Ecotoxicology and Environmental Safety 154 (2018) 1–5

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Toxicity of different fatty acids and methyl esters on *Culex quinquefasciatus* larvae

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ARTICLE INFO

Keywords:
Insecticide
Mosquito
Linoleic acid
Acetylcholinesterase

ABSTRACT

The Culex quinquefasciatus mosquito is a vector of several diseases, and its control has been performed with synthetic insecticides, which may have human and environmental side effects. Thus, the use of new and safe molecules are important, and this study evaluated the toxicity of active substances against this mosquito. The oleic, linoleic, linolenic, palmitic and stearic acids and their respective methyl esters were tested against fourth instar C. quinquefasciatus larvae. Oleic, linoleic and linolenic acids had LC50 values of 8.58, 10.04 and 19.78 mg/L, respectively. Histological analysis showed that these three compounds caused cell instability with an increase in the number of vesicles in the fat body and in the midgut cells. Based on these results, glucose, triglyceride, and protein levels were evaluated after 1 h of acid exposure. These compounds decreased in insects treated with linoleic acid. Linolenic acid also caused a significant increase in acetylcholinesterase activity. These results show that oleic, linoleic, and linoleic acids have a lower LC50 for C. quinquefasciatus, affecting its metabolism and the morphology of midgut and fat body.

1. Introduction

The mosquito *Culex quinquefasciatus* is a vector of bancroftian filariasis and different types of encephalitis (WHO, 1987). *C. quinquefasciatus* it typically controlled using pyrethroids, carbamates and organophosphates insecticides, leading to resistance (Yadouléton et al., 2015; Richards et al., 2017). Natural products from seeds and fruit are rich in oils with many fatty acids that can act against mosquitoes, which has led to their increased use of alternative molecules to control these insects (Bosch et al., 2000; Reifenrath, 2005; Cantrell et al., 2011; Jones et al., 2012).

Plant oils contain multiple fatty acids; the most commonly found are palmitic, stearic, oleic, linoleic, and linolenic acids (Erdemoglu and Kusmenoglu, 2003; Korul'kina et al., 2004). Rahuman et al. (2008) found variations in sub-lethal concentrations (LC_{50}) of oleic acid against mosquito larvae ranging from 7.66 mg/L to 18.20 mg/L. Moreover, these acids may be changed to other molecules, such as the methyl esters (Suarez et al., 2007). The larvicidal, insecticidal, and repellent actions of fatty acids against mosquitoes have been reported (Skinner et al., 1970; Puritch et al., 1981; Osborne and Henley, 1982; Bosch et al., 2000; Reifenrath, 2005; Ali et al., 2012; Perumalsamy et al., 2015).

However, little is known about the effects these natural products have on insects when used as larvicidal agents. Sharma et al. (2011) have shown that two phytoextracts produce significant alterations in the biochemical profiles of carbohydrate, lipid, and protein in anopheline and culicine larvae. Silva et al. (2016) reported changes in the total protein content of *C. quinquefasciatus* larvae after exposure to methyl esters from sunflower oil, as well as potential interference with the activity of Na^+/K^+-ATPase membrane transporter. Protein content and acetylcholinesterase activity decrease in *C. quinquefasciatus* larvae exposed to nanoemulsion of eucalyptus oil (Sugumar et al., 2014).

Inhibition of synaptic transmission is mainly caused by the inhibition of acetylcholinesterase, the main mode of action for many insecticides, including organophosphates and carbamates (Raymond et al., 1998; Alout et al., 2012). Acetylcholinesterase (AChE) is a key enzyme that hydrolyzes the neurotransmitter acetylcholine (ACh) in cholinergic synapses of neurons (Hematpoor et al., 2016). In mosquitoes, insecticide resistance is usually caused by inhibition of the target enzymes in the resistant strains (Yu, 2014).

Here we evaluated the larvicidal activity of oleic, linoleic, linolenic, palmitic, and stearic acids and their respective methyl esters in fourth instar larvae of *C. quinquefasciatus*.

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https://doi.org/10.1016/j.ecoenv.2018.02.009
Received 21 September 2017; Received in revised form 7 January 2018; Accepted 2 February 2018

2. Materials and methods

2.1. Obtaining fatty acids and their respective methyl esters

Oleic acid, methyl oleate, palmitic acid, methyl palmitate, stearic acid, methyl stearate, linoleic acid, methyl linoleate, linolenic acid and methyl linolenate were obtained from Sigma-Aldrich® (U.S.A.).

2.2. Mosquitoes

The *C. quinquefasciatus* larvae were reared in the Insect Disease Vectors Laboratory at the *Campus* Centro-Oeste Dona Lindu, Federal University of São João Del-Rei, according to Gerberg (Gerberg, 1979).

2.3. Bioassays

The larvicidal bioassay was performed according to the World Health Organization (WHO, 1981) standard protocols. To determine the sublethal concentrations, fourth instar larvae of *C. quinquefasciatus* were taken in 3 batches of 20 in 100 mL of fatty acids solution and their respective methyl esters in 0.5% dimethyl sulfoxide (DMSO) for 24 h. Bioassays were conducted at three different concentration (1, 10 and 100 mg/L) with three replications for each treatment. Larvae mortality was monitored after 24 h of exposure in all replicates of each dosage. The control larvae were exposed to water with 0.5% DMSO. The temperature was 26 ± 1 °C throughout tests.

2.4. Histology

C. quinquefasciatus larvae at fourth instars were exposed to LC_{50} of fatty acids and their respective methyl esters for 1 h. The control was exposed to unchlorinated water with 0.5% DMSO. Then, larvae were decapitated, and the respiratory siphon was removed. The thorax and abdomen were transferred to Stefanini fixative solution (Stefanini et al., 1967) for 48 h. Samples were dehydrated in a graded ethanol series and embedded in historesin (Leica®). Slices (4 μm) were stained with haematoxyline and eosin. Another set of slices from the same larvae were submitted to histochemical tests of mercury bromophenol blue for total protein (Pearse, 1968).

2.5. Biochemistry

Groups of the 50 fourth instar larvae of *C. quinquefasciatus* were exposed to 100 mL of LC_{50} of oleic acid, linoleic acid, linolenic acid or methyl linolenate for 1 h. The control group was exposed to 100 mL of unchlorinated water with 0.5% DMSO. The larvae were homogenizedin 10 mL 20 mM Tris buffer pH 7.3 and centrifuged for 3 min at 1500*g*, and the supernatant was used for the determination of protein, glucose, and triglyceride (TAG) contents.

Glucose concentration was measured by the Trinder method using glucose oxidase Trinder (1969). The supernatant (10 μL) was pipetted into a small test tube, glucose oxidase reagent (1.5 mL) was added and the test tube was incubated at 37 °C for 15 min. Then the test tube was steeped in boiling water for 150 s to stop the reaction. The absorbance was read immediately using a spectrophotometer at a wavelength of 505 nm.

The proteins were determined according to Hartree (1972) using 30 μL aliquot of the supertant obtained from 50 larvae was modified to accommodate small sample volumes. The assay was carried out in small glass tubes by use of bovine serum albumin (standard ranged from 0.3 to 10 mg/mL) as standard. By means of semi-microcuvettes, all samples were read at 650 nm. Samples were assayed in duplicate or triplicate.

TAG determination according to Megraw et al. (1979). The supernatant (10 μL) was pippetted into a small test tube. The glycerol released by the hydrolysis of the triacylglycerol contained in the serum, catalyzed by lipoprotein lipase and reproduced by the action of glycerol glycerol-3-phosphate, which is oxidized to dihydroxyacetone and hydrogen peroxide in presence of glycerolphosphate oxidase. A coupling reaction between the hydrogen, 4-aminoantipyrine and ESPAS is catalyzed by peroxidase producing the quinoneimine that has maximum absorption at 540 nm.

2.6. Analysis of acetylcholinesterase (AChE) activity

The larvae of the control group and that exposed to LC_{50} of acid linolenic were homogenized in a motor-drive Teflon Potter-Elvehjem homogenizer and ultracentrifuged at 100,000 × *g* for 1 h at 4 °C. The supernatant was used to determine AChE activity by colorimetric methods (Ellman et al., 1961). This was based on the measure of the velocity of production of thiocholine through hydrolysis of an analog substrate for AChE; acetylthiocoline. Thiocoline reacts with so called Ellman Reagent (DNTB or 5,5′-dithiobis-2-nitrobenzoic acid 3 mM), forming a mixture of disulfides and a yellow anion (nitrobenzoate) with intense absorption at 415 nm.

2.7. Statistical analysis

All experiments were carried out in triplicate. The lethal concentrations were calculated by regression analysis using the Probit model (DL50 software) at a 5% significance level. The quality of fitted models was checked by residual analysis with chi-square test at 5% of significance level.

The larval mortality and biochemical test results were submitted to analysis of variance (ANOVA), followed by Dunnett multiple comparison tests. The analyses were performed considering 95% of significance and a p-value < 0.05 using GraphPad software (Prism, version 5).

3. Results

3.1. Mortality rates of larvae exposed to the fatty acids and methyl ester

Only oleic, linoleic, and linolenic acids showed a high larvicidal effect in 100 mg/L, with 95.0%, 100% and 97.22% larval mortality respectively, whereas for their methyl esters, the mortality rates were low (< 40.83% for methyl linolenate) (Fig. 1).

The adjusted model, median lethal concentration (LC_{50}), confidence interval at 95%, and quality of adjusted model are summarized in Table 1. Oleic, linoleic and linolenic acids had larvicidal activity against *C. quinquefasciatus*, with LC_{50} ranging from 8.58 and 190.78 mg/L. These acids were classified as effective and methyl linolenate as low effectiveness according to the classification of Komalamisra et al. (2005) (Table 1). The other compounds tested were not effective, with

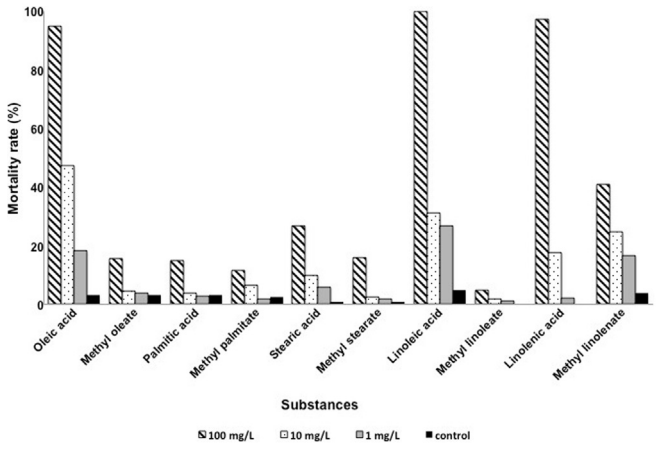

Fig. 1. Mortality (%) of fourth instar larvae of *Culex quinquefasciatus* after exposure to the fatty acids and methyl esters for 24 h.