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SCIENTIFIC NOTES

Larvicidal Effect of Medicinal Plants Against *Aedes aegypti* (L.) (Diptera: Culicidae) in Mexico

FILIBERTO REYES-VILLANUEVA[1], OTHÓN J. GONZALEZ-GAONA[2] & MARIO A. RODRÍGUEZ-PEREZ[3]

[1]*Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Laboratorio de Entomología, Apdo. Postal 105, San Nicolás de los Garza, Nuevo León, 66450, México. E-mail: freyes@fcb.uanl.mx*
[2]*Instituto Tecnológico de Ciudad Victoria, Blvd.. Emilio Portes Gil 1301 Pte. Ciudad Victoria, Tamaulipas, México. E-mail: othonjavier@hotmail.com*
[3]*Centro de Biotecnología Geonómica, Instituto Politécnico Nacional, Blvd. Del Maestro Esquina Elías Piña, Col. Narciso Mendoza, Reynosa, Tamaulipas, México. E-mail: drmarodriguez@hotmail.com*

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Efeito Larvicida de Plantas Medicinais no Controle de *Aedes aegypti* (L.) (Diptera: Culicidae) no México

RESUMO – Alguns fitoquímicos apresentam compostos tóxicos que podem ser explorados no controle de larvas de mosquitos. Sendo assim, o efeito larvicida de extratos aquosos a 0,05% (peso:volume) de 14 plantas medicinais foram avaliadas para o controle de larvas de *Aedes aegypti* (L.) no México. Os bioensaios foram conduzidos com larvas nos estágios iniciais do quarto instar mediante a submersão das larvas nos extratos. Resultados preliminares com as plantas testadas mostraram que a maior mortalidade foi observada com extratos de macerados e de plantas inteiras. As mortalidades médias obtidas para *Solanum nigrescens* Martens & Galeotti, *Operculina pteripes* (G.Don) O'Donell e *Phoradendron tamaulipensis* Trel. foram 55; 17,5 e 5,8% respectivamente. Posteriormente, foram realizadas bioensaios com plantas de *S. nigrescens* coletadas mensalmente no campo para avaliar o efeito sazonal na atividade larvicida com diferentes partes da planta. Somente extratos obtidos das raízes maceradas (mortalidade de 83 a 100%) ou inteiras (mortalidade de 88 a 98%) foram letais às larvas de *A. aegypti*. As mortalidades mensais médias obtidas com extratos de raízes maceradas ou inteiras de *S. nigrescens* foram 91,6 e 93,3% respectivamente.

PALAVRAS-CHAVE – *Aedes aegypti*, plantas medicinais, extrato aquoso, *Solanum nigrescens*.

ABSTRACT – Some phytochemicals comprise toxic compounds that can be exploited in the control of mosquito larvae. Therefore, larvicidal effect of aqueous extracts of 14 medicinal plants at 0.05% (weight: volume) was evaluated against *Aedes aegypti* (L.) in Mexico. Bioassays were conducted with early fourth instars submerged in plant infusions to ingest the potential insecticide compounds. A preliminary bioassay for all plants showed that the highest mortality occurred in extracts from crushed and whole plants. The mean mortality for *Solanum nigrescens* Martens & Galeotti, *Operculina pteripes* (G.Don) O'Donell and *Phoradendron tamaulipensis* Trel. was 55, 17.5 and 5.8% respectively. Then, monthly bioassays with fresh field-collected *S. nigrescens* were conducted to evaluate seasonal variation in larvicidal activity with different plant parts. Only extracts of crushed (83-100% mortality) and entire (88 – 98% mortality) root were lethal to *A. aegypti* larvae. Monthly average mortality was 91.6 and 93.3% for crushed and whole root extracts of *S. nigrescens*. These findings can be exploited to developed ecologically friendly products to be integrated with other control tactics in dengue vector control program in Mexico.

KEYWORDS – *Aedes aegypti*, larvicidal, medicinal plants, aqueous extract, *Solanum nigrescens*.

Toxicity of phytochemicals in mosquitoes was first reported by Campbell *et al*. 1933**.** In a review paper, Sukumar *et al*. (1991) summarized a list of 104 (out of 344 tested) plant species from 49 families that possessed either larvicidal, pupicidal, and/or adulticidal activity on *Aedes aegypti* L. Recent papers from all over the world have documented the toxic effect of plant extracts on *A. aegypti* larvae (Tare *et al*. 2004, De Lima *et al.* 2006, Promsiri *et al.* 2006), but most species of plants from Mexico have not yet been examined for its activity on *A. aegypti.* More than 1,000 species of plants are used for the treatment of human diseases, but less than 20% of all plant species have been investigated (García-Alvarado *et al*. 2001).

Dengue disease, transmitted by *A. aegypti* is a worldwide public health threat. In Mexico, mortality

and morbidity rates are increasing year after year (Martínez *et al.* 2001). *A. aegypti* control program is entirely based on the use of synthetic chemical insecticides and is becoming expensive, due to insecticide resistance problems (Rawlins 1998, Macoris *et al*. 2003). Thus, *A. aegypti* control program would benefit if it were complemented with environmentally safe native plants or plant products that could serve as natural insecticides. The aim of this paper is to report the larvicidal effect of some Mexican medicinal plants with emphasis in *S. nigrescens* on *A. aegypti*, and to determine seasonality in the larvicidal activity.

Fourteen plant species currently used in traditional medicine were collected in Ahome, El Fuerte, and Choix municipalities of the state of Sinaloa, Mexico in June-July 2004 (Table 1). Selection of plants was based on interviews with local traditional healers. Voucher specimens were deposited in the herbarium of the Universidad Autónoma de Nuevo León (UANL) in Monterrey, NL, Mexico. Taxonomic authentication was confirmed by specialists. All plants were dried for two weeks under laboratory conditions at 26 ± 2 ^{o}C, 60 ± 10% RH, and a photoperiod of 14:10-h (L:D).

Table 1. Mortality (%) of *Aedes aegypti* larvae (mean ± SEM) after exposure to aqueous extracts of fourteen Mexican medicinal plants

Species	Family	Aqueous extract type	
		Crushed plant	Entire plant
Solanum nigrescens Martens & Galeotti	Solanaceae	46.7±7.8	63.4±9.5
Operculina pteripes (G. Don O'Donnell)	Convolvulaceae	23.3±4.7	11.7±6.4
Phoradendron tamaulipensis Trel.	Viscaceae	10.0±4.1	1.7±3.8
Dorstenia drakeana L.	Moraceae	5.1±1.2	0
Tephrosia nicaraguensis Oersted	Leguminosae	5.0±2.6	0
Mirabilis jalapa L.	Nyctaginaseae	5.0±3.0	1.6±0.5
Coutarea pterosperma (S. Watson) Standl.	Rubiaceae	1.7±0.9	1.7±0.7
Euphorbia colorata Engelm	Euphorbiaceae	1.7±0.5	1.6±0.8
Ricinus communis L.	Euphorbiaceae	1.6±1.1	1.6±1.2
Montanoa arborescens (D.C.) Sch.-Bip	Compositae	1.6±0.4	1.6±0.8
Amni majus L.	Apiaceae	1.6±1.0	0
Karwinskia humboldtiana (Roem & Schult.) Zucc.	Rhamnaceae	0	1.7±1.3
Lactuca serriola L.	Compositae	0	1.6±0.9
Rhus schiedeana Schlecht	Anacardiaceae	1.6±0.8	0

Plant extracts for each species were prepared as infusions. Some extracts were obtained from crushed plants by using a blender, while others were prepared as infusions from the entire plant or part of it. Regardless of how each plant was processed (crushed, whole plant, or part of the plant) an infusion was prepared as follows: 50 ml of boiling water were mixed with 2.5 g of plant material, cooled down at room temperature (at 26 ± 2^{o}C) for 2 h in a 250 ml flask. From this 5% infusion, a 100-fold dilution was prepared using 1 ml of the extract, diluted with 99 ml of distilled water to have 0.05% infusions. Preliminary bioassays were done with infusions prepared from crushed and complete plants. Once the most toxic plant species were detected, bioassays were focused on *S. nigrescens* due to this exhibited the highest larvicide activity and only in infusions from roots. Therefore, monthly bioassays with *S. nigrescens* root infusions were performed with recently field-collected plants each month during a year to determine a likely seasonal variation in larvicidal activity.

A. aegypti larvae from a local strain mosquito colony with no insecticide history were used in the bioassays. The colony was established in 2002 with specimens originally collected in Monterrey, NL located at Northeastern Mexico, and it is at the Universidad Autonoma de Nuevo Leon. After egg hatching, 400 first instars were poured into an enamel pan containing 1 L distilled water. Three ml of a 5% alfalfa and potbelly pig chow mixture (2:1) were added to each pan, and then at 48 h after hatching. Adults were fed with 5% sucrose solution in cotton pads. Twenty fourth instar *A. aegypti* larvae were placed directly into a plastic cup containing 100 ml of the

plant infusion. During each bioassay, the infusion from the crushed root was used as one treatment, and the infusion from the entire root as another. In each bioassay there were three replicates of the treated larval group, and the control (only distilled water). Larval mortality rates were registered only 24 h after exposure to the plant infusion, and the values corrected using the Abbott's equation (Abbott 1925). To evaluate the seasonal variation in the activity of the plants, larval mortality was assessed monthly during one year. The mortality data for the twelve months were compared using a χ^2 Kruskal-Wallis test (Schlotzhauer & Littel 1987).

Out of fourteen plants assessed against *A. aegypti* larvae, those displaying the highest mortality as infusions from crushed and entire plant were *S. nigrescens* with 47 and 63%, *Operculina pteripes* with 23 and 12%, and *P. tamaulipensis* with 10 and 2%, respectively (Table 1). *S. nigrescens* exhibited the highest larvicidal effect on *A. aegypti* (63%); in addition when different parts of *S. nigrescens* were tested individually on *A. aegypti*, dead larvae (92%) were found only in root infusions, and differences were not seen in mortality rates during a year (χ^2 = 11.00, df = 5, P >0.05) (Table 2). On average, the monthly larval mortality rate was similar (χ^2 = 1.37, df = 1, P = 0.24) in both types of infusions from crushed (93%) and entire roots (92%).

Table 2. Seasonal variability in the mortality (%) of *Aedes aegypti* larvae (mean ± SEM) exposed to aqueous extracts of crushed or entire roots of *Solanum nigrescens*.

Month	Crushed root	Entire root
January	88.3±0.03	96.6±0.03
February	88.3±0.02	96.6±0.02
March	88.3±0.07	98.3±0.02
April	100±0.00	88.3±0.07
May	88.3±0.07	93.3±0.03
June	100±0.00	90.0±0.03
July	93.3±0.04	90.0±0.06
August	90.0±0.03	90.0±0.05
September	100±0.00	94.8±0.03
October	88.3±0.04	95.0±0.00
November	91.6±0.02	90.0±0.05
December	83.3±0.02	96.6±0.02

Solanaceae plants possess alkaloids of medicinal value (Maiti *et al*. 2002) and are abundant in Latin-American tropics. Indeed, in Guatemala, leaf extracts of *Solanum nigrescens* are used for the treatment of dermatophytoses and against bacterial respiratory infections (Cáceres *et al*. 1991a, 1991b), while leaf decoction is effective to cure vaginal candidiasis (Giron *et al*. 1991). Although leaf extract of *S. trilobatum* is an effective oviposition deterrent and skin repellent against *Anopheles stephensi* (Rajkumar & Jebanesan 2005), the leaf extract from *S. nigrescens* has none effect on *A. aegypti* larvae. In summary, the *S. nigrescens* root is toxic to *A. aegypti* larvae and the plant is abundant in Mexico, however nothing is known on the active compound responsible for its larvicidal activity. These compounds could be developed into ecologically friendly products to be integrated with other control tactics such as chemical insecticides (temephos) that are being currently used in dengue vector control program in Mexico.

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