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BIOLOGY

Larvicidal activity of the crude methanolic extract from leaves of *Clibadium surinamense* against *Aedes aegypti*

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ABSTRACT: *The continuous use of synthetic insecticides for controlling the arboviral vector Aedes aegypti has led to the natural selection of mosquito populations resistant to different chemical groups. Thus, plant-derived compounds have emerged as a viable alternative for vectorcontrol. This study determined whether the crude methanolic extract (CME) from leaves of Clibadium surinamense has larvicidal activity against Ae. aegypti. Third- and fourth-instar Ae. Aegyptilarvae were kept in recipients containing 99 mL of water and 1mL of ethanol-diluted CMEat concentrations of 250, 500, 750, and 1000 ppm. The control group contained 99 mL of water and 1 mL of ethanol. Three trials were performed in triplicate for each group.After 24 hours of treatment, the LC_{50} and LC_{90} values were determined to be 283 and 430 ppm, respectively, according to one-way analysis of variance. In conclusion, we have demonstrated for the first time that the CME from leaves of C. surinamense show larvicidal activity against Ae. aegypti under laboratory conditions.*
Key words: *Culicidae, Cunambi, botanical larvicide, plant extract.*

Ação larvicida do extrato de folhas de *Clibadium surinamense* sobre *Aedes aegypti*

RESUMO: *O uso contínuo de inseticidas sintéticos para o controle do mosquito vetor de arbovírus, Aedes aegypti, tem levado à seleção natural de populações resistentes a diferentes grupos químicos. Assim, compostos derivados de plantas surgiram como uma alternativa viável para o controle desses vetores. Desse modo, este estudo foi realizado para determinar se o extrato metanólico bruto (CME) das folhas de Clibadium surinamense possui atividade larvicida contra Ae. aegypti. Para isso, Larvas de terceiro e quarto instar de Ae. aegypti foram mantidas em recipientes contendo 99 mL de água e 1 mL de CME diluído em etanol nas concentrações de 250, 500, 750 e 1000 ppm. O grupo controle continha 99 mL de água e 1 mL de etanol. Três ensaios foram realizados em triplicata para cada grupo. Após 24 horas de observação, de acordo com a análise de variância de uma via,os valores de CL50 e CL90 foram de 283 e 430 ppm, respectivamente. Em conclusão, demonstramos pela primeira vez que o CME das folhas de C. surinamense apresenta atividade larvicida contra Ae. aegypti em condições de laboratório.*
Palavras-chave: *Culicidae, Cunambi, larvicida botânico, extrato vegetal.*

INTRODUCTION

The etiological agents of human viral diseases transmitted by mosquito vectors are among the main causes of morbidity and mortality in Brazil and in the world and thus are of great public health concern (MOTA et al., 2016). Among the mosquito vectors, *Aedes aegypti* (LINNAEUS, 1762) is characterized as an important carrier of arboviruses (MUKTAR et al., 2016). According to CHOUIN-CARNEIRO et al. (2016), this vector is capable of transmitting the pathogens that cause diseases such as dengue, Zika, chikungunya, and yellow fever.

The control of these mosquitoes is largely based on the use of chemical insecticides, such as organochlorines, organophosphates, carbamates, pyrethroids, and synthetic larvicides. However, aside from causing environmental damage, the constant use of these synthetic chemicals has resulted in the emergence of both resistant insect and pathogen populations (BROGDON & MCALLISTER, 1998; MACORIS et al., 2003; DINIZ et al., 2014).

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The rich biodiversity of flora in Brazil has increasingly attracted the attention of researchers hoping to find new sources of plant extracts for the development of drugs for various purposes (COELHO et al., 2009; GARCEZ et al., 2013; TORRES et al., 2015). According to MUKANDIWA et al. (2015), plant extracts can be an excellent alternative for the control of insect populations, as they degrade more rapidly in nature than synthetic insecticides do and thuscan delay the development of resistance in mosquitoes (ROEL, 2001).

Clibadium, a genus of flowering plants native to South America (TJITROSOEDIRDJO, 2002), is used widely by several populations in the Amazon for predatory fishing owing to its ichthyotoxic activity. Cunaniol and cunaniol acetate are the most abundant neurotoxic compounds found in extracts of *Clibadium surinamense* (COSTA et al., 2006; HAMOY, 2011; SANTOS et al., 2016). Considering that *Clibadium* spp. has neurotoxic effects, this study was carried out to verify if the crude methanolic extract (CME) from leaves of *C. surinamense* has larvicidal activity against *Ae. aegypti*.

MATERIALS AND METHODS

Sampling and breeding of Ae. aegypti

Using ovitrap-type laying traps, eggs and larvae of *Ae. aegypti* were collected in different districts of the municipality of Castanhal– PA, Brazil, and distributed to recipients containing 50% distilled water and 50% water from the original breeding site. The larvae were fed crushed fish feed (Mega Food Bits®, Campinas, SP, Brazil).

Once the larvae had reached the pupal stage, they were transferred to distilled water in plastic cups (50 mL).These were then placed in plastic cages covered with nylon screens until the emergence of adults. The mosquitoes were then fed a 10% glucose solution that had been soaked into a cotton swab.

Females were fed horse bloodonce a week, using the artificial feeding system Glytube (COSTA-DA-SILVA et al., 2013) with adaptations. After hematophagy, the females were transferred to plastic cups (50 mL) containing a piece of moistened filter paper for the oviposition stage. Larvae from the F1 generation were used for the bioassays and were kept at an average temperature of 27 °C (heated room) under a photoperiod of 12 hours.

Botanical material

Leaves of *C. surinamense* were obtained from the Federal University of Pará (UFPA) - Belém, Brazil. The exsiccate is registered in the Herbarium Amazônia Oriental under the number 185502.

Chemical analysisof the dry extract

The dry CME was chemically analyzed for the presence of saponins (negative), organic acids (negative), reducing sugars (negative), polysaccharides (negative), proteins and amino acids (negative), phenols (negative), tannins (positive), and phenolics such as depsides, and depsidons (negative), coumarin (positive), and anthraquinones (negative). The chemical analysis, which was carried out in accordance with the Manual for Phytochemical and Chromatographic Analysis of Plant Extracts (BARBOSA, 2001), was performed at the Phytochemical Laboratory of the UFPA.

Bioassays

The larvicidal activity of the CME was determined using the methodology proposed by the World Health Organization (WHO, 2005) with slight modifications. Third- and fourth-instar larvae (n=25for each experimental group) were kept in recipients containing 99 mL of water and 1 mL of different concentrations of CME (250, 500, 750, and 1000 ppm) diluted in ethanol. A control group of larvae was placed in a flask containing 99 mL of water and 1 mL of ethanol without CME. Three trials were performed in triplicate for each group. The 50% and 90% lethal concentrations (LC_{50} and LC_{90}) were determined 24 hours after treatment through linear regression analysis with one-way analysis of variance (ANOVA).

The number of dead larvae was recorded at 6, 12, 24, 48, 72, 96, and 120 hours after exposure to the CME. The remaining live larvae were observed for another 8 days to note the emergence of adults. Mortality was considered when the larvae showed the total absence of movement even after mechanical stimulation or did not maintain diving and then climbing to the surface of the solution.

Statistical analysis

One-way ANOVA was performed to evaluate the effects of the CME treatments on larval mortality and the emergence of *Ae. aegypti* adults, and the Shapiro–Wilk and homoscedasticity assumptions were tested using the Levene test (ZAR, 2010). The significance of differences was determined with the Tukey test using the R program, and descriptive statistical analysis was applied to evaluate the larval metamorphosis time. Differences with a P value of less than 0.05 were considered statistically significant. The LC_{50} and LC_{90}values were determined through Probit analysis, with a 95% confidence interval, using R software.

RESULTS

The CME of *C. surinamense* leaves showed toxicity toward third- and fourth-instar larvae of *Ae. Aegypti*, with the results differing statistically atthe concentrations of 750 and 1000 ppm (Figure 1). The LC_{50} was 283 ppm and the LC_{90} was 430 ppm (Figure 2). There was a significant difference in larval mortality among the treatment concentrations analyzed (F-test (4.40) = 67.25; P = 0.000). All larvae in the control group emerged as adults by the end of the experiment. Compared with the number of dead larvae in the 250 ppm CME-treated group, there were on average 29.8 more dead larvae in the 1000 ppm group (P = 0.00, according to the Tukey test), 28.6 more dead larvae in the 500 ppm group (P = 0.00, Tukey test), and 28.4 more dead larvae in the 750 ppm group (P = 0.00, Tukey test) (Figure 1).

Significant differences were also observed among the different treatments in terms of the number of adults emerging (F-test (4.43) = 54.53; P = 0.000). Compared with the number of adults that emerged in the control group, there were on average 11.7 less in the 250 ppm group (P = 0.02, Tukey test), 24.5 lessin the 500 ppm group (P = 0.000, Tukey test), 24.7 less in the 750 ppm group (P = 0.00, Tukey test), and 25.6 less in the 1000 ppm group (Figure 3).

DISCUSSION

In this study, we have demonstrated for the first time the larvicidal effect of the CME from *C. surinamense* leaves against *Ae. aegypti*, with anLC_{50} of 283 ppm and LC_{90} of 430 ppm. To the best of our knowledge, there are no other published studies that have reported the LC_{50} of the CME of dry leaves of *C. surinamense* for any species of genus *Aedes*.

For many decades, the riverside populations of the Amazon have used the leaves of *Clibadium* spp. as floating baits for fishing. Once the plant is ingested, the fishdevelop convulsions and remain on the water's edge where they are easily removed by hand by the fishermen.

It has already been demonstrated that the leaves of *C. surinamense* have an abundance of cunaniol and cunaniol acetate, which are polyacetylene alcohols present not only in the leaves but also in the fruits of this plant genus. These compounds are powerful stimulants of the central nervous system, and their convulsant activity has been demonstrated in mammals and fish to involve the GABAergic system (COSTA et al., 2006; HAMOY et al., 2018). Because mosquitoes of the genus *Aedes* also have GABA as a neurotransmitter, they are vulnerable to the neurotoxic activities of cunaniol and

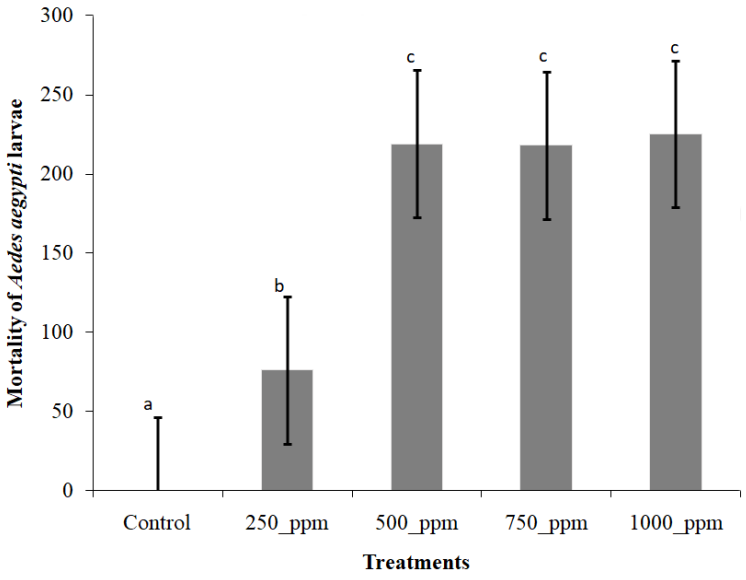

Figure 1 - Mortality of *Aedes aegypti* larvae treated with 250, 500, 750, and 1000 ppm of a crude methanolic extract from *Clibadium surinamense* leaves. Error bars represent the standard error with a 95% confidence interval. Different letters above the bars indicate that the differences between the means were significant according to the Tukey test.

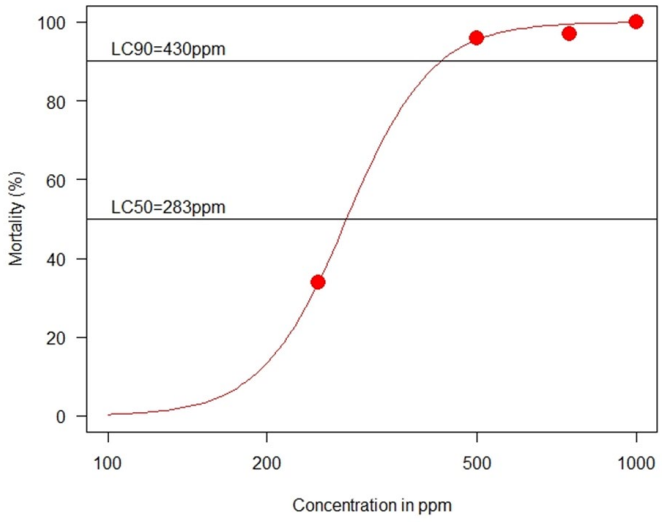

Figure 2 - Mortality curve of *Aedes aegypti* larvae treated with various concentrations of the crude methanolic extract of *Clibadium surinamense* leaves.

cunaniol acetate. The insecticidal effect of the leaves of *Clibadium* spp. has already been reported by FILGUEIRAS et al. (2011), who demonstrated the effective toxicity of the *Clibadium sylvestre* extract toward the aphid species *Myzuspersicae*.

Phytochemical analysis of leaves and fruits of *C. sylvestre* revealed tannins to be the most common secondary metabolites in this plant (FILGUEIRAS et al., 2011). Tannins have several known biological functions, such as larvicidal activity (NERY et al., 2009), which was demonstrated by Silva et al. (2004) using an extract from *Magonia pubescens*. In our present study, tannins were also reported in the CME from *C. surinamense* leaves, suggesting that these secondary metabolites may also be involved in the observed larvicidal activity.

In a review by PAVELA (2015) on the larvicidal effect of essential oils from plants of different continents, the oils from 122 plant species showed high activity against various insect larvae, where $LC_{50} \leq 100$ ppm was the main criterion for the determination of larvicidal efficacy. Based on this criterion, the larvicidal effect of the CME from *C. surinamense* leaves against *Ae. Aegyptic* annot be considered asbeing high, as the LC_{50} was 283 ppm. However, despite the adoption of the LC_{50}value as a determining parameter for the larvicidal effect of plant extracts, PAVELA (2009) had also shown that the essential oils of *Thymus vulgaris*, *Satureja hortensis*, and *Thymus satureioides*, which had very similar LC_{50}values (33, 36, and 44 ppm, respectively), differed in their biological effects. That is, they interfered with the population dynamics of *Culex quinquefasciatus* (SAY, 1823) to different degrees. Therefore, a plant extract can still be useful for the control of mosquito vectors even if its LC_{50} is greater than 100 ppm.

PAVELA (2015) also stated that the concentration required to achieve maximum larval mortality depends on several factors, such as the route of intoxication, environmental temperature, larval stage, and the mechanism of action of the bioactive compounds in the extract. Because the toxic effects of *Clibadium* spp. are obtained through ingestion of the leaves or their extracts (FILGUEIRAS et al., 2011; HAMOY, 2011), the deleterious effects observed in this study had likely occurred after the larval ingestion of the extract.

BOHM & STUESSY (1981) were the first to isolate three types of flavonoid derivatives from *Clibadium* spp.: kaempferol, quercetin, and quercetagetin. Subsequently, some studies have demonstrated that kaempferol and quercetin have larvicidal activity against *Ae. aegypti* (OCHIENG et al., 2010; PONTUAL et al., 2012).

The use of plant compounds to control mosquito larvae is an interesting direction to take with the goal find new insecticidal options. Therefore, it is a field of study that has been gaining attention and traction in recent decades, in lockstep with research producing efficient and safe insecticides for the environment and humans (MUKANDIWA et al., 2015; TORRES et al., 2015).

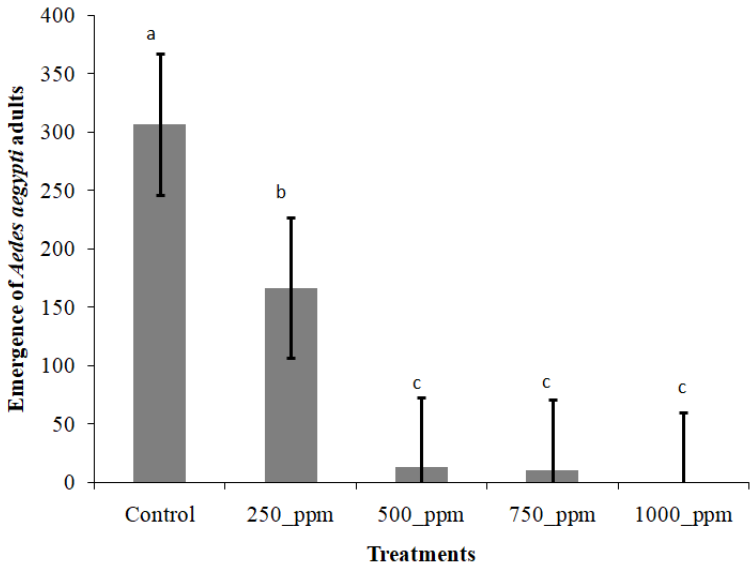

Figure 3 - Number of *Aedes aegypti* adults emerging after treatment of the larvae with 250, 500, 750, and 1000 ppm of the crude methanolic extract of *Clibadium surinamense* leaves. Error bars represent the standard error with a 95% confidence interval. Different letters above the bars indicate that the differences between the means were significant according to the Tukey test.

Thus, this study highlights an alternative natural product that can be used to control *Ae. aegypti*. According to WHO (2005) recommendations, three steps (viz., laboratory, small-scale, and large-scale field tests) need to be performed to validate a larvicide. This present research represents the laboratory phase, and we plan to move ahead to the second phase with small-scale field tests.

Generally, studies on the insecticidal properties of *C. surinamense* are still in the developing stages, requiring more research for the chemical evaluation and identification of phytocompounds with larvicidal and/or pupicidal activity (e.g., for interfering with the emergence of adults) as well as elucidation of their mechanisms of action. Therefore, more research is needed to gain a better understanding of the effects of CMEs from *C. surinamense*, especially against *Ae. aegypti*.

CONCLUSION

The crude methanolic extractfrom leaves of *C. surinamense* showed larvicidal activity against *Ae. Aegypti* at all concentrations tested under laboratory conditions. There was also a significant difference in the emergence of adult forms among the analyzed treatments, especially at the CME concentration of 1000 ppm. This is the first-ever report that the CME from *C. surinamense* leaves has larvicidal activity against *Ae. aegypti*.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. Funding sponsors had no role in the study design, collection, analysis, and data interpretation; during the writing of this manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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