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

Martha Celina González-Güereca[1], Isaías Chairez-Hernández[1], Román Alberto López-Muñoz[1], and Baldomero Esquivel-Rodríguez[2]

1. Instituto Politécnico Nacional, CIIDIR-Durango, Mexico

2. Universidad Nacional Autónoma de México, Instituto de Química, Mexico

Abstract: Sewer cockroaches have become an important pest in the health sector due to the microorganisms that it carries over its body. They contaminate food and mainly affect the respiratory tract. Most of the chemical insecticides used to control this pest, affect people's health and the cockroaches have become more resistant. The use of aromatic plants is a friendly alternative to the environment in pest control. In this investigation, the repellent effect of residual extracts (hydrolates) of oregano from three municipalities of Durango were evaluated. Control cockroaches were given water and those in the test groups, oregano hydrosols. The survival of the insects was evaluated daily for four weeks. Survival data obtained were successful for the hydrosols from the municipalities of Mezquital and Nombre de Dios with similar chemical composition. In addition to the repellent effect of the hydrosols, an insecticidal effect was also manifested. The analysis of variance of the bioassays showed significant differences for a confidence interval of 95%. The results of Kaplan-Miers survival analysis, showed oregano hydrosols with higher thymol contents, were more effective. Oregano hydrosols (*Lippia graveolens*) containing mainly thymol, are effective in controlling *Periplaneta americana* and could reduce insecticide contamination.

Key words: sewer cockroach, oregano hydrosols, repellency, survival

1. Introduction

The sewer cockroach: *Periplaneta americana* (Linneus, 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, 2]. It lives in garbage collectors and in dark and humid places such as cisterns, drains, light registers and in any crack in walls and floors. To control of this pest, chemical insecticides are mainly used due to their rapid effect, but the pest has become resistant to many of them. In addition, there is greater environmental contamination and elimination of beneficial insects such as worms, pollinators and others [3]. Currently, alternatives are still being sought to use plants with insecticidal capacity to control different pests and there are many botanic families that have been studied for this purpose. Regarding the insecticidal and acaricidal effect of the genus *Lippia*, to which oregano (*Lippia graveolens*) belongs, there are numerous reports that support this activity with essential oils or extracts from the leaves [4, 5]. Oregano hydrosols are residues derived from the extraction of essential oil by steam stripping, which contain many of the compounds contained in their essential oils, but in lower concentrations. Several of its biological properties are like those produced by essential oils of the same plant [6]. These hydrosols obtained in large

Corresponding author: Martha Celina González-Güereca, Master in Science; research areas/interests: natural products/control of urban pests, varroa, and phytopatogenic fungi of chili peppers. E-mail: celina.ciidirdgo@gmail.com.

volumes are wasted 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 differents ecological conditions versus adults and nymphs of *Periplaneta americana*.

2. Material and Methods

2.1 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 presented different ecological condition (Table 1). The hydrosols were obtained through the process of extracting the essential oil from samples, by steam distillation. The hydrosols were separated from the essential oil and packed in glass containers with a plastic lid and placed in the shade, at room temperature in a cool and dry place.

Table 1 Ecological conditions of oregano collection sites [12-14].

Site	Geographical coordinates	Altitude (m)	Climate	Rainfall (mm)	Vegetation of type
El Mezquital	23º29’42.6’’N, 104º25’55.1’’W	1588	Temperate and sub-humid	120-150	Tropical deciduous forest
Nombre de Dios	23º48’22.1’’N, 104º02’22.4’’W	1949	Temperate semi-dry	90-120	Grassland
Lerdo	25º41’04.3’’N, 103º51’01.6’’W	1377	Very dry	30-60	Xeric scrub

2.2 Breeding Cockroaches

In 2020 adult cockroaches and ootheca were collected from the sewers and placed in 1.0 × 0.5 × 0.3 m glass box, with a mesh-coverd lid. Egg cartons were placed inside the boxes, which were initially moistened every third day, then 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.

2.3 Chemical Analysis

The hydrosols were analyzed in an Agilent Technologies 7890B gas chromatograph, packed with a 30 m × 250 μm × 0.25 μm 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. Compound identification was performed by comparison with NIST 14 library spectra [7].

2.4 Bioassays

The bioassays with nymphs were carried out as the different stages of the second and third generation were obtained during 2020 to 2022. On the first generation, only adult specimens were evaluated (Table 2). Coockroach sex was not considered in the experiment.

Table 2 Bioassays with adult cockroaches and nymphs of three generations.

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

Plastic boxes of 7 L capacity were used to carry out the bioassay. in. Six perforations of 2 mm were made on the lids. The bioassays were performed in triplicate for each treatment. Control cockroaches were used in each group (T = control), which were only provided with drinking wáter. For the other groups, were provided with the oregano hydrosols from the three sites (I = Mezquital, II = Nombre de Dios, III = Lerdo).

The test treatments were applied in a volumes of 15 mL on 10X15X1 cm sponges. Weekly was added 8 mL of the respective treatment, depending on the survival of the insects. The survival of the insect was measured assessed daily during four weeks. A completely random analysis of variance was performed among different conditions 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.

3. Results and Discussion

3.1 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 γ-Terpinene. In the hydrosols of the municipalities of Mezquital and Nombre de Dios, thymol was found in high concentrations, which has strong insecticidal properties (Table 3). This could explain the more effective repellent and insecticidal effect. In general, the compounds present in the Lerdo hydrolate were found in very low concentrations and thymol was not detected; which also suggest that this plant could have a different chemotype due to different ecological conditions [8, 9] and as a result of previous mining.

Table 3 Major compounds detected in hydrosols by GC/MS.

	Compound (% Area under the curve)											
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	0.00	0.00
Lerdo	4.57	14.52	3.59	14.19	5.92	6.19	6.41	3.67	0.00	0.00	2.49	8.31
Nombre de Dios	2.18	15.67	0.60	1.11	1.15	0.97	5.48	3.29	59.44	0.54	0.00	0.00

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

3.2 Bioassays

Initially, after introducing the hydrosols in the test boxes, the cockroaches remained at the opposite end, except for those treated with Lerdo's hydrosol. Later they died gradually.

The anális of variance showed significant differences ($p < 0.05$) for the different ecological conditions, as well as for the different generation and instars of the cockroaches. In LSD test, the survival variable of the homogeneous groups, showed differences in the instars of the cockroaches. Adult cockroaches, generation 1-instar 2 and generation 3-instar 1 were different from generation 3 cockroaches from instar 1 and instar 2 and different from generation 3-instar 2 and from instar 3-generation 1 (Fig. 1). These results showed that under the laboratory conditions evaluated, the youngest instars of the cockroaches and adult, were more susceptible to hydrosols.

The LSD test with the variable of hydrosols applied and their survival, showed very marked differences between the hydrosol from Nombre de Dios and from Mezquital. The same case occurred with those of the control group and the Lerdo hydrosol, which were similar (Fig. 2). These results showed that the chemical composition of the hydrosols affected the degree of mortality of cockroaches, mainly due to the presence of thymol in high concentrations and carvacrol, even in low concentrations. These compounds were not detected in the hydrosol from the municipality of Lerdo, under the operating conditions of the analysis.

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 a low mortality very similar to the control. With the other hydrosols, cockroach mortality was very high, mainly for the

hydrosol from the municipality of the El Mezquital (Fig. 3).

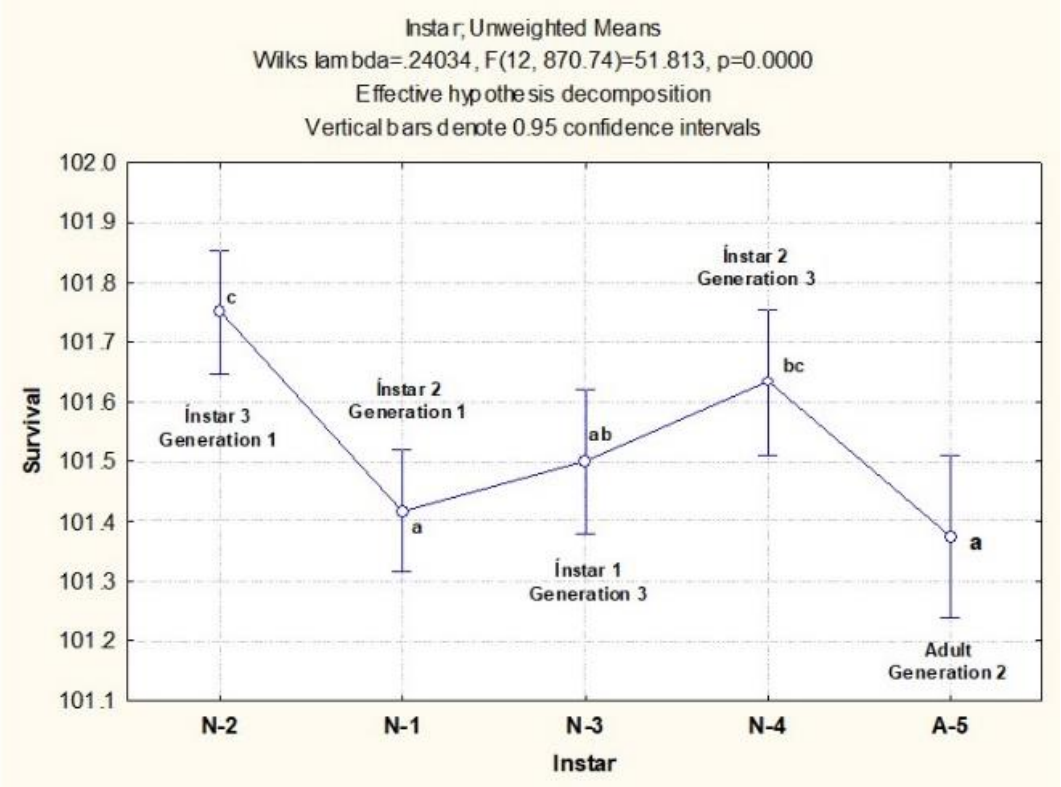

Fig. 1 Fisher LSD test for comparison of means for survival and instars.

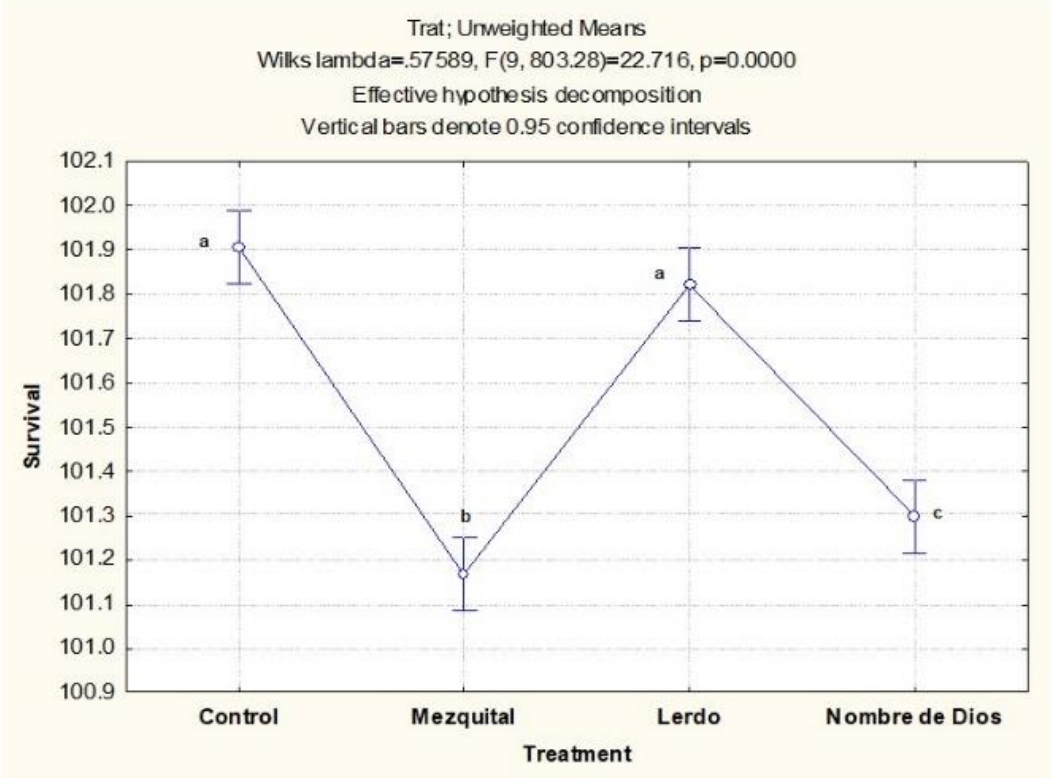

Fig. 2 Fisher LSD test for comparison of means for survival and treatments.

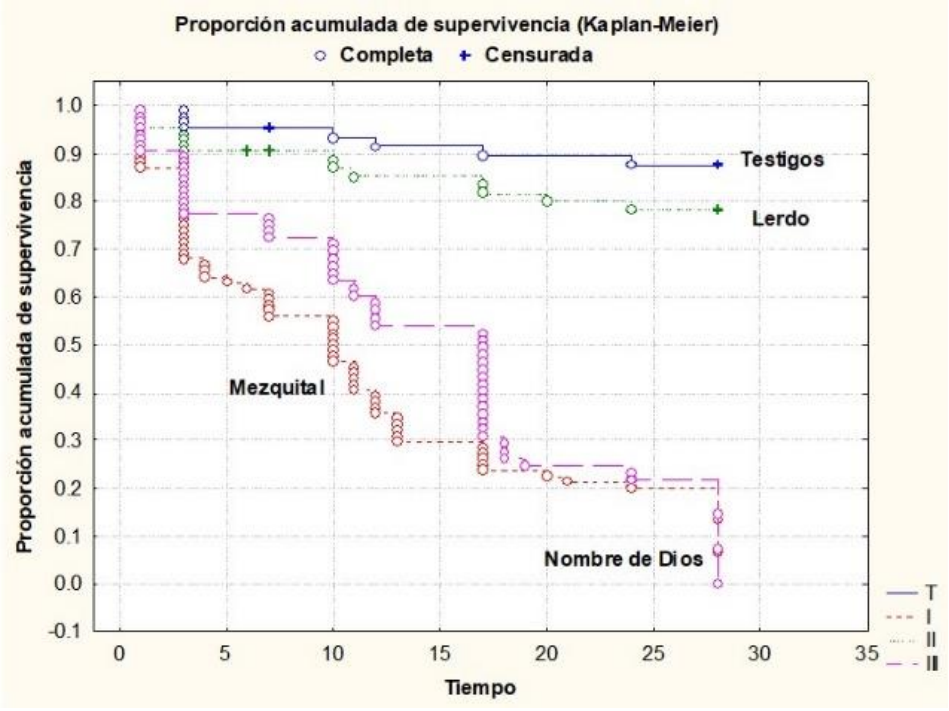

Fig. 3 Cumulative proportion survival (Kaplan-Mier) for cockroaches instars.

Regarding the cumulative proportion survival of the cockroaches of different instars, the nymphs of generation three died faster than those of the first generation and then the adult cockroaches (Fig. 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.

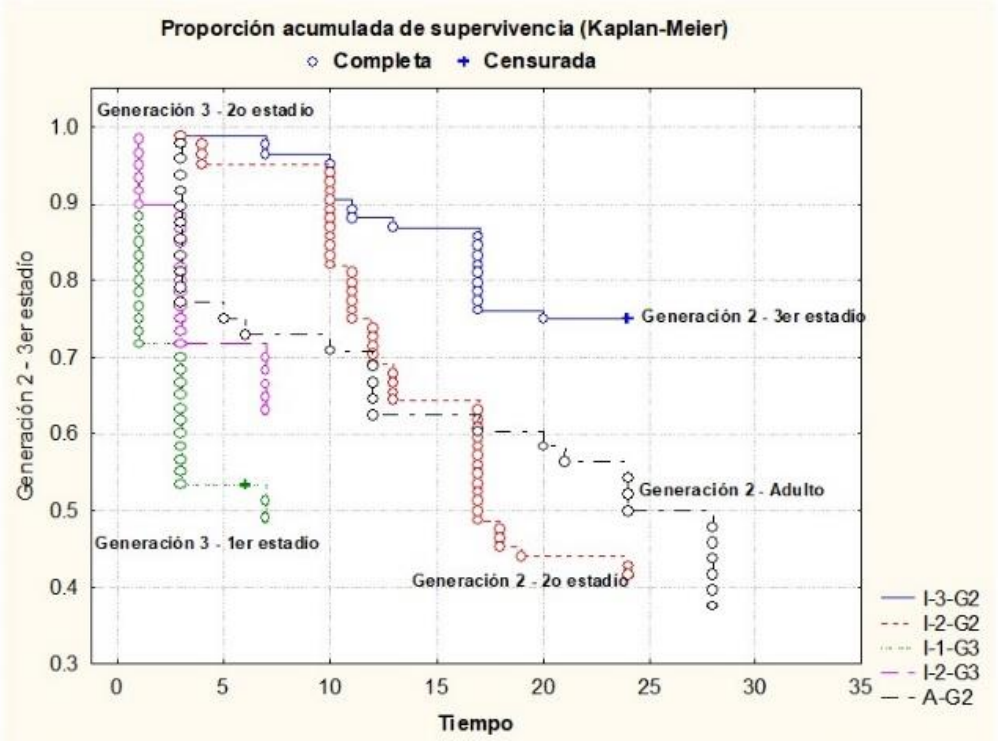

Fig. 4 Cumulative proportion survival (Kaplan-Mier) for hydrosols.

These results can be used to develop strategies to control the populations of and have a good management of the pest [10]. In order to have options in the control of this pest, it is important to also evaluate the hydrosols of other essential oils, reported for the control of *P. americana* [11].

4. Conclusions

The Mezquital and Nombre de Dios hydrosols caused repellency at the beginning of the experiment, followed by death. The nymphs of third generation were the most susceptible to the treatments of Mezquital and Nombre de Dios hydrosols. Mezquital hydrosol has shown an evident mayor insecticide effect over cockroaches, maybe due to the higher concentration content in plants growing at places with higher temperatures and humidity. The chemical composition of these, also influenced the repellent and toxic effect of cockroaches, mainly due to the presence of thymol in high concentrations. It is important to validate the corresponding toxicological studies, so that these residues can be applied in the control of the populations of *Periplaneta americana*. The residues from distillation of oregano essential oils, can be used for their insecticidal action. Its application can be alternated with other insecticides to reduce environmental contamination.

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References

[1] J. Guzmán and A. Vilcinskas, Bacteria associated with cockroaches: health risk or biotechnological opportunity? *Applied Microbiology and Biotechnology* 104 (2020) 10369-10387, cited 2023 16 Feb, available online at: https://doi.org/10.1007/s00253-020-10973-6.pdf.

[2] C. Schapheer, G. Sandoval and C. A. Villagra, Pest cockroaches may overcome environmental restriction due to anthropization, *J. Med. Entom*. 55 (2018) (5) 1357-1364, doi: 10.1093/jme/tjy090.

[3] K. L. Arruda, L. D. Vailes, V. P. Ferriani, A. B. Santos, A. Pomés and M. D. Chapman, Cockroach allergens and asthma, *J. Allergy Clin Immunol*. 107 (Mar. 2001) (3) 419-428, doi: https://doi.org/10.1067/mai.2001.112854.

[4] C. Cicera J., D. O. Duarte-Leite, C. F Alves-Nonato, N. K. Gomes de Carvalho, D. Alves-Ribeiro and J .G. Martins da Costa, Traditional use of the genus *Lippia* sp. and pesticidal potential: A review, *Biocat. Agric. Biotech.* 40, cited 2023 11 Jul., doi: https://doi.org/10.1016/j.bcab.2022.102296.

[5] M. Martinez-Velázquez, R. Rosario-Cruz, G. Castillo-Herrera, J. M. Flores-Fernández, A.H. Álvarez and E. Lugo-Cervantes, Acaricidal effect of essential oils from *Lippia graveolens* (Lamiales: Verbenaceae), *Rosmarinus officinalis* (Lamiales: Lamiaceae), and *Allium sativum* (Liliales: Liliaceae) against *Rhipicephalus* (Boophilus) *microplus* (Acari: Ixodidae), *J. Med. Entom.* 48 (Jul. 2011) (4) 822-827, doi: 10.1603/me10140. PMID: 21845941.

[6] J. N. Govil and S. Bhattacharya, *Recent Progress in Medicinal Plants*: *Essential Oils I*, Studium Press LLC, U.S.A., 2013, pp. 119-140.

[7] E. E. Stashenko and J. R. Martínez, Algunos aspectos prácticos para la identificación de analitos por cromatografía de gases acoplada a espectrometría de masas, *Scientia Chromatographica* 2 (2010) (1) 29-47.

[8] L. M. Calvo-Irabien, V. Parra-Tabla, V. Acosta-Arreola, F. Escalante Erosa, L. Díaz-Vera, G. R. Dzib and L. M. Peña-Rodríguez, Phytochemical diversity of the essential oils of Mexican Oregano (Lippia graveolens Kunth) populations along an Edapho-Climatic Gradient, *Chem. Biodivers.* 11 (2014) 1010-1021, available online at: https://onlinelibrary.wiley.com/doi/pdf/10.1002/cbdv.201300389.

[9] H. Nasirian and A. Salehzadeh, Control of cockroaches (Blattaria) in sewers: A practical approach systematic review, *J. Med. Entomol*. 56 (2019) (1) 181-191, doi: 10.1093/jme/tjy205.

[10] D. R. Oliveira, G. G. Leitâo, H.R. Bizzo, D. Lopes, D. S. Alviano, C. S. Alviano and S. G. Leitâo, Chemical and antimicrobial analysis of essential oils of *Lippia origanoides*, HBK *Food Chemistry* 2007: 236-240.

[11] N. Subooni, M. Shahid and Abduraheem K. Insecticidal activities of some essential oils against American cockroach *Periplaneta americana* (L.), *Int. J. Adv. Res. Biol. Sc.* 8 (2021) (7), doi: http://dx.doi.org/10.22192/ijarbs.2021.08.07.019.

[12] Comisión Nacional del Agua (CONAGUA), Perspectiva de precipitación acumulada mensual (mm) 2018, Cited 2023 5 May, available online at: https://smn.conagua.gob.mx/es/climatologia/pronostico-cl

imatico/precipitacion-form.

[13] Mapa vegetación, Estado de Durango, México. cited 2023 5 May, available online at: https://paratodomexico.com/estados-de-mexico/estado-durango/vegetacion-durango.html.

[14] M S. González-Elizondo, M. González-Elizondo and M. A. Márquez-Linares, *Vegetación y Ecorregiones de Durango*, Instituto Politécnico Nacional, 2007, p. 220.