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Evaluation of leaves and flowers acetone extracts of *Lantana camara* Linn as larvicidal agents against *Anopheles arabiensis* and *Culex quinquefasciatus* larvae

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Abstract
Lantana camera belonging to the family Verbenacea, is a cosmopolitan invasive species with several medicinal uses in traditional medication system. It can be used to cure health problems such as; antiulcer, analgesic, anti-inflammatory, antimicrobial, anthelmintic, anticancer, antifungal. Therefore, the current study was aimed to evaluate the larvicidal effect of acetone extract of L. *camara leaves* and flowers against *Anopheles arabiensis* and *Culex quinquefasciatus* larvae. Results showed that larvicidal activities of L. *camara leaves* and flower acetone extracts against the 4th instars larvae of A. *arabiensis* and C. *quinquefasciatus* after 24-hour exposure at concentrations of 85.0, 74.38, 63.75, 53.13and 21.25ppm showed a range of mean mortality of 17% - 97% on A. *arabiensis* and 18% - 92% on C. *quinquefasciatus* larvae. The probit analysis showed that, the LC50's was 34.67ppm and 32.35 ppm for *A. arabiensis* and *C. quinquefasciatus* larvae, respectively, while the LC95's was 79.43ppm and 89.12 ppm, followed the same order of larvae. The flower acetone extract at concentrations of 99.12, 86.73, 74.34, 61.95and 49.56ppm showed a range of mean mortality of 37% - 78% on A. *arabiensis* and 35% - 92% on C. *quinquefasciatus* larvae. The probit analysis showed that, the LC50's were 66.06ppm and 60.25 ppm for C. *quinquefasciatus* and A. *arabiensis* larvae, respectively, while the LC95's were 104.71ppm and 102.32ppm, followed the same order of larvae. According to the obtained LC50 values, it was clear that, the acetone 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. Current results confirm that the use of Lantana camara leaves and flowers extracts in mosquito control instead of synthetic insecticides could be a better approach to reduce the control cost and environmental pollution.

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

Introduction

Lantana camara Linn, belonging to the family Verbenaceae, is an evergreen ornamental perennial shrub, native to tropical America, but it is now cultivated in many other parts of the world and it can be used to treat health problems such as; antiulcer, analgesic, anti-inflammatory, antimicrobial, anthelmintic, anticancer, antifungal [1].

The use of artificial chemicals and the rising incidence of resistance in the mosquito larvae has led to the development of new approaches for mosquito larvae control. Biological pesticides provide a substitute to the synthetic insecticides due to its very low environmental pollution, low toxicity to mankind [2]. Natural products and compounds from different plants families are best option as biological control agents as they are less harmful to environment and non-target organisms. Several extracts have been evaluated for new and promising larvicides [3]. *Lantana viburnoides sp.* extracts demonstrate larvicidal activity for managing various mosquito habitats even in their semi-purified form, nevertheless, crude extracts and some fractions had higher larvicidal activity to the pure compounds. Such compounds can be used as distinct markers in the active extracts belonging to the genus Lantana [4]. 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 larvae and pupa damage (with disconnected alimentary canal and head loss) in addition to failure to pupate on color pupa and morphogenesis termination of *A. arabiensis* and *C. quinquefasciatus*. Such results confirm that botanical substances in mosquito control instead of synthetic insecticides could reduce the control cost and environmental pollution [5]. *Lantana camara* aculeata active extracts were evaluated against the 4th instar larvae of three mosquito species namely *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. These extracts showed potent larvicidal efficacy due to the presence of active ingredients including; tannins, alkaloids, flavonoids, anthocyanin, quinines, triterpenoids, flavonoids, saponin and steroids [6]. Extract from the leaves of *L. camara* was found to possess larvicidal activity while extract from flowers of the plant showed repellent activity against adult mosquitoes [7]. Another study concluded that the larvicidal potential of fractions of hexane, ethyl acetate, and methanol leaf extract of *L. camara*. Based on this study, 3rd instar larvae of *A. aegypti* was most susceptible to ethyl acetate fraction compared to other fractions with LC_{50} of 564, 102, and 84 ppm at 24, 48, and 72 hours respectively. The active compounds were screened and found to be flavonoids, steroids, saponins, and glycosides [8]. *L. camara aculeata* extracts showed promising activity in mosquito control and very much feasible commercially to be used in stagnant water bodies which are the breeding grounds for mosquitoes [9, 10]. The current study aimed to evaluate the larvicidal effect of acetone extract of *L. camara* leaves and flowers against *Anopheles arabiensis* and *Culex quinquefasciatus* larvae.

Materials and Methods

Preparation of Acetone extracts

Fresh *Lantana camara* flowers and leaves were powdered using an electric blender, and then 10g of the powdered samples were taken and homogenized with 100 ml of acetone. 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 were separated from their predators that accidentally collected with them, and were immediately used in the bioassay tests. Susceptibility tests followed WHO (1996) [11] 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± 3ºC) and each test was based on three replications. After 24 hours, in each test cup, the dead larvae were counted.

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* leaves and flower acetone 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* leaves at concentrations of 85.0, 74.38, 63.75, 53.13 and 21.25ppm showed a range of mean mortality of 17% - 97% on *A. arabiensis* and 18% - 92% on *C. quinquefasciatus* larvae. The probit analysis showed that, the LC_{50}'s was 34.67ppm and 32.35 ppm for *A. arabiensis* and *C. quinquefasciatus* larvae, respectively, while the LC_{95}'s was 79.43ppm and 89.12 ppm, followed the same order of larvae. The acetone flower extract at concentrations of 99.12, 86.73, 74.34, 61.95 and 49.56 ppm showed a range of mean mortality of 37% - 78% on *A. arabiensis* and 35% - 92% on *C. quinquefasciatus* larvae. The probit analysis showed that, the LC_{50}'s were 66.06ppm and 60.25 ppm for *C. quinquefasciatus* and *A. arabiensis* larvae, respectively, while the LC_{95}'s were 104.71ppm and 102.32ppm, followed the same order of larvae. According to the obtained LC_{50} values, it was clear that, the acetone 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 acetone extract of Lantana leaves on *A. arabiensis* and *C. quinquefasciatus* larvae after 24 hour

C. quinquefasciatus		*A. arabiensis*		Log- Conc	Conc. (ppm)
Probit	Mortality %	Probit	Mortality %		
6.41	92	.886	97	1.929	85.0
6.04	85	5.92	82	1.871	74.38
5.84	80	5.84	80	1.804	63.75
5.58	72	5.52	70	1.725	53.13
4.08	18	4.05	17	1.327	21.25
0.93		0.93			R2
4.14		4.14			slope
1.53		1.533			x-coefficient
32.35 ppm		34.67 ppm			LC50
89.12 ppm		79.43 ppm			LC95

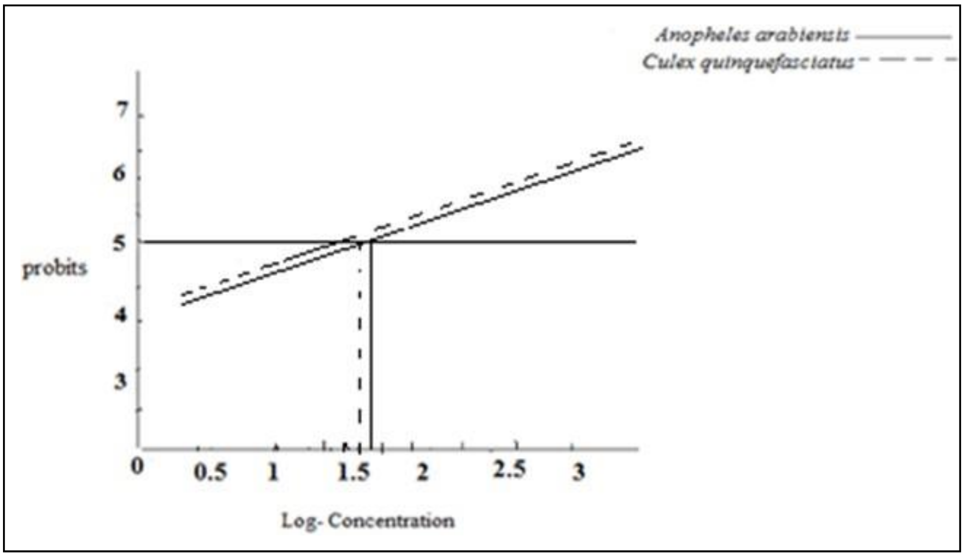

Fig 1: Log- Probity curve of effect of acetone extract of *L. camara* Leaves on *A. arabiensis* and *C. quinquefasciatus* larvae after 24 hour

Table 2: The larvicidal effect of acetone extract of Lantana flower on *A. arabiensis* and *C. quinquefasciatus* larvae after 24 hour

C. quinquefasciatus		*A. arabiensis*		Log- Conc	Conc. (ppm)
Probit	Mortality %	Probit	Mortality %		
6.41	92	5.77	78	1.996	99.12
5.44	67	5.58	72	1.938	86.73
5.31	62	5.05	52	1.871	74.34
5.18	57	4.80	42	1.792	61.95
4.61	35	4.67	37	1.695	49.56
0.84		0.92			R2
5.03		3.89			slope
3.96		2.05			x-coefficient
60.25 ppm		66.06 ppm			LC50
102.32 ppm		104.71 ppm			LC95

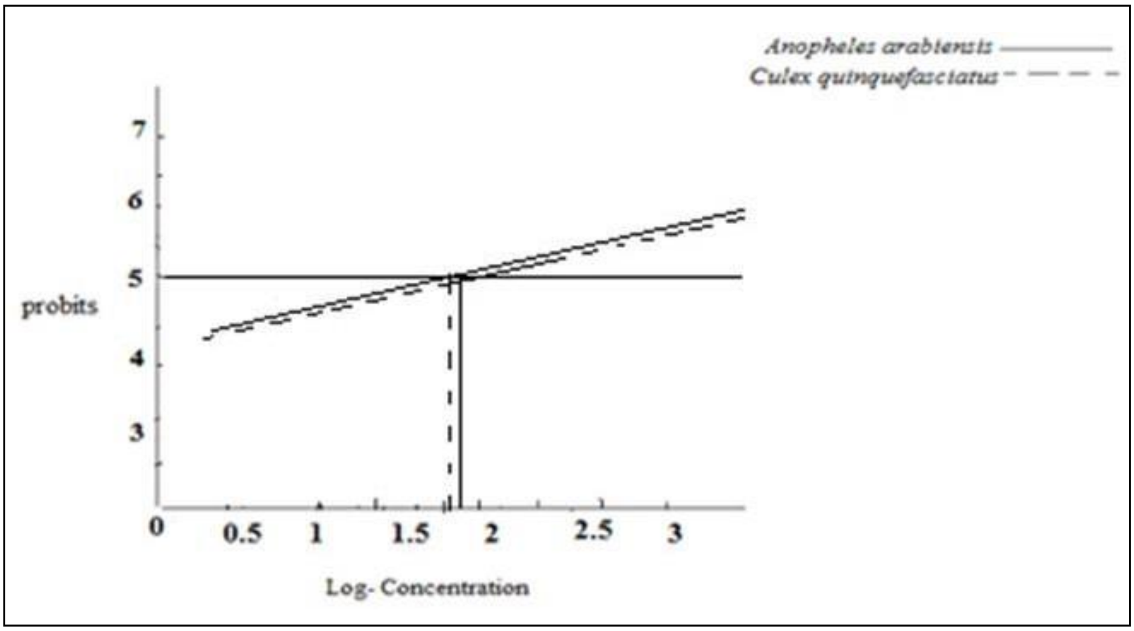

Fig 2: Log-Probity curve of effect of acetone extract of *L. camara* flower on *A. arabiensis* and *C. quinquefasciatus* larvae after 24 hour

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 acetone 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*	
	Acetone			
Plant parts	LC_{50}	LC_{95}	LC_{50}	LC_{95}
L. camara leaves	34.67	79.43	32.35	89.12
L. camara flower	66.06	104.71	60.25	102.32

Discussion

Some medicinal plants species have been used traditionally as natural insecticides before the discovery of synthetic organic insecticides [12]. Current results obtained in this study about larvicidal activity of *Lantana camara* were consistent with results found by Babita *et al*, 2014.They tested the *Anopheles stephensi* larvicidal bioassay using different extracts of *Lantana camara* and other plants and their results showed larvae malfunction of some physiological processes. They concluded that extracts of this plant showed potent larvicidal efficacy and can be considered for further investigation [13, 14]. Previous results was in agreement with the current study results which found that the obtained LC_{50} values, the acetone 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. 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. [5]. These results were consistent with result 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. In addition, mosquito larvicidal activity and phytochemical screening of methanol and ethanol extract of leaves and flowers of *L. camara* Linn have been evaluated [16]. Larvicidal effect on 3 and 4 instar larvae of mosquito species *Aedes aegypti* and *Culex rd th quinquefasciatus* have been investigated in a dose dependent manner for 24 h.

With 1.0 mg/ml concentration of extracts of *L. camara* maximum mortality was observed in *Aedes aegypti* exposed for 24 h. In the case of *Culex quinquefasciatus* the mortality was maximized when the concentration increased to 3.0mg/ml [15]. Due to huge consequences of synthetic insecticides, plants may be alternative sources of mosquito control agents. [16] and the current study has been consistent with such conclusion. Therefore, current results confirm that the use of *Lantana camara* leaves and flowers extracts in mosquito control instead of synthetic insecticides could be a better approach to reduce the control cost and environmental pollution.

Conclusion

Acetone extract of *L. camara* leaves showed the LC_{50} values, it was clear that, the acetone 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. Therefore, current results confirm that the use of *Lantana camara* leaves and flowers extracts in mosquito control instead of synthetic insecticides could be a better approach to reduce the control cost and environmental pollution.

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