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Larvicidal and repellent activity of tetradecanoic acid against *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say.) (Diptera:Culicidae)

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ABSTRACT

Objective: To investigate the larvicidal and repellent efficacy of tetradecanoic acid against *Aedes aegypti* (*Ae. aegypti*) L. and *Culex quinquefasciatus* (*Cx. quinquefasciatus*) Say (Diptera: Culicidae). **Methods:** Larvicidal efficacy of tetradecanoic acid was tested at various concentrations against the early third instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*. The repellent activity was determined against two mosquito species at three concentrations viz., 1.0,2.5 and 5.0 ppm under the laboratory conditions. **Results:** The tetradecanoic acid was found to be more effective against *Cx. quinquefasciatus* than *Ae. aegypti* larvae. The LC_{50} values were 14.08 ppm and 25.10 ppm, respectively. Tetradecanoic acid showed lesser repellency against *Ae. aegypti* and *Cx. quinquefasciatus*. The highest repellency was observed in higher concentration of 5.0 mg/cm^2 provided 100% protection up to 60 and 90 min against *Ae. aegypti* and *Cx. quinquefasciatus* respectively. **Conclusions:** From the results it can be concluded the tetradecanoic acid is a potential for controlling *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes.

1. Introduction

Mosquito-borne diseases not only cause high levels of morbidity and mortality, but also inflict great economic impact, including loss in commercial and labor output, particularly, in tropical and subtropical countries. However, no part of the world is free from these diseases[1]. Mosquitoes, like many other biting flies, are important pests which transmit many diseases like dengue and encephalitis. These are serious diseases that some cases cause death. Many chemicals are available for controlling mosquitoes, but most of them are not selective and may even harm beneficial insects. Also, many of the mosquito species have developed at least a partial resistance to the chemicals. In recent years, many researchers have been looking for new biological insecticides. Different organisms, such as plants and bacteria, have been used for the development of new products[2]. However, only a few components and formulations of biological origin are available commercially in the world[3]. Mosquitoes are responsible for the spread of more diseases than any other group of arthropods. The incidence of dengue, one of the mosquito-transmitted diseases, has increased tremendously in recent years. *Aedes aegypti* (*Ae. aegypti*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*) are principle vectors responsible for many diseases[4,5].

An obvious method for preventing the spread of these diseases is to control mosquito vector population by insecticides and synthetic agents, which have been developed and employed in the field with considerable success. However, resistance and environmental damage caused by synthetic agents have prompted alternative pest management strategies[6]. In this regard, there is an urgent need for the development of techniques that would provide more efficient insect control, incur ill effects on the non-target population, and are easily degradable[7]. Rahuman *et al*[8] have reported that a bioassay-guided fractionation of the acetone extract of *Feronia limonia* dried leaves afforded a potent mosquito larvicide, identified as n-hexadecanoic acid and found to be effective against fourth instar larvae of *Anopheles stephensi* (*An. stephensi*). 1,5-Diphenyl-1-pentanone (A) and 1,5-diphenyl-2-penten-1-one (B) compounds isolated from *Stellera chamaejasme* showed strong contact activity and very good anti-feedant activity[9] against *Ashbya gossypii*. Seven flavonoid compounds were isolated and identified from the flowers of *Abutilon indicum*[10]; Chaubal *et al*[11] have reported that the

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alicyclic polyalcohol, which was found to be D-pinitol (=3-O-methyl-D-chiro-inositol; 1), isolated from the aerial parts of acetone extract of *Acacia nilotica* showed chronic toxicity against *Ae. aegypti* and *Cx. quinquefasciatus* fourth instar larvae.

Vitex negundo has larvicidal activity against the mosquito species *Cx. quinquefasciatus*, and Pushpalatha and Muthukrishnan[12] and Rahuman *et al*[8] also found that n-hexadecanoic acid in *Feronia limonia* dried leaves was effective against fourth instar larvae of *Cx. quinquefasciatus*, *An. stephensi*, and *Ae. aegypti*. Repellency properties of nepetalactone (cyclopentanoid monoterpene) isolated from the catnip plant, *Nepeta cataria*, against 17 species of insects were reported by Eisner[13]. The active components dymalol, Nymania-3, and triterpenes isolated from the extract of *Dysoxylum malabaricum* act as an oviposition repellent and/or deterrent to *An. stephensi*[14]. 1, 8-cineole compound isolated from the leaves of essential oils of *Hyptis martiusii* showed pronounced insecticidal effect against *Ae. aegypti* larvae and *Bemisia argentifolii*[15]; thymol, alpha-amyrin, carvacrol+beta-caryophyllene isolated from the petroleum ether fraction of *Thymus capitatus* were proven to have high larvicidal potency against the *Culex pipiens*[16].

Tetracyclic triterpenoid showed activity against entomopathogenic nematodes from insects[17] and 22, 23-dihydronimocinol (1) and desfurano- 6alpha-hydroxyazadiradione (2) were isolated from a methanolic extract of the fresh leaves of *Azadirachta indica*, which showed mortality for fourth-instar larvae of *An. stephensi*[18]. Mosquito larvicidal activity of plant-derived isolated compounds have been reported such as spipnoohine, pipyahyine, leptostachyol acetate, diterpenoid furans 6alpha-hydroxyvouacapan-7beta, 17beta-lactone, 6alpha, 7beta-dihydroxyvouacapan-17beta-oic acid and methyl 6alpha, 7beta-dihydroxyvouacapan-17beta-oate, rotenone and tetranortriterpenoids[19,20]. Butler[21] working with *Ae. aegypti* (L.), *Monodelphis domestica* and horn fly, *Haematobia irritans* (L.), identified 120 semiochemicals with structures indicative of insecticidal activity from commercially available flavor and fragrance compounds.

In view of the recently increased interest in developing biodegradable insecticides as an alternative to chemical insecticide, this study was undertaken to assess the larvicidal and repellent activity of the tetradecanoic acid from *Ae. aegypti* eggs extract against the medically important mosquito vectors, *Ae. aegypti* and *Cx. quinquefasciatus*.

2. Materials and methods

2.1. Test compound

Tetradecanoic acid was procured from Sigma, USA and ethanol was used as solvent to prepare the stock solution. The stock solution was diluted further to get the required concentrations for the bioassays.

2.2. Test organisms

The mosquitoes, *Ae. aegypti* and *Cx. quinquefasciatus* were reared in the vector control laboratory, Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder at 3 : 1 ratio. Adults were provided with 10% sucrose solution and one week old chick for blood meal. Mosquitoes were held at (28 ± 2) ℃, 70%-85% relative humidity, with a photo period of 14 h light, 10 h dark.

2.3. Larvicidal bioassay

The larvicidal activity of the plant crude extracts was evaluated as per the method recommended by WHO[22]. Batches of 25 third instar larvae were transferred to a small disposable test cups, each containing 200 mL of water. The appropriate volume of dilution was added to 200 mL water in cups to obtain the desired target dosage (concentration ranging from 10 to 30 ppm), starting with the lowest concentration. Six replicate were set up for each concentration and an equal number of control were set up simultaneously using tap water. To this 1 mL of appropriate solvent was added. The LC_{50} value was calculated after 24 h by probit analysis[23].

2.4. Repellent activity

The repellent study was following the method of WHO[24]. Three-day-old blood-starved female *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes (100) were kept in a net cage (45 cm × 30 cm × 45 cm). The volunteer had no contact with lotions, perfumes or perfumed soaps on the day of the assay. Arms of volunteer, only 25 cm^2 dorsal side of the skin on each arms was exposed and the remaining area covered by rubber gloves. The crude extract was applied at 1.0, 2.5 and 5.0 mg/cm^2 separately in the exposed area of the fore arm. Only ethanol served as control. The time of the test dependent on whether the target mosquitoes day-or night biters. *Ae. aegypti* was tested during the day time from 07:00 to 17:00, while *Cx. quinquefasciatus* was tested during the night from 19:00 to 05:00. The control and treated arm were introduced simultaneously in to the mosquito cage, and gently tapping the sides on the experimental cages, the mosquitoes were activated. Each test concentration was repeated six times. The volunteer conducted their test of each concentration by inserting the treated and control arm in to the same cage for one full minute for every five minutes. The mosquitoes that landed on the hand were recorded and then shaken off before imbibing any blood; making out a 5 minutes protection. The percentage of repellency was calculated by the following formula.

$$\% \text{ Repellency} = [(T_a - T_b)/T_a] \times 100$$

Where T_a is the number of mosquitoes in the control group and T_b is the number of mosquitoes in the treated group.

2.5. Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90}, regression equation and other statistics at 95% confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL), and *chi*-square values were calculated using the Statistical Package of Social Sciences (SPSS) 12.0 software. Results with P<0.05 were considered to be statistically significant.

3. Results

In the present investigation, the toxicity of teradecanoic acid was tested against *Ae. aegypti* and *Cx. quinquefasciatus* larvae (Tables 1). The data were recorded and statistical data regarding LC_{50}, LC_{90}, regression equation, LCL, UCL and *chi*-square values were calculated. The LC_{50} value of tetradecanoic acid against early third instar larvae of *Cx. quinquefasciatus* was 14.08 ppm and against *Ae. aegypti* value was 25.10 ppm, respectively. No mortality was observed in control. *Chi*-square values were significant at $P<0.05$ level. The repellency of tetradecanoic acid against *Ae. aegypti* and *Cx. quinquefasciatus* under laboratory condition is given in Table 2. The highest repellency was observed in higher concentration of 5.0 mg/cm^2 provided 100% protection up to 60 and 90 min against *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. In repellency results, increases in the concentration of the compound from 1.0 to 5.0 mg/cm^2 were found to increase the repellency time. The tetradecanoic acid used in this study did not cause skin irritation, hot sensations or rashes on the arms of the test volunteers during the study period.

4. Discussion

This study showed that tetradecanoic acid has significant larvicidal and repellent activity against *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes. Hierro *et al*[25] reported such differences in activity of geraniol and citronellol (structural isomers of terpene alcohols) on herring worm, *Anisakis simplex* (Rudolphi), larvae. Although all terpene alcohols showed toxicity to both mosquito species, the LC_{50} and LC_{90} values varied within terpene alcohols, depending on the insect species. Similar responses were observed for ketones and carboxylic esters. Mosquito response also varied between structural isomers, such as geraniol and nerol, within the terpene alcohols, and between α-damascone and δ-damascone within the ketone ionones. Similar response variation was observed within the carboxylic ester isomers methyl jasmonate and methyl dihydrojasmonate. Therefore, insect response cannot be predicted solely on the basis of molecular structure. Ten of these 16 compounds that showed activity were from natural sources and are components of essential oils. Geranyl acetone, citronellol, nerol and geraniol were the most toxic compounds to *Ae. aegypti* and *Anopheles quadrimaculatus*, and are constituents of essential oils. The insecticidal properties of essential oils containing these compounds against several insects including mosquitoes have been reported[26,27].

Although a majority of the researchers identified the constituents of essential oils, very few evaluated the toxicity of individual constituents, and this is the first study to examine the toxicity of synthetic flavor and fragrance industry-utilized semiochemicals against mosquitoes. Considering their flavor and fragrance use, and the low molecular weight and repellant and attractant properties exhibited by these compounds, authors evaluated their

Table 1

Larvicidal activity of tetradecanoic acid against the mosquito *Ae. aegypti* and *Cx. quinquefasciatus*.

Mosquito	Concentration (ppm)	Mortality (%)	Regression equation	LC_{50}(ppm)		LC_{90}(ppm)		χ^2
				Mean	LCL-UCL	Mean	LCL-UCL	
Ae. aegypti	30	99.6±0.2	Y= -184.3+9.32X	25.10	24.16-26.07	29.32	28.00-31.85	15.401*
	28	78.5±0.4						
	26	56.8±0.2						
	24	27.1±0.6						
	22	19.6±0.6						
	20	10.4±0.8						
	Control	0.0±0.0						
Cx. quinquefasciatus	20	99.2±0.4	Y= -88.77+9.27X	14.08	14.08-15.70	19.11	17.90-20.93	11.461*
	18	81.4±0.6						
	16	58.2±0.2						
	14	31.8±0.2						
	12	20.4±0.6						
	10	11.2±0.4						
	Control	0.0±0.0						

*Significant at $P<0.05$.

Table 2

Repellent activity of tetradecanoic acid on *Ae. aegypti* and *Cx. quinquefasciatus* (X±SD).

Mosquitoes	Concentration (mg/cm^2)	Percentage of repellency 15 min	30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min
Ae. aegypti	1.0	100.00±0.00	89.89±4.08	76.62±4.08	66.66±5.27	50.00±5.27	23.33±4.08	10.00±6.62	Nil	Nil
	2.5	100.00±0.00	100.00±0.00	86.66±3.33	76.67±4.08	66.66±5.27	53.33±6.23	36.66±6.23	13.32±6.23	Nil
	5.0	100.00±0.00	100.00±0.00	100.00±0.00	90.99±4.08	73.33±4.08	63.33±3.33	50.00±5.27	36.66±3.33	26.66±4.08
Cx. quinquefasciatus	1.0	100.00±0.00	100.00±0.00	90.33±4.08	73.33±6.23	45.99±4.08	28.66±4.08	13.33±6.23	Nil	Nil
	2.5	100.00±0.00	100.00±0.00	100.00±0.00	86.66±4.08	67.66±4.08	39.66±3.33	26.66±6.66	10.00±4.08	Nil
	5.0	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	87.33±5.26	72.00±3.33	56.66±4.08	39.99±4.08	23.33±4.08

larvicidal and repellent activity. In addition, some compounds are also reported to have insect host-seeking behavior effects and contain insect sex pheromone components[28].

Morais *et al*[29] also reported that main components methyleugenol and alpha-copaene for *Croton nepetaefolius* (LC_{50} of 84 ppm); alpha-pinene and beta-pinene for *Croton argyrophyloides* (LC_{50} of 102 ppm); and alpha-pinene, beta-phelandrene, and transcaryophyllene for *Croton sonderianus* (LC_{50} of 104 ppm) and *Croton zenhtneri* exhibited higher larvicidal activity with an LC_{50} of 28 ppm against *Ae. aegypti*. Siddiqui *et al*[30] have reported that the compounds spipnoohine (1) and pipyahyine (2) isolated from the petroleum ether extract of dried ground whole fruits of *Piper nigrum* exhibited toxicity at 35.0 and 30.0 ppm, respectively, against fourth-instar larvae of *Ae. aegypti*. Park *et al*[31] have also reported the compounds retrofractamide (0.039 ppm), pipercide (0.1 ppm), guineensine (0.89 ppm), pellitorine (0.92 ppm) and piperine (5.1 ppm) derived from the fruits of *Piper nigrum* against *Ae. aegypti* third instar larvae. Prenylated xanthones, tetracyclic phenols and saponins are reported to be effective in controlling mosquito *Ae. aegypti*, the vector of yellow fever[32]. Mosquito larvicidal activity of crude carbon-tetra-chloride extract of *Solanum xanthocarpum* fruits was the most effective with LC_{50} values of 5.11 ppm after 24 h and 1.27 ppm after 48 h of treatment against *An. stephensi*[33]. Saponin compound was separated from the *Agave sisalana* leaves and used at 100 ppm, which causes 100% mortality of *Cx. quinquefasciatus* larvae after 3-4 days[34]. Earlier report showed that the compound like diterpenoid furans 6alpha-hydroxyvouacapan-7beta, 17beta-lactone (1), 6alpha, 7beta-dihydroxyvouacapan-17beta-oic acid (2) and methyl 6alpha, 7beta-dihydroxyvouacapan-17beta-oate (3) from seeds of *Pterodon spolygalaeflorus* exhibited LC_{50} values of 50.08, 14.69, and 21.76 μg/mL against fourth instar *Ae. aegypti* larvae, respectively[35].

The bioactive compound *Azadirachtin* isolated from *Azadirachta indica* showed complete ovicidal activity in eggs of *Culex tarsalis* and *Cx. quinquefasciatus* exposed to 10 ppm concentration[36]. Zani *et al*[37] reported the larvicidal activity of isolated compound thiophene derivatives from the ethanol extract of *Tagetes minuta* showed LC_{50} value of 1.0 mg/L on *Aedes fluviatilis*. The toxic effect of neolignans compound separated from *Piper decurrens* showed maximum activity on mosquito[38-45]. Earlier authors reported that the isolated compound neemarin from *Azadirchta indica* exhibited LC_{50} and LC_{90} values were 0.35 and 1.81 mg/L for *An. stephensi* and 0.69 and 3.18 mg/L for *Cx. quinquefasciatus*[46]; leptostachyol acetate compound isolated from the roots of *Phryma leptostachya* with LC_{50} values of 0.41, 2.1, and 2.3 ppm against third instar larvae of *Culex pipiens* pallens, *Ae. aegypti*, and *Ochlerotatus togoi*[47]; vilasininoid and two havanensinoids were isolated from the chloroform fractions of the methanol extracts of the root barks of *Turraea wakefieldii* and *Turraea floribunda* showed LD_{50} values of 7.1, 4.0, and 3.6 ppm, respectively against third instar larvae of *Anopheles gambiae*[19].

In conclusion, results of this research indicate that semiochemical tetradecanoic acid should be more fully examined as potent insecticides useful in mosquito control programs. Given the vital need for environmentally safe chemicals, the field efficacy, mode of action, persistence, effects on natural enemies and feasibility of using these compound in mosquito control programs warrant further attention.

Conflict of interest statement

We declare that we have no conflict of interest.

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