International Journal of Mosquito Research 2020; 7(5): 43-47

International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2020; 7(5): 43-47

www.dipterajournal.com
Received: 19-07-2020
Accepted: 23-08-2020

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Efficacy of leaves and flowers ethanol extracts of the invasive species *Lantana camara* Linn as a Mosquito larvicidal

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Abstract
Lantana camara is a perennial shrub belongs to the family Verbanaceae, and is considered to be one of the most invasive plant species worldwide. It is well known of having many phytoconstituents of medicinal value which can be exploited as eco-friendly biocontrol agents against harmful insects. Therefore, the current study aimed to evaluate the larvicidal effect of ethanol extract of *L. camar*a leaves and flowers against *Anopheles arabiensis* and *Culex quinquefasciatus* larvae. Results showed that concentrations of flower extracts reflected an LC50 of 15.84 ppm for *A. arabiensis* and 21.37 ppm for *C. quinquefasciatus*, while, it reflected an LC_{95} of 50.11 ppm for *A. arabiensis* and 63.09 ppm for *C. quinquefasciatus*. Also, leaves extracts reflected an LC_{50} of 9.54 ppm for *A. arabiensis* and 5.01 ppm for *C. quinquefasciatus*, while, it reflected an LC_{95} of 21.11 ppm for both *A. arabiensis* and *C. quinquefasciatus*. According to the obtained LC_{50} values, ethanol extract of *L. camara* leaves showed relatively higher larvicidal potentiality against *C. quinquefasciatus* larvae than *A. arabiensis* larvae. Also, leaves extract showed more potent effect on larvae of both species than flower extract. In addition, some morphological changes were noticed on both species when they submitted to higher extracts of leaves and flower. Changes include larva and pupa damage with disconnected alimentary canal and head loss, as well as to failure to pupate on color pupa and morphogenesis termination of *A. arabiensis* and *C. quinquefasciatus*. Finally, the change in the larval color was higher when submitted to leaves than flower extracts. Current study concludes that the use of *Lantana camara* active substances in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution.

Keywords: Ethanol extracts, *Lantana camara***,** *A. arabinoses*, *C. quinquefasciatus*

Introduction

Lantana camara Linn, a member of the family Verbenaceae, is an evergreen aromatic perennial shrub, native to tropical America, but it is now cultivated in many other parts of the world [1]. However, it has been reported as extremely weedy and invasive species causing large economic and environmental losses in many countries [2, 3], including the southern highlands of Saudi Arabia [4].

L. camera has been reported as an important medicinal plant containing some phytoconstituents with several medicinal uses in folk and traditional therapeutic purposes. The plant can be used for the treatment of wider disorders such as; antiulcer, analgesic, anti-inflammatory, antimicrobial, anthelmintic, anticancer, antifungal, wound healing, and its oil is sometimes applied as an antiseptic for the treatment of skin itches, leprosy and scabies [5]. However, *L. camara* phytoconstituents are known to be toxic to livestock such as cattle, sheep, horses, dogs and goats [6]. The active substances causing toxicity in grazing animals is pentacyclic triterpenoids which result in liver damage and photosensitivity [7]. Also, several studies have addressed the toxicity of *L. camara* to humans, particularly in case of ingestion its green unripe fruits [8]. However other studies have found that ingestion of *L. camara* fruit poses no risk to humans [9]. Regarding risks of *L. camara* on vegetation, it has been found that the plant excretes allelopathic chemicals which reduce the growth of surrounding plants by inhibiting germination and root elongation [10]. Mosquitoes are considered the most dangerous insects worldwide, and the problem is not the insect itself, but because it is the transmitter of many serious public diseases including; malaria, filariasis, Japanese encephalitis, and dengue as well as yellow fever and other forms of encephalitis [11].

Among the chief vectors of some serious diseases are; *Anopheles arabiensis* and *Culex quinquefasciatus*. The most efficient way to stop those diseases from further spread is to control mosquitos especially at the larval stage by using insecticides. However, using conventional synthetic insecticides proved to cause environmental pollution and to developed physiological resistance among mosquitoes [12]. Therefore, it is necessary to search for an alternative, more potent, cost-effective and eco-friendly mosquito vector control agents, such as biological substances of botanical origin. *L. camara* is well known of having many bioactive compounds of medicinal value which can be exploited as eco-friendly biocontrol agents against harmful insects. For this, the aim of the current study is to evaluate the larvicidal effect of ethanol extract of *L. camara* leaves and flowers against *Anopheles arabiensis* and *Culex quinquefasciatus* larvae.

Materials and methods

Preparation of Ethanol and Acetone extracts

Fresh flowers and leaves of *Lantana camara* were used. Extracts of flowers and leaves were powdered using an electric blender, and then 10g of the powdered samples were taken and homogenized with 100 ml of ethanol. The supernatant containing the plant extract of each part was then transferred to a measuring cylinder and after the solvent evaporated, powder residue collected and weighed before used for bioassay.

Mosquito larvae

Mosquito larvae that have been collected for the purpose of the current study were separated from their predators that accidentally collected with them, and were immediately used in the bioassay tests. Susceptibility tests followed WHO (1996) [13] protocol. Twenty larvae of *A. arabiensis* and *C. quinquefasciatus* of the third or early fourth instar were placed in 300 ml cups which were adjusted to 250-300 ml tap water. From the stock concentrations, about 1, 1.5, 2, 2.5, and 3 ml extract were added to these cups, but not to the control. Experiments were run at the room temperature (26+ 3 ºC) and each test was based on three replications. After 24 hours, in each test cup, the dead larvae were counted, put in glass slices in order to be photographed to monitor the morphological changes (especially changes in color, status of digestive tract and the separation of some body parts). A digital microscope provided with camera was used for documentation of these observed changes.

Statistical analysis

The mean larval mortality after 24 hours taken as Y variable and was subjected against the corresponding concentrations (X variable) to the regression analysis by using Microsoft excel 2010. The regression lines were created to determine the lethal concentrations of 50% and 95% (LC_{50} and LC_{95}) on *A. arabiensis* and *C. quinquefasciatus* larvae.

Results

The larvicidal activities of *L. camara* flower and leaves extracts against the 4th instars larvae of *A. arabiensis* and *C. quinquefasciatus* after 24 hour were presented in (Table, 1 and Figure 1) and (Table, 2 and Figure, 2), respectively. The *L. camara* flower at concentrations of 33.83, 27.06, 20.3, 13.53 and 6.77 ppm showed a range of mean mortality of 31% - 73% on *A. arabiensis* and 15% - 78% on *C. quinquefasciatus* larvae. The probit analysis showed that, the LC_{50}'s was 15.84 ppm and 21.37ppm for *A. arabiensis* and *C. quinquefasciatus* larvae, respectively, while the LC_{95}'s was 50.11 ppm and 63.09 ppm, followed the same order of larvae. The ethanol leaves extract at concentrations of 21.11, 16.88, 12.66, 8.44 and 4.22 ppm showed a range of mean mortality of 30% - 95% on *A. arabiensis* and 48% - 95% on *C. quinquefasciatus* larvae. The probit analysis showed that, the LC_{50}'s were 5,01 ppm and 9,54 ppm for *C. quinquefasciatus* and *A. arabiensis* larvae, respectively, while the LC_{95}'s were 21.11 ppm and 21.11 ppm, followed the same order of larvae. According to the obtained LC_{50} values, it was clear that, the ethanol extract of *L. camara* leaves showed relatively higher larvicidal potentiality against *C. quinquefasciatus* larvae than *A. arabiensis* larvae. Also, leaves extract was more potent than the flower extract.

Table 1: The larvicidal effect of Ethanol extract of *L. camara* leaves on *A. arabiensis* and *C. quinquefasciatus* larvae after 24 hour

C. quinquefasciatus		*A. arabiensis*		Log- Conc	Conc. (ppm)
Probit	Mortality %	Probit	Mortality %		
6.64	95	6.64	95	1.324	21.11
5.88	81	6.04	85	1.227	16.88
5.67	75	5.39	65	1.102	12.66
5.13	55	4.87	45	0.926	8.44
4.95	48	4.48	30	0.625	4.22
0.83		0.90			R^2
2.216		2.997			slope
3.348		2.365			x-coefficient
5.01 ppm		9.54 ppm			LC50
21.11 ppm		21.11 ppm			LC95

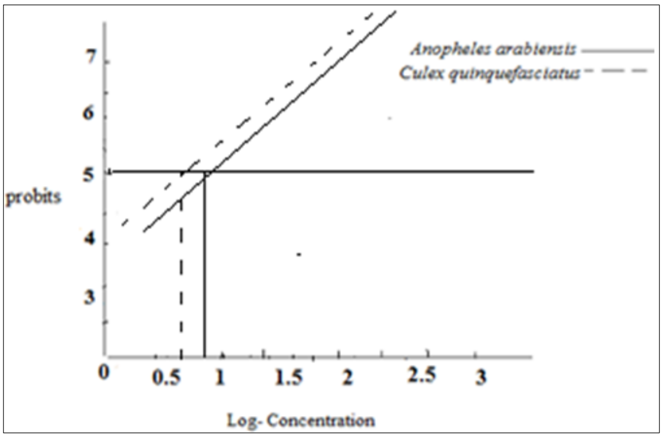


Fig 1: Log- Probity curve of effect of ethanol extract of *L. camara* Leaves on *A. arabiensis* and *C. quinquefasciatus* larvae after 24 hour

Table 2: The larvicidal effect of Ethanol extract of *L. camara* flower on *A. arabiensis* and *C. quinquefasciatus* larvae after 24 hor

C. quinquefasciatus		*A. arabiensis*		Log- Conc	Conc. (ppm)
Probit	Mortality %	Probit	Mortality %		
5.77	78	5.61	73	1.529	***33.83***
5.30	65	5.33	63	1.432	***27.06***
4.82	43	5.15	56	1.307	***20.3***
4.50	31	4.69	38	1.131	***13.53***
3.96	15	4.50	31	0.831	***6.77***
0.94		***0.93***			R2
2.47		***1.6***			slope
1.787		3.062			x-coefficient
21.37 ppm		15.84 ppm			LC_{50}
63.09 ppm		50.11 ppm			LC_{95}

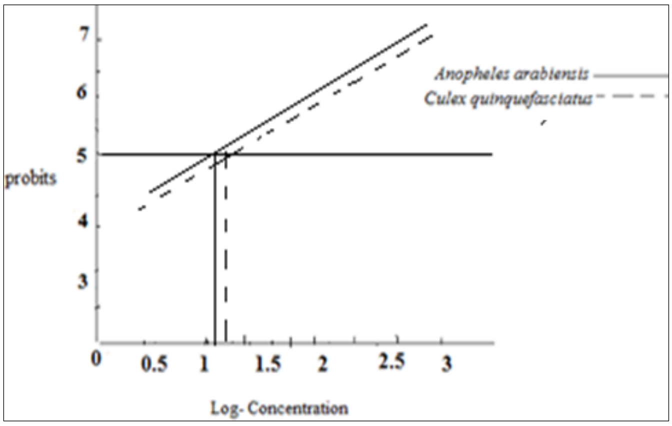


Fig 2: Log- Probity curve of effect of ethanol extract of *L. camara* flower on *A. arabiensis* and *C. quinquefasciatus* larvae after 24 hour

Efficient the relatives

The study showed that the plants extracts the efficient relative according to the obtained LC_{50} values, it was clear that, the ethanol extracts of *L. camara* leaves showed relatively higher larvicidal potentiality against *A. arabiensis* and *C. quinquefasciatus* larvae.

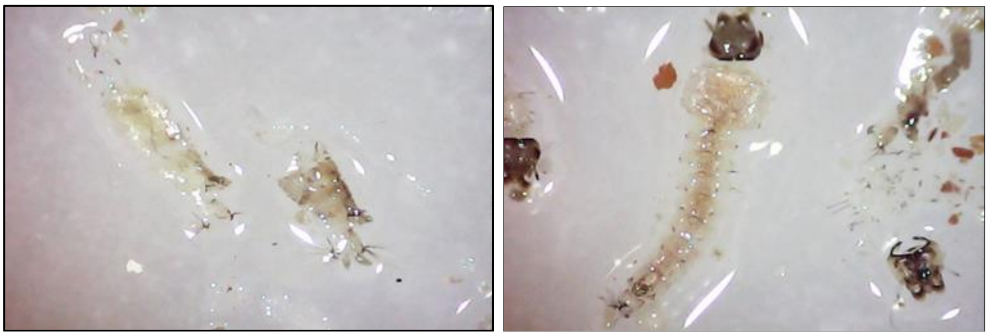
Table 3: Efficient the relatives

preparation	*A. arabiensis*		*C. quinquefasciatus*	
	Ethanol			
Plant parts	LC_{50}	LC_{95}	LC_{50}	LC_{95}
L. camara leaves	9.54	21.11	5.01	21.11
L. camara flower	15.84	50.11	21.37	63.09

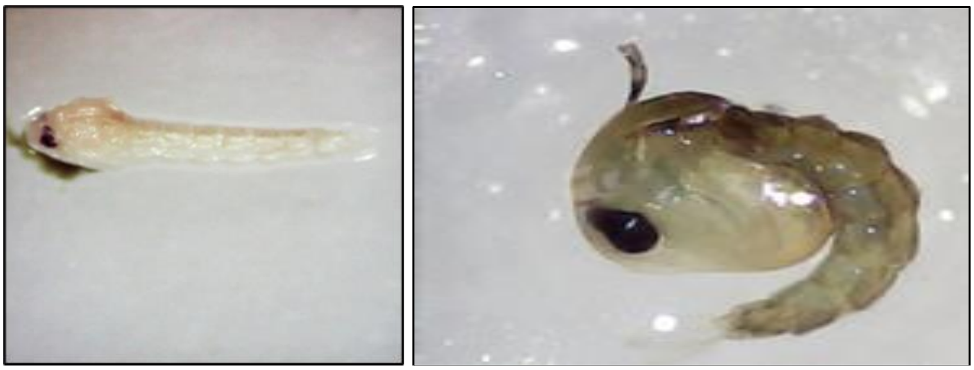
The damage caused by the Ethanol extracts of *L. camara* leaves and flower on *A. arabiensis* and *C. quinquefasciatus* larvae after 24 hour:

After 24 hour of applying *L. camara* leaves and flower extracts, a lethal effect and some morphological changes were noticed on both mosquito species when they submitted to higher extracts of leaves and flower. Changes include larva and pupa damage (with disconnected alimentary canal and head loss), in addition to failure to pupate on color pupa and termination of morphogenesis of *A. arabiensis* and *C. quinquefasciatus*. (Plate, 1and 2). Finally, the change in the larval color was higher when submitted to leaves than flower extracts.

Control Anopheles larva | Control Culex larva | Larvae swelled



Bright color larva and damage with disconnected alimentary canal and head



Bright color pupa and termination of morphogenesis | Normal pupa

Plate 1: Damage caused by the of Ethanol extract of *L. camara* leaves extracts on *A. arabiensis* and *C. quinquefasciatus* larvae after 24 hours

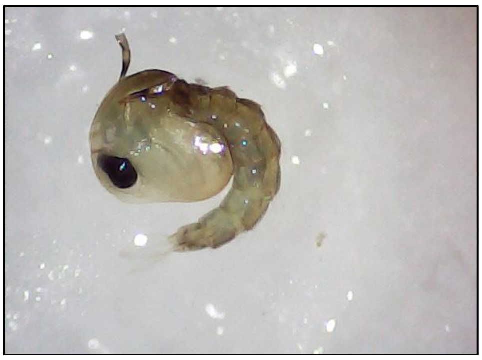
Control Anopheles larva

Control Culex larva

Larvae swelled and damage with disconnected alimentary canal and head loss

Bright color larva

Bright color pupa and termination of morphogenesis



Normal pupa

Plate 2: Damage caused by the of Ethanol extract of *L. camara* flower extracts on *A. arabiensis* and *C. quinquefasciatus* larvae after 24 hour.

Discussion

Mosquito larvicidal activity of methanol and ethanol extract of leaves and flowers of *Lantana camara* belongs to the family of Verbanaceae have been evaluated in the present study. Larvicidal effect against 3 and 4 instar larvae of mosquito species *Anopheles arabiensis* and *Culex quinquefasciatus* larvae have been investigated in a dose dependent manner for 24 h.

Current results obtained in this study about larvicidal activity of *Lantana camara* were constant with results found by Babita *et al.* [14]. They tested *Anopheles stephensi* larvicidal bioassay using different extracts of two different plants *Lantana camara* and *Barringtonia racemosa*, using solutions of Petroleum ether, Chloroform and Ethyl acetate. The most active extract against late third or early fourth instar larvae of *A. stephensi*, were found to be the petroleum ether extract of *L. camara* and Ethyl acetate extract of *B. racemosa*. Larvae malfunction of some physiological processes was noticed and may be referred to the fact that, ethanol may provoke some potent chemicals from the raw oil, which caused damage in the connective and other tissues of the digestive tracts of the tested larvae. Similar findings of larvae malfunction were reported when subjected to *Ricinus communis* extracts. *R. communis* is known to stop the cell division proliferating tissues in insects and disrupt their physiological functions [15]. Nearly half of the larvae were decapitated as a result of being submitted to ethanol extract of castor raw oil, and this may be due to the effect of its constituents that led to releasing tissues that connect the head to the thorax. Also, 100% of the larvae were noticed to be swelled [15]. The percentage mortality of the different mosquito species was tested after 24 hrs of exposure to different concentration of the leaf extract. The extracts of this plant showed potent larvicidal efficacy and can be considered for further investigation [16].

The researchers to control for the effects of extraneous variables. After the 24 hours of observation, researchers found out that the mortality rate of all the methanolic extracts that were prepared from *Lantana camara* leaves were not significantly effective against the larvae causing only 0-20% mortality on all of the treatments except the 60% on trial 1 of the treatment 3 with 4mg/mL concentration. The following concentrations did not manage to kill all or majority of the larvae. The larvae had survived and managed to grow into pupa and some turned into mosquitoes [17]. The damage caused by *Lantana camara* in current study is similar to the result obtained by Kehail *et al.* [18] whom studied the damages caused by castor seed oil and its ethanol extracts on *Anopheles arabiensis* larvae, Gezira State, Sudan [18]. They concluded that the activities of the plant extracts against mosquito species depend on the solvent used in extracting the phytochemicals responsible in the responses [18, 19]. Previous results were consistent with results obtained in current study which generally confirms that phytoconstituents could be an alternative source as mosquito larvicidal because they constitute a potential source of bioactive chemicals and free from harmful impacts.

Conclusion

Ethanol extract of *L. camara* leaves showed relatively higher larvicidal potentiality against *C. quinquefasciatus* larvae than *A. arabiensis* larvae. Also, leaves extract showed more potent effect on larvae of both species than flower extract. In addition, some morphological changes were noticed on both species when they submitted to higher extracts of leaves and flower. Changes include larva and pupa damage (with disconnected alimentary canal and head loss) in addition to failure to pupate on color pupa and stop morphogenesis of *A. arabiensis* and *C. quinquefasciatus*. Therefore, use of such botanical substances in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution.

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