# Larvicidal Activity of Essential Oils from Brazilian Plants against *Aedes aegypti* L.

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Aedes aegypti *L. is the major vector of dengue fever, an endemic disease in Brazil. In an effort to find effective and affordable ways to control this mosquito, the larvicidal activities of essential oils from nine plants widely found in the Northeast of Brazil were analyzed by measurement of their* $LC_{50}$. *The essential oils were extracted by steam distillation and their chemical composition determined by GL-chromatography coupled to mass spectroscopy. The essential oils from* Cymbopogon citratus *and* Lippia sidoides*, reported in the literature to have larvicidal properties against* A. aegypti*, were used for activity comparison. The results show that* Ocimum americanum *and* Ocimum gratissimum *have* $LC_{50}$ *of 67 ppm and 60 ppm respectively, compared to 63 ppm for* L. sidoides *and 69 ppm for* C. citratus. *These results suggest a potential utilization of the essential oil of these two* Ocimum *species for the control of* A. aegypti.

Key words: larvicidal activity - mosquito control - *Aedes aegypti* - essential oils - dengue

Dengue fever is endemic over large areas of tropics and subtropics. Outbreaks of dengue have repeatedly occurred in Brazil over the last 10 years. The etiological agent is an arbovirus and the major vector is the *Aedes aegypti* mosquito, which is found in 3600 Brazilian municipalities (Cives 2002). While most patients are asymptomatic, reinfection with different serotypes of dengue viruses may lead to hemorrhagic fever with high mortality. During outbreaks, public health authorities in Brazil have standardized the use of aerolized pyrethroid insecticides that can cause allergies. This measure only partially controls the mosquito population since it eliminates the adult flying insects but does not eliminate the breeding places. In these breeding sites, the larvicide used is usually the organophosphorate Temephos, although very slightly toxic may cause headaches, loss of memory, and irritability (NICC 2003).

Secondary metabolites of plants, many of them produced by the plant for its protection against microorganisms and predator insects are natural candidates for the discovery of new products to combat *A. aegypti*. Several studies have on focused natural products for controlling *Aedes* mosquitoes as insecticides and larvicides, but with varied results (Consoli et al. 1988, Perich et al. 1995, Jayaprakasha et al. 1997, Sathiyamoorthy et al. 1997, Chariandy et al. 1999, Pizarro et al. 1999, Rahuman et al. 2000, Markouk et al. 2000, Ciccia et al. 2000, Tsao et al. 2002). The repellency to *A. aegypti* of essential oils from orange peel (Ezeonu 2001), thyme and clove (Barnard 1999) and components of essential oils such as eugenol, cineole, and citronellal (Hummelbrunner & Isman 2001) was determined in laboratory tests. Studies with *Lippia sidoides* (Carvalho et al. 2003) and *Cymbopogon citratus* (Sukumar et al. 1991) essentials oils suggest that they are promising as larvicides against *A. aegypti*.

In the present study essential oils of nine plants commonly found in Northeastern Brazil were tested against third instar *A. aegypti* larvae in a search for effective and affordable natural products to be used in the control of dengue.

## MATERIALS AND METHODS

*Plant material* - Citrus fruits such as lemon and orange were purchased in local markets and other plants were collected in the Medicinal Plants Garden of the Federal University of Ceará (UFC), Brazil. Taxonomic identification of plants was performed by botanists of the Prisco Bezerra Herbarium, Department of Biology, UFC, where voucher specimens are deposited. Eight plants commonly found in the Northeast of Brazil (*Alpinia zerumbet, Citrus limonia, Citrus sinensis, Syzygium jambolana, Ocimum americanum, Ocimum gratissimum, Hyptis suaveolens*) were evaluated in addition to *C. citratus* and *L. sidoides*, which were used for comparison. The essential oils were extracted by steam distillation in a Clevenger-type apparatus (Craveiro et al. 1976). The oils were extracted from the aerial parts (leaves and branches) of the plants and for the *Citrus* species fruit peels were used.

*Essential oil analysis* - The oils were analyzed using a Hewlett-Packard 5971 GC/MS instrument employing the following conditions: column: Dimethylpolysiloxane DB-1 coated fused silica capillary column (30 m x 0.25 mm); carrier gas: He (1 ml/min); injector temperature: 250°C; detector temperature: 200°C; column temperature: 35–

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180ºC at 4ºC/min then 180–250ºC at 10ºC/min; mass spectra: electron impact 70 eV. The identification of the constituents was performed by computer library search, retention indices and visual interpretation of the mass spectra (Craveiro et al. 1984, Adams 2001). The identified constituents are listed in their order of elution from a non-polar column in Table I.

*Larvicidal bioassay* - Larvae of *A. aegypti* were collected from a mosquito colony maintained at Nuend (Núcleo de Controle de Endemias Transmissíveis por Vetores – Secretaria de Saúde do Estado do Ceará). For the assay, the essential oil of the plants was placed in a 50 ml beaker and DMSO (0.3 ml) was used to solubilize the oil in water (19.7 ml) that contained 50 larvae (third instar). With each experiment, a set of controls using 1% DMSO and untreated sets of larvae in tap water, were also run for comparison. Mortality was recorded after 24 h of exposure during which no nutritional supplement was added.

The experiments were carried out 28 ± 2$^{o}$C. Each test comprised of three replicates with four concentrations (500, 250, 100, 50 ppm). Data were evaluated through regression analysis. From the regression line, the $LC_{50}$ values were read representing the lethal concentration for 50% larval mortality of *A. aegypti*.

## RESULTS AND DISCUSSION

The results from the *A. aegypti* larvicidal assay using nine different plants, common in the Northeast of Brazil, are shown in Table II. The most active essential oils against third instar larvae of *A. aegypti* were those of *O. gratissimun* ($LC_{50}$ 60 ppm), *O. americanum* ($LC_{50}$ 67 ppm), *L. sidoides* ($LC_{50}$ 63 ppm), and *C. citratus* ($LC_{50}$ 69 ppm). Skumar et al. (1991) reported that *C. citratus* causes significant growth inhibition and mortality in later developmental stages of *A. aegypti*. The analysis of the essential oil of this plant from the state of Ceará, showed that its major components are geranial (60.3%) and neral (39.7%). *L. sidoides* essential oil and its main constituent thymol were shown to be very active against *A. aegypti* larvae (Carvalho et al. 2003). Previous studies of *O. americanum* showed that solvent extracts from the whole plant have ovipositional deterrence against *A. aegypti* (Sukumar et al. 1991). Matos (2000) reported that *O. gratissimum* essential oil displays antifungal (*Aspergillus* and *Trichoderma*) and antibacterial (*Staphylococcus*) activities. Both eugenol and *O. gratissimum* oil presented anthelmintic activity against *Haemonchus contortus*, the main nematode of ovines and caprines in Northeastern Brazil (Pessoa et al. 2002). The citrus oils, although they have insecti-

TABLE I

Main constituents of essential oils from Brazilian plants tested for larvicidal activity against *Aedes aegypti*

| Constituents [a] | KI [b] | Plants [c] / Percentage composition | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Az | Cl | Cs | Cc | Hs | Sj | Ls | Oa | Og |
| α-Thujene | 929 | 8 | | | | | | | | |
| α-Pinene | 939 | 3 | 2.2 | | | 2.1 | 1.1 | | | 1 |
| β-Pinene | 974 | 3.1 | 6.6 | | | 10.7 | 5.5 | | | 3 |
| Sabinene | 975 | 9.2 | | | | | | | | |
| β-Myrcene | 991 | 1.4 | 0.9 | | | | 3.9 | 0.9 | | 0.7 |
| α-Terpinene | 1018 | 3.2 | | | | | | | | |
| p-Cymene | 1027 | 12.9 | | | | | | 8.6 | | |
| Limonene | 1029 | 3.1 | 82 | 98 | | 1.9 | 6.4 | | | |
| 1,8-Cineole | 1032 | 17.9 | | | | 44.2 | | 1.3 | | 32.7 |
| Z-Ocimene | 1039 | | | | | | 27.2 | | | 6.2 |
| E-Ocimene | 1048 | | | | | | 12.2 | | | |
| γ-Terpinene | 1062 | 14.5 | | | | | | 1.6 | | |
| 4-Terpineol | 1177 | 17.8 | | | | | | | | |
| α-Terpineol | 1189 | 0.7 | 6.4 | | | | 1.3 | | | 0.6 |
| Neral | 1238 | | | | 39.7 | | | | | |
| Geranial | 1267 | | | | 60.3 | | | | | |
| Thymol | 1272 | | | | | | | 80.8 | | |
| Citronellal | 1284 | | | | | | | | | |
| Z-Methyl-Cinnamate | 1300 | | | | | | | | 8.8 | |
| Eugenol | 1359 | | | | | | | | | 43.7 |
| β-Citronellol | 1365 | | | | | | | | | |
| E-Methyl-Cinnamate | 1379 | | | | | | | | 70.9 | |
| Trans-Caryophyllene | 1421 | 0.8 | | | | 14.5 | 4.7 | 5.1 | 4.6 | 4.1 |
| Trans-Bergamoptene | 1435 | | | | | | | | 6.8 | |
| Germacrene-D | 1472 | | | | | 5.1 | | | | |
| Bicyclogermacrene | 1488 | | | | | 7.7 | | | | |
| β-Selinene | 1491 | | | | | | | | | 4 |
| Spathulenol | 1557 | | | | | 3.7 | | | | |

*a*: the compounds are displayed in order of elution from a non polar column, *b*: retention indices in the chromatographic column, *c*: symbols represent the first letters of botanical names (Az: *Alpinia zerumbet*; Cl: *Citrus limonia;* Cs: *Citrus sinensis*; Cc: *Cymbopogon citratus*; Hs: *Hyptis suaveolens*; Sj: *Syzygium jambolana*; Ls: *Lippia sidoides*; Oa: *Ocimum americanum*; Og: *Ocimum gratissimum*).

TABLE II

Larvicidal activity of essential oils from Brazilian plants against third instar larvae of *Aedes aegypti* after 24 h of exposure

| Botanical name of the plant | Voucher number | Family | $LC_{50}$ (ppm) |
|---|---|---|---|
| *Alpinia zerumbet* (Pers.) Burtt & Smith | 30308 | Zingiberaceae | 313 |
| *Citrus limonia* Osbeck | - | Rutaceae | 519 |
| *Citrus sinensis* Osbeck | - | Rutaceae | 538 |
| *Syzygium jambolana* DC | 29042 | Myrtaceae | 433 |
| *Lippia sidoides* Cham. | 25149 | Verbenaceae | 63 |
| *Ocimum americanum* L. | 17611 | Labiadae | 67 |
| *Ocimum gratissimum* L. | 23929 | Labiadae | 60 |
| *Hyptis suaveolens* Poit | 23934 | Labiadae | 261 |
| *Cymbopogon citratus* Stapf. | 23358 | Gramineae | 69 |

$LC_{50}$: oil concentration that kills 50% of *A. aegypti* larvae.

cidal activities (Ezeonu et al. 2001), and *H. suaveolens*, that is used as mosquito repellent (Palsson & Jaenson 1999), were not effective in the larvicidal test. Supavarn et al. (1974) tested 36 vegetable extracts on *A. aegypti* and found that 11.1% were capable of producing mortality at a concentration of 500 ppm but only 2.8% produced the same effect at a concentration of 100 ppm. In conclusion, the essential oils of *O. americanum* and *O. gratissimun* were shown to be as potent as *L. sidoides* and *C. citratus* in the larvicidal activity against *A. aegypti* and caused 100% mortality at a concentration of 100 ppm. These results are very promising in creating new effective and affordable approaches to the control of *Aedes* mosquito and, thus, of dengue fever.


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