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Toxic effect and genotoxicity of the semisynthetic derivatives dillapiole ethyl ether and dillapiole *n*-butyl ether for control of *Aedes albopictus* (Diptera: Culicidae)

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ABSTRACT

Two derivatives of dillapiole, dillapiole ethyl ether (1KL39-B) and butyl ether-*n* dillapiole (1KL43-C), were studied for their toxicity and genotoxicity against *Aedes albopictus*, to help develop new strategies for the control of this potential vector of dengue and other arboviruses, because it is resistant to synthetic insecticides. Eggs and larvae exposed to different concentrations of 1KL39-B (25, 30, 50, 70, and 80 μg/mL) and of 1KL43-C (12.5, 20, 25, 30 and 40 μg/mL) exhibited toxicity and susceptibility, with 100% mortality. The LC_{50} was 55.86 ± 1.57 μg/mL for 1KL39-B and 25.60 ± 1.24 μg/mL for 1KL43-C, while the LC_{90} was 70.12 μg/mL for 1KL39-B and 41.51 μg/mL for 1KL43-C. The gradual decrease in oviposition of the females of the G_1 to G_4 generations was proportional to the increase in concentrations of these compounds, which could be related to the cumulative effect of cell anomalies in neuroblasts and oocytes ($P < 0.05$), including micronuclei, budding, multinucleated cells and nuclear bridges. These findings showed that both 1KL39-B and 1KL43-C can serve as potential alternatives in the control of *A. albopictus*.

1. Introduction

Aedes (*Stegomyia*) *albopictus* Skuse, 1894 is a mosquito with the potential to transmit 26 arboviruses, including *Dengue virus* (DENV), *Chikungunya virus* (CHIKV) and *Zika virus* (ZIKV) [1–6]. Dengue affects over 50 million people every year in several countries, accounting for high morbidity among the sick. [7] Although there is no record of the involvement of *A. albopictus* in the transmission of arboviruses to humans in Brazil, this species is of great epidemiological importance in this transmission process in Asia and Africa [8–11]. Furthermore, *A. albopictus* can serve as a bridge between the wild and urban cycles of yellow fever because of its ease of adapting to different environments [12].

A. albopictus is native to Asia and its spread around the world began in 1980 [12,13]. The first record of this mosquito in Brazil was in Rio de Janeiro in 1986 [14] and in the Amazon region in 1996 [15]. It has a rural habitat [16,17], but it is also found in peri-urban regions and is generally associated with areas with vegetation [18]. The introduction of people in these areas increases the risk of arbovirus transmission by *A. albopictus* [3].

Synthetic insecticides are commonly used to control mosquito vectors, with the main ones being organochlorines, organophosphates, carbamates and pyrethroids [19–22]. These insecticides block sodium and potassium channels [23,24], act as GABA receptor antagonists [23,25] or inhibit the enzyme acetylcholinesterase, leading to accumulation of acetylcholine [26,27], which interferes with the transmission of nerve impulses, causing the death of the mosquito [23,25,26]. However, mosquitoes acquire resistance to most synthetic insecticides used [23,24,26–28].

Natural plant-based compounds, such essential oils or their components have been studied as alternatives in vector control [29–31]. Dillapiole is a compound found in the essential oil of the long pepper plant (*Piper aduncum*), which has been tested for its potential in the control of *A. aegypti* [32]. Another approach is to use semisynthetic compounds of dillapiole and other natural products, to improve the physical and chemical properties of these substances and to achieve better efficacy [32–35].

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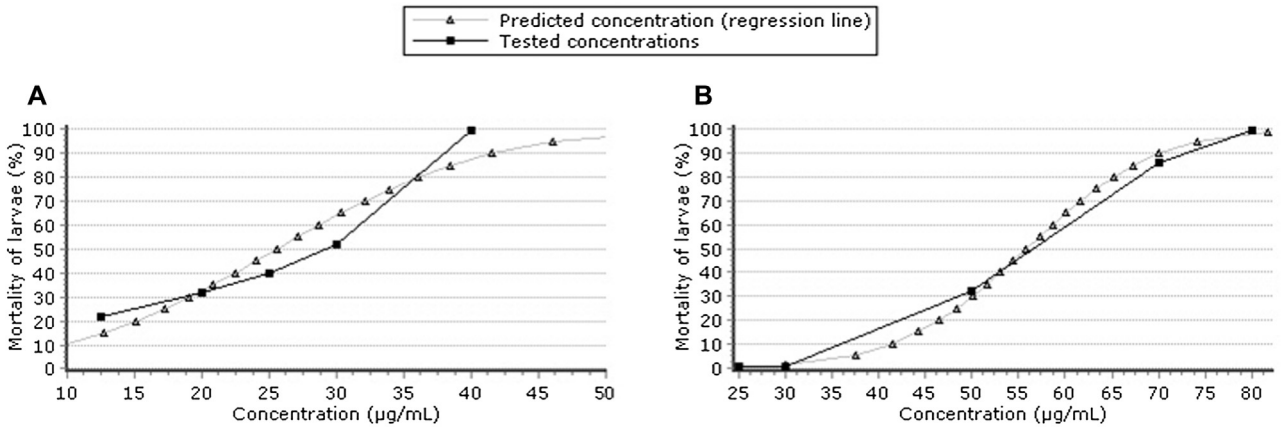

Fig. 1. Probit analysis of mortality of *A. albopictus* larvae after 24 h of exposure to 1KL39-B and 1KL43-C. A – experimental doses of 1KL39-B (25, 30, 50, 70 and 80 μg/mL) and dose predicted from linear regression; B – experimental doses of 1KL43-C (12.5, 20, 25, 30 and 40 μg/mL) and dose predicted from linear regression.

Table 1
Average and ±standard deviation of eggs of *Aedes albopictus* females exposed to 1KL39-B and 1KL43-C, and untreated controls, along different generations.

Compound	Concentration (μg/mL)	Generation			
		G_1	G_2	G_3	G_4
Control	0	69.0 ± 1.0	66.0 ± 3.4	63.3 ± 3.2	62.6 ± 2.5
1KL39-B	50	50.3 ± 1.5	44.0 ± 4.5	28.3 ± 4.1	14.0 ± 3.3
	70	51.3 ± 3.2	45.3 ± 4.5	34.0 ± 1.0	27.3 ± 2.5
1KL43-C	12.5	33.0 ± 2.0	34.3 ± 4.0	15.6 ± 3.2	14.0 ± 4.1
	20	51.3 ± 2.5	46.3 ± 2.0	30.0 ± 4.5	14.0 ± 4.5

Cytogenetics has helped to characterize anomalies in mosquito cells exposed to genotoxic substances in both *in vitro* and *in vivo* assays [32,35]. The evaluation of nuclear abnormalities uses tools such as the micronucleus test *in vivo*, considered standard in genotoxicity assays [32,35,36], where it is a quick and easy way to determine the genotoxic effects of certain compounds on a given organism [37].

Considering the data in the literature, semisynthetic derivatives of dillapiole as well as dillapiole, at concentrations over 80 μg/mL are toxic to *A. aegypti*, but when used at much lower concentrations, DNA damage is observed, along with a decrease in fertility [35]. *Aedes albopictus* lacks similar data regarding alternative insecticides for vector control for mosquitoes in general. Accordingly, the aim of the present study was to evaluate the genotoxicity of 1KL39-B and 1KL43-C in neuroblasts and oocytes of exposed specimens of *A. albopictus*, and also to determine if the frequency of alterations was related to a decrease in fertility of individuals exposed to these compounds and of their descendants.

2. Materials and methods

2.1. Derivatives of dillapiole

Two semisynthetic compounds, dillapiole ethyl ether (1KL39-B) and dillapiole *n*-butyl ether (1KL43-C), were obtained by the addition of the radicals ethyl (-CH_2-CH_3) and *n*-butyl (-CH_2-CH_2-CH_2-CH_3), after successive isomerization, oximercuration, epoxidation and oxidation, according to Pinto et al. [34].

2.2. Collection of material

Larvae of *A. albopictus* were collected in the Aleixo neighborhood (03° 05′ 29.1″ S, 59° 59′ 40.7″ W) of Manaus, state of Amazonas, Brazil in order to fix a colony. The specimens were analyzed according to the taxonomic identification key of Consoli and Lourenço-de-Oliveira [38]. The samples were reared using Tetra Cichlid food under standard insectarium conditions at 26 °C and 70% relative humidity, at Coordenação de Sociedade, Ambiente e Saúde (CSAS), Instituto Nacional de Pesquisas da Amazônia (INPA). After the emergence of adult individuals, males were fed with glucose solution (5%) and females with hamster (*Mesocrisetus auratus*) blood to obtain the G_1 generation. This progeny were used to produce subsequent generations (G_2, G_3 and G_4).

2.3. Toxicological analysis

Larvae and eggs of *A. albopictus* were exposed to 1KL39-B and 1KL43-C for determination of the 50 and 90% lethal concentration (LC_{50} and LC_{90}) of each compound. In the larvicidal evaluation, 50 3rd instar larvae individuals (G_1 generation) were used, divided into five replicates (n = 10 larvae), for each treatment tested, along with a negative control using 1% DMSO. We used five concentrations of 1KL39-B (25, 30, 50, 70, and 80 μg/mL) and of 1KL43-C (12.5 20, 25, 30 and 40 μg/mL), on the basis of response of similar organisms reported in the literature [32,35], and the larvae were exposed for 24 h and then examined for mortality. In the ovicidal evaluation, we used the same procedure as above with the larvae. Mortality was evaluated considering each concentration to which specimens of eggs and larvae were exposed, and the mortality curves were used to determine the LC_{50} and LC_{90}.

2.4. Counting eggs of generations G_1 to G_4

The eggs from the mating of 10 couples were obtained at the end of each generation of *A. albopictus* (G_1 to G_4) treated with 1KL39-B (50 and 70 μg/mL) or 1KL43-C (12.5 and 20 μg/mL) for 4 h (bioassay), at concentrations below the LC_{50}, previously determined as described in section 2.3. Each couple was placed in an individual container, for mating. After oviposition, the eggs of each female (n = 10) of each treatment were counted under stereo microscope.