Biotemas, 28 (4): 105-112, dezembro de 2015
ISSNe 2175-7925

http://dx.doi.org/10.5007/2175-7925.2015v28n4p105

Repellent activity of *Cnidoscolus phyllacanthus* Mart. and *Ricinus communis* L. extracts against *Aedes aegypti* L. oviposition behavior

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Submetido em 04/11/2014
Aceito para publicação em 23/09/2015

Resumo

Atividade repelente de extratos de *Cnidoscolus phyllacanthus* Mart. e *Ricinus communis* L. sobre o comportamento de oviposição de *Aedes aegypti* L. Fêmeas de *Aedes aegypti* põem seus ovos em qualquer lugar úmido e com acúmulo de água. Métodos alternativos com potencial de repelência à oviposição de insetos reduz a infestação contribuindo para controlar epidemias. Avaliou-se a influência de extratos de *Cnidoscolus phyllacanthus* e *Ricinus communis* sobre o comportamento de oviposição de *A. aegypti*. A repelência a oviposição foi avaliada a partir das concentrações letais CL_{50} e CL_{90} obtidas no teste com larvas deste vetor após 24 horas de exposição. O teste de preferência para oviposição de *A. aegypti* foi baseado em duas situações: teste de múltipla escolha e teste sem chance de escolha. Os resultados do Índice de Atividade de Oviposição (IAO) para o teste de múltipla escolha, para ambos *R. communis* e *C. phyllacanthus*, foram negativos. A CL_{90} de *R. communis* e *C. phyllacanthus* apresentaram atividade repelente de oviposição, sendo confirmado pelo IAO = -1. Diferença significativa entre as médias dos tratamentos foi verificado no teste com escolha tanto para *R. communis* quanto para *C. phyllacanthus*. No teste sem chance de escolha, os mosquitos ovipositaram independente do tipo de substrato. A partir dos valores de IAO, todos os produtos apresentaram ação repelente à oviposição de *A. aegypti*.

Palavras-chave: Comportamento de oviposição; Dengue; Extratos de plantas; Vetor

Abstract

Female *Aedes aegypti* lay their eggs on nearly any moist substrate. Methods with potential to repel oviposition may reduce infestation, thereby contributing to control of epidemics. We evaluated the influence of *Cnidoscolus phyllacanthus* and *Ricinus communis* plant extracts on the oviposition behavior of *A. aegypti.* Lethal concentrations were first determined in experiments with larvae after 24 h of exposure, after which LC50 and LC90 were used to test oviposition repellency. The experiment consisted of an oviposition preference test based multiple-choice and no-choice assays. The Oviposition Activity Indices (OAIs) from the multiple-choice test using both *R. communis* and *C. phyllacanthus* were negative, suggesting oviposition repellent and deterrent activity. The LC_{90} of both plant extracts deterred oviposition by this vector, as demonstrated by an OAI = value of -1. In the choice study, mean oviposition values were significantly different between *R. communis* and *C. phyllacanthus*. In the absence of choice, mosquitoes laid eggs independent of the substrate. In conclusion, our OAI values indicate that all substrates used repelled oviposition by *A. aegypti*.

Key words: Dengue; Oviposition behavior; Plant extracts; Vector

Introduction

The control of mosquitoes remains a challenge even after continuous use of synthetic insecticides by the public health sector. The main challenge is development of insecticidal resistance in mosquito vectors, which enables mosquitoes to continue transmitting vector borne diseases in endemic areas (SINGH; MITTAL, 2014). *Aedes aegypti* has epidemiological importance as the main vector of causative agents of yellow fever and dengue. These viral diseases affect many humans, and are serious public health problems throughout the world (FORATTINI, 2002).

Aedes aegypti is a species found in close association with human habitats, and readily enters buildings to feed and to rest. Adult females preferentially feed on humans; other vertebrate species constitute only a small proportion of their blood meals. Unlike many other mosquito species, *A. aegypti* is a day-biting mosquito (JANSEN; BEEBE, 2010). *Aedes aegypti* feeds more than once between successive oviposition events. Moreover, the eggs are the most resistant form in the life cycle and may become quiescent in inhospitable environments (SILVA et al., 2004). Females preferentially lay eggs in man-made or artificial containers including water tanks, flower vases, potted plant bases, discarded tires, buckets, or other containers typically found around or inside the home (JANSEN; BEEBE, 2010). This aspect has made dengue increasingly common, including the occurrence of an “epidemic mosaic”, which includes simultaneous outbreak of numerous viral serotypes (NATAL, 2002).

Vector control strategies typically include the use of synthetic insecticides. The intense use of insecticides has caused substantial numbers of resistant populations which have been described in several Brazilian cities, including Campinas-SP (CAMPOS; ANDRADE, 2001), the Federal District (CARVALHO et al., 2004) Ceará (LIMA et al., 2006) and Paraíba (BESERRA et al., 2007). Thus, alternative methods for control of insects of medical importance deserve greater attention (SIMAS et al., 2004).

Different plant extracts and oils have been studied as potential mosquito oviposition deterrents (PRAJAPATI et al., 2005; RAJKUMAR; JEBANESAN, 2009; SWATHI et al., 2010; WARIKOO et al., 2011; EL-GENDY; SHAALEN, 2012; PHASOMKUSOLSIL; SOONWERA, 2012). Organisms residing in plants that coexist with insects and other microorganisms are commonly natural sources of insecticidal, antimicrobial, fungicidal and allelopathic substances (ROEL, 2001).

Cnidoscolus phyllacanthus is a plant belonging to the Euphorbiaceae family. Structurally, it is composed of several chemical compounds, including favelina methyl ester, which according to Endo et al. (1991a; 1991b) has cytotoxic activities. *Ricinus communis* (Euphorbiaceae), popularly known as the castor oil plant, among other substances contains the alkaloid ricinine, a potentially insecticidal substance (LEITE et al., 2005). The present study evaluated the potential of *C. phyllacanthus* and *R. communis* to repel *A. aegypti* oviposition behavior.

Materials and Methods

Samples of *A. aegypti* populations were collected in the Monte Santo neighborhood, Campina Grande, Paraíba State, Brazil. Eggs were collected twice with one month between collections, using 50 ovitraps placed both inside and outside of five houses per block, for a total of ten blocks.

Establishment and maintenance of *Aedes aegypti* in the laboratory

Laboratory bioassays were conducted in a temperature-controlled room (26 ± 2 °C and 12 h photoperiod) in the entomology laboratory at the Center for Insect Systematics and Bioecology the State University of Paraíba (UEPB). Eucatex wood sheet vanes containing *A. aegypti* eggs from the field were dried for 48 h after collection, then placed in white plastic trays (40 x 27 x 7.5 cm) and filled one third of capacity with distilled water. After hatching, 100 mg / tray of ornamental fish food (Goldfish growth) was added. Adults were kept in cages (~200 per cage) made of wooden frames covered with organza type fabric (40 cm x 40 cm x 30 cm). Mosquitoes were fed a 20% of honey solution, and females were blood fed using quails (*Coturnix japonica*) three times a week for 30 min per feeding. After feeding, a disposable cup containing 200 mL distilled water covered with a plastic funnel coated with a paper filter was places inside of each cage as oviposition substrate.

Collection and preparation extracts of solutions

The seeds used for extraction of the *C. phyllacanthus* oil were provided by the Agricultural and Technical School of the State University of Paraiba, Lagoa Seca-PB. *Ricinus communis* seeds were collected in the city of Esperança-PB. The seeds were washed, milled, and subjected to hydraulic cold pressing to extract oils. The fixed oils were stored in glass bottles covered with aluminum foil, and stored in the refrigerator until the beginning of the experiments.

The repellent effect of *C. phyllacanthus* and *R. communis* extracts was evaluated using the following lethal doses: LC_{50} =0.28 µL/mL; LC_{90}=1.48 µL/mL and LC_{50} = 0.26 µL/mL; LC_{90} = 0.029 µL/mL. Doses were verified with a larvicidal activity bioassay (CANDIDO et al., 2013). Dilutions of the oils were made in a 1:1 ratio (extract: emulsifier). Tween 20 with water was used for controls. The preference test for oviposition of *A. aegypti* was based on a) a multiple choice test and b) a no-choice test, as follows.

Multiple-choice test

Oviposition repellency activity of *C. phyllacanthus* and *R. communis* extracts were evaluated separately in two identical experiments using a randomized block experimental design with three treatments LC_{50} and LC_{90}, and water replicated six times. Each replicate consisted of a cage made of a wooden screened frame (40.0 cm x 40.0 cm width x 30.0 cm depth) containing ten male and ten female *A. aegypti*. Mosquitoes were offered a 20% honey solution and females were blood fed using quails for a period of 30 minutes, for three days in a row prior to initiating oviposition behavior assays. After the third blood feeding, each of the three treatment solutions (LC_{50}, LC_{90} or distilled water) in 200 mL plastic cups were placed inside each cage. In each cup, a funnel plastic coated with a filter paper served as oviposition substrate. Cages were checked daily for a period of 72 h, at which time the filter paper was removed and the number of eggs was counted using a stereomicroscope.

No-choice test

In this test we evaluated oviposition preference following a randomized experimental design, with four replicates per treatment, each replicate consisting of a wooden screened cage (20 cm^3) containing ten male and ten female *A. aegypti*. For each replicate there was only one oviposition substrate available for female mosquitoes. We used the LC50 and LC90 from *C. phyllacanthus* and *R. communis* oil as experimental substrates, and water as control. Cages were checked daily following the same procedures as in the multiple-choice test.

Data analysis

Attractiveness or repellency of the solutions for *A. aegypti* oviposition and preference in multiple-choice test were compared using a Friedman test ($P < 0.05$), and a Kruskal-Wallis test ($P < 0.05$) was used to compare results for the no-choice test. The oviposition activity index (OAI) was determined with the following formula (KRAMER; MULLA, 1979).

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

in which Nt = number of eggs in the test solution and Nc = number of eggs in the control solution. According to these authors the OAI + ≥ 0.3 indicates attractiveness, while OAI ≤ 0.3 indicates that the solution is repellent to oviposition (TIKAR et al., 2014).

Results

In the multiple-choice test with *R. communis* and *C. phyllacanthus*, we observed a clear preference for oviposition in the control solution, where 457 eggs (91%) were deposited in the *R. communis* test, and 108 eggs (73%) were deposited in the *C. phyllacanthus* test (Figure 1). In the test using *R. communis* extract, females select oviposition site based on substrate type (LC_{50}, LC_{90} or water). In this treatment 12 eggs were laid in one other group, in the LC_{50} extract; though the egg number seems small comparatively, this solution repellency with an OAI of -0.9 (Table 1). For all treatments the LC_{90} of *R. communis* showed oviposition deterrent activity against of *A. aegypti,* which was confirmed by an OAI = -1, where egg-laying was not observed (Figure 1).

FIGURE 1: Oviposition response of *Aedes aegypti* females with in multiple-choice tests with lethal concentrations LC_{50} and LC_{90} of *Ricinus communis* and *Cnidoscolus phyllacanthus*.

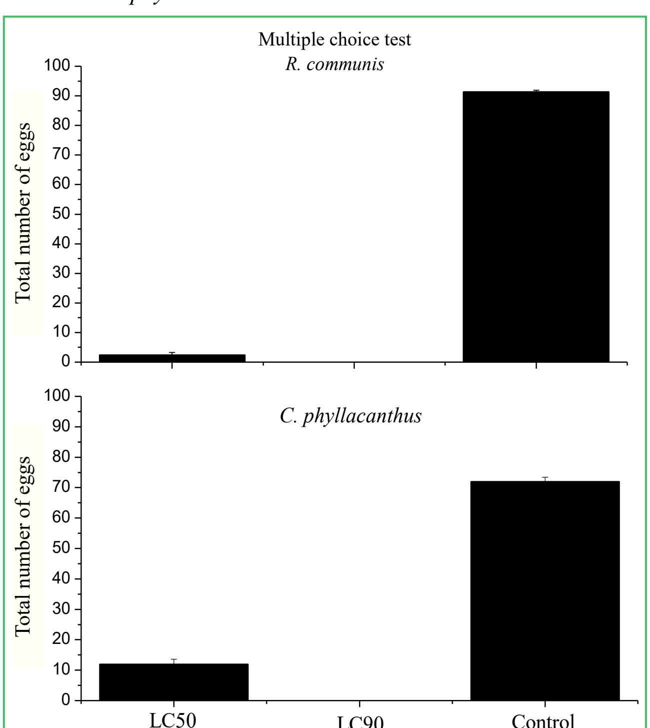


TABLE 1: Comparison of the mean number *Aedes aegypti* eggs laid in substrates with plant extracts in multiple-choice test. Analyzed using Friedman test (α = 0.05), with respective chi-square values (x^2), degrees of freedom (gl), means values and OAIs.

	Ricinus communis	***Cnidoscolus phyllacanthus***
Mean LC_{50}	1.78	1.94
Mean LC_{90}	1.67	1.72
Mean (water)	2.56	2.33
X^2	9.50	5.63
gl	2	2
OAI	LC_{50}=-0.9 LC_{90}=-1	LC_{50}=-0.7 LC_{90}=-1
*p-value	0.009	0.06

* Values significant at $p < 0.05$.

Cnidoscolus phyllacanthus had lower *A. aegypti* oviposition repellant activity than did *R. communis* (Figure 1). However, even though a greater number of eggs were laid in the *C. phyllacanthus* LC50 solution, the extracts still significantly reduced *A. aegypti* oviposition compared to controls.

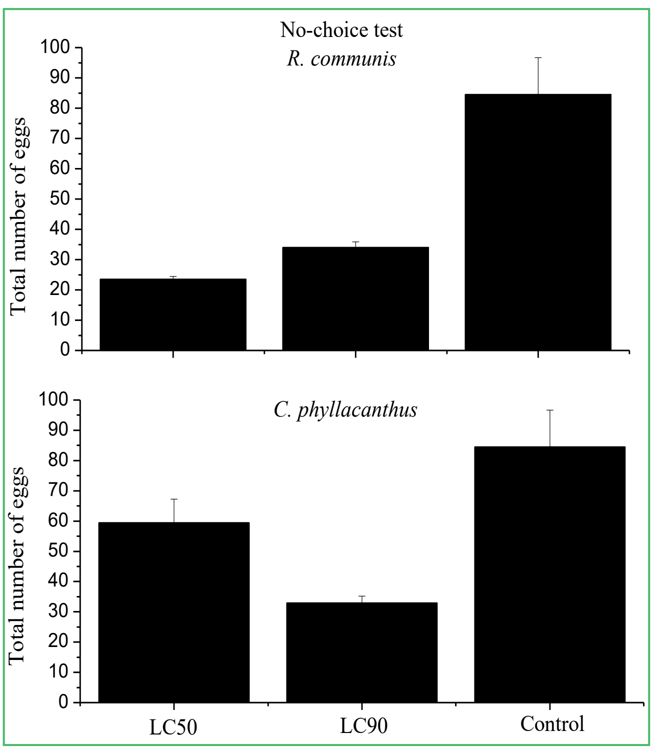
Eggs were also laid in the *C. phyllacanthus* LC50 oviposition substrate, though only in two blocks. Despite differences found in the average numbers of eggs via a Friedman test (Table 1) for *C. phyllacanthus*, the deterrent bioactivity of the product against *A. aegypti* oviposition is not invalidated because there were more eggs in this treatment compared to controls (OAI = -0.7). Similar behavior was observed for the LC_{90} substrate, which showed repellent activity for all experimental blocks (OAI = -1) as in the R. communis tests (Table 1).

The OAI values were negative in the multiple-choice test for both *R. communis* and *C. phyllacanthus*. Both extracts showed repellent effects against *A. aegypti* oviposition, with OAI values lower than -0.3 (Table 1). There were no significant differences between treatments in tests with no choice (x^2 = 0.77, $P \leq 0.05$). Oviposition was observed for all concentrations (Figure 2), demonstrating that females vectors may oviposit in environments less favorable to larval development when there is no clean water available. Although oviposition did occur in *R. communis* and *C. phyllacanthus* extract solution treatments, these treatments also showed repellent activity since LC_{50} and CL_{90} values were both negative (Table 2), in agreement with prior results for choice tests.

Ricinus communis oil at LC_{90} had higher repellent activity than did the LC_{50} despite the increased number of eggs, as indicated by OAI values: LC_{50} = -0.5, and LC_{90} = -0.4 (Table 2).

Aedes aegypti was indifferent to solution type in the no-choice test ($p = 0.77 > 0.05$) (Table 2). The deterrent effect of the lethal concentrations (LC_{50} and LC_{90}) of *R. communis* and *C. phyllacanthus* were similar to controls (Figure 2).

FIGURE 2: Oviposition response of female *Aedes aegypti* in no-choice tests using lethal concentrations LC50 and LC90 of *Ricinus communis* and *Cnidoscolus phyllacanthus*.



Although some eggs were laid in the experimental group solutions, the *C. phyllacanthus* LC_{50} and LC_{90} values indicate oviposition repellent activity against *A. aegypti* (-0.1 and -0.4) for LC50 and LC90 respectively (Table 2). Data from the multiple-choice vs. no-choice tests demonstrate that, when given a choice, *A. aegypti* avoided treatment solutions and preferred no-treatment controls (Figures 1 and 2).

TABLE 2: Comparison of the mean number *Aedes aegypti* eggs laid in substrates with plant extracts in no choice tests. Analyzed using Kruskall-wallace test ($\alpha = 0.05$), with respective chi-square values (x2), degrees of freedom (gl), mean values and OAIs.

	Ricinus communis	***Cnidoscolus phyllacanthus***
Mean LC_{50}	14.5	12.7
Mean LC_{90}	12.7	14.7
Mean (water)	14.7	14.5
X^2	0.505	0.500
gl	2	2
OAI	LC_{50}= -0.5 LC_{90}= -0.4	LC_{50}= -0.1 LC_{90}= -0.4
*p-value	0.77	0.77

* Value significant at $p<0.05$.

Discussion

The OAIs in both multiple-choice and no-choice tests were negative for both *R. communis* and *C. phyllacanthus*. According to Hwang et al. (2003), the OAI ranges from -1 to +1; positive values indicate an attraction or stimulation for oviposition, while negative values indicate repellent or deterrent activity. Values equal to or greater than +0.3 indicate that the material is attractiveness, while values equal to or lower than -0.3 indicate repellency. The differences in OAI values were greater in the no-choice test than in choice tests. According to Beserra et al. (2010), *A. aegypti* females are somewhat flexible with regard to variation in substrate quality, and will oviposit in less favorable sites such as polluted water sources such as raw sewage, when a more appropriate environment for offspring survival is unavailable.

Similar results were observed for *Earias vittella* (Fab) (Lepidotera, Noctuidae) subjected to methanol extracts from *Azadirachta indica* and *Melia azedarach* seeds in the multiple-choice test; females preferred to place a greater number of eggs in the control solution substrate (GAJMER et al., 2002). Chen et al. (1996) also observed that oviposition of *Plutella xylostella* (L.) (Lepidoptera, Acrolepiidae) was reduced by the presence of aqueous extract from *M. azedarach* fruits, with a reduction of 49.6%, 86.6% and 93.5% in freechoice tests, and 46.2%, 72.1% and 80.2% in no-choice tests. Therefore, the results indicate similarity of the oviposition behavior of *P. xylostella* and *E. vittella*, in both tests who underwent to plants extracts.

Nathan et al. (2005) evaluated repellent or deterrent activity of plants on other groups of insects and verified a strong oviposition repellent activity in *M. azedarach* seed and leaf methanol extracts on *Anopheles stephensi* Liston (Diptera, Culicidae) in the laboratory. Medeiros et al. (2005) found 100% deterrent effects of different aqueous extracts of *Sapindus saponaria* L. (Sapindaceae) and *Enterolobium contortisiliquum* Vell. (Fabaceae) fruits, and *Trichilia pallida* Sw (Meliaceae) leaves on *P. xylostella* oviposition in kale leaf discs. The effect of aqueous extracts of *Aspidosperma pyrifolium* Mart. Bark showed higher repellency than *M. azedarach* and *Azadirachta indica* (almond) fruit extracts on *P. xylostella* oviposition (TORRES et al., 2006). In this study, *R. communis* deterred oviposition by *A. aegypti* to a greater extent than did *C. phyllacanthus*.

The use of oviposition deterrents and attractants to modify mosquito oviposition behavior has an important role in mosquito control programs. The selection of an oviposition site by gravid mosquito females is a critical factor that determines species proliferation and population densities, and dispersion in different geographical areas (TIKAR et al., 2014). Plant extracts and oils have been reported by several authors to act as mosquito oviposition deterrents. The action of various oils have been studied for use against *A. aegypti* , *C. quinquefasciatus*, and *Anopheles stephensi* (PRAJAPATI et al., 2005); Elango et al. (2010) observed that leaf extracts in different solvent deterred oviposition by *C. tritaeneorynchus*. The role of essential oils as oviposition-altering compounds has also been in studied in *A. aegypti* (WARIKOO et al., 2011).

Although the number of eggs in the no-choice test was significantly higher than in the multiple-choice test, the deterrent effect of these extracts on adult *A. aegypti* is not disregarded. In one study with different types of oviposition substrates in which mosquitoes were confined in cages containing only one substrate, there were also a greater number of eggs in the unfavorable treatments compared with multiple-choice assays (BESERRA et al., 2010). When only one choice is available, Tauil (2002) suggest that the adaptive capacity of *A. aegypti* in different environmental situations, including those considered unfavorable for production of offspring (e.g., the LC_{50} and LC_{90} plant extracts used in this study) female vectors adapt to the conditions available.

According to Lopes et al. (1993), *A. aegypti* shows no preference for container type, but prefers environments with water that does not contain high levels of pollutants. In the present work, the females preferred to oviposit in the control solution containing only water. An environment considered favorable for oviposition is associated with conditions that the medium presents that foster development and survival of immature mosquitoes. Factors such as the color of the vivarium, organic matter, and other substances that favor the development of the immature mosquitoes may serve as stimuli to the female when choosing a location for oviposition (CONSOLI; LOURENÇO-DE-OLIVEIRA, 1994). Humidity and rainfall have overt impacts on mosquito survival and ecology, and other climatic factors such as photoperiod and wind velocity may also be influential. However, the domestic nature of this species probably exerts more influence on its distribution than either environmental factor (JANSEN; BEEBE, 2010). Generally speaking, the greater the repellency the lower the infestation, thus reduction or elimination of egg-laying causes a reduction in the number of insects. Our study concludes that *C. phyllacanthus* and *R. communis* repel oviposition by *A. aegypti*. These results should encourage the search for new active natural compounds, offering an alternative to synthetic repellents and insecticides from other medicinal plants.

Acknowledgements

The authors would like to thank Paraíba State University for the opportunity to develop this research through the "Incentive Program of Postgraduate Studies and Research"-PROPESQ/UEPB.

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