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Larvicidal and Repellent Activities of *Cestrum nocturnum* (leaves) Extracts against the Mosquito Vector, *Culex antennatus* Becker (Diptera: Culicidae)

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ABSTRACT

The present study investigated the larvicidal and repellent activities of *Cestrum nocturnum* (leaves) different extracts against Rift Valley Fever vector, *Culex antennatus*. The obtained results showed that the petroleum ether extract was the most effective extract against *Cx. antennatus* larvae followed by chloroform, acetone, and ethanolic extracts. The LC_{50} values of petroleum ether extract recorded 179.4, 164.2, and 148.7 ppm against *Cx. antennatus* third larval instar after 24, 48, and 72 h., respectively. On the other hand, at 6.67, 3.33, and 1.67 mg/cm^2, ethanolic extract induced a degree of repellency equal to 93.1, 90.1, and 60.2% within the 4h post-treatment, respectively. Generally, petroleum ether extract proved high efficacy as repellents. At 3.33mg/cm^2 petroleum ether extract produced the highest protection (100.0%) during the entire testing period of 4h post-treatment. Moreover, the protection (93.3, 90.7 and 80.8%) obtained at 2.67, 1.67 and 0.833 mg/cm^2, respectively, compared with 100.0% repellency for DEET at a dose1.8 mg/cm^2. So, *Ce. nocturnum* tested extracts can be considered as new promising controlling agents against the mosquito vector, *Cx. antennatus*.

INTRODUCTION

The control of mosquitoes is an important public health concern around the world. In Egypt, *Culex antennatus* has a wide distribution and it is the main vector of the Rift Valley fever virus (Meagan *et al,* 1980; Darwish and Hoogastrall, 1981). Immature stages of mosquitoes are attractive targets for pesticides because they breed in water and thus are easy to deal with them in this habitat (Johnson and Singh, 2017). Mosquito eggs, larvae, and pupae are usually targeted using organophosphates, insect growth regulators, and microbial agents. Indoor residual spraying and insecticide-treated bed nets are also employed (Lees *et al,* 2014; Benelli, 2015). However, these chemicals have negative effects on human health and the environment, as well as induce resistance in a number of mosquito species (Hemingway and Ranson, 2000). Natural products represent a large family of diverse chemical entities with a wide variety of biological activities that have multiple uses (Amer *et al,* 2019). Historically, plants have supplied the chemistry for over 25% of prescription drugs used in human medicine (Cox and Balick, 1994), and such biologically active plants have also provided leads to natural insecticides. Plants are a rich source of bioactive organic chemicals and synthesize a number of secondary metabolites to serve as defense chemicals against attack.

These chemicals may serve as insecticides, antifeedants, oviposition deterrents, repellents, growth inhibitors, juvenile hormone mimics, moulting hormones, as well as attractants (Murugan *et al,* 1996; Koul, 2005). Moreover, botanical pesticides offer an advantage over synthetic pesticides as they can be much less toxic, less prone to the development of resistance and more easily degradable. Various plant species have been exploited throughout the world to control mosquito populations (Muthukrishnan *et al,* 1997).

The present study aimed to evaluate the larvicidal activity of *Cestrum nocturnum* 70% ethanol, acetone, chloroform and petroleum ether extracts against the mosquito vector, *Cx. antennatus*.

MATERIALS AND METHODS

The Origin and Laboratory Maintenance of The Mosquito Colony*:*

Culex antennatus larvae obtained from Medical Entomology Research Center and reared for five generations, in the insectary of medical entomology at the Department of Zoology Faculty of Science Al-Azhar University using the procedure described by Hassanain *et al,* (2019).

Extraction of Plant Materials*:*

Cestrum nocturnum (leaves) after collected from natural habitat, they were left to dry at room temperature (27-31°C) for 5 to 10 days according to the plant species and pulverized to powder separately in a hammer mill. The extraction was performed using 70% ethanol, acetone, chloroform, and petroleum ether solvents. One hundred grams of powder from each part of the plant for each solvent separately was extracted five times with 300 ml of aqueous 70% ethanol, acetone, chloroform and petroleum ether at room temperature. After 24 h., the supernatants were decanted, filtrated through Whatman filter paper No. 5.and dried in a rotary evaporator at 40 ºC for (2–3) hours to ethanol and (40–60) minutes to other solvents. The dry extracts were weighed and kept in a deep freezer (-4ºC) till used for experiments (Hassan *et al,* 2014).

Larvicidal Activity of Tested Plant Extracts*:*

In order to study the toxicity of the concerned plant extracts, the tested material of the ethanolic extracts was dissolved in 0.1ml of 70% ethanol, while the tested material of acetone, chloroform, and petroleum extracts was dissolved in 2 drops of $Tween_{80}$ as an emulsifier to facilitate the dissolving of tested material in water. Different range of concentrations of each concerned extract was prepared in order to detect mortalities. All tested materials were performed in 250 ml. of dechlorinated tap water contained in 350 ml plastic cups. Then, third 3rd instar larvae (25 larvae) were put immediately into plastic cups contained different concentrations of extracts. At least three replicates were usually used for each tested concentration. All plastic cups were incubated under controlled conditions at a temperature of 27±2°C, relative humidity 70±10%, and 12-12 light-dark regime for 24, 48, and 72 h. and subsequently, mortality was recorded, Control larvae received 0.1 ml of 70% ethanol or 2 drops of $Tween_{80}$ in 250 ml water. Percentage of mortalities were corrected according to Abbott's formula (Abbott, 1925). The statistical analysis of the data was carried out according to the method of lentner *et al,* (1982). LC_{50} was calculated using multiple linear regression (Finney, 1971).

Repellent Activity:

Standard cages (20×20×20cm) were used to test the repellent activity of plant extracts. Different weight from each extract was dissolved in 2ml (70% ethanol, acetone, chloroform and petroleum ether with a drop of $Tween_{80}$ separately) in glass 4×4cm to prepare different concentrations. The concentration was directly applied onto 5×6cm of the ventral surface of pigeon after removed feathers from the abdomen to evaluate the repellency against *Cx. antennatus*. After 10 minutes of treatment, the treated pigeons were placed in the cages containing *Cx. antennatus* starved females 5-7 d-old for three hours. Control tests were carried out alongside the treatments using ethanol or water. Each test was repeated three times to get a mean value of repellent (Hassan *et al*, 2014). After treatments, the number of fed and unfed females were counted and calculated according to Abbott, (1925).

RESULTS

Data given in table (1) indicate the toxic effect of ethanolic extract of *Cestrum nocturnum* (leaves) against the 3rd instar larvae of *Culex antennatus*. The highest concentration 2000 ppm induced 90.8 and 93.2% larval mortality after 24 and 48 hours increased to complete larval mortality 100% after 72 hours. The concentration 1500 ppm caused larval mortality percent 80.0, 85.2, and 90.8 after 24, 48, and 72 hours, respectively. Meanwhile, the concentrations 1000, 500, 250 and 125 ppm caused larval mortality 64.0, 38.8, 29.2 and 14.8% after 24 hours; 65.2, 40.0, 33.2 and 17.2% after 48 hours and 66.8, 45.2, 37.2 and 20.0% after 72 hours, respectively. The acute mortality was 49.2, 44.0, 37.2, 26.8, 12.0 and 5.2% at the concentrations 2000, 1500, 1000, 500, 250 and 125ppm. However, the chronic mortality was 100.0, 90.8, 66.8, 45.2, 37.2 and 20.0% at 2000, 1500, 1000, 500, 250 and 125ppm, while the survival potential was 0.0, 9.2, 33.2, 54.8, 62.8 and 80.0% at the same concentrations.

Table 1: Toxic effect of ethanolic extract from leaves of *Ce. nocturnum* on the 3rd instar larvae of *Cx. antennatus*.

Conc. (ppm)	Larval mortality %			Acute mortality %	Chronic mortality %	Survival potential %
	24 h.	48 h.	72 h.			
2000	90.8	93.2	100.0	49.2	100.0	0.0
1500	80.0	85.2	90.8	44.0	90.8	9.2
1000	64.0	65.2	66.8	37.2	66.8	33.2
500	38.8	40.0	45.2	26.8	45.2	54.8
250	29.2	33.2	37.2	12.0	37.2	62.8
125	14.8	17.2	20.0	5.2	20.0	80.0
Control	0.0	0.0	0.0	0.0	0.0	100.0

Conc. = Concentration; ppm = particle per million; h. = hours; Acute mortality = mortality after 12 hours; Chronic mortality = mortality calculated after 72 hours; Survival potential = 100- Chronic mortality.

Data given in table (2) indicate the toxic effect of the acetone extract of *Ce. nocturnum* (leaves) against the 3rd instar larvae of *Cx. antennatus.* After 24 hours from the treatment, the highest concentration 1500 ppm induced 92.0% larval mortality, while the lowest concentration 75 ppm induced the lowest larval mortality 6.8%. At 1000, 500, 250, and 125 ppm the larval mortality recorded 76.0, 49.2, 40.0 and 22.8%, respectively. While, after 48 hours the larval mortality increased to 94.8% at the highest concentration 1500 ppm, while it recorded 85.2, 65.2, 48.0, 26.8, and 9.2% at 1000, 500, 250, 125, and 75 ppm, respectively. On the other hand, after 72 hours a complete larval mortality 100.0% recorded at the concentration 1500 ppm, while the lowest concentration 75 ppm caused 12.0% larval mortality. At the concentrations 1500, 1000, 500, 250, 125 and 75ppm, the acute mortality recorded 51.2, 38.8, 26.8, 21.2, 10.8 and 4.0, while; chronic mortality was 100.0, 88.0 72.0, 53.2, 28.0 and 12.0, respectively. The survival potential % was 0.0, 12.0, 28.0, 46.8, 72.0 and 88.0 obtained at the same concentrations vs. 100.0% for the untreated group.

Table 2: Toxic effect of acetone extract from leaves of *Ce. nocturnum* on the 3rd instar larvae of *Cx. antennatus*.

Conc. (ppm)	Larval mortality %			Acute mortality %	Chronic mortality %	Survival potential %
	24 h.	48 h.	72 h.			
1500	92.0	94.8	100.0	41.2	100.0	0.0
1000	76.0	85.2	88.0	38.8	88.0	12.0
500	49.2	65.2	72.0	26.8	72.0	28.0
250	40.0	48.0	53.2	21.2	53.2	46.8
125	22.8	26.8	28.0	10.8	28.0	72.0
75	6.8	9.2	12.0	4.0	12.0	88.0
Control	0.0	0.0	0.0	0.0	0.0	100.0

See foot note of table (1).

Data given in table (3) indicated the toxic effect of chloroform extract of *Ce. nocturnum* (Leaves) against the 3rd instar larvae of *Cx. antennatus*. The highest larval mortality 94.8% occurred at the concentration 1000ppm and the lowest mortality 8.0% occurred at the concentration 50ppm after 24 hours of treatment, while; the concentrations: 800, 600, 400, 200 and 100 ppm induced mortality percent 84.0, 72.0, 60.0, 37.2 and 26.8, respectively. On the other hand, the larval mortality after 48 hours increased to 98.8, 88.0, 78.8, 65.2, 40.0, 28.0 and 10.8% at 1000, 800, 600, 400, 200, 100 and 50 ppm. After 72 hours the mortality was 100.0, 96.0, 82.8, 68.0, 49.2, 30.8 and 16.0% at the concentrations 1000, 800, 600, 400, 200, 100 and 50 ppm, respectively. At the concentrations 1000, 800, 600, 400, 200 and 100 ppm, the acute mortality recorded 52.0, 45.2, 36.0, 17.2 and 13.2 %, while; chronic mortality % was 100.0, 96.0, 82.8, 68.0, 49.2, 30.8 and 16.0, respectively. The survival potential was 0.0, 4.0, 17.2, 32.0, 50.8, 69.2 and 84.0 % obtained at the same concentrations vs. 100.0% for the untreated group.

Table 3: Toxic effect of chloroform extract from leaves of *Ce. nocturnum* on the 3rd instar larvae of *Cx. antennatus*.

Conc. (ppm)	Larval mortality %			Acute mortality %	Chronic mortality %	Survival potential %
	24 h.	48 h.	72 h.			
1000	94.8	98.8	100.0	52.0	100.0	0.0
800	84.0	88.0	96.0	45.2	96.0	4.0
600	72.0	78.8	82.8	36.0	82.8	17.2
400	60.0	65.2	68.0	30.8	68.0	32.0
200	37.2	40.0	49.2	17.2	49.2	50.8
100	26.8	28.0	30.8	13.2	30.8	69.2
50	8.0	10.8	16.0	0.0	16.0	84.0
Control	0.0	0.0	0.0	0.0	0.0	100.0

See foot note of table (1).

Data recorded in table (4) indicated the toxic effect of petroleum ether extract of *Ce. nocturnum* (leaves) against the 3rd instar larvae of *Cx. antennatus*. The highest concentration 500ppm induced 93.2% larval mortality after 24 hours increased to complete larval mortality of 100% after 48 hours. The concentration 400, 300, 200, 100, 50 and 25 ppm caused larval mortality 92.0, 84.0, 64.0, 38.8, 24.0 and 10.8% after 24 hours, respectively. Meanwhile, the concentrations 400, 300, 200, 100, 50 and 25 ppm caused larval mortality percent 93.2, 85.2, 66.8, 44.0, 26.8 and 12.0 after 48 hours, respectively. On the other hand, there was an increase in the larval mortality after 72 hours as it recorded 96.0, 89.2, 69.2, 46.8, 30.8, and 13.2% at 400, 300, 200, 100, 50, and 25 ppm, respectively.

Acute mortality % recorded 57.2, 46.8, 44.0, 37.2, 20.0, 8.0 and 2.8 at the concentrations 500, 400, 300, 200, 100, 50 and 25ppm. However, the chronic mortality % was 100.0, 96.0, 89.2, 69.2, 46.8, 30.8 and 13.2 at 500, 400, 300, 200, 100, 50 and 25ppm, while the survival potential recorded 0.0, 4.0, 10.8, 30.8, 53.2, 69.2 and 86.8% at the same concentrations.

Table 4: Toxic effect of petroleum ether extract from leaves of *Ce. nocturnum* on the 3rd instar larvae of *Cx. antennatus*.

Conc. (ppm)	Larval mortality %			Acute mortality %	Chronic mortality %	Survival potential %
	24 h.	48 h.	72 h.			
500	93.2	100.0	100.0	57.2	100.0	0.0
400	92.0	93.2	96.0	46.8	96.0	4.0
300	84.0	85.2	89.2	44.0	89.2	10.8
200	64.0	66.8	69.2	37.2	69.2	30.8
100	38.8	44.0	46.8	20.0	46.8	53.2
50	24.0	26.8	30.8	8.0	30.8	69.2
25	10.8	12.0	13.2	2.8	13.2	86.8
Control	0.0	0.0	0.0	0.0	0.0	100.0

See foot note of table (1).

As shown in table (5), the petroleum ether extract was the most effective extract against *Cx. antennatus* larvae followed by chloroform, acetone, and ethanolic extracts. The LC_{50} values of petroleum ether extract recorded 179.4, 164.2, and 148.7 ppm against *Cx. antennatus* third larval instar after 24, 48, and 72 h., respectively.

Table 5: Relative efficiency of *Ce. Nocturnum* (leaves) different extract against *Cx. antennatus* 3rd instar larvae.

Time (Hours)	Extracts	LC_{50} (ppm)	Slope (b)	R^2
24	Ethanol 70%	836.4	0.039	0.965
	Acetone	620.2	0.054	0.925
	Chloroform	398.8	0.084	0.944
	Petroleum ether	179.4	0.177	0.909
48	Ethanol 70%	755.25	0.04	0.962
	Acetone	487.2	0.054	0.848
	Chloroform	354.9	0.087	0.937
	Petroleum ether	164.2	0.180	0.923
75	Ethanol 70%	664.9	0.041	0.968
	Acetone	417.8	0.055	0.832
	Chloroform	297.9	0.085	0.925
	Petroleum ether	148.7	0.178	0.904

R^2: Correlation Coefficient; see footnote of table (1).

The repellent activity of *Ce. nocturnum* tested extracts against starved *Cx. antennatus* females varied according to the doses used (Table 6). At doses 6.67, 3.33, and 1.67 mg/cm^2, ethanolic extract induced a degree of repellency equal to 93.1, 90.1 and 60.2% within the 4h post treatment, respectively. It is obvious from the obtained results in table (6) that petroleum ether extract proved high efficacy as repellents. The repellent activity at 3.33mg/cm^2 produced the highest protection 100.0% during the entire testing period of 4h post-treatment. Moreover, the protection (93.3, 90.7 and 80.8%) obtained at 2.67, 1.67 and 0.833mg/cm^2, respectively, compared with 100.0% repellency for DEET at a dose1.8mg/cm^2.

Table 6: Repellent activity of tested extracts from leaves of *Ce. nocturnum* against *Cx. antennatus* females.

Extracts	Dose (mg/cm^2)	No. of tested females	Fed females		Unfed females		Repellency %
			No.	%	No.	%	
Ethanol 70%	6.67	51	3	5.9	48	94.1	93.1
	3.33	59	5	8.5	54	91.5	90.1
	1.67	44	15	34.1	29	65.9	60.2
Acetone	6.67	48	2	4.2	46	95.8	95.3
	3.33	42	3	7.1	39	92.9	92.0
	1.67	57	14	24.6	43	75.4	72.3
Chloroform	3.33	67	3	4.5	64	95.5	95.0
	1.67	61	6	9.8	55	90.2	89.3
	0.833	72	14	19.4	58	80.6	78.8
Petroleum ether	3.33	58	0	0.0	58	100.0	100.0
	2.67	51	3	5.9	48	94.1	93.3
	1.67	49	4	8.2	45	91.8	90.7
	0.833	53	9	17.0	44	83.0	80.8
DEET	1.8	25	0.0	0.0	25	0.0	100.0
Control	0.0	42	36	85.7	6	14.3	0.0

DISCUSSION

The findings of the present study revealed that the toxicity of *Cestrum nocturnum* tested extracts against 3rd instar larvae of *Culex antennatus* was varied according to the solvent used in the extraction and concentration of the extract. The larval mortality percent was increased by increasing extract concentration for all extracts tested. Based on LC_{50} values, the petroleum ether extract was the most effective extract against *Cx. antennatus* larvae followed by chloroform, acetone and ethanolic extracts. These results are in consistent with the previously mentioned suggestions of (Sukumar *et al*, 1991; Maurya *et al*, 2009). Several plant extracts other than those used in the present study had been tested against different species of mosquitoes by many authors worldwide. The activity of *Ce. nocturnum* tested extracts against *Cx. antennatus* larvae were in agreement with the results obtained by Vahitha *et al*, (2002) using leaf extracts of *Pavonia zeylanica* and *Acacia ferruginea* against the late third instar larvae of *Cx. quinquefasciatus*, where the LC_{50} values recorded 2214.7 and 5362.6 ppm; Jeyabalan *et al*, (2003) using methanol extracts of *Pelargonium citrosa* leaves against *Anopheles stephensi*, where the larval mortality recorded 98.0% with the highest dose of 4% plant extract; Prabakar and Jebanesan, (2004) using extracts from five species of Cucurbitacious plants, *Momordica charantia*, *Trichosanthes anguina*, *Luffa acutangula*, *Benincasa cerifera* and *Citrullus vulgaris* against the late third larval age of *C*x. *quinquefasciatus*, where the LC_{50} values after 24h were 465.85,567.81, 839.81, 1189.30 and 1636.04 ppm and Maurya *et al*, (2009) using petroleum ether extract from leaves of a widely grown medicinal plant, *Ocimum basilicum*, against *An. stephensi* and *Cx. quinquefasciatus*, where the petroleum ether extract from leaves of *O. basilicum* was found to be the most effective against the larvae of both mosquitoes than other extracts with LC_{50} values of 8.29, 4.57; 87.68, 47.25 ppm and LC_{90} values of 10.06, 6.06; 129.32, 65.58ppm against *A. stephensi* and *Cx. quinquefasciatus* after 24 and 48 h of treatment. Also, these results are in consistent with those obtained by Sakthivadivel *et al*, (2014) who found that aqueous fruit extract of *Wrightia tinctoria* exhibited highest larvicidal activity against the filarial vector *Cx. quinquefasciatus* with LC_{50} value of 0.17% after 24 and 48 h and Samuel *et al*, (2014) who mentioned that, *Ipomoea cairica* and *Ageratina adenophora* extracts were found to be effective against third instar larvae of *Cx. quinquefasciatus* causing 77-100% mortality at 48 h.

There was considerable variation in the repellent activity of the various plant materials and this may reflect the complexity of the chemical composition of their constituents (Bisseleua *et al,* 2008). In the present study, all the concentrations of *Ce. nocturnum* tested extracts exhibited repellency effect against the starved female adults of *Cx. antennatus*. The repellent activity depends on the solvent used in the extraction and the dose of the extract. The petroleum ether extract was the most effective extract which evoked 100.0% repellency or biting deterrency at the dose 3.33 mg/cm^2. The present results of repellency effects caused by *Ce. nocturnum* extracts come in an agreement with those results reported by Govere *et al,* (2000) using extracts of fever tea (*Lippia javanica*), rose geranium (*Pelargonium reniforme*) and lemongrass (*Cymbopogon excavatus*) against *An. arabiensis*; Kim *et al,* (2002) using ethanol extract of fruits from *Foeniculum vulgarea* against hungry *Aedes aegypti* females; Yang *et al*, (2004) who obtained that, the repellent activity of methanol extracts of *Cinnamomum cassia* bark, *Nardostachys chinensis* rhizome, *Paeonia suffruticosa* root-bark and *Cinnamomum camphora* at the dose of 0.1 mg/cm^2 was 91.0, 81.0, 80.0 and 94.0% comparable to DEET (82.0%) against starved *Ae. aegypti*; Govindarajan *et al,* (2014) using extracts from *Delonix elata* against malaria vector *An. stephensi* and they reported that both leaf and seed methanol extracts showed maximum efficacy at the highest concentration of 5.0mg/cm^2 they provided over 210 and 180 min. protection and Shehata, (2018) who found that at 3.33, 1.67, 0.83, and 0.42 mg/cm^2 *Deverra triradiata* tested extracts showed a variable degree of repellency against *An. sergentii, Cx. pipiens* and *Cx. antennatus*, however hexane extract was the most effective extract with RD_{50} equal to 0.704, 1.122, and 0.92 mg/cm^2 against *An. sergentii, Cx. pipiens* and *Cx. antennatus* starved females.

Conclusion:

Based on LC_{50}, petroleum ether extract of *Cestrum nocturnum* tested extracts was the most effective in larvicidal and repellent activity against *Culex antennatus* than those of chloroform, acetone, and ethanolic extracts. So the tested extracts used can be considered as new promising controlling agents for the mosquitoes, *Cx. antennatus*.

REFERENCES

Abbott, WS, 1925. A method for computing the effectiveness of an insecticide. *Journal of Economic Entomology*. 18:265-277.

Amer, MS, Hasaballah, AI, Hammad, KM, Shehata AZI, Saeed, MS, 2019. Antimicrobial and antiviral activity of maggots extracts of *Lucilia sericata* (Diptera: Calliphoridae). *Egyptian Journal of Aquatic Biology and Fisheries*. 23(4):51-64.

Benelli, G, Bedinis, FG, Cosci, F, Cioni, PL, Amira, S, Benchikh, F, Laouer, H, Giuseppe, DG, Conti, B, 2015. Mediterranean essential oils as effective weapons against the West Nile vector *Culex pipiens* and the *Echinostoma* intermediate host *Physella acuta*: what happens around? An acute toxicity survey on non-target mayflies. *Parasitology Research*. 114:1011-1021.

Bisseleua, HBD, Gbewonyo, SWK, Obeng-Ofori, D, 2008. Toxicity, growth regulatory and repellent activities of medicinal plant extracts on *Musca domestica* L. (Diptera: Muscidea). *African Journal of Biotechnology*. 7(24):4635-4642.

Cox, PA, Balick, MJ, 1994. The ethnobotanical approach to drug discovery. *Scientific American.* 270:82-87.

Darwish, M, Hoogstraal, H, 1981. Arboviruses infesting human and lower animals in Egypt., A review of thirty years of research. *Journal of the Egyptian Public Health Association.* 56:1-112.

Finney, DJ, 1971. Probit analysis Third edition. Cambridge Univ. Press. 333p.

Govere, TA, Durrheim, DN, Du, TN, Hunt, RH, Coetzee, M, 2000. Local plants as repellents against *A. arabiensis*, in Mpumalanga Province, South Africa. *Central African Journal of Medicine.* 46(8):213-216.

Govindarajan, M, Rajeswary, M, Sivakumar, R, 2014. Repellent properties of *Delonix elata* (L.) Gamble (Family: Fabaceae) against malaria vector *Anopheles stephensi* (Liston) (Diptera: Culicidae). Article in press, *Journal of the Saudi Society of Agricultural Sciences, King Saud Univ. Saudi Arabia*.

Hassan, MI, Fouda, MA, Hammad, KM, Tanani, MA, Shehata, AZ, 2014. Repellent effect of *Lagenaria siceraria* extracts against *Culex pipiens*. *Journal of the Egyptian Society of Parasitology*. 44:243-248.

Hassanain, NA, Shehata, AZ, Mokhtar, MM, Shaapan, RM, Hassanain, MA, Zaky, S, 2019. comparison between insecticidal activity of *Lantana camara* extract and its synthesized nanoparticles against *Anopheline* mosquitoes. *Pakistan Journal of Biological Sciences.* 22(7):327-334.

Hemingway, J, Ranson, H, 2000. Insecticide resistance in insect vectors of human disease. *Annual Review of Entomology.* 45:371-391.

Jeyabalan, D, Arul, N, Thangamathi, P, 2003. Studies on effects of *Pelargonium citrosa* leaf extracts on malarial vector, *A. stephensi* Liston. *Bioresource Technology*. 89(2):185-189.

Johnson, AD, Singh, A, 2017. Larvicidal activity and biochemical effects of Apigenin against Filarial Vector *Culex quinquefasciatus*. *International Journal of Life-Sciences Scientific Research.* 3(5):1315-1321.

Kim, DH, Kim, SI, Chang, KS, Ahn, YJ, 2002. Repellent activity of constituents identified in *Foeniculum vulgare* fruit against *Ae. aegypti* (Diptera: Culicidae). *Journal of Agricultural and Food Chemistry*. 50(24):6993-6996.

Koul, O, 2005. Insect Antifeedants. CRC Press, Boca Raton, FL.

Lees, RS, Knols, B, Bellini, R, Benedict, MQ, Bheecarry, A, Bossin, HC, 2014. improving our knowledge of male mosquito biology in relation to genetic control programmes. *Acta Tropica.* 132: 2-11.

Lentner, C, Lentner, C, Wink, A, 1982. Studentis t- distribution tables. In Geigy scientific Tables Vol. 2. International Medical and Pharmaceutical information, Ciba- Geigy Limited, Basal, Switzerland.

Maurya, P, Sharma, P, Mohan, L, Batabyal, L, Srivastava, CN, 2009. Evaluation of the toxicity of different phytoextracts of *Ocimum basilicum* against *Anopheles stephensi* and *Culex quinquefasciatus*. *Journal of Asia-Pacific Entomology*. 12:113-115.

Meagan, JM, Khalil, GM, Hoogstraal, H, Adham, FK, 1980. Experimental transmission and field isolation studies implicating *C. pipiens* as a vector of Rift Valley virus in Egypt. *The American Journal of Tropical Medicine and Hygiene*. 80:1405-1410.

Murugan, TS, Babu, R, Jeyabalan, D, Kumar, SN, Sivaramkrishnan, S, 1996. Antipupational effect of neem oil and neem seed kernel extract against mosquito larvae of *A. stephensi* (Liston). *Journal of the Entomological Research*. 20:137-139.

Muthukrishnan, J, Pushalatha, E, Kasthuribhai, A, 1997. Biological effect of four plant extracts on Culex quinquefasciatus larval stages. *Insect Science and Its Application*. 17:389-394.

Prabakar, K, Jebanesan, A, 2004. Larvicidal efficacy of some Cucurbitacious plant leaf extracts against *C. quinquefasciatus* (Say). *Bioresource Technology*. 95(1):113-114.

Sakthivadivel, M, Gunasekaran, P, Annapoorani, JT, Samraj, DA, Arivoli, S, Tennyson, S, 2014. Larvicidal activity of Wrightia tinctoria R. BR. (Apocynaceae) fruit and leaf extracts against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). *Asian Pacific Journal of Tropical Disease*. 4(1):373- 377.

Samuel, L, Lalrotluanga, Muthukumaran, RB, Gurusubramanian, G, Senthilkumar, N, 2014. Larvicidal activity of *Ipomoea cairica* (L.) Sweet and *Ageratina adenophora* (Spreng.) King & H. Rob. plant extracts against arboviral and filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae). *Experimental Parasitology*. 141:112-121.

Sukumar, K, Perich, MJ, Boobar, LR, 1991. Botanical derivatives in mosquito control: A review. *Journal of the American Mosquito Control Association*. 7(2):210-237.

Vahitha, R, Venkatachalam, MR, Murugan, K, Jebanesan, A, 2002. Larvicidal efficacy of *Pavonia zeylanica* L. and *Acacia ferruginea* D.C. against *C. quinquefasciatus* Say. *Bioresource Technology*. 82(2):203-204.

Yang, YC, Lee, EH, Lee, HS, Lee, DK, Ahn, YG, 2004. Repellency of aromatic medicinal plant extracts and a steam distillate to *Ae. aegypti*. *Journal of the American Mosquito Control Association.* 20(2):146-149