Synergistic Potential of Dillapiole Combined With Pyrethroids against Mosquitoes

Erika Oliveira Gomes, Sergio Massayoshi Nunomura, Osvaldo Marinotti and Wanderli Pedro Tadei

Abstract

The synergistic potential of dillapiole, a phenylpropanoid isolated from Piper aduncum, in a combination with pyrethroids was evaluated. Although dillapiole has already been proposed as a synergist for a range of insecticides, it has not been tested for controlling disease vector mosquitoes. Aedes aegypti and Anopheles albittarsis mosquitoes were exposed to Cypermethrin, α-cypermethrin and dillapiole. Tests were conducted in the bottles were impregnated with pyrethroid or pyrethroid plus dillapiole. Bottles with dillapiole-insecticide combinations were prepared in the same way as the insecticide-only impregnated bottles. The TL50 and TL90 values of dillapiole/pyrethroid combinations were significantly different from those obtained with pyrethroid or dillapiole applied separately. The data indicates potential for dillapiole to be used in public health programs directed to mosquito control. The potential advantages of using dillapiole as a synergist and the consequent reduction in the quantity of applied insecticide include lowered cost of applications, decreased environmental impact and improved insecticide resistance management.

Keywords
Anopheles; Aedes; Dillapiole; Pyrethroid; Synergism

Introduction

Vectors of human diseases include anopheline mosquitoes, which are vectors of parasites causing malaria and culicine mosquitoes such as Aedes aegypti which transmit the viruses that cause dengue, chikungunya, zika and yellow fever. The high rates of morbidity and mortality associated with these diseases encourage studies to develop and test novel means of controlling vector mosquito populations. Biologically active compounds extracted from plants indicate the possibility of replacing or reducing the use of chemical insecticides [1-8].

Some plant extracts, for example, acting as insecticide synergists, containing compounds that are inhibitors of insect P450 cytochromes responsible for the metabolism of xenobiotics, including insecticides. These include phytochemicals containing methane dioxy rings such as dillapiole, piperamides, and furanocoumarins [1,9]. Since the 1940s, many natural and synthetic compounds with insecticide-synergistic action have been used commercially [2,10-16].

Plants of the genus Piper have been investigating for their antibacterial, antifungal and insecticidal properties [17-19]. One compound in particular, Dillapiole, extracted from Piper aduncum and its derivatives have been investigated for their insecticde-synergier action [2,12-14,20-25]. In this work, we evaluated synergistic potential of dillapiole, combined with synthetic insecticides (cypermethrin and α-cypermethrin), against Aedes aegypti and Anopheles albittarsis adult mosquitoes.

Material and Methods

Extraction of essential oil and isolation of dillapiole

Aerial parts (leaves and twigs) of Piper aduncum were collected in the Bosque da Ciência, Instituto Nacional de Pesquisas da Amazônia - INPA, Manaus - AM, Brazil. Piper aduncum L. 1753, was identified by the INPA Herbarium personnel, according to the existresses of the species already deposited in the Institution. Collected plant materials were kept for 5 days at about 23°C and 45% relative humidity. The dried plant material was milled and volatile oil extracted by hydro distillation using a Clevenger-type apparatus [26]. A total of 6.1g of essential oil were obtained from 490 g of starting dry plant, yielding 1.25%. Dillapiole, was isolated from the oil by flash column chromatography over silica gel, eluted with hexane/ethyl acetate (98:2). Dillapiole was identified by high resolution gas chromatography with flame ionization detection (GC-FID) and gas chromatography coupled to a mass spectrometer (GC-MS).

Insecticides: The commercially available insecticides used in this study were: Cypermethrin: Cipermetrina CE 200, registered by Fersol Ltda, composed of (R,S)-alpha-cyano-(3-phenoxyphenyl)methyl 3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylate; and α-Cypermethrin: AlphaGold SC 200, registered by Bayer CropScience Ltda, composed of cis (R) and (S) enantiomorphic isomers of alpha-cyano-3-oxo-3-phenylbut-2-enyl-2,2-dimethyl-2-cyclopropanecarboxylate.

Mosquitoes

Anopheles albittarsis Lynch-Aribalzaga, 1878: The specimens of A. albittarsis used in this work refer to the sensu lato species [27,28]. Collected in the town of Cacau Pirera, municipality of Iranduba, Amazonas, Brazil. Mosquitoes were collected at night with the aid of a flashlight and captured using a suction apparatus (Castro device). The captured specimens were transferred to paraffin waxed paper cups, stored in a polystyrene (Styrofoam) box and transported to the Malaria and Dengue Laboratory, National Institute of Amazonian Research (INPA) in Manaus, where they were identified and allowed to rest and feed on sugar: water (1:1). Anopheles albittarsis mosquitoes were kept in the laboratory for at least 24 hours prior to the bioassays.

Aedes (Stegomyia) aegypti Linnaeus, 1762: Aedes aegypti were obtained from a permanent colony, maintained at the temperature of 26 ± 2 °C with relative humidity of 80% in the Laboratório de Vetores de Malária e Dengue of the Instituto Nacional de Pesquisas da Amazônia. Eggs were placed in plastic trays with approximately one liter of water and hatched larvae fed a 1: 1 mixture of fish meal and liver powder [29]. Three day old mosquitoes were used in the tests.
Bioassays: CDC bottle bioassays [27] were performed in 250 mL bottles with screw cap (Schott Inc.). Bottles were impregnated with 1 mL of acetone containing pyrethroid and/or dillapiole to disperse the products homogeneously, and dried for 24 h to ensure that the solvent evaporated completely. Negative control bottles were impregnated with only solvent (acetone). Mortality was never observed in the negative control tests. The concentrations of commercial chemical insecticides were calculated according to the manufacturer’s specifications and the internal area for the bottles (mean value of 275.5 cm²).

Tests were performed in triplicate; forty-five mosquitoes were used for each sample, with 15 specimens per bottle, the survival rate and mortality were assessed every 15 min for 90 min. First, pyrethroids and dillapiole were evaluated individually. Cypermethrin was tested at 0.0015, 0.003, 0.004 and 0.006 µg/cm², whereas α-cypermethrin at 0.0001, 0.0006, 0.001 and 0.002 µg/cm². Dillapiole was tested at 0.007, 0.07 and 0.73 µg/cm² for Aedes aegypti and 0.004, 0.007, 0.02 and 0.04 µg/cm² for A. albitarsis. Test of synergism was conducted combining the commercial insecticides with dillapiole. The registered mortality of mosquitoes Aedes and Anopheles was subjected to analysis with non-linear model with the following equation:

\[ y = \frac{1}{1 + \exp(a \cdot \log(x) + b)} \]

Where, \( y \) is the proportion of dead mosquitoes (deaths/45) and \( x \) is the time in minutes. Statistical analyses were performed with the software R 3.0.2. TL50 is defined as the time that causes 50 % of mortality in the mosquito population and accordingly, TL90 is the time necessary to cause 90 % of mortality.

Results

Initial tests with A. aegypti and cypermethrin, in quantities ranging between 0.0015 and 0.006 µg/cm², showed mortality varying from 40% to 100% in 45 min. Dillapiole at 0.007 to 0.730 µg/cm², resulted in A. aegypti mortality between 24% and 100% in 45 min. Based on these results, synergism tests were performed with cypermethrin at 0.0015 mg/cm² and dillapiole at 0.07 mg/cm².

A. aegypti mortality following exposure to only cypermethrin (0.0015 mg/cm²), only dillapiole (0.07 mg/cm²), or cypermethrin plus dillapiole ((0.0015 mg/cm² and 0.07 mg/cm² respectively) was determined (Figure 1). At 15 and 30 minutes the mortality values were observed in the assays with mixed compounds (Cypermethrin/Dillapiole) was more than double the sum of the mortalities caused by exposure to Cypermethrin and dillapiole separately (Cypermethrin + Dillapiole). The association Cypermethrin/dillapiole caused 100% mortality in 45 min, while living mosquitoes were still present at 90 minutes of exposure in the assays performed with each separate compound. The cypermethrin/dillapiole association modulated the time these compounds act upon mosquitoes, shortening in approximately half the time necessary to kill, as shown in lethal time causing 50 % (TL50) and 90% of mortality (TL90) (Table 1).

The susceptibility of A. albitarsis to α-cypermethrin at concentrations ranging from 0.0001 to 0.002 µg/cm² showed mortalities from 13% to 44% at 45 min. Dillapiole in amounts of 0.04 to 0.004 µg/cm² resulted in percent mortality ranging between 36% and 22% at 45 min. For the study of synergism, the amounts of 0.0006 µg/cm² for α- α-cypermethrin and 0.0400 µg/cm² for dillapiole were selected.

Similar to the observations with Aedes aegypti, the association α-cypermethrin/dillapiole increased mortality of A. albitarsis at all times between 30 minutes and 90 minutes of exposure (Figure 2). No statistically significant differences were noted at 15 minutes. The association α-cypermethrin/dillapiole caused 100% mortality between 60 and 75 min, while living mosquitoes were still present at 90 minutes of exposure in the assays performed with each separate compound. The association of these two compounds shortened the time necessary to kill mosquitoes, estimated as TL50 and TL90 (Table 1). These results further support our conclusion that dillapiole is useful in targeting mosquitoes when used together with pyrethroids.
Discussion

Mosquitoes are important vectors of parasitic, viral pathogens and serious nuisance pests to humans and animals. Therefore the management of mosquito populations and control of mosquito-borne disease transmission are of prime importance to public health and welfare. The most widely-used mosquito control strategies at present use various formulations of chemical insecticides to kill larvae and adults [28,29]. However, these tools are not always efficient, applicable, or available. Increased resistance of mosquitoes to chemical insecticides and the cost of these interventions make them prohibitive in many settings [30-33].

Considering these drawbacks, pursuing informed, efficient ways of using chemical insecticides are essential. In the formulation process it is important to choose the best compounds, including synergists in order to achieve safety, environmental and economic benefits. Our results support the conclusion that dillapiole is useful for public health applications targeting mosquitoes when used together with pyrethroids [34-36].

Acknowledgments

We thank CNPq for awarding a fellowship (Process No. 553235/2005-2), CTPetro, PIATAM project (Potenciais Impactos e Riscos Ambientais na Indústria de Petróleo e Gás na Amazônia), FAPEAM (Fundação de Amparo à Pesquisa do CTPetro, PIATAM project (Potenciais Impactos e Riscos Ambientais na Indústria de Petróleo e Gás na Amazônia), CAPES/Pró-Amazônia for financial support. We are also grateful to Fundação de Vigilância em Saúde do Amazonas – FVS/Secretaria de Estado da Saúde do Amazonas – SUSAM, for helping with the testing and mosquito collections.

References


11. Motsail RL (1992) The synergistic activity of various selected chemicals on the insecticides carbofuran and malathion against brine shrimp larvae. Department of Agricultural and Environmental Sciences, University of Newcastle upon-Tyne, UK.


Author Affiliations

1Laboratory of Malaria and Dengue, National Research Institute of the Amazon – INPA, Brasil
2Coordination of Technology and Innovation, National Research Institute of the Amazon – INPA, Brasil
3MTEKPrime, 132 Woodcest Lane, Aliso Viejo, CA 92656, USA

Submit your next manuscript and get advantages of SciTechnol submissions

- 80 Journals
- 21 Day rapid review process
- 3000 Editorial team
- 5 Million readers
- Quality and quick review processing through Editorial Manager System

Submit your next manuscript at www.scitechnol.com/submission