SCIENTIFIC NOTE

# Larvicidal Activity of *Copaifera reticulata* Ducke Oil-Resin against *Culex quinquefasciatus* Say (Diptera: Culicidae)

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Atividade Larvicida do Óleo-Resina de *Copaifera reticulata* Sobre *Culex quinquefasciatus* Say (Diptera: Culicidae)

RESUMO - A evolução de resistência de *Culex quinquefasciatus* Say aos inseticidas sintéticos e a modificação da suscetibilidade desse mosquito aos piretróides, estimularam estudos sobre a atividade de plantas inseticidas, como alternativa para o seu controle. Neste trabalho foram realizados ensaios biológicos para verificar a atividade larvicida do óleo-resina de *Copaifera reticulata* sobre *C. quinquefasciatus*. Para a realização dos bioensaios o óleo-resina foi solubilizado com dimetilsulfóxido (DMSO), na proporção de 0,4 ml do DMSO para 24,6 ml de água destilada. Para cada estádio, os bioensaios foram realizados em copos descartáveis, contendo 100 ml de solução na qual colocavam-se 20 larvas, com quatro réplicas para ajuste das concentrações letais. As leituras de mortalidade foram feitas após 48h de exposição das larvas à solução. O controle foi feito em água destilada e DMSO, com o mesmo número de réplicas dos bioensaios. Os experimentos foram realizados numa câmara biológica, climatizada a 28 ± 1°C, umidade relativa de 80 ± 5 % e fotofase de 12h e posteriormente, no campo. Foram consideradas mortas as larvas que não possuíam movimentos. O óleo-resina de *C. reticulata* demonstrou atividade larvicida para todos os estádios de *C. quinquefasciatus*. As $LC_{50}$ encontradas para larvas de 1º, 2º, 3º e 4º estádios foram de 0,4; 0,9; 39 e 80 ppm, e as $LC_{99}$ foram de 15; 15; 50 e 180 ppm, respectivamente.

PALAVRAS-CHAVE: Inseticida botânico, elefantíase, controle, Culicinae

ABSTRACT - The appearance of resistance of *Culex quinquefasciatus* Say to chemical insecticides and the modification of the susceptibility of this mosquito to pyrethroid has stimulated studies on the activity of plants as alternative for its control. In this study, bioassays were carried out to verify the larvicidal activity of the oil-resin of *Copaifera reticulata* on *C. quinquefasciatus.* The bioassays were carried out with the oil dissolved in dimethylsulfoxide (DMSO), at the proportion of 0.4 ml of DMSO to 24.6 ml of distilled water. For each instar, the bioassays were carried out in plastic glasses with 100 ml solution using 20 larvae, wit four replications to adjust the lethal concentrations. The mortality observations were made after 48h exposure of the larvae to the solution. The control was carried out in distilled water and DMSO, with the same number of replications as the bioassays. The experiments were carried out in a biological chamber, controlled at 28 ± 1°C, 80 ± 5% relative humidity and 12h light. The larvae without movement were considered dead. The *C. reticulata* oil-resin demonstrated larvicide activity for all the *C. quinquefasciatus* instars. The $LC_{50}$ found for the $1^{st}$, $2^{nd}$, $3^{rd}$ and $4^{th}$ larval instars were: 0.4, 0.9, 39 and 80 ppm, and the $LC_{99}$ were 15, 15, 50 and 180 ppm, respectively.

KEY WORDS: Botanical insecticide, elephantiasis, control, Culicinae

*Culex quinquefasciatus* Say is the main transmitter of bancroftian filariasis in the world and is the only vector in the Americas in very humid areas. In Brazil, transmission is restricted to the states of Alagoas, Pará and Pernambuco (Rocha & Fontes 1998). Filariasis is caused by *Wucheria bancrofti* (Cobbald), a helminth that lives in the lymph glands and vessels that provokes edemas by lymph obstruction. It is responsible for the more severe clinical manifestations in the lower limbs (elephantiasis) and the scrotum (World Health Organization 1992,1994).

The high *C. quinquefasciatus* population density in the cosmotropical area has triggered several interventions by the public health authorities using wide synthetic insecticide application as the main means of combat and control.

However, the inefficiency of the organophosphate and carbamate insecticides (Bracco *et al.* 1999, Hemingway *et al.* 1985, Failloux *et al.* 1994), along with the need for safer methods regarding toxicity to man and the environment has stimulated the search for new means of control. Oil-resin or plant extracts are an alternative with potential for use.

Studies have shown the potential of plants for use in *C. quinquefasciatus* control, such as *Piper nigrum* (Chahad & Boof 1994), *Azadirachta indica, Rhazya sticta* and *Syzygium aromaticum* (Mishra *et al*. 1995, Su & Mulla 1998, Das *et al*. 1999, El-Hag *et al*. 1999), *Agave americana* and *Kaempferia galanga* (Dharmshaktu *et al.* 1987, Choochote *et al*. 1999, Pizarro *et al*. 1999), *Atriplex halimus* (El-Gougary 1998, Massoud & Labib 2000) and *Commiphora molmol* (Pitasawat *et al*. 1998).

The objective of this study was to investigate insecticide substances in plants to find an alternative for *C. quinquefasciatus* control. After previous studies with dozens of plant species, *Copaifera reticulata* (Leguminosae) presented the greatest potential. The oil-resin of this plant has been used up to now only as a phytotherapeutic, anti-inflammatory and antimicrobial of the respiratory, urinary and dermatological systems and as a healer for ulcers and wounds (Corrêa1984, Veiga Jr & Pinto 2002).

The *C. reticulata* oil-resin used in the bioassays was obtained in August 2001 in Jacundá, PA, by exudation through a perforation of approximately 70 cm in the base of the trunk of the plant (about 50 cm above soil level). The oil-resin was placed in amber-colored glass flasks until the bioassays with *C. quinquefasciatus*.

The *C. quinquefasciatus* larvae used in the tests were reared in plastic basins with water from the public water supply, similar to the methodology by Silva *et al.* (1998) in an acclimatized chamber with temperature 28 ± 1°C moisture 80 ± 5% and 12h photoperiod.

The stock solutions were prepared by weighing the *C. reticulata* oil-resin on analytical scales using dimethylsulfoxide as solubilizer (DMSO). The quantity of DMSO used to prepare the solution had been determined previously by tolerance experiments with *C. quinquefasciatus* larvae to find the non-lethal concentrations. These were observed at a proportion of 0.4 ml DMSO to 24.6 ml distilled water.The stock solution was prepared at the 500 ppm concentration, using 500 mg *C. reticulata* oil-resin dissolved in 16.2 ml DMSO and the volume completed to 1000 ml with distilled water. The other solutions were obtained from this by dilution with distilled water to the required concentrations. Later 100 ml of the solutions with appropriate concentrations were transferred to disposable plastic cups to carry out the tests, in which the larvae were placed with the help of a disposable plastic pipette. In the laboratory 20 larvae were used at each instar for each experiment, with four replications, and the same number for their respective controls, using only DMSO at the proportion of 0.4 ml to 24.6 ml distilled water. The laboratory experiments were carried out in an acclimatized chamber under the same conditions as the mosquito rearing. In the field, the experiments were carried out in six different types of recipients, considered as potential breeding-grounds (ceramic pot, cement and asbestos vase, plastic bottle, tire and glass aquarium) with two replications, placing 1.5 liters of the solution in each one. One hundred 4th-instar *C. quinquefasciatus* larvae were placed in each one of the recipients. The same quantity of larvae was used for the control, placed in the DMSO and distilled water solution keeping the proportion of the laboratory experiments.

In the laboratory, the mortality was read 48h after the start of the tests and after 24, 48 and 72h in the field. The larvae were considered dead when there was total absence of movement, with darkening on the body and head capsule. The lethal concentrations were interpoled by the Probit analysis by the Statistical Product and Service Solution program (SPSS) only for the laboratory tests.

The mixture of oil-resin with DMSO and distilled water was milky-white in color, semi transparent with a balsamic smell. The results of the larval mortality assessed after 48h of exposure in the laboratory are shown in Table 1.

Table 1. Susceptibility of *C. quinquefasciatus* larvae to *C. reticulata* oil-resin after 48h exposure. (n = 100 larvae)

| Larval stages | $LC_{50}$ (ppm) (CI 95%) | $LC_{99}$ (ppm) (CI 95%) |
|---|---|---|
| 1ª | 0,40 (0,29-0,46) | 15 (13.6-17,09) |
| 2ª | 0,91 (0,67-0,98) | 15 (13.6-17,09) |
| 3ª | 39 (41,98-36,02) | 50 (46,01-53,00) |
| 4ª | 80 (79,01-83,99) | 180 (170,03-190,01) |

CI 95% - Confidence interval at 95% probability. Obs: there was no mortality in the control group.

The *C. reticulata* oil-resin acted on all the *C. quinquefasciatus* larval stages. The 4th instar larvae were the most tolerant to the lethal concentrations, indicating this concentration for use in the combat interventions of this mosquito. In the field, the larva mortality was total after 72h of contact with the solution in all the types of potential breeding grounds tested. However, in the glass, plastic and tire breeding grounds, 100% death occurred after 24h exposure. This is an interesting fact because these types of recipients are predominant in urban areas and thus there are perspectives and possibilities for successful use of the oil-resin. After 24h exposure in the recipients – ceramic, cement and cement-asbestos – the mortality was respectively, 90, 91 and 95%, and after 48h, it was 95, 95 and 98%.

Studies were not found in the literature on the larvicidal properties of *C. reticulata* oil-resin for culicids. However, studies with other plants taking as base their lethal concentrations and indication for use, served as reference for the potentiality of *C. reticulata* oil-resin for use in *C. quinquefasciatus* control. Pizzarro *et al.* (1999) studied the activity of the dehydrated gross extract and saponine fraction of *Agave sisalana* and estimated the lethal concentration $LC_{50}$, $LC_{90}$ and $LC_{95}$ for 3rd instar *C. quinquefasciatus*, that were 183, 408 and 512 ppm, respectively. These concentrations were much higher than those reported in this study, but these authors suggested its use for control of this mosquito. Based on the lethal concentrations, *C. reticulata* presented the most promising form, and its potential for use increased when referring to its non toxic activity for hepatic cells demonstrated by Brito *et al.* (2000) in rats.

The larvicidal activity of plants in the Philippines was studied by Monzon *et al.* (1994) by the gross aqueous extract for 3rd and 4th *C. quinquefasciatus* instars and presented extremely high lethal concentrations and $LC_{90}$ of 37, 28, and 24g/100ml water, respectively, for *Lansium domesticum, A. indica, Eucalyptus globosus* and *Codiaeum variegatum*. This corresponded to a concentration one thousand times greater than reported in this study.

Chahad & Boof (1994) assessed the toxic effect of *P. nigrum* extracts obtained by the soxhlet methanolic and acetonic methods on 4th-instar *C. quinquefasciatus* larvae after 36h exposure and reported $LC_{99}$ of 41.6 and 35.7 ppm, respectively. The results of the black pepper extracts were inferior but compatible with the results obtained with the *in natura C. reticulata* oil-resin.

The *C. reticulata* oil-resin $LC_{50}$ for the 4th *C. quinquefasciatus* instar was 80 ppm. This larvicidal activity was similar to the isolated main ingredient, lemonine, from the *Citrus reticulata* seed in India by Jayaprakasha *et al*. (1997). However, the $LC_{50}$ of *C. reticulata* oil-resin for the same instar of this mosquito presented an inferior result to the isolated main ingredient, carvacrol, from the Egyptian plant *Thymus capitatus* whose lethal concentration was 100 ppm (Mansour *et al.* 2000).

In Thailand, the larvicidal activity of *K. galanga* extractions was studied with several solvents in 4th instar *C. quinquefasciatus* larvae where the hexane fraction had a $LC_{50}$ of 42.3 ppm (Choochote *et al.* 1999). This result was slightly lower than that reported for the *in natura C. reticulata* oil-resin solution.

*C. reticulata* presented lower lethal concentration ($LC_{50}$ and $LC_{99}$) for *C. quinquefasciatus* than the corresponding concentration for the second most promising plant, *Magonia pubescens*, studied by Silva *et al.* (1996) and Guimaraes (2001) with 3rd instar *Aedes aegypti* L.

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