UNACH,

approved by the University Council during the session held on October 11, 2001, points out that the options to obtain the degree are:

- Recognition of academic achievement
- Recognition of academic achievement with written congratulations or honorable mention
- Thesis evaluation (research)
- General knowledge assessment
- Evaluation through supervised technical assistance
- Evaluation by general graduation examination (EGEL-CENEVAL)

Students with a bachelor's degree GPA higher than 9 are entitled to graduation by academic achievement. Those who choose the graduation option through the EGEL-Medicine have averages below nine. The annual reports of the EGEL have been available online since 2016, although in the case of the Universidad Autónoma de Chiapas, the results are reported together for the two campuses: II, located in Tuxtla Gutiérrez, Chiapas, and IV, in Tapachula, Chiapas. This data can have a great impact on the evaluation of this institution since both faculties have different accreditation processes at the time of writing this article. The data of this study correspond only to Campus II.

The Inter-institutional Commission for the Training of Human Resources for Health (CIFRHS) is the body in charge of regulating entry to medical specialization programs in Mexico. Per the Official Mexican Standard NOM-001-SSA3-2012 for the organization and operation of medical residences, doctors are selected by the ENARM, which is applied annually following the call issued. Passing this exam does not guarantee admission, as each hospital can set its selection criteria.

Therefore, the objective of this study was to describe the performance of university graduates of the Autonomous University of Chiapas with a bachelor's degree in Human Medicine using the EGEL evaluations in the years 2018-2019 and ENARM in the years 2016-2020.

METHODOLOGY

The universe corresponded to the graduates of the School of Human Medicine Dr. Manuel Velasco Suárez, C-II of the UNACH. The sample was selected for convenience and made up of the total number of students who obtained passing grades in the EGEL evaluation and graduated during the 2018-2019 period, with a total of 997 graduates.

We manually captured the information, considering the passing grades in each area of the evaluation according to the scale provided by CENEVAL:

- Unsatisfactory (S) 700-999 points
- Satisfactory (TDS) 1000-1049 points
- Outstanding (TDSS) 1150-1300 points

In addition, an analysis of the averages obtained in the ENARM by the UNACH graduating doctors is presented. These data were obtained from the academic reports presented by CIFHRS from 2016 to 2020, clarifying that they do not correspond to those who presented the EGEL, since that is a different analysis. The data were captured in Excel spreadsheets and then processed with the SPSS statistical program version 25.

RESULTS

Data were collected from 997 graduates who reached the passing grade in the EGEL-Medicine, in an age range of 23 to 35 years, with a mean of 27.80 and a standard deviation of 1.505. 51.1% (509) of the sample are men, and 48.9% (488) are women. Of the total sample, 70.11% corresponded to graduates of the 1993 study plan and the rest (29.89%) to the 2013 plan.

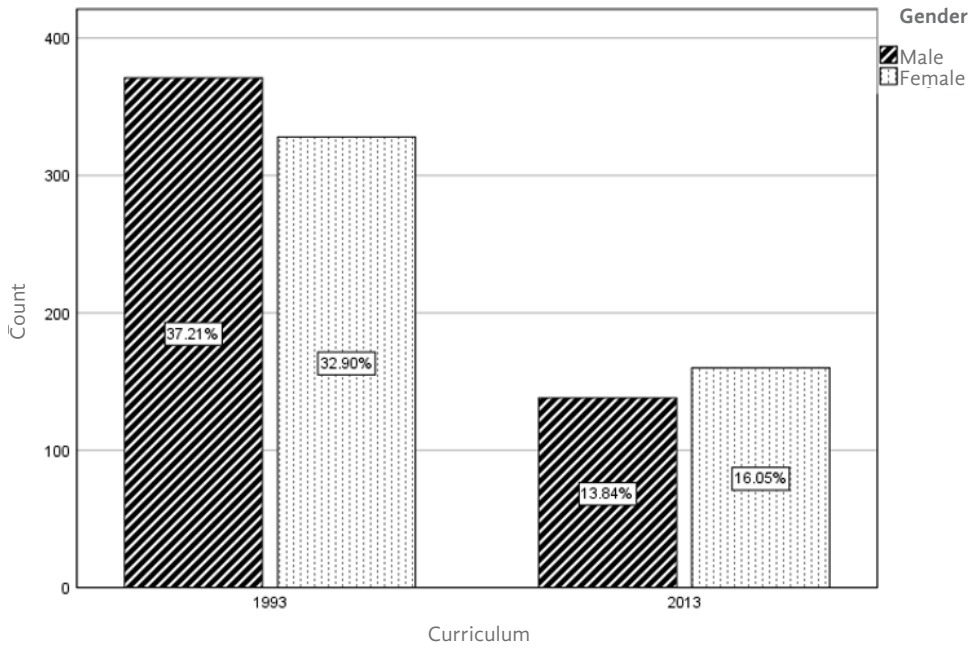


Figure 1. Distribution of total graduates concerning curriculum and gender. Source: Elaboration from the Graduation Department's institutional archives de Titulación. School of Human Medicine "Manuel Velasco Suárez, Campus II UNACH"

Regarding the year of graduation, 75.1% (749) of graduates graduated in 2019 and 24.9% (248) in 2018.

GPAs obtained during the bachelor's degree for both curricula ranged from 7.45 to 8.99, with a mean of 8.5305 and a standard deviation of 0.26059.

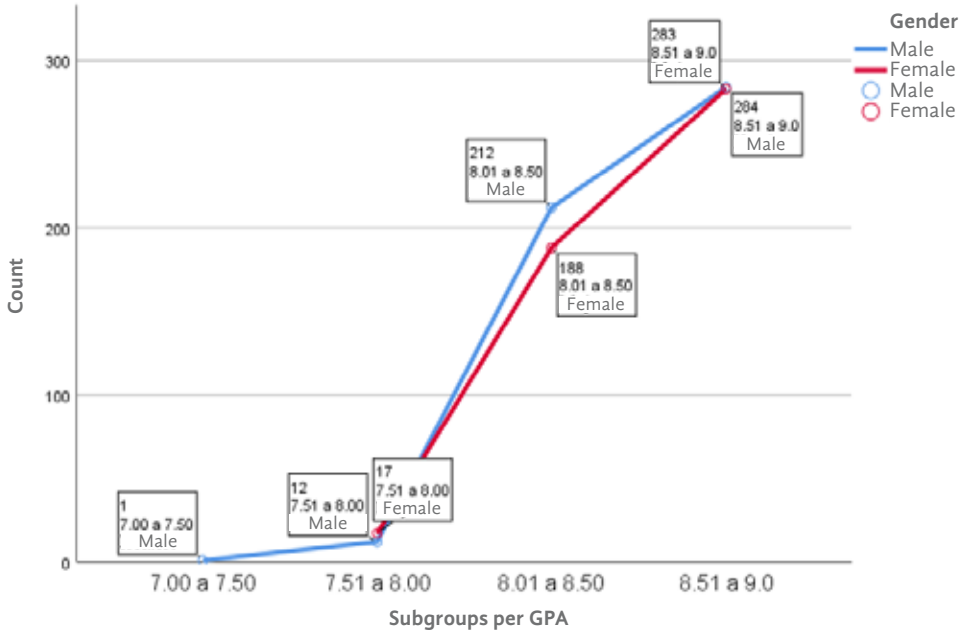
Table 1

Grade Point Average of Graduates

	Frequency	Percentage (%)
7.00 a 7.50	1	0.1
7.51 a 8.00	29	2.9
8.01 a 8.50	400	40.1
8.51 a 9.00	567	56.9
Total	997	100

Source: Elaboration from the Graduation Department's institutional archives de Titulación. School of Human Medicine "Manuel Velasco Suárez, Campus II UNACH"

Table 1 groups by GPA ranges of graduates, observing that the group with the highest frequency (56.9%) were those who obtained a bachelor's degree GPA between 8.51 to 9.0 (567), followed by the range between 8.01 to 8.50, with 40.1% (400).



Source: Elaboration from the Graduation Department's institutional archives de Titulación. School of Human Medicine "Manuel Velasco Suárez, Campus II UNACH"

Figure 2 shows that, according to the GPA, those with the lowest scores (7.1 to 8.0) correspond to 30 graduates, of whom 17 are women. In the following groups, the male-to-female ratio was comparable. Regarding the opinion obtained in the EGEL-Medicine, 95.6% of the graduates showed a satisfactory performance, and 4.4% of the students presented an outstanding performance.

Table 2

The ratio between GPA, EGEL-Medicine result, and Curriculum

		Dictamen CENEVAL				Total
Average	Plan of study	Satisfactory	* Group Total %	Outstanding	*Group Total %	
7.00 a 7.50	1993	1	0.14	0	0.00	1
	2013	0		0	0.00	
7.51 a 8.00	1993	24	3.43	1	0.14	25
	2013	4	1.34	0	0.00	4
8.01 a 8.50	1993	272	38.91	10	1.43	282
	2013	112	37.58	6	2.01	118
8.51 a 9.00	1993	378	54.08	13	1.86	391
	2013	162	54.36	14	4.70	176
Total	**1993**	**675**	**96.57**	**24**	**3.43**	**699**
	2013	**278**	**93.29**	**20**	**6.71**	**298**
	Total	953	95.59	44	4.41	997

* The percentages presented were calculated concerning the total curriculum, corresponding to 1993 (699) and 2013 (298) respectively.

Source: Prepared from institutional files of the Degree Department School of Medicine "Dr. Manuel Velasco Suárez, Campus II." UNACH

Table 2 shows the distribution of graduates concerning the study plan and opinions obtained in the EGEL-Medicine. Of the 1993 study plan graduates, 96.56% (675) were satisfactory and 3.43% (24) outstanding. In the 2013 study plan, graduates with satisfactory grades were 93.29% (278) and 6.71% (44) outstanding.

Table 3
Overall results by areas evaluated in the EGEL-Medicine

	Health promotion		Medical diagnosis		Treatment and evolution		Sociomedicine	
	N	%	N	%	N	%	N	%
Unsatisfactory	**345**	**34.6**	60	6	19	1.9	50	5
Satisfactory	622	62.4	**901**	**90.4**	760	76.2	794	79.6
Outstanding	29	2.9	31	3.1	**217**	**21.8**	153	15.3
Total	996	99.9	992	99.5	996	99.9	997	100
Lost	1	0.1	5	0.5	1	0.1		

Source: Prepared from institutional files of the Degree Department School of Medicine "Dr. Manuel Velasco Suárez, Campus II." UNACH.

Regarding the areas evaluated in the examination, it was observed that medical diagnosis is the best performing in graduates (90.4%), followed by treatment and evolution (76.2%), both with a satisfactory opinion. However, it is the area of treatment and evolution in which graduates reach the highest number of outstanding opinions (21.8%). Health promotion is the area with the lowest performance of unsatisfactory opinions.

ENARM

From the available open database, records were obtained in terms of the number of supporters by Faculties and Schools of Medicine in the period between 2016 and 2020.

Table 4
ENARM results during the period 2016-2020 of the graduates of the Universidad Autónoma de Chiapas

Year	National Ranking	Sustaining	Accepted	Average grade	National Average	Acceptance %
2016	34	609	113	62.347	76.06	18.56
2017	50	758	134	62.78	62.05	17.68
2018	45	794	154	63.22	62.1	19.4
2019	67	968	172	60.054	60.13	17.77
2020	66	1028	344	58.5	58.3	33.46

Source: Own elaboration with data obtained from the annual academic reports CIFRHS (2016-2020)

114 Mexican universities applied the ENARM during 2016 and 2017, while in 2018, there were 122. In 2019, 131 participated, and in 2020 it increased to 136. Not all graduates of national universities present the ENARM on an annual basis. Table 4 indicates that the UNACH has dropped in the national ranking of universities (although, the number of universities increases year by year) the average achieved in the ENARM has also dropped. The percentage of accepted students increased by more than 10 percentage points on the last record.

Table 5

Ranking of Mexican universities by GPA obtained by applicants accepted into the ENARM from 2016 to 2020

Place	Institution	2020	2019	2018	2017	2016	Average
1	Univ. Panamericana	69.76	74.498	76.7	77.02	73.46	74.2876
2	ITESM. - Campus Cdmx	65.93	68.622	70.75	69.4	68.348	68.61
3	Univ. Aut. De Querétaro	63.58	66.241	67.98	70.93	68.371	67.4204
4	Univ. La Salle	63.72	66.873	69.22	69.13	67.779	67.3444
5	Univ. De Guanajuato	63.48	66.193	68.59	69.47	65.106	66.5678
6	Univ. Aut. De B.C., U. Mexicali	62.32	65.865	67.95	68.87	66.177	66.2364
7	Univ. Aut. De Coahuila, U. Saltillo	62.72	64.901	69.27	68.34	65.87	66.2202
8	Univ. Aut. De Aguascalientes	62.85	66.781	67.93	67.52	65.739	66.164
9	Univ. Aut. De Coahuila, U. Torreón	63.02	65.168	68.43	68.24	65.924	66.1564
10	Univ. Aut. De Chihuahua	61.96	64.711	67.54	67.81	66.419	65.688
11	Univ. Aut. De B.C., U. Tijuana	62.27	64.717	67.39	68.2	65.614	65.6382
12	Univ. Aut. De Nuevo León	61.39	63.88	66.04	69.05	67.509	65.5738
13	Univ. De Guadalajara, U. Los Altos	61.76	62.786	65.22	67.24	68.669	65.135
14	Univ. Aut. De Yucatán	62.14	64.952	65.96	66.43	65.269	64.9502
15	Univ. Aut. Del Edo. De México	62.1	63.722	66.51	66.4	64.965	64.7394
16	Univ. Aut. De San Luis Potosí	64.07	68.144	70.09	61.76	59.602	64.7332
17	Univ. De Sonora	62.37	64.056	66.52	66.69	62.954	64.518
18	UNAM FM CU	61.67	63.935	65.85	65.77	64.409	64.3268
19	Univ. Cuauhtemoc - Plantel San Luis Potosi	60.11	64.639	67.3	66.43	62.183	64.1324
20	Univ. De Guadalajara	61.8	64.1	66.18	64.3	64.123	64.1006
21	Univ. De Monterrey	62	65.31	66.56	67.51	58.85	64.046
22	Univ. Aut. De B.C., U. Ensenada	60.96	61.996	65.27	64.88	64.81	63.5832
23	Univ. De Guadalajara, U. Cd. Guzmán	59.75	63.901	63.6	65.02	64.238	63.3018
24	Univ. De Colima	61.14	62.059	63.98	63.8	64.922	63.1802
25	Univ. Del Valle De México - Campus Hermosillo	57.62	60.048	61.66	64.93	71.556	63.1628
26	Univ. Aut. De Hidalgo	60.16	61.673	63.63	65.7	63.531	62.9388

27	Univ. Aut. De Nayarit	59	61.305	63.64	66.48	64.254	62.9358
28	Univ. Anáhuac	59.98	62.249	64.09	64.57	62.675	62.7128
29	Univ. Cuauhtémoc - Campus Aguascalientes	58.64	62.571	65.12	63.81	62.539	62.536
30	Univ. Las Américas Puebla	60.18	61.257	65.05	65.23	60.794	62.5022
31	Univ. De Celaya	59.36	61.098	63.69	65.5	62.641	62.4578
32	Univ. Aut. De Morelos	60.26	62.981	65.4	62.67	60.409	62.344
33	Univ. De Guadalajara Centro Universitario De La Costa	58.77	61.161	63.75	64.77	61.909	62.072
34	Univ. Mich. De San Nicolás De Hidalgo (Zamora)	59.67	62.294	63.55	62.86	61.333	61.9414
35	Univ. Aut. Metropolitana, U. Xochimilco	60.09	61.538	63.1	63.47	61.075	61.8546
36	Univ. Latina De México	58.62	60.903	62.72	63.66	63.36	61.8526
37	Univ. Montemorelos	59.26	59.922	63.61	64.77	61.131	61.7386
38	Esc. Médico - Militar Sedena	60.22	64	55.56	66.56	62.148	61.6976
39	Univ. Aut. De B.C. - Campus Valle De Las Palmas	59.4	61.43	64.22	62.7	60.576	61.6652
40	Univ. Aut. De Cd. Juárez	58.83	60.994	63.03	63.43	61.701	61.597
41	IPN Esc .Sup. Medicina	59.25	61.117	62.74	62.8	61.629	61.5072
42	Univ. Veracruzana, U. Cd. Minatitlán	59.22	61.324	62.03	62.77	62.055	61.4798
43	Univ. Del Valle De México, Campus Qro	57.88	60.649	63.14	63.48	61.971	61.424
44	**Universidad Autónoma de Chiapas**	**58.5**	**60.054**	**63.22**	**62.78**	**62.347**	**61.3802**
45	Centro Est. Univ. Xochicalco, U. Tijuana	59.4	61.482	63.26	62.48	60.113	61.347
46	Univ. Aut. De Zacatecas	58.94	60.723	62.47	62.36	62.047	61.308
47	Univ. Aut. De Guadalajara	58.51	61.148	63.7	62.1	61.025	61.2966
48	Univ. Veracruzana, U. Poza Rica	57.36	59.725	62.95	63.47	62.845	61.27
49	Univ. Popular Aut. Del Edo. Puebla	58.72	60.801	62.72	62.38	61.576	61.2394
50	Univ. Juárez Del Edo. Durango, U. Gómez Palacio	59.21	59.608	62.94	63.07	61.347	61.235
51	Univ. Del Mayab	59.62	62.326	62.73	63.86	57.213	61.1498
52	Univ. Del Valle De México - Campus Zapopan	58.32	60.781	62.25	63.45	60.925	61.1452
53	UNAM Fes Iztacala	58.91	60.666	62.66	61.87	60.242	60.8696
54	Univ. Veracruzana, U. Veracruz	58.66	60.089	60.94	63.19	61.242	60.8242
55	Univ. Veracruzana, U. Xalapa	59.32	60.388	61.83	61.71	60.762	60.802
56	Univ. La Salle - Campus Cd. Victoria	58.14	60.026	61.34	62.36	59.923	60.3578
57	Univ. Del Valle De Atemajac	57.28	61.126	62.25	62.32	58.508	60.2968
58	UNAM Fes Zaragoza	59.14	59.818	61.7	61.09	59.661	60.2818
59	Univ. Juárez Del Edo. Durango, U. Durango	57.97	59.986	62.34	61.6	59.346	60.2484
60	Univ. Aut. De Campeche	58.48	58.957	62.34	60.89	59.516	60.0366

61	Univ. De Quintana Roo	57.69	57.738	59.59	60.45	64.591	60.0118
62	Univ. Aut. Benito Juárez De Oaxaca	58.11	59.515	60.65	60.9	59.547	59.7444
63	Univ. Aut. De Tamps, U. Matamoros	55.71	57.42	60.16	63.52	61.788	59.7196
64	Univ. Aut. De Durango - Campus Zacatecas	55.94	57.093	59.06	64.23	62.25	59.7146
65	Univ. Aut. De Sinaloa	56.77	60.326	62.34	60.42	58.034	59.578
66	B. Univ. Aut. Puebla (Puebla)	58.09	59.129	60.9	60.04	59.394	59.5106
67	Univ. De Durango - Campus Cd. Juarez	52.35	56.938	58.52	65.86	63.416	59.4168
68	Univ. Cuauhtemoc - Plantel Guadalajara	56.36	57.625	62.02	63.59	57.333	59.3856
69	Univ. Aut. De Tamps, U. Tampico	58.33	61.367	63.43	56.89	56.553	59.314
70	Univ. Guadalajara Lamar - Campus Vallarta	56.96	58.994	59.06	60.78	60.72	59.3028
71	Centro Est. Univ. Xochicalco, U. Ensenada	56.36	58.18	61.2	61.16	59.469	59.2738
72	Univ. West Hill Institute S.C.	56.62	58.193	60.48	61.75	58.993	59.2072
73	Univ. Aut. De Guerrero	55.48	57.011	58.87	62.91	61.631	59.1804
74	Univ. Mich. De San Nicolás De Hidalgo (Morelia)	57.74	59.26	60.99	59.89	57.954	59.1668
75	B. Univ. Aut. Puebla (Tehuacán)	58.21	58.443	60.97	58.52	59.629	59.1544
76	Centro Est. Univ. Xochicalco Campus Mexicali	59.33	62.873	65.31	59.45	48.444	59.0814
77	Univ. Del Valle De México - Campus Cd. Reynosa	57.75	58.442	61.2	60.59	56.064	58.8092
78	Univ. Veracruzana, U. Cd. Mendoza	57.24	58.436	59.78	59.11	59.461	58.8054
79	Univ. Del Valle De México - Campus Cd. Victoria	55.9	58.047	58.1	57.8	63.627	58.6948
80	Univ. Guadalajara Lamar - Campus Inglaterra	56.95	59.108	61	56.48	59.737	58.655
81	Univ. Del Noreste	55	56.541	59.48	59.59	62	58.5222
82	Univ. Aut. De Guadalajara, Campus Tabasco	57.16	58.608	61.46	58.27	57.085	58.5166
83	Univ. Vasco De Quiroga - Campus Santa Maria	56.9	59.845	60.23	58.92	56.61	58.501
84	Ipn Esc. Nal. Med. Y Homeopatía	57.29	57.786	59.83	59.65	57.919	58.495
85	Univ. Quetzalcóatl De Irapuato	56.52	57.693	60.25	59.73	58.005	58.4396
86	Estudios En El Extranjero	55.65	58.017	59.51	58.87	58.664	58.1422
87	Univ. Regional Del Sureste	56.71	57.687	59.72	58.51	57.865	58.0984
88	Univ. Cristobal Colon - Veracruz	55.29	57.118	60.77	58.76	58.045	57.9966
89	Ipn Centro Interdisciplinario De C. Salud	57	57.173	59.25	58.96	57.17	57.9106
90	Univ. Latinoamericana	56.33	57.07	58.75	58.72	58.15	57.804
91	Univ. Del Valle De México - Campus Saltillo	55.49	55.454	59.07	60.29	57.825	57.6258
92	Univ. Del Valle De México - Campus Villahermosa	52.97	56.489	61.77	59.2	57.246	57.535
93	Univ. Aut. De Tlaxcala	55.17	55.452	57.62	61.43	57.907	57.5158

94	Univ. Olmeca	53.37	55.051	55.48	58.97	60.067	56.5876
95	Univ. Justo Sierra	54.6	55.371	58.53	58.37	56.049	56.584
96	Univ. Juárez Aut. De Tabasco	54.17	55.918	58.12	57.27	55.78	56.2516
97	Univ. Juárez Aut. De Tabasco División Académica	53.82	54.293	57.24	55.9	59.644	56.1794
98	Univ. Aut. De Durango - Campus Durango	53.59	55.012	55.76	58.02	55.948	55.666
99	Esc. Med. Tominaga Nakamoto	53.22	54.583	57.28	56.23	55.322	55.327
100	Inst. Est. Superiores De Chiapas -Campus Tuxtla Gutierrez	54.01	55.011	56.53	54.93	54.26	54.9482
101	Univ. Aut. España De Durango	55.07	52.973	57.12	56.43	52.042	54.727
102	Univ. México-Americana Del Norte	52.76	53.358	55.23	55.17	55.395	54.3826
103	Inst. de Ciencias y Estudios Sup. De Tamaulipas	51.72	53.628	55.28	56.41	54.728	54.3532
104	Inst. Est. Superiores De Chiapas - Campus Tapachula	52.66	51.564	54.05	54.22	54.744	53.4476
105	Inst. Est. Superiores Tepeaca	53.16	54.432	53.86	54.08	50.749	53.2562
106	Univ. Hipócrates	52.02	51.844	52.82	54.34	55.022	53.2092
107	Esc. Libre De Homeopatía De México	52.85	51.569	53.62	54.39	50.597	52.6052

Source: Own elaboration with data obtained from the annual academic reports CIFRHS (2016-2020)

For Table 5, only those universities with available data for the aforementioned years were taken into account, bringing the total to 107 (out of 136). When performing the global analysis, the maximum corresponded to 74.28 and the minimum to 52.60, with a range of 21.68, mean of 60.75, variance of 13.14, and standard deviation of 3.6.

The Universidad Autónoma de Chiapas ranks 44th according to the average obtained in the years analyzed in the ENARM. The maximum average obtained in that period corresponded to 63.22 and the minimum to 58.5, with a mean of 61.38, a range of 4.72, a variance of 3.26, and a standard deviation of 1.80.

DISCUSSION

In the same way as Guerrero, Romero, and Noriega (2015), the male population in our study was greater than the female, contrary to the national report for the EGEL-Medicine 2018 and 2019. Regarding the average graduation from the bachelor's degree, the national report in 2018 indicated that 66% and in 2019 68% of the supporters obtained an average between 8.0 and 9.0, unlike this study, where 97% of the sample obtained averages in this same range. Regarding the opinion obtained in the EGEL-Medicine, 95.6% of the graduates presented a satisfactory performance, a figure higher than the

national percentage (47.6% in 2018 and 53.3% in 2019), 4.4% of the students presented an outstanding performance (7.4% in 2018 and 12.1% in 2019).

We highlight that, at UNACH, unlike other universities, students with a GPA rate higher than 9.0 obtain their degrees by academic merit. That is why we cannot compare the results obtained with those reported by authors such as Guerrero, Romero, and Noriega (2015), who found that graduates with averages above 9.0, obtained outstanding opinions more frequently, although in this study it could also be seen that graduates with a GPA between 8.51 to 8.99, also obtained the highest scores and outstanding opinions in the EGEL-Medicine.

In the evaluation by areas, the best performing at the national level corresponds to medical diagnosis with 62% of satisfactory opinions in 2018 and 69.2% in 2019, against 90.4% of the population studied, followed by treatment and evolution with 52.3% in 2018 and 57.1% in 2019 against 76.2% of the graduates of UNACH campus II. The area of *health promotion* was the lowest performing, as 34.6% of graduates obtained an unsatisfactory opinion, which is lower than the national one (60.7% in 2018 and 56.1% in 2019). At the national level, *health promotion* is the area with the highest number of graduates with an outstanding opinion, medical diagnosis in 2018 (15.1%) and *Health promotion* in 2019 (20.3%). For medical graduates of UNACH C-II, in the area of treatment and evolution, 21.8% of the opinions were outstanding.

In the work carried out by Ramiro *et al* (2017), which studies trends in the percentage of accepted students per institution during 2001-2016, it was observed that the national universities with the highest number of accepted students are the Universidad Panamericana, Universidad Autónoma de San Luis Potosí, Instituto Tecnológico de Estudios Superiores de Monterrey, Universidad la Salle, and Universidad de Guanajuato. The lowest number of accepted correspond to the Centro de Estudios Universitarios Xochicalco Mexicali, Universidad Autónoma de Durango, Escuela Libre de Homeopatía, Instituto de Estudios Superiores Tepeaca, and Universidad México-Americana del Norte In this study, the average of those accepted per institution in the years 2016-2021, was taken as a reference, since the percentage of accepted and graduates taking the exam are extremely different between institutions, however, in this analysis great coincidences were observed regarding the universities with the best averages in the last five years concerning the analysis carried out by Ramiro *et al* (2017); where he also mentions that the percentage of acceptance from 2001 to 2016 in the Universidad Autónoma de Chiapas was 21%.

CONCLUSIONS

Although there are bodies dedicated to unifying criteria for the operation of educational institutions in medicine, to raise and maintain quality standards, the curricula and the results in national regulatory evaluations are so disparate that they represent difficulties for comparison.

In the case of the Autonomous University of Chiapas, the statistical data corresponding to the ENARM are granted jointly and not by campus, as in the case of other universities, which makes it even more difficult to follow up on graduates, since, even in the accreditation processes, campuses present different opinions.

Regarding the graduation GPA with the opinions of the EGEL, comparing the results with other studies was not possible, since the graduates of the UNACH with the highest average have different graduation process options. The results obtained in this study show a better performance concerning the national average, although in the area of health promotion, it is clear that the students' performance was not satisfactory, so it is necessary to improve the curriculum. It was also observed that the graduates with higher averages, the majority obtained outstanding opinions in the EGEL-Medicine.

Evaluating students' knowledge is a challenge at all levels of education and as the curricula are so different between institutions, the analysis of the results of the EGEL-Medicine and ENARM could help to make adjustments in all the institutions that teach the degree.

The number of universities with a degree in Medicine increases year after year, which has contributed to UNACH dropping in the national ranking in the ENARM, however, the averages of graduates continue to be slightly above the national average. The average in the ENARM obtained by UNACH graduates in the years 2016-2020 is slightly higher than the national average of 61.3 vs 60.7.

RECOMMENDATIONS

- To obtain a better picture regarding the performance of UNACH graduates at ENARM, it is necessary that, as in other institutions, the number of accepted students and their GPAs are reported by campus, which must be managed by UNACH before CIFRHS.
- It is necessary to place greater emphasis on the teaching of knowledge in the area of health promotion since it is the area of lowest performance of graduates.
- Given the new modality of choosing specialty positions, carrying out the statistical analysis of the averages obtained by area of knowledge in the ENARM could be of help to university graduates, since it will

allow those who have a specialty in mind to focus on the study of the areas in which their peers have the greatest deficiencies, allowing to increase the educational quality of the institution.

- The number of graduates and graduates who took the ENARM varies greatly concerning the institution and the year, therefore, the analyses of trends in terms of average obtained by the examinees could present a better picture concerning the performance of the graduates by institution, it is suggested that future analyses take this variable into account.

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Remittances and financial inclusion in municipalities of Northeast Mexico: A Bayesian spatiotemporal analysis

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— Abstract—

This paper aims to analyze the spatiotemporal distribution of remittances in the municipalities of northeastern Mexico given the effect of financial services and therefore focuses on studying the relationship between the flow of remittances and financial inclusion, considering the existing infrastructure and network of banking services in the municipalities of the region, while arguing that financial inclusion can support the accumulation of resources through savings and allow access to credit, insurance and other types of financial products. The results show that the concentration of resources in institutions and municipalities with a greater presence of banking institutions can lead to the expansion of small businesses, the generation of income derived from the development of micro-businesses and productive investment, especially in low-income segments, and that this is associated with the receipt of remittances, as well as highlighting the importance of understanding the complex interactions between international migration, remittances, and the economy.

Keywords:

Financial inclusion; additive log-linear models; remittances.

This longitudinal cohort study aims to analyze the spatial distribution of remittances in the municipalities of northeastern Mexico conditioned by the effect of the levels of financial inclusion observed. Remittances were quantified by Banco de Mexico in the balance of payments based on the municipal distribution of income from family remittances in quarterly series estimated in millions of dollars. Financial inclusion was quantified from the total number of bank branches reported by the Economic Information System of Banco de Mexico within the operational information module of commercial banking for the municipalities of northeastern Mexico, a region made up of Tamaulipas, Nuevo León, and Coahuila. The analysis period covers the years 2015 to 2020.

Therefore, the relationship between remittance flows and financial inclusion was analyzed, considering the infrastructure and economic network present in the municipalities of the northeastern region of Mexico. It is, therefore, an analysis of the economic flows and how these financial resources transit, resources from the work of migrants that are sent to their places of origin, where they could have a family and/or consumption relationship in Mexico. Remittances can be understood as one more instrument that supports the financial inclusion of families receiving remittances. The integration into the banking or financial system of a part of the migratory circuits causes the concentration of resources in institutions and municipalities, where both the reception of remittances and bank branches are concentrated, with the reduction of the dispersive effects of resources in the municipalities with less presence of the banking system.

Financial inclusion makes it possible in principle, the opportunity to accumulate resources via savings, and secondarily allows making payments or receiving them with collateral, which would eventually lead to the possibility of accessing credits, insurance, and financial products of greater complexity (Buchenau, 2010). Experience and studies show the correlation between access to financial services and obtaining credit, associated with the expansion of small businesses, the generation of other income, and productive investment in low-income segments (Dittus and Klein, 2011).

BACKGROUND

Recognizing the complex relationships between international migration and its economic counterpart, the issuance of remittances leads to the analysis of the behavior that remittances describe in the spatial and temporal dimensions. Although the behavior of remittances at any scale is similar, of course with variations, one interpretation of remittances is to give them a role as an agent of dependence and motivator for international migration; from the explanation of the cyclical performance of the economy, in the

case of concrete of the Mexico-USA circuit, the flow of remittances has a dynamic behavior, dominated by defined cycles, which implies a certain vulnerability; this is by what has been expressed in recent years by the figures of Banco de Mexico.

The connection between migration and financial services is largely determined by remittances and the possibility of accessing these resources in a secure market with convenient conditions for those who send and those who receive. The flow of remittances is identified as a starting factor, within a particular vision of local development, capable of promoting the development of productive projects and of direct connection with consumption and indirect with the productive and commercial sectors. In particular, remittances have been identified as a support lever for reducing poverty and migration as a means of occupying surplus labor.

There are countless examples of actions undertaken such as the Tres por uno program (3X1 for migrants) and investment in family businesses; to some extent, collective remittances (intermittent and under-served) are connected to problems in planning and complications in their use; at the family level it is where subtle aspects such as banking and financial education (initial aspects of financial inclusion) play a role in the management of local businesses.

Access to remittances, from a broad and theoretical perspective, would reduce vulnerability and, in the long term, dependence on external resources, however, market conditions and low integration with the financial sector cause more complex problems for the management and use of remittances. One of the characteristics of Financial Inclusion (FI) is its use in economic theory and, of course, in public policies, where specific institutions of national governments seek to integrate migrants and their families into the management of financial instruments, specifically to access, knowledge, and management of technologies associated with banking, (cell phones, institutional apps among others).

Inclusive financial systems consider the user or potential user as a connoisseur of operations and the logic of financial transactions. They consider the user a central actor who intuits that approaching financial institutions and their services can be of benefit. On the other hand, there are the operators or financial intermediaries who do not stop to observe the client, nor their condition or situation their objective is to attract attention through efficiency in the use of products and services, while traditional banking systems act based on corporate interests so they avoid dealing with specific social strata, strata that are integrated into new models of the global market, through public or private financial actors that to consolidate themselves offer efficient, cheap, immediate, and safe financial products and services.

Despite being a widespread demographic component in much of the national territory, migration is selective due to economic (strategic definition) and family issues, and in turn, the economic response that is the counter-flow of resources is given using transfers within financial systems. With information from Banco de Mexico, we now know which municipalities receive remittances. Regardless of the amount, the disaggregated data are a source of direct and frequent information on the place "where the remittances arrive". The previous references were "*households that declared receiving remittances*" for 2000 and 2010 of the Migration Intensity Index (IIM-CONAPO) and the different INEGI household income surveys. Therefore, the opportunity of reference under another source that is opposed to what was established by initial sources provides a greater capacity for interpretation, allowing to detail the circuit that remittances follow in the country and, of course, the medium, to the levels of financial inclusion of the spaces associated with the counterflow that, together with the amount, frequency, and sequence of the information, will allow a greater analysis of the impact. Despite the above, it is recognized by different studies that their role is consumption and, to a lesser extent, investment (Canales, E. 2006).

In the consumption line, it is important to note that it is mostly generated in the same place of receipt of remittances, so the measurement of that economic impact is concentrated and decreases by distance and time from these points, but without distinguishing the exit or stay from the municipal demarcation. Specifically, the reception takes place in medium to larger localities where financial services are located (banks, remittances, exchange houses, among others) that are chosen by users to have this service (Pérez C., 2015).

Communication-information technologies in this area, through cell phones, have taken two paths, their use as an element of economic potential for the traditional financial sector and, above all, for the characteristics of the inclusive financial and social system (BID, 2009). In addition to being a technological platform, it is an operational instrument of the market and the network through which monetary volumes flow. The World Bank identified the dimensions in Latin America where 98% of the territory has mobile signal reception, and 84% of households have mobile telephone service (Consultative Group to Assist the Poor, The World Bank Group, 2010).

Other existing elements for financial inclusion (FI) are defined by the political openness of governments to strengthen inclusive public policies; integrating specialized international organizations and an academic, theoretical, practical, and methodological debate on this issue in different public and private forums that acquires prominence. The FI is intended to have strategic nuances in national economies in times of digital services (Alfaro, 2010).

The distribution of the population in rural and/or remote areas increases the difficulties of relocation for simple financial transactions, such as receiving payments or subsidies. Reports show that access to Financial Services (FS) is a critical factor in increasing the quality of life, since they overcome contact barriers between people or microenterprises, strengthen their condition by reducing their vulnerability and directly reduce costs for procedures, add security and reduce cash handling times, facilitate the reception of remittances and transfers (BID, 2010b).

In some Latin American countries, programs were undertaken that promoted financial inclusion in a mandatory manner, which included actions at three levels: a) Change regulatory frameworks, b) Acquire resources for the supply of FS that include alternative products to traditional ones, adaptable to the needs of disadvantaged populations and c) Support and advise for the sustainability of financial companies. This means the application of subsidies intended to offset the costs of SF supply. It is about integrating new technologies that, in the form of channels and tools, allow us to support and provide efficient, fast, and low-cost services.

A developed financial system is crucial for economic growth; transaction costs, in addition to institutional weaknesses, restrict the integration, performance, and functionality of the finances of very low-income households, the microenterprise sector, and rural economies (Central Bank of Brazil, 2010). Access to financial services invigorates activities in local economies, generates new capacities that optimize resources, raise revenues, and integrate a greater number of actors, by eliminating imperfections in markets, from the local to the national level.

The above is marked by the positive correlation between access to financial services and economic growth (Levine, Loayza, and Beck, 2000). By the end of 2010, the G-20 promoted the Global Alliance for Financial Inclusion[1] with the formation of a group for FI and a subgroup for Innovation. Given the consensus on the importance of FI, there is talk of "banking the unbanked". Asmundson (2011) mentions that SFs comprise

1 Historical overview: informal savings and credit groups have antecedents in the 15th century. In Germany in the 1800s, a model of cooperative finance emerged, with adaptations that were replicated in the 20th century in rural areas of Latin America. In order to increase access to agricultural credit, state institutions were created (with losses due to subsidies and low recovery). By 1970 "microcredit" and the integration of small women's associations were created; by the 1980s, with greater methodological capacity, new models with sustainability were created and by the 1990s the concept of microcredit changed to microfinance. The transition to multi-product models took place, taking advantage of the unregulated market, reducing costs and expanding into the payment of remittances and the collection of savings. Geographic distribution and risk diversification took place, thus serving a group of strong potentials, increasing the number of clients (rural and urban), innovations and the beginning of regulation. The transition to inclusive financial systems is due to the need for access to quality instruments and fair costs, which reduced negative aspects.

significant components such as the transaction to obtain a financial good, with variations in transactions (real estate, consumer, insurance, and banking), and the intermediation that financial services make for the use of money in a productive way.

FI connects elements such as regulatory frameworks, adjusted product offerings (payments, savings, credit, insurance), coverage capacity, access to broad channels (traditional and modern), quality services at reasonable prices, safety, and efficiency potential, as well as the promotion of financial education and culture, along with information transparency formats (Pérez C., 2015).

The relationship between financial culture and technological exclusion are two important factors, given that the presence and distribution of formal banking institutions in the regions involved with SF and specifically with the payment of remittances is enhanced, in a market with low regulation, on the other hand, a high supply of SF increases the vulnerability of the plaintiffs, because the regulations that are fundamental in the action of small or informal financial institutions, increases the risks in the protection, execution of guarantees or tax burdens that distort the control of interest rates and directed loans (Gardev & Rhyne, 2011).

The lack of regulatory frameworks that prevent the risk of money laundering, known as "know your customer"[2], especially limits access to SF by the low-income population, and also by those who exercise informal productive activities, since they lack documents that prove their activity or their person to open an account. Another complication associated with the above is the reasonable and fair integration of costs to the provision of services to users.

The FI seen as a public policy that aims to shape an inclusive financial system that makes available to low-income people financial services (transactions, remittances, savings, microcredit, credit card, housing credit, and insurance) which implies changing the vision and management of financial institutions, regulators, supervisors and operators, it is about "restructuring" the idea of the financial services market towards simple models in technological conditions of broad support, developing institutions that allow the control and review of actors, generating levels of reliable and updated information, as well as with open regulatory frameworks that allow the connection of instruments and actors to improve and increase competition (Mas & Siedek 2008).

The target market of the FI should be the population with the lowest resources or income, the base of the social pyramid, which is looking for a

2 Know your client.

way to incorporate it into the benefits of economic growth through access to basic services, payments, and remittances. A public policy of FI must seek the arrival of more users and instruments to financial systems, and reduce their costs and limitations for households, people, and companies (Siedek, 2007, 2007b). On the other hand, according to Pérez (2015), remittances are the expression of contemporary international economic networks and their social relations, which are established between the places of origin and destination of migration. Its dynamics are associated with the objective of the initial migration, the number of people, the temporality of the process, and the spatial dispersion of migrants in the labor sectors and family consumption, among other elements.

At the national level and for income accounting in the banking sector, a significant growth in remittances is shown, which gives it an outstanding weight, due to its management at the local or regional level, it tends to be assumed that they affect the economies of the recipient countries, that there is a generalized impact, but this only occurs at the scale of the recipient households. In general, sending resources to migrant communities constitutes a family transfer wage fund, so its contribution to national economic growth and development is usually marginal.

The logic of sending remittances does not correspond to that of an investment or savings fund, but to a family income, which is usually destined to the satisfaction of basic needs, which contributes to reducing social vulnerability, to generating support during critical situations.

The circulation and use of remittances have constituted a financial circuit, part of which is immersed in a rural context, with high rates of social deterioration and the productive fabric, while on the other hand, there is an economy that demands labor with higher wage conditions even in informal and/or irregular conditions. It is common that, since they are peasant economies, remittances impact production destined for self-consumption and the local market that has a different rationality, which by the exogenous injection of income generates new scales of polarization that transcend the spatiotemporal dimension. Therefore, the territorial scope and geographical scale are differentiating elements of the use and impact of remittances.

Another differentiating feature of remittances is the magnitude and impact they have in dispersed communities where this income, as well as its circulation, are fundamental for their survival. It is important to note that remittances have a sociocultural function to maintain and reinforce traditional practices and values, which strengthen the social cohesion, culture, and identity of the people in which they are inserted in the migratory circuits (collective remittances).

Sources of Information

The data used for the development of this research were obtained from the tabulations of the Economic Information System, published by Banco de Mexico and are available at https://www.banxico.org.mx/SieInternet/ In particular, the variables related to family remittance income and the total number of bank branches were processed, in both cases the variables are disaggregated at the municipal level.

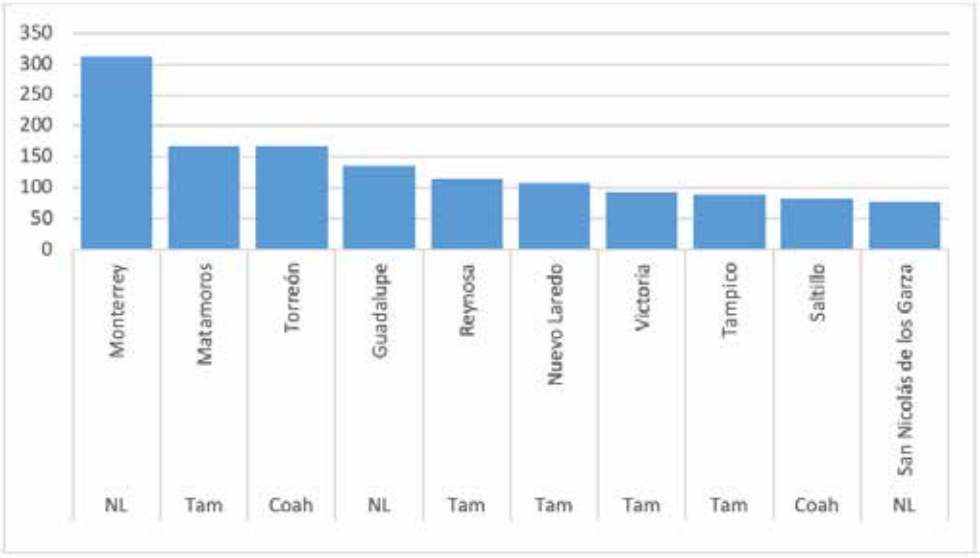
REMITTANCE AND FINANCIAL INCLUSION IN MEXICO

According to the World Bank, the global flow of remittances in 2010 was 440 billion dollars, with Mexico in third place, surpassed only by India and China. In recent years, Banco de Mexico has recorded two important trends in the flow of remittances, one between 2004 and 2007, where the annual volume of remittances increased along with the number of transactions, however, as of 2008, these parameters fell to normal levels. The second trend began after 2014, when a sustained growth of remittances was observed until 2020, with an annual growth of 11.4%, marking a record growth for five consecutive years, its amount for that last year is equivalent to more than 875 billion pesos, which is higher than the federal budget of Mexico approved for 2021 by the Secretariats of Public Education, Health, Labor and Social Security, Welfare, and Culture as a whole. According to bank estimates, 0.7% of remittances arrive in cash or kind while 77.1% are collected in non-bank institutions (BBVA, 2021).

	2015	2016	2017	2018	2019	2020
Nuevo León	126.6	125.9	167.1	180.3	179.5	189.4
Coahuila	135.6	140.6	172.3	192.8	207.3	226.9
Tamaulipas	196.6	187.8	207.0	226.0	242.7	259.7

Graph 1. Evolution of remittances per capita in northeastern Mexico, 2015 to 2020. Source: Own elaboration with BANXICO information. Remittances per capita in annual dollars.

It should be noted that the average dollars per operation had a similar behavior during the years analyzed. In Mexico, the distribution by state shows unequal regional patterns, where only nine states concentrate more than 60% of remittances, a trend that is repeated within the states due to infrastructure conditions and connection with migratory circuits. In the case of the northeastern region of the country and only for the years 2019 and 2020, there was an increase in the amount received in Nuevo León of 6.7% (from 960 to 1,024 million dollars), Tamaulipas with an increase of 8.2% (from 873 to 945), as well as Coahuila, which showed a growth of 11.1% (from 640 to 711 million dollars) (BBVA, 2021).



Graph 2. Municipal remittances in the northeastern region of Mexico in 2020. Source: own elaboration based on data from BANXICO and the Population and Housing Censuses, INEGI. Remittances per capita in dollars

Graph 1 shows the time evolution of remittance amounts per capita at the state level. The figures allow us to have an idea of the dispersion of the phenomenon among the entities of northeastern Mexico, where it is evident that over the period 2015-2020, remittances increased steadily, going from a regional average of 149 dollars per person-year to an average of 220 dollars per person-year. This increase in the number of remittances was also reflected in the number of receiving municipalities, while in 2015, a total of 23 municipalities of the 132 that make up the region did not receive remittances; for 2020, only one municipality, San Nicolás in Tamaulipas did not receive remittances, a municipality in which there are neither remittances nor ATMs, much fewer bank branches where the collection of remittances can be effective, so we assume that they are addressed to the surrounding municipalities (Banxico database, consulted on 02/20/2020).

Regarding the net flow of remittances at the municipal level, during 2020, it is observed that the most important municipalities in the region receive the highest amount of remittances, which may be an indicator of the existence of a greater proportion of their population working in the United States. Thus, the municipalities of Monterrey, Matamoros, Torreon, Guadalupe, and Reynosa receive the highest amounts of remittances (see graph 2); for example, Monterrey received 312 million dollars during 2020, the average for the region was 20.2 million dollars per municipality for the same year. Although remittances reach practically all municipalities, as can be seen in map 1, there are large differences in the spatial and temporal distribution of remittances between the municipalities of the region, although a spatial pattern in the distribution of these cannot be distinguished.

BAYESIAN SPATIAL ANALYSIS

The spatiotemporal modeling of municipal remittances is done through the adjustment of a series of latent Gaussian models that include the application of parametric, non-parametric models, and spatiotemporal interaction models. The linear predictor of the latent Gaussian model is defined from a link function:

$$\eta = g(E(Y \mid \eta))$$

where η represents the linear predictor, g is a binding function, and Y represents an indexed sequence of the formula:

$$Y(i,t) = \left\{ y(i,t) \mid (i,t) \in \Re^2 \times \Re \right\}$$

defined as a stochastic process, where each $y(i,t)$ represents the remittances captured by the municipality $i = 1,...,2\ 456$, at year $t = 1,...,6$ (Blangiardo & Cameletti, 2015: 235), which are modeled by a Gaussian distribution, $Y_{it} \sim N(\eta_{it}, \sigma^2{}_t)$ where η_{it} represents the expected value of remittances in the municipality i at year t and σ^2 the variance.

Under these criteria, the Bayesian model is defined by a hyperparameter layer, a latent Gaussian field, and a likelihood function that captures the marginal distribution defined through an exponential family. The formulation of spatiotemporal models comprises a classic parametric model, a non-parametric dynamic model, and four variations of the spatiotemporal interaction model with structured and unstructured effects. The classic parametric formulation of spatiotemporal models defines the linear predictor η_{it} as:

$$\eta_{it} = \beta_0 + u_i + v_i + (\beta + \delta_i)t \quad (1)$$

The non-parametric dynamic model uses a formulation that allows it to manipulate temporal trends through parameters that independently control the structured ρt and unstructured φt temporal effects (Bivand, Gómez-Rubio & Rue, 2015), so its linear predictor is given by:

$$\eta_{it} = \beta_0 + u_i + v_i + \rho_t + \varphi_t \quad (2)$$

Spatiotemporal interaction models expand parametric and non-parametric models by introducing a γ_{it} term, capable of capturing the interaction between space and time, whereby its linear predictor takes the form:

$$y_{it} = \eta_{it} = \beta_0 + u_i + v_i + \rho_t + \varphi_t + \gamma_{it} \quad (3)$$

In models (1) to (3), β_0 represents the average level of remittances received in the municipalities of northeastern Mexico, u_i represents the spatially structured random effects, and the parameter v_i the spatially unstructured effect.

In model (1), each spatial unit has a spatial intercept given by $(\beta_0+u_s+v_s)$ and a slope $(\beta+\delta_s)$ representing the temporal trend (Blangiardo & Cameletti, 2015), while in model (2) the structured temporal trend ρt is modeled as a random walk (rw(1)) distributed as:

$$\rho_t \mid \rho_{t-1} \sim N\left(\rho_{t-1}, \sigma^2\right)$$

Whereas the unstructured temporal trend φt follows a previously interchangeable Gaussian distribution N(0, 1/τφ) (Schrödle & Held, 2011). Finally, in equation (3) the spatiotemporal interaction term γ_{st} follows a Gaussian distribution given by $\gamma_{it} \sim Normal\ (0, 1/\tau_\gamma)$, with a precision matrix given by $\tau_\gamma R_\gamma$.

In model (3), the term γ_{it} specifies the interaction between spatial and temporal effects, structured and unstructured, with which type I to IV interaction models are defined. The type I interaction controls the unstructured spatial and temporal effect, vi and ρt, the type II interaction defines the variation between the unstructured spatial effect vi and the structured temporal effect φt, the so-called type III interaction between the structured spatial effect ui and the unstructured temporal effect ρt and finally, the type IV interaction controls the variation between the structured spatial effect ui and the structured temporal effect φt.

MODELS OF SPATIOTEMPORAL INTERACTION

The six competing models were evaluated based on a statistical selection criterion known as Akaike. The selection of the model, within the set of alternative models, was made based on the implementation of algorithms that allow discrimination between models. In this way, the Akaike information criterion (AIC) provided a measure of the goodness of fit of the model while penalizing the degree of complexity of the model (Akaike, 1974). The AIC selection criterion consisted of choosing from among the alternative models, the model that achieves the smallest AIC, which is estimated in terms of the likelihood function and the number of parameters estimated by the model.

Among the set of six adjusted alternative models, the Akaike criterion was estimated for each of them. The results are presented in Table 1 and show that the minimum AIC was reached by the type II spatiotemporal interaction model, with an advantage of more than a thousand points over its closest competitor, the classic parametric model, so that the type II model proved to be the best option to model the spatiotemporal evolution of per capita remittances in the municipalities of northeastern Mexico.

Table 1
Akaike information criteria, estimated models

	Model	AIC
PAR	$y_{it} = \beta_0 + u_i + v_i + (\beta + \delta_i)t$	2,780.18
NOP	$y_{it} = \beta_0 + u_i + v_i + \rho_t + \varphi_t$	3,224.90
Tipo I	$y_{it} = \beta_0 + u_i + v_i + \rho_t + \varphi_t + \gamma_{it}$	3,018.47
Tipo II	$y_{it} = \beta_0 + u_i + v_i + \rho_t + \varphi_t + \gamma_{it}$	1,724.09
Tipo III	$y_{it} = \beta_0 + u_i + v_i + \rho_t + \varphi_t + \gamma_{it}$	3,221.36
Tipo IV	$y_{it} = \beta_0 + u_i + v_i + \rho_t + \varphi_t + \gamma_{it}$	3,226.32

Source: Own elaboration based on R-INLA estimates

Table 2 presents the subsequent estimates of the type II spatiotemporal interaction model for the fixed effects and random effects, where it is observed that the fixed effect α=1.59 estimates the average of the logarithm of per capita remittances received by the municipalities of northeastern Mexico, which reached an average observed amount of 200 dollars per person per

year, throughout the entire period analyzed (see table 2), which is why α significantly underestimates the average of municipal per capita remittances. This underestimation effect can be attributed to the spatial weighting carried out to adjust the spatiotemporal effects observed between municipalities with high and low levels of remittance reception, which is associated with the model borrowing information from neighboring municipalities to reduce variance and improve the estimation of a specific municipality (see map 2), while the effect of the β parameter shows the existence of a positive relationship between per capita remittances received and municipal financial inclusion, the estimated value of β shows that the increase at one point in municipal financial inclusion would imply a growth of 0.22 points in municipal per capita remittances.

About the estimated random effects for the type II interaction model, the mean of the posterior density of the structured spatial effect u_i was found to be 60% larger than the estimated mean of the unstructured spatial effect v_i, showing that the effect of the spatial dispersion of remittances per capita between municipalities depends significantly on the spatial structure and the distance between municipalities. Regarding the average estimated value of the temporal effects, by the spatiotemporal interaction model type II, it is observed in Table 2 that the unstructured temporal effect reaches a value much higher than that presented by the rest of the estimated effects, both temporal and spatial, which indicates that the temporal evolution of remittances has a greater relative importance than that presented by the spatial variations and that these follow a temporal pattern not subject to the variations of the calendar.

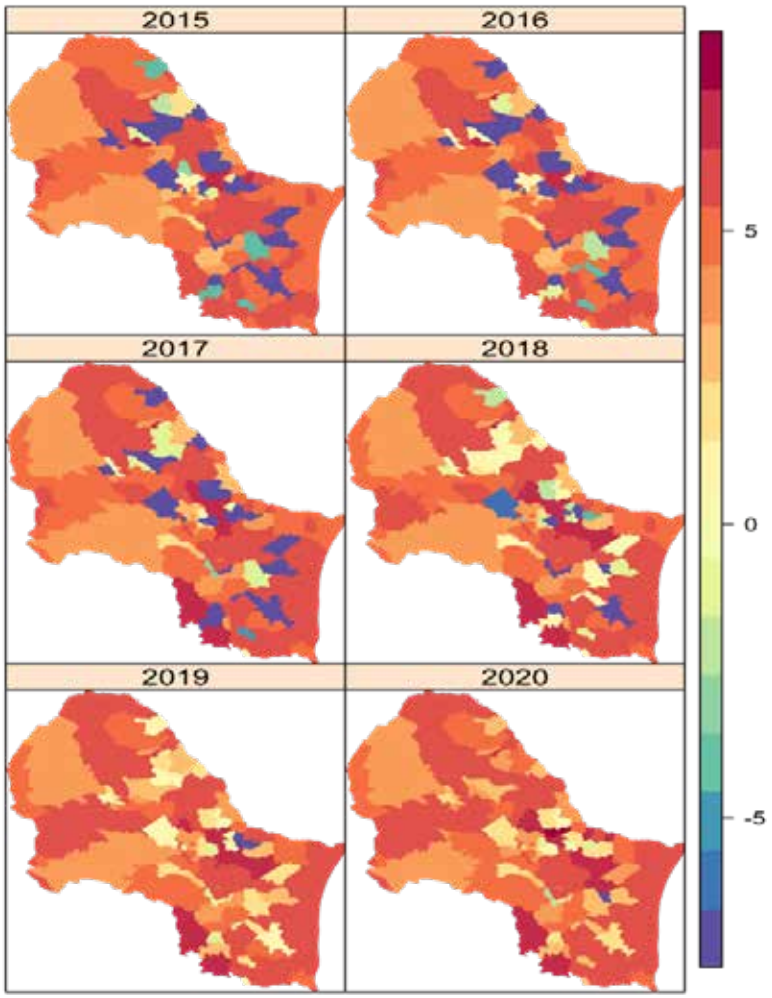
Table 2
Subsequent estimates, type II spatiotemporal interaction model

Fixed effects	mean	sd	Q1	Q2	Q3
α	1.598	0.197	1.211	1.598	1.985
β	0.288	0.197	-0.098	0.288	0.675
Random effects	mean	sd	Q1	Q2	Q3
Structured spatial effect	2274.63	2285.92	209.51	1606.61	8295.67
Unstructured spatial effect	1857.59	1835.37	128.37	1316.22	6707.52
Structured temporal effect	7.46	4.65	1.83	6.40	19.44
Unstructured temporal effect	21157.93	21330.11	1690.21	14889.58	78121.28
Spatiotemporal interaction effect	0.65	0.07	0.52	0.65	0.79

Source: Own elaboration based on R-INLA estimates

The spatiotemporal distribution of remittances per capita received by the municipalities of northeastern Mexico, during the years 2015 to 2020, are

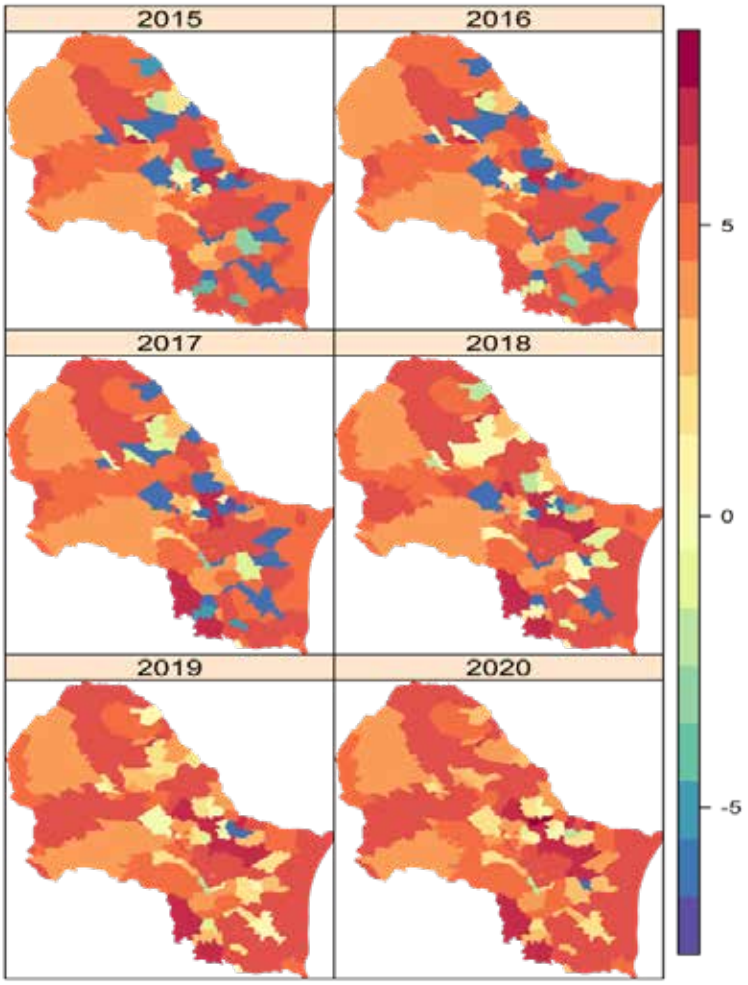
presented in map 1, while the estimates, obtained by the spatiotemporal interaction model type II, for municipal remittances per capita can be seen in map 2. Maps 1 and 2 present the contrast between observed and estimated per capita remittances, both on a logarithmic scale. Map 2 presents the estimates of the subsequent linear predictor of per capita remittances made by the type II spatiotemporal interaction model, given the observed level of municipal financial inclusion present in the municipalities of northeastern Mexico during the period studied, in the same map it is possible to observe the effect of the smoothing made by the model.



Map 1. Observed municipal remittances, northeastern region of Mexico 2015 to 2020. Source: Own elaboration based on R-INLA estimates. Logarithmic scale remittances

The spatial dispersion of the natural logarithm of remittances per capita observed in the municipalities of northeastern Mexico, over the period studied, shows a sustained increase in the number of municipalities that received

remittances between 2015 and 2020. While in 2015 the total number of municipalities that received remittances reached 109 of the existing 132 by 2020 the number rose to 131. Growth in the number of remittance-receiving municipalities in the northeastern region increased as did the number of bank branches and the average per capita amount of remittances received by municipalities (see Chart 1).

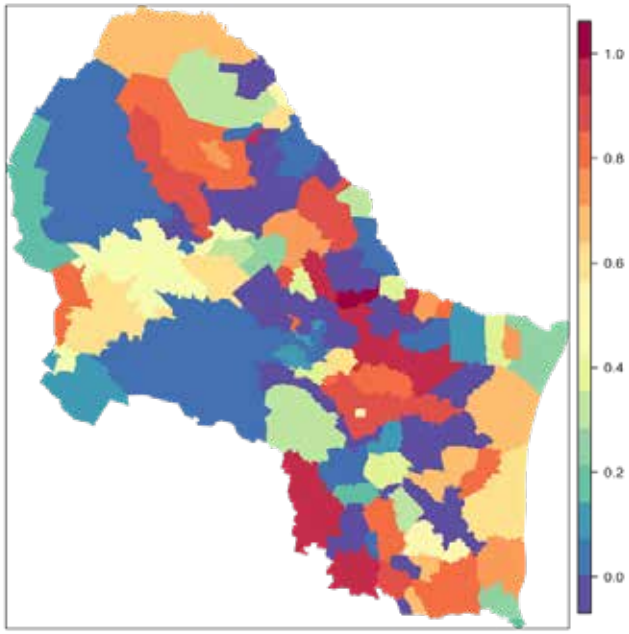


Map 2. Subsequent average estimate of municipal per capita remittances, according to type II interaction model. Source: Own elaboration based on R-INLA estimates. Logarithmic scale remittances

In general terms, a spatial pattern of concentration or dispersion of remittances between the analyzed municipalities cannot be distinguished (see maps 1 and 2), a situation that reveals a democratization in the reception of remittances for the northeastern region of the country, except perhaps a small region, of low reception formed by the municipalities of Dr. Coss, Los Aldama, General Treviño, and Melchor Ocampo (located in Nuevo León),

which seems to consolidate over the period analyzed. In any case, it is a set of basically rural municipalities with low population density, a situation that could somehow explain the behavior of remittances.

The type II spatiotemporal interaction model generates the subsequent distribution of municipal per capita remittances for the analyzed period, given the levels of financial inclusion observed so that it is possible to create a probability map in terms of the risk associated with the reception of a level of per capita remittances, defined from a given threshold. Map 3 presents the subsequent conditional probability that a municipality receives a level of remittances per capita higher than the regional average. Municipalities in red to orange shades show a high probability of receiving remittances above the region's average, while municipalities in blue to green shades have a low probability of obtaining remittances above the regional average, which draws a spatial pattern. In general, municipalities in blue are located in rural areas that are difficult to access, while municipalities in red, in general, correspond to urban areas.

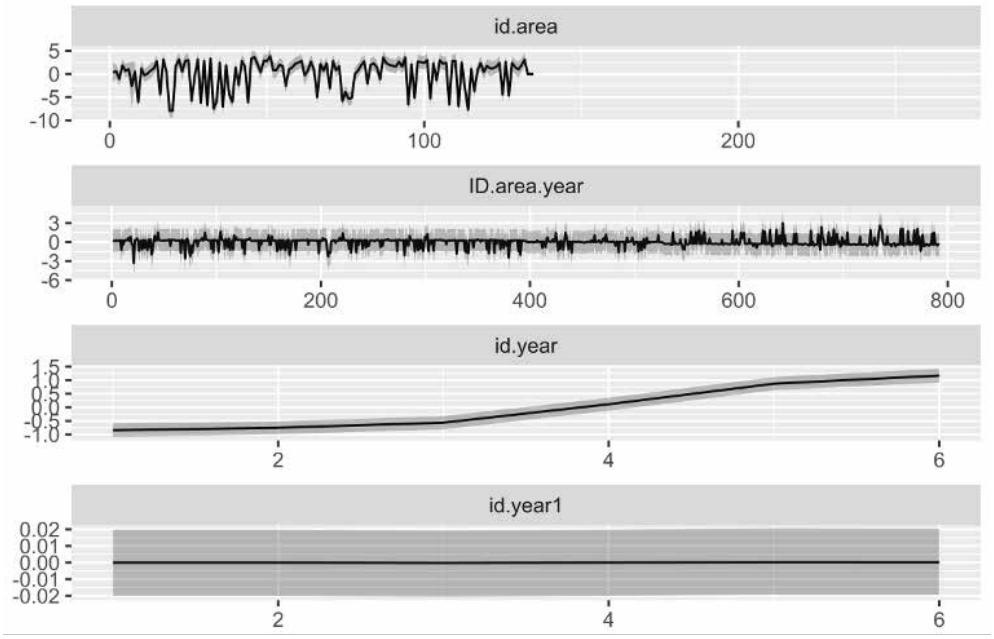


Map 3. Probability. Source: Own elaboration based on R-INLA estimates

Graph 3 presents the estimates of the random spatial and temporal structured and unstructured effects of municipal per capita remittances for the analyzed period, for the type II interaction model. Graph 3.a shows the variations of the structured spatial effects that model the spatial dependence associated with the level of remittances received by the 132 municipalities that make up the northeastern region. In graph 3.b you can see the unstruc-

tured spatial effect, it is an interchangeable (unstructured) random effect that models the uncorrelated noise between geographical areas, that is, it models the random variations not captured by the spatial structure, for the 132 municipalities throughout the six years analyzed (i.e. 792 estimates).

Graph 3.c presents the structured temporal effect for the six years analyzed, while graph 3.d shows the unstructured temporal effect, which captures the random temporal variations that cannot be modeled by the calendar. The increase observed in Graph 3.c captures the temporal growth of the average value of remittances, while the value of the estimated parameter of unstructured temporal effects reflects the inability of the model to capture such effects.



Graph 3. Spatial and temporal random effects of the type II interaction model. Source: Own elaboration based on R-INLA estimates

The reduction achieved in the AIC by the type II interaction model implies that the γit parameter efficiently captures the interaction between spatial and temporal effects (Schrödle & Held, 2011). When the variations between spatial effects and between temporal effects are relatively large (i.e. within each of them), compared to the estimated variation between the spatial and temporal effects (see Table 2), the estimate of the interaction term γit is of lower magnitude (Martins *et al.*, 2012) than that achieved by the temporal and spatial effects, so, the interaction term can adequately model the spatiotemporal dependence.

DISCUSSION

The contribution of this work revolves around the analysis of the spatio-temporal evolution of municipal remittances in the states of northeastern Mexico, and in a quantitative contribution to determine the importance of financial inclusion in the territorial dispersion of remittances. The analysis carried out through the above model allows us to recognize a specific behavior in the transmission of resources resulting from migration in that period, it is transcendental to recognize the capacity in the analysis of the past and to be able to recognize the conditions of the remittance market in the region, as well as the elements to be considered of the value of the information that Banco de México is now systematizing in the face of the relevance of the migration process in general and in a specific way of the transfer market from abroad.

The presence of bank branches and financial institutions, also called "dispersers" in the different municipalities will then have an impact on remittance recipients in municipalities without branches, as well as a potential increase in remittances in their regional total. Financial inclusion as a national policy responds to a need caused by migration (also created by structural deficiencies) for which it reproduces and uses the conditions established by the concentration market in cities with low investment for new branches and generates a transfer of costs for the use and management of certain associated technologies.

The integral process of inclusion also requires a greater development in communication infrastructure (digital channels), an expansion of telecommunication technologies, and a functional management for users that translates into a section of financial education that is not an issue present in most of the user population of some levels of financial services, although superficial management of a majority of users can be inferred.

The model shows the levels of concentration of the amounts in the institutions associated with the financial system, thinning remittances from other channels such as pocket money. It should be considered that in parallel there is an unregistered market for the transit of resources that may be temporary and others in kind, the former associated with collective or social expenses, for financing public works or festivities in migrant localities, which it does not define as current expenditure and the latter are gifts that are delivered directly to families that may include appliances, tools, cars, among other objects, without economic import registration, as well as payment of taxes.

CONCLUSIONS

The estimation of the spatiotemporal models was possible thanks to the use of Laplace's integrated nested approximation implemented in the INLA package and available in R language. The use of INLA provides significant computational advantages over other algorithm-based packages such as the Monte Carlo Markov chain (MCMC), that result in a significant reduction in processing time.

The interdisciplinary work between mathematics and social sciences, specifically between statistics and geo-demography, allows us to recognize the opportunity, and potential of the analysis beyond the numerical result of the model and the spatial expression, since the association was indispensable as the assimilation of terms of and in both areas to be able to coordinate the interpretations, so it was direct the adjustment of the process for the mathematical analysis in accessible variables, which closely express the selected conceptual elements, this as part of the recognition of a great context resulting from poverty and economic inequality in the country.

The model forms a robust basis for establishing a series of direct behaviors of the financial services infrastructure associated with transfers originating in another country and resulting from migration. The transit of remittances through these institutions has caused the concentration of these financial services in cities and in the banking sector by reducing their passage through other ways than transfers to commercial banking accounts. This concentration in cities and institutions occurs despite having large population segments to be served, especially low-income and in non-urban contexts as postulated by financial inclusion; the banking sector requires multiple elements for the installation and continuity of branches in medium or small cities, so the installation of new branches is an exercise that does not respond to the financial inclusion of the aforementioned groups but to a market vision of the demanders of these financial services.

Despite the concentration of remittances, the increase they have expressed does not fully respond to elements of inclusion or expansion of financial services, the dynamics of these respond more to a labor market in the destination of migration and to family economic behavior in the places of origin (consumption). The demand for financial services associated with remittances, such as bank accounts, has developed innovations and flexibilizations, to achieve positioning in the segment of families with migrants has allowed the integration of new banking actors and intermediaries in connection with remittances and through banking financial services manage to get these resources in a short time and advantages within the transfer market.

In the northeastern region, the weight of the metropolitan area of Monterrey, the major urban areas of Saltillo and Ciudad Victoria is evident,

following the border areas such as Matamoros, Reynosa, Nuevo Laredo, and Piedras Negras, in addition there is a trend that accompanies remittances together with bank branches in areas with rural and industrial profiles, where it can be the reason for subsequent research exercises on their population profile and especially migratory.

Finally, this type of research and the use of statistical and spatial tools can allow financial service providers to identify not only unattended population or territorial segments, but also specific profiles of the instruments demanded and the potential connection with other intermediaries to form a service and network costs and offer cross-products such as health insurance, credits, payments to third parties and other savings.

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A C A D E M I C

P A P E R

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Review: University Memories. Images of the past and voices of the present[1]

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The contemporary history of the Universidad Autónoma de Chiapas is permeated by different moments and symbolic places that confer, to the space where they took place, the importance of being remembered. Therefore, the book University Memoirs. Images of the past and voices of the present (UNACH, 2022) addresses these social spheres to think about the past, as well as the reflection around the foundational moments where the correlation between tradition and future projects are analyzed with a current perspective.

In this sense, the book brings together researchers and actors of university life, who have been part of the beginnings of the institution, and whose main interest is to delve into the role of UNACH in shaping today's Chiapas society. Through photographs, interviews, and anecdotes, these actors narrate their experience of how a rich university history was shaped and managed to convey, with acuity and reflection, an honest and realistic vision of university life and culture. In addition, this work proposes to carry out this retrospective exercise through the recognition of university identity and identification of substantive transformations. From this perspective, as Edensor (1997) pointed out, "memory is shaped through iconic values that originate and create meanings in social terms for collective memory".

The work consists of 24 articles and is divided into three sections: genesis, consolidation, and the future. The first, "Vital integration. The first schools", addresses the origins of UNACH and its development in the first decades of its existence, and corresponds to the main academic structures of the second half of the twentieth century. The second "Paths of recognition. 48 years of history with society", analyzes the growth of the institution in recent decades and its role in Chiapas society, the development and sustained growth of the Universidad Autónoma de Chiapas, and the memorial sites. Finally, the third, "The university and its transformation for the future", reflects prospectively on the challenges and opportunities of higher education that the highest educational institution in the state of Chiapas in the 21st century is facing.

This work, which houses 48 years of institutional life and is approaching its fiftieth anniversary, reflects the evolution of the university in academic, scientific, cultural, and service terms to Chiapas society.

University memories. Images of the past and voices of the present emphasize the imprint of knowing the past and knowing who we are by resignifying the

genesis of our alma mater. The dynamic that permeates the book and governs the work is the strong commitment of young people to materialize the construction of a higher education institution and a message of celebration and commitment from UNACH.

Finally, the reader will find in these pages that university identity springs into the scene as a kind of equation between gratitude and recognition. *University memories. Images of the past and voices of the present* edited by the same house of studies is available in print and digital format as part of the Paperless Letters Collection of the *Scientific Dissemination Journal Espacio I+D: Innovación más desarrollo*, in open access for free consultation and download.

REFERENCES

Edensor, T. (1997). National identity and the politics of memory: remembering Bruce and Wallace in symbolic space. Environment and Planning D: Society and space, (29), 175-194.