Journal of
NUCLEAR
Technology in Applied Science
ISSN 2314-8209 e-ISSN 2314-8217

J. Nucl. Tech. Appl. Sci., Vol. 7, PP. 151 : 159 (2019)

Toxicological and Repellent Effects of Lantana camara (Verbenaceae) and Eucalyptus citriodora (Myrtaceae) Extracts against Rift Valley Fever Vector, Culex antennatus (Becker) (Diptera: Culicidae)

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Received: 21/02/2019
Accepted: 07/04/2019

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ABSTRACT

Culex antennatus (Becker) play an important role in transmission of Rift Valley Fever virus in the Nile Delta of Egypt. The present study aimed to evaluate the larvicidal activity of ethanol 70% and hexane extracts from *Lantana camara* and *Eucalyptus citriodora* leaves against the *C. antennatus* third larval instar. In addition, the repellent activity of the tested extracts against *C. antennatus* starved females was examined. The obtained results revealed that, hexane extract of tested plants was more effective against 3rd instar larvae of *C. antennatus* than ethanolic extract. The LC_{50} values of hexane extracts recorded 84.4, 158.6; 71.3, 129.1 and 64.0, 113.3 ppm after 24, 48 and 72 hours post treatment for *L. camara* and *E. citriodora*, respectively. Also, the tested ethanolic and hexane extracts of *L. camara* and *E. citriodora* (leaves) evoked a variable degree of repellency against *C. antennatus* starved females. The ethanolic extract exhibited 86.2, 68.8, 57.9 and 74.1, 59.0, 47.3% protection for *L. camara* and *E. citriodora* at 3.33, 1.67 and 0.83 mg/cm^2. Potent repellency (92.5%) achieved by *L. camara* hexane extract at 1.67 mg/cm^2 through the 3h post treatment, Also, *E. citriodora* (leaves) hexane extract showed 88.1, 77.8 and 53.7% repellency at 3.33, 1.67 and 0.83 mg/cm^2.

KEYWORDS

Larvicidal, Repellent, Culex antennatus, Lantana camara, Eucalyptus citriodora.

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INTRODUCTION

Culex antennatus (Becker) is one of the most important vectors in Egypt; it is the main vector of Rift Valley Fever virus during an outbreak in the Nile Delta of Egypt. 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 conventional chemical insecticides, including organochlorides, pyrethroids mainly the deltamethrin and organophosphates such as malathion and fenthion as last resort for vector control **(Ravaomanarivo *et al.*, 2014)**. The usages of these synthetic compounds are not only environmentally polluting but also have concomitant hazardous effects to non-target organisms and to human health **(Raharimalala *et al.*, 2012)**. In addition, the extensive use of synthetic insecticides **(Yang *et al.*, 2002)**, resistance to the widely used chemical insecticides and the appearance of the secondary pests have stimulated many institutions and researchers to be engaged with the search for some environmentally safe control agents in order to avoid the disadvantages and hazards of the synthetic insecticides. A great part of efforts have been achieved for the investigation and re-examination of plant sources to obtain natural compounds which may act as growth regulation, fecundity suppression, male sterility, loss of flying ability, immune depression and enzyme inhibition repellent or anti-feedant characteristics **(Su and Mulla, 1998; Thomas and Callaghan, 1999)**. These compounds are called Plant-natural products, which are considered as defensive means against the animals and insects which attack the plants **(Perveen *et al.*, 2008)**. Thus, the aim of the present study was to evaluate the larvicidal and repellent activities of ethanol 70% and hexane extracts from *Lantana camara* and *Eucalyptus citriodora* leaves against the Rift Valley Fever Vector, *C. antennatus*.

MATERIALS AND METHODS

Culex antennatus culture

The Rift Valley fever vector, *Culex antennatus* larvae were collected from Shubramunt, Giza, Egypt and maintained continuously for several generations in Medical Entomology Insectary, Animal house using the standard procedures described by **Adham *et al.* (2003)**.

Plant collection and preparation of crude extract

Lantana camara and *Eucalyptus citriodora* collected during April 2017 from Sadat City, Egypt away from sun rays were left to dry at room temperature (25-30ºC) for 5 to 10 days and pulverized to powder separately in a hammer mill. The extraction was performed using ethanol 70% and hexane. One hundred grams of powder from *L. camara* and *E. citriodora* (leaves) for each solvent separately were extracted and filtered five times using 300ml of ethanol 70% and hexane at room temperature. After 24 h., the supernatants were decanted, filtrated through whatman filter paper (No. 5) and dried in a rotary evaporator. The dry extracts were weighed and kept at -4ºC till using for experiments.

Larvicidal activity

The tested material of the ethanolic extracts was dissolved in 0.1ml of ethanol 70%, while the tested material of hexane extract was dissolved in 2 drop of $Tween_{80}$. Different concentrations of each extract were performed in 250ml. of dechlorinated tap water contained in 300ml plastic cups. Then, third instar larvae (25 larvae) were put immediately into plastic cups contained different concentrations of extracts. Three replicates were usually used for each tested concentration. Control larvae received only 0.1 ml of ethanol 70% or 2 drop of $Tween_{80}$ in 250ml water **(El-Sheikh *et al.*, 2012)**.

Mortalities were recorded every 24 hours and indicated by a failure to respond to mechanical stimulation **(Williams *et al.*, 1986)**. However, acute

mortalities are mortalities in the first 12 hours and chronic mortalities are mortalities calculated after 72 hours of treatment. Mortality was calculated using Abbott's equation formula.

Repellent activity

Standard cages (20×20×20cm) were used to test the repellent activity of ethanolic and hexane extracts from *L. camara* and *E. citriodora* (leaves). Different weights from each extract were dissolved in 2ml (ethanolic and hexane with a drop of $Tween_{80}$ separately) in glass 4×4cm to prepare different concentrations. The concentration was directly applied onto 5×6cm of ventral surface of pigeon after feathers removal from the abdomen to evaluate the repellency against *C. antennatus* compared with commercial repellent DEET (N. N. diethyl-meta- toulamide) (Johnson Wax Egypt) as a positive control. After 10 minutes, the treated pigeons were placed in the cages (20×20×20cm) containing *C. antennatus* starved females (5- 7d-old) for three hours. Control tests were carried out alongside with the treatments using ethanol or water. After treatments, the number of fed and unfed females were counted and calculated according to Abbott (1925): Repellency % = [% A - % B / 100 - % B] × 100 (where: A = percent of unfed females in treatment and B = percent of unfed females in control).

Statistical analysis

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).

RESULTS

As shown from the results, the highest larval mortality recorded after 24 hours by ethanolic extract from leaves of *L. camara* and *E. citriodora* was 69.2 and 62.8% at the highest concentrations (500 and 800 ppm), while the lowest mortality was 24.0 and 18.8% at the lowest concentrations (25 and 50 ppm) (Table 1). However, the concentrations 500, 400, 300, 200, 100, 50 and 25 ppm from *L. camara* ethanolic extract induced 86.8, 70.8, 60.0, 49.2, 42.8, 33.2, 25.2 and 100.0, 92.0, 68.0, 53.2, 50.8, 40.0, 28.3% larval mortality, respectively, after 48 and 72 hours (Table 1). On the other hand, at 800, 700, 600, 500, 400, 300, 200, 100 and 50 ppm from *E. citriodora* ethanolic extract induced larval mortality percent equal to 89.2, 84.0, 61.2, 58.8, 42.8, 37.2, 33.2, 29.2, 22.8 and 100.0, 90.8, 76.0, 65.2, 52.0, 46.8, 37.2, 34.8, 25.2% after 48 and 72 hours, respectively, compared with 0.0% larval mortality in the control group. *L. camara* and *E. citriodora* (leaves) ethanolic extract recorded a variable acute mortality percent depending on the concentration of the extract, as it increased with the concentration of two extracts increasing. Ethanolic of *L. camara* (leaves) recorded 18.8, 22.8, 24.0, 30.8, 34.8, 37.2 and 46.8 acute mortality percentages at 25, 50, 100, 200, 300, 400 and 500 ppm, respectively (Table 1). Meanwhile, acute mortality percent recorded by ethanolic of *E. citriodora* (leaves) were 41.2, 34.8, 32.0, 26.8, 25.2, 24.0, 22.8, 21.2 and 17.2% at 800, 700, 500, 400, 200, 100 and 50 ppm, respectively (Table 1). The survival potential recorded 0.0, 9.2, 24.0, 34.8, 48.0, 53.2, 62.8, 65.2 and 74.8% at 800, 700, 600, 500, 400, 300, 200, 100 and 50 ppm by *E. citriodora* (leaves) ethanolic extract and 0.0, 8.0, 32.0, 46.8, 49.2, 60.0 and 72.0% at 500, 400, 300, 200, 100, 50 and 25 ppm by ethanolic of *L. camara* (leaves), respectively, compared with 100.0% survival potential percent in the untreated groups.

After 24 hours both hexane extracts from leaves of *L. camara* and *E. citriodora* exhibited larval mortality percent equal to 92.0, 77.2, 64.0, 62.8, 50.8, 32.0, 25.2, 16.0, 14.8 and 88.0, 74.8, 57.2, 42.8, 33.2, 26.8, 21.2, 10.8% at 160, 140, 120, 100, 80, 60, 40, 20, 10 and 300, 250, 200, 150, 100, 50, 25, 12.5 ppm, respectively (Table 1). In addition, after 72 hours post treatment the larval mortality were 100.0, 90.8, 82.8, 76.0, 61.2, 50.8, 33.2, 22.8, 18.8 and 100.0, 86.8, 70.8, 54.8, 48.0, 38.8, 29.2, 18.8%

at 160, 140, 120, 100, 80, 60, 40, 20, 10 and 300, 250, 200, 150, 100, 50, 25, 12.5 ppm, respectively, for hexane extracts of *L. camara* and *E. citriodora* (leaves).

Table (1) : *Toxic effect of L. camara leaves ethanolic extract on 3rd the instar larvae of C. antennatus.*

Plant Sp.	Extract	Conc. (ppm)	Larval Mortality (%)			Acute Mortality (%)	Chronic mortality (%)	Survival potential (%)
			24h.	48h.	72h.			
L. camara	Ethanol 70%	500	69.2	86.8	100.0	46.8	100.0	0.0
		400	52.0	70.8	92.0	37.2	92.0	8.0
		300	45.2	60.0	68.0	34.8	68.0	32.0
		200	42.8	49.2	53.2	30.8	53.2	46.8
		100	34.8	42.8	50.8	24.0	50.8	49.2
		50	28.0	33.2	40.0	22.8	40.0	60.0
		25	24.0	25.2	28.0	18.8	28.0	72.0
		Control	0.0	0.0	0.0	0.0	0.0	100.0
	Hexane	160	92.0	100.0	100.0	58.8	100.0	0.0
		140	77.2	86.8	90.8	42.8	90.8	9.2
		120	64.0	74.8	82.8	40.0	82.8	17.2
		100	62.8	69.2	76.0	37.2	76.0	24.0
		80	50.8	56.0	61.2	32.0	61.2	38.8
		60	32.0	42.8	50.8	25.5	50.8	49.2
		40	25.2	30.8	33.2	18.8	33.2	66.8
		20	16.0	21.2	22.8	13.2	22.8	77.2
		10	14.8	17.2	18.8	9.2	18.8	81.2
		Control	0.0	0.0	0.0	0.0	0.0	100.0
E. citriodora	Ethanol 70%	800	62.8	89.2	100.0	41.2	100.0	0.0
		700	49.2	84.0	90.8	34.8	90.8	9.2
		600	42.8	61.2	76.0	32.0	76.0	24.0
		500	36.0	58.8	65.2	26.8	65.2	34.8
		400	33.2	42.8	52.0	25.2	52.0	48.0
		300	30.8	37.2	46.8	24.0	46.8	53.2
		200	25.2	33.2	37.2	22.8	37.2	62.8
		100	24.0	29.2	34.8	21.2	34.8	65.2
		50	18.8	22.8	25.2	17.2	25.2	74.8
		Control	0.0	0.0	0.0	0.0	0.0	100.0
	Hexane	300	88.0	100.0	100.0	45.2	100.0	0.0
		250	74.8	81.2	86.8	42.8	86.8	13.2
		200	57.2	65.2	70.8	33.2	70.8	29.2
		150	42.8	48.0	54.8	29.2	54.8	45.2
		100	33.2	45.2	48.0	22.8	48.0	52.0
		50	26.8	34.8	38.8	18.8	38.8	61.2
		25	21.2	26.8	29.2	16.0	29.2	70.8
		12.5	10.8	13.2	18.8	9.2	18.8	81.2
		Control	0.0	0.0	0.0	0.0	0.0	100.0

Con.: Concentration, ppm: part per million and h.: hours.

Acute mortality percent attained by *L. camara* and *E. citriodora* (leaves) hexane extracts recorded 58.8, 42.8, 40.0, 37.2, 32.0, 25.5, 18.8, 13.2, 9.2 and 45.2, 42.8, 33.2, 29.2, 22.8, 18.8, 16.0, 9.2% at 160, 140, 120, 100, 80, 60, 40, 20, 10 and 300, 250, 200, 150, 100, 500, 25, 12.5 ppm, respectively. From LC_{50} values it is obvious that, hexane extract of *L. camara* and *E. citriodora* (leaves) was more effective than ethanolic extract (Table 2).

Table (2) : *Lethal concentrations of L. camara and E. citriodora leaves extracts against C. antennatus third .*

Extract	Time (hours)	LC_{50} (ppm)		LC_{95} (ppm)		Slope (b)		R^2	
		L. camara	*E. citriodora*	*L. camara*	*E. citriodora*	*L. camara*	*E. citriodora*	*L. camara*	*E. citriodora*
Ethanol 70%	24	318.5	686.1	864.0	1579.0	0.0825	0.0504	0.9517	0.9342
	48	203.1	394.8	586.4	907.4	0.1174	0.0878	0.9814	0.9505
	72	143.1	316.4	457.8	779.3	0.143	0.0972	0.9625	0.980
Hexane	24	84.4	158.6	171.0	339.0	0.5194	0.2493	0.9829	0.9814
	48	71.3	129.1	152.5	299.1	0.5543	0.2648	0.9964	0.9677
	72	64.0	113.3	144.0	285.7	0.5628	0.261	0.988	0.9841

Also, tested ethanolic and hexane extracts of *L. camara* and *E. citriodora* (leaves) evoked a variable degree of repellency as shown in table (3). Potent repellency (92.5%) achieved by *L. camara* hexane extract at 1.67mg/cm^2 through the 3h post treatment, the ethanolic extracts of *L. camara* and *E. citriodora* exhibited 86.2, 68.8, 57.9 and 74.1, 59.0, 47.3% protection at 3.33, 1.67 and 0.83 mg/cm^2. Also, *E. citriodora* (leaves) hexane extract showed 88.1, 77.8 and 53.7% repellency at 3.33, 1.67 and 0.83 mg/cm^2, compared with 100.0% repellency for DEET at 1.8mg/cm^2 (Table 3).

Table (3) : *Repellent activity of tested L. camara and E. citriodora leaves extracts against C. antennatus starved females.*

Plant Sp.	Extract	Dose (mg/cm^2)	No. of tested females	Fed Females		Unfed Females		Repellency (%)
				No.	%	No.	%	
L. camara	Ethanol 70%	3.33	45	6	13.3	39	86.7	86.2
		1.67	63	19	30.2	44	69.8	68.8
		0.83	59	24	40.7	35	59.3	57.9
	Hexane	1.67	55	4	7.3	51	92.7	92.5
		0.83	58	11	19.0	47	81.0	80.4
		0.41	47	12	25.5	35	74.5	73.6
E. citriodora	Ethanol 70%	3.33	60	15	25.0	45	75.0	74.1
		1.67	53	21	39.6	32	60.4	59.0
		0.83	49	25	51.0	24	49.0	47.3
	Hexane	3.33	61	7	11.5	54	88.5	88.1
		1.67	65	14	21.5	51	78.5	77.8
		0.83	58	26	44.8	32	55.2	53.7
DEET	----	1.8	49	0	0.0	49	100.0	100.0
Control	----	0.0	61	59	96.7	2	3.3	0.0

DISCUSSION

Earlier authors reported that the bio insecticides, particularly those are derived from plant origin, have been increasingly evaluated in controlling the population of insects' pest **(Suresh *et al.*, 2017)**. Crude or partially purified plant extracts are rich source of bioactive chemicals, less expensive and highly efficacious for the control of dipterans. Several studies concerning with the effect of several plant extracts on different mosquitos species were performed by many authors worldwide. *Culex antennatus* has been reported to be prone to infection by Rift Valley Fever virus in the Nile Delta of Egypt. In the present study, ethanol 70% and hexane extracts of *Lantana camara* and *Eucalyptus citriodora* leaves exhibited larvicidal activity against 3rd instar larvae of *C. antennatus*. These results were corroborate with the findings of **Vahitha *et al.* (2002)** who reported that, LC_{50} values of *Pavonia zeylanica* and *Acacia ferruginea* leaf extracts was 2214.7 and 5362.6 ppm against late third instar larvae of *C. quinquefasciatus*, **Prabakar and jebanesan (2004)** used 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. quinquefasciatus*. The LC_{50} values after 24 h were 465.85, 567.81, 839.81, 1189.30 and 1636.04 ppm. Similar results were reported by **Nathan *et al.* (2005)** who concluded that, azadirachtin was most potent in all experiments against the malaria vector, *A. stephensi* L. and produced almost 100% larval mortality at 1 ppm concentration, **Coria *et al.* (2008)** for extracts from *Melia azedarach* on *A. aegypti*. Also, **Maurya *et al.* (2009)** have reported that, the petroleum ether extract from leaves of *Ocimum basilicum* was found to be the most effective against the larvae of both mosquitoes, *A. stephensi* and *C. quinquefasciatus* 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, 65.58 ppm against *A. stephensi* and *C. quinquefasciatus* after 24 and 48 h of treatment, **Madhua *et al.* (2010)** used *Curcuma aromatica* rhizomes extracts on *C. quinquefasciatus* larvae. They recorded that, the efficacy of petroleum ether extract seemed to be effective with LC_{50} and LC_{90} values of 11.4 and 18.0 ppm, this was in agreement with **Sakthivadivel *et al.* (2014)** who found that, aqueous fruit extract of *Wrightia tinctoria* exhibited highest larvicidal activity against the filarial vector, *C. quinquefasciatus* followed by aqueous leaf extract with LC_{50} values of 0.17% and 0.09%; 0.21% and 0.11% after 24 and 48 h, **Samuel *et al.* (2014)** who mentioned that, *Ipomoea cairica* and *Ageratina adenophora* extracts were found to be effective against third instar larvae of *C. quinquefasciatus* causing 77-100% mortality at 48 h. Also, these results are in consistent with those obtained by **Asiry *et al.* (2017)** for ethanolic leaf extracts from four plants, *Citrullus colocynthis*, *Artemisia annua*, *Pergularia tomentosa* and *Rhanterium epapposum* selected from Hail region, northern Saudi Arabia, against the larval stages of *Ae. aegypti*. Where, the ethanolic extracts of both *R. epapposum* and *A. annua* were more toxic to the 4th instar larvae of *Ae. aegypti* compared to the other two plants and **Nasir *et al.*, (2017)** used essential oils from some medicinal plants against *Aedes albopictus*. Where, Ginger was more effective with the lowest LC_{50} values after 8 and 16h followed by peppermint, basil, eucalyptus and neem. In addition, basil was efficacious after 24 and 48h.

On the other hand, all doses of plant extracts used in the present study exhibited repellent activity against the starved female adults of *C. antennatus*. The repellent activity was varied according to solvent used in extraction and the dose of the extract. These results indicate that, the petroleum ether extraction of *L. camara* and *E. citriodora* leaves was more effective in exhibiting the repellent action against the mosquito tested as compared with ethanolic extraction. Many plant extracts and essential oils manifest repellent activity against different mosquito species and the present results are in accordance with such results obtained by **Choi *et al.* (2002)** testing the essential oils of *Eulcalyptus globulus*, *Lavender offici-*

nalis, *Rosemarinus officinalis* and *Thymus vulgaris* against *C. pipiens*, **Jeyabalan *et al.* (2003)** using methanol extracts of *Pelargonium citrosa* against *A. stephensi*, **Tuetun *et al.* (2004)** using extracts of *Apium graveolens* seeds against *Ae. aegypti*, **Yang *et al.* (2004)** using methanol extracts from twenty three aromatic medicinal plant species against *Ae. aegypti* female, **Prajapati *et al.* (2005)** using essential oils extracted from ten medicinal plants against *A. stephensi* and *C. quinquefasciatus* and **Chio and Yang (2008)** using neem tree (*Azadirachta indica*) oil against the Asian tiger mosquito (*Ae. albopictus*). Similar observation was also recorded by **El- Sheikh *et al.* (2012)** who used methanolic extract of *Tribulus terrestris* (leaves and seeds) against the malarial vector, *A. arabiensis* and they reported that, The seeds extract was more effective in exhibiting the repellent action (100.0%) against the mosquito tested as compared with the leaves extract (79.5%) at the dose 1.0 and 2.0mg/cm^2 compared with (100.0%) of commercial formulation, N. N. diethyl- meta- toulamide (DEET), **Adhikari and Chandra (2014)** who recorded that, petroleum ether leaf extract of *Swietenia mahagoni* against *A. stephensi* showed repellency up to 2h after treatment and **Govindarajan *et al.* (2014)** who used extracts from *Delonix elata* against malaria vector *A. stephensi* and they reported that, both leaf and seed methanol extracts showed maximum efficacy at the highest concentration of 5.0 mg/cm^2 they provided over 210 and 180 min. protection.

CONCLUSION

From the present study we conclude that, *Lantana camara* and *Eucalyptus citriodora* extracts proved promising larvicidal and repellent agents against Rift Valley Fever vector, *Culex antennatus* (Becker) in the laboratory. Further, in near future we need to initiate studies leading to find the bioactive compounds in *L. camara* and *E. citriodora* which may responsible for larvicidal and repellent activity.

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