Potential use of *Piper nigrum* ethanol extract against pyrethroid-resistant *Aedes aegypti* larvae

Utilização em potencial do extrato alcoólico de *Piper nigrum* como larvicida em *Aedes aegypti* resistente a piretróides

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**ABSTRACT**

Fractionation of *Piper nigrum* ethanol extract, biomonitored by assays on pyrethroid-resistant *Aedes aegypti* larvae yielded isolation of the larvicidal amides piperolein-A and piperine. Comparing LC₅₀ values, the ethanol extract (0.98 ppm) was the most toxic, followed by piperolein-A (1.46 ppm) and piperine (1.53 ppm).

**Key words:** *Piper nigrum*. *Aedes aegypti*. Piperamides. Larvicide.

**MATERIAL AND METHODS**

**Plant material.** Unripe *Piper nigrum* fruits were harvested from plants grown in the municipality of Monte Alegre, State of Pará, Brazil (02°00´S, 54°06´W), in the Amazon basin. Taxonomic identification of the plant was performed by Ricardo Secco, a botanist at the Emilio Goeldi Museum, Belém, Pará, where the voucher specimen was deposited (MG 156.772).

**Plant extract.** The dried fruits (1.4kg) of *Piper nigrum* were crushed and extracted exhaustively using ethanol at room temperature. The combined filtrate was concentrated in vacuum to yield 10% as a dark viscous extract. The crude...
ethanol extract (100g) was sequentially partitioned into hexane (20.5g), dichloromethane (54.5g), ethyl acetate (10g) and water-soluble (15g) portions for subsequent bioassay. Piperine was isolated by overnight precipitation of ethanol extract, resulting in 1% yield. The partitions and piperine were assayed against *Aedes aegypti* larvae. Bioassay-guided fractionation revealed that the dichloromethane partition was the most active one; hence, it was selected for further studies.

**Fractionation of the dichloromethane partition.** This was carried out by chromatography on XAD-16 resin using methanol and ethyl acetate as the eluting solvents. The active fraction PNPC17 (LC$_{50}$ = 2.3ppm) eluted with methanol was subjected to preparative thin layer chromatography using hexane/ethyl acetate (7:3) as the developing solvent to produce five bands (A to E). Biologically active band D (LC$_{50}$ = 1.0ppm) was further purified on silica gel with hexane/ethyl acetate (9.5:0.5) as the eluant, to yield the most active compound, which was PNPC17D-20A (LC$_{50}$ = 1.46ppm). The structure of this compound was determined as piperolein-A by spectroscopic analysis.

**Bioassay test.** The larvicides were a slightly modified version of the standard protocol described by the World Health Organization. The total volume in the test solutions was modified from the standard 250 to 20 ml. The volume modification was necessary to enable bioassaying of minor fractions resulting from the chromatography procedure. Bioassays were carried out using a cypermethrin-resistant colony of *Aedes aegypti* (NPPN colony) that was established from the adult survivors of a diagnostic dose of cypermethrin, (i.e. 37mg ia/m$^2$). Ten third-instar mosquito larvae were placed in 19.9ml of distilled water and 100µl of ethanol solution containing the test fractions was added to each cup (50ml), which was shaken lightly to ensure a homogeneous test solution. The toxicity of each test fraction was determined with four to ten concentrations ranging from 0.1 to 1,000ppm. The control was prepared with 19.9ml of distilled water and 100 µl of ethanol to which larvae were added. The assay was conducted at room temperature in triplicates. Mortality was recorded 24 hours after treatment. The LC$_{50}$ and LC$_{99}$ values were calculated using the Probit analysis software (SPSS).

**RESULTS**

The susceptibility level of pyrethroid-resistant *Aedes aegypti* larvae for extracting fractions and piperine from *Piper nigrum* was determined. The ethanol extract was more active on third-instar *Aedes aegypti* larvae (LC$_{50}$ = 0.98 ppm, LC$_{99}$ = 2.72ppm) than was piperine (LC$_{50}$ = 1.53ppm, LC$_{99}$ = 10.6ppm) or the hexane, dichloromethane, ethyl acetate or aqueous fractions. Bioassay-guided fractionation of the dichloromethane fraction produced an active crude fraction, and further chromatographic purification yielded piperolein–A (LC$_{50}$ = 1.46ppm, LC$_{99}$ = 4.22ppm) as the major active compound.

**DISCUSSION**

The ethanol extract of *Piper nigrum* fruits exhibited potent larvicidal activity against the cypermethrin-resistant strain of *Aedes aegypti*. The main larvicidal constituent of the extract was identified as the piperidine amide piperolein-A. Piperine, the main constituent of the fruit, presented an LC$_{50}$ equivalent to piperolein-A activity.

The fractionation of *Piper nigrum* ethanol extract proved to be unnecessary for isolating the larvicultural compound, because the activity was gradually lost during the fractionation process. These findings are in agreement with Miyakado et al., who reported the use of piperamide mixtures to increase the larvicidal activity in a pyrethroid-resistant *Culex quinquefasciatus* mosquito strain. Furthermore, piperine has been reported to enhance the bioavailability of several drugs, possibly by inhibiting drug metabolizing enzymes and/or increasing their absorption. Possibly, the toxicity of *Piper nigrum* ethanol extract towards pyrethroid-resistant strains of *Aedes aegypti* is increased by its piperine content. The use of crude *Piper nigrum* ethanol extract as the larvicide decreased its cost by avoiding the purification processes. This is the first report of *Piper nigrum* larvical activity on a pyrethroid-resistant strain of *Aedes aegypti*.

In conclusion, the larvicide property of crude *Piper nigrum* ethanol extract indicates that its compounds did not confer cross-resistance to pyrethroid-resistant strains. The extract could be useful for managing field populations of pyrethroid-resistant *Aedes aegypti* larvae when the other control methods are not promptly accessible. The pronounced taste of the extracts on non-target organisms and the environment, and regarding formulations to improve the insecticide potency and stability, are needed for these naturally occurring mosquito larval control agents to be used in practice.

**REFERENCES**


