Uttar Pradesh Journal of Zoology

Volume 45, Issue 4, Page 152-158, 2024; Article no.UPJOZ.3253
ISSN: 0256-971X (P)

Larvicidal Efficiency of *Citrus maxima* against *Aedes aegypti* and *Aedes albopictus*

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Authors' contributions

This work was carried out in collaboration between Both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2024/v45i43918

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3253

Original Research Article

Received: 15/12/2023
Accepted: 18/02/2024
Published: 22/02/2024

ABSTRACT

Mosquitoes transmit serious human diseases, causing millions of deaths every year and developed resistance to chemical insecticides resulting in rebounding vectorial capacity. Plants are the alternative sources of mosquito control agents. In the present study, various concentrations of 10, 20, 30, 40, and 50 mg of *Citrus maxima* (Brunnich) Merr. were tested against the third instar larvae of *Aedes aegypti* (Linn) and *Aedes albopictus* (Skuse). The highest larval mortality was found in the extract of *C. maxima* against third-instar larvae with P value = 0.0287. This study concluded that the increase in concentration of *C. maxima* extract increased the mortality of *Aedes aegypti* and *Aedes albopictus*.

Keywords: Mosquito; plants; C. maxima; A. aegypti; A. albopictus.

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1. INTRODUCTION

"Mosquitoes are vectors responsible for the transmission of various diseases, such as malaria, yellow fever, dengue fever and other infections. These diseases pose a major problem in disease-prevalent countries and are presently rampant owing to amplified globalization, urbanization, and global warming" [1,2]. "More than 50 million people are at risk of dengue virus exposure worldwide" [3]. "Annually, there are 2 million infections,500000 cases of dengue hemorrhagic fever and 12,000 deaths" [4]. "Many approaches have been developed to control mosquitoes. To prevent mosquito-borne disease and to improve the quality of the environment and public health, mosquito control is essential. The current mosquito control approach is based on synthetic insecticides. Even though they are effective, they create many problems like insecticide resistance, pollution, and toxic side effects on human beings. Biological control is used as an alternative to currently employed larvicides for minimizing the mosquito population, which provides an effective and environmentally friendly approach to bring down mosquito population under the bottom level. Unfortunately, the mosquitoes developed resistance against the chemical larvicides" [5].

"One of the most effective alternative approaches under the biological control program is to explore the floral biodiversity and enter the field of using safer insecticides of botanical origin as a simple and sustainable method of mosquito control. Plants are a rich source of alternative agents for the control of mosquitoes because they possess bioactive chemicals that act against the limited number of species including specific target insects and are eco-friendly. Traditionally, plant-based products have been used in human communities for many centuries to manage insects. Plant-based products do not have any hazardous effects on the ecosystem. About 2000 species of terrestrial plants have been reported for their insecticidal properties. In recent times, chemicals derived from plants have been projected as weapons of future mosquito control programs, as they are shown to be ecologically friendly. Plant extracts are safer for non-target organisms including man therefore, plant-based formulations would be more feasible from an environmental perspective than synthetic mosquitocide" [6].

Citrus maxima, the pomelo (also called pummel or shaddock) belongs to the Rutaceae family and it is a medium-sized but the largest of all Citrus species with large leaves, flowers, and fruits. The leaves are large evergreen oblong to elliptic with winged petioles. The flowers and fruits are borne singly. It contains a high amount of vitamin C.

Given the recently increased interest in developing plant-origin insecticides as an alternative to chemical insecticides, this study was undertaken to assess the larvicidal potential of the leaves of *C. maxima* against the dengue vectors *Aedes aegypti* and *Aedes albopictus.*

2. MATERIALS AND METHODS

2.1 Preparation of *C. maxima* Leaf Extract

Fresh *C. maxima* leaves were collected. The leaves were shade-dried, powdered in a blender, sifted and stored in an air-tight bottle. Exactly 200g of *C. maxima* leaf powder was extracted at 45℃ using 500 ml of methanol in a Soxhlet apparatus. The extraction was continued for a period of 8hrs. The extract was concentrated using a vacuum desiccator and the extract yield was about 22.6 g.

2.2 Procurement of *Aedes aegypti* and *Aedes albopictus* Eggs

Aedes aegypti and *Aedes albopictus* eggs were procured from CRME (Centre for Research in Medical Entomology), Madurai-2, in the form of egg- cards. The egg cards were stored in a well-aerated container, without giving access to ants. The egg card was placed in non-chlorinated tap water taken in a clean paper cup. The egg hatched out in about 48 h and the larvae were transferred to a fresh cup with about 1000 ml of tap water. The larvae were allowed to moult till the third instar.

2.3 Larvicidal Assay

In the larvicidal assay, 3rd instar larvae of *A. aegypti* and *A. albopictus* (25 numbers each) were exposed to test concentrations of 10, 20, 30, 40, and 50 mg of methanol extract of *C. maxima*. The number of dead larvae at the end of 24 hrs and 48 hrs were recorded and the mortality percentage was calculated. Five replicates were performed.

3. RESULTS

Larvicidal activity of *C. maxima* methanol leaf extract against *A. aegypti* and *A. albopictus* was noted. The mean and percentage mortality of *A. aegypti* and *A. albopictus* mosquito larvae treated with the various concentrations of plant extract of *C. maxima* after 24 hrs has been furnished in Table 1 and also depicted in Fig. 1.

Effects of *C. maxima* on 3rd instar of *A, aegypti* after 24 hrs, at 10 mg/ml concentration the mean mortality of *C. maxima* was found to be 6.54 ± 0.20 likewise 20, 30, 40, and 50 mg/ml concentration, the mean mortality was found to be 8.26 ± 0.23, 10.14 ± 0.17, 13.6 ± 0.15, and 15.2 ± 0.13 respectively. As the concentration increased the mean mortality also increased.

Similarly, the effect of *C. maxima* on the 3rd instar of *A. albopictus* after 24 hrs, at 10 mg/ml concentration the mean mortality was 2.56 ± 0.09 likewise 20, 30, 40, and 50 mg/ml concentration, the mean mortality was found to be 3.82 ± 0.10, 5.66 ± 0.08, 9.71 ± 0.12 and 12.24 ± 0.11 respectively. As the concentration increased the mean mortality also increased.

The result shows that the *C. maxima* has more larvicidal activity against A. aegypti when compared with *A. albopictus*. The percentage mortality at 10, 20, 30, 40 and 50 mg/ml concentration was found to be 26.16, 33.04, 40.56, 54.4, and 60.08 respectively. As the concentration increased the percentage mortality also increased.

Similarly, the mean and percentage mortality of two species of mosquito larvae treated with the *C. maxima* methanol leaf extract at various concentrations after 48 hrs are presented in Table 2 and Fig. 2. Effect of *C. maxima* on 3rd instar of *A. aegypti* after 48 hrs, at 10, 20, 30, 40 and 50 mg/ml concentration, the mean mortality was found to be 1.62±0.9,6.2±0.18, 7.8±0.12, 10.1±0.08, and 11.4±0.10 respectively. As the concentration increased the mean mortality also increased (Table 2).

Effect of *C. maxima* on 3rd instar of *A. albopictus* after 48 hrs, at 10 mg/ml concentration, the mean mortality was 1.02±0.02 likewise at 20, 30, 40 and 50 mg/ml concentration, the mean mortality was found to be 4.63±0.11, 5.8±0.15, 7.3±0.18 and 9.1±0.20, respectively. As the concentration increased the mean mortality also increased (Table 2).

Figs. 1 and 2 explain the relationship between the concentration of leaf extract and the percentage of mortality. The P-values of *A. aegypti* and *A. albopictus* larval mortality in different concentrations of *C. maxima* extract are shown in Table 1. The P-value of A. aegypti after 24 hrs was 0.0287 which was higher than the *A. albopictus.*

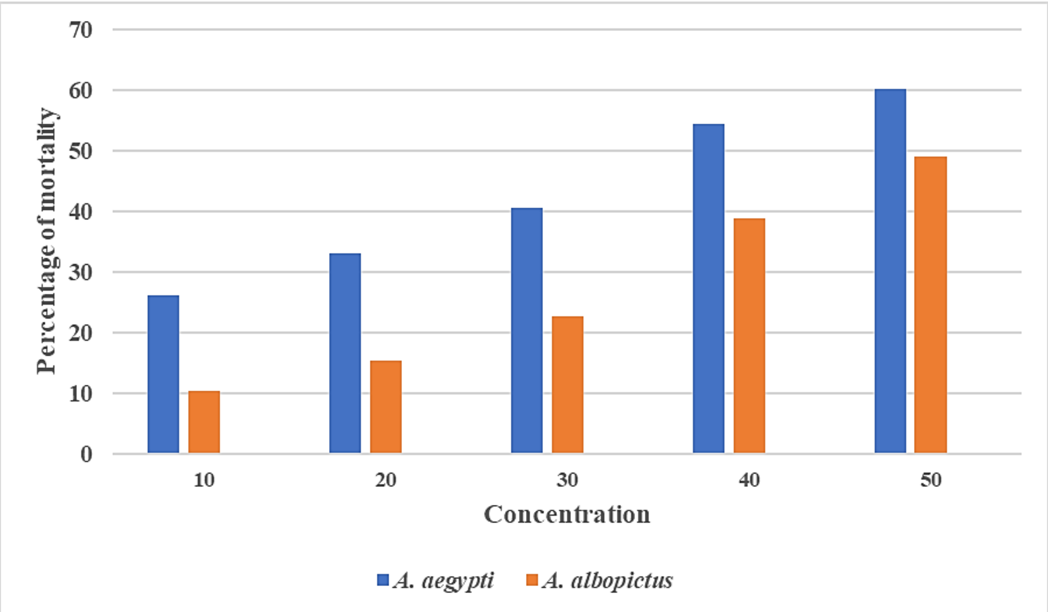

Fig. 1. Graph showing the mortality of different concentrations of leaf extract of *C. maxima* against third- instar larvae of *A. aegypti* and *A. albopictus* after 24 hrs

Table 1. Larvicidal activity of different concentrations of leaf extract of *C. maxima* against third instar larvae of *A. aegypti* and *A. albopictus* after 24 hrs

Mosquito species	Conc (mg/l)	Mean mortality	%Mortality	Regression equation	P-value
Aedes aegypti	10	6.54±0.20	26.16	y = 0.223x + 4.022	0.028752796
	20	8.26±0.23	33.04	R^2 = 0.9819	
	30	10.14±0.17	40.56		
	40	13.6±0.15	54.4		
	50	15.02±0.13	60.08		
Aedes albopictus	10	2.56±0.09	10.24	y=0.2525x – 0.777	0.013041939
	20	3.82±0.10	15.28	R^2 = 0.9628	
	30	5.66±0.08	22.64		
	40	9.71±0.12	38.84		
	50	12.24±0.11	48.96		

Table 2. Larvicidal activity of different concentrations of leaf extract of *C. maxima* against third instar larvae of *A. aegypti* and *A. albopictus* after 48 hrs

Mosquito species	Conc (mg/l)	Mean mortality	%Mortality	Regression equation	P-value
Aedes aegypti	10	1.62±0.9	6.48	y=0.2346x + 0.386	0.014587205
	20	6.2±0.18	24.8	R^2 = 0.9441	
	30	7.8±0.12	31.2		
	40	10.1±0.08	40.4		
	50	11.4±0.10	45.6		
Aedes albopictus	10	1.02±0.02	4.08	y=0.2525x – 0.777	0.009463811
	20	4.63±0.11	18.52	R^2 = 0.9628	
	30	5.8±0.15	23.2		
	40	7.3±0.18	29.2		
	50	9.1±0.20	36.4		

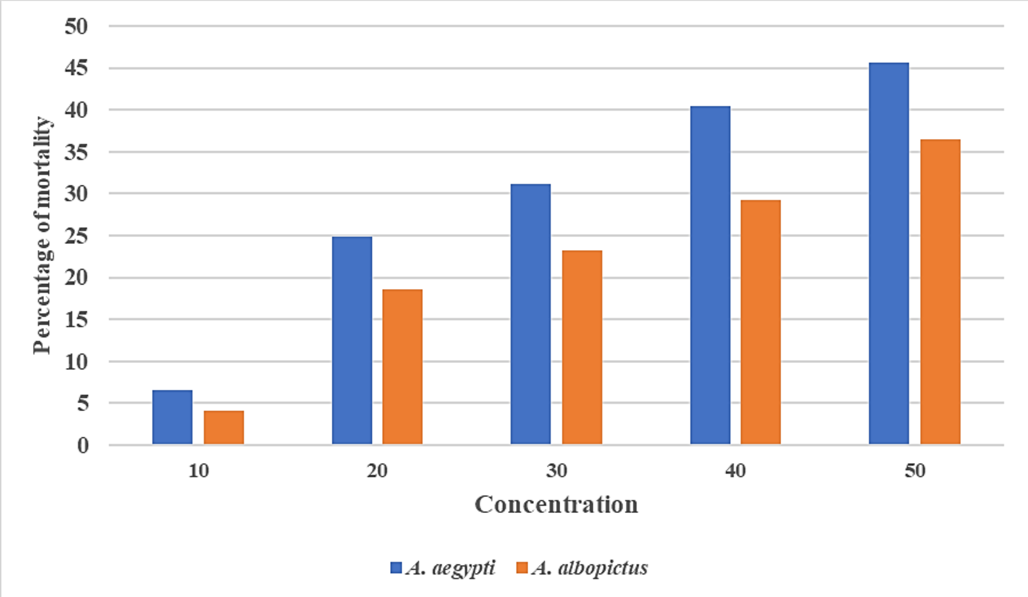

Fig. 2. Graph showing the mortality of different concentrations of leaf extract of *C. maxima* against third- instar larvae of *A. aegypti* and *A. albopictus* after 48 hrs

4. DISCUSSION

"Mosquitoes in the larval stages are attractive targets for pesticides because mosquitoes breed in water, which makes it easy to deal with them in this habitat. The use of conventional pesticides in the water sources, however, introduces many risks to people and the environment. Vector control is facing a serious threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides or the development of newer insecticides. However, due to the continuous increase in the resistance of mosquitoes to familiar synthetic insecticides, better alternative means are sought. The development of insecticide resistance in populations of *A. aegypti* indicates the need for the search for safe and effective alternative measures. Natural pesticides, especially those derived from plants, are more promising in this aspect" [7].

"Nowadays, the control of mosquitoes at the larval stage is focused on plant extracts. The advantage of targeting mosquitoes at the larval stage is that they cannot escape from their breeding sites until the adult emerges and it also reduces the overall pesticide use to control adults by serial application of adulticidal chemicals. The methanol extract of *Cassia fistula* exhibited LC50 values of 17.97 and 20.57 mg/l against *Anopheles stephensi* and *Aedes aegypti* respectively. For the ethanol and methanol extracts of *Annona reticulata* the LC50 value was about 9.996 mg/l and 6.9184 mg/l regarding ethanol and methanol respectively" [8]. Similar results are observed in the present study.

"The larvicidal activity of *A. reticulate* leaf crude extract at different concentrations shows a 100% mortality rate of larvae was observed at 4, 10, 25, 50, 100 and 200 ppm concentrations of crude extract. Present results are in agreement with these findings as nearly 100% mortality was observed after 48 hours of treatment" [9]. "Identifying plant-based insecticides that are efficient as well as suitable and adaptive to local ecological conditions, biodegradable, and have widespread insecticidal properties will work as a new weapon in the arsenal of insecticides and the future may act as a suitable alternative product to fight against mosquito-borne diseases" [10].

"The larvicidal activity depend on the presence of several bioactive chemicals in different parts of the plant" [11]. The presence of cytotoxic compound saponin was observed and the presence of saponin along with other phytoconstituents may be the reason for 100 percent mortality observed with reference to the extracts of the tested plant.

The ethanol extract of *A. muricata* root presented LC50 of 42.3 µg/mL against *A. Aegypti* [12] while isolated compounds had lower LC50 values, such as annonacin, annonacin *A. annonacin* 4-OAc, with LC50 values of 2.65, 10.80 and 6.20 μg/mL, respectively [13,14]. “There is no doubt about the potential of natural products extracted from plants for the control of mosquito vectors of human diseases such as arboviruses. However, despite the vast literature confirming the larvicidal action of plant extracts and their isolated constituents, no commercial plant-based larvicide is available at this time” [15].

5. CONCLUSION

Today environment safety is considered to be of paramount importance. An insecticide does not need to cause high mortality on target organisms, but it should be acceptable and eco-friendly in nature. Phytochemicals may serve as these are relatively safe, inexpensive and readily available in many parts of the world. Several plants are used in traditional medicines for mosquito larvicidal activities in many parts of the world.

The larvicidal efficacy of plant extract *C. maxima* was studied wherein mean mortality and percentage mortality of both *A. aegypti* and *A. albopictus* larval populations were noted at different concentrations after 24 hrs and 48 hrs periods of exposure. Both mean mortality and percentage mortality increased as the concentration increased. Our findings show that leaf extract *C. maxima* can be developed as an eco-friendly larvicide, since it is useful to control *A. aegypti* and *A. albopictus* populations.

ACKNOWLEDGEMENT

Authors thanks to the Manonmaniam Sundaranar University, Thirunelveli for financial support, internet facilities and laboratory facilities provided for our campus.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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