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Article

Insecticide activity of sodium ricinoleate on *Aedes* (Stegomyia) *aegypti* (Linnaeus, 1762)

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Karla Porto[1]*, Antonia Railda Roel[2], Hemerson Pistori[3], Alessandra N. Souza[4], Alexandre A. Machado[5], Lucas R. R. Souza[6]

[1]Programa de Mestrado em Biotecnologia, Universidade Católica Dom Bosco, Av. Tamandaré, 6000, 79117-900, Campo Grande-MS, Brasil, Phone 55 067 3312.3702. E-mail: portokra@gmail.com, [2]Programa de Mestrado em Biotecnologia, Universidade Católica Dom Bosco, Av. Tamandaré, 6000, 79117-900, Campo Grande-MS, Brasil. E-mail: arroel@ucdb.br, [3]Departamento de Pesquisa, Universidade Católica Dom Bosco, Av. Tamandaré, 6000, Jd. Seminário 79117-900, Campo Grande-MS, Brasil. E-mail: pistori@ucdb.br, [4]Centro de Pesquisas do Pantanal, R. Nove, 305 - Cpa III 78068-410, Cuiabá-MT, Brasil. E-mail: nutricionistaalessandra@yahoo.com.br, [5]Departamento de Pesquisa, Universidade Católica Dom Bosco, Av. Tamandaré, 6000, Jd. Seminário 79117-900, Campo Grande-MS, Brasil. E-mail: amachado.net@gmail.co, [6]Instituto de Química, Universidade Federal do Mato Grosso do Sul, Av. Senador Filinto Muller, 1555, Vila Ipiranga 79070-900, Campo Grande, MS, Brasil. E-mail: lucas_roel@gmail.com*

Abstract

This research has been dedicted to developing an insecticide taken from plants to combat the major vector of dengue fever virus in Brazil. Sodium ricinoleate, derived from the oil of *Ricinus communis* L., presented with insecticidal activity in preliminary tests carried out in Bioprospection, using native plants. The objective was to evaluate the deleterious effects of sodium ricinoleate on *A. Aegypti*, and to determine the lethal concentration (CL). The experiment was carried out with the third instar larvae, 25 per treatment, in quadruplicate. Concentrations of solution were tested: 1% (1 $mg.dL^{-1}$), 0,5% (0,5 $mg.dL^{-1}$), 0,1% (0,1 $mg.dL^{-1}$), 0,05% (0,05 $mg.dL^{-1}$) and 0,01% (0,01 $mg.dL^{-1}$), respectively, diluted in water. To calculate the lethal concentrations (LC) the mortality of larvae was observed 24hours after the treatments. It is concluded that sodium ricinoleate causes 90% mortality of the larvae of *A. aegypti* in concentrations up to 0,2025 $mg.dL^{-1}$. Sub lethal doses, lower than CL_{50}, in *A. aegyti* larvae causes total mortality of larvae or pupae stage, no adults emerge from these treatments.

Additional keywords: active ingredients, dengue, pests control, ricinoleic acid.

Introduction

Dengue fever virus, an epidemic in many parts of Brazil, is transmitted by the mosquito *Aedes aegypti*, currently also responsible for the transmission of simultaneous chikungunya virus (CHIKV) (Nuckols *et al.* 2015). The high rate of ground population in the cities and increasing accumulation of artificial breading of larvae, favours the proliferation of this insect, especially in hot and rainy seasons.

The repetitive use of chemical synthetic insecticides to control *A. aegypti*, in the last 20 years, and the lack of regularity of educational campaigns, has provided favourable conditions to increase its area of distribution and survival. This causes the appearance of *A. aegypti* populations with phenotype resistant (Andrade 2008). To control *A. aegypti* larvae Themephos insecticide was used for many years but this only resulted in resistance, in many parts of Brazil. This situation has led to deployment to campaigns to monitoring mosquitoes susceptibility to chemical synthetic insecticides (Campos and Andrade 2001, Braga and Valle 2007).

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Some products have been studied for this insect control, with emphasis on plant extracts, essential oils of citronella, eucalyptus, geranium and soybean (Masetti and Maini 2006). The potential of insecticide from plants provides use of molecules with specific activity in the control of insect vector diseases, which by their complex composition causes a reduction in environmental risk (Porto *et al.* 2008). These normally fit in like more safe insecticides in pest control, relatively harmless to non-target organisms, and thus not interfering with biological control (Oliveira and Bleicher 2006).

The castor oil plant *Ricinus communis* (Linnaeus, 1753), a very common plant in several regions of Brazil. It is used in the production of industrial lubricants, resins, paints, cosmetics and medicines and also used for biodiesel production. The main component of this oil is ricinoleic acid, which has molecules that have flexible properties and unusual fatty acids (Silva 2005). In the oil seed there is a protein, ricin, which is one of the most potent natural toxins that exist, with insecticidal properties for ants *Atta sexdens rubropilosa* (Cazal *et al.* 2009). However, the main product is castor oil, which generally has 90% of ricinoleic acid, but ricinine is retained in the oil cake (Hoffman *et al.* 2007).

The aim of this study was to evaluate sodium ricinoleate's potential as an insecticide, lethal doses, and it's effect on the biological cycle of *A. aegypti*.

Material and Methods

Creation of *A. aegypti*

The eggs were collected using the ovitrapas trap, in Campo Grande, Mato Grosso do Sul, according to the methodology of Zoonosis Control Center (CCZ). The creation was maintained in a climatized chamber BOD (SPLabor, SP500), adjusted to the temperature 28±2ºC, fotophase 14 hours, in Entomology laboratory of Universidade Católica Dom Bosco, UCDB. Larvae was kept in dechlorinated water and pH 7,0 with sodium bicarbonate was maintained.

The larvae was fed daily with 0,02 mg of normal, crushed, cat food. The pupae was transferred to cages made of wood and covered with fine woven plastic until adults. The adults were fed with an 8% sweet solution. The female was fed with pidgeon blood for an hour and a half, twice a week, according methodology adapted to Laranja (2006).

Tests to determine Lethal Concentration (LC)

The test to determine Lethal Concentration (LC) was developed with third instar larvae from the first generation in the laboratory. The larvae was separated in groups of 25 individuals, in a solution of 25 mL each treatment, in four repetitions per treatment. Maintaining the proportion of one individual for 1 mL of solution. The dechlorinated water was used for the negative control, and Rotenone (Sigma-Aldrich) in concentrations 0,01 $mg.mL^{-1}$ (10 ppm) was used for the positive control, figure 1. The concentrations were tested: 1% ($1mg.dL^{-1}$); 0,5 (0,5 $mg.dL^{-1}$); 0,1% ($0,1mg.dL^{-1}$); 0,05% (0,05 $mg.dL^{-1}$) e 0,01% (0,01 $mg.dL^{-1}$) of Sodium Ricinoleate (37% ricinoleic acid), to calculate the Lethal Concentrations (LC_{10}, LC_{50} and LC_{90}). It was considered, after 24 hours, that the larvae showed no reaction when tested with the pipette Pasteur. The experimental design was completely randomized with four repetitions, each treatment contained 100 larvae, or 25 larvae per repetition.

The test to determine the effect of the sub lethal doses

The tests have been conducted at concentrations of 0,1% (0,1 $mg.dL^{-1}$); 0,05 (0,05 $mg.dL^{-1}$) and 0,01% (0,01 $mg.dL^{-1}$) of Sodium ricinoleate (37% ricinoleic acid) for observation of smaller doses, to determine lethality or other deleterious effects on larvae and pupae. These doses were determined based in LC50 determined in pre tests, as described before. The larvae was separated in groups of 25 individuals in a solution of 25 mL each treatment, in four repetitions per treatment. The experimental design was completely randomized with four repetitions; each treatment contained 100 larvae, or 25 larvae per repetition.

Statistical analysis

The result obtained from the data from lethal concentration was analyzed by the Probit Method, using the software Leora® (POLO 97355947870655352).

The data of larval and pupal duration was registered in hours. The data of larval and pupae mortality was transformed in percentage.

Results and Discussion

The lethal concentration (LC_{50}) for sodium ricinoleate in this population was 0,1125 $mg.mL^{-1}$ (0,1100 at 0,1140 $mg.mL^{-1}$). For control the mortality 90% of this population only 0,2025 $mg.mL^{-1}$ (0,2000 at 0.2040 $mg.mL^{-1}$), was necessary (Table 1, Figure 1).

According to Mendonça *et al.* (2005) oils from *Anacardium occidentalis*, *Copaifera langsdorffii*, *Carapa guianensis*, *Cymbopogon winterianus* and *Ageratum conyzoides* have high activities with LC_{50} values of 0,145, 0,041, 0,057, 0,098 and 0,148 $mg.mL^{-1}$, respectively. The most active ethanolic extract tested was that from the stem of *Annona glabra* which presented an CL_{50} value of 0,027 $mg.mL^{-1}$.

After establishing the level of lethal concentration, it was observed, that in sub lethal doses, the larvae didn't become pupae for a long time, or they died in the pupae stage. Many effects from plants on insects were related, as a repellent, egg-laying inhibition, feeding inhibition, deformations in pupae and adults and mortality (Barreto 2005). However it was necessary to consider the effects in sub lethal dosage on insect, which can cause a low reproductive rate (Shaalan *et al.* 2005). The substance Grandsin, was isolated from *Piper solmasianum* C. (Piperaceae) causing larval mortality CL_{50} 0,150 $mg.mL^{-1}$ and changes in the anterior-middlegut, with intense tissue destruction and cell disorganization (Leite *et al.* 2012).Furtado *et al.* (2005) indicated that the essential oil of *Vanillosmo psisarborea* Baker and *O. gratissima* L. is composed of substances with larvicidal properties for *A. aegypti*, with CL_{50} of 0,0159 $mg.mL^{-1}$and CL_{50}de 0,0958 $mg.mL^{-1}$, respectively.

Table 1. Lethal Concentration (LC) of Sodium Ricinoleate on *Aedes aegypti* larvae.

CL_{10} (IC 95%) $mg.dL^{-1}$	**CL_{50} (IC 95%) $mg.dL^{-1}$**	**CL_{90} (IC 95%) $mg.dL^{-1}$**
0,0225 (0,02012 –0,02479)	0,1125 (0,11012– 0,11487)	0,2025 (0,20012 – 0,20487)

IC 90% - 95% Confidence Interval

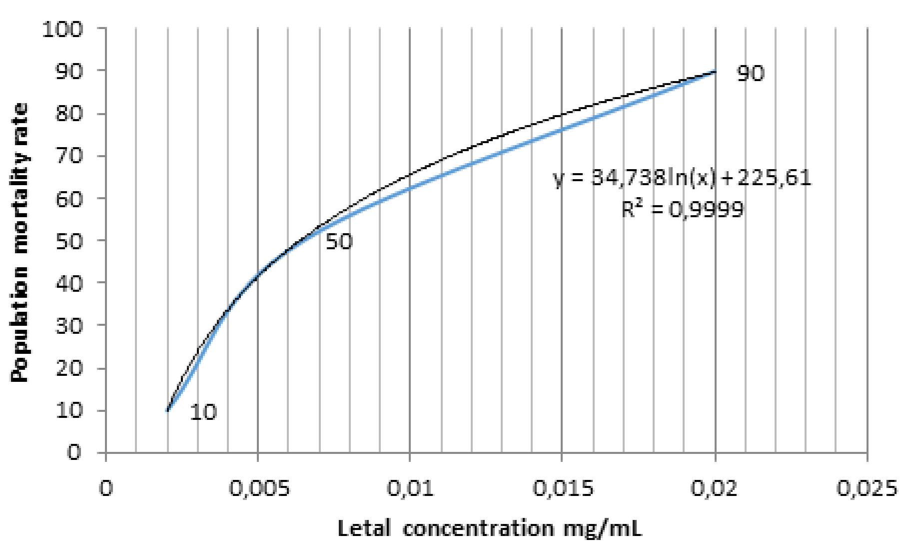

Figure1. Lethal concentration (LC) in mg.mL -1 rotenone on larvae of *Aedes aegypti*.

The concentrations of sodium ricinoleate used in this study to control *A. aegypti* larvae proved to be a potential insecticide, for dengue fever vector. This product has the advantage of being a surfactant, having an affinity with water, which gives the characteristic of a higher solubility (Dabdoub *et al.* 2009).

On treatment with concentration of lower lethal doses (0,1, 0,05 e 0,01 $mg.mL^{-1}$) changes were observed in the total cycle total of *A. aegypti*. The mortality level of the larvae was 99, 97 and 84% in the concentration of 0,1, 0,05 e 0,01 $mg.mL^{-1}$ respectively. While in the negative control only 2% of larvae died. The larvae on treatment took for 400 hours to die, while in the negative control took 168 hours.

There was an increase of larvae duration in all treatments with 336, 192, 384 h, while the control was 98 h. Statistical analysis was not possible due to only one larvae transforming into pupae in 0,01 $mg.mL^{-1}$ treatment. However, the larvae phase had a longer period of time in all the treatments 336, 192 and 384 h, in relation to control, within 98 h, in average. All the pupae died in all of the treatments, but in the control the adults emerged from all pupae, not one died (Table 2).The variation of larval duration for each treatment was observed, but no statistical analysis was carried out.

Some extracts from plants may reduce viability of eggs, nymphs, larvae and pupae, which makes them important for the reproduction of the insects, which enables the development of formulations and broadens its usage time (Corrêa and Salgado 2011).

Conclusion

Sodium Ricinoleate (37%) is an efficient insecticide using a low concentration in the larvae phase, can cause total mortality at the end of the life cycle of the *A. aegypti*, as no adults will emerge.

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Table 2. Effect of sub lethal doses of Sodium ricinoleate on the development and the mortality of larvae and pupae of *Aedes aegypti* L. (Culicidae).

$mg.dL^{-1}$	Larvae duration (hours)	Larvae Mortality (%)	Pupae Mortality (%)
0,1	336±36,0(n=1)	99	100
0,05	192±68,93 (n=3)	97	100
0,01	384±114,8 (n=16)	84	100
Control	168±14,0 (n=98)	2	0

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