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Extracts of Potential Plants in the Control of the *Aedes aegypti* Population

Extratos de Plantas Potenciais no Controle da População de *Aedes aegypti*

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Abstract

The dengue fever is a febrile illness transmitted by mosquitoes *Aedes aegypti* to humans. The control of dengue dissemination is a hard challenge since the human population is presenting a considerably increase. Brazilian plants are known to control mosquitoes larvae due to their insecticides properties. This work had the objective to evaluate the success of plants extracts on the *Aedes aegypti* population. It was hypothesized that the studied plant extracts influence the population size of *Aedes aegypti*. It was hoped that at least one of these extracts is 100% efficient on the mosquito larvae combat, thus contributing to Brazilian Health Programs for the local Dengue eradication. Five plants extracts were studied against *Aedes aegypti* larvae populations under laboratory conditions. The plants were macerated and 4 concentrations for each plant were obtained using distillate water. Each concentration of each extract was tested on 10 Aedes larvae with 3 repetitions, for the experiment and positive and negative control on larvae were tested, using alcohol and mineral water, respectively. The count of dead larvae was daily done. The Ricinus communis L. extract was the best extract against Aedes *aegypti*, killing 100% of their larvae, followed by *Mimosa Tenuiflora* and *Ipoema eriocalyx* that achieved DL50 at a concentration of 20%. These results suggest the use of the *Ricinus communis L.* extract on the control of *A. aegypti* population in order to extinct the Dengue fever and to help the government healthy programs with this disease.

Keywords: Mosquitoes larvae. Dengue Fever. Medicinal Plants.

Resumo

A Dengue é uma doença transmitida por mosquitos Aedes aegypti para humanos. Seu controle é uma tarefa árdua, uma vez que a população humana tem apresentado crescimento considerável. Entretanto, plantas brasileiras são indicadas para controle de larvas deste mosquito em função de suas propriedades inseticidas. Este trabalho objetivou avaliar o sucesso de extratos de plantas no controle da população de Aedes aegypti. Foi proposta a hipótese de que os extratos das plantas estudados influenciam no tamanho da população de Aedes aegypti. Esperou-se que pelo menos um destes extratos fosse 100% eficiente no combate às larvas de mosquito, de forma a contribuir para Programas de saúde que visam erradicação da Dengue. Cinco extratos de plantas foram estudados no controle do Aedes aegypti sob condições laboratoriais. As plantas foram maceradas e quatro concentrações foram obtidas de cada uma, utilizando água destilada. Cada concentração foi testada em dez larvas de Aedes com três repetições no experimento e se testou um controle positivo e negativo nas larvas, usando álcool e água destilada, respectivamente. A contagem de larvas mortas foi feita diariamente. O extrato da Ricinus communis L. foi o de maior sucesso no controle de Aedes aegypti, matando 100% das larvas, seguido da Mimosa Tenuiflora e Ipoema eriocalyx, que atingiram DL50 em uma concentração de 20%. O uso de extrato de Ricinus communis L é, portanto, a ferramenta mais indicada para o controle de A. aegypti, auxiliando potencialmente na erradicação da Dengue, e dando diretrizes para programas governamentais de saúde dos países com esta doença.

Palavras-chave: *Larvas de Mosquitos. Dengue. Plantas Medicinais.*

1 Introduction

The dengue fever is a febrile illness transmitted by the mosquitoes *A. aegypti, A. albopictus* e *A. polynesiensis* (WENGLER, 1991). The man is the main host of this illness. Its incubation period could vary from three to 15 days. After that, the virus might spread over all the body and it could be isolated from the human blood and its tissue (GUBLER, 1998). The infected individual could have a variety of symptoms, such as light fever (classic mode of the illness) to a crash syndrome and hemorrhagic fever (MONATH, 1994).

The vectors present high biological adaptation and genetic variability and they occur mainly at urban areas. The mosquitoes are holometabolous, with the egg phase followed by 4- aquatic larvae stages, pupae and adults (REY, 2011; MARCONDES, 2011). After the blood sucking, the mosquito female lay from 100 to 300 eggs, each laying time and it could do two to eight laying eggs, depending of the species. The eggs rupture occur from two to four days by average temperature of 26 °C. After 10 to 20 days, the larvae become pupae and these ones become adults from one to three days (TRIPLEHORN; JONNSON, 2015; MARCONDES, 2011; RUPPERT; FOX; BARNES, 2005; NEVES, 2016).

The control of dengue dissemination is a hard challenge since the human population are presenting a considerably

increase. According to Tauil (2006) before starting a kind of control activity, government should take into consideration the education conditions of the population and the absence of public policies of the cities. Almost 70% of the notified dengue cases are at municipalities with more than 50.000 individuals (TAUIL, 2006; MACIEL-DE-FREITAS et al., 2007), increasing the opportunities for the mosquitoes development and difficulting its control.

Vectors populations can be suppressed over large areas rapidly by the use of chemical substances, such as space sprays released from low-flying aircraft (WHO, 2009). The DDT was the most used insecticide of the 40 decade, but in 1946 flies from Italy and other insects presented high resistance to it (WONDJI et al., 2008).

The chemical insecticides were intensively used in Africa to combat Malaria disease (MABASO et al., 2004), however mosquitoes started to present resistance to them (BROOKE, 2001). In addition, Chemical elements leave residues on environment (CHMIELEWSKY; BEREJKA, 2008). Trying to solve this problem, the authorities started to test alternative methods, such as Gama radiation to sterilize male mosquitoes (CHMIELEWSKY; BEREJKA, 2008), physical or mechanic control with laying traps and the Biological Control (PAULA et al., 2011) with the use of plants extracts (MARTINEZ, 2002).

According to Roel (2001) the use of plant extracts to control mosquitoes is preferable once insects present more difficult in establish resistance to plants in comparison to chemical elements. Moreover, plants are renewable resources, rapidly degraded and present low economical cost.

Silva et al. (2015) analyzed the plant extract *Ziziphus joazeiro*, *Magonia pubescens* and *Jatropha curcas* on the control of fruits flies, showing that *Ziziphus joazeiro and Magonia pubescens* were the most efficient on larvae mortality (E%= 100%).

Other Brazilian studies showed the efficiency of the *Magonia pubescens* on the control of *Aedes aegypti* in all its phases of development (SILVA et al., 2004). The caffeine kills 100% of mosquitoes larvae at 1,0 mg/ml between 24 and 48 hours of the product exposition (LARANJA ET AL., 2006; GUIRADO; BICUDO, 2007; GUIRADO, 2004). Fernandes et al. (2001) showed that the pepper extract (*Piper nigrum)* killed 100% of the mosquitoes larvae.

Other Brazilian plants are known to have insecticides properties such as *Melia azedarach L*. (Cinnamon) (ARAÚJO et al., 2009). Selvaraj and Mosses (2011) analyzing the control of cinnamon on Anopheles, Culex e Aedes larvae, showed that the extract killed 83% of the larvae in at least 24 hours of exposition.

The *Copaifera spp*. extract were used as repellent against *A. aegypti* mosquito, showing 95% of efficiency for more than 9 hours of use by humans (RIBAS; CARRENO, 2010).

This work had the objective of evaluating the success of five plants extracts on the *A. aegypti* populations. The hypothesis was that the plant extracts influence the population size of *A. aegypti*. We hoped that at least one of this extracts was 100% efficient on the combat of the larvae of the mosquito, thus contributing to Brazilian Health Programs for the local eradication of Dengue.

2 Material and Methods

2.1 Study area

This work was carried out in July 2015 at the Federal Institute of Sergipe, São Cristóvão Municipality (11º01' S e 37º12' W), with altitude of 20 m. The region presents hot and humid weather with the rainy season from March to August (autumn and winter) (INPE, 2018). The local climate is Tropical raining as with a dry summer, following Koppen classification.

2.2 Data collection

The extract of the plants studied were 5: *Ricinus Communis L.* (Malpighiales: Euphorbiaceae); *Ipomoea eriocalyx Meins.* (Solanales: Convolvulaceae); *Croton heliotropiifolius Kunth* (Euphorbiales:Euphorbiaceae); *Momordica charantia L.* (Curcubitales: Curcubitaceaes) and *Mimosa tenuiflora (Wild) Poir. (Fabales: Fabaceae*). 100 g of leaves of each plant were collected, cleaned with a damp cloth and macerated with a mortar. Secondly, 500ml of distillate water was added, following Cruz et al. (2000). The extracts were conditioned in bottles covered by aluminum foil that were kept in boxes in darkness for 72 hours. After that, all the extracts were filtered using a coated paper and were identified and kept in amber bottles of 500 ml. From the extracts, we obtained four concentrations using distillate water: 20%, 10%, 5% e 2.5%.

We used Aedes larvae at five instar, given by Osvaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil, from the lineage Rockfeller/colony (12/02/2015). 10 larvae were collected and transferred to each petri plate. In short we had: 20 treatments (water extract of 5 plants with 4 concentrations) with 3 repetitions, totaling 60 petri plates and 600 mosquito larvae for the total plants extracts used. We tested the positive control C+ (alcohol 70%) on 30 larvae (10 for each petri plate) and the negative control (C-)(mineral water) on 30 larvae (10 for each petri plate). The count of dead larvae was daily done. We tested a positive control and a negative control using only the water.

2.3 Data analysis

To verify the letal dose for 50% of the population sampled (LD50) Generalized Linear models were done (GLM) with binomial error distributional, followed by the properly testing of waste that were adjusted when they are not assumed and we used the ligation function Logit. In the model, we obtained as dependent variable, the total of individuals per treatment

divided by the total of dead individuals in function of the explanatory variables (each plant extract and their respective concentrations) and we analyzed the interaction between these variables. This model was done at R software (R CORE TEAM, 2014).

3 Results and Discussion

The positive control (C+) (alcohol) caused 100% of the larvae mortality and the mineral water (Negative control; C-) caused 0% of mortality (Table 1). The alcohol is a larvae dehydrator agent and the water is a common environment for the mosquito reproduction.

Table 1 - Number of dead larvae in function of time and of the positive and negative control

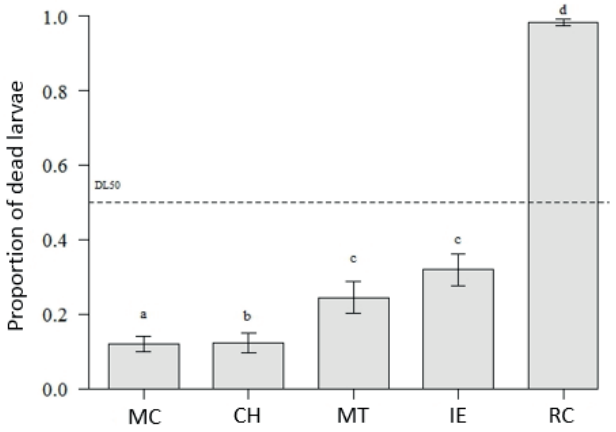
Period	C +	C -
24h	10 10 10	0 0 0
48h	10 10 10	0 0 0
72h	10 10 10	0 0 0

Source: Research data.

The studied plants were selected based on the popular knowledge of their bio-activity as toxic plants or medicine ones. This was a way to value the popular knowledge and promote agroecological alternatives for the *A. aegypti* control.

Considering all the studied plants, the *Ricinus communis L.* (Mamona) was the most effective in the mortality of the mosquito larvae, followed by *Ipoema eriocalyx* (Corda de viola), *Mimosa tenuiflora* (Jurema Preta), *Croton heliotropiifolius* (Velame) and *Momordica charantia* (Melão-de-são-caetano) (Figure 1).

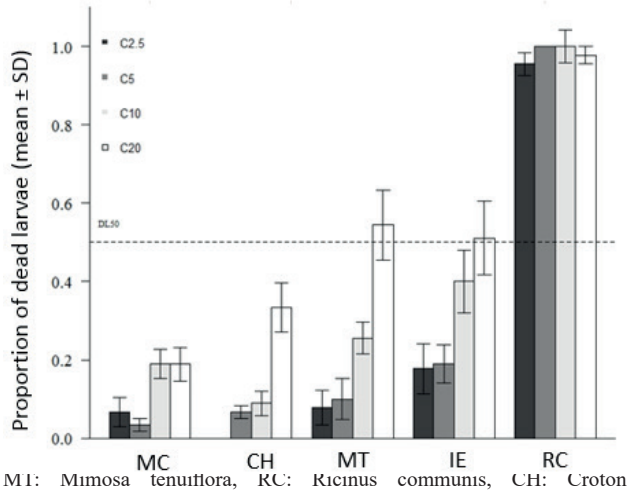
Figure 1 - Efficiency of the studied plants on the control of *Aedes aegypti*, considering all the concentrations



MT: *Mimosa tenuiflora*, RC: *Ricinus communis*, CH: *Croton heliotroppifolius*, IE: *Ipoema eriocalyx*, MC: *Momordica charantia*.
Source: Research data.

However, when the extracts were analyzed individually, considering each concentration, it was observed that the *Ipoema eriocalyx* and *Mimosa Tenuiflora* were satisfactory (LD 50) by the concentration of 20%, showing that these plants are effective on the *A. aegypti* control at high concentrations (Figure 2). Similar results were find by David Junior et al. (2010), with 100% of *A. aegypti* mortality when the larvae were exposed to concentrations higher than 20 % of the *Azadirachta indica* leaves.

Figure 2 - Effect of different plants extract concentrations on the population control of Aedes aegypti larvae



MT: Mimosa tenuiflora, RC: Ricinus communis, CH: Croton heliotroppifolius, IE: Ipoema eriocalyx, MC: Momordica charantia.
Source: Research data.

Andrade et al. (2007) evaluated the effect of *Ricinus communis* (L.) seed extract, at 1%, 5% e 10% concentrations on adults of *Tenebrio molitor* (L.) and showed that the insects mortality tax increased as well as the plant extract concentration increased, and at 10 % of concentration after 120 hours, all the insects were dead.

Kehail et al. (2017) observed the effect of the 3g/L aqueous-extract (137.4 ppm; 6.87% polar extract) of R. communis persisted for eight days for Anopheles and Culex larval populations. Means of 62.8 and 47.5% were obtained for Anopheles and Culex, respectively. Mortality of Anopheles and Culex ranged between 7.5- 100% for the former species, and 5- 100% for the latter. The LD50 were 74.1 and 84.8 ppm, respectively, whereas the LD95s, following the same order, were 121.7 and 122.2 ppm.

Ipoema eriocalyx studied here presented positive effect on the mosquito mortality witch was previously unknown at literature. Considering similar plants to *Ipomoea cairica*, the essential oil of their leaves was pointed as a good larvicide against *Culex tritaeniorhynchus, Aedes aegytpi, Anopheles stephensi* and *Culex quinquefasciatus* (THOMAS et al., 2004).

Take into consideration the popular knowledge, parts of the *Ipomoea cairica plants* such as leaves and roots are used for the treatment of cutaneous eruptions, hepatitis, syphilis, presenting laxative, anti-inflammatory and anti-

rheumatic effects. Other studies showed its antioxidant and antimicrobial actions (WEILER 2010; TOGNON; PETRY, 2012; AURORA et al., 2013). Other Convolvulaceae used as larvicide against *Aedes aegypti* is the *Operculina macrocarpa* (LUNA et al., 2005).

At a 20% concentration of *Croton heliotropiifolius* aqueous extract, it was observed a significant quantity of dead larvae. Silva and Richtmam (2006) tested the effect of the essential oils of the leaves *Croton heliotropiifolius*, *Croton pulegiodorus*, *Hyptis fruticosa*, *Hyptis pectinata* and *Lippia gracilis* on *Aedes aegypti*, suggesting their larvicide effects as an alternative for mosquito population control.

The *Momordica charantia* presented low larvae mortality in this study. However, Cordeiro et al. (2010) showed good results of these leaves alcohol extract in the control of eggs and larvae of goats nematodes.

In this study we showed the first data of *Mimosa tenuiflora* on the *Aedes aegypti* control. This species was very successful in this management at a 20% of concentration, achieving DL50 (Figure 2). Medeiros (2007) using infusion of the bark of Mimosa hostilis obtained 45.25% mortality of the larvae of Aedes aegypti and the bark in decoction 83.5% of mortality after 24 hours.

The oil of *C. langsdorffii* was also a good larvicide against the *A. aegypti*. The CL50 of this oil for the 1°, 2°, 3° and 4° larvae stage was respectively of 0,5; 1,0; 95 and 70 ppm (SILVA et al. 2001). Zanon (2001) observed that the oil of *C. langsdorffii* presented larvicide effect against the *Cx. quinquefasciatus*. The CL50 of the *C. langsdorffii* oil, for the 1°, 2°, 3° and 4° stages were, respectively of 0,4; 0,9; 39 and 80 ppm. The CL100 for the same stages were15; 15; 50 and 180 ppm.

Considering the exposition time, during the first 24 h, the *Ricinus communis L.* was very toxic to Aedes larvae, killing all of them at the concentrations of 20, 10 and 5%. Even at the 2,5% concentration, this plant action was satisfactory, killing 8 of the 10 mosquito larvae on each petri dish. In addition, the *Ipoema eriocalyx* was similarly efficient at a 20% concentration (10 dead larvae), followed by the *Mimosa Tenuiflora* (7 dead larvae), *Croton heliotropiifolius* (5) and *Momordica charantia* (4).

Sakthivadivel and Daniel (2008) achieved CL50 of 24h, using concentrated solution up to 200 ppm at 4° stage larvae of *Cx. quinquefasciatus*, but using *D. horrida* leaves alcohol extract against *Aedes aegypti* larvae, this plant extract was not satisfactory. On the other hand, Mendonça et al. (2005) verified the *C. langsdorffii* extract positive action on 4° larvae stage, presenting CL50 of 41 µg/ml after 48 h of exposition.

While the *Ricinus communis L.* killed the larvae instantly, the plants *Ipoema eriocalyx, Croton heliotropiifolius*, *Mimosa Tenuiflora* and *Momordica charantia* killed the larvae with success progressively at a 72 h interval, at 20% and 10% of concentration. However, at 5% and 2,5% concentrations, the toxicity test showed low plants efficiency on the larvae control (Figure 2).

The *Momordica charantia* and the *Croton heliotropiifolius* were the only plants that in all of the concentrations (20%), (10%), (5%) e (2,5%) did not exceed the DL50. On the other hand, the *Mimosa Tenuiflora*, *Ipoema eriocalyx* and the *Ricinus communis L.* were successful on the larvae control. While the *Mimosa Tenuiflora and Ipoema eriocalyx* only achieved the complete larvae mortality at 20% concentration, with the other concentrations being down to the DL50, the *Ricinus communis L.* extract was successful as larvicide for all the concentrations tested here (Figure 2).

4 Conclusion

The dengue fever was recorded in Brazil, mainly at Atlantic forest biome, followed by the dry region of the country (Agreste) and finally at Savanna biome, with less incidence.

Probably, due to the intervention of the healthy government programs, the dengue records oscillated during the year of this study.

The *Ricinus communis L.* extract studied here was the best extract against *A. aegypti*, killing 100% of their larvae, followed by *Mimosa Tenuiflora* and *Ipoema eriocalyx* that achieved DL50 at a concentration of 20%. The *Croton heliotropiifolius* and the *Momordica charantia* were the less effective extracts against these larvae, not achieving the DL50.

These results suggest the use of the *Ricinus communis L.* extract to control *A. aegypti* population in order to extinct the Dengue fever and to help the government healthy problems around the world.

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