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Attractive activity of plant extracts for the oviposition of *Aedes aegypti* L. (Diptera: Culicidae)

Actividad atractiva de extractos vegetales para la oviposición de Aedes aegypti L. (Diptera: Culicidae)

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ABSTRACT

Aedes aegypti is one of the most important culicidae species for public health, since it transmits the viruses for yellow fever, dengue fever, chikungunya and zika. With the purpose of analyzing the effect of plant extracts on the oviposition of this vector in ovitraps, 100 ovitraps were set containing an attractive plant substrate and a pressed wooden palette vertically installed on the pot for female oviposition. The traps were randomly distributed in 10 neighborhoods of Crato, Ceará, Brazil. To determine the best extract, water from the supply network, distilled water, sewage and aqueous extracts of plant leaves from guava (*Psidium guajava* L.), papaya (*Carica papaya* L), cassava (*Manihot esculenta* Grantz), cashew tree (*Anacardium occidentale* L.), sorghum (*Sorghum bicolor* L., Moench) and grass hay (*Cynodon nlemfluensis* Vanderyst) were used. The substrates were prepared at the Laboratory of Agricultural Entomology at the Center of Agricultural Sciences and Biodiversity of the Federal University of Cariri (UFCA). Leaves were collected from the plants and crushed using a blender and then mixed with water at a concentration of 50 g of each substrate per liter of water. The material was stored for seven days in a dark room to facilitate the reactions and avoid the photoinactivation of active compounds. To determinate the best concentration, guava leaves were used subject to the same treatment of the first experiment, at concentrations of 0.5, 1; 5, 10, 25, 50, and 75 g of substrate per liter of water. Traps with sewage water showed the highest attractiveness for the oviposition of *A. aegypti* and the extract of guava leaves showed the highest percentage of egg attractiveness for the vector mosquito. These results can be useful to guide the adoption of new entomological surveillance and control methods of *A. aegypti.*

Key words: Ovitraps. Oviposition attraction. Alternative control.

RESUMEN

Aedes aegypti es una de las especies de culícidos más importantes para la salud pública, pues es transmisor de los virus de la fiebre amarilla, dengue, chikungunya y zika. Intentando analizar el efecto de extractos vegetales sobre la ovoposición de ese vector en trampas de ovoposición, fueron instaladas 100 trampas conteniendo en su interior sustrato atrayente de un vegetal y una paleta de madera prensada insertada en la posición vertical de la pared del vaso para que la hembra realice la postura. Las trampas fueron distribuidas, al azar, en 10 barrios de Crato-CE. Para determinar el mejor extracto fueron utilizadas agua de la red de suministro, agua destilada, desagüe y extractos acuosos de las hojas del guayabo (Psidium guajava L.), papayo (Carica papaya L.), mandioca (Manihot esculenta Crantz), anacardo (Anacardium occidentale L.), sorgo (Sorghum bicolor L., Moench) y heno de la gramínea (Cynodon nlemfluemsis Vanderyst). Los sustratos fueron preparados en el Laboratorio de Entomología Agrícola, en el Centro de Ciencias Agrarias y de Biodiversidad (CCAB), de la Universidad Federal de Cariri (UFCA). Las hojas fueron extraídas de la planta y enseguida, trituradas en una licuadora y mezcladas con agua en concentración de 50 g de cada sustrato por litro de agua. El material pasó siete días en ambiente oscuro para facilitar las reacciones y evitar la foto inactivación de los ingredientes activos. Para determinar la mejor concentración fueron utilizadas hojas del guayabo sometidas a las mismas etapas del primer experimento, aplicadas en las concentraciones de 0,5; 1; 5; 10; 25; 50 y 75 g del sustrato por litro de agua. Trampas cebadas con agua de desagüe manifiestan mayor atractividad para la ovoposición del A. aegypti y el extracto de la hoja del guayabo presenta mayor porcentaje de atractividad de huevos del mosquito vector. Estos resultados pueden ser útiles para orientar la adopción de métodos de vigilancia entomológica con ovotrampas y control del A. aegypti.

Palabras clave: *Ovotrampas, Atrayentes de ovoposición, Control alternativo.*

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Introduction

Aedes aegypti is a species of international concern, since it is the vector of four important arboviroses: yellow fever, dengue, chikungunya and zika, caused by the FAV, DENV, CHICKV and ZIKAV viruses, respectively (Health Ministry, 2016a). Dengue has had an increase of incidence estimated at 30 times over the last 50 years, with growing geographic expansion to new countries (WHO, 2012). The latest calculation of global dengue incidence in developed and developing regions of the world, including Latin America and the Caribbean, indicated that approximately 40% of the global population is at risk of infection by DENV (Chadee and Martinez, 2016). Currently zika is also the focus of much concern due to the recent discovery of its possible association with severe neurological morbidities, especially microcephaly.

Over the years, the fight against these arboviroses was focused on eliminating the vector or reducing infestation indexes to very low levels using chemical insecticides as the main measure, since only the vaccine against the yellow fever virus was available. In 2016, the vaccine against DENV became available on the market; however there is no estimation of its public distribution, and controlling the vector still is the main tool available.

Many studies indicate the failure of the traditional control method, due to the resistance to insecticides developed by the vector, its continuous dissemination and occurrence of epidemics (Lima *et al*., 2015). Therefore, efforts are being made to search for alternatives such as biological control, vegetable bioagents (Garcez *et al*., 2013), predator animals and bacteria with antiviral and transgenic action (Lima *et al.,* 2015).

Mechanical strategies such as the use of ovitraps have been tested. “Ovitraps are considered as a sensitive and cost-effective method to detect the presence of *A. aegypti*, mainly when the infestation is low and the larvae index surveys are not much (*sic*) effective” (Brasil, 2001). Based on this knowledge, researchers try to improve them in terms of structure or by the addition of synthetic chemical or natural substrates (Jahan and Sawar, 2013).

One of the natural substrates that is most commonly added to the traps is the hay solution, since it increases the attraction of *A. aegypti* females compared to water without any substrates. Santos *et al*. (2010) observed that other vegetable extracts also attract them. Thus, the aim of this study was to analyze the effect of vegetable extracts on *A. aegypti* in ovitraps in the municipality of Crato, Ceará, Brazil.

Material and Methods

On March and April 2016, 100 ovitraps were installed in 10 randomly selected neighborhoods in the municipality of Crato, after consulting with the Brief Survey of the *A. aegypti* Infestation Index (BSAaII). The ovitraps were made using a black 400 mL polypropylene pot, a 3 x 11 cm pressed wood pallet (Eucatex type) inserted vertically against the wall of the pot for the deposition of eggs, immersed in water or extracts.

Production of aqueous vegetable extracts

The extracts were prepared at the Laboratory of Agricultural Entomology of the Center of Agricultural Sciences and Biodiversity (CCAB) of the Federal University of Cariri (UFCA), in Crato. Fresh leaves of guava plants (*Psidium guajava* L.), papaya plants (*Carica papaya* L.), cassava plants (*Manihot esculenta* Crantz), cashew plants (*Anacardium occidentale* L.), sorghum (*Sorghum bicolor* L. Moench) and grass hay (*Cynodon nlemfluemsis* Vanderyst), collected from the experimental area of UFCA. The material from each species was crushed in a domestic blender and the resulting powder was dissolved in water (50 g L^{-1}). After this process, the solution was matured for seven days in the dark in order to facilitate the chemical reactions and avoid the photoinactivation of active ingredients of the extracts.

To evaluate the activity of the extracts, an experiment with a completely randomized design was established; the treatments were water from the supply network (control treatment), distilled water, sewage water, and aqueous extracts from the leaves of the guava plant, papaya plant, cassava plant, cashew plant, sorghum and grass hay, distributed in 10 replications, totalizing 90 experimental plots.

Determining the best extract concentration with the greatest attractive activity

After evaluating the attractive potential of the extracts, the ideal concentration was determined for the extract that showed the best performance. The experiments were conducted according to the same stages as previously described, using concentrations of 0.5, 1.5, 10, 25, 50 and 75 g L^{-1}.

Statistical analysis

For the first experiment, the statistical analyses were conducted using the Statistical Analysis System (SAS) program. The hypothesis test was calculated, using the two-sample t-test statistical procedure for means at 5% probability. For the second experiment, the different concentrations of the aqueous extract of guava plant leaves were analyzed using Tukey's test at 5% probability, allowing the comparison of the attractiveness potential of the extracts as well as of the concentrations. To determine the best extract concentration, the mean number of *A. aegypti* eggs was considered in relation to the most efficient extract concentrations, using the following model: $y = ae^{-bx}$, where y is the mean number of vector eggs and x is the extract concentration.

The oviposition response from females to the different types of substrates was determined by the Oviposition Attractiveness Index (OAI) suggested by Kramer and Mulla (1979). This index varies from -1 to 1; positive values correspond to attractive or stimulating substances, while negative values indicate a repelling or inhibitory action. OAI may be obtained using the following formula:

$$\text{OAI} = \frac{Nt - Nc}{Nt + Nc}$$

Where:
OAI: Oviposition attractiveness index;
Nt = Mean number of eggs of the test solution;
Nc = Mean number of eggs of the control treatment.

Results and Discussion

Attractive activity of substrates added in ovitraps traps for *A. aegypti*

The mean number of eggs deposited varied from 11.5 ± 9.5 (papaya leaf extract) to 21.4 ± 11.1 (guava leaf extract). The highest egg deposition was observed in ovitraps with sewage water (68.2 ± 20.8). The number of deposited eggs was higher in those with distilled water than in the others containing sorghum, hay, cashew plant, cassava plant and papaya plant extracts. A difference was observed ($P<0.05$) among the evaluated extracts, except for the sorghum, cashew plant and cassava plant extracts, which did not differ from each other (Table 1).

Table 1. Mean number of eggs by oviposition substrate, standard deviation, oviposition attractiveness index (OAI), obtained from different aqueous plant extracts at the concentration of 50 g L^{-1}.

Treatments	Eggs	CI 95%		OAI (-1 to 1)
		IL	SL	
Supply network water	18.5 ± 5.7 c	16.6	20.4	0.0
Distilled water	18.7 ± 8.9 c	16.8	20.6	0.0
Sorghum	15.9 ± 10.7 d	14.0	17.8	-0.1
Hay	18.6 ± 10.9 c	16.7	20.4	0.0
Cashew	14.0 ± 8.6 d	12.1	15.9	-0.1
Cassava	15.0 ± 8.7 d	13.1	16.9	-0.1
Papaya	11.5 ± 9.5 e	9.6	13.4	-0.2
Guava	21.4 ± 11.1 b	19.5	23.3	0.1
Sewage	68.2 ± 20.8 a	66.3	70.1	0.6

Means followed by the same letter do not differ from each other according to the t test for two samples using the confidence interval procedure with 95% confidence.

Treatments with positive OAIs that differed from the supply network water treatment – the control treatment – were those with sewage water and the aqueous extract from the guava plant leaves, showing values of 0.6 and 0.1, respectively, while the hay extract showed the same OAI as the control, Table 1.

Nunes *et al*. (2011), evaluating the attractiveness of ovitraps in the state of Amapá, observed that based on the positivity index of these traps, those with the hay infusion solution were the most attractive or stimulating for the oviposition by females. This result was different from that of this study, which did not find a significant difference between hay extract and natural water.

As to the higher oviposition attractiveness of sewage water, this study found a similar result to that found by Beserra *et al*. (2010). These authors observed that the vector is able of developing in aquatic environments with elevated levels of pollution, also showing a positive correlation between the polluted water and oviposition rate (mean of 415 eggs in polluted water and 113.2 in water without chlorine). They suggested that *A. aegypti* develops in environments with high levels of pollution, such as domestic sewage due to a high concentration of organic matter, which serves as food for the insect during its larval stage.

Extracts of the leaves of papaya, cashew, cassava and sorghum plants caused repellence to the oviposition of this insect, showing values of -0.2 for the papaya plant and -0.1 for the other substrates. In terms of percentage, the papaya plant extract caused 37.8% repellence to this insect compared to the control. Studies with isolated bioagents from these species could be conducted in order to obtain a repellent against this vector. El Maghrbi and Hosni (2014), evaluating the influence of plant extracts on the oviposition of *Ancilus fluviatilis* and *Culex quinquefasciatus,* highlighted that acetone extracts from papaya seeds had a repelling action (55.6%) for *C. quinquefasciatus* oviposition at 100 mg L^{-1}.

C. papaya has two important biological components, chymopapain and papain. There is also presence of caricain and glycine endopeptidase (Ahmad *et al.,* 2011). Studying the effect of the extract from the leaves and seeds of *C. papaya* on *A. aegypti* larvae, Wahyuni (2015) showed that the larvicide effect occurred due to the presence of secondary metabolic compounds such as saponins, flavonoids and triterpenoids.

Chitolina *et al.* (2016) observed a difference between the number of eggs laid on raw sewage, with 526.50 eggs, in relation to the positive control (distilled water), with 409.83 eggs. This is in agreement with this study, since the mosquito adapted to the current sanitary conditions, that is, it preferred to lay its eggs in environments in an environment that was considered as not preferred for oviposition by this insect, which has been described in the past by the specialized literature, when it used to choose clean water.

Among the oviposition substrates, it was observed that the ones obtained from guava plant leaves had more eggs laid by this vector (21.4), differing statistical from the other plant substrates. Santos *et al.* (2010) observed a mean oviposition of 25 eggs when they used the leaf extract from the cashew plant to obtain the eggs from this mosquito in the city of Recife, state of Pernambuco, while in this study a mean of 14 eggs was found with the extract from the same plant in the city of Crato, Ceará. In percentage terms, the guava leaves showed the highest oviposition, at 22.20%, while the aqueous extract from papaya leaves showed 11.93%, with the lowest contribution to attract oviposition (Figure 1).

Prajapati *et al*. (2005), investigating 10 essential oils for oviposition activity, observed that the *Cinnamomum zeylanicum* J. Presl essential oil was highly efficient for the oviposition of *Anopheles stephensi*, *A. aegypti* and *C. quinquefasciatus* mosquitos. Using a multiple choice test with *Ricinus communis* L. and *Cnidoscolus phyllacanthus* Pohl., Candido and Beserra (2015) observed a clear preference for oviposition in the control solution (water and Tween 20), where 457 eggs (91%) were laid in comparison to *R. communis,* and 108 eggs laid (73%) in *C. phyllacanthus*.

As to the ideal concentration of the extract with the highest attractiveness, (guava leaf), the best results were with 0.5, 1.0, 5.0 and 10 g L^{-1}, the last had the best performance. Increasing the concentration beyond this value did not result in a higher oviposition rate. Curiously, a significant reduction in the number of laid eggs occurred, exhibiting a loss of efficiency as the extract concentration increased (Figure 2).

In oviposition essays with *Melanochyla fasciculiflora* Koch. extracts used for *A. albopictus,* the mosquito preferred to deposit its eggs on the control solution (distilled water with 1 mL of 10% acetone). The number of eggs laid by *A. albopictus* was reduced as the *M. fasciculiflora* and *Gluta renghas* concentrations increased. However, only the *M. fasciculiflora* extracts showed significant

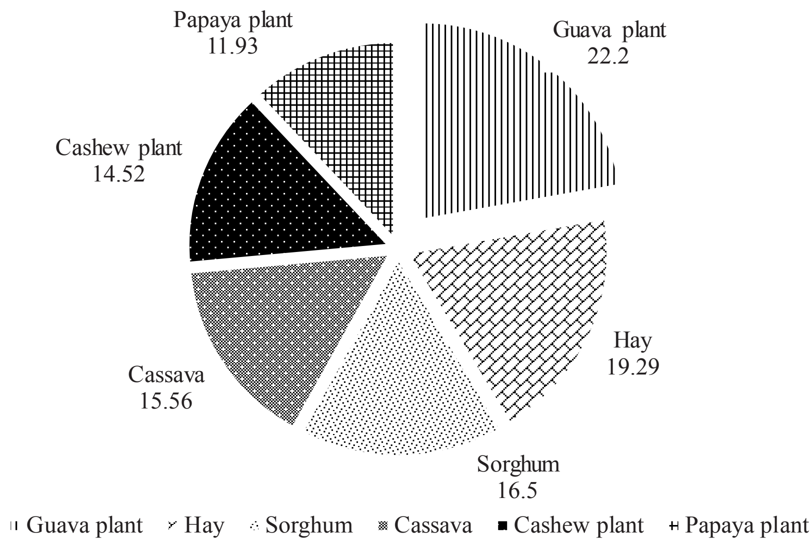

Figure 1. Ratio of eggs collected in traps according to the type of extract. Crato, Ceará, 2016.

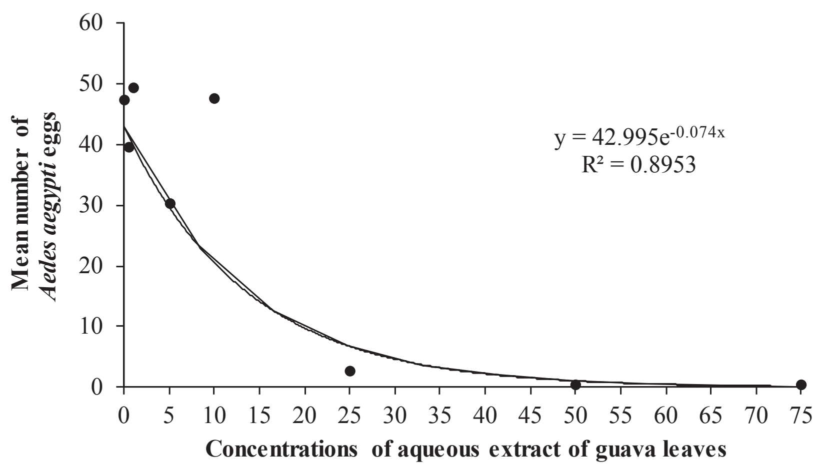

Figure 2. Mean number of *A. aegypti* eggs in relation to the concentrations of aqueous extracts from guava plant leaves. Crato, Ceará, 2016.

effects compared to the control treatment. At the lowest dose (LC_{25}) of *M. fasciculiflora*, RE reached 57%. However, the *G. renghas* extract needed a concentration of LC_{50} to reach a RE of 50% (Zuharah *et al*. 2015). Both results are in agreement with this study, since as the concentration increases, oviposition repellence for the vector insect occurs.

Reegan *et al.* (2015), analyzing oviposition prevention in India, reported that the hexane extract from *Limonia acidissima* L. showed a 100%repellent action to oviposition in all tested concentrations (62.5-500 ppm) against *C. quinquefasciatus* and *A. aegypti*. The study of Elimam *et al.* (2009) on the efficiency of the concentrations of the leaf extract of *Calotropis procera* Ait. To control *Anopheles arabiensis* and *C. quinquefasciatus* observed that the extract showed an oviposition prevention action of 100% for *A. arabiensis,* and 90.6% for *C. quinquefasciatus* compared to the control treatment. However, when all concentrations were available without any control (free choice), oviposition prevention was not observed except at a high concentration (1,000 ppm), and the maximum number of eggs was laid at the lowest concentration of 200 ppm.

Table 2 shows that OAI for the 50 and 75 g L^{-1} concentrations was -1, attesting that the higher the concentration of the guava leaf extract, the lower will be its efficacy to attract oviposition. Coria *et al.* (2008), studying the effects of the extracts of leaves and fruits of *Melia azedarach* L. on the oviposition of *A. aegypti,* observed that the leaf extracts showed maximum repulsive effect for the oviposition at a concentration of 1 g L^{-1}.

The guava tree has many uses in traditional medicine, since the main component of its leaves is the essential oil (90.3%), which is rich in caryophyllene, nerolidol, β-bisabolene, aromadendrene, p-selinene,

Table 2. Oviposition Attractiveness Index (OAI) of A. aegypti submitted to different concentrations of the aqueous extract of guava leaves. Crato, Ceará, 2016.

Concentrations (g L^{-1})	OAI
Water	0.0
0.5	-0.1
1.0	0.0
5.0	-0.2
10.0	0.0
25.0	-0.9
50.0	-1.0
75.0	-1.0

α-pinene and 1,8-cineol; triterpenoids (oleanolic acid, ursolic, catecholic, guavavolic, maslinic acid) and β-sitosterol (Vendruscolo *et al*., 2005). Probably, some of these constituents from the guava plant leaves caused greater attractiveness for the females to deposit their eggs on this substrate, and the essential oils acted as the oviposition attraction agent.

Abugri *et al.* (2016), analyzing the raw extract from *S. bicolor* leaves, showed that these extracts contained significant amounts of apigeninidine, luteolinidin, 7-methoxy-apigeninidine, 5-methoxy-apigeninidine, 5-methoxyluteolinidin, 7-methoxyluteolinidin, 5,7-dimethoxypigeninidine and 5,7-dimethoxyluteolinidin. Abugri *et al.* (2013), analyzing the bioactivity and nutritive compounds of *S. bicolor,* reported that the leaves were mainly constituted by carotenoids, flavonoids and phenolic acids, with small amounts of chlorophyll, lycopene and β-carotene. The fatty acid profiles from the leaves showed palmitic, stearic, oleic and linoleic acid.

Al-Rofaai *et al*. (2012), studying the ovicidal and larvicidal effect of methanolic extracts from the leaves of *M. esculenta* on *Trichostrongylus colubriformis,* stated that the effects found occurred due to the presence of tannins in the leaves of this plant. Chaves *et al.* (2010), who analyzed the chemical constitution of *A. occidentale* extracts, reported that three steroid mixtures were isolated from the ethanol extract from the barks of the stem of this plant, one constituted by palmitate, oleate and sitosterol linoleate; another composed of sitosterol and stigmasterol and the third including 3-O-β-D- galactopyranoside from sitosterol and 3-O-β-D-galactopyranoside from stigmasterol (Chaves *et al*., 2012). A mixture of anacardic acids with an unsaturated chain and 3-O-β-D-glucopyranoside from sitosterol was also isolated.

Conclusions

Traps with sewage water showed the highest attractiveness for the oviposition of *A. aegypti*.

The extract from guava plant leaves showed the highest attractiveness percentage for the eggs of the vector mosquito.

The extracts with repelling action warrant further study of their constituents responsible for the referred effect and potential application against the vector.

The results obtained may be useful to guide the adoption of entomological surveillance methods using ovitraps and to control *A. aegypti.*

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