Fatty acid-rich volatile oil from *Syagrus coronata* seeds has larvicidal and oviposition-deterrent activities against *Aedes aegypti*

Leilane M.M. Santos, Jéssica S. Nascimento, Mirela A.G. Santos, Nadja B. Marriel, Patrícia C. Bezerra-Silva, Suyana K.L. Rocha, Alexandre G. Silva, Maria T.S. Correia, Patrícia M.G. Pavia, Gustavo F. Martins, Márcia V. Silva, Thiago H. Napoleão

*Departamento de Bioquímica, Centro de Ciências, Universidade Federal de Pernambuco, Cidade Universitária, Recife 50670-420, Pernambuco, Brazil*

*Departamento de Química Fundamental, Centro de Ciências Exatas da Natureza, Universidade Federal de Pernambuco, Recife 50670-901, Pernambuco, Brazil*

*Núcleo de Bioprospecção da Caatinga, Instituto Nacional do Semiárido, Campina Grande 58429-970, Paraíba, Brazil*

*Departamento de Antibióticos, Centro de Ciências, Universidade Federal de Pernambuco, Cidade Universitária, Recife 50670-420, Pernambuco, Brazil*

**Article Info**

**Article history:**
Received 18 April 2017
Received in revised form 30 May 2017
Accepted 30 May 2017
Available online 31 May 2017

**Keywords:**
Larvicide
Ovicidal assay
Oviposition
Fatty acids
Essential oil

**Abstract**

This work evaluated the potential of a volatile oil extracted from *Syagrus coronata* seeds for the control of *Aedes aegypti*. The oil was extracted by hydrodistillation, characterized by gas chromatography-mass spectrometry (GC-MS), and evaluated for larvicidal and ovicidal activities, as well as for influence on the choice of oviposition site by females. The effects of the oil on swimming activity of larvae were also investigated. The oil extraction showed a yield of 0.41% and GC-MS revealed that 98.42% of the composition corresponded to the following fatty acids: octanoic acid (40.55%), decanoic acid (17.39%) and dodecanoic acid (40.48%). The oil promoted the death of *A. aegypti* larvae, with LC50 of 21.07 ppm, but had no ovicidal action. The octanoic, decanoic and dodecanoic acids showed larvicidal activity with LC50 of 51.78, 24.01 and 19.72 ppm, respectively. The swimming activity of larvae incubated with the oil during 1 and 3 h was significantly (*p* < 0.05) lower than that of control (0.2% Tween 80, v/v) larvae. The *S. coronata* oil and the octanoic acid (both at 50 ppm) showed a deterrent effect on oviposition. In conclusion, the essential oil of *S. coronata* seed was able to promote death of *A. aegypti* larvae and exerted a deterrent effect on pregnant females. The results indicated that the larvicidal activity is due to the action of decanoic and dodecanoic acids while the oviposition deterrent effect is probably linked to the presence of octanoic acid.

© 2017 Elsevier Ltd. All rights reserved.

**1. Introduction**

*Aedes aegypti* is a mosquito belonging to the Culicidae family and known to be the vector of the viruses that cause dengue, chikungunya and Zika virus disease. Dengue is an illness characterized by high fever, severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands or rash [50]. It is among the most important viral illnesses disseminated by arthropods throughout the world and is a major public health concern since the incidence of the more severe forms has increased in the last decades [9,39]. Recent estimates indicate that 3.9 billion of people in 128 countries are at risk of acquiring dengue and 390 million dengue infections occur every year, of which 294 million manifest clinically the symptoms [9,10,50].

Outbreaks of chikungunya (a rheumatic disease that can influence life quality of infected people for weeks, months, or years) have recently emerged in Africa, Americas and Asia, as well as in some European countries [14,51]. Currently, about 60 countries and territories are experiencing outbreaks of Zika (characterized by mild headaches, maculopapular rash, fever, malaise, conjunctivitis, and joint pains). In addition, associations between Zika virus infection and Guillain-Barré syndrome as well as fetal microcephaly have been reported [11,29,30,52–54].

The use of chemical insecticides (such as organophosphates),...
insect growth regulators, and microbial agents for controlling mosquitoes have been the main strategy adopted by public health programs to control the incidence of the diseases mentioned above [5]. However, the emergence of mosquito populations resistant to the conventional insecticides has been recorded in several parts of the world [16,26,28]. In addition, these compounds are able to promote serious damages to the environment and human health [5]. In this context, several studies have been conducted evaluating compounds with potential to be used in control of mosquito-borne diseases, mainly natural products, which are biodegradable and usually less toxic or non-toxic to the environment [48].

The essential (volatile) oils are mixtures of lipophilic compounds, usually with strong odor [3]. They can be stored in special secreting structures, such as secretory ducts and glands trichomes, and are found in all organs and tissues of plants [31]. The physiological importance of essential oils has been linked to pollination, plant-plant interactions, and protection against microorganisms, herbivores and predators [27]. These oils are broadly studied as alternative insecticides against mosquitoes [4,13,20,42,43,45] and strategies for their biotechnological application have been evaluated. A study on the larvicidal activity of a formulation containing Citrus sinensis essential oil showed that a gelling nanostructured system improved the oil solubility in water and then can be used as delivery vehicle [17]. A nanoemulsion containing the Pterodon emarginatus oil, considered non-toxic for mammals, also showed larvicidal activity and it was suggested that the mechanism of action might involve reversible inhibition of acetylcholinesterase [32].

The oviposition-deterrent and repellent activities of essential oils against A. aegypti are also well documented. The presence of an oviposition deterrent is useful to avoid the laying of eggs in potential breeding sites and thus to minimize the spread of the disease in a given area [6,44]. Some essential oils have shown repellent activity equivalent to that of N,N-Diethyl-3-methylbenzamide (DEET), which is the most common active ingredient in insect repellents [7,46]. A repellent cream formulation containing essential oils from camphor, cinnamon, citronella, lemongrass, lime, orange, neem, basil, Vetex, Lantana, eucalyptus, and clove was repellent against A. aegypti, promoting 100% protection until 3 h at field conditions, revealing the synergistic effects between its components [37].

Syagrus coronata (Arecaceae), popularly known as “ouricuri” or “licuri” is a palm typically found in the Caatinga (semiarid region of Brazilian northeast), enduring prolonged drought and flowering and fruiting during a long period of the year [15]. Its uses in culinary (mainly the fruit oil), construction, folk art, fuel, and medicine have been reported but the predominant value is linked to the almond’s (mainly the fruit oil), construction, folk art, fuel, and medicine have been reported but the predominant value is linked to the almond’s usage [38]. The economical and social importance of S. coronata fruits stimulated us to evaluate the presence of volatile oil in the seeds of this plant and its possible biotechnological potential. In this sense, this work reports the characterization and evaluation of a volatile oil from S. coronata seeds for larvicidal and ovicidal activities as well as to effects on oviposition of A. aegypti.  

2. Materials and methods

2.1. Plant collection

Seeds of S. coronata were collected in March 2014 at the Vale do Catimbau National Park (PARNA do Catimbau; 08°30’02.3” S - 37°20’31” W) in Pernambuco, Brazil, with authorization (number 16,806) of the Instituto Chico Mendes de Conservação da Biodiversidade (ICMbio) from Brazilian Ministry of Environment. Voucher specimen (number 90,470) is deposited at the herbarium “Dârdano de Andrade Lima” from the Instituto Agronomico de Pernambuco, Recife, Brazil.

2.2. Volatile oil

The powder of S. coronata seeds was submitted to hydro-distillation in a Clevenger-type apparatus for 4 h. The volatile oil obtained was then dried over anhydrous sulphuric acid and stored in sealed vials protected from the light at ~20 °C. The extraction of the volatile oil from S. coronata seeds showed a yield of 0.41%. For use in the assays, a stock solution was prepared by dissolving 0.01 g of the oil in 100 mL of 0.2% (v/v) Tween 80 in distilled water.

2.3. Chromatography analysis

2.3.1. Quantification of oil components

Gas Chromatography (GC) analyses were performed in order to determine the relative proportions of the components of the oil. These analyses were carried on a Thermo Trace GC Ultra (Thermo Scientific, Milan, Italy) equipped with a flame ionization detector (FID) and a VF-5 fused silica capillary column (ValcoBond 30 m × 0.25 mm i.d.; film thickness 0.25 mm). Nitrogen at a flow rate of 1 L/min and 30 psi inlet pressure was employed as a carrier gas. The oven temperature program was: initially 40 °C, held for 2 min, increased to 230 °C at 4 °C/min, and then held for 5 min. Injector and detector temperatures were set to 250 °C and 280 °C, respectively. The sample (1 μL) was injected splitless. The relative amount of each component was estimated according to the corresponding peak area expressed as a percentage of the total peak areas of the chromatogram. Analyses were carried out in triplicate to provide a standard deviation.

2.3.2. Identification of the compounds

In order to identify the compounds present in the oil, which contained fatty acids, the sample was submitted to an esterification according to the Standard Method ISO 5509:2000 and purification process [22,24,25]. Samples (before and after esterification) were submitted to GC analyses coupled to mass spectrometry (GC-MS). These analyses were carried out using an Agilent 5975C Series GC/MSD (Agilent Technologies, Palo Alto, USA) quadrupole instrument equipped with an Agilent J&W non-polar DB-5 fused silica capillary column (30 m × 0.25 mm i.d.; film thickness 0.25 μm). For each sample (n = 3), 1 μL was injected in split mode (50:1) with the injector temperature set to 250 °C. GC oven temperature was set to 40 °C, held for 2 min, increased to 230 °C at 4 °C/min, and then held for 5 min. Helium (He) carrier gas flow (1 mL/min) was maintained at a constant pressure of 7.0 psi. MS Source and quadrupole temperatures were set to 230 °C and 150 °C, respectively. Mass spectra were taken at 70 eV (in El mode) with a scanning speed of 1.0 scans from m/z 35—350.

The identification of the individual esters was carried out by comparison with previously reported values of retention indices, obtained by co-injection of oil samples and C9—C30 linear hydrocarbons, and calculated according to the equation of Van den Dool and Kratz. Subsequently, the MS data acquired for each component were matched with those stored in the mass spectral library of the GC–MS system (MassFinder 4, NIST08 and Wiley Registry™ 9th Edition) and with other published mass spectral data [1]. All chemicals and solvents used were of Analytical Grade purity or greater.

2.4. Bioassays with A. aegypti

2.4.1. Insects

The insect colony used in the experiments belongs to Rockefeller strain. The rearing room was kept at 25—27 °C and 75—80% humidity, with a 12:12 light—dark photoperiod. A. aegypti eggs...
were hatched in distilled water in plastic bowls at a temperature range of 25–27 °C and cat food (Whiskas®) was offered to larvae. Adults were maintained in cages (30 × 30 × 30 cm) and were fed with 10% glucose solution. Females took blood meal from chicken blood acquired from local farms and offered using an artificial feeder.

2.4.2. Larvicidal activity

When reaching the early fourth stage (L4), the larvae were exposed to the 0.2% Tween 80 solution (control) or to only distilled water. After each period, the larvae (all live) were transferred to a Petri dish (90 × 100 mm) filled with 20 mL of test solution. The swimming activity of the larvae was recorded for 15 min with a charge-coupled device camera and digitally transferred to a computer equipped with video-tracking software (VideoTrack System, Viewpoint LifeSciences, Montreal, Canada). The camera was positioned 30 cm from the arena. The swimming activity level (in pixels) was determined and the bioassays were carried out under light–dark photoperiod. Larvae that were unable to reach the surface solution or did not respond to mechanical stimulus were considered dead [49]. Three independent experiments were performed in duplicate. Larvicidal assays were also performed at these same conditions using the octanoic, decanoic, and dodecanoid acids (Sigma-Aldrich, USA) at concentration ranges 45–60 ppm, 15–30 ppm, and 10–30 ppm, respectively.

2.4.3. Swimming bioassay

In each assay, twenty A. aegypti larvae (L4) were exposed during 1, 3, 5, 7 and 24 h to the S. coronata volatile oil at the LC50/48 h (test), to the 0.2% Tween 80 solution (control) or to only distilled water. After each period, the larvae (all live) were transferred to a Petri dish (90 × 100 mm) filled with 20 mL of test solution. The swimming activity of the larvae was recorded for 15 min with a charge-coupled device camera and digitally transferred to a computer equipped with video-tracking software (VideoTrack System, Viewpoint LifeSciences, Montreal, Canada). The camera was positioned 30 cm from the arena. The swimming activity level (in pixels) was determined and the bioassays were carried out under incandescent light at a temperature of 25 ± 2 °C.

2.4.4. Ovicidal activity

The assay was performed according to Santos et al. [41]. A. aegypti eggs on filter papers, stored for a maximum of 1 month at 25–27 °C, were selected by considering their integrity using a stereomicroscope (Leica M80). The stock solution of the oil was diluted in filtered tap water to provide test solutions in the concentration range of 15–30 ppm. The final volume of each larvicidal assay was 20 mL of test solution or negative control (0.2%, v/v), Tween 80 in distilled water) and contained 25 larvae. Mortality rate (%) was determined after 24 and 48 h of incubation at 25–27 °C and 12:12 light–dark photoperiod. Larvae that were unable to reach the surface solution or did not respond to mechanical stimulus were considered dead [49]. Three independent experiments were performed in duplicate. Larvicidal assays were also performed at these same conditions using the octanoic, decanoic, and dodecanoid acids (Sigma-Aldrich, USA) at concentration ranges 45–60 ppm, 15–30 ppm, and 10–30 ppm, respectively.

2.4.5. Oviposition assay

Oviposition assay was performed according to Santos et al. [41]. A total of 25 A. aegypti gravid females (3 days after blood feeding) were transferred to a cage containing two plastic vessels (diameter 10 cm): one containing 20 mL of 0.2% (v/v) Tween 80 solution in distilled water (control) and the other containing 20 mL of a S. coronata volatile oil solution at 50 ppm (test). The vessels were placed diagonally at opposite corners of the cage. A piece of filter paper was placed in each vessel to provide a support for oviposition. The females were maintained at 27 ± 0.5 °C with 73± 0.4% relative humidity for 14 h in the dark. After this period, eggs deposited in each vessel were manually counted with the aid of a stereomicroscope. Two independent experiments were performed, each with eight replicates. The oviposition response (OR) and the oviposition active index (OAI) were calculated as follows:

\[
OR(\%) = 100 \times \frac{A}{A + B}
\]

\[
OAI = \frac{A - B}{A + B}
\]

where A corresponds to the number of eggs laid in test vessel and B corresponds to the number of eggs laid in test vessel. OAI value higher than +0.3 indicates attractant effect while OAI lower than −0.3 indicates repellent/deterrent effect [23]. Oviposition assay was also performed at these same conditions using the octanoic acid at 50 ppm.

2.5. Statistical analysis

Standard deviations (SD) were calculated using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA) and data were expressed as mean of replicates ± SD. One-way fixed-effects ANOVA (significance at p < 0.05) and Tukey’s test were conducted using the IBM® SPSS® Statistics version 24 (IBM Corp.). The linear regressions and the concentrations required to kill 50%, 90% and 99% of larvae (LC50, LC90, LC99) in 48 h were established by probit analysis with a reliability interval of 95% also using the IBM® SPSS® Statistics software.

3. Results and discussion

GC-MS analyses revealed that the volatile oil from S. coronata seeds was mainly composed by fatty acids. It was identified three compounds, corresponding to 98.42% of the oil (Table 1) being the octanoic acid (40.55%) and the dodecanoid acid (40.48%) the majoritarian components.

Plant-derived insecticides have been evaluated against several species of disease vectors and are considered cost-effective methods for use in integrated pest management, with lower hazard to people and the environment in comparison with synthetic insecticides [36]. In this scenario, we evaluated the hypothesis that the S. coronata volatile oil could be a potential insecticidal agent for use in control of A. aegypti.

Mortality was not observed after 24-h incubation. However, the oil was effective in promoting the death of A. aegypti larvae after 48 h, with a LC50 value of 21.07 ppm. Other informations on the larvicidal activity of the oil are available in Table 2 and no mortality was observed in control. The essential oils from Dendropanax morbifera, Clausena anisata, Croton rhammifoloides, and Eugenia brejoensis were less active against A. aegypti larvae than the S. coronata oil since they showed LC50 of 62.32, 130.19, 89.03, and 214.7 ppm, respectively [12,19,40,42]. The essential oil from Piper corcovadensis leaf was slightly less toxic to the larvae (LC50: 30.52 ppm) while the oils from leaf, stem, and inflorescence of Piper marginatum showed larvicidal activity (LC50 of 23.8, 19.9 and 19.9 ppm, respectively) similar to that determined by us for the S. coronata oil [2,43].

The effects of the octanoic, decanoic and dodecanoid acids on larvae survival were then determined. The results can be seen in Table 2 and reveal that the octanoic acid showed a LC50 value about 2.45 times higher than that determined for the oil while decanoic and dodecanoid acids showed a larvicidal effect statistically similar (p > 0.05) to the oil, which indicates that these two last are responsible for the larvicidal activity. Perumalsamy et al. [33] reported that fatty acids were able to kill A. aegypti larvae. These
authors also reported that the degree of saturation, the side chain length, and the geometric isomerism of fatty acids may influence the toxicity to larvae; in addition, they showed that the acetylcholinesterase was the main target of oleic and palmitic acids while the octopaminergic system was affected by the elaidic, arachidic, and behenic acids.

Since inhibition of acetylcholinesterase activity has been reported as an action mechanism of fatty acids and this may lead to impairment of the motility of larvae, we evaluated whether the *S. coronata* oil at LC50/48h would be able to affect swimming of the *A. aegypti* larvae exposed for 1–24 h (Fig. 1), periods in which larval death does not occur. It was observed that the swimming activity of larvae exposed to the oil was not significantly (p > 0.05) different among the incubation periods but was significantly (p < 0.05) lower than those in water and negative control treatments at 1 h and 3 h of incubation. On the other hand, the swimming activity of larvae incubated with the solvent (Tween) for 1 h was higher (p < 0.05) than that determined for larvae incubated in only water; the motility decreased to levels similar to those of larvae treated with water or the oil since 5-h incubation time. The high motility of larvae in the first hours in presence of Tween may be a reaction to the presence of a foreign substance. Taking this possibility, the results indicate that the *S. coronata* oil interfered with this ability of larvae in reacting to Tween presence. Compounds with this property may be important, for example, in use as an additive together with another insecticide. Alterations in the swimming activity of mosquito juveniles (larvae, pupae) may affect several activities, such as breathing, foraging, refuge seeking and predator evasion [47].

Eggs of *A. aegypti* have also been considered promising targets for mosquito control since, similarly to the larvae, they are also confined in the aquatic environments [35]. Thus, we evaluated the ovicidal activity of the *S. coronata* essential oil. However, the oil did not reduce the hatching rate at any of the concentrations tested, in comparison with the control (Table 3).

Table 1
Identification of constituents of the volatile oil of *Syagrus coronata*.

<table>
<thead>
<tr>
<th>N°</th>
<th>Compounda</th>
<th>Retention indexes</th>
<th>Content (as % of total oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Octanoic acid</td>
<td>1195 1167</td>
<td>40.55 ± 2.41</td>
</tr>
<tr>
<td>2</td>
<td>Decanoic acid</td>
<td>1378 1364</td>
<td>17.39 ± 0.62</td>
</tr>
<tr>
<td>3</td>
<td>Dodecanoic acid</td>
<td>1573 1565</td>
<td>40.48 ± 1.82</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>98.42</td>
</tr>
</tbody>
</table>

a Constituents listed in order of elution on a non-polar DB-5 column.
b Retention indices calculated from retention times in relation to those of a series of C9–C30-alkanes on a 30 m DB-5 capillary column.
c Values taken from Ref. [1].

Table 2
Larvicidal activity of volatile oil from *S. coronata* seeds and octanoic acid against *A. aegypti*.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Oil concentration (ppm)</th>
<th>LC50 [confidence interval]</th>
<th>LC90</th>
<th>LC99</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. coronata</em> oil</td>
<td>21.07 [19.95–22.18] a</td>
<td>32.56 34.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Octanoic acid</td>
<td>51.78 [48.76–54.81] b</td>
<td>64.10 66.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decanoic acid</td>
<td>24.01 [23.31–24.71] a</td>
<td>28.98 29.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dodecanoic acid</td>
<td>19.72 [18.47–20.97] a</td>
<td>31.09 33.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Lethal concentrations required to 50% (LC50), 90% (LC90) and 99% (LC99) of larvae in 48 h were calculated by probit analysis with a reliability interval of 95%. No mortality was detected in negative control (0.2%, v/v, Tween 80 in distilled water). Different letters indicate significant (p < 0.05) difference between the larvicidal effect observed for the treatments.

![Fig. 1. Swimming activity of *Aedes aegypti* larvae incubated during 1, 3, 5, 7 and 24 h in only water, with 0.2% (v/v) Tween 80 in distilled water (control) or with the *Syagrus coronata* volatile oil at the LC50/48h (21.07 ppm). The bars represent the mean ± SD of the pixels detected by the software during 15 min. Different letters indicate significant differences (p < 0.05).](image-url)
The results from oviposition assay demonstrated that the A. aegypti gravid females laid their eggs preferentially in the control vessel (Fig. 2A). The OAI index was −0.35 indicating deterrent effect of the S. coronata oil at 50 ppm. Other essential oils, extracted from different plant parts, have shown oviposition-deterrent effects such as those extracted from C. rhamnifoiloides leaves, Ettlingera elatior flowers, Cananga odorata flowers, Cymbopogon citratus stem, Cymbopogon nardus stem, Eucalyptus citriodora leaves, Ocimum basilicum leaves and Syzygium aromaticum flowers [8, 34, 40].

In the oviposition assay with the octanoic acid, the females also laid their eggs preferentially in the control vessel (Fig. 2B) and the OAI was −0.31, also revealing an oviposition-deterrent effect. It was previously reported that the decanoic acid also possess a deterrent effect [21] while the dodecanoic acid exerted an attractive action, with OAI indexes of +0.37 and +0.54 in assays at 10 and 100 ppm [18]. Indeed [8], reported that compounds constituted by oxygenated hydrocarbon chains are able to elicit responses in the antennae of A. aegypti females. Thus, the oviposition-deterrent effect of the S. coronata oil may be linked to the responses elicited in the sensilla of the antennae by the octanoic acid and the decanoic acid, whose concentrations account for 58% of the oil composition, enough to overlay the attractive action of dodecanoic acid.

4. Conclusion

The essential oil extracted from S. coronata seeds is able to promote death of A. aegypti larvae and to exert oviposition-deterrent effect on gravid females. The oil also interfered with the larval motility pattern that occurred in response to the presence of the foreign substance Tween 80 in the larval motility pattern that occurred in response to the presence of the antennae by the octanoic acid and the decanoic acid, whose concentrations account for 58% of the oil composition, enough to overlay the attractive action of dodecanoic acid.

References
