VOLUME 10 (SPECIAL ISSUE 2) 2024

ISSN 2454 - 3055

Manuscripts under Special Issue are published under the theme "Modern Aspects of Applied Biology"

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INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 10 (Special Issue 2), 17-24 (2024)

International Journal of Zoological Investigations

Contents available at Journals Home Page: www.ijzi.net

Editor-in-Chief: Prof. Ajai Kumar Srivastav

Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Anti-larvicidal Efficacy of Leaves of *Sphaeranthus amaranthoides* Burm on *Culex quinquefasciatus* Say (Diptera:Culicidae)

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Received: 14th September, 2024; **Accepted:** 5th October, 2024; **Published online:** 5th November, 2024

https://doi.org/10.33745/ijzi.2024.v10ispl2.004

Abstract: Mosquitoes are well-known vectors that spread a variety of diseases all over the world. It is widely acknowledged that mosquito-borne diseases have a global influence on public health. Mosquitoes transmit dangerous human diseases, leading to the deaths of millions of people each year. *Culex quinquefasciatus* is responsible for several diseases, including West Nile Virus, Japanese Encephalitis, and filariasis. Mosquito larval control is simple since the target specificity of the larvicide employed can be ensured. At the same time, it has several negative consequences, and mosquitoes tend to develop resistance to it. As a result, it is past time to start using plant extracts to make biopesticides that can effectively control mosquitoes. Plants could serve as an alternate source of mosquito repellents. The objective of this study was to see how effective *S. amaranthoides* Burm.f. Leaf extracts in Hexane, chloroform, and methanol extracts were checked against *Cu. Quinquefasciatus* larvae. A preliminary phytochemical study revealed the potential ingredient in the plant that is responsible for its larvicidal activity. The data was statistically analyzed, including standard deviation, test of significance, and LC_{50} and LC_{90} calculations. We may infer that the hexane extract of *S. amaranthoides* Burm.f. leaf is the most effective based on the results of the studies.

Keywords: Anti-larvicidal, Mosquito-borne diseases, *Culex quinquefasciatus*, *Sphaeranthus amaranthoides*

Citation: Gillella Vedavati, Manoj Dhanraj K. and Joyce Priyakumari: Anti-larvicidal Efficacy of Leaves of *Sphaeranthus amaranthoides* Burm on *Culex quinquefasciatus* Say (Diptera:Culicidae). Intern. J. Zool. Invest. 10(Special Issue 2): 17-24, 2024.

https://doi.org/10.33745/ijzi.2024.v10ispl2.004

Introduction

Mosquitoes being annoying, biting and blood-sucking, have been a great menace to humans for thousands of years. Mosquito has elongated piercing and sucking mouthparts which are helpful in sucking host blood. Infected mosquitoes are vectors known to transmit pathogens such as arboviruses, malaria protozoa, bacteria, and filarial worms to the animals they feed upon, and cause several serious diseases including malaria, dengue, filariasis, yellow fever, Zika fever, Chikungunya, Japanese encephalitis, Rift Valley fever and West Nile fever (Lundstrom

et al., 2011). Mosquito-transmitted diseases have caused severe health impacts on humans, livestock, and wild animals throughout history, which impose economic burdens as well. Nearly 50% of the world population is prone to these vector-borne diseases. Mosquito-borne diseases account for 17% of all infectious diseases and cause more than one million deaths per year (Wilson and Schlagenhauf, 2016).

Culex mosquitoes are called common house mosquitoes that feed on humans, domestic animals, and birds, and are well-known for medical and veterinary concerns. *Culex* mosquitoes are persistent vectors of arboviruses such as St. Louis encephalitis virus, western and eastern equine encephalitis virus, West Nile virus, Japanese encephalitis virus, filarial nematodes, and avian malarial parasite, *Plasmodium spp*. In 2018, WHO reported that 51 million cases of *Culex*-transmitted lymphatic filariasis. *Cx. quinquefasciatus*, *Cx. Pipiens*, *Cx. Vishnu*, *Cx. salinarius*, *Cx. tarsalis*, *Cx. Perexiguus*, *Cx. modestus*, etc., are some important vectors of public health burden.

Control of the mosquito population is the appropriate control strategy for preventing mosquito-borne diseases and reducing the public health burden. Synthetic chemical insecticides are extensively used to control mosquito vectors. Insecticides are directly applied to the mosquito breeding sites to kill eggs, larvae, pupae and adults. Since chemicals are cost-effective and easy to use for large mosquito breeding sites, chemical control of mosquitoes is comparatively the widely preferred measure (Gericke *et al*., 2002). The disadvantages of synthetic chemical insecticides pushed scientists to seek an alternative measure that should overcome the drawbacks of synthetic chemical insecticides. Biological control measures of mosquitoes as an alternative are effective, particularly the insecticides of plant origin. Plants produce secondary metabolites known as phytochemicals against insects and pathogenic microorganisms, which act as repellents, growth regulators, feeding deterrents, and toxins (Hargreaves *et al*., 2000). Hence, in the present investigation, anti-larvicidal efficacy of leaves of *Sphaeranthus amaranthoides* Burm on *Culex quinquefasciatus* Say was studied.

Materials and Methods

Collection of Plant material:

Sphaeranthus amaranthoides was collected from Sankarankoil, Tamil Nadu, India (Latitude: 9.181414; Longitude: 77.535324). The taxonomical description of plant species was performed by taxonomist Dr. Mutheeswaran, Xavier Research Foundation, Palayamkottai, Tamil Nadu, India. The receipt specimen was kept in the herbarium of Madras Christian College, Chennai, Tamil Nadu, India.

Preparation of Solvent extracts:

Collected plant materials were thoroughly washed in running water and then dried in shade for 5-7 days. Dried plant materials were coarsely powdered using an electric blender. First, the plant powder (150 g) was soaked in 500 ml of hexane for 72 h. After 72 h, plant powder–soaked hexane was filtered using Whatman No 1 filter paper. The filtered solvent was concentrated in a rotary vacuum evaporator to obtain the crude extract. Next, the plant powder was similarly soaked in chloroform and methanol solvents to obtain their extracts, respectively. Hexane, chloroform, and methanol extracts from the plants were prepared for the experiments.

Test animal Mosquitoes:

Eggs of *Cx. Quinquefasciatus* were collected from the Cooum River, Chennai, India and maintained at the laboratory of the Department of Zoology, Madras Christian College, Chennai, Tamil Nadu, India. The eggs hatched into larvae after 2–3 days, which were transferred to enamel trays containing water. The larvae were fed with dog biscuits and Brewer's yeast (3:2). The pupae emerging out from larvae after 4–5 days were immediately collected and transferred to cups filled with water. These cups with pupae were placed inside the screened cages (23×23×32 cm

in dimension), in which the adult mosquitoes would emerge after 2–3 days. A cotton wick moistened in 10% sucrose solution was kept in a jar to feed adult mosquitoes. In order to collect eggs, the adult mosquitoes were not given sucrose for 12 h, after which they were provided membrane blood feeding for an hour. After 3 days, the ovitrap was kept inside the cages to collect the eggs laid, which were shifted to the enamel trays for further experimentation. Different life stages of mosquitoes were obtained from laboratory cultures for this study, which were free from exposure to pathogens, insecticides or repellents. The rearing conditions were: 28±1°C; 70–75%, relative humidity, and 11±0.5 h photoperiod.

Ovicidal bioassay:

Twenty-five eggs of *Cx. Quinquefasciatus* were collected and exposed to different concentrations viz., 62.5, 125, 250 and 500 ppm of hexane, chloroform and methanol extracts. Each crude extract concentration has five replicates for the study. DMSO (Dimethyl Sulfoxide) in water as solvent control as well as water control were separately used. A microscope was used to observe the egg hatchings. The percentage of ovicidal efficacy was observed at 120 h post-treatment using the formula:

Per cent of ovicidal activity= (Number of unhatched eggs)/ (Total number of eggs introduced) ×100.

Larvicidal bioassay:

The larvicidal efficacy of the crude extracts of different plant extracts was assessed using the method with little variations. Different concentrations viz., 62.5, 125, 250 and 500 ppm of hexane, chloroform and methanol extracts were used. Five replicates were prepared for each concentration. Third-instar larvae of *Cx. Quinquefasciatus* (n=20) were used in each replicate. Solvent and positive controls were prepared as mentioned above for the larvicidal assay. The numbers of dead larvae in each replicate were recorded after 24 h of exposure. Larvae were confirmed as dead if they did not react to stimulus or did not come to the water surface. The percentage of larval mortality was estimated for each concentration using the following formula:

Percentage of larval mortality= (Number of dead larvae)/(Number of larvae introduced) ×100

Pupicidal bioassay:

Pupicidal assay was performed as per the standard protocol with little variations. Hexane, chloroform and methanol plant extracts were made in concentrations of 62.5, 125, 250 and 500 ppm. Five replicates of each concentration were used. Twenty pupae of *Cx. Quinquefasciatus* were taken in each replicate. Solvent and positive controls were prepared as described for the ovicidal assay. The dead pupae in the replicate were counted and recorded after 24 h of exposure. Pupae were confirmed as dead if they did not react to stimulus. The percentage of pupal mortality at each concentration was estimated using the following formula:

Percentage of pupal mortality= (Number of dead pupae)/(Number of pupae introduced) ×100

Statistical analysis:

Replication data are used to calculate the mean values and standard deviations. Mortality values were subjected to US EPA probit analysis software (1.5 version) to calculate the lethal concentration values (LC_{50} and LC_{90}). 95% Confidence limits were calculated with upper limits and lower limits, and Chi-square values were calculated using SPSS version 11.5 (Statistical software package). One-way Analysis of Variance was used to find out the significance of the treatments. The means were separated with Tukey's test of multiple comparisons by using SPSS software (version 11.5).

Results

The present study revealed the anti-larvicidal efficacy of leaves of *Sphaeranthus amaranthoides* Burm on *Culex quinquefasciatus*. Among the hexane, chloroform, and methanol extracts of *S. amaranthoides*, the hexane extract of *S.*

Table 1: Ovicidal activity of various extracts of *S. amaranthoides* on *Cx. quinquefasciatus*

S. No.	Extracts	Concentrations (ppm)			
		62.5	125	250	500
1	*Sa*HE	7 ±0.5*	17±0.5*	34±0.8*	63±0.5*
2	*Sa*CE	6 ±0.8*	11±0.8*	27±0.5*	56±0.8*
3	*Sa*ME	5±0.7*	11±0.8*	26±0.8*	46±0.8*

Means are separated by Tukey's test of multiple comparisons, One-way Analysis of Variance (ANOVA). *p≤0.5, level of significance.

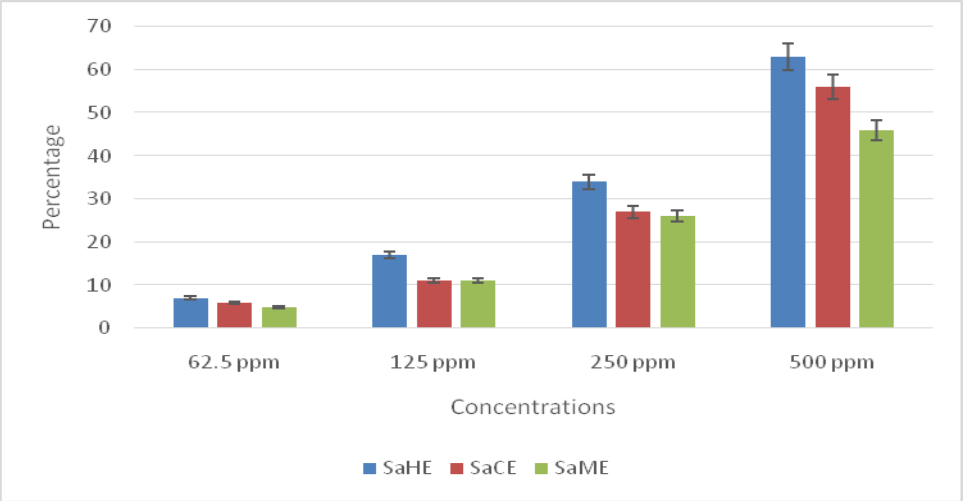

Fig. 1: Ovicidal activity of various extracts of *S. amaranthoides* on *Cx. quinquefasciatus.*

amaranthoides exhibited the most efficient mosquitocidal activities against *Cx. quinquefasciatus*.

Ovicidal activity of various extracts of S. amaranthoides against Cx. quinquefasciatus:

The hexane (*Sa*HE) and chloroform (*Sa*CE) extracts of *S. amaranthoides* exhibited ovicidal efficacy of 63% and 56%, respectively, at 500 pm concentrations against *Cx. quinquefasciatus*. The methanol extract of *S. amaranthoides* (*Sa*ME) expressed 46% ovicidal activity at 500 pm concentrations against *Cx. quinquefasciatus*. Table 1 and Figure 1 depicts the ovicidal efficacy of hexane, chloroform, and methanol extracts of *S. amaranthoides.*

Larvicidal activity of various extracts of S. amaranthoides against Cx. quinquefasciatus:

The hexane extract of *S. amaranthoides* exhibited the highest larvicidal efficacy against *Cx. quinquefasciatus* with LC_{50} and LC_{90} values of 218.178 and 183.210 ppm, respectively. The methanol extract of *S. amaranthoides* showed the larvicidal efficacy with LC_{50} and LC_{90} values of 555.584 and 4535.857 ppm, respectively. Chloroform extract of *S. amaranthoides* revealed considerably higher larvicidal efficacy against *Cx. quinquefasciatus* with LC_{50} and LC_{90} values of 908.937 and 9388.107 ppm, respectively (Table 2; Figs, 2, 3).

Pupicidal activity of various extracts of S. amaranthoides against Cx. quinquefasciatus:

Hexane extracts of *S. amaranthoides* exhibited the most significant pupicidal activity against *Cx. quinquefasciatus*, with LC_{50} values of 240.266 and LC_{90} values of 1461.305 ppm. Chloroform extracts showed pupicidal activity with LC_{50} values of 372.348 and LC_{90} values of 1770.714 ppm. Methanol extracts showed pupicidal activity

Table 2: Lethal concentration (in ppm) of *S. amaranthoides* extracts against the larvae of *Cx. quinquefasciatus*

Extract	Mosquitoes	LC_{50}	95% Confidence Limit		LC_{90}	95% Confidence Limit		Intersept ± SE	Slope ± SE	X^2
			LL	UL		LL	UL			
Hexane	*Cx. quinquefasciatus*	218.178	176.375	278.305	183.210	1065.997	4674.891	1.7 ± 0.4	1.3 ±0.1	0.7*
Chloroform		908.937	585.043	2148.483	9388.107	3394.131	78574.102	1.2 ± 0.5	1.2 ± 0.2	0.3*
Methanol		555.584	410.068	919.231	4535.857	2152.075	18037.980	1.1 ± 0.5	1.4 ± 0.2	0.03*

LC_{50} = lethal concentration that kills 50% of the exposed larvae; LC_{90} = lethal concentration that kills 90 % of the exposed larvae; LL = lower limit; UL = upper limit. *p≤0.05, level of significance of chi-square values

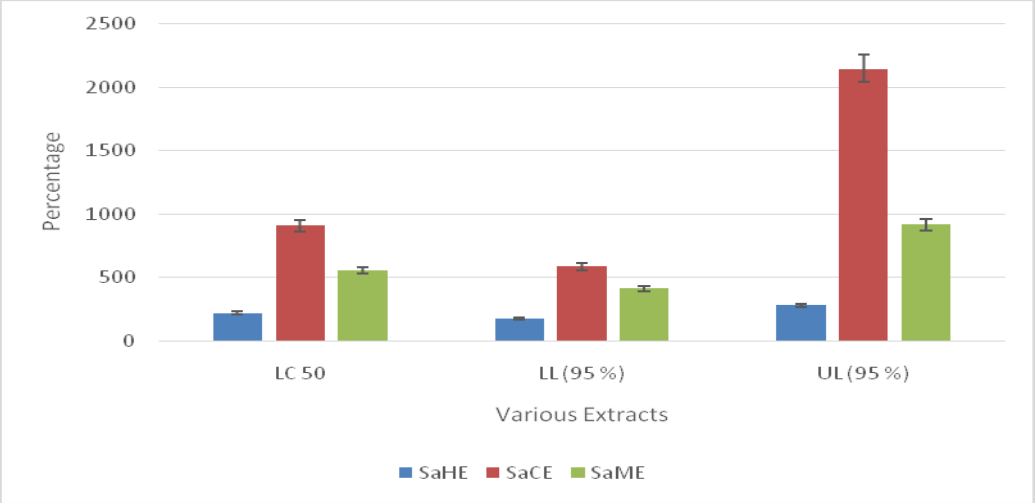

Fig. 2: LC_{50} (in ppm) of *S. amaranthoides* extracts against the larvae of *Cx. quinquefasciatus.*

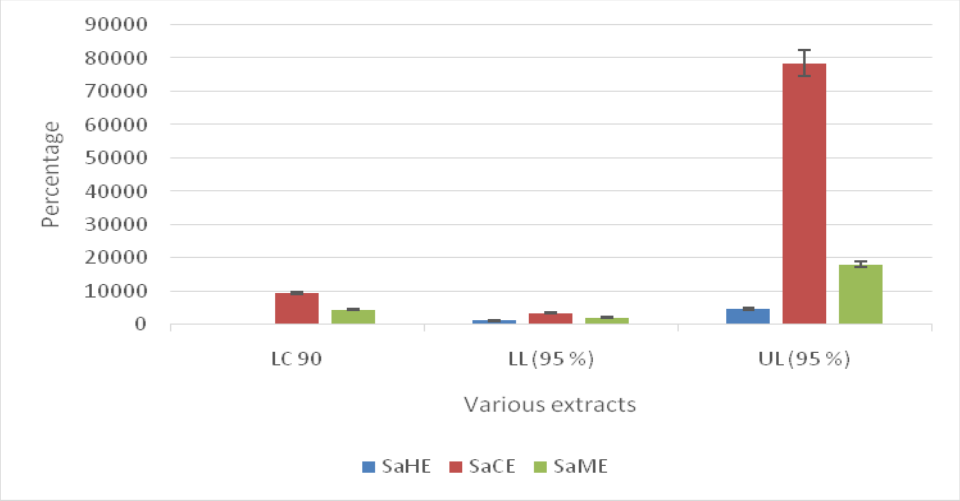

Fig. 3: LC_{90} (in ppm) of *S. amaranthoides*extracts against the larvae of *Cx. quinquefasciatus.*

Table 3: Lethal concentration (in ppm) of *S. amaranthoides* extracts against the Pupae of *Cx. quinquefasciatus*

Extract	Mosquitoes	LC_{50}	95% Confidence Limit		LC_{90}	95% Confidence Limit		Intersept ± SE	Slope ± SE	X^2
			LL	UL		LL	UL			
Hexane	*Cx. quinquefasciatus*	240.266	185.238	333.349	1461.305	821.533	4588.875	0.1± 0.7	1.8±0.3	0.1*
Chloroform		372.348	287.896	551.302	1770.714	1006.497	5266.066	1.2±0.5	1.2±0.2	1.4*
Methanol		532.803	357.730	1228.371	4511.052	1710.429	49982.582	1.2±0.7	1.3±0.3	0.6*

LC_{50} = lethal concentration that kills 50 % of the exposed Pupae; LC_{90} = lethal concentration that kills 90 % of the exposed Pupae;LL = lower limit; UL = upper limit. *p≤0.05, level of significance of chi-square values

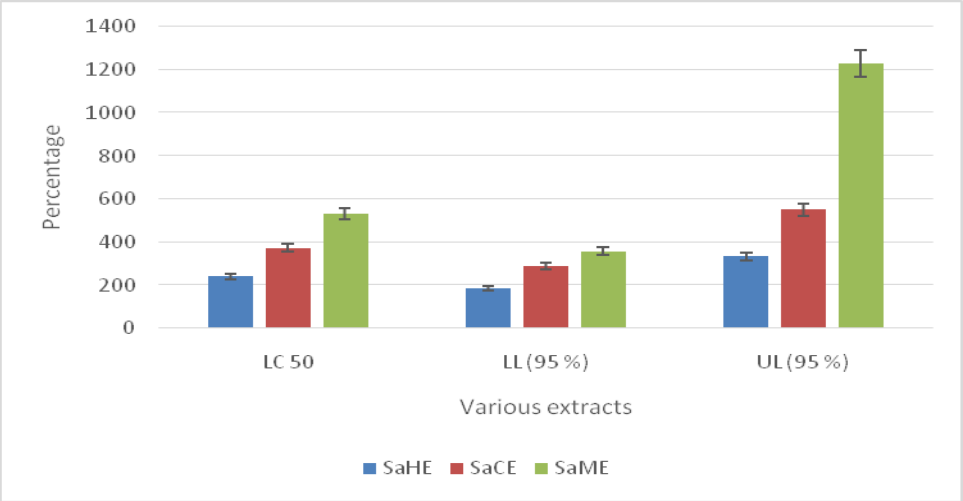

Fig. 4: LC_{50} (in ppm) of *S. amaranthoides* extracts against the Pupae of *Cx. quinquefasciatus.*

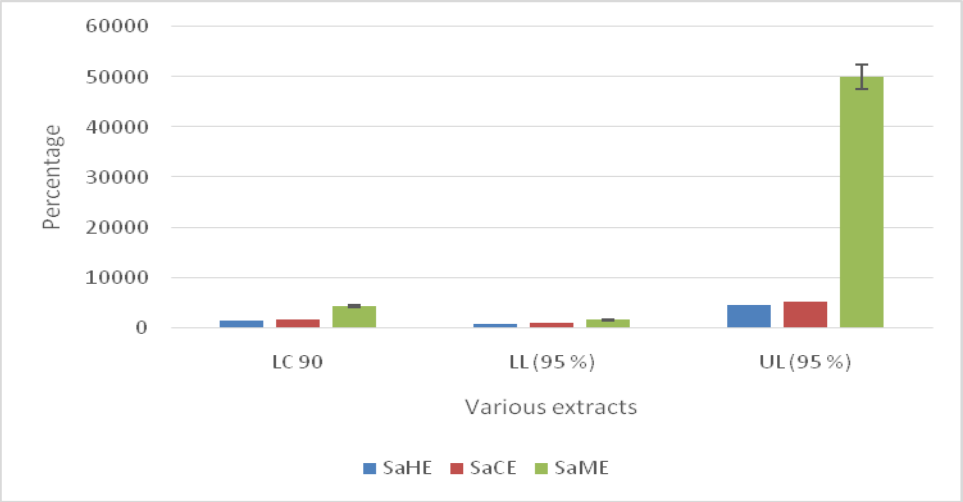

Fig. 5: LC_{90} (in ppm) of *S. amaranthoides* extracts against the Pupae of *Cx. quinquefasciatus.*

with LC_{50} values of 532.803 and LC_{90} values of 4511.052 ppm, respectively (Table 3, Figs. 4, 5).

Discussion

Synthetic chemical pesticides have been largely used against mosquito vectors. Their prolonged and excessive usage led to several serious issues, such as drug resistance, biomagnification, environmental pollution, and toxicity to humans and non-target organisms. These disadvantages impose an urgent requirement for eco-friendly and target-specific pesticides for mosquito control. Phytochemicals obtained from various plants, shrubs, and trees were reported to have high control efficacy against important mosquito vectors (Shaalan *et al*., 2005).

The most potent ovicidal efficacy against *Cx. quinquefasciatus* eggs were exhibited by hexane extract of *S. amaranthoides* with 63% at 500 ppm. Next, the chloroform extract of *S. amaranthoides* presented 56% ovicidal activity against *Cx. quinquefasciatus* eggs at 500 ppm. In addition, the methanol extract of *S. amaranthoides* also provided 46% of ovicidal activity against *Cx. quinquefasciatus* eggs at 500 ppm. Comparatively, methanol extract of *Cocculus hirsutus* inhibited up to 86% and 100% of egg hatching at 500 and 1000 ppm, respectively. Govindarajan *et al*. (2011) demonstrated that *Ervatamia coronaria* extract exhibited 100% ovicidal efficacy against *Cx. quinquefasciatus* at 250 ppm. Panneerselvam *et al*. (2013) reported that 150 ppm of methanol extract of *A. paniculata* inhibited 100% egg hatchability of *An. Stephensi* eggs. Methanolic extract of *Terminalia chebula* produced 100% ovicidal activity at 300 ppm against *An. stephensi*, *Ae.aegypti*, and *Cx. quinquefasciatus* eggs (Veni *et al*., 2017).

The hexane extract of *S. amaranthoides* at 500 ppm showed 67% potential against *Cx. quinquefasciatus* third-instar larvae after 24 h of exposure with the LC_{50} and LC_{90} values of 218.178 and 1830.210 ppm, respectively. It has also been reported that ethanolic crude extract of Duranta leaves exhibited 100% larvicidal efficacy against all instars of *Culex pipiens*. Liu *et al*. (2018) revealed that ethanolic extracts of *Curcuma longa* root showed 100% larval mortality at 1000 ppm concentration after 24 h. The aqueous extract of *Piper longum* showed larvicidal efficacy with LC_{50} and LC_{90} values of 50.81 and 104.7 ppm, respectively, against *Ae. aegypti*, and with LC_{50} and LC_{90} values of 133.42 and 279.60 ppm, respectively, against *An. stephensi*. Krishnappa and Elumalai (2012) reported that ethonolic extract of *Gliricidia sepium* presented high larvicidal activity with LC_{50} and LC_{90} values of 121.79 and 231.98 ppm, respectively, against *An. stephensi*. Ethyl acetate extract of *Alangium salviifolium* (L.f.) Wangerin fruit pericarp displayed 100% larvicidal activity against *Cx. quinquefasciatus* larvae at 72 h exposure with LC_{50} of 3.60 ppm (Mondal *et al*., 2022).

The hexane extracts of *S. amaranthoides* expressed potent pupicidal efficacy with LC_{50} values of 240.266 ppm, and LC_{90} values of 1461.305 ppm. Similarly, Subarani *et al*., (2013) stated that aqueous extract of *Catharanthus roseus* presented the LC_{50} values of 118.08 and 146.2 against pupae of *An. stephensi* and *Cx. quinquefasciatus*, respectively. In the present study, the methanol extract of *S. amaranthoides* presented suicidal efficacy with LC_{50} and LC_{90} values of 235.2 and 1198.2, respectively, against Cx*. quinquefasciatus*. Hexane extracts of *Foeniculum vulgare* Mill., *Tagetes minuta* L., and *Cosmos bipinnatus* Cav exerted pupicidal activity with LC_{50} of 1.07, 1.12 and 1.16 mg/ml, respectively, against *Cx. quinquefasciatus*, while extracts of *F. vulgare*, *T. minuta*, and *C. bipinnatus* showed pupicidal efficacy with LC_{50} of 1.11, 1.14 and 1.31 mg/ml, respectively (Modise and Ashafa, 2016). *Clerodendrum philippinum* Schauer exhibited pupicidal activity against *Ae. aegypti* and *An. stephensi* with 51 ± 0.5% and 58 ± 0.8%, respectively, at 600 ppm concentration (Dhal *et al*., 2018). Hexane and dichloromethane fractions of *Helicteres velutina* K. Schum exhibited pupicidal activity against *Ae. aegypti* with LC_{50} of 0.12 and 8.85 mg/ml, respectively (Araujo

Fernandes *et al*., 2021). The present study revealed that *S. amaranthoides* leaves can be used to make environmentally friendly larvicides.

Conclusion

This study demonstrated the critical need for innovative solutions to stop the global threat of mosquito-borne diseases. The study focuses on the potential of leaf extracts from *S. amaranthoides* Burm.f, as a promising biopesticide for successfully controlling *Culex quinquefasciatus* larvae. The findings provided a more environmentally responsible and sustainable alternative to conventional pesticides by proving the potential of plant-based alternatives. This study provides the path for additional research into utilising natural substances to lessen the negative effects of mosquito-borne diseases on public health worldwide.

Acknowledgements

The authors are grateful to the authorities at Department of Zoology at Madras Christian College for providing lab facilities for the duration of this study.

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