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Short Communication

Different susceptibilities of *Aedes aegypti* and *Aedes albopictus* larvae to plant-derived products

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Abstract

Introduction: *Aedes aegypti* and *Aedes albopictus* are important vectors that transmit arboviruses to human populations. **Methods:** Natural products were obtained and tested against larvae collected from the field in Fortaleza, capital of Ceará state. **Results:** The essential oils of *Syzygium aromaticum* (*Ae. aegypti* LC_{50} = 32.7 ppm and *Ae. albopictus* LC_{50} = 138.1 ppm) and *Croton nepetaefolius* (*Ae. aegypti* LC_{50} = 81.7 ppm and *Ae. albopictus* LC_{50} = 76.1 ppm) showed the most intense larvicidal activity. **Conclusions:** The essential oils and methyl esters showed greater larvicidal activity than did the ethanol extracts.

Keywords: Larvicidal activity. *Aedes*. Botanical products. Mosquito-borne virus.

Mosquitoes are major vectors of several diseases, transmitting pathogens to more than 700 million people annually[1]. Dengue is a mosquito-borne viral infection whose incidence has increased dramatically worldwide in recent decades. The dengue virus is transmitted mainly by *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) and *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae), which are adapted to both tropical and temperate regions[2].

Difficulties related to the production of effective vaccines against chikungunya, Zika, and dengue viruses have led to more emphasis on vector control. For many years, the control of *Ae. aegypti* and *Ae. albopictus* populations has involved the use of synthetic insecticides such as organochlorines, organophosphates, and pyrethroids. However, the indiscriminate and frequent use of these substances has caused insecticide resistance, environmental pollution, destabilization of ecosystems, and toxic hazards to humans and other non-target organisms[3].

In the past decade, research using botanical products as pesticides has increased, since these substances are potentially a rich source of bioactive compounds that are also biodegradable. Extracts and essential oils obtained from plants have been suggested as alternative sources of substances for insect control, because some are selective, biodegrade to nontoxic products, and have few effects on non-target organisms and the environment. These products are thought to contain insecticidal phytochemicals that are predominantly secondary metabolites produced by plants to protect themselves against herbivorous insects[4].

In the present study, essential oils, ethanol extracts, and seed oil methyl esters of nine plants were tested against third and fourth instars of *Ae. aegypti* and *Ae. albopictus* larvae. We analyzed their different susceptibilities and discovered a natural product useful for the control of infections transmitted by these mosquitoes.

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Leaves of *Anadenanthera colubrina* (Vell.) Brenan (Fabaceae), *Momordica charantia* L. (Cucurbitaceae), and *Sterculia striata* St. Hil. et Naud (Malvaceae) were collected in Piauí state, to prepare the ethanol extracts. We purchased oils of sunflower (*Helianthus annuus* L., Asteraceae), linseed (*Linum usitatissimum* L., Linaceae), and rapeseed (*Brassica napus* L., Brassicaceae) from a local drugstore, to obtain lipids and prepare the fatty acid methyl esters. Finally, we extracted essential oils from the leaves of *Cymbopogon citratus* (DC) Stapf (Poaceae) and *Croton nepetaefolius* Baill (Euphorbiaceae), and flower buds of *Syzygium aromaticum* (L.) Merrill & Perry (Myrtaceae). The above products were tested against the third and fourth instars of *Ae. aegypti* and *Ae. albopictus* larvae, to evaluate their potential larvicidal activity. We deposited reference plant specimens in the Graziela Barroso Herbarium and Parnaíba Delta Herbarium—both of which are associated with the Federal University of Piauí—and they were identified by their botanists.

The essential oils were extracted by hydrodistillation in a Clevenger apparatus[5]. Leaves of *M. charantia, A. colubrina*, and *S. striata* were dried in the shade and subjected to cold extraction with ethanol for 72 h, repeating the process three times. To obtain the extracts, the solutions were processed in a rotary evaporator for complete elimination of the solvent. The percentage of extraction was calculated by the ratio between weight of the extract / dry weight of the leaves, multiplied by 100.

The oils of *H. annuus*, *L. usitatissimum*, and *B. napus* were methylated: 500 mg of lipid, hexane (5 mL), and 0.1 M KOH in methanol (5 mL) were mixed and then added to 30 cm test tubes. The tubes were placed in a water bath at 50 °C for 1 h. Hexane (5 mL) and 5% HCl solution (15 mL) were added, and the mixture was transferred to a separating funnel, where the hexane phase, containing the methyl esters, was separated and dried with Na_2SO_4 to produce the corresponding fatty acid methyl esters.

Ae. aegypti and *Ae. albopictus* eggs were collected using ovitraps in the field in March 2016, during activities of the Fortaleza Dengue Control Program. The traps were sent to the Entomology Laboratory at the Federal University of Ceará, where eggs were removed and placed in dishes to hatch. The resulting larvae were maintained under laboratory conditions (25 ±2 °C, 80 ± 10% relative humidity, and 12:12 h light-dark photoperiod) in plastic containers with 1000 mL of distilled water and fed with fish feed, until they reached the third and fourth instars.

The third and fourth instars of *Ae. aegypti* and *Ae. albopictus* larvae (20 specimens of each) were exposed to different concentrations (50, 100, 250, and 500 mg/L) of the ethanol extracts, methyl esters, and essential oils, according to standard methods for testing larvicidal activity, per the World Health Organization[6] with slight modifications. The bioassays were conducted at the Chemistry Laboratory of the Federal Institute of Education, Science and Technology of Ceará (Maracanaú Campus), where the larvae were observed for mortality after 24 h and 48 h. Three replicates were carried out simultaneously for each concentration. During the assays, no food was offered to the larvae.

A generalized linear model with binomial response (logistic regression) was applied, followed by an analysis of variance (ANOVA) and multiple comparison of the means. The level of statistical significance (p-value) was 0.05.

The strongest larvicidal effects against *Ae. aegypti* larvae, among the tested natural products, were shown by the essential oils of *S. aromaticum* and *C. nepetaefolius,* for which the concentrations that caused 50% mortality (LC_{50}) were 32.7 ppm and 81.7 ppm, respectively (**Table 1**). Regarding *Ae. albopictus*, the essential oil of *C. nepetaefolius* had greater larvicidal effect (LC_{50} = 76.1 ppm) than did *S. aromaticum* essential oil (LC_{50} = 138.1 ppm) (**Table 2**).

The fatty acid methyl esters from the three oils showed differences in larvicidal activity. The most active was from

TABLE 1: Larvicidal activity of different concentrations of natural plant products on *Aedes aegypti* larvae and the respective LC_{50} after 24 h.

Plant species	Natural Product	Concentration (ppm)				LC_{50} (ppm)
		50	100	250	500	
A. colubrina	Ethanol extracts	0.8 (0.0 ± 15.5)^{bc}	5.8 (1.8 ± 17.0)^{cd}	6.7 (2.3 ± 18.0)^{b}	5.0 (1.4 ± 16.0)^{c}	> 1000
M. charantia		0.8 (0.0 ± 15.5)^{bc}	1.7 (0.2 ± 13.1)^{c}	4.2 (1.1 ± 15.0)^{b}	40.0 (27.4 ± 54.1)^{b}	542.1
S. striata		1.7 (0.2 ± 13.1)^{b}	5.8 (1.8 ± 17.0)^{cd}	5.0 (1.4 ± 16.0)^{b}	14.2 (6.9 ± 26.9)^{c}	> 1000
H. annuus	Fatty acid methyl esters	9.2 (3.7 ± 21.0)^{bc}	9.2 (3.7 ± 21.0)^{cd}	20.0 (11.1 ± 33.5)^{ab}	43.3 (30.3 ± 57.4)^{b}	554.9
L. usitatissimum		0.0 (0.0 ± 100.0)^{abc}	0.8 (0.0 ± 15.5)^{cd}	5.0 (1.4 ± 16.0)^{b}	14.2 (6.9 ± 26.9)^{c}	753.5
B. napus		11.7 (5.2 ± 24.0)^{bc}	20.8 (11.7 ± 34.4)^{d}	34.2 (22.3 ± 48.4)^{a}	73.3 (59.4 ± 83.8)^{a}	342.8
C. citratus	Essential oils	10.0 (4.2 ± 22.0)^{bc}	12.5 (5.8 ± 25.0)^{cd}	6.7 (2.3 ± 18.0)^{b}	15.0 (7.5 ± 27.9)^{c}	> 1000
C. nepetaefolius		25.8 (15.5 ± 39.8)^{c}	64.2 (50.0 ± 76.3)^{b}	100.0 (0.0 ± 100.0)^{ab}	100.0 (0.0 ± 100.0)^{abc}	81.7
S. aromaticum		63.3 (49.1 ± 75.5)^{a}	88.3 (76.0 ± 94.8)^{a}	100.0 (0.0 ± 100.0)^{ab}	100.0 (0.0 ± 100.0)^{abc}	32.7

Different letters in the same column indicate significant differences ($p < 0.05$). **LC_{50}:** medial lethal concentration; **ppm:** part per million.

TABLE 2: Larvicidal activity of different concentrations of natural plant products on *Aedes albopictus* larvae and respective LC_{50} after 24 h.

Plant species	Natural Product	Concentration (ppm)				LC_{50} (ppm)
		50	100	250	500	
A. colubrina	Ethanol extracts	0.0 (0.0 ± 100.0)[a]	0.0 (0.0 ± 100.0)[ab]	9.2 (3.0 ± 25.1)[c]	0.8 (0.0 ± 27.4)[cd]	> 1000
M. charantia		24.2 (12.4 ± 41.7)[a]	34.2 (20.0 ± 51.8)[ab]	7.5 (2.1 ± 23.2)[c]	45.8 (29.7 ± 62.9)[bd]	795.4
S. striata		17.5 (7.9 ± 34.5)[a]	22.5 (11.3 ± 39.9)[b]	25.0 (13.0 ± 42.6)[c]	13.3 (5.3 ± 29.9)[c]	> 1000
H. annuus	Methyl esters	13.3 (5.3 ± 29.9)[a]	21.7 (10.7 ± 39.0)[b]	32.5 (18.7 ± 50.2)[bc]	61.7 (44.1 ± 76.6)[ab]	> 1000
L. usitatissimum		12.5 (4.8 ± 28.9)[a]	0.0 (0.0 ± 100.0)[ab]	27.5 (14.9 ± 45.2)[c]	47.5 (31.2 ± 64.4)[bd]	502.2
B. napus		18.3 (8.4 ± 35.4)[a]	49.2 (32.6 ± 65.9)[ab]	61.7 (44.1 ± 76.6)[ab]	87.5 (71.1 ± 95.2)[a]	181.4
C. citratus	Essential oils	21.7 (10.7 ± 39.0)[a]	18.3 (8.4 ± 35.4)[b]	17.5 (7.9 ± 34.5)[c]	23.3 (11.8 ± 40.8)[cd]	> 1000
C. nepetaefolius		37.5 (22.7 ± 55.1)[a]	60.0 (42.5 ± 75.2)[a]	100.0 (0.0 ± 100.0)[ac]	100.0 (0.0 ± 100.0)[acd]	76.1
S. aromaticum		18.3 (8.4 ± 35.4)[a]	44.2 (28.3 ± 61.4)[ab]	79.2 (61.9 ± 89.9)[a]	100.0 (0.0 ± 100.0)[acd]	138.1

Different letters in the same column indicate significant differences ($p < 0.05$). **LC_{50}:** medial lethal concentration; **ppm:** part per million.

B. napus (LC_{50} = 342.8 ppm against *Ae. aegypti*, **Table 1**; and LC_{50} = 181.4 ppm against *Ae. albopictus*, **Table 2**), showing medium activity.

Increases in the concentrations of the ethanol extract of *M. charantia,* methyl esters of *H. annuus, L. usitatissimum*, and *B. napus*, and essential oils of *C. nepetaefolius* and *S. aromaticum* increased *Ae. aegypti* larval mortality (**Table 1**). However, only methyl esters of *H. annuus* and *B. napus* and the essential oils of *C. nepetaefolius* and *S. aromaticum* had increasing larvicidal effect against *Ae. albopictus*, with rising concentrations (**Table 2**). However, increased exposure, from 24 h to 48 h, was significant only for *Ae. albopictus* (**Table 3**) (*Ae. aegypti*, p = 0.869).

The damage caused to humans and the environment by the extensive use of synthetic insecticides beginning in the second half of the 20th century to combat the transmission of numerous mosquito-borne pathogens has prompted the need to find new strategies to control these vectors[7]. Thus, integrated mosquito management is an eco-friendly way to control immature forms of the mosquito vectors, based on the use of natural products, such as phytochemicals. Unlike conventional insecticides, which are based on a single active ingredient, plant-derived insecticides typically contain botanical blends of chemical compounds that act synergistically on both behavioral and physiological processes[1].

The LC_{50} values of the essential oils of *S. aromaticum* and *C. nepetaefolius* against *Ae. aegypti* larvae in this study contrast with those obtained by previous studies[8], which showed that their LC_{50} values against *Ae. aegypti* larvae were 92.5 ppm and 66.0 ppm, respectively. In another study[9], the main chemical constituents of *C. nepetaefolius* were methyleugenol and alpha-copaene, with an LC_{50} value of 84 ppm against *Ae. aegypti*, similar to our results. The larvicidal activity of *S. aromaticum* against *Ae. aegypti* was previously reported[10] (LC_{50} = 93.56 ppm), similar to our findings, and the chemical analysis revealed the presence of eugenol (65.99%) and caryophyllene (28.32%). These results may have been influenced by site-specific differences in the tested larval strains and essential oil composition.

Ae. aegypti and *Ae. albopictus* larvae showed different levels of sensitivity to the natural products tested. These differences might be related to factors such as the intraspecific and interspecific differences that naturally occur in these mosquitoes, which are subject to different environmental selection pressures, thus influencing their resistance/susceptibility.

The fatty acid compositions of several edible oils were analyzed by Kamal-Eldin and Andersson[11]. They found that sunflower oil's main constituents were linoleic (56.78%) and oleic acid (33.7%), while linseed oil contained linolenic (57.8%), linoleic (15.7%), and oleic (17.7%) acids; and rapeseed oil contained oleic (60.1%), linoleic (21.4%), and linolenic (11.4%) acids. To investigate these differences, Du et al.[12] compared the fatty acid composition of these oils. Several fatty acid methyl esters were isolated via chromatography-mass spectrometry using *Pharbitis purpurea* seed oil, and these were evaluated for acaricidal activity. Methyl oleate was the most active. Thus, enhanced biocidal activity could explain the higher insecticidal activity of *B. napus*, which contains more oleic acid.

The larvicidal activity of a product is usually improved by increasing its concentration and exposure time. Exploration of plant extracts with significant larvicidal activity should be based on using different plant parts, associated with the extraction of various solvents[13]. In our study, this variability may have contributed to the reduced larvicidal effect of *A. colubrina*, *M. charantia*, and *S. striata*, since only their ethanol extracts were tested.

Generally, plant essential oils are recognized as important natural sources of insecticides and can be composed of complex mixtures of substances at different concentrations. In this study, the essential oils of *C. nepetaefolius* and *S. aromaticum* showed significant larvicidal activity against *Ae. aegypti*, while

TABLE 3: *Aedes albopictus* larval mortality (%) after 24 h and 48 h of exposure to natural plant products.

Plant species	Natural Product	*Ae. albopictus*	
		24h	48h
A. colubrina	Ethanol extracts	0.8 (0.0 ± 16.9)^{d}	4.2 (1.0 ± 15.6)^{e}
M. charantia		10.4 (4.3 ± 23.1)^{d}	45.4 (31.7 ± 59.8)^{cd}
S. striata		0.8 (0.0 ± 16.9)^{d}	38.3 (25.5 ± 53.0)^{d}
H. annuus	Methyl esters	21.3 (11.7 ± 35.4)^{cd}	43.3 (29.9 ± 57.8)^{cd}
L. usitatissimum		14.2 (6.7 ± 27.5)^{d}	29.6 (18.2 ± 44.2)^{d}
B. napus		35.8 (23.4 ± 50.5)^{bc}	72.5 (58.0 ± 83.5)^{ab}
C. citratus	Essential oils	8.8 (3.3 ± 21.1)^{d}	31.7 (19.9 ± 46.3)^{d}
C. nepetaefolius		61.3 (46.6 ± 74.1)^{a}	87.5 (74.5 ± 94.4)^{a}
S. aromaticum		56.7 (42.2 ± 70.1)^{ab}	64.2 (49.5 ± 76.6)^{bc}

Different letters in the same column indicate significant differences ($p < 0.05$). LC_{50}: medial lethal concentration.

Ae. albopictus larvae were very susceptible to the essential oil of *C. nepetaefolius*, as shown by LC_{50} values < 100 ppm.

Methyl esters, essential oils, and other lipophilic compounds are more active larvicides than polar extracts of plants, based on facilitated transport through insect cell walls and cytoplasmic membranes[14]. Despite the low larvicidal effect of the methyl esters of *H. annuus, L. usitatissimum*, and *B. napus* observed in this study, oils with high oleic acid content should be preferably evaluated. Among the essential oils, *S. aromaticum* was the most active and *Ae. aegypti* showed higher susceptibility than *Ae. albopictus* to this oil.

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Conflict of Interest: The authors declare that we have no conflict of interest.

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