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Sewer cockroach; Oregano hydrosols; Repellency; Survival

### **Abbreviations**

LSD: Least Significant Difference

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# **Repellency of Oregano** (Lippia graveolens HBK): Against Periplaneta americana (Blattodea: Blattidae) from **Durango, Mexico in Vitro**

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## Abstract

Background: The sewer cockroach is one of the important pests in the health sector due to the microorganisms they carry in their bodies, contaminate food, and mainly affect the respiratory tract. Most chemical insecticides applied to control this pest affect people's health and in addition to the fact that cockroaches have already become resistant to many of these products. An environmentally friendly alternative in pest control is the use of aromatic plants. The repellency of residual oregano hydrosols from three municipalities of Durango was evaluated in this research. The control groups drank drinking water, and the test groups oregano hydrosols. Insect survival was monitored daily for four weeks.

Results: The survival results were successful for the hydrosols of similar chemical composition from Mezquital and Nombre de Dios. These hydrosols additionally produced an insecticidal effect. The analysis of variance of the bioassays showed significant differences for a confidence interval of 95%. The results of the Kaplan-Miers survival analysis showed that oregano hydrosols with higher thymol content were more effective.

Conclusions: Oregano hydrosols (Lippia graveolens HBK) contain mainly thymol, are effective in controlling Periplaneta americana, and could reduce insecticide contamination.

# Introduction

### The sewer cockroach

Periplaneta americana (Linnaeus, 1758) is a pest insect of medical, economic, and veterinary interest. It transmits various diseases caused by bacteria, fungi, parasitic worms, protozoa, and viruses [1-3]. It lives in garbage collectors and dark and humid places such as cisterns, drains, light registers, and in any hole in walls and floors. Chemical insecticides are the most widely used products to control this pest due to their rapid effect, but the pest has become resistant to many of them. In addition, they caused environmental contamination and the elimination of beneficial insects such as worms, pollinators, and others [4]. Currently, alternatives to use plants with insecticidal action in the control of different pests continue to be studied, and many botanical families that have been studied for this purpose. Regarding the insecticidal and acaricidal effect of the genus Lippia, to which oregano (Lippia graveolens) belongs, many reports support this insecticidal activity, and its essential oils and leaf extracts have been evaluated [5,6]. Oregano hydrosols are by-products derived from the extraction of essential oils using steam distillation. They contain many of the same compounds found in their essential oils but in lower concentrations. Several of its biological properties are similar to those produced by essential oils of the same plant [7]. These hydrosols obtained in large volumes are eliminated and thrown away without looking for a potential use based on their chemical composition. This research aimed to evaluate the repellence of oregano hydrosols from three different ecological conditions versus adults and nymphs of Periplaneta americana.

# **Material and Methods**

### Wild oregano collection

Oregano was collected in September 2018 in three locations in the municipalities of El Mezquital, Nombre de Dios, and Lerdo belonging to the state of Durango, Mexico. These sites present different ecological conditions (Table 1). The hydrosols were obtained through steam distillation of the essential oil of oregano. The hydrosols were separated from the essential oil. They were stored in glass containers with a plastic lid and placed in the shade, at room temperature, and in a cool and dry place.

Table 1: Ecological conditions of oregano collection sites [19-21].								
Site	Geographical Co-ordinates	Altitude (m)	Climate	Rainfall (mm)	Vegetation of Type			
El Mezquital	23°29'42.6''N, 104°25'55.1''W	1588	Temperate and sub-humid	120-150	Tropical deciduous forest			
Nombre de Dios	23°48'22.1"N, 104°02'22.4"W	1949	Temperate semi-dry	90-120	Grassland			
Lerdo	25°41'04.3"N, 103°51'01.6"W	1377	Very dry	30-60	Xeric scrub			

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#### **Breeding cockroaches**

In 2020 adult cockroaches and ootheca were collected from the sewers and placed in a 1.0x0.5x0.3 m glass box, with a mesh lid. Egg cartons were placed inside the brood boxes, which were initially moistened every third day, subsequently once or twice a week, depending on the temperature of the breeding chamber. They were provided with water and food made with cornmeal, yeast, honey, powdered sugar, powdered milk, and pork fat.

#### **Chemical analysis**

The hydrosols were analyzed in an Agilent Technologies 7890B gas chromatograph, packed with a  $30mx250 \mu mx0.25 \mu m$  HPS ms column. A temperature ramp was used and the sample was injected in split mode: 50:1. The gas chromatograph was coupled to an Agilent Technologies 5977D mass spectrometer with Scan 30-600 m/z. The identification of compounds was performed by comparison with NIST 14 library spectra [8].

## **Bioassays**

The bioassays with nymphs were carried out during the period from 2020 to 2022, in different stages of the first and third generations of cockroaches. In the second generation, only adult specimens were evaluated (Table 2). Cockroaches' sex was not considered in the experiment. In the bioassay 7L capacity plastic boxes were used, with six 2 mm diameter perforations in the lids. Bioassays were performed in triplicate for each treatment. In each bioassay, a group of control cockroaches (T) was used, and only drinking water was supplied. Three test groups were formed to which oregano hydrosols were supplied (I = Mezquital, II = Nombre de Dios, and III = Lerdo). The test treatments were applied in a volume of 15 mL on 10X15X1 cm sponges. Weekly was

added 8 mL of the respective treatment, according to the survival of the insects. The survival of the insect was measured and assessed daily for four weeks. A completely random analysis of variance was performed between the hydrosols from different sites: Mezquital, Nombre de Dios, and Lerdo, and nymphs' instars: Generation 3 (instar 1, instar 2), Generation 1 (instar 2, instar 3) and Generation 2 (Adult). Survival data were analyzed using the Kaplan-Miers test in the Statistica Ver. 7 software.

<b>Tuble 2:</b> Dioussu's with utual coefficience and nymphs of three generations.	Table 2: Bioassa	ys with adult cockroaches and nymphs of three generation	ons.
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Instar	2	3	1	2	Adults
Generation	1	1	3	3	2
Total number of specimens used with hydrosols (I, II, III)	63	63	45	45	36
Total number of specimens used as control (T)	21	21	15	15	12

## **Results and Discussion**

#### Chemical composition of hydrosols

Common chemical compounds found in all three hydrosols were myrcene, cymene, limonene, eucalyptol, linalool, terpineol, caryophyllene, humulene, thymol, carvacrol, terpinolene, and  $\gamma$ -terpinene. The concentration of thymol in Mezquital and Nombre de Dios hydrosols was high, and this compound has potent insecticidal activity (Table 3). This result could explain the higher repellent and insecticidal effects of Mezquital and Nombre de Dios hydrosols. In general, the concentration of the compounds detected in the Lerdo hydrosol was low, and thymol was not detected. These results suggest that the wild oregano collected near the mine is a different chemotype due to ecological diversity. It could also be the result of previous mining activities [9-11]. Therefore, its insecticidal and repellent actions are not good.

Table 5. Major compounds detected in oregano nytrosols by GG/MG/	Table 3: Major	compounds d	letected in	oregano h	ydrosols b	y GC/MS).
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				Com	pound (% Are	ea under the c	urve)					
Municipality	1	2	3	4	5	6	7	8	9	10	11	12
Mezquital	0.94	8.05	0.25	0.22	0.79	0.91	5.34	3.27	69.77	0.89	0	0
Lerdo	4.57	14.52	3.59	14.19	5.92	6.19	6.41	3.67	0	0	2.49	8.31
Nombre de Dios	2.18	15.67	0.6	1.11	1.15	0.97	5.48	3.29	59.44	0.54	0	0

1: Myrcene; 2: p-Cymene; 3: Limonene; 4: Eucalyptol; 5: Linalool: 6: 4-Terpineol; 7: Caryophyllene; 8: Humulene; 9: Thymol; 10: Carvacrol; 11: Terpinolene; 12:  $\gamma$ -Terpinene

#### **Bioassays**

Initially, after introducing the hydrosols in the test boxes, the cockroaches remained at the opposite end, and later they died gradually, except for those treated with Lerdo's hydrosol. The analysis of variance showed significant differences (p<0.05) for the different ecological conditions, as well as for the different generations and instars of the cockroaches. In the Least Significant Difference (LSD) test of Fisher, the survival variable of the homogeneous groups, showed differences in the instars of the cockroaches. Adult cockroaches, instar-2 generation 1 and instar-1 generation 3. Other groups were those of generation 3 of instar-2 and generation 1 of instar-3 (Figure 1).





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These compounds were not detected in the hydrosol from the municipality of Lerdo, under the operating conditions of the analysis. These results support the studies carried out by other authors on the most active components contained in plants with insecticidal, acaricidal, and repellent activity, such as thymol and carvacrol, and their precursors p-cymene and  $\gamma$ -terpinene. Other active compounds present are trans-cinnamaldehyde and  $\alpha$ -terpineol [12]. The survival analysis of the accumulated Kaplan-Meier proportion for the cockroaches showed that the bioassays carried out with hydrosols from the municipality of Lerdo produced low mortality similar to the control. With the other hydrosols, cockroach mortality was very high, mainly for the hydrosol from the municipality of the Mezquital (Figure 3).



Regarding the cumulative proportion survival of the cockroaches of different instars, the nymphs of generation three died faster than those of the first generation, the same as the adult cockroaches (Figure 4). The cumulative proportion surviving concerning the time, most of the test insects died 17 days into the experiment, especially between the first and second day of experimentation. The effectiveness of insecticidal plants depends on the presence and concentration of their chemical compounds that induce different properties. With the responsible use of those plants, a synergistic or selective insecticidal, repellent, or inhibitory effect can be obtained against some pests, then could help maintain the biodiversity of beneficial entomological species [13,14]. The hydrosols of other essential oils reported in the control of *P. americana* [15] and *Blatella germanánica*, could be evaluated to have more options for control of these pests [16].



Other authors have evaluated the insecticidal effect of *Mentha pulegium* and *Melissa officinalis* hydrosols, with low contents of carvacrol and thymol, against the aphid *Myzus persicae* (Sulzer, 1976), and found lower mortality of the insects, compared to that caused by the hydrosol of *Origanum majorana* contains 78% carvacrol and that produced a mortality of 15% [17]. The Turkish oregano (*Origanum acutidens*) with 87% carvacrol, also had a mortality of 68.3 % and 36.7 % respectively on *Sitophilus granarius* and *Tribolium confusum* [18]. This thymol isomer also has very similar properties, which is why oreganos from both Lippia and Origanum genus, could be excellent candidates for the control of various pests. These results can be

used to develop new strategies to control cockroach populations and have good pest management.

#### Conclusions

The Mezquital and Nombre de Dios hydrosols caused repellency at the beginning of the experiment, followed by death. The nymphs of the third generation were the most susceptible to the treatments of Mezquital and Nombre de Dios hydrosols. The chemical composition of the hydrosols of these plants influenced the repellent action and toxic effect of cockroaches, mainly due to the thymol content in high concentrations in plants that develop in places with higher humidity and warm temperatures, as occurred with the oregano hydrosol from Mezquital, and oregano hydrosol from Nombre de Dios. Evaluation of toxicological studies of oregano distillation residues should guarantee its safe application to control *Periplaneta americana* populations. In this way, the residues from the distillation of essential oils from oregano and probably from other aromatic plants can be used and applied alternately with other insecticides to reduce environmental contamination and the toxic effects that derive from it.

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