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Bio-efficacy of dodecanoic acid on larvicidal, ovicidal, pupicidal, and repellent activities against malarial vector *Anopheles stephensi* (Liston) (Diptera: Culicidae)

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Abstract
Mosquitoes are the important vectors of deadly diseases including Malaria, Dengue, Filariasis, etc. Malaria is caused by the malarial parasite Plasmodium falciparum. The major vectors belong to Anopheles, Aedes, Culex, and Mansonia species. These deadly vectors are commonly controlled by pesticides. But continuous use of pesticides causes resistance to develop in these vector agents. There is an urgent need to control these vector populations. Most novel alternative are semiochemicals. Semiochemicals are signaling chemicals used to carry information between living organisms, which cause changes in their behaviour. Semiochemicals are known for attractive and repellent insect activity; however, some of these also contain insecticidal properties. The Dodecanoic acid is a novel Semiochemical. It is one of the saturated fatty acid commonly found in both animal and vegetable fats and is frequently used in cosmetics, soaps, perfumes and flavorings etc. It is having the property of hypercholesterolemia among the saturated fatty acids. The Dodecanoic acid has not been fully evaluated against the malarial vector Anopheles stephensi. The present study aims to evaluate the bio-efficacy of Dodecanoic acid on larvicidal, ovicidal, pupicidal and repellent activities against the malarial vector Anopheles stephensi.

Keywords: larvicidal, ovicidal, pupicidal, repellent, *Anopheles stephensi*, dodecanoic acid

Introduction
Insect vectors, especially mosquitoes, are responsible for spreading devastating parasites and pathogens causing serious diseases, including malaria, yellow fever, dengue, filariasis and, more recently Zika virus [1]. Tropical areas are more vulnerable to parasitic diseases and the risk of contracting arthropod borne illnesses is increased due to climate change and intensifying globalization [2]. *Anopheles stephensi* acts as vector of *Plasmodium* parasites, which are responsible for malaria in tropical and subtropical areas worldwide [3]. *An. stephensi* is a major vector of malaria worldwide, and has been shown to be directly responsible for about 40‑50 percent of the annual malarial incidence [2]. Malaria afflicts 36 percent of the world people i.e, 2020 million in 107 countries and territories situated in the tropical and subtropical regions [4]. Currently, malaria management is a big challenge due to the presence of insecticide-resistant strains as well as to the development of *plasmodium* species highly resistant to major antimalarial drugs [5]. Young instar populations of mosquitoes are the targets of the majority of control programs, since focus on killing adults may only temporarily reduce the population and has higher operational costs [6]. Most common one technique available for the control of human vector mosquitoes is the use of synthetic chemical insecticides, but synthetic insecticides are high toxic on non-target organisms and give the negative effect on environment as well as over long time use of synthetic chemical pesticides leads mosquitoes to develop the resistance against chemical pesticides [7]. In this regard the alternative method to control these vector population is by means of Semiochemicals.
Semiochemicals are organic compounds used by insects to convey specific chemical messages that modify behavior or physiology [8]. Semiochemicals have different molecular weights depending on carbon chain. They are biologically active at very low concentration in the environment, thus their chemical characterization is complicated. Semiochemicals are the advanced cost effective, harm less way and easily applicable in the field and are used to lessen mosquito population [9]. They do not develop vector resistance and are normally harmless to the non target organisms. In addition, the Semiochemicals are often environmentally safe, with rapid biodegradation, and are non-toxic to humans and other mammals and also possesses pleasant smell. The active ingredients in these compounds are of relatively low cost because of their extensive worldwide use as fragrances and flavoring compounds [10]. Therefore, Semiochemicals can be excellent candidates for replacing conventional insecticides.
Lauric-acid or Dodecanoic-acid is a saturated fatty acid with a 12-carbon atom chain, thus falling into the medium chain fatty acids. It is a white powdery solid with a faint odour of bay oil or soap. Lauric acid, as a component of triglycerides, comprises about half of the fatty acid content in coconut oil [11]. Lauric acid has the

strongest antimicrobial activity among all saturated fatty acids against gram-positive bacteria and some viruses and fungi [12]. The Lauric acid has not been fully evaluated against the malarial vector *Anopheles stephensi*. So the present experiment aims to evaluate the bio efficacy of Dodecanoic acid against the malarial vector *Anopheles* mosquito.

Materials and methods

1. Test compound

Lauric 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 concentration for the bio-assays.

2. Test organism

The mosquitoes, of *An. stephensi* 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 percent sucrose solution and one week old chick for blood meal. Mosquitoes were kept at (28±2 ^{O}C), 70%-85% relative humidity, with a photo period of 14 h light, 10 h dark.

3. Larvicidal bioassay

The larvicidal activity of the plant crude extracts was evaluated as per the method recommended by WHO [13]. Batches of 25 third instar were transferred to a small disposable test cups, each containing 200 ml of water. The appropriate volume of dilution was added to 200ml water in cups to obtain the desired target dosage concentration ranging from (5 to 30 ppm), starting with the lowest concentration. Five replicates were set up for each concentration and an equal number of controls were set up simultaneously using tap water. To this 1 ml of appropriate solvent was added. The LC_{50} value was calculated after 24h by probit analysis separately [14].

4. Ovicidal activity

The method of Su and Mulla [15] and was slightly modified and used to test the ovicidal activity. The eggs of *An.stephensi* were collected from vector control laboratory, Department of Zoology, Annamalai University. The different compounds were diluted in ethanol to achieve various concentrations. Before treatment the egg rafts of *An.stephensi* were counted under microscope individually. Eggs of this mosquito species (100 numbers of 12-18h old) were exposed to different concentrations of the compounds until they hatched or died. After treatment the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. Each experiment was replicated five times along with appropriate control. The hatch rates were assessed 48 h post treatment by following formula.

$$\% \text{ of egg hatchability} = \frac{\text{Number of hatched larvae}}{\text{Total number of eggs}} \times 100$$

5. Pupicidal activity

Testing of Dodecanoic acid for the pupicidal activity was carried out separately for the pupa of *An. stephensi* at different concentrations of (20-45) ppm by preparing the required stock solution by following standard procedure WHO [16]. The desired concentration of the test solution was achieved by adding 1.0ml of an appropriate solution to 100 ml of tap water. Five replicates for each concentration were maintained. 25 numbers of pupae were introduced into the disposable plastic cups. The plastic cups were obtained from the laboratory colony. Ethanol was used as a control. The pupal mortality in both in treated and control were recorded after 24 h. The mortality of mosquito pupae was recorded according to the following criteria WHO [13]. Which were incapable of rising to the surface or did not show the characteristic living reaction when water disturbed has discoloration, an unnatural position or rigor. The corrected percentage of mortality was calculated by applying Abbots formula Abbott [17].

Mortality (%) = (Number of dead individuals/number of treated individuals) x100 .

6. Repellent activity

The repellent study was following the method of WHO [18]. Three days old blood starved female *An.stephensi* mosquitoes (100) were kept in a net cage (45cm×30cm×45cm). The volunteer had no contact with lotions, perfumes, or perfumed soaps on the day of the assay. Arms of the volunteer, only 25 cm^2 dorsal side of the skin on each arm was exposed and the remaining area covered by rubber gloves. The crude extract was applied at 1.0, 2.0 and 4.0 mg/cm^2 separately in the exposed area of the fore arm. Only ethanol served as control. The time of the test depend on whether the target mosquitoes day or night biters. *An. stephensi* were tested during the night from 17.00 to 04.00 h. 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 five times. The volunteer conducted their test of each concentration by inserting the treated and control arm into the same cage at a same time for one full minute for every 5 min. The mosquitoes that landed on the

hand were recorded and then shaken off before it imbibing any blood; making out a 5 minute protection. The percentage of repellency was calculated by the following formula.

% repellency = $[(Ta - T_b) / T_a] \times 100$

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

7. 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.

Result and Discussion

In the present experiment the toxicity of Dodecanoic acid, was tested for larvicidal, Ovicidal, pupicidal and repellent activities against the malarial vector *An. stephensi.* The result for larvicidal activity is shown in Table 1. The data were recorded and the statistical data regarding LC_{50}, LC_{90}, Regression equation, LCL, UCL, and *chi*-square values were calculated. The LC_{50} and $LC_{90,}$ values for larvicidal bio-assay of Dodecanoic acid were 14.723, 27.071, LC_{50} 95% (LCL-UCL) confidence limit value are 13.598-15.911 and LC_{90} 95% (LCL-UCL) confidence limit values are 25.540-29.631, and chi-square (x^2) value is 2.510 respectively. No mortality was observed in control.

Table 1: Larvicidal activity of Dodecanoic acid against the malarial mosquito, *Anopheles stephensi.*

Compound	Concentration (ppm)	Mortality (%) ± S.D	Regression equation	LC_{50}	Lc_{50} (ppm) LCL-UCL	LC_{90}	Lc_{90} (ppm) LCL-UCL	x^2
Dodecanoic acid	30 25 20 15 10 5 control	96 ± 1.73^f 84 ± 2.34^e 68 ± 2.82^d 48 ± 3.08^c 36 ± 2.36^b 15 ± 1.22^a 0.00	Y=-1.511+0.102X	14.786	13.598-15.911	27.331	25.540-29.631	2.510

Value represents mean ± S.D of five replications; Mortality of the larvae observed after 24h of exposure period. Chi square values are not significant at $p<0.05$ level LCL-lower confidence limit UCL- upper confidence limit.

The present results were also comparable with the previous authors as Rahuman *et al.* [19], reported the acetone solvent extract from *Feronia limonia* having the strong larvicidal activity and found to be effective against fourth instar larvae of *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*, with LC_{50} of 129.24, 79.58 and 57.23 ppm, and the compound was determined as n-hexadecanoic acid. Saurav *et al.* [20] evaluated the larvicidal property of actnobacterial compound 5-(2,4-dimethylbenzyl) pyrrolidin-2-one (DMBPO) which was extracted and isolated from *Streptomyces* VITSVK5 sp and tested against the *Anopheles stephensi* Liston, and *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). The results shown the LC_{50} value of crude extract as 88.97 ppm where as maximum efficacy was observed in the actnobacterial compound DMBPO with LC_{50} value of 88.97 ppm against *Anopheles stephensi*. Baranitharan *et al*. [21], reported the isolated compound 11-octadecenoic acid methyl ester and evaluated its larvicidal activity against *Ae. aegypti*, *An. stephensi* and *Culex quinquefasciatus* with LC_{50} values of 23.90, 22.32 and 20.51 ppm. Kumar *et al.* [22], evaluated the In-vitro elicitation of an important compound Conessine for its larvicidal activity against the *An.stephensi*. The isolated compound revealed a strong larvicidal activity against *An.stephensi* Liston with LC_{50} and LC_{90} values being 1.93 and 5.67 ppm, respectively.

The results of ovicidal activity are shown in Table 2. The eggs of *An.stephensi* treated with different concentrations viz., (1.0ppm, 1.5 ppm, 2.0 ppm, 2.5 ppm, 3.0 ppm 3.5 ppm and 4.0 ppm) of Dodecanoic acid. Percent hatch of eggs placed in control medium was 96.4 percent whereas, with 1.0, 1.5, 2.0, and 2.5 ppm concentrations, it was 74.3 percent, 52.5 percent, 28.2, 10.8 percent and with 3.0 ppm, 3.5ppm and 4.0 ppm doses, egg hatching was completely arrested. These results clearly revealed that the toxicity of Dodecanoic acid was dependent on its concentration which will determine the egg hatchability.

Table 2: Ovicidal activity of Dodecanoic acid against the eggs of *Anopheles stephensi.*

Egg hatchability (%)		
		Concentration (ppm)

Total	Control	1.0	1.5	2.0	2.5	3.0	3.5	4.0
100	96.4±*0.52*	74.3±*1.54*	52.5±*1.64*	28.2±*2.34*	10.8±*1.16*	NH	NH	NH

NH- no hatchability (100% mortality)

Similar reports were evaluated by previous authors as, Cheah *et al.* [23], evaluated the Larvicidal, oviposition, and ovicidal effects of *Artemisia annua* (Asterales: Asteraceae) against *Aedes aegypti*, *Anopheles sinensis*, and *Culex quinquefasciatus*. In the ovicidal assay, the percentage hatchability of eggs after treatment with 500 ppm of *Artemisia annua* extract was significantly lower than the control, with values of 48.84 ± 4.08, 38.42 ± 3.67, and 79.35 ± 2.09 % for *Aedes aegypti*, *Anopheles sinensis*, and *Culex quinquefasciatus*, respectively. Tennyson *et al.* [24], evaluated Ovicidal activity of *Ageratum houstonianum* Mill. (Asteraceae) leaf extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). The minimum concentration at which maximum egg mortality rate of 80 percent and above obtained was 10.0 mg/L in the case of methanol and ethyl acetate against *Anopheles stephensi* and *Aedes aegypti* respectively and 5.0 mg/L in ethyl acetate extract against *Culex quinquefasciatus*. One hundred per cent egg mortality was obtained only in ethyl acetate extract at 20.0 mg/L against *Aedes aegypti*. Veni *et al.* [25], evaluated the Ovicidal and larvicidal efficacy of *Crataeva magna* against the *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. Ovicidal bioassay conducted with five different extracts confirmed the higher efficacy of methanol extracts exerted the zero hatchability at 240 ppm with *An. stephensi*, 320 ppm with *Ae. aegypti* and 400 ppm with methanol and ethyl acetate extract of *Cx. quinquefasciatus* after 48h of exposure. Veni *et al.* [26], evaluated the larvicidal and ovicidal activity of *Terminalia chebula* Retz. (Family: Combretaceae) medicinal plant extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. Mean percent hatchability of the ovicidal activity was observed 48 h post treatment. The percent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. All the five solvent extracts showed moderate ovicidal activity; however, the maximum egg mortality (zero hatchability) was observed in the methanol extract of *T.chebula* at 200 and 250 ppm against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* showed 100 percent mortality at 300 ppm respectively.

For pupicidal activity the results are shown in Table 3. The pupae were treated with the different concentration of Dodecanoic acid (20-45) ppm. The LC_{50} and $LC_{90,}$ values were 29.129, 41.563, LC_{50} 95% (LCL-UCL) confidence limit value are 27.914-30.258 and LC_{90} 95% (LCL-UCL) confidence limit value are 39.837-43.766, *chi-square* (x^2) value is 3.003 respectively. No mortality was observed in control.

Table 3: Pupicidal activity of Dodecanoic acid against the malarial mosquito, *Anopheles stephensi.*

Compound	Concentration (ppm)	Mortality (%) ± S.D	Regression equation	LC_{50}	Lc_{50} ppm LCL-UCL	LC_{90}	Lc_{90} (ppm) LCL-UCL	x^2
Dodecanoic acid	45	94±1.68^f	Y=-3.002+0.103X	29.129	27.914-30.258	41.563	39.837-43.766	3.003
	40	84±2.54^e						
	35	76±2.18^d						
	30	58±1.58^c						
	25	34±2.34^b						
	20	14±1.24^a						
	control	0.00						

Value represents mean ± S.D of five replications; Mortality of the pupae observed after 24h of exposure period. Chi-square values are not significant at p<0.05 level, LCL-lower confidence limit UCL- upper confidence limit.

Similar reports were revealed by earlier authors as Ragavendran *et al.* [27], reported *Aspergillus terreus* fungus showed pupicidal activity against *Anopheles stephensi* and *Aedes aegypti* where the LC_{50} and LC_{90} values were 25.228 and 10.536 and 140.48 and 63.76 µg ml^{-1}. Vivekanandhan *et al* [28], evaluated the toxicity of *Fusarium oxysporum* extract alone and in binary combinations with temephos, on larvae and pupae of *An.stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. They found the binary combination of temephos plus *F. oxysporum* extract (1:1 ratio) was highly toxic to larvae of *An.stephensi* LC_{50}: 35.927 µg/ml, *Ae. aegypti* LC_{50}: 20.763 µg/ml and *Cx. quinquefasciatus*, LC_{50} 51.199 µg/ml. For pupae LC_{50} values were 38.668, 26.394, and 72.086 µg/ml. Magesh *et al.*[29], evaluated the efficacy of *Acalypha fruticosa* extracts as mosquito ovicidal, larvicidal and pupicidal agents against three vector mosquitoes *Aedes aegypti*, *Cx quinqueficiatus*, and *An.stephensi* for 24h. The chloroform extract showed highest activity against *Cx. quinquefasciatus* larvae with the LC_{50} value of 189.22 ppm. The pupicidal activity also showed the same trend at 500 ppm. The hexane extract showed highest activity against *An.stephensi* and *Ae.aegypti* with LC_{50} values of 61.38ppm and 184.67 ppm. Madhiyazhagan *et al.* [30], evaluated the Phytochemical Profiling and Mosquitocidal Properties of Grape Fruit Pedicel Extract against Malarial, Dengue and Filarial Vectors. After 24h the mortality was noted and Lethal Concentration LC_{50} was calculated against *An.stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. The LC_{50} of *An.stephensi* were 133.263ppm, 178.275ppm, 235.619ppm, 284.472ppm and 380.630ppm for I, II, III, IV Instar and pupae. Similarly, LC_{50} for *Ae. aegypti* were 89.093ppm (I Instar), 196.560ppm (II Instar), 241.043ppm (III Instar), 323.565ppm (IV Instar) and 363.515ppm (pupae) and for *Cx. quinquefasciatus* were 190.073ppm, 261.693ppm, 295.404ppm, 289.067ppm and 348.430ppm for I Instar, II Instar, III Instar, IV instar and Pupae, respectively.

The Dodecanoic acid was also tested for its repellent activity against the malarial vector *An. stephensi.* The results were shown in Table 4. The highest repellency was observed in higher concentrations of 4.0mg/cm^2 provide 100 percent protection up to 150 minutes against *An. stephensi.* The repellency results clearly indicate that as the concentration of the compound is increased from 1.0mg/cm^2 to 4.0mg/cm^2 the repellency time also get increased.

Table 4: Repellent activity of Dodecanoic acid against malarial vector, *Anopheles stephensi.*

Compound	**Concentration (mg/cm^2)**	**Percent of repellency in time interval**								
		15 min	30 min	60 min	90 min	120 min	150 min	180 min	210min	240 min
Dodecanoic acid	1.0	100.00±0.00	92.08±4.02	78.62±4.04	67.64±5.26	48.00±5.24	23.21±4.06	10.00±6.58	Nil	Nil
	2.0	100.00±0.00	100.00±0.00	87.64±3.32	74.62±4.06	62.66±5.25	51.31±3.32	34.66±6.22	15.28±6.22	Nil
	4.0	100.00±0.00	100.00±0.00	100..00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	87.23±5.22	69.78±3.32	54.64±4.07

Mean ± SD value of the replications.

Similar results were also reported by the earlier authors as Deepa *et al*.[31], reported the methanol extract of *Polygala arvensis* had strong repellent action against mosquitoes as it provided 100 percent protection against *An. stephensi, Ae. aegypti and Cx. quinquefasciatus* for 280 min. Govindarajan *et al*. [32], evaluated the effects of leaf and seed, extracts with different solvents of hexane, ethyl acetate, benzene, chloroform and methanol of *Delonix elata* on repellent activity against the malaria vector mosquito *An.stephensi.* Plant crude extracts of *D. elata* were applied at 1.0, 2.5, 5.0 mg/cm^2 separately in the exposed forearm of volunteers. Among the tested solvents, both the leaf and the seed methanol extracts showed maximum efficacy. The highest concentration of 5.0 mg/cm^2 provided over 210 and 180 min protection for the leaf and seed extracts, respectively. Mathalaimuthu *et al*. [33], evaluated the *Coleus aromaticus* leaf extract fractions as ovicides, larvicides and repellents against *Anopheles*, *Aedes* and *Culex* mosquito vectors. Among different fractions the high repellence of methanol fraction 4 tested at 2.5 mg/cm^2 was observed in "arm in cage" tests for at least 320 min. They hypothesized that 11-octadecenoic acid, methyl ester was the main constituent responsible for the mosquitocidal and repellent activity of *C.aromaticus* fractions. Pirmohammadi *et al.* [34], evaluated the Chemical Composition and Repellency Effect of *Ferulago Angulate* Plant Against Malaria Vector, *An.stephensi*. The mean assessed protection time and efficacy for plant was 60 and 100 minutes respectively. ED_{50} and ED_{90} values for this plant were 18.12 and 93.19 µl /cm^2 respectively. Fouda *et al*. [35], evaluated the Magnesium Oxide Nanoparticles (Mg-NPs) Fabricated by *Penicillium chrysogenum* against *An.stephensi*. The biogenic MgO-NPs exhibit high efficacy against different larvae instar and pupa of *An.stephensi*, with LC_{50} values of 12.5–15.5 ppm for I–IV larvae instar and 16.5 ppm for the pupa. Additionally, 5 mg/cm^2 of MgO-NPs showed the highest protection percentages against adults of *An.stephensi*, with values of 100 percent for 150 min and 67.6 percent ± 1.4 percent for 210 min.

Conclusion

In conclusion the results revealed that the Semiochemical Dodecanoic acid, showed effective larvicidal, ovicidal, pupicidal and repellent activities against the malarial vector, *Anopheles stephensi*. The Semiochemical Dodecanoic acid possesses the potential to act a mosquitocide. The Semiochemical Dodecanoic acid should be further explored upto the molecular level. So that it can act as a novel biocide in future. As our environment needs these eco-friendly chemicals, for their efficacy, action mode, persistence, their effect on natural enemies and the feasibility of such compounds, so that they may be employed in control programs.

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Conflict of Interest

We declare that we have no conflict of interest.

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