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Insecticidal and genotoxic potential of two semi-synthetic derivatives of dillapiole for the control of *Aedes* (*Stegomyia*) *aegypti* (Diptera: Culicidae)

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ABSTRACT

The effects of two semi-synthetic dillapiole derivatives, ethyl-ether dillapiole and n-butyl ether dillapiole, on eggs and larvae of *Aedes aegypti* were studied in view of the need for expansion and renovation of strategic action to control this mosquito – the vector of Dengue virus –, which currently shows a high resistance to chemical insecticides. Eggs and third-instar larvae of *A. aegypti* that had been exposed to different concentrations of these two compounds showed toxicity and susceptibility, with 100% mortality. Classical cytogenetic assays showed genotoxicity caused by the two compounds in *A. aegypti* from the cumulative effect of nuclear abnormalities, indicating that these derivatives may be potential alternatives to control *A. aegypti*.

1. Introduction

Dengue is a major arbovirus that infects about 50 million people per year worldwide [1]. Its main vector is the mosquito *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762). Because a vaccine against different types of viruses is lacking, and in view of the occurrence of several cases of resistance to chemical insecticides, as well as the increase in registered cases during periods of epidemic dengue in the world [2–10], new alternative methods are necessary to effectively control this mosquito. Bio-insecticides or plant extracts as repellent substances have been a viable and effective alternative for biological control of insect pests and/or vectors [2,11–14].

Piper aduncum (family, Piperaceae) has been used to control larvae of *A. aegypti* in Argentina, Bolivia, Peru and Brazil [15–17]. The essential oil extracted from this plant has bio-insecticidal effects [18]. It contains dillapiole [18,19], with potential application to control *A. aegypti* [12,18,20–23]. Dillapiole has a highly stable chemical structure, which offers potential for production of semi-synthetic derivatives as alternative sources to enhance the action and the physicochemical properties of agents with confirmed pharmacological or bio-insecticidal activity [24–27]. Semi-synthetic derivatives synthesized from dillapiole were most effective as insecticide (adulticide) in *Aedes atropalpus*, when compared with the parent compound (dillapiole), according Belzile et al. [28], and in adults of *A. aegypti* according Pinto et al. [25]. Moreover, the small structural variations that occur among the monoterpenes *R*-carvone, *S*-carvone, *R*,*S*-carvone, *R*-limonene, *S*-limonene, *R*,*S*-menthol, γ-terpinene and 3-carene, culminate in a large variation in toxicity of these compounds to larvae of *A. aegypti* [29]. This indicates the potential of manipulation of natural substances with high stability, such as dillapiole, for the synthesis of new compounds with improved activity.

Bioassays for evaluating the genotoxicity and mortality of dillapiole in *A. aegypti* were successfully performed by Rafael et al. [23], showing genotoxic effects of the insecticide in samples of *A. aegypti*. We evaluated the insecticidal and genotoxic activity of two semi-synthetic derivatives of dillapiole against larvae of *A. aegypti*, in order to understand the toxic and genotoxic activity of these substances in this mosquito.

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2. Materials and methods

2.1. Material used

Specimens of *A. aegypti* were collected in the neighborhood of Puraquequara (03°03′06.95″ S and 59°52′31.38″ W), municipality of Manaus, Amazonas, Brazil. The samples were used to form colonies (generations F1, F2, F3 and F4) in the insectary of the Coordenação de Sociedade, Ambiente e Saúde (Coordination of Society, Environment and Health) – CSAS, Instituto Nacional de Pesquisas da Amazônia (National Institute for Amazonian Research) – INPA/Ministério da Ciência, Tecnologia e Inovação (Ministry of Science, Technology and Innovation) – MCTI. Ethyl-ether dillapiole (1KL39-B) and n-butyl-ether dillapiole (1KL43-C) compounds (Fig. 1) were assayed against *A. aegypti* individuals in our biological tests. After isomerization, oxy-mercuration, epoxidation, bis-hydroxylation (oxidation) reactions with dillapiole, Pinto et al. [25] produced a series of derivatives, among which 1KL39-B and 1KL43-C. Dimethyl sulfoxide (DMSO) at concentrations of 2% was used as a solvent to solubilize the derivatives in water.

2.2. Bioassays of toxicity and genotoxicity

Two independent bioassays with the F1 generation of *A. aegypti* were conducted. In Bioassay I we established two lethal concentrations (LC) 50% (LC_{50}) and 90% (LC_{90}) for both compounds 1KL39-B and 1KL43-C, causing mortality of eggs and third-instar larvae exposed for 24 h. In Bioassay II we evaluated acute genotoxicity (larval neuroblasts) and residual genotoxicity (oocytes of adults emerged from these larvae) after exposure of the third-instar larvae to the compounds for 4 h in the aquatic phase.

Bioassay I: We used the compounds 1KL39-B at concentrations of 40, 50, 60, 70 and 80 μg/mL, and 1KL43-C at concentrations of 12.5, 20, 25, 30 and 40 μg/mL to treat eggs and larvae, respectively. A total of 200 eggs and 200 larvae were partitioned into five replicates ($n = 40$) for each concentration. Exposure was continued for 24 h. After this time, eggs and larvae were transferred to vessels containing tap water. After evaluation of the mortality caused by each concentration in the samples of eggs and larvae, the final mortality curve and the values of LC_{50} and LC_{90} were established.

Bioassay II: We selected the concentrations of 70 and 80 μg/mL for 1KL39-B and 20 and 30 μg/mL for 1KL43-C, which were higher than the LC_{50} value established in Bioassay I. A total of 200 third-instar larvae, divided into five replicates ($n = 40$) for each concentration were transferred to vessels containing tap water and exposed to both semi-synthetics for 4 h. Then, 30 larvae were used to prepare cytological slides and the remaining larvae were transferred to cups with tap water, where they were maintained until adulthood. Upon reaching the adult phase, 30 female mosquitoes were used to prepare cytological slides and the remaining larvae were used for formation of couples ($n = 30$ males, 30 females), and crossed in order to obtain the next generation (F2). The steps developed in this bioassay were repeated for generations F3 and F4, the next generation emerging from the eggs laid by couples of the previous generation. At the end of each generation the average oviposition for each treatment was evaluated.

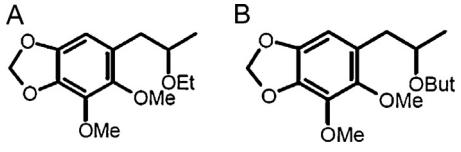

Fig. 1. (A) Ethyl-ether dilapiole. (B) n-Butyl ether dilapiole (Pinto, personal communication).

2.3. Genotoxic activity at the cellular level

Genotoxic effects of the compounds 1KL39-B and 1KL43-C were evaluated by classical cytogenetics. The cytological preparations were prepared with the spreading method [30], with cerebral ganglia of the third-instar larvae and ovaries of adult females emerged from larvae exposed to the semi-synthetics in Bioassay II. The F1 progeny obtained from crosses of individuals exposed to two concentrations of 1KL39-B and 1KL43-C, received the same treatments to obtain the F2 and subsequent generations (F3 and F4). The exposure of *A. aegypti* to these products for 4 h in four successive generations aimed to highlight the potentially deleterious effects in different and successive generations of *A. aegypti*.

Neuroblasts ($n = 10{,}000$) and oocytes from ovaries ($n = 10{,}000$) in interphase nuclei were analyzed for normality or occurrence of structural and morphological abnormalities (buds, micronuclei, poly-nuclei and other malformations). Moreover, 300 neuroblasts and 300 oocytes were analyzed for normality or the occurrence of aberrant structures and morphological abnormalities (chromosomal breaks, fragmentation or chromosomal bridges, micronuclei and other chromosomal malformations). Analysis of the interphase nuclei and of the nuclei in division across the four generations tested (F1, F2, F3 and F4), was performed to investigate differences between them.

2.4. Statistical analysis

The lethal concentrations (LC_{50} and LC_{90}) of the compounds 1KL39-B and 1KL43-C on the samples of *A. aegypti* exposed for 24 h (Bioassay I) were determined from the Probit analysis [31], by use of StatPlus® portable v5.8.4. The differences in oviposition average of individuals, and in the average frequency of abnormal nuclei between the test concentrations and between the generations of each treatment (Bioassay II) were evaluated for statistical significance by means of two-way analysis of variance (two-way ANOVA, $P < 0.05$) and Tukey's test ($P < 0.05$), both performed with the aid of GraphPad Prism 6.0.

3. Results

The mortality of eggs laid by *A. aegypti* exposed to the semi-synthetics 1KL39-B and1KL43-C was 100%, after a 24 h exposure to the lowest concentrations of both compounds (40 μg/mL of 1KL39-B and 12.5 μg/mL of 1KL43-C). The LC_{50} and LC_{90} observed from the mortality curves plotted for exposure of third-instar larvae of *A. aegypti* for 24 h were 61.8 ± 1.9 and 89.0 ± 1.7 μg/mL for the treatment with 1KL39-B, respectively, while for treatment with 1KL43-C the LC_{50} and LC_{90} values were 18.6 ± 1.9 and 27.1 ± 2.3 μg/mL, respectively (Fig. 2).

In Bioassay II we used 1KL39-B at concentrations of 70 and 80 μg/mL and 1KL43-C at 20 and 30 μg/mL. Both tests were

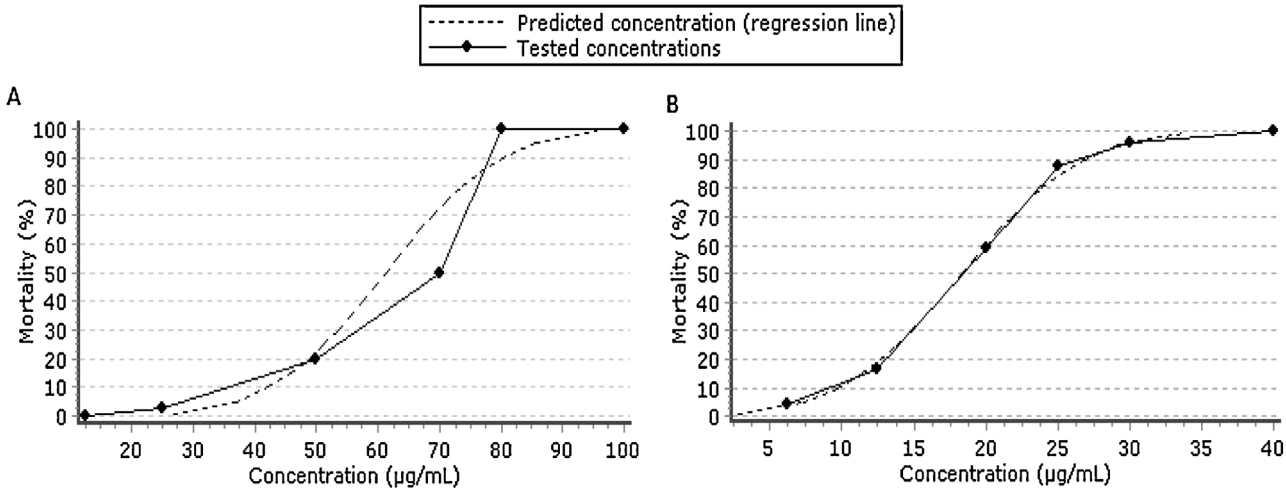

Fig. 2. Probit analysis to determine the lethal concentration (LC) against larvae of *A. aegypti* exposed for 24 h to semi-synthetic derivatives of dillapiole. (A) Concentration vs. mortality after treatment with 1KL39-B; (B) Concentration vs. mortality after treatment with 1KL43-C.