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Larvicidal Activity of Native Plant Extracts From the Araripe National Forest on *Aedes aegypti*

Francisco R. de Azevedo[1], Glauber C. Maciel[1], Gilberto B. Oliveira e Silva[1], Francisco de O. Mesquita[1] & Antonio C. Leite Alves[2]

[1] Department of Agrarian Sciences and Biodiversity, Federal University of Cariri, Crato, CE, Brazil

[2] Department of Plant Science, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

Correspondence: Francisco R. de Azevedo II, Department of Agrarian Sciences and Biodiversity, Federal University of Cariri, Crato, CEP: 63.133-520, CE, Brazil. Tel: 55-889-9950-4498. E-mail: roberto.azevedo@ufca.edu.br

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Abstract

Aiming to verify the insecticidal potential of 16 native plants from the Araripe National Forest (ANFO) on L3 *Aedes aegypti* larvae in laboratory conditions, were researches performed in controlled conditions of temperature, air relative humidity and photophase, in a type B.O.D. climatized chamber, executed in period from August/2016 to May/2017. The adopted experimental design was completely randomized, represented by ethanolic extracts obtained from 16 native plants, besides the witness without application and with pyriproxyfen as chemical insecticide, conducted with four replicates in each plant extract. The application of the extracts was performed only once, and at 24, 48 and 72 hours, after the infestation, was determined the effects of the extracts on larval mortality. The extracts of *Amargoso*, *oil'tree*, *Lacre*, *Cajui*, *Louro smelling*, *Field Rosemary*, *Murici truthful*, *Janaguba* and *laranjinha* provoke mortality above of 90% to the larvae of *Aedes aegypti* after three days of exposure, in the dose of 50 mL of the extracts. After 24 hours of exposure, the ethanolic extracts from barks and leaves of *Field Rosemary* and of *laranjinha* killed all the larvae. On the other hand, the extracts with higher larvicide potential were evaluated at different doses (*i.e.*, 12.5, 25, and 50 mL/L) in a 9 × 3 factorial scheme, with four replicates. The ethanolic extract of the leaves of *Louro smelling* is the most efficient, because in any dosage it eliminates all the larvae of *Aedes aegypti*, thus demonstrating to be an excellent vegetable larvicide in the control of this vector after three days of exposure, whereas the bark extract of the *oil'tree* is the least effective. The alkaloids and flavonoids are present in the leaves of the *Louro smelling*.

Keywords: vegetables insecticides, biotechnology, dengue

1. Introduction

The Araripe National Forest (ANFO) is located in the Araripe Plateau, South of the Ceará state, being the first National Forest created in Brazil, with an area of 97.019.320 acres, presenting a tabular relief, varying from 760 to 920 meters, yearly average rainfall of 1.000 mm and temperature varying from 15 to 25 ºC, covering part of the municipalities of Crato, Barbalha, Jardim and Santana of Cariri (IBAMA, 2010).

Due to its location and biodiversity of native plants, this area presents a great importance due to the ecological functions, presenting vegetation of the Cerrado, Caatinga and Atlantic Forest biomes, and areas of transition phytophysiognomies between the two extremes (Ceara, 2018).

The *Aedes aegypti* is a species of international interest for its ability in transmitting to humans four important arbovirus diseases: yellow fever, dengue, Zika and Chikungunya. The last one, recently, has been spreading to all continents (Lima & Camara, 2018).

This vector of epidemiological importance in the Americas (Kay et al., 2010), found a favorable climate and propitious conditions to its rapid expansion in disorderly-created cities, with deficient water supply and poor urban

cleansing. Added to these factors are the utilization of disposable recipients, which serve as artificial breeders for the mosquito (Heintze, Garrido, & Kroeger, 2007; Sakthivadivel & Daniel, 2008).

In Brazil, the mosquito is distributed throughout the entire country, with register of the circulation of the four serotypes of dengue virus (DENV), a situation which raised the risk of occurrence of the four severe manifestations of the disease, deaths and lethality (Ballenger-Browning & Elder, 2009).

In spite of the advancements of the control centers and the researches, there is still no exist efficient preventive vaccine against the dengue. In the same manner, no etiological therapy or effective chemoprophylaxis are available. One of the main effective measures in the action against dengue consists in vector control, an action of collective responsibility, which demands the effort of the whole society (Câmara, Theophilo, & Santos, 2007), but several technologies have been developed as alternatives in the control of this vector as selective monitoring of infestation, social measures, dispersion of chemical insecticides, new biological control agents and molecular techniques for population control of mosquitoes, however, the technologies in development demand evaluation of the effectiveness, feasibility and costs for implementation as complementary strategies to the actions already advocated by the National Dengue Control Program. Therefore, the integration of different compatible and effective vector control strategies, considering available technologies and regional characteristics, seems to be a viable method to try to reduce mosquito infestation and the incidence of arboviruses transmitted by them (Zara et al., 2016).

There are several researches with native and introduced plants in Brazil with insecticidal potential of the larvae from *A. aegypti*, such as *Copaifera reticulata* Ducke (Geris et al., 2008); *Magonia pubescens* Saint Hill (Arruda, Cavasin, and Silva (2008), *Jatropha curcas* L., *Pedilanthus tithymaloides* Poit*, Phyllanthus amarus* Schum*, Euphorbia hirta* Linn and *Euphorbia tirucalli* Linn (Rahuman et al., 2008), *Persea americana* Mill (Carvalho et al., 2011), *Annona coriacea* Mart (Dill, Pereira, & Costa, 2012), *Neoregelia compacta* (Mez) and *Aechmea fasciata* (Lindley) (Guimarães et al., 2013), *Piper aduncum* Linn (Oliveira et al., 2013), *Eugenia jambolana* Lam (Sobral-Souza et al., 2013) and *C. reticulata* (Valotto et al., 2014).

Botanical products such as plant extracts and their proven insecticidal effects through scientific research have a wide range of active ingredients in their metabolism. These in turn act synergistically, thus being able to attract or repel insects. Therefore, due to these intrinsic characteristics, they can be used in integrated management systems of this vector, as one of the alternatives directed to the control and monitoring of populations of these insects (Navarro-Silva, Marques, & Duque, 2009).

Generally, the insecticidal activity was attributed to the secondary metabolites of the plants. Indeed, the secondary metabolism is utilized by the plants as protection against the insects, arthropods and other phytophagous microorganisms (Belchior et al., 2012). Therefore, the secondary metabolites are natural candidates for the discovery of new products that might be utilized in insect control.

Substances synthesized in the secondary metabolism of the plants present biological action directly against agents attack or plants resistance induction, due to elicitors characteristics present in the active principles of the plants in the Araripe National Forest. The production of these chemical components has as the function to protect the plants against herbivores, pathogen attack, as well as benefiting them in competition with other vegetables (Silva et al., 2010; Vinale et al., 2014).

Some studies were already performed with extracts of native plants from the ANFO, with medical purposes (Pereira et al., 2014). However, works performed in the Cariri, related to the insecticide potential of these plants in the control of vectors are incipient. Those that exist are work with *Drosophila melanogaster* Linn (Sobral-Souza et al., 2013; Pinho et al., 2014) and with *Trypanossoma cruzi* and *Leishmania brasiliensis* Viannia (Leite et al., 2013). Therefore, the aim of this work was to evaluate the larvicidal potential of the ethanolic extracts of barks and leaves of native plants from the Araripe National Forest, according to Table 1 below, on the *A. aegypti*.

These plant species have been chosen to be studied as candidates for botanical larvicides because they have been widely used by Cariri family farmers and communities around the forest that use them for medicinal purposes to control some diseases.

2. Materials and Methods

2.1 Plant Selection and Extraction Plant Selection

Scientific literature queries and previous visits to the outskirts of the Araripe National Forest (ANFO) were performed in order to elaborate a list of the possible species with insecticidal potential on the larvae of the *A. aegypti* mosquito (Table 1).

Table 1. Species of native plants found in the ANFO and the respective parts utilized in the experiments with ethanolic extracts

Common name	Scientific name	Family	Utilized part
Praiba	*Simarouba amara* Aubl.	Simaroubaceae	Bark
Earth pau	*Qualea parviflora* Mart.	Vochysiaceae	Bark
Candeeiro	*Vanillosmopsis arborea* Baker	Asteraceae	Bark
Alecrim of field	*Baccharis dracunculifolia* DC.	Asteraceae	Bark and Leaf
Oil's tree	*Copaifera langsdorffii* Desf.	Fabaceae	Bark and Leaf
Lacre	*Visenya* sp.	Sterculiaceae	Leaf
Cajui	*Anacardium humile* Saint Hill	Anacardiaceae	Leaf
Louro smelling	*Ocotea* sp.	Lauraceae	Leaf
Faveira	*Dimorphandra gardineriana* Tehl	Leguminosae	Bark
Amargoso	*Andira vermífuga* Mart.	Leguminosae	Leaf
Amescla	*Protium heptaphyllum* March	Burseraceae	Bark
Maniçoba	*Manihot* sp.	Euphorbiaceae	Bark
Visgueiro	*Parkia platycephala* Benth	Mimosaceae	Bark
Janaguba	*Himatanthus drasticus* Mart.	Apocynaceae	Bark and Leaf
Murici truthful	*Byrsonimasericea* DC.	Malpighiaceae	Bark
Larajinha of bush	*Xanthoxylum gardneri* Engl.	Rutaceae	Bark and Leaf

Note. The number of plants and extracts depended of the epoch of occurrence, and, therefore, throughout the ten months of research, were tested several parts of these plants.

Selected plants were collected from the Araripe National Forest (ANFO) on February 24, April 28, June 30, and August 31, 2017 and separated into bark and leaves. Then, the parts of the plants were dried in a forced ventilation hothouse at 40 ºC, and grinded with a domestic blender, obtaining a dry powder. The plant extracts were obtained following the methodology adapted from Silva et al. (2017), through of the addition of 500 g of the powder with 1.500 mL of ethanol solvent. Following the methodology adapted from Carvalho et al. (2011), this mixture remained during 72 hours at ambient temperature and in the absence of light and; afterwards, the suspension was filtered with a cloth strainer mesh and separated, obtaining the crude ethanolic extract, which was immediately utilized in the study.

2.2 Traps System

For the collection of the mosquito eggs, 100 craft traps (oviposition traps) were installed in strategic spots, that is, those with known dengue transmission and presence of vector outbreaks. The oviposition trap was made utilizing a small dark vase for attraction of the female-vector, in which, was introduced about from 300 mL of water obtained from faucet of the municipal supply system, and a wood pallet of 10x3 cm of porous texture for the fixation of the eggs.

The traps remained installed in the residencies per a period of five days, after which, the material was collected and taken at the Agricultural Entomology Laboratory of the FUCA, in the Center of Agricultural and Biodiversity Sciences (CCBS), of the Federal University of Cariri (FUCA), in the county of Crato, for the obtaining of the larvae. Afterwards, the identification of the insect was performed through morphological observations of the larvae and mounting of adults on lamina, based on the identification keys elaborated by Forattini (2002).

2.3 Experimental Design

The larvicide experiments were conducted under controlled conditions, at a mean temperature of 25±1 °C, photophase of 12 hours in a type B.O.D. (Biochemical Oxygen Demand). Climatized chamber, being with the air relative humidity monitored by thermo-hygrometer and maintained at 70±10%, from august/2016 to may/2017, being the collections of eggs and the obtaining of the larvaes realized weekly during the period in which the research was conducted.

3rd instar larvae (L3) were used and were placed in coffe cups with the capacity of 50 mL, and in each replicate were put 10 live larvaes. It was used 50 mL of each crude extract per liter of water, since, in works with essential oils, usually, are utilized 1 to 5% per liter of water. Moreover, the chemical insecticide pyriproxyfen was used as positive control at the recommended dose by the manufacturer (*i.e.*, 0.01 g/L). Conversely, distilled wated was used as negative control.

The extracts were put in 1 L beakers containing distilled water, agitated with glass stick and, afterwards, was added 50 mL of the solution (extract/pyriproxyfen+water) in each little cup. The evaluations were realized at 24, 48 and 72 hours after the exposure of the larvaes, and were considered dead those, which did not react to the mechanical stimulation of a tweezers or a brush. The extracts that caused a mortality equal or superior to 90%, were subjected to a new evaluation in smaller concentrations.

To evaluate the best concentration of the extracts, the completely randomized design (CRD) was adopted, in a 9 × 3 factorial scheme, represented by the nine best extracts obtained and the dosages inferior to 50 mL, doubled, being 12.5; 25 and 50 mL/L of water, conducted with four replicates per each extract and dosage. Were utilized 3rd instar larvae (L3), rather than 4rd instar larvae (L4) because they could become pupae during the conduction of the experiment because it is the last larval stage, in the same coffee cups-little utilized in the first test, and in each replicate 10 live larvae were also exposed. The evaluation was performed three days after the exposure of the larvae, and were considered dead those which did not react to the mechanical stimulation of tweezers or a brush.

2.4 Phytochemical Screening

To perform a preliminary phytochemical study of Louro smelling extract, initially, the dry ethanolic extract of the leaves was obtained through evaporation under reduced pressure. Following that, 200 mg of the dry ethanolic extract were added in test tubes. The phytochemical study for the identification of the secondary metabolites was based in the methodology of Kokate, Purohit, and Gokhale (2008). The phytochemical tests were performed for the identification of alkaloids, phenolics in general, flavonoids, tannins, triterpenes, steroids and saponins.

The analysis was carried out in the Chemistry Laboratory of the Federal Rural University of Pernambuco, Serra Talhada Academic Unit, using 10 grams of the dried and crushed extract. This was placed into an Erlenmeyer flask and suspended with distilled water to the 100 mL mark of the flask. The mixture was stirred for 30 min and then collected by simple filtration with cotton. Into eight test tubes were added 3 mL each of the aqueous extract filtered as follows:

Tube 1: Tannins—3 drops of 2% gelatin solution saturated with NaCl were added and there was no precipitate.

Tube 2: Saponins—the tube was shaken vertically for 30 min and after 5 min the foam persisted.

Tube 3: Triterpenes—Extraction was performed with CHCl 3. The organic phase was separated from the aqueous phase slowly, three drops of sulfuric acid. There was no change in color;

Tube 4: Steroids—Extraction was performed with CHCl 3. The organic phase separated from the aqueous phase was added 1 mL of acetic anhydride and slowly three drops of sulfuric acid. There was no change in color.

Tube 5: Phenols—3 drops of 3% FeCl 3 solution were added. A dark-colored top ring was formed.

Tube 6: Flavonoids—Mg shavings were added and then five drops of HCl slowly. Formation of dark solution.

Tube 7: Alkaloids—three drops of Dragendorff's reagent were added. An orange precipitate was formed.

Tube 8: White (reference).

2.5 Statistical Analysis

The results were subjected to analysis of variance by the F test, and the means were compared by the Tukey's test ($p < 0.05$). For the processing of the data, was utilized the free version of the SISVAR 5.6 software, Build 86-DEX-UFL Alivre (Ferreira, 2011).

3. Results and Discussion

Out of the 16 evaluated plant species of the Araripe National Forest over the *A. aegypti*, nine caused larval mortality equal or superior to 90% (Table 2), and in the search for better alternatives in the control of Dengue, the utilization of these 9 plants is a viable option. This alternative has been highly researched in the last decades, mainly due to its lower impact to human health and to the environment, besides representing a method of easy obtaining. Medicinal plants are utilized throughout the world for many years, with wide utilization in agriculture as botanical insecticides (Bueno & Carvalho, 2010; Carvalho et al., 2011).

After 24 hours of exposure, the leaf extract of the *Louro smelling* and the bark extracts of the *alecrim* and *laranjinha* caused 100% larval mortality, not statically differing from the leaf extracts of the *amargoso* or the bark extracts of the *Oil's tree* and the *janaguba*. Geris et al. (2008) tested the *Oil's tree* oil against larvae of *A. aegypti,* which demonstrated an excellent response, reaching an average of 90% larval mortality in the first day of treatment.

Similarly, Oliveira et al. (2013) verified that the concentration of 1.000 ppm of the *Piper aduncum* L. extract, caused 100% mortality in the larvae of this vector at 72 h. Valoto et al. (2014) also verified that the 3-*β*-acetoxylabdan-8(17)-13-dien-15-oic acid, in the dose of 9 ppm, extracted from the medicinal plant *Copaifera reticulata* Ducke, a species of the same genus of the *Oil's tree* found in the ANFO, caused the death of larvae by midgut cell destruction, through the cytoplasmic vacuolization, cell and nuclear hypertrophy, brush border degeneration, apical vesicle formation with releasing of cytoplasmatic content, epithelium stratification and folding of the peritrophic matrix; however, in the present research, no were did or performed histological studies.

Table 2. Average number of dead larvae±SE and mortality efficiency in percentage of 3rd instar *Aedes aegypti* larvae subjected to different ethanolic extracts of native plants from the Araripe National Forest in the dose of 50 mL/L, and pyriproxyfen in the dosage of 0.01 g/L, at three exposure times

Treatments	Exposure time			Efficiency
	24 h	48 h	72 h	
Distilled water	0.3[1]±0.3 e	0.3±0.3 ef	0.5±0.5 a	0
Amargoso (Leaf)	6.8±0.6 ab	3.3±0.6 abcdef	0±0 a	100
Praiba (Bark)	1±0.6 de	6.5±1.2 ab	1.5±0.9 a	88.9
Earth pau (Bark)	0±0 e	0.3±0.3 ef	1.3±1.3 a	5.6
Candeiro (Bark)	0±0 e	5.5±1.7 abcd	0.5±0.3 a	55.6
Oil's tree (Leaf)	1.3±0.8 de	8±0.7 a	0±0 a	91.7
Lacre (Leaf)	3.8±0.6 bc	6±0.8 abc	0.3±0.3 a	100
Cajuí (Leaf)	0.3±0.3 e	7.3±1 ab	1.8±0.6 a	91.7
Louro smelling (Leaf)	10±0 a	0±0 f	0±0 a	100
Oil's tree (Bark)	5.8±1.3 ab	0.5±0.3 ef	0.3±0.3 a	61.11
Faveira (Bark)	2.8±1 bcde	2.8±1 bcdef	1±0.7 a	61.11
Amescla (Bark)	4±0.4 bc	2.3±0.5 bcdef	0.5±0.5 a	63.9
Maniçoba (Bark)	4.3±1.2 bc	1.3±0.5 def	0±0 a	50
Alecrim (*)	10±0 a	0±0 f	0±0 a	100
Visgueiro (Bark)	2.8±1.1 bcde	4.3±1.6 abcde	0.8±0.5 a	75
Janaguba (Leaf)	4.5±1 abc	3.8±1.5 abcdef	0.3 ±0.3 a	83.3
Murici truthful (Bark)	3.3±1.7 bcde	5±1.4 abcd	1.3±0.6 a	94.4
Janaguba (Bark)	6.5±1.2 ab	1.8±0.9 cdef	1.3±0.6 a	94.4
Laranjinha (*)	10±0 a	0±0 f	0±0 a	100
Pyriproxyfen	1.5±0.29 cde	2.0±0.58 cdef	1.50±0.29 a	41.2
C.V. (%)	**19.09**	**23.03**	**26.79**	**-**

Note. [1]Means followed by the same letter in the columns are not significantly different by Tukey's test at 5% probability*(Bark and Leaf). SE = Standard Error.

Most of the tested ethanolic extracts of native plants from the Araripe National Forest presented larvicidal activity against the *A. aegypti* (Table 2), considering the total efficiency of the tree exposure periods, varying from 50 to 100% mortality, except for the bark extract of the *Earth pau*, which caused only 5.6% mortality, whereas the pyriproxyfen growth regulator reached 41.2%.

The pyriproxyfen is not a product, which directly kills the mosquito larvae, but it interrupts the normal development process of the insect in the 4rth larval instar for the beginning of the pupal stage, leading to pupal mortality and preventing the emergence of adult mosquitoes. The pupal and larval stages of 4th instar are the most susceptible, but, in the present research, 3rd instar larvae were utilized. Although it is yet possible to verify larvae after the application of the SumiLarv®, these usually die in the pupa/adult stage, thus preventing the appearing of adult mosquitoes, what was observed in the present research.

Resende and Gama (2016), when applying the pyriproxyfen in water tanks (45 liters), glass flasks (5 liters) and plastic buckets (20 liters), in the control of this vector, observed that the persistence was of 45 days and 90 days for the final concentration of 0.01 and 0.05 ppm of pyriproxyfen, respectively, and that the pupal mortality was significantly higher than the larval and adult mortality for all tested recipients and concentrations.

One of the least effective extracts in this exposure time was that obtained from the leaves of *cajuí*, with only 0.3% mortality, not statistically differing from the distilled water, but showed high larvicidal activity at 48 hours. Porto et al. (2008) also did not verify a toxic effect of the ethanolic fraction of the leaves of this species over this mosquito vector.

At 48 hours of exposure, there was higher mortality when the larvae were subjected to the bark extracts of the *Praiba* and to the leaf extracts of the *Oil's tree*, *lacre* and *cajuí*. In this same exposure time, Barreto et al. (2006), observed that the crude ethanolic extract of the fruit husk of the soapberry (*Sapindus saponaria* L.) causes total or partial destruction of epithelial cells, high vacuolization, hypersecretion of the epithelial cells and paving of the midgut epithelium in the larvae of this vector.

When utilizing the ethanolic extract of the same plant species, Arruda, Cavasin, and Silva (2008), observed that the morphological alterations started after four hours of treatment, and included the loss of mitochondrial crests and the thickening of the peritrophic matrix. With the increase in the exposure time, new ultra-structural alterations were evidenced, highlighting the complete destruction of mitochondria, cytoplasmic vacuolization, loss, reduction or disruption of the microvilli and nuclear alterations.

At 72 hours, all extracts were not statistically different within each other and the witnesses, demonstrating that in the third day of exposure. Therefore, the highest larval mortalities occurred at 24 and 48 hours of exposure, being more efficient at 24 hours for some of the evaluated plants.

Guirado and Bicudo (2009), when utilizing coffee grounds, which contain caffeine, in the control of this insect, reported that this vegetable by-product, in the dose of 1.000 ppm, causes 100% mortality after 24 to 48 hours of exposure, presenting persistence in the environment for 7 months. Bansal et al. (2012) observed that at 24 and 48 hours, it was necessary to apply 63.2 and 128.10 mg of the extract of fresh leaves of *algarroba* (*Prosopis juliflora*) respectively, in order to cause 90% mortality.

According to Guimarães et al. (2013), the highest efficiency of the crude extracts of ethyl acetate of *Neoregelia compacta* (Mez) and *Aechmea fasciata* (Lindley) over the L3 instar of *A. aegypti* is obtained with the concentration of de 200 μg mL^{-1}, eliminating the larvae in less than a day, and consequently preventing its growth.

Beserra et al. (2014), observing positive results obtained with the larvicidal use of leaf and bark extracts of *Jatropha curcas* over *A. aegypti*, noticed that the action of the insecticide was significant over the 3rd instar larvae of this disease-vector insect.

It is noted, therefore, that the most efficient ethanolic extracts were obtained from the leaves of *amargoso*, *lacre*, and *Louro smelling*, as well as the bark and leaf extracts of *alecrim of field* and *laranjinha*, which caused 100% mortality after three cumulative days of exposure. In a concentration of 100 ppm of the extract of *Annona coriacea* Mart., Dill, Pereira, and Costa (2012) observed a residual effect of 100% larval mortality during a period of 15 days

Of the nine plant extracts, analyzed in differents dosages varying from 12.50 to 50 mL/L of ethanolic extracts extracted from different native plants (Table 3), only the leaf extract from the louro smelling, when compared with the remaining extracts, it obtained 100% of mortality. Already, the leaf extracts of *alecrim of field*, *cajuí* and *larajinha* were the least effective when applied in concentrations smaller than 50 mL/L.

Table 3. Average number of dead larvae of *Aedes aegypti* subjected to nine ethanolic extracts of native plants from the Araripe National Forest, in three dosage (mL/L), after three days of exposure

Dose	A*	OT*	LS*	JA*	LA*	LAJ*	MT*	CA*	AF*
12.5	7.0ABb	5.7BCb	10Aa	3.0CDb	6.7ABa	0.2Ec	2.2Eb	1.15Eb	0.0Eb
25.0	9.5Aab	9.50Aa	10Aa	4.0Bb	6.8ABa	3.2BCab	2.2BCb	2.0BCb	0.2Cab
50.0	9.5Aab	10.0Aa	10Aa	10Aa	9.5Aa	5.7Ba	9.2Aa	6.75ABa	7.2Aba
C.V.%	24.93								

Note. A*= *Amargoso*; OT* = *Oil's tree* (leaves); LS*= *Louro smelling*; JA*= *Janaguba*; LA*= *Lacre*; LAJ*= *Laranjinha*; MT*= *Murici truthful*; CA*= *Cajui*; AF*= *Alecrim of field*; Means followed by the same uppercase letter in the column and lowercase in the line do not differ within each other by Tukey's test at 5% probability.

The presented results are in accordance with the studies of Ndione et al. (2007), and Dua et al. (2009), when both evaluated the toxic effects of the oil extract of *Azadirachta indica* A. Juss, under several doses, against the larvae

of this vector. These authors verified a mortality of approximately of 96 and 100% of the larval population only in the first day of exposure, reaching total mortality of the population after seven days of exposure. Nevertheless, from the lowest concentration (2 mg/L), a mortality under 50% of the population was obtained.

The ethanolic extracts of *A. coriacea* and *A. mucosa* (Jacq.) presented 100% of mortality in 0.1 mg/mL. *A. crassiflora* Mart. presented mortality superior to 90% in 1.0 mg/mL, in the methanolic crude extract, hexane and dichloromethane extracts and in the hexane fraction. The hydroalcoholic, ethyl acetate and chloroform fractions did not present insecticidal activity. In the species *A. dioica* Saint Hill and *Cardiopetalum calophyllum*, with crude extract, the mortality was inferior to 50%. Therefore, *A. crassiflora, A. coriaceae and A. mucosa*, in methanol and hexane, are promising in the future development of biocides for the control of the dengue vector (Costa et al., 2013).

The data of this work are in agreement with the results presented by Silva et al. (2014), where these authors evidenced that the plant extracts demonstrated potentially promising with regard to the larval control of *A. aegypti*.

The phytochemical tests with the Dragendorff, Mayer's and Wagner's reactives were positive, pointing to the presence of alkaloids in the ethanolic extract of the louro smelling (Table 4). This result is in accordance with the literature, inasmuch as the aporphine alkaloids were identified in the species *Ocotea* sp. (Zanin & Lordello, 2007). The presence of phenolic compounds in *Ocotea* sp. extracts was highlighted due to the capacity antioxidant. Thus, in the present research it is believed that the secondary metabolites that caused larvals mortality of *A. aegypti* were the alkaloids and the flavonoids, for being present in high concentrations in the ethanolic extract.

The test with gelatin's solution saturated with sodium chloride for the identification of the tannins was positive, and the test of flavonoids through of the Shinoda reaction was also positive. The test of the triterpenes class was positive, through the Salkowski reaction, but the test of the steroids was negative, through the Libermann-Burchard reaction and the foam test for saponins indenticatin was negative.

The extract of *Louro smelling* was chosen for phytochemical screening because it was the only one of the extracts tested that killed all *A. aegypti* larvae at all doses applied (Table 3).

Table 4. Phytochemical screening of the ethanolic extract of the leaves of *Louro smelling* (*Ocotea* sp.). Crato-CE, 2019

Secondary metabolites	Method	Result*	Observation
Alkaloids	Dragendorff	+++	Brown precipitate
	Wagner	++	Brown precipitate
	Mayer	+	Beige precipitate
Phenolic	$FeCl_3$	++	Gray precipitate
Tannins	Gelatin	++	White precipitate
Flavonoids	Shinoda	+++	Red precipitate
Triterpenes	Salkowski	+	Red intense phase
Steroids	Libermann-Burchard	-	No significant change
Saponins	Foam	-	No formation of foam

Note. *(-) absence; (+) low concentration; (++) average concentration; (+++) high concentration. Observation: The phytochemical testes were compared with the white (transparent green solution).

4. Conclusions

The Araripe National Forest presents a great potential with bioinsecticide plants of the tested plants, such as: the *Amargoso*, *Oil's tree*, *Lacre*, *Cajuí*, *Louro smelling*, *Alecrim of field*, *Murici truthful*, *Janaguba* and *laranjinha* cause mortality above 90% to the larvae of *Aedes aegypti* after three days of exposure in the dosage of 50 mL of the extracts.

After 24 hours of exposure, the ethanolic extracts from barks and leaves of the *alecrim of field* and the *laranjinha* kill all the larvae of *Aedes aegypti* in the dosage of 50 mL.

The ethanolic extract of the leaves from *Louro smelling* is the most efficient, because, in any dosage, it eliminates all the larvae of *Aedes aegypti*, thus demonstrating to be an excellent vegetable larvicide in the control of this vector after three days of exposure.

The alkaloids and flavonoids are present in high concentrations in the leaves of the *Louro smelling*.

The bark extract of the *Earth pau* is the least effective in the control of *Aedes aegypti* even after three days of exposure.

References

Arruda, W., Cavasin, G. M., & Silva, I. G. (2008). Estudo ultraestrutura do efeito da toxicidade do extrato da *Magonia pubescens* (st. hil.) no mesêntero de larvas de *Aedes aegypti* (L.) (Diptera: Culicidae). *Revista de Patologia Tropical, 37*(3), 255-267. https://portais.ufg.br/up/63/o/2008_37_3_255_267.pdf

Ballenger-Browning, K., & Elder, J. P. (2009). Multi-modal *Aedes aegypti* mosquito reduction interventions and dengue fever prevention. *Tropical Medicine and International Health, 14*(12), 1542-1551. https://doi.org/10.1111/j.1365-3156.2009.02396.x

Bansal, S. K., Singh, K. V., Sharma, S., & Sherwani, M. R. K. (2012). Laboratory observations on the larvicidal efficacy of three plant species against mosquito vectors of malaria, Dengue/Dengue Hemorrhagic Fever (DF/DHF) and lymphatic filariasis in the semi-arid desert. *Journal of Environmental Biology, 33*(1), 617-621.

Barreto, C. F., Cavasin, G. M., Silva, H. H. G., & Silva, I. G. (2006). Estudo das alterações morfo-histológicas em larvas de *Aedes aegypti* (Diptera, Culicidae) submetidas ao extrato bruto etanólico de *Sapindus saponária* Lin (Sapindaceae). *Revista de Patologia Tropical, 35*(1), 37-57.

Beserra, F. P., Aguiar, R. W. S., Carvalho, E. E. N., Borges, J. C. M., & Vale, B. N. (2014). *Jatropha curcas* L. (Euphorbiáceae) como novo bioinseticida: análise fitoquímica preliminar e atividade larvicida contra *Aedes aegypti* (Diptera: culicidae). *Revista Amazônia Science & Health, 2*(3), 17-25.

Belchior, C., Del-Claro, K., & Oliveira, P. S. (2012). Seasonal patterns in the foraging ecology of the harvester ant *Pogonomyrmex naegelli* (Formicidae, Myrmicidae) in a Neotropical savanna: Daily rhythms, shirt in granivory, and homerage. *Arthropod-Plant Interact, 6*(4), 571-582. https://doi.org/10.1007/s11829-012-9208-1

Bueno, V. S., & Andrade, C. F. S. (2010). Avaliação Preliminar de Óleos Essencias de Plantas como Repelentes para *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae). *Revista Brasileira de Plantas Medicinais, 12*(2), 215-219. https://doi.org/10.1590/S1516-05722010000200014

Câmara, F. P., Theophilo, R. L. G., & Santos, G. T. (2007). Estudo retrospectivo (histórico) da dengue no Brasil: características regionais e dinâmicas. *Revista da Sociedade Brasileira de Medicina Tropical, 40*(2), 192-196. https://doi.org/10.1590/S0037-86822007000200009

Carvalho, G. H. F., Silva, H. H. G., Cunha, L. C., & Silva, I. G. (2011). Atividade inseticida do extrato bruto etanólico de *Persea americana* (Lauraceae) sobre larvas e pupas de *Aedes aegypti* (Diptera, Culicidae). *Revista de patologia tropical, 40*(4), 348-361.

Ceará. (2018). *Perfil básico municipal do Crato-2018*. Retrieved from http://www.ipece.ce.gov.br/publicacoes/perfil_basico/pbm-2018/Crato.pdf

Costa, M. S., Pereira, M. J. B., Oliveira, S. S., Sousa-Junior, P. T., Dall'Oglio, E. L., & Lves, T. C. (2013). Anonáceas provocam mortalidade em larvas de *Aedes aegypti* (Diptera: Culicidae). *Revista Brasileira de Biociência, 11*(2), 184-190.

Dill, E. M., Pereira, M. J. B., & Costa, M. S. (2012). Efeito residual do extrato de *Annona coriacea* sobre *Aedes aegypti*. *Arquivos do Instituto Biológico, 79*(4), 595-601. https://doi.org/10.1590/S1808-16572012000400017

Dua, V. K., Pandey, A. C., Raghavendra, K., Gupta, A., Sharma, T., & Dash, A. P. (2009). Larvicidal Activity of Neem Oil (*Azadirachta indica*) Formulation against Mosquitoes. *Malaria Journal, 8*(1), 1-6. https://doi.org/10.1186/1475-2875-8-124

Ferreira, D. F. S. (2011). A computer statistical analysis system. *Ciência e Agrotecnologia, 35*, 1039-1042. https://doi.org/10.1590/S1413-70542011000600001

Forattini, O. P. (2002). *Culicidologia médica: identificação, biologia e epidemiologia* (2nd ed., p. 864). São Paulo, EDUSP

Geris, R., Silva, I. G., Silva, H. H., Barison, A., Rodrigues-Filho, E., & Ferreira A. G. (2008). Diterpenoids from *Copaifera reticulata* Ducke with larvicidal activity against *Aedes aegypti* (L.) (Diptera, Culicidae). *Revista do Instituto de Medicina Tropical, 50*(1), 25-28.

Guimarães, M. G. A., Martins, K. S., Carvalho, M. A., Kersten, V. A., Vieira, R. R., & Maleck, M. Ação dos extratos de *Neoregelia compacta* (Mez) L.B. Smith e *Aechmea fasciata* (Lindley) Baker sobre as formas imaturas de *Aedes* (Stegomyia) *aegypti*. *Revista Fitos, 8*(2), 73-160.

Guirado, M. M., & Bicudo, H. M. C. B. (2009). Alguns aspectos do controle populacional e da resistência a inseticidas em *Aedes aegypti* (Diptera, Culicidae). *Bepa: Epidemiológica Paulista, 6*(64), 5-14.

Heintze, C., Garrido, M. V., & Kroeger. A. (2007). What do community-based dengue control programs achieve? A systematic review of published evaluations. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 101*(4), 317-325. https://doi.org/10.1016/j.trstmh.2006.08.007

IBAMA. (2010). *Áreas de Proteção Ambiental.* Retrieved from http://www.ibama.gov.br

Kay, B. H., Tuyet-Hanh, T. T., Le, N. H., Quy, T. M., Nam, V. S., Hang, P. V., … Ryan, P. A. (2010). Sustainability and cost of a community-based strategy against *Aedes aegypti* in Northern and Central Vietnam. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 82*(5), 822-830. https://doi.org/10.4269/ajtmh.2010.09-0503

Kokate, C. K., Purohit, A. P., & Gokhale, S. B. (2008). *Pharmacognosy* (42th ed., p. 635). Nirali Prakashan: Pune.

Lima, V. H. F., & Camara, T. N. L. (2018). Natural vertical transmission of dengue virus in *Aedes aegypti* and *Aedes albopictus*: A systematic review. *Parasites & Vectors, 11*(77), 2-8.

Leite, N. F., Sobral-Souza, C. E., Albuquerque, R. S., Brito, D. I. V., Lavor, A. K. L. S., Alencar, L. B. B., ... Coutinho, H. D. M. (2013). Atividade antiparasitária in vitro e citotóxica de cariofileno e eugenol contra *Trypanossoma cruzi* e *Leishmania brasiliensis*. *Revista Cubana de Plantas Medicinales, 18*(4), 522-528.

Navarro-Silva, M. A., Marques, F. A., & Duque, J. E. L. (2009). Review of semiochemicals that mediate the oviposition of mosquitoes: A possible sustainable tool for the control and monitoring of Culicidae. *Revista Brasileira de Entomologia, 53*(1), 1-6. https://doi.org/10.1590/S0085-56262009000100002

Ndione, R. D., Faye, O., Ndlaye, M., Dieye, A., & Afautou, J. M. (2007). Toxic effects of neem products (*Azadirachta indica* A. Juss) on *Aedes aegypti* Linnaeus 1762. *African Journal of Biotechnology, 6*(24), 2846-2854. https://doi.org/10.5897/AJB2007.000-2454

Oliveira, G. L., Cardoso, S. K., Lara Júnior, C. R., Vieira, T. M., Guimarâes, E. F., Figueiredo, L. S., ... Kaplan, M. A. C. (2013). Chemical study and larvicidal activity against *Aedes aegypti* of essential oil of *Piper aduncum* L. (Piperaceae). *Anais da Academia Brasileira de Ciências, 85*(4), 1227-1234. https://doi.org/10.1590/0001-3765201391011

Pereira, P. S., Barros, L. M., Brito, A. M., Duarte, A. E., & Maia, A. J. (2014). Uso da *Myracroduon urundeuva* (lentisco) por los agricultores en el tratamiento de enfermedades. *Revista Cubana de Plantas Medicinales, 19*(1), 51-60.

Pinho, A. I., Wallau, G. L., Nunes, M. E. M., Leite, N. F., Tintinho, S. R., Cruz, L. C., ... Franco, J. L. (2014). Fumigant Activity of the *Psidium guajava* var. Pomifera (Myrtaceae) Essential Oil in *Drosophila melanogaster* by Means of Oxidative Stress. *Oxidative Medicine and Cellular Longevity, 1*, 1-8. https://doi.org/10.1155/2014/696785

Porto, K. R. A., Roel, A. R., Silva, M. M., Coelho, R. M., Scheleder, E. J. D., & Jeller, A. H. (2008). Atividade larvicida do óleo de *Anacardium humile* Saint Hill sobre *Aedes aegypti* (Linnaeus, 1762) (Diptera, Culicidae). *Revista da Sociedade Brasileira de Medicina Tropical, 41*(6), 586-589. https://doi.org/10.1590/S0037-86822008000600008

Rahuman, A. A., Gopalakrishnan, G., Venkatesan, P., & Geetha, K. (2008). Larvicidal activity of some Euphorbiaceae plant extracts against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitology Research, 102*(5), 867-873. https://doi.org/10.1007/s00436-007-0839-6

Sakthivadivel, M., & Daniel, T. (2008). Evaluation of certain insecticidal plants for the control of vector mosquitoes *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*. *Applied Entomology and Zoology, 43*(1), 57-63. https://doi.org/10.1303/aez.2008.57

Silva, M. L. C., Costa, R. S., Santana, A. S., & Koblitz, M. G. B. (2010). Compostos fenólicos, carotenóides e atividade antioxidante em produtos vegetais. *Semina: Ciências Agrárias, 31*(3), 669-682. https://doi.org/10.5433/1679-0359.2010v31n3p669

Silva, S. L. C., Gualberto, S. A., Carvalho, K. S., & Fries, D. D. (2014). Avaliação da atividade larvicida de extratos obtidos do caule de *Croton linearifolius* Mull. Arg. (Euphorbiaceae) sobre larvas de *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae). *Revista Biotemas, 27*(2), 79-85. https://doi.org/10.5007/2175-7925.2014v27n2p79

Silva, T. I., Leite, A. C. A., Azevedo, F. R., Marco, C. A., Santos, H. R., & Alves, W. S. (2017). Efeito larvicida de óleos essenciais de plantas medicinais sobre larvas de *Aedes aegypti* L. (Diptera: Culicidae). *Revista verde de Agroecologia e Desenvolvimento Sustentável, 12*(2), 256-260. https://doi.org/10.18378/rvads.v12i2.4672

Sobral-Souza, C. E., Leite, N. F., Cunha, F. A. B., Pinho, A. I., Albuquerque, R. S., Carneiro, J. N. P.,... Coutinho, H. D. M. (2013). Cytoprotective effect against mercury chloride and bioinsecticidal activity of *Eugenia jambolana* Lam. *Arabic Journal Chemistry, 7*(1), 165-170. https://doi.org/10.1016/j.arabjc.2013.10.003

Tafurt-García, G., Jiménez-Vidal, L. F., & Calvo-Salamanca, A. M. (2015). Capacidade antioxidante e conteúdo de fenóis totais de *Hyptis* spp., *P. heptaphyllum*, *T. panamensis*, *T. rhoifolia*, e *Ocotea* sp. *Revista Colombiana de Química, 44*(2), 28-33. https://doi.org/10.15446/rev.colomb.quim.v44n2.55217

Valotto, C. F. B., Silva, H. H. G., Cavasim, G., & Geris, R. (2014). Atividade larvicida do ácido 3-β-acetoxylabdan-8(17)-13-dien-15-óico isolado da planta medicinal *Copaifera reticulata* (Leguminosae) sobre *Aedes aegypti* (Diptera, Culicidae) evidenciada pelas alterações morfohistológicas. *Revista de Patologia Tropical, 43*(3), 375-384.

Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Woo, S. L., Nigro, M., Marra, R., ... Lorito, M. (2014). Trichoderma Secondary Metabolites Active on Plants and Fungal Pathogens. *The Open Mycology Journal, 8*(1), 127-139. https://doi.org/10.2174/1874437001408010127

Zanin, S. M. W., & Lordello A. L. L. (2007). Alcalóides aporfinóides do gênero Ocotea (Lauraceae). *Química Nova, 30*(1), 92-98. https://doi.org/10.1590/S0100-40422007000100020

Zara, A. L. S. A., Santos, S.M., Fernandes-Oliveira, E. S., Carvalho, R. G., & Coelho, G. E. (2016). Estratégias de controle do *Aedes aegypti*: Uma revisão. *Epidemiologia e Serviços de Saude, 25*(2), 391-404.