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The use of Annona squamosa leaf as an alternative in the control of *Aedes aegypti*

A utilização da folha de Annona squamosa como uma alternativa no controle de *Aedes aegypti*

El uso de la hoja de Annona squamosa como alternativa en el control de *Aedes aegypti*

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Abstract
Changes in human behavior towards the environment, barriers to urban development, globalization and climate change are some of the factors that contribute to the occurrence and spread of infectious diseases. The incident affects three arboviruses in Brazil, Chikungunya, Dengue and Zika, which are widely disseminated by mosquito vectors distributed throughout the country, related to humans, causing major public health problems. In the absence of effective vaccines and specific treatments, it is important to maintain and integrate ongoing entomological and epidemiological surveillance to guide the country's control and prevention approaches against these arboviroses. The Annonaceae family has been extensively studied to utilize many species as a source of insecticidal compounds with different modes of action on insects. Therefore, this work aimed to develop a natural, economical and sustainable product using Annona squamosa leaf extract as a resource against Aedes aegypti larvae. From the results, one can prove the hypothesis that the natural insecticide developed from the leaf extract of the fruit of the conde (Annona squamosa) is very effective and also effective in reducing the mortality of Aedes aegypti.
Keywords: Natural products; Arboviroses; Extracts;

Resumo
Mudanças no comportamento humano em relação ao meio ambiente, barreiras ao desenvolvimento urbano, globalização e mudanças climáticas são alguns dos fatores que contribuem para a ocorrência e disseminação de doenças infecciosas. O incidente atinge três arbovírus no Brasil, Chikungunya, Dengue e Zika, que são amplamente disseminados por mosquitos vetores Distribuídos por todo o país, relacionados aos seres humanos, causando grandes problemas de saúde pública. Na ausência de vacinas eficazes e tratamentos específicos, é importante manter e integrar a vigilância entomológica e epidemiológica em curso para orientar as abordagens de controle e prevenção do país contra essas arboviroses. A família Annonaceae tem sido extensivamente estudada para utilizar muitas espécies como fonte de compostos inseticidas com diferentes modos de ação sobre insetos. Portanto, este trabalho teve como objetivo desenvolver um produto natural, econômico e sustentável, utilizando o extrato de folhas Annona squamosa como recurso contra larvas de Aedes aegypti. A partir dos resultados, pode-se comprovar a hipótese de que o inseticida natural desenvolvido a partir do extrato da folha da fruta do conde (Annona squamosa) é muito eficaz e também eficaz na redução da mortalidade de Aedes aegypti.
Palavras-chave: Produtos naturais; Arboviroses; Extratos.

Resumen
Los cambios en el comportamiento humano hacia el medio ambiente, las barreras del desarrollo urbano, la globalización y el cambio climático son algunos de los factores que contribuyen a la aparición y propagación de enfermedades infecciosas. El incidente afecta a tres arbovirus en Brasil, el Chikungunya, el Dengue y el Zika, que son ampliamente diseminados por mosquitos vectores distribuidos por todo el país, relacionados con los seres humanos, causando importantes problemas de salud pública. A falta de vacunas eficaces y tratamientos específicos, es importante mantener e integrar la vigilancia entomológica y epidemiológica permanente para orientar los enfoques de control y prevención del país contra estas arbovirosis. La familia Annonaceae ha sido ampliamente estudiada para utilizar muchas especies como fuente de compuestos insecticidas con diferentes modos de acción sobre los insectos. Por lo tanto, este trabajo tuvo como objetivo desarrollar un producto natural, económico y sostenible, utilizando el extracto de hojas de Annona squamosa como recurso contra las larvas de Aedes aegypti. A partir de los resultados, se puede comprobar la hipótesis de que el insecticida natural desarrollado a partir del extracto de hoja del fruto de la oreja (Annona squamosa) es muy eficaz y también efectivo para reducir la mortalidad del Aedes aegypti.
Palabras clave: Productos naturales; Arboviroses; Extractos.

1. Introduction

Aedes aegypti (Linnaeus, 1762) is a viral vector that causes dengue, chikungunya, and Zika viruses (Vasconcelos, 2015). In the absence of effective vaccines and specific treatments, it is important to maintain and integrate ongoing entomological and epidemiological surveillance to guide the country's control and prevention approaches against these arboviroses.

A. aegypti mosquitoes have four biological stages: egg, larva, pupa and adult takes about 10 days to complete a cycle, the first three being aquatic. The eggs are white and, in the first 24 hours, will have a shiny black appearance; under adequate temperature and humidity conditions, their embryonic development is completed in 48 hours (Feitosa et al., 2017). Population control actions occur mainly in the larval stage, when it is most vulnerable.

The code fruit (Annona squamosa) belongs to the family Annonaceae, this is a family that deserves prominence for being currently the botanical family most studied and used for alternative pest control and are as Acentogenins, ie, the active ingredient found in the bark of the branches, roots and seeds of these plants of this family that shows the effect against pest insects. (Paz et al., 2018).

Therefore, this work aimed to develop a natural, economical and sustainable product using the leaf extract of the fruit of conde or pine cone (Annona squamosa) as a resource against Aedes aegypti larvae. From the results, one can prove the hypothesis that the natural insecticide developed from Annona squamosa leaf extract is very effective and also effective in killing Aedes aegypti.

2. Methodology

To test the mortality of Aedes aegypti larvae, an extract of Annona squamosa leaves, commonly known as conde fruit, was used. For the preparation of the aqueous and ethanolic extracts, the leaves were collected for bioassays in Umarizal/RN at the cooler hours of the day, removed from the top of the plant and left to dry for 2 weeks.

For the extract preparation, an acrylic beaker with a movable blade fixed at the bottom was used, in which the harvested part was triturated. The grinding was done in different amounts of solute, using equal amounts of solvent for different extracts, 1 liter of distilled water for aqueous extracts and 500 ml of alcohol for ethanolic extracts. As recommended by (EMBRAPA, 2006), the aqueous substances were left to stand for 24 hours in a place without light, and then the ethanolic extract was left to stand for 120 hours for better extraction of active ingredients. After this period, the material is filtered and placed in a container. There were 3 replicates in the experiment, it is important to note that two experiments were performed for each control substance (Santos et al., 2012). The first is the indirect application and the second is the direct application of

the extract on Aedes aegypti larvae.

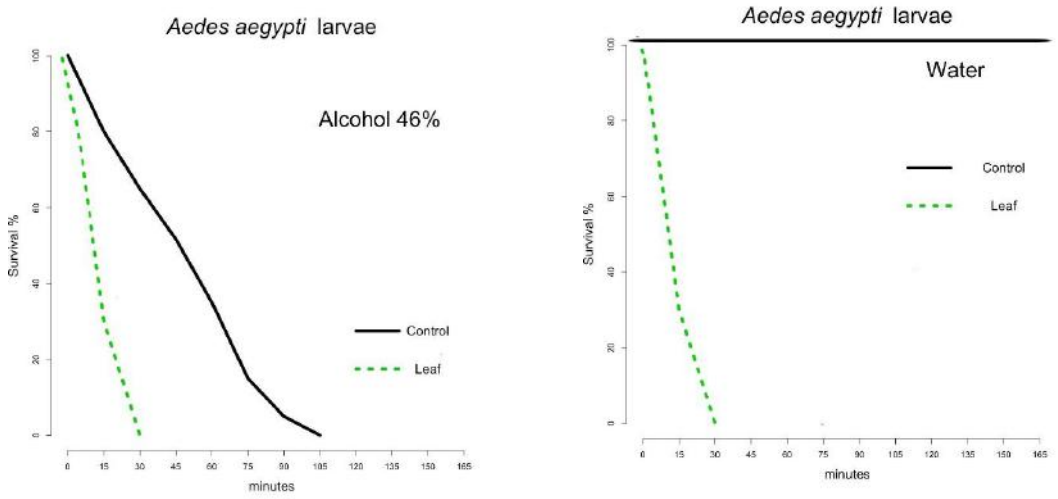
In the experiment, 4 containers were used to contain 40 Aedes aegypti larvae, and in each container 20 Aedes aegypti larvae were placed. In these containers 100 ml of distilled water and 10 drops of natural insecticide were placed, and the control group was distilled water. This is consistent with Cruz et al. (2012) for experiments testing larval mortality of Aedes aegypti. In the second experiment, 20 larvae and two well plates were used with 10 Aedes aegypti larvae on each plate. In the first plate, 10 Aedes aegypti larvae were distributed as one unit in each compartment and one drop of distilled water-based extract was applied to each larva, and in the second container, one drop of distilled water was applied to each larva. Mortality/survival of the Aedes aegypti larvae was observed.

In experiments with ethanolic extracts with Aedes aegypti larvae, we used 4 containers to store 40 larvae. We put 20 Aedes aegypti larvae in each container, in which we put 100 ml of distilled water and 10 drops of natural insecticide, and a control group of 46% alcohol. In the second experiment, we used 20 larvae of the dengue mosquito and two well plates with 10 larvae in each plate. In the first plate, 10 Aedes aegypti larvae were evenly distributed in each compartment and one drop of ethanolic extract was added to each larva, and in the second container, one drop of 46% alcohol is dripped on each larva. Mortality/survival of dengue mosquito (Aedes aegypti) larvae was observed.

3. Results and Discussion

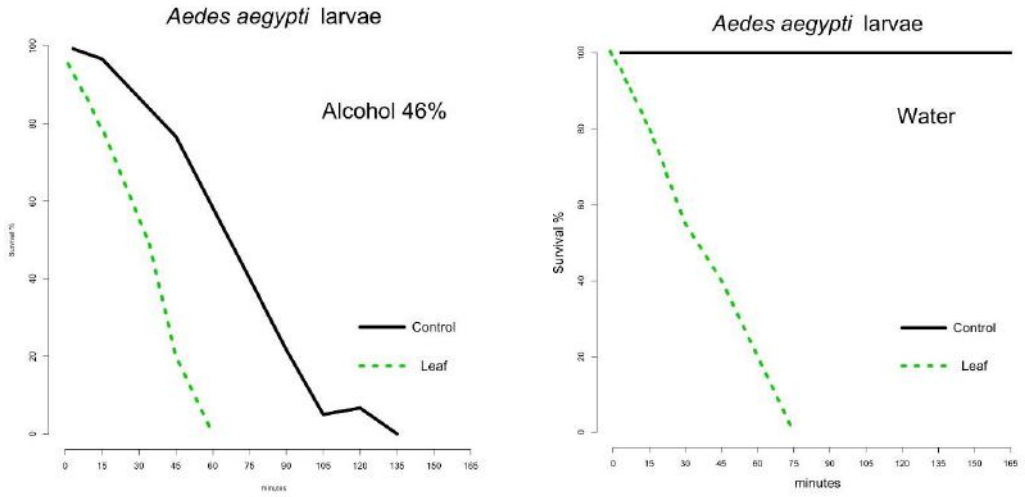
Figure 1 represents the mortality rate in minutes with the test made with the ethanolic extract, figure 2 represents the mortality rate in minutes made with the aqueous extract.

Figure 1. It represents a direct application of insecticides on the adult larvae of the Aedes aegypti.



* Mortality of Aedes aegypti larvae subjected to direct application of Annona squamosa extracts dissolved in 46% alcohol and distilled water. Source: Authors.

Figure 2. It represents an indirect application of insecticides on the adult larvae of the Aedes Aegypti.



* Mortality of Aedes aegypti larvae subjected to indirect application of Annona squamosa extracts dissolved in 46% alcohol and distilled water. Source: Authors.

Figure 1 shows that the insecticides had an average mortality time in yellow-fever mosquito larvae with aqueous extract in direct application of 30 minutes, in ethanolic extract of 30 minutes, the control had a mortality time of 1h45min.

Figure 2 shows that the insecticides had an average mortality time on Aedes aegypti larvae with aqueous extract in indirect application of 1h15min, in ethanolic extract of 60 minutes, the control had a mortality time of 2h15min. The result of the water mortality time shows the veracity of the insecticidal action in Annona squamosa leaf.

From the results, it was possible to prove the veracity of the hypothesis, that the natural insecticides developed from the extract of Annona squamosa leaves are very efficient, indicating that they are more effective than the industries in the question of mortality rates of yellow-fever mosquito larvae (Aedes aegypti).

Proving that Annona squamosa also has the component Acetogenin which constitutes a class of promising natural products as prototypical insecticidal agents, being found in the barks of branches and roots, roots and especially in the seeds of plants of the Annonaceae family (Bermejoet al., 2005; Castillo-Sánchezet al., 2010).

The insecticidal activity is due to the presence of the substance Acetogenins that act in the mitochondria, inhibiting NADH -ubiquinone and oxidoreductase, hitting the metabolism, causing insect death (Zafra-Poloet al., 1996; Lümmen, 1998).

4. Conclusion

Bioinsecticides made from Annona squamosa leaves have proven to be very effective in combating yellow-fever mosquito larvae. The use of this natural insecticide is effective, bringing benefits to the population, since sweetsop based natural insecticides are naturally bioactive and show cytotoxic activity. The use of natural pesticides is an alternative when related to the use of synthetic chemicals for the same purpose, because the developed product becomes more economical and accessible than synthetic pesticides, besides contributing to human health.

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