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## Presentation of the Content

In the first article we present, *Isolation and identification of Enterobacterales present in dog feces in the city of Merida, Yucatan* by BASTO-MIJANGOS, Harold N., CAAMAL-LEY, Angel D., PUC-FRANCO, Miguel A. and VARGAS-GONZALEZ, Alberto, with adscription in the Universidad Autónoma de Yucatán, as following article we present, *Evaluation of dehydration parameters of habanero chili (Capsicum chinense jacq.) by tray method, for the conservation of seasonal fruits* by GUTIERREZ-PEÑA, Esteban, RENDON-SANDOVAL, Leticia, LLANILLO-NAVALES, Jesus Gerardo and MARIN-RAMOS, Martha, with adscription in the Tecnológico Nacional de México / Instituto Tecnológico Superior de Huatusco, as following article we present, *Identification of sources of resistance in tomato to Phytophthora infestans at Mexico* by ARELLANO-RODRÍGUEZ, Luis Javier, RODRÍGUEZ-GUZMÁN, Eduardo, PADILLA-GARCIA, José Miguel and LEPIZ-ILDEFONSO Rogelio, with adscription in the Universidad de Guadalajara, as the last article we present *Supramolecular self-assembly studies and spectroscopic analysis of oligomers used for the removal of pollutants from wastewaters* by RAMÍREZ-SALAS, Virginia, MORENO-MARTÍNEZ, Beatriz Eugenia, ORDOÑEZ-PACHECO Luis Daniel and ALARCÓN-RUIZ, Erika, with adscription in the Tecnológico Nacional de México / Instituto Tecnológico de Ciudad Madero / Instituto Tecnológico de Nuevo León.

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## Isolation and identification of Enterobacterales present in dog feces in the city of Merida, Yucatan

### Aislamiento e identificación de Enterobacterales presentes en heces de perros en la ciudad de Mérida, Yucatán

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#### Abstract

The close coexistence between humans and pets such as dogs has increased the risk of transmission of infectious diseases (zoonoses) caused by Enterobacterales. The ingestion of food and water sources contaminated with animal feces matter constitutes the main mechanism of dissemination of these diseases. The objective of the study was to determine the prevalence of Enterobacterales in stool samples from domestic and street dogs collected in the city of Mérida, Yucatán. For this, 30 stool samples from canines (15 domestic dogs and 15 street dogs) were collected. The bacterial samples were seed on McConkey agar and salmonella-shigella agar (after enrichment in tetrathionate broth). Likewise, microorganisms were identified by biochemical tests: citrate, MIO, LIA, urea, TSI, catalase and oxidase. The most outstanding findings was a high percentage of dogs infected with *Salmonella* spp., the animals were of both domestic and street origin. This is alarming given the potential risk of zoonosis for the population.

Fecal, Zoonosis, *Salmonella*

#### Resumen

La convivencia estrecha entre el ser humano y animales de compañía como los perros a incrementado el riesgo de transmisión de enfermedades infecciosas (zoonosis) producidas por Enterobacterales. La ingesta de alimentos y fuentes de agua contaminados con materia fecal se constituyen como el principal mecanismo de disseminación de estas enfermedades. El objetivo del estudio consistió en determinar la prevalencia de Enterobacterales en muestras de heces de perro domésticos y callejeros recolectadas en la ciudad de Mérida, Yucatán. Para esto, se recolectaron 30 muestras de heces de caninos (15 perros domésticos y 15 callejeros). Las muestras fueron cultivadas en agar McConkey y agar salmonella-shigella (posterior a enriquecimiento en caldo tetratonato). Así mismo, se identificaron los microorganismos mediante pruebas bioquímicas: citrato, MIO, LIA, urea, TSI, catalasa y oxidasa. El hallazgo más sobresaliente fue que se encontró un porcentaje elevado de perros infectados con *Salmonella* spp., los animales eran tanto de origen doméstico como callejero. Esto resulta alarmante ante el riesgo potencial de zoonosis para la población.

Fecal, Zoonosis, *Salmonella*

**Citation:** BASTO-MIJANGOS, Harold N., CAAMAL-LEY, Angel D., PUC-FRANCO, Miguel A. and VARGAS-GONZALEZ, Alberto. Isolation and identification of Enterobacterales present in dog feces in the city of Merida, Yucatan. ECORFAN Journal-Ecuador. 2022. 9-17: 1-6

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## Introduction

In recent years, Enterobacterales infections have gained relevance in global public health. These bacteria are the cause of infections in different anatomical sites, which is why they are an important cause of morbidity and mortality. They usually affect the population of all ages, although with greater consequences in children, immunosuppressed patients and the elderly. (Procop *et al.*, 2017).

The most frequent infections by Enterobacterales are gastrointestinal, whose main etiological agents are: *Salmonella*, *Yersinia*, *Escherichia coli* pathotypes, among others. (Cinquelpalmi *et al.*, 2013) These microorganisms cause fever, diarrhea, tenesmus, hematochezia, leukopenia, splenomegaly, etc. they can even cause systemic illnesses and death for those who are not diagnosed and treated promptly. (Procop *et al.*, 2017)

The impact of these microorganisms is great; In the case of *Salmonella* spp alone, it is estimated that it causes 93.8 million cases of gastroenteritis and 155,000 deaths each year. (Riveros & Ochoa, 2015).

Outbreaks of infections with epidemiological relevance of many of these microorganisms have been recorded. During 2011, in Germany, the emergence of multiple infections by enterohaemorrhagic *Escherichia coli* (O104:H44) was reported, involving 4,000 cases of bloody diarrhea, 850 cases of Hemolytic Uremic Syndrome (HUS) and 50 deaths. (Grade *et al.*, 2012).

In Mexico, the impact of these organisms on the health of the population can also be observed; For example, it is estimated that 70,000 cases of salmonellosis arise in the country every year. (Contreras *et al.*, 2007). In a study carried out in the state of Yucatán in Mexico, it was found that of 463 cases of diarrhea in hospitalized infants, 28% were caused by *Escherichia coli* pathotypes and 12% by *Salmonella* spp. (Patzi *et al.*, 2015)

Ingestion of animal feces contaminated food and water sources is an important transmission mechanism for Enterobacterales.

Not only human feces can serve as a vehicle for the dissemination of these microorganisms, but also that from animals, such as cattle, birds, reptiles, cats and dogs, since they are considered potentially harmful for humans. (Procop *et al.*, 2017; Silva *et al.*, 2014; Wang *et al.*, 2014)

The close coexistence between humans and domestic animals, as in the case of dogs, has increased the risk of transmission of infectious diseases (zoonosis). Dog feces can contain bacteria that are harmful to humans, so their presence is a hygiene and public health problem. (Ferreira *et al.*, 2017; Himsworth *et al.*, 2010)

Jay *et al.*, (2014), reported the presence of 13 *Salmonella enterica* serovars in feces samples of street dogs collected in the United States and Mexico. This will indicate the possibility of pollution in cultivation areas directly or indirectly, through the presence of feces in water sources.

In a study conducted in Japan by Teruyoshi & Ikejima (1980), *Plesiomonas shigelloides* was isolated from 37 street dogs (3.8%), suggesting that this is an important reservoir with the potential for transmission to humans.

Despite the risk of zoonoses that dogs and their waste, there are no studies in Yucatan focused on the isolation and identification of Enterobacterales in dog feces. This represents a problem because the real situation that is occurring in the field is not known. State, therefore it is impossible to develop effective strategies to maximize their impact.

The objective of the study was to determine the prevalence of Enterobacterales in feces samples of domestic and street dogs collected in the city of Mérida, Yucatán. The study represents a decisive moment for future research focused on the identification of pathogenic species in animal waste in the state, as well as the identification of their virulence factors.

## Methodology

Enterobacterales species were identified in feces samples from street and domestic dogs by traditional biochemical tests.

## Sampling

Convenience samples were taken from 30 dog feces on public roads (15 from domestic dogs and 15 from street dogs). The samples were collected in each of the 5 zones of the city of Mérida (north, south, center, east and west). In each zone, 3 samples of domestic dogs and 3 of street were collected. 15 g of fresh feces (per sample) were sent refrigerated to the laboratory within a period of no more than 24 h.

## Microbiological culture

With a sterile bacteriological loop, the samples were seeded by the pentagon method on MacConkey agar (MCDLab, Mexico). They were incubated in a bacteriological oven (Riossa series: ECML. México®), at 37°C for 24 hours.

Likewise, tubes were inoculated with CTT tetrathionate broth (BD Bioxon, Becton Dickinson. México®), for the selective enrichment of *Salmonella* spp. They were incubated at 37°C for 24 hours. Subsequently, the CTT was seeded on *Salmonella*-*Shigella* agar (MCDLab, Mexico), by the pentagon method and incubated at 37°C for 24 hours.

## Identification of microorganisms

From the developing cultures, Gram staining of the colonies was performed, as well as catalase and oxidase tests (diagnostic ID, Mexico). Next, biochemical tests were carried out: citrate, Mobility Indole Ornithine medium (MIO), Lysine and Iron Agar (LIA), urea and Iron-Triple Sugar Agar (TSI), for the biochemical identification of Enterobacterales. The biochemical tests were incubated at 37°C for 24 hours, after which time they were read and the results were analyzed based on the biochemical reaction tables proposed by both Procop *et al.* (2017), Cowan & Steel (2003) and the ABIS online platform (ABIS online, bacterial identification software, 2022).

Finally, the presumptive isolates of *Salmonella* spp. were confirmed using Difco A-I polyvalent antiserum (Becton Dickinson., USA), for *Salmonella enterica*.

## Survey application

In addition to the feces samples, an interview was conducted with the owners of each domestic dog in order to find out aspects of the habits of their pets (feeding, contact with feces from other animals, contact with other dogs, etc.) and the careful handling of your waste.

## Statistic analysis

The results obtained were grouped according to the type of dog (domestic or street) and compared using Fisher's exact F test through the statistical software Past4.05, an  $\alpha = 0.05$  was used.

## Results

A total of 30 stool samples were seeded in culture media in order to identify existing Enterobacterales through phenotypic tests. Of these, *Escherichia coli* and *Salmonella* spp. (Table 1)

Microorganism	Percentage
<i>Escherichia coli</i>	86.7%
<i>Salmonella</i> spp.	20.0%
<i>Proteus</i> spp.	16.7%
<i>Citrobacter</i> spp.	16.7%
<i>Enterobacter</i> spp.	16.7%

**Table 1** Percentages of bacterial isolates from dog fecal samples

The samples from which *Salmonella* spp. they belonged to both street dogs (4 samples) and domestic dogs (2 samples). The origin of the samples according to the collection area were that 2 were from the south, 2 from the east and 2 from the west.

The comparison of the data on the isolates of *Salmonella* spp, based on two sets (domestic and street), was made using Fisher's exact F test. This revealed that there is no significant difference ( $p=0.6513$ ) in the presence of *Salmonella* spp., in domestic and street dogs.

Regarding the data obtained in the survey carried out on the owners, it is worth noting that both dogs with isolates of *Salmonella* spp. They have contact with other dogs. One of the two domestic dogs also has contact with the feces of other animals or relatives, in addition, in both cases the dogs are fed homemade food.

In general, it was observed that, of the domestic dogs, 46.7% was related to congeners, while 53.3% had contact with feces from other dogs or animals, in addition, 33% were fed homemade food.

## Discussions

Table 1 shows a high percentage of *Escherichia coli* isolates, this is an expected aspect because this microorganism is part of the canine gastrointestinal microbiota. (Beutin, 1998). However, the strains were cryopreserved for future serotyping studies because some are of clinical importance for humans and have been reported in samples from canines. (Jay *et al.*, 2014).

Among the data, the high percentage of isolates of *Salmonella* spp. (20%), this data was higher than that reported by Jay *et al.*, (2014), where they pointed out the isolation of the same microorganism in 9.2% of feces samples from street dogs studied. For its part, it was similar to that indicated by a study carried out in Canada by Leonard *et al.*, (2011). The group of researchers reported that 23% of the dogs studied had at least one positive stool culture for *Salmonella* spp.

Bacteria of the *Salmonella* genus have been described as causing gastroenteritis, typhoid fever, bacteremia, osteomyelitis and meningitis. (Riveros & Ochoa, 2015). This bacterium presents resistance to different temperatures, pH, desiccation, changes in osmolarity and nutrients; which allows it to survive and disperse in soils. (Contreras *et al.*, 2007).

In this study it was observed that domestic animals infected with *Salmonella* spp. they were fed homemade food, which could be a source for the acquisition of this bacterium. In this regard, Leonard *et al.*, (2011), found that a risk factor for the acquisition of *Salmonella* spp. in canines it is the consumption of both raw and cooked food.

The high percentage of *Salmonella* spp. it is alarming; It has been described that dogs can be asymptomatic carriers of pathogenic serotypes for humans such as: Typhimurium and Enteritidis; consequently, this situation poses a great risk of zoonosis for humans.. (Leonard *et al.*, 2011).

Finally, no significant differences were found in the number of dogs with *Salmonella* spp. depending on the group to which they belong (domestic or street). This is worrisome as differences were expected to exist as domestic dogs are better cared for by their owners. However, the findings indicate that, in the study group, both domestic and street dogs are equally infected. It is necessary to carry out a study with a larger population sample to obtain more information to confirm whether this finding can be generalized to dogs in the city of Mérida and the reasons why this occurs.

## Acknowledgement

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## Conclusions

Dogs have become a fundamental part of human life; however, their feces residues are a potential reservoir of Enterobacterales, some of them recognized as pathogens for humans. The disposal of canine feces must be done in a hygienic manner to avoid potential zoonosis. Likewise, it is essential to develop studies that provide a broader overview of the risks and consequences of pet waste in the state.

## Financing

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## Evaluation of dehydration parameters of habanero chili (*Capsicum chinense jacq.*) by tray method, for the conservation of seasonal fruits

## Evaluación de parámetros de deshidratación de chile habanero (*Capsicum chinense jacq.*) por método de charolas, para la conservación de frutos de temporada

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### Abstract

Dehydration is a food preservation procedure that, by eliminating all free water from it, prevents microbial activity and reduces enzymatic activity, which allows us to give the dehydrated product a long shelf life. This project aims to find the optimal parameters of dehydration in which the data is easy to use for the control of the shelf life of habanero chili, this being a simplified method that allows the producer to adapt techniques to guarantee the commercialization of their product. Due to its high degree of pungency or itching, it has a variety of uses in the food industry, its national average yield of habanero pepper is around 12 (Ton / ha) (Inforural, 2020).

**Dehydration, Conservation, Useful life, Optimization**

### Resumen

La deshidratación es un procedimiento de conservación de alimentos que al eliminar la totalidad del agua libre de este, impide la actividad microbiana y reduce la actividad enzimática lo que nos permite darle una larga vida de anaquel al producto deshidratado. El presente proyecto pretende encontrar los parámetros óptimos de deshidratación en la que los datos sean de fácil manejo para el monitoreo de la vida útil de chile habanero, siendo este un método simplificado que permita al productor adaptar técnicas para garantizar la comercialización de su producto. Por su grado elevado de pungencia o picor tiene una diversidad de usos en la industria alimentaria, su rendimiento promedio nacional de chile habanero es alrededor de 12 (Ton/ha) (Inforural, 2020)

**Deshidratación, Conservación, Vida útil, Optimización**

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## Introduction

The present investigation consists of obtaining data that will help to evaluate the parameters of dehydration of habanero chili (*Capsicum chinense jacq.*) by tray method, for the conservation of seasonal fruits, the interest in obtaining technical scientific data is derived from the fact that in the region of the central zone of the State of Veracruz and specifically in the region of Huatusco and surrounding municipalities where there are several cultivation areas and the producers do not have the strategies for the conservation of their product, which is normally marketed in semi-ripe fruits so its shelf life is very short in addition to being a seasonal product.

According to (Arias 2000) some of the most common causes of losses during harvesting are: unqualified personnel for production and harvesting, inadequate maturity, poor selection of the product, inappropriate handling boxes, mechanical damage, inopportune time of harvest, excessive harvesting period, exposure of the product to the sun, excessive permanence of the harvested product in the field, poor sanitary conditions, deficient, to all these problems one of the solutions to improve profits of producers is to offer their products only in seasons, because they do not have more practical methods of preserving them.

Within good post-harvest handling practices (López 2003), it indicates that once fruits and vegetables are harvested, they need to be prepared for sale, either in the orchard, at retail, wholesale or supermarket chains, some have focused their techniques to develop new products such as sauces, however many companies or even direct consumers demand this product to develop their own by-products with peculiar characteristics, which necessarily requires the beginning of another value chain to complete them in the design of new products. Dehydration is a good alternative so that they can have a physical product and adapt it to the needs that producers require.

## Problem

Mexico excels in the generation of chili varieties in the world; around 90% of the chili consumed worldwide is of Mexican origin.

Other producing countries are China, Indonesia, Turkey, Spain, United States and Nigeria. (Macías *et al*, 2017). It is of great importance to look for conservation alternatives by giving it an added value.

## Objective

Experimental evaluation of the drying process of habanero chili (*Capsicum chinense Jacq.*) by the tray method, in order to obtain the ideal parameters for its conservation and handling.

## Specific objectives

- Characterization of habanero peppers at different degrees of maturity, considering the following aspects (Degree of Maturity, Length (mm) Width (mm) Weight (g) Density (gr/ml).
- Adequacy of specific dehydration conditions.
- Determination of drying curves for habanero chili slices.

## Theoretical Framework

Dehydration is the reduction of the amount of water by treating the food by artificial heat (previously heated air, hot surfaces, etc.), i.e., artificially or industrially. Foods that can be dehydrated are fruits, vegetables, legumes, mushrooms, spices, milk and eggs. Drying is the reduction of the amount of water by treating the food under environmental conditions (sun, wind, etc.), i.e., in a natural or artisanal way. (De Michelis *et al*, 2015).

Habanero peppers (*Capsicum chinense*) are traditionally produced in the Yucatan Peninsula: Campeche, Yucatan, and Quintana Roo. Traditional open field yields vary from 10 to 40 tons per hectare.

## Fruit Characteristics

The habanero chili is a herbaceous plant or shrub, branched, reaching a size of up to 2.5 m high. Immature specimens of habanero peppers are green in color, but their color varies at maturity (Macías *et al*, 2017). The most common colors are orange, semi-mature and red at maturity.



According to scientific research, the origins of habanero peppers, is in the area from southern Brazil to northern Argentina, through eastern Bolivia and western Paraguay (Macías *et al*, 2017).

### Itching

Capsaicin, the main capsaicinoid, stimulates the mucous membrane of the stomach, increasing salivary secretion and peristalsis (contractions of the intestine that move food forward), which stimulates appetite.(Nancy Lau *et al*, 2011).

In addition, hot peppers intensify nasal and tear secretion, as well as gastric juices. Also, capsaicin has an anti-inflammatory and anti-irritant effect.

### Size

When we talk about fruit quality we refer to its appearance and different sensory characteristics such as size according to (Maldonado *et al*, 2020) the quality of the fruit is related to the appearance where it is described that several morphologies can be found where bell-shaped, elongated, square, triangular and round shapes stand out. The size ranges from 1.14 to 9.88 cm and the weight varies between 0.46 and 24.2 g.

- Preserving food for long periods of time. Once the amount of water present in a food has been reduced, it will be possible to keep it for months or even years in airtight jars without it deteriorating.
- Reduced storage space in your pantry. Dehydration can be a good idea to store more food in less space.
- Preserve food properties. Properly dehydrated foods lose water, but retain most of their nutrients that other preservation or cooking methods might alter.
- Experiment with new textures. You can choose to thinly slice foods to reduce drying times, or leave them whole to rehydrate when you are ready to use them. Also, you can pulverize vegetables to easily add them to cold soups, smoothies, infusions, etc. and reduce the preparation time of your menus.

- Consume more fruits and vegetables. Having a variety of dehydrated fruits and vegetables on hand can be stimulating when cooking and allows you to add more plant-based ingredients to your dishes.
- Have healthier snacks on hand. Dried fruits and vegetables can be used to make snacks or to make homemade granola with nuts and oats. You can also prepare salty or sweet raw crackers, light and nutritious.
- Dehydration is an ideal method to take advantage of fruits and vegetables to give them added value and prolong their shelf life.

### Methodology

The present project was implemented in the facilities of the Food Industry Engineering laboratory at the Instituto Tecnológico Superior de Huatusco in the state of Veracruz, carrying out one of the most used processes for the preservation of food and seasonal fruits.

### Identification of the problems of habanero peppers

First, the problem of the product was identified, which consisted of the fact that this product is marketed in a way that sometimes affects the stability of its composition or tends to decompose faster due to the conditions in which it is exposed. Therefore, a drying of habanero peppers was carried out in an industrial dehydrator model FD-1, applying a variation of time-temperature ratio and taking into account the moisture losses that are perceived in the samples placed in the dehydrator before and after drying in order to reach the desired optimum point which will allow us to give better handling to the product after processing and give it a longer shelf life.

Some of the fruit drying procedures were investigated, and which degree of maturity was the most optimal for dehydration, either by any of the existing types of drying, but choosing the most feasible for the fruit. A fundamental aspect when considering post-harvest handling of fruit is that they are still alive.

In this sense, the harvested fruit continues breathing, ripening in some cases and initiating senescence processes, all of which implies a series of structural, biochemical and component changes that are specific to each fruit (Arias, 2000).

### Fruit Dehydration

Within food engineering there is a range of products with great interest as an object of study, among them are dehydrated products, according to (Perez, 2008). To preserve food, external and internal factors, temperature, oxygen, relative humidity and/or water activity ( $a_w$ ), light for preservation, the most appropriate packaging and shelf life must be considered. Dehydration and drying are the most widely used preservation methods throughout the history of mankind. In the past, fruits, grains, vegetables, meats and fish were dried in the sun to provide food in times of scarcity.

Although the objective of both is to reduce the amount of water in fresh food, what differentiates them is the method used to do so (Pedro *et al.*, 2016). Dehydration is generally understood as the operation by which water is totally or partially removed from the substance containing it. This definition can be applied to solids, liquids and gases. And as it is expressed it can serve to describe various unit operations such as evaporation, adsorption, etc.

The habanero chili should be washed after being cut and avoid handling it if it had contact with other objects, this is to prevent the spread of microorganisms which produce fungi even after dehydration. It is advisable to wash hands thoroughly, as well as to disinfect the area where the practices are carried out. Samples were taken of each habanero chili fruit cut into slices of different weights in order to experiment with different degrees of maturity and testing with different temperatures and times.

### Fruit selection

The fruits with the best quality in terms of size, pigmentation and different degrees of ripeness were selected to collect data by drying test, saving them in an Excel database to later represent them through linear graphs, moisture losses, comparison of weights and percentage of loss in a desiccator (Gómez, 2010).

(Gómez, 2010) The fruit is presented between 120 and 140 days after transplantation whose shape is bell-shaped with three locules on average (Estrada, 2018) ripen red, orange, yellow and even white. Verifying all these aspects, fruits with different maturity times were collected.

### Classification

Subsequently, they were sorted by maturity grade considering their organoleptic characteristics of color and texture



**Figure 1** Degrees of Maturity

They are selected by differentiating the color and size, then they are cut into slices and weighed on the scale, taking the observations of the weight of each sample that is cut from different fruit to have a better perception according to the drying and thus obtain relevant data that gives us the loss of these samples.

### Characterization.

The characterization consists of identifying each sample of habanero peppers at different degrees of maturity, considering the following aspects (Degree of Maturity, Length (mm) Width (mm) Weight (g) Density (gr/ml), Weight (g) Density (g/ml), Weight (g) Density (g/ml), Length (mm) Width (mm) Weight (g) Weight (g) Density (g/ml)



**Figure 2** Characterization

## Dehydration

The samples were introduced in a tray dehydrator in order to dehydrate by convective method different presentations of habanero peppers (slices and whole peppers, variables of analysis are: the color at harvest time according to the previous characterization, as well as the optimum dehydration temperatures and times.



**Figure 3** Characterization

## Dry samples

After drying, depending on the time and temperature, the samples were introduced into the dehydrator and allowed to cool down a little to avoid burn injuries. Once the equipment cools down a little, we use tweezers to remove the samples from the dehydrator to be weighed on the analytical balance and calculate the moisture loss obtained by our sample.



**Figure 4** Dry samples

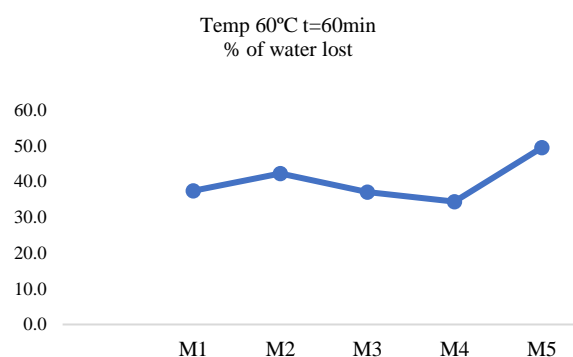
## Results

Once the data of the samples were registered, we proceeded to make our database to get the results of moisture loss of our product, to later be represented in tables and make the comparison of all the data obtained through graphs, each sample was assigned a letter to identify them accurately to observe the behavior they presented after drying and was also given a number since each sample is of different weight and they were ordered from the largest to the smallest. Each sample was cut from a different fruit in terms of maturity and size.

The following tables show the results obtained in our evaluation of parameters for the dehydration of the habanero chili fruit according to the processes carried out. It is worth mentioning that each table specifies the process number, the initial weight (I) of each sample before placing it in the dehydrator, the final weight (F) once the sample was dried, the loss in grams (gr) obtained, the drying loss in percentage (%), the loss of the product in the desiccator (D) and finally the final percentage.

Sample	Weight(I)	Weight (F)	Loss(gr)	% of weight lost
M1	2,86	1,79	1,07	37,4
M2	2,51	1,45	1,06	42,2
M3	1,73	1,09	0,64	37,0
M4	1,63	1,07	0,56	34,4
M5	0,89	0,45	0,44	49,4

**Table 1** Table of data for the first test (slice samples)



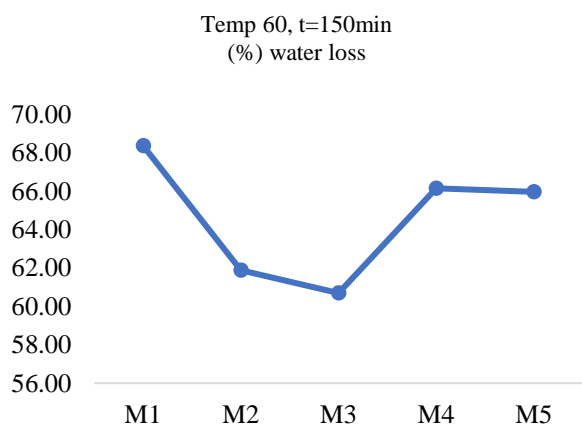
**Graph 1** Second Test Graph (Samples in Slices)

The variation of the water loss of each of the samples is observed.

Each sample lost water according to its weight and size; the larger the sample, the less water was lost. The following table shows the data obtained in the second slice drying test.

Sample	Weight(I)	Weight (F)	Loss (gr)	Drying loss (%)
M1	2,34	0,74	1,6	68,38
M2	2,23	0,85	1,38	61,88
M3	2,01	0,79	1,22	60,70
M4	1,95	0,66	1,29	66,15
M5	1,94	0,66	1,28	65,98

**Table 2** Table of data from the second test (samples in slices).



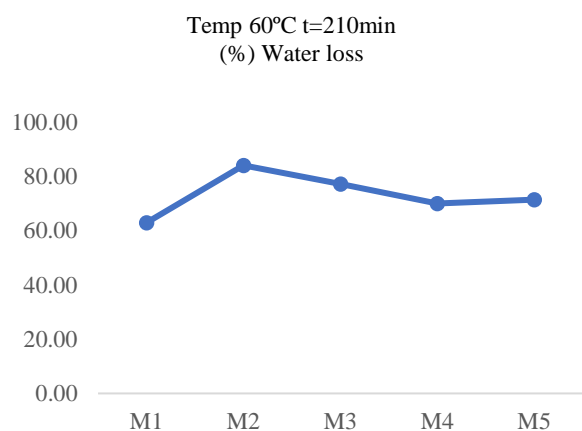
**Graph 2** Second Test Graph (Slice Samples)

The second drying operation in slits is shown, where the progress obtained is observed, but the time was extended, as they are the first tests that were carried out, optimal results were not yet obtained in our tests.

The following table shows the test that was carried out by increasing the exposure time at the same temperature.

Rajas	Weight(I)	Weight (F)	Loss(gr)	Drying loss (%)
M1	1,97	0,73	1,24	62,94
M2	1,57	0,25	1,32	84,08
M3	1,27	0,29	0,98	77,17
M4	1,2	0,36	0,84	70,00
M5	1,19	0,34	0,85	71,43

**Table 3** Table of data third test (samples in slices)



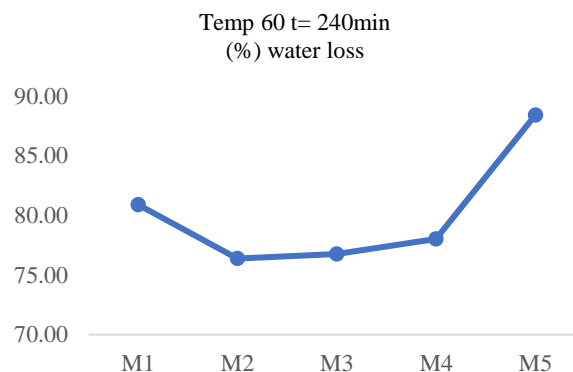
**Graph 3** Third Test Graph (Slice Samples)

It can be seen in the previous graph that up to 70% of moisture was eliminated from the slice samples, the processes were continued until the desired parameter of this project was obtained. The data obtained from the drying of habanero peppers were analyzed, showing that the less time a process takes, the more feasible the product is; when dehydrating whole peppers, more time is needed, or another factor that influences is the low temperature to which the sample is exposed.

Rajas	Weight(I)	Weight (F)	Loss(gr)	Drying loss (%)
M1	1,83	0,35	1,48	80,87
M2	1,82	0,43	1,39	76,37
M3	1,72	0,4	1,32	76,74
M4	1,41	0,31	1,1	78,01
M5	1,38	0,16	1,22	88,41

**Table 4** Table of data fourth test (Sliced Samples)

This sample shows the most favorable results in terms of water loss in the process, but the disadvantage was the time.



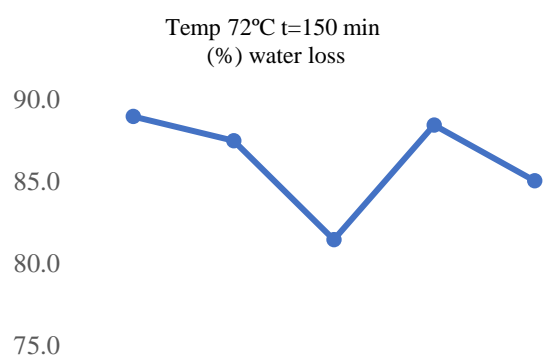
**Graph 4** Graph Fourth Test (Slice Samples)

The following data shown in the following table are from the penultimate drying process carried out at a temperature of 72 °C for a time period of 2 hours 30 minutes.

Integrals	Weight(I)	Weight (F)	Loss(gr)	Drying loss (%)
M1	2,27	0,25	2,02	89,0
M2	1,92	0,24	1,68	87,5
M3	1,78	0,33	1,45	81,5
M4	1,3	0,15	1,15	88,5
M5	1,74	0,26	1,48	85,1

**Table 5** Table of data fifth process (Samples in Raja)

In this process with temperature change, up to 88% water loss was obtained from the habanero samples, so an alternative was to dry small samples in slices at the temperature represented in the table above, the data are presented in the following graph.



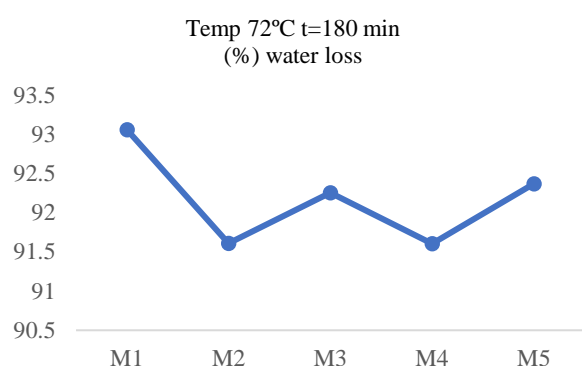
**Graph 5** Fifth Process Graph (Sample in slits)

Comparing the previous results with those of these last tests, an improvement in the drying process can be observed, the temperature was increased to reduce the process time and thus obtain better results in terms of the fact that the fruit must lose at least 90 to 95% of water to give better performance in terms of shelf life, the more water is eliminated, the more fungal growth is avoided.

The following table shows the most relevant results of this project.

Integrals	Weight(I)	Weight (F)	Loss(gr)	Drying loss (%)
M1	1,44	0,1	1,34	93,05
M2	1,43	0,12	1,31	91,60
M3	1,42	0,11	1,31	92,25
M4	1,31	0,11	1,20	91,60
M5	1,31	0,1	1,21	92,36

**Table 6** Sixth process data table (Sliced samples)



**Graph 6** Graph Sixth Process (Slice Samples)

Our drying process favored in every aspect in which the dehydration operation time was reduced and we obtained the ideal parameter desired in this project.

## Conclusions

It is successfully concluded that our process used in the drying of habanero chili (*Capsicum chinense jacq*) was acceptable in the results that were intended to be obtained to preserve the product in storage issues once it is harvested and also to be marketed. For this reason, several tests were carried out to avoid damage to the product and thus obtain a better quality and shelf life.

The graphs and tables show the average water loss of the fruit from the first test to the last, which was the most accurate in terms of results. According to the averages obtained, it was concluded that the most acceptable drying prognosis was 93% water loss in the dehydrated product, which is the average demand at which dehydrated foods or products should be processed.

It was also observed that starting the drying tests, very real results were not obtained, and with each process carried out, very low data were obtained to the drying standard, due to the fact that the samples were exposed to very low temperatures, observing in the whole samples that they tended to lose less water than the samples in slits.

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## Identification of sources of resistance in tomato to *Phytophthora infestans* at Mexico

### Identificación de fuentes de resistencia a *Phytophthora infestans* en jitomate en México

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#### Abstract

Late blight caused by *Phytophthora infestans*, devastating disease in tomato (*Solanum lycopersicum* L.) worldwide. The present study was carried out to identify sources of resistance in wild genotypes of *Solanum lycopersicum* var. *cerasiforme* collected in Mexico, and experimental varieties. San Marzano and Rio Grande were susceptible controls, and the resistant control, accession LA2533 *pimpinellifolium*. Field and greenhouse trials were established for exposure to natural infections. Incidence and severity of the disease and the area under the curve of the disease (AUDPC) were estimated. In laboratory, leaf samples were inoculated with six pathogen strains isolated from potato plants, from Valle de México. It was calculated necrotic area percentage (NAP), sporulation degree (SD) and index disease (ID). Wild populations V115, 319 and 327 had similar behavior to resistant control, followed by experimental genotypes 1-1, 3-1, 1-12, 2-29, 3-31 and 2-14. In separated leaflets inoculated test with Toluca's strains, V115 highlighted, followed by 3-3, 1-12, 2-29, 3-6, 1-1, 2-14 and 319 and moderated resistance were showed by 3-1, 3-31, LA2533, 3-33 and 327. Susceptible control San Marzano obtained higher values for AUDPC, NA, SD and ID.

*Late blight, Solanum lycopersicum* var. *cerasiforme*, genotypes, disease resistance, AUDPC

#### Resumen

El tizón tardío causado por *Phytophthora infestans*, enfermedad devastadora en tomate (*Solanum lycopersicum* L.) a nivel mundial. El presente estudio se realizó para identificar fuentes de resistencia en genotipos silvestres de *Solanum lycopersicum* var. *cerasiforme* colectados en México, y variedades experimentales. San Marzano y Río Grande fueron los controles susceptibles, y el control resistente, la accesión LA2533 *pimpinellifolium*. Se establecieron ensayos de campo y de invernadero para la exposición a infecciones naturales. Se estimaron la incidencia y la gravedad de la enfermedad y el área bajo la curva de la enfermedad (AUDPC). En laboratorio, se inocularon muestras foliares con seis cepas del patógeno aisladas de plantas de papa, provenientes del Valle de México. Se calculó el porcentaje de área necrótica (NAP), el grado de esporulación (SD) y el índice de enfermedad (ID). Las poblaciones silvestres V115, 319 y 327 tuvieron un comportamiento similar al testigo resistente, seguidas por los genotipos experimentales 1-1, 3-1, 1-12, 2-29, 3-31 y 2-14. En el ensayo de inoculación de folíolos separados con cepas de Toluca, destacó V115, seguido de 3-3, 1-12, 2-29, 3-6, 1-1, 2-14 y 319 y mostraron resistencia moderada 3-1, 3-31, LA2533, 3-33 y 327. El control susceptible San Marzano obtuvo valores más altos para AUDPC, NA, SD e ID.

**Tizón tardío, *Solanum lycopersicum* var. *cerasiforme*, genotipos, resistencia a la enfermedad, AUDPC**

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## Introduction

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, stands out for its destructive capacity on tomato (red tomato) and potato crops, which causes that worldwide about one billion dollars are spent annually in the application of products for its control (Abreu *et al.*, 2008). In Sinaloa, Mexico, losses of up to 100% were reported in the 1967-68, 1978-79, 1980-81 (Retes, 1982) and 1991-92 cycles (Félix *et al.*, 2004). Following the onset of infection, entire tomato crops can be destroyed in 7 to 10 days (Merk *et al.*, 2012).

Infection and development of this pathogen are optimal under conditions of high relative humidity (91 to 100 %) and temperatures between 15 and 20 °C (Agrios, 2005; Leyva *et al.*, 2013).

*P. infestans*, belonging to the Oomycetes group, reproduces both sexually and asexually, behaving as a heterothallic organism that requires two types of mating, called A1 and A2 (Fry and Goodwin, 1997, Alarcón *et al.*, 2013). However, homothallic types (A1/A2) with different allo-enzymatic genotypes also occur in Chapingo, Mexico (Alarcón *et al.*, 2013).

The Toluca Valley, Mexico, has been proposed as the site of origin of this pathogen (Grünwald and Flier, 2005; Romero *et al.*, 2011) considering that the sexual form of the pathogen, reported by Graham, *et al.* (1959), had not been observed elsewhere. The pathogen population exhibits diverse virulence characteristics; all enzyme alleles known to date have been detected and populations from this locality contain all DNA fingerprinting bands (Fry and Goodwin, 1995; Andrivon, 1996). Thus, the world's greatest genotypic diversity of *P. infestans* is found in the Mexican central highlands and the Chapingo area, east of Lake Texcoco, is the second center of diversity of the oomycete (Alarcón *et al.*, 2013).

A practical and economical method regarding disease control is based on the use of resistant genotypes, which has led to an intensified search for genetic resistance, using wild species for this purpose (Frías *et al.*, 2001; Barquero *et al.*, 2005a; Barquero *et al.*, 2005b; Scott and Gardner, 2007).

Chunwongse *et al.* (1998), Gardner and Shoemaker (2004), Robertson and Labate (2007), mention the existence of three genes that condition resistance to specific races against *P. infestans* in tomato: Ph1, Ph2 and Ph3, from different populations and accessions of *Solanum* (= *Lycopersicon*) *pimpinellifolium*, located on chromosomes 7, 10 and 9, respectively. Foolad *et al.* (2006) found in this species a new gene (Ph5) located on chromosome 1 in accession PSLP153 of *S. pimpinellifolium* which confers resistance to no less than five different races of *P. infestans* (US8, US13, US14, US15 of A2 mating type), and numerous highly resistant selections of *S. habrochaites* (= *L. hirsutum*) have been made (Scott and Gardner, 2007).

In Mexico, the search for resistance to late blight is focused on potato accessions, so in tomato there is little scientific information available regarding the generation or discovery of resistant varieties. However, there is potential in native varieties and in wild populations of *S. lycopersicum* var. *cerasiforme*, widely distributed in Mexico from Sinaloa to the Yucatan Peninsula (Chavez *et al.*, 2011), present in abandoned and cultivated fields, in tropical dry forests, coastal sites on the Pacific slope (300-1,100 m) of the Sierra Madre Occidental; with the largest populations collected at altitudes between 0 and 1,200 m (Sánchez *et al.*, 2006; Álvarez *et al.*, 2009).

Based on the above, the objective of this study was to identify sources of resistance to *P. infestans* among three wild genotypes of tomato *Solanum lycopersicum* var. *cerasiforme* (Dunal) Spooner, Anderson and R.K. Jansen collected in Mexico, and nine advanced lines, through exposure to natural and artificial infections using strains from the Valley of Mexico.

## Materials and methods

### Plant material

Since 2002, wild populations of tomato *S. lycopersicum* var. *cerasiforme* (Dunal) Spooner, G. J. Anderson et R. K. Jansen were collected in different regions of the country with the support of the National System of Phylogenetic Resources (SINAREFI-SAGARPA).



As a result of the characterization and evaluation of the wild populations during the years 2004 to 2008 in field and greenhouse conditions, in the experimental area of the Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA) of the University of Guadalajara, located in Las Agujas, municipality of Zapopan, Jalisco, Mexico at 20° 44' 44" N latitude, 103° 54' 62" W longitude and an altitude of 1,567 m asl, resistance and/or tolerance responses against late blight attack were observed, where populations V115, 319 and 327 stood out (Arellano *et al.*, 2013).

To confirm the above results, these 3 wild populations were used: V115 (originating from Veracruz, Mexico), 319 and 327 (originating from Nayarit, Mexico), in addition, nine experimental tomato materials developed through crosses, backcrosses and selection from the open-pollinated variety "Rio Grande" and a wild plant originating from Nayarit, Mexico were included; the denominations for the selected lines were: 1-1, 1-12, 2-14, 2-29, 3-1, 3-3, 3-6, 3-31 and 3-33.

The Rio Grande and San Marzano varieties were used as controls, and the accession LA2533 belonging to the *Solanum pimpinellifolium* species, provided by the Tomato Genetics Resource Center (TGRC) of the University of California-Davis in Davis, California, reported to have genetic resistance to races 0 and 1 of *P. infestans* (Chetelat and Rick, 1998).

Exposure to natural infections. During the Spring-Summer 2012 cycle, three trials were established in the CUCBA experimental area: Trial I in a field area with a history of tomato planting; Trial II in an open field where corn had been grown; and Trial III in a greenhouse. Seedling production was carried out under greenhouse conditions, placing the seeds in 200-cavity polystyrene trays filled with a mixture of peatmoss (Sphagnum) and coconut fiber 50-50% vol/vol. At the beginning, they were irrigated with simple water (one week) and after the seedlings emerged they were irrigated with Steiner's Universal nutrient solution (Rodríguez, 2004) at 0.3 atm of concentration until transplanting, after 35 to 40 days.

The trials were established in a randomized complete block design with 2, 6 and 5 replications respectively for trial I, II and III. The experimental unit consisted of 5 plants per plot, with a plant spacing of 0.40 m and a row spacing of 1.20 m. In these trials, no fungicides were applied; cultivation tasks such as manual weeding, foliar fertilization and chemical pest control (whitefly and mites) were carried out.

Late blight occurred during the months of September and October, with daily minimum temperatures of 12 and 14 °C and relative humidity of 80 to 90%, especially during the night and early morning hours (6 am to 10 pm). Taking into consideration the total number of plants in each experimental unit, and in order to determine the average severity of each experimental unit, visual readings of the percentage of disease severity were taken every seven days, according to the International Potato Center scale (Henfling, 1987): 1=0%, 2=3%, 3=10%, 4=25%, 5=50%, 6=75%, 7=90%, 8=97%, and 9=100%. According to Forbes *et al.* (2014), when the number of plants per plot is low, some researchers take severity data on a per plant basis. However, there is little evidence that this process confers any advantage, with the aggravating factor that it requires a significant amount of additional time. For this reason, CIP recommends taking data simply at the plot level.

Finalizing canopy damage ratings when the varieties used as susceptible controls reached 95 to 100% of leaf area damaged by *P. infestans* (Frías *et al.*, 2001). In order to compare the behavior of each material, the area under the disease progress curve (ABCPE) was calculated with the data obtained each week in the experimental plots, to obtain a single data for statistical comparisons (Shaner and Finney, 1977; Campbell and Madden, 1990):

$$AUDPC = \sum_{i=1}^n \left[ \frac{Y_{i+1} + Y_i}{2} \right] [X_{i+1} - X_i] \quad (1)$$

Where:

Y<sub>i</sub>= Percentage foliage of affected tissue at each reading,

X<sub>i</sub>= Time in days from transplanting to the time of evaluation, and

n = Total number of observations

Separated leaflet test. This test included six strains of *P. infestans* isolated from potato crops and characterized by Dr. Héctor Lozoya Saldaña and Dr. Norma M. Alarcón Rodríguez (Alarcón *et al.*, 2014) from the Universidad Autónoma Chapingo, Mexico (Table 1). In experimental fields of this University, during the 2008 and 2009 rainfed crop cycles, isolates of *P. infestans* were randomly collected from simple, young, leaf and stem lesions, taking the tissue with the lesion once it appeared due to natural infection of the pathogen.

The diseased tissues were placed on healthy potato slices (var. Alpha) disinfested with 2% sodium hypochlorite in water (v/v) in petri dishes (10 cm diameter) to allow mycelial growth through the tissue at room temperature. On the third day, mycelial growth was observed microscopically, and the presence of the pathogen was confirmed by morphological observations on fixed preparations. The purified mycelium was transferred to solid agar-centene medium and incubated at 21 °C for characterization (Grünwald *et al.*, 2001).

Compatibility was determined by crossing the *P. infestans* isolates with a known compatible type strain (A1 or A2) provided by the Programa Internacional Cooperativo del Tizón Tardío Tardío de la Papa (PICTIPAPA A. C.), Metepec, Mexico. On agar-centene medium, in a Petri dish (10 cm in diameter), the unknown strain and the known type were planted on opposite sides so that they would grow towards the center of the dish. After two to three weeks at 21 °C, the presence of oospores where the mycelia crossed with the A1 type identified the unknown isolate as A2, and crossing with the A2 type indicated that the unknown isolate was A1. If the same isolate formed oospores when crossed with the two known type strains, it was considered homothallic (Gilchrist *et al.*, 2009).

Genotype identification by allozyme was performed using the method described by Goodwin *et al.* (1995). Mycelium of two to three weeks of active growth was transferred to a microcentrifuge tube with 30 µl of sterile distilled water and macerated with a plastic drill bit. After resting for 5 min, 10 µl was taken and placed on cellulose acetate plates (Titan III, Helena Laboratories). Electrophoresis was performed in Tris-Glycine buffer (pH 8.5).

Glucose-6-phosphate isomerase (Gpi) and Peptidase (Pep) enzymes were used, and each enzyme was revealed according to the methodology reported by Hebert and Beaton (1993). Parallel to field sowings of tomato materials established in greenhouses (experiment III), leaflets from the 5th and 6th subterminal leaves, fully developed, were taken from plants 6 to 8 weeks after transplanting. Subsequently, they were placed in transparent plastic boxes of 20×20×6.5 cm, in abaxial position, on a sterile paper towel placed on a metal sieve. To each box, 100 ml of sterile water was previously added. Each leaflet was inoculated with 25 µl of sporangium suspension, at a concentration of 40,000 sporangia ml<sup>-1</sup>. The boxes with inoculated leaflets were placed in the laboratory at 18±1 °C and a photoperiod of 16 h light, as proposed by Barquero *et al.* (2005b) and Xuan and Byung (2007).

The test was done in duplicate and for each material 10 leaflets were used. The degree of resistance of the genotypes was determined by calculating the disease index described by Jeger *et al.* (2001), Pérez *et al.* (2001), and Barquero *et al.* (2005b), where three variables are considered in each leaflet with the following scales: leaflet necrotic area (NA): 1=no symptoms, 2=necrotic spots at the drop site, 3=necrotic spots of 2 mm, 4=1 cm, 5=50%, 6=51-65%, 7=66-75%, 8=76-85%, and 9=>85%; degree of sporulation (GE): 0=no sporulation, 1=Some sporangia in necrotic area, 2=50% of necrotic area with sporangia and 3=100% of necrotic area with sporangia. Each leaflet received a disease index (DI) value corresponding to the product of the necrotic area and the degree of sporulation, considering that values from 1 to 9 correspond to highly resistant genotypes, 10 to 18 moderately resistant and 19 to 27, highly susceptible genotypes (Barquero *et al.*, 2005b).

### Statistical analysis

The data of the variable AUDPC, obtained from the three trials of exposure to natural infections in the field and greenhouse, were tested for normality using the Univariate procedure of SAS 8.1 and the NORMAL option based on the Shapiro-Wilks test.

When the data did not meet the assumption of normality according to the test, transformations were performed on the ABCPE data, where the square root of ABCPE+1 allowed the values to adjust to a normal distribution. For the variables studied in the field, greenhouse and leaflet test, analysis of variance (ANOVA) was carried out individually and in combination. To separate genotypes into groups based on resistance, tolerance or susceptibility to late blight, a Tukey multiple means test was carried out for each observed variable. The statistical package SAS (Statistical Analysis System 8.1) was used.

## Results and discussion

### Exposure to natural infections

Analyses of variance for the variable Area Under the Disease Progress Curve in the three trials indicated highly significant differences ( $P \leq 0.01$ ) among genotypes. The three wild populations, in the three trials were grouped together with the resistant control LA2533 (Table 2), followed by the 9 improved experimental materials and only in trial I genotype 3-31 reached similar levels to the susceptible genotypes. The V115 and 319 populations had lower ABCPE values than LA2533 in all three trials, 19 to 76 % lower and 82 to 94 % lower than the susceptible varieties.

When comparing the trials among themselves, the highest ABCPE values were found in the greenhouse (trial III), followed by trial I, where tomato had been grown in previous cycles, and thirdly in trial II, where corn had been grown previously.

In the combined analysis (Table 2), a clear separation between genotypes was observed. Group "d" corresponds to population V115 with the highest degree of resistance shown through experiments, followed by populations 319 and 327 (significance group cd) and accession LA2533 (group c). In group b, with intermediate resistance, were the experimental materials; and finally in group a, the susceptible controls, with the highest ABCDE values. The higher this value, the more susceptible the variety (Forbes *et al.*, 2014). The data indicated that significant interaction between genotypes and trials varied in the response of genotypes in relation to the evaluation site.

Graph 1 shows that populations V115 and 319, showed a similar response in the three conditions, while population 327 showed greater susceptibility to the pathogen under greenhouse conditions, as did eight of the experimental varieties. On the other hand, variety 3-31 and accession LA2533 showed an increase of the disease in the sites with a history of tomato. In contrast, Rio Grande and San Marzano showed a lower level of disease in field conditions with a history of tomato cultivation and a very high susceptibility under greenhouse conditions, which could be due to very favorable conditions for pathogen expression, including greater leaflet development, influencing pathogen variability (Stewart *et al.*, 1983), in addition to favoring pathogen expression due to the predominant humidity and temperature inside the greenhouse.

In field trials infected by late blight races, their aggressiveness, virulence and amount of inoculum are usually unknown and many climatic changes occur during the test season that can influence the behavior of pathogen-resistant genotypes by strongly depending on the environment (Michalska *et al.*, 2011).

In the present study, the greenhouse did not have equipment for internal environmental control, so that during the months of September and October, during the night and early morning hours, temperatures tended to drop (below 15°C) and the predominant relative humidity was above 90%, favoring the presence of dew on the upper part of the greenhouse (roof and plastic walls), allowing the wetting of the leaves for a period of more than eight hours. According to Henfling (1987), late blight sporangia only form when relative humidity is above 95%; furthermore, if water (dew, dew, rain) is present on the leaf surface for a minimum of two hours, zoospores germinate and penetrate.

The response of the genotypes to the natural infection of *P. infestans* in the field and greenhouse conditions of Zapopan, Jalisco were consistent, in terms of the relative behavior among genotypes, which led to consider the wild populations of var. *cerasiforme* as the most resistant to the pathogen, together with accession LA2533.

### Separated leaflet test

Significant differences (P0.01) were obtained between genotypes in the ANOVA, while between strains and genotypes there was no interaction in the 3 variables evaluated (AN, GE, and IE), indicating that the genotypes presented a similar response when inoculated with the different strains.

The lowest values of necrotic area were found in the V115 population, and the experimental materials 1-12, 2-29, 3-3 and 3-6, while in degree of sporulation (DS) the lowest values were obtained in V115 and 3-3; with the exception of 327 and San Marzano, the other 10 materials had intermediate values (Graph 2). Genotypes V115, 3-3, 3-6, 1-12, 2-29, 1-1 and 2-14 were in the highest resistance group because they had the lowest disease index. Materials 319, 3-1, 3-31, LA2533, 3-33 and 327 were placed with an intermediate disease index (Graph 2).

According to Barquero *et al.* (2005b), disease index (DI) values from 1 to 9 correspond to highly resistant genotypes such as V115, 3-3, 3-6, 1-12, 2-29, 1-1, 2-14; from 10 to 18, medium resistance: 319, 3-1, 3-31, LA2533, 3-33 and 327; and from 19 to 27, highly susceptible genotypes such as the San Marzano (SM) control.

When analyzing the aggressiveness of the strains, expressed in the three variables evaluated, genotypes V115, 3-3, 1-12, 2-29, presented the lowest values, indicating greater resistance to the six strains with which they were inoculated. For genotype 2-14 the highest value of necrotic area and degree of sporulation was with strain 24. In general, strain 13 was the most aggressive for V115, 3-3, 319, 327 and strain 10 was the least aggressive. On average, strains 23, 24 and 31 were the most aggressive for the rest of the materials.

When subjected to the action of different strains in the leaflet test, the wild population V115 showed lower levels of necrotic area and sporulation than the resistant control, being characterized by maintaining its high resistance even when subjected to the action of the strains from Chapingo.

This response is of interest if it is considered that in the central part of Mexico is where the greatest diversity of this pathogen is found worldwide, while in other areas of Mexico and the world there are populations of late blight with low or moderate genotypic diversity (Grünwald *et al.*, 2001 and Flier *et al.*, 2003).

These low values could indicate that the plant suppresses the action of the fungus on its foliage and at the same time reduces its sporulation capacity, while in the resistant control LA2533 the necrotic area has a higher value but a degree of sporulation similar to V115, which could indicate that the pathogen manages to affect the foliage of the plant and in response the plant tries to suppress its sporulation by developing a hypersensitivity reaction around the infected tissue. This hypersensitivity reaction is intended to protect or immunize the rest of the plant against a potential second infection (Lemus, 2009).

The two wild populations of var. *cerasiforme* from Nayarit showed intermediate levels in the disease index, close to the value of the resistant control and well below the value presented by the susceptible control. Population 319 showed less necrotic area and less sporulation than LA2533 and a lower disease index than LA2533, but higher than V115, while population 327 showed a lower necrotic area value but a higher sporulation index value, which places it with a higher disease index. In ABCPE the positioning of these two populations is reversed, 327 with higher resistance than 319.

When comparing the values obtained in natural infections (field) and those obtained with artificial inoculation of strains from the Chapingo area, the experimental materials showed greater resistance to artificial infections, and some of them (3-3, 3-6, 1-12, 2-29, 1-1 and 2-14) were superior to the resistant control LA2533 and genotypes 319 and 327.

These differences may be due to the fact that possibly the experimental materials present specific resistance to certain strains of the pathogen and when subjected to field evaluations with high pathogen pressures and climatic changes during the development of the same, made it possible to break the resistance.

Since specific resistance in plants allows a clear differentiation between races of a pathogen, since it is effective against certain specific races of the same and ineffective against others (Lemus, 2009). Lesion size (% necrotic leaf area) and spore density (degree of sporulation) values measured in separate leaflet tests apparently correlate well with ABCPE values obtained in field experiments for late blight (Singh and Birhman, 1994). However, Vleeshouwers *et al.* (1999), using these two tests on several *Solanum* species, found no significant differences between them.

A disadvantage of selecting resistant genotypes under field evaluations versus selection under laboratory conditions is that the latter is less related to the environmental conditions prevailing in agricultural areas; since field resistance is directly evaluated including interactions of climatic conditions, cultural practices, pesticides, and other diseases (Horneburg and Becker, 2011). In many reported experiments with selection under artificial conditions, only one or a few strains of *Phytophthora infestans* have been applied to tomato (Michalska and Pazio, 2005).

To date, there is very little work reported on this crop using these methodologies (Foolad *et al.*, 2008). However, laboratory trials can help breeders to discard part of the germplasm to minimize field treatments, which is necessary for the correct selection of resistant lines (Stewart *et al.*, 1983; Dorrance and English, 1997). Laboratory conditions are defined and stable conditions, a single strain is applied, they usually have complex virulence and high aggressiveness and a high concentration of inoculum is used (Michalska *et al.*, 2011).

Therefore, if we want to have reliable results on the genetic resistance of some genotypes to late blight, it is recommended to evaluate this resistance with strains from the Valley of Mexico, since in numerous studies the identification of resistance in wild species has been based on inoculations in artificial environments, or with exposure to limited genotypes of the pathogen (Lozoya *et al.*, 2006).

## Conclusions

It was confirmed that the three wild populations of *Solanum lycopersicum* var *cerasiforme* have high resistance to *P. infestans*, both in field and greenhouse tests and with exposure to strains from the Valley of Mexico, which can be incorporated into breeding programs as sources of resistance to late blight for the generation and development of commercial varieties.

The experimental materials showed a consistent response, intermediate in resistance, both in field and greenhouse trials, as well as in the leaflet test. Among them, it is possible to consider genotype 1-12 as a viable germplasm source, with comparable levels of ABCPE, necrotic area and degree of sporulation to LA2533.

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## Supramolecular self-assembly studies and spectroscopic analysis of oligomers used for the removal of pollutants from wastewaters

### Estudios de autoensamblaje supramolecular y análisis espectroscópico de oligómeros empleados para la remoción de contaminantes en aguas residuales

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#### Abstract

The oil spills in the oceans have caused severe damage, many of these are irreversible generating the loss of marine ecosystems, such is the case of the British Petroleum company in 2010. Therefore, there is a need to produce materials that contain or prevent the spread of the hydrocarbon in the ocean, in addition to being able to recover the crude oil quickly and effectively. Various materials have been designed focused on environmental remediation, specifically in the treatment of contaminated water. In this work, organogelling materials were synthesized from alkoxides such as Methyl 4-hydroxybenzoate, Propyl 4-hydroxybenzoate, Ethyl 4-hydroxybenzoate and the alkyl halides 1-bromohexadecane and 1-bromotetradecane, all of them analyzed by FTIR spectroscopy. In addition, gelation tests were performed in protic, aprotic and fatty acid solvents. Organogels, have a solid appearance at the nanoscale and extends into a liquid phase. Consequently, if there is a close contact between the solvent and the nanogel structure, a highly effective surface is obtained, providing a kind of solid phase in contact with highly polluting liquids. The importance of this work lies in the feasibility of using oligomers as removers or sequestrants of unwanted contaminants in effluents.

#### Oligomers, Contaminants, Remediation

#### Resumen

Los derrames de petróleo en los océanos han ocasionado severos daños, muchos de estos son irreversibles generando la pérdida de ecosistemas marinos, tal es el caso de la empresa British Petroleum en el año 2010. Por ello, existe la necesidad de producir materiales que contengan o impidan el esparcimiento del hidrocarburo en el océano, además de poder recuperar el crudo de manera rápida y efectiva. Se han diseñado diversos materiales enfocados a la remediación ambiental, específicamente en el tratamiento de aguas contaminadas. En este trabajo, se sintetizaron materiales organogelantes a partir de alcóxidos como el Metil 4-hidroxibenzoato, Propil4-hidroxibenzoato, Etil 4-hidroxibenzoato y los haluros de alquilo 1-bromohexadecano y 1-bromotetradecano, todos ellos analizados mediante espectroscopía FTIR. Además, se realizaron pruebas de gelificación en solventes próticos, apróticos y ácidos grasos. Los organogeles, tienen una apariencia sólida a nanoescala y se extiende en una fase líquida. En consecuencia, si existe un contacto cercano entre el solvente y la estructura del nanogel, se tiene una superficie altamente efectiva, proporcionando una especie de fase sólida en contacto con líquidos altamente contaminantes. La importancia de este trabajo radica en la factibilidad de emplear los oligómeros como removedores o secuestrantes de contaminantes no deseados en efluentes.

#### Oligómeros, Contaminantes, Remediación

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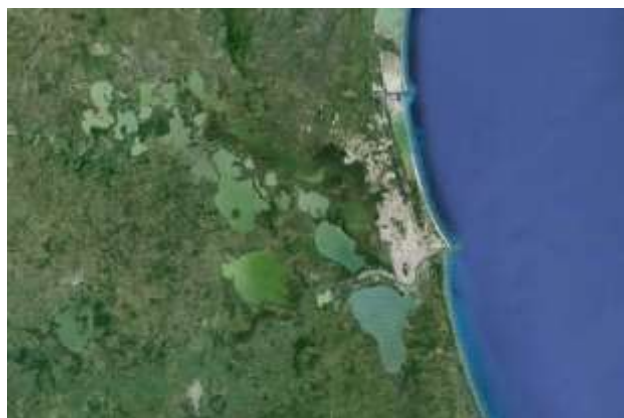
† Researcher contributing as first author.

## 1. Introduction

Water is one of the most precious natural resources in the world and in spite of being so abundant in our planet, only 3% is contemplated for human consumption while 97% is distributed in seas and oceans. As a consequence of this problem and considering the unforeseen environmental accidents of this nature, there is a need to create materials to contain crude oil spills in the sea and thus recover it quickly and efficiently. The contamination problem in southern Tamaulipas and northern Veracruz affects both northern Veracruz (Pueblo Viejo) and the municipalities of Tampico, Cd. Madero and Altamira in Tamaulipas. The effects of the contamination have been on health, where in 1995 there were 700 cases of cholera, and on fish production, especially in the lagoon of Pueblo Viejo<sup>[1]</sup>.

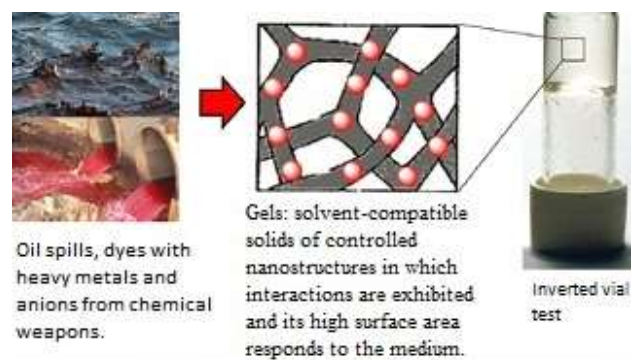
Geomorphological aspects are determinant in the system's problems. Sediments originating in the Sierra Madre Oriental reach this zone, which tend to be deposited over geological time in low-gradient zones. On the other hand, the existing low gradient allows seawater to enter the estuary at high tide conditions, which generates an interaction between suspended solids, of fluvial origin, and dissolved solids, of marine origin<sup>[1]</sup>.

Likewise, the coastal waters of the Gulf of Mexico show increasing levels of contamination derived from the discharge of industrial waters from the Altamira area, mainly<sup>[1]</sup>.



**Figure 1** Aerial view of the Tamesí River Lagoon System, the city of Tampico, and the Gulf of Mexico  
 Source Google Earth, Ponce and Aguilar 2019<sup>[1]</sup>.

The importance of this work lies in obtaining environmentally friendly materials, which allow the fast, efficient and accurate removal of pollutants in water bodies or effluents and thus reduce the level of contamination of the same, coming from carboxylic acids and amides which are susceptible to form bonds by physical forces, specifically hydrogen bonds, specifically hydrogen bridges, as well as pi-pi attractions and Van der Waals<sup>[II]</sup> forces from the hydrocarbon chains that allow access to the oil constituents in a selective way that are possible to create selective systems of non-polar organic liquids and oils that are capable of achieving a gelation of a specific mixture containing two phases. Some researchers have succeeded in obtaining low weight molecules capable of gelling in oil spills, dyes, heavy metals and chemical weapons anions Figure 2.



**Figure 2** Self-assembled gel-phase materials for environmental remediation  
 Source Okesola and Smith 2016<sup>[111]</sup>

Organogelators exhibit thermotropic properties and this generates potential interest in their application as sensors, templates for nanostructure fabrication, biochemistry, rheology modifiers. Although many types of oligomers have been developed, there is little reference to the ability of gelators to selectively self-assemble in one solvent into another solvent in a given mixture. This is interesting when one of the solvents in the mixture is water. Oligomers possess self-assembly properties and form fibrillar objects in different organic solvents.

A gelling agent is a substance that when added in very small proportions, less than 1%, to a liquid transforms it into a gel. When the liquid used is an organic solvent, the gel is called organogel.

Simple organic molecules self-organize into supramolecular polymers of a fibrillar nature, whose structure is maintained by non-covalent interactions. Oligomers derived from metal alkoxides, as well as alkyl halides, retain the fibrillar structure without collapsing upon solvent removal, a property only attributed to oligomers containing chiral carbons in the structure. In this work, oligomers without chiral carbons were designed, the materials were analyzed by FTIR characterization technique to identify functional groups and due to this condition, self-assembly and thermo-reversibility tests allowed obtaining the latent heats of each molecule.

Organogels can be distinguished from hydrogels by their predominantly organic solvent. The gelation process involves the self-assembly of low molecular weight organogels (LMOG) to give a fibrillar polymer-like appearance, which immobilize the organic solvent forming a three-dimensional network of cross-links or entangled chains for chemical and physical gels<sup>[IV]</sup>.

Fibers are stabilized by weak interactions such as hydrogen bridges, Van der Waals forces. Many factors such as steric effects, rigidity and polarity can counteract self-assembly. Control over the gelation process and the conception of new gelling molecules remain important challenges in the search for new organogelators<sup>[VI,VI]</sup>.

## 2. Methodology for the synthesis of molecules

### 2.1 Synthesis of C1C16, C2C14, C3C16 molecule

– Method of preparation of the oligoether:

The reagents were used as received: methyl 4-hydroxybenzoate (Aldrich), N,N-dimethylformamide (DMF), potassium carbonate K<sub>2</sub>CO<sub>3</sub>, alkyl bromide (1-bromohexadecane, 1-BHD) and nitrogen as inerte gas (Figure 3). The oligomer was synthesized by Williamson's method from a phenolic derivative (alkoxybenzoates) and an alkyl halide.



**Figure 3** Reaction equipment using an inert médium  
*Own source*

A solution was prepared with the corresponding alkoxide in dimethylformamide (DMF) to which potassium carbonate was added. After having reacted for 2 hours at a temperature between 60-65°C, the corresponding bromide (1-bromohexadecane) was added and allowed to react for a further 8 hours at the same temperature. At the end of the reaction, the contents of the flask were allowed to cool to room temperature or immediately poured into a beaker containing very cold water with ice (Figure 4).



**Figure 4** Obtaining the C1C16 oligomer  
*Own Source*

The product is recovered with the formation of a white material, which crystallizes immediately and is filtered (Figure 5). The filtrate is left to dry on a crystallizer for 3 to 4 days in a desiccator to eliminate any percentage of moisture (Figure 6).



**Figure 5** Washing and crystallization of the oligomer derived from Alkoxybenzoates and alkyl halides  
*Own Source*



**Figure 6** Washing and crystallization of oligomer derived from alkoxybenzoates and alkyl halides  
*Own source*

### 3. Characterization by FTIR infrared spectroscopy C1C16, C2C14, C3C16 and gelation tests.

#### 3.1 FTIR Characterization

This spectroscopy is based on the absorption of IR radiation by vibrating molecules. Two basic categories of vibrations can be distinguished: tensile and bending vibrations. Tension vibrations are changes in the interatomic distance along the bond axis between two atoms. Bending vibrations are caused by changes in the angle formed by two bonds. The samples were characterized in a Fourier transform spectroscopy (FTIR) apparatus. The effect of the hydrocarbon chain of the ester group in the organogel will be examined using FTIR spectroscopy. The analyzed molecules presented functional groups such as the double bonds of the aromatic ring,  $-\text{COOR}$ ,  $-\text{CH}_3$ ,  $-\text{CH}_2$ . The synthesized oligomers were evaluated in a Fourier Transform Infrared Spectrophotometer Perkin Elmer Spectrum 100 model with the ATR technique with diamond reflection unit and a resolution of  $4\text{ cm}^{-1}$  and 16 scans.

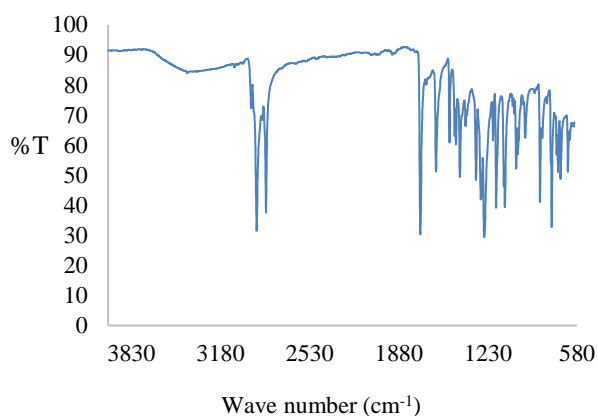
#### 3.2. Gelation tests

Gelation tests were performed in duplicate on C1C16, C2C14, C3C16 molecules in different solvents, starting from an initial concentration of 5%. The molecules were dissolved in diethylether DMF, methanol, ethanol, heptane, propylene carbonate, ethyl acetate, isopropanol, hexane, olive oil, castor oil and propylene glycol. The gelation tests were performed by heating the oligomer at the indicated concentration until a homogeneous solution was formed, leaving a transparent solution inside the vial and allowing it to cool for a few seconds (in some samples for minutes) at room temperature, observing different behaviors: gel, precipitates and homogeneous solutions. When the gel state was obtained, the inverted vial method was used and its formation was verified.

## 4. Results

### 4.1 Infrared Results

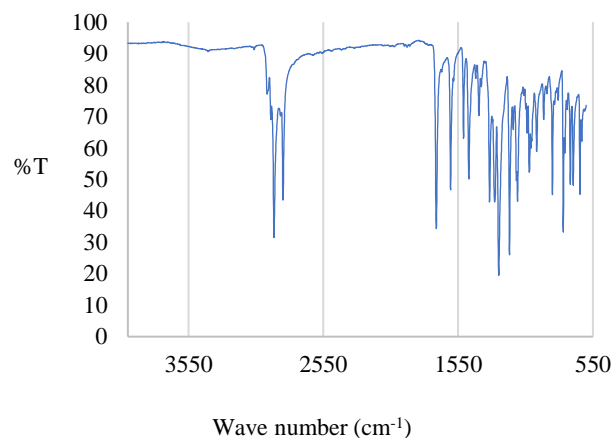
The characteristic groups of the synthesized molecules C1C16, C2C14 and C3C16 are observed in graph 1; two middle bands are presented which correspond to the elongations of the C-H bonds, these appear in the range of 3020-2850  $\text{cm}^{-1}$  attributed to the  $-\text{CH}_3$  and  $-\text{CH}_2$  groups whose bending at 1497-1350  $\text{cm}^{-1}$ , provide the certainty of the existence of these groups; at 762  $\text{cm}^{-1}$  it was possible to determine the effect of the long alkyl chains; the ester group at 1725  $\text{cm}^{-1}$ , the aromatic ring at 1609  $\text{cm}^{-1}$  has an average C=C bond elongation band corresponding to the vinyl ether group, the ether group is located in the range of 1310-1000  $\text{cm}^{-1}$ ; a strong split band of asymmetric elongation of the C-O-C group in the vinyl ethers appears in the range of 1275-1230  $\text{cm}^{-1}$  and is corroborated by the appearance of the symmetric elongation signal at 1075- 1020  $\text{cm}^{-1}$ .



**Graph 1** Fourier Transform infrared spectrum of C1C16 molecule  
*Own Source*

The following spectrum (graph 2), containing information about the function groups shows the infrared spectra of the C3C16 molecules, it is observed that the band size increases as the chain length increases in the oligoether, the characteristic band of the methyl and methylene groups at 2973-2833  $\text{cm}^{-1}$ , at 1712  $\text{cm}^{-1}$  the ester group and the double bond of the aromatic ring 1604  $\text{cm}^{-1}$  at 1511 and 1475  $\text{cm}^{-1}$  a shift of the bends is observed for methyls and methylenes, this is attributed to the increase in the size of the alkyl chain for the ester group. The band attributed to the ether groups is observed in the 1150-1085  $\text{cm}^{-1}$  range which is generated by the asymmetric stretches. This band is usually observed near 1125  $\text{cm}^{-1}$ .

Finally, the ether group band occurs at 1280 and 1264  $\text{cm}^{-1}$ . It is noticeable in all three spectra that as the alkyl chain increases the spectral band shifts increasing from 1255 to 1259  $\text{cm}^{-1}$ .



**Graph 2** Fourier Transform infrared spectrum of C3C16 molecule  
*Own Source*

### 4.3 Results of the gelation tests

The synthesized materials were subjected to gelation tests in different solvents initially at a single concentration equal to 5%, each test in duplicate, with the purpose of verifying the solvents in which the C1C16, C2C14, C3C16 molecules presented gelation and self-assembly processes.

The importance of this test lies in the fact that it is possible that by designing organogels from non-chiral carbons, it will be possible to create molecules that present resistance to collapse at the moment of making the templates, or by being used as sequestering agents of environmental pollutants, it will be possible to easily remove them from the toxic or harmful structures that are contained in the effluents or polluted bodies of water.

Once the gelation result was obtained, the samples were prepared again in these solvents varying the concentrations from 1 to 10%, in 1 ml vials mixed with each solvent (0.6 ml). The organogel was left to stand at room temperature for 20 minutes and was gently heated until a homogeneous solution was obtained, then it was slowly cooled and kept at 10°C (Figure 7). The gelation capacity was evaluated using the vial inversion methodology and stored for 24 hours at 20°C; the vial was inverted for 1 hour and if the material did not flow, it was considered gelled (Tables 1 to 3).



**Figure 7** Gelation of the C3C16 molecule  
*Own Source*

C1C16 (5%)		
	Vial 1	Vial 2
Diethylether	I do not gel	I do not gel
DMF	I do not gel	I do not gel
Cyclohexane	I do not gel	I do not gel
Methanol	If I gel	If I gel
Ethanol	I do not gel	I do not gel
Heptane	I do not gel	I do not gel
Propylene carbonate	I do not gel	I do not gel
Ethyl acetate	I do not gel	I do not gel
Isopropanol	I do not gel	I do not gel
Hexane	I do not gel	I do not gel
Olive oil	I do not gel	I do not gel
Castor oil	I do not gel	I do not gel
Propylene glycol	I do not gel	I do not gel

**Table 1** Results of gelation tests at 5% of C1C16  
*Own Source*

C2C14 (5%)		
	Vial 1	Vial 2
Diethylether	I do not gel	I do not gel
DMF	I do not gel	I do not gel
Cyclohexane	I do not gel	I do not gel
Methanol	If I gel	If I gel
Ethanol	I do not gel	I do not gel
Heptane	I do not gel	I do not gel
Propylene carbonate	If I gel	If I gel
Ethyl acetate	I do not gel	I do not gel
Isopropanol	I do not gel	I do not gel
Hexane	I do not gel	I do not gel
Olive oil	I do not gel	I do not gel
Castor oil	I do not gel	I do not gel
Propylene glycol	I do not gel	I do not gel

**Table 2** Results of gelation tests at 5% C2C14  
*Own source*

C3C16 (5%)		
	Vial 1	Vial 2
Diethylether	I do not gel	I do not gel
DMF	Si gelifico	Si gelifico
Cyclohexane	I do not gel	I do not gel
Methanol	If I gel	If I gel
Ethanol	If I gel	If I gel
Heptane	I do not gel	I do not gel
Propylene carbonate	I do not gel	I do not gel
Ethyl acetate	I do not gel	I do not gel
Isopropanol	I do not gel	I do not gel
Hexane	I do not gel	I do not gel
Olive oil	I do not gel	I do not gel
Castor oil	I do not gel	I do not gel
Propylene glycol	I do not gel	I do not gel

**Table 3** Results of gelation tests at 5% of C3C16  
*Own Source*

With the results obtained, it was possible to determine that the oligomers that presented gelation. The organogelation was carried out in different organic solvents showing reversibility of the materials and exhibiting that the self-assembly is carried out by supramolecular forces, since when performing such tests and forming the organogels they presented the phase change when an increase in temperature was applied, returning to the colloidal state as it cooled down.

It was observed that the solvents of similar chain to the oligomer did not gel and were soluble as toluene, cyclohexane, hexane and pentane; on the other hand, the solvents used of short polar chains, such as methanol, ethanol, isopropanol, acetonitrile, propylene carbonate and diethylenetriamine presented gelation.

## 5. Funding

This work was financed by PRODEP with code ITCMAD-PTC-12 and own resources were also used. Some reagents, supplies and equipment were provided by the Instituto Tecnológico de Nuevo León and the Instituto Tecnológico de Ciudad Madero.

## 6. Conclusions

The self-assembled materials gave rise to the formation of fibers of different lengths when varying the primarily polar solvent, this is shown in the images whose morphology varies from short fibers as in alcohols to ribbons, in solvents whose polar factor increased. In the molecules studied in this work, the forces driving self-assembly can be originated through  $\pi$ - $\pi$ , dipole-dipole and Van der Waals interactions. The length of the alkyl chains causes solvent compatibility and gelation efficiency. As future work, it is intended to collect effluent liquids from the conurbation area to measure the efficiency of these materials in the removal of environmental pollutants.

As future work, it is necessary to perform gelation tests on gasoline, petroleum oil, kerosene, phenols and other common compounds found in water bodies and effluents in order to carry out the application of the materials.

These water bodies and effluents will be georeferenced in order to determine the water and effluent sampling sites in the metropolitan area and to be able to apply the supramolecular oligomers with the proper treatment of the samples.

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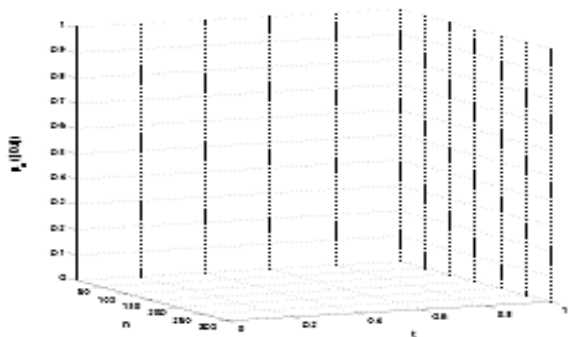
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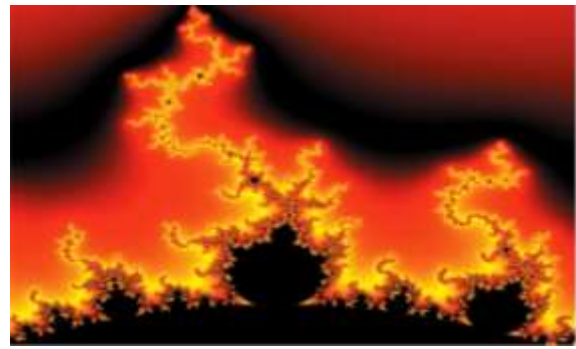
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