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ORIGINAL ARTICLE

Inhibition of *Candida albicans* by oregano (*Lippia* spp.) essential oil from Municipality of Rodeo, Durango, México.

Inhibición de *Candida albicans* por aceite esencial de orégano (*Lippia* spp.) del Municipio de Rodeo, Durango, México.

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A B S T R A C T

One of the main opportunistic fungi in the world is *Candida albicans*, belong of the *Cryptococcaceae* family, it can present in an oval way with an average size of 2 to 4 microns, is the main cause of infections in the human body of candidiasis type. In Mexico in 2015 there were 177,394 cases of vaginal candidiasis between the ages of 19 to 29 years, 2,695 cases occur in the State of Durango. The essential oil of oregano (*Lippia* spp.) of the municipality of Rodeo, Dgo. Has a 70 % concentration of thymol, is one of the main natural antifungals, since it changes the permeability of the cell membranes causing the filtration of chemical constituents vital for metabolism. The experimental work was monitoring inhibition halos with three different concentrations (150 ppm, 250 ppm and 350 ppm), reading every 24, 48 and 72 hours to identify suitable time and concentration. A unifactorial analysis was used in a Tukey test and an F test for significance performed in the statistical program SAS (Statistical Analysis System), finding as a result a contact time of 48 hours with a suitable dosage of 250 ppm inhibiting 73.8 % to the fungus.
Keywords: Oregano oil, *Lippia* ssp., Thymol, Carvacrol and *Candida albicans*.

R E S U M E N

Uno de los principales hongos oportunistas en el mundo es *Candida albicans*, este es de la familia *Cryptococcaceae*, puede presentarse de forma ovalada con un tamaño promedio de 2 a 4 micrones, es la principal causa de infecciones en el cuerpo humano de tipo candidiasis. En México en 2015 hubo 177,394 casos de candidiasis vaginal entre las edades de 19 a 29 años, en el Estado de Durango se presentaron 2,695 casos. El aceite esencial de orégano (*Lippia* spp.) del municipio de Rodeo, Dgo. Tiene una concentración del 70 % de timol, es uno de los principales antifúngicos naturales, ya que cambia la permeabilidad de las membranas celulares provocando con ello la filtración de constituyentes químicos. Vital para el metabolismo. El trabajo experimental consistió en monitorear halos de inhibición con tres concentraciones diferentes (150 ppm, 250 ppm y 350 ppm), leyendo cada 24, 48 y 72 horas para identificar el tiempo y la concentración adecuado. Se utilizó un análisis unifactorial en una prueba de Tukey y una prueba F de significancia en el programa estadístico SAS (Statistical Analysis System), encontrando como resultado un tiempo de contacto de 48 horas con una dosis adecuada de 250 ppm inhibiendo el 73.8 % al hongo.
Palabras clave: Aceite de orégano, *Lippia* ssp., Timol, Carvacrol y *Candida albicans*.

INTRODUCTION

Oregano in Mexico is found in large proportions in the states of Durango, Chihuahua, Sonora, Zacatecas, etc. In general, its commercial value is due to its characteristics as spice, condiment and medicinal properties (Bonilla *et al*., 2011). It has greater importance in the industry and pharmaceutical for its essential oil, which is used as a fragrance in soaps, perfumes, cosmetics, flavorings; in addition, it preserves antibacterial, antifungal, antiparasitic, antimicrobial and antioxidant properties (Scazzocchio *et al*., 2016).

During the last years a dramatic increase in the incidence of infections caused by yeast has been reported, mainly the vaginal infections caused by *Candida albicans* L. called candidiasis. This opportunistic fungus is usually found in the body in a controlled manner, the problems are caused when it begins to proliferate due to the increase of humidity causing infections. In Mexico in 2014, 24 % of women have been victims of candidiasis, an infection caused by this proliferation of this fungus (IMSS, 2016).

Candida albicans, is a fungus and like most of them, its optimum temperature of growth is 37 ºC (body temperature), (Apares, 2016). Candidiasis is responsible for 80 to 90 % of vaginal yeast infections and the prevalence of these is between 5 and 20 % in non-pregnant and asymptomatic pregnant women (García, 2014). The development of new techniques of alternative organic medicines leads us to the study of essential oils, which contain physicochemical properties capable of inactivating microbial growth. In the leaves of oregano, we can find two main components, thymol that has the ability to decrease the spread of fungi and in the case of carvacrol that inhibits the growth of bacteria (Lara *et al*., 2016; SEMARNAT, 2015).

The information generated will contribute to the development in the social character due to the addition of information of different organic alternatives such as oregano oil, using it as a medical treatment, in the environmental character due to the decrease in production of inorganic antiseptics in order to use them to inactivate infections (ISP, 2010).

In addition, there are economic advantages due to the reduction of costs in the treatments, consequent to the low cost of the realization of the essential oil being another factor of economic development the increase in the production activity and obtaining local oregano where in the current region it is potential producer of this same plant ceasing to be a plant without exploitation (Menassé, 2016).

The present project aims to determine the optimal dose of essential oil of oregano (*Lippia* ssp.), from the municipality of Rodeo in the state of Durango, to inactivate the *Candida albicans* fungus causing vaginal infection.

MATERIALS AND METHODS

The present investigation was developed in the facilities of the Technological University of Rodeo of the municipality of Rodeo that is formed by a territorial extension of 1,389.3 km² and is located at a height of 1,340 meters above sea level and its geographic coordinates are 25.1705 °N, 104.5364 °W.

To determine the concentration and time of contact phase suitable for oil (*Lippia* ssp.), to inhibit *Candida albicans* a series of laboratory procedures was carried out in the general microbiology area of the Technological University of Rodeo. First the discs impregnated whith *Lippia spp.* essential oil were prepared, for this filter paper discs were impregnate with essential oil diluted with alcohol 96° at final concentration of 150, 250 and 350 part per million subsequently, blood agar was prepared replicating the methodology proposed by Rodríguez-Díaz *et al*. (2017); Lopez-Colombo *et al.* (2013), mention that it should be taken into account in the dilution of blood in the culture medium, it is necessary to neutralize the blood bactericidal properties and possible antimicrobial treatment received by the patient.

Subsequently, *Candida albicans* was cultured in human blood agar petri dishes using the diffusion disc method, it is a key tool to detect the presence of polymicrobial infections, subsequently 3 discs with the same dose were introduced to 3 Petri dishes and perfectly covered the edges with Parafilm^{TM} for labeling, then they were placed in the incubator calibrated at 37 °C., this process is repeated every 24, 48 and 72 h. At 72 hours, for the disposable of the culture dishes they were inactivated in autoclave at 15 pounds for 15 minutes wrapped in Kraft paper.

RESULTS AND DISCUSSION

The results of the monitoring data in the inhibition zones at three different times are presented in a unifactorial analysis with a Tukey test and a F test for significance performed in the SAS statistical program (Statistical Analysis System), (Stacciarini and Pace, 2017).

Table 1, shows the structure of the statistical analysis that was performed with the monitoring data of the inhibition zones, where it is realized that 108 data were read at each of the 3 levels (24, 48 and 72 hours) in the three different concentrations (150, 250 and 350 parts per million). A Tukey test was carried out, data shown below.

Table 1. Database information in SAS system.
Tabla 1. Información de la base de datos en el sistema SAS.

Procedure	Class	Levels	Values
ANOVA	B	3	150
			250
			350

An F-test of 0.0001 was obtained, which has shown in table 2 the significance of the monitoring of the data with a 95 % confidence interval showing that the levels of the treatment levels are different, discarding the null hypothesis focused on the similarity of the means in each treatment where I throw a mean of 1.667778, among the observed halos (Table 2). Demonstrating that the treatments studied are different by approving an alternative hypothesis.
In Table 3, we can observe the value of F for significance where a value of 0.001 was obtained by eliminating a null hypothesis and affirming an alternative hypothesis, implying that the treatments of the statistical model (150, 250 and 350) are different in a 95 % confidence interval.

Table 2. Tukey test in SAS (Statistical Analysis System).
Tabla 2. Prueba de Tukey en SAS (Sistema de análisis estadístico).

Source	DF	Sume of squares	Scuare of the mean	F-value	Pr > F
Model	2	20.58326667	10.29163333	167.70	<0.0001
Error	105	6.44360000	0.06136762		
Total, corrected	107	27.02686667			
	Rsquare	Coef. Variance	Root MSN	Media	
	0.761585	14.85359	0.247725	1.667778	

Table 3. F test with a confidence interval of 95 %.
Tabla 3. Prueba F con un intervalo de confianza del 95 %.

Process de ANOVA	
Alpha	0.05
Degrees of freedom error	105
Average of square error	0.061368
Critical value of the studentized range	3.36216
Minimum significant difference	0.1388

Based on the value of alpha is the difference between the means of the dosages by grouping them in two and thus obtaining the variabilities between these.
In Table 4 the alpha value of 0.05 is detected in our analysis, based on this the variability between treatments is determined. In this table, a greater significant difference is observed in the 150 ppm treatment, the other concentrations, no favorable response was observed (Figure 1).

It can be seen that at level 150 a minimum reading of 43 mm and a maximum reading of 129 mm was obtained, in the graph shown a mean, average and an equal median. Demonstrating the graph, at the 150 ppm level, a series of data with a standard deviation of 0.50285 and behavior in stable inhibition zones.

Table 4. Tukey Student's Range Test (HSD) for Y.
Tabla 4. Prueba de rango T-Student's de Tukey (HSD) para-Y.

b-Comparisons	Difference between means	Edge of confidence at al 95 % Simultaneus		
250 – 350	0.42000	0.28118	0.55882	***
250 – 150	1.06167	0.92285	1.20048	***
350 – 250	-0.42000	-0.55882	-0.28118	***
350 – 150	0.64167	0.50285	0.78048	***
150 – 250	-1.06167	-1.20048	-0.92285	***
150 – 350	-0.64167	-0.78048	-0.50285	***

Important comparisons of the 0.05 level are indicated by ***

Compared to the mustache graph of the level 150 ppm in the graph of 250 ppm has a mode, average and unequal medians, focusing the average of data an on the data of smaller size and obtaining a minimum reading of 178 mm and a maximum reading of 268 mm, which gives us a more extensive range of data than in the first level.
In the last 350 ppm level, a very deferential mustache plot has been developed since we obtain a larger interval between the minimum and maximum reading

of the data than in the other descriptions, with the minimum level being 113 mm and the maximum level being 211 mm. Unevenly, the mean and median drifted toward the higher data readings in the concentrations.
The diameters of the inhibition zones can be compared in the 48 and 72 hour readings. The first time the fungus was inhibited, it is noticed that contamination begins in the circumferences of the halos. Therefore, 72 hours is ruled out as a possible contact phase time for essential oil of oregano (*Lippia* ssp.).

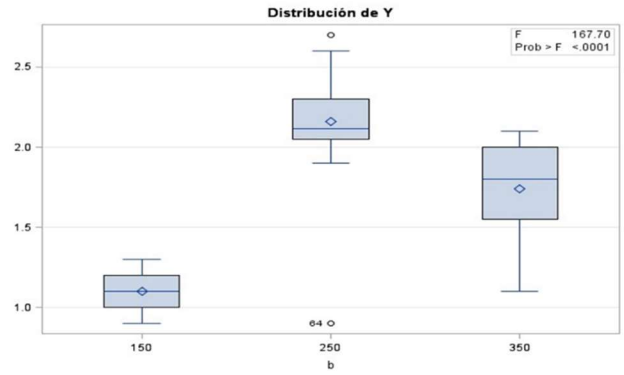

Figure 1. Distribution of the fungal load of the 3 concentrations of oregano oil used.
Figura 1. Distribución de la carga fúngica de las 3 concentraciones utilizadas de aceite de orégano.

It is possible to notice the behavior of the halos in the three different reading times where similar results are observed in the 24 and 48 hours in the concentrations of 15 and 25 %. In the dosage of 150 ppm (15 %) and 250 ppm (25 %), a difference of 10 mm was found in the first reading point at 24 hours, in the second reading point at 48 hours a difference is noticed 4 mm, demonstrating that there is no significant difference in the reading of data in question at 24 and 48 hours of monitoring in the inhibition halos, Hazen (2013), also showed that dimethyl sulfoxide (DMSO) inhibits the growth of *Candida* yeast, concludes that adding DMSO can provide misleading minimum inhibitory concentration (MIC), in the present study the essential oil of oregano (*Lippia* ssp.) was used, which consisted in diluting concentrations of 15 % and 25 % in a time of 24 and 48 hours, by means of contact.
The results of the tests of determination of the capacity of inhibition to the fungus *Candida albicans* by contact phase of essential oil of oregano in its main components (thymol and carvacrol), unlike de Benites (2015), it was observed that the ethanolic extract of *Caesalpinia spinosa* (Tara) had an inhibitory effect *in vitro* against *Candida albicans*, when using the different concentrations (25 %, 50 %, 75 % and 100 %), and this effect increases in relation directly proportional to the concentrations used in the study, giving as MIC 50 %.

This similarity in the reading of the radio of halos can be due to the similarity in the climatic characteristics of the two states and in turn the characteristics of the species of oregano.
In the work of a simple method for reading inhibition of *Escherichia coli* using an experimental work similar to the present study, they focused on demonstrating the results with a series of comparative graphs reading the monitors in mm as a unit of measurement and obtaining up to 50 % inhibition in *Candida albicans* being this a great sensitivity to the antibiotic rifampicin (Bonilla *et al*., 2011). Citing Saleh and Abu-Dieyeh (2021), they used PJ-WS-LE as extract where it showed the best efficacy, its various concentrations showed good inhibition of the four strains tested (*Escherichia coli, Proteus mirabilis, S. aureus and B. subtilis*). All bacteria showed a dose-dependent response against the extract with the largest zone of inhibition with 50 mg / ml of PJ-WS-LE extract.
In an investigation for Lopez-Rivera (2018), it showed that the inhibition halos for *Origanum vulgare* of 30.8 mm and for amikacin 30.0 mm, against *Escherichia coli*. The essential oil of *Origanum vulgare* with amikacin has a difference between both halos of inhibition of 0.8 mm. Finding a slightly higher value in favor of the combination of the oil of *Origanum vulgare* and amikacin, which is not significant compared to the present investigation with the inhibition of *Candida albicans* by the contact phase method (Jasso, 2011). In an investigation developed by Medina-De La Cruz *et al*. (2021), found that the statistical analysis showed that between the control without diluent and the one with DMSO at 1.25 % there is no statistically significant difference, therefore, the diluent does not affect the growth of the cells. An important data is that in all the strains there was a difference between the controls and the concentrations of essential oil of *C. mexicana* tested. Although for the *S. cerevisiae* strain, isolate NY66 and isolate NY62, the second concentration used, no longer shows statistical differences with respect to the previous concentration tested, this information was obtained somewhat similarly, but this is attributed to the fact that oregano oil develops high percentages of thymol and carvacrol, as shown in the present investing.
In a work realized by Acosta *et al*. (2019), made an aqueous extract of *Caesalpinia spinosa* (Tara) the

measurement of the halos, they were: 2 % (3.4 mm), 5 % (3.6 mm), 10 % (4.4 mm), 15 % (4.6 mm), and 20 % (5.2 mm). For the hydro-alcoholic extract of the rhizomes of *Polypodium picnocarpum* C. (Calaguala) the measure of the halos were 2 % (2.6 mm), 5 % (2.0 mm), 10 % (2.4 mm), 15 % (2.8 mm) and 20 % (2.2 mm). For the synergistic activity, the measurement of the inhibition zones were 2 % (3.2 mm), 5 % (3.0 mm), 10 % (4.4 mm), 15 % (3.8 mm) and 20 % (3.2 mm), similar to the present work obtaining a comparison of the mustache graph of the 150 mm level in the 250 mm graph has an unequal fashion, media and median (Cárdenas y Quintana, 2017).

In an investigation for Ismail *et al*. (2012), demonstrated that the crude extract of *Geranium wallichianum*, obtained 40 mm inhibition halo, twice the average of the halos obtained in our study with 50 % *Pelargonium graveolens* oil. It also determined the minimum inhibitory concentration of the crude extract of *Geranium wallichianum*, of 110.8 µm/ml (0.1108 mg/ml). In the present study a similar average of 43 mm was obtained from the halos obtained, but in essential oil of oregano (*Lippia* ssp.), by contact phase in its main components (thymol and carvacrol).

CONCLUSION

The essential oil of the leaf of *Lippia* ssp., from the community of Héroes de México in the municipality of Rodeo, Dgo., Showed important antifungal activity in the blood agar diffusion test against *Candida albicans*. Where he obtained an R squared of 0.761585 a coefficient of variance with 14.85359 and a mean of 1.667778, in the respective milestones.

Due to the results obtained by the F test of significance and Tukey, a difference was found between the three treatments and in turn a lower variance between the dose readings of 150 parts per million, having an average inhibition of fungus of 24 % in its circumference of the inhibition halo.

Comparing the percentages of inhibition of the two possible doses between 150 and 250 ppm, the latter obtained a greater amount of inhibition due to the characteristics of variance between data and amounts of concentrations, it was concluded that it is more advisable to use the concentration of 150 ppm , because there is no significant difference in the results of the means between treatments and especially the behavior of the readings is more stable, obtaining a smaller data reading interval in the treatment of 150 ppm and there is a variance between the results of the minor reads.

In obtaining an optimal time there was no problem since in Table 1, it is clearly observed the increase in the readings in 48 hours and a decrease in the follow-up at 72 hours, obtaining in this hour a visible contamination in the circumferences of the inhibition halos that eradicate the work of oregano essential oil.

Author Contributions

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Conflict of interest

The authors declare that they have no conflict of interest.

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