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Research Article

Larvicidal and Repellent efficacy of some of the weed plant extracts against *Culex quinquefasciatus* Say

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Abstract: The indiscriminate use of synthetic insecticides over years to control mosquitoes has caused an array of adversities to human beings as well as to nature and to a greater extent it has inflicted damages to the natural environment and disturbed ecological balances. Insecticides in plant origin play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community levels. The present study tested the larvicidal activity of methanolic extracts of four plants viz., *Uvaria narum* A. DC. (Annonaceae), *Morinda pubescens* Sm. (Rubiaceae), *Caesalpinia pulcherrima* (Linn.) Sw. (Leguminosae) and *Leea indica* (Burm. f.) Merr. (Vitaceae) and repellent activity of *Uvaria narum* against *Culex quinquefasciatus* Say. From the tested plants it is observed that methanolic extracts of *U. narum* possesses the highest larvicidal activity and ensure 100% protection. The findings of the present investigation revealed that the methanolic extracts of the selected plants have remarkable larvicidal activity against *Cx. quinquefasciatus*.

Keywords: Mosquitoes, larvicidal activity, plant extracts, repellent activity.

1. Introduction

Mosquitoes are one of the medically important insects as they are known to spread dreadful diseases like filariasis, Japanese encephalitis, malaria, dengue fever, yellow fever, chikungunya, etc. More than 100 species of mosquitoes are reported to be capable of transmitting diseases to humans to date. Most effective method to reduce the transmission of vector-borne diseases is through controlling the vectors. Indiscriminate use of synthetic insecticides against mosquitoes poses substantial risk to non-target organisms and pollutes the environment.

Application of synthetic insecticides though, efficacious against the target species, vector control is facing a threat due to the development of resistance to chemical insecticides resulting in rebounding vectorial capacity[1]. The environmental protection act of 1969 has framed a set of rules and regulations to check the application of chemical control agents in nature. Alternative methods to interrupt the transmission of vector-borne diseases without any residual effect include usage of plant extracts.

Culex quinquefasciatus Say, a vector of lymphatic filariasis is widely distributed and inflicts common chronic manifestation[2]. The eradication or control of this vector is an important step to preventing and controlling filariasis[3]. Phytochemicals derived from plant sources have larvicidal activity, insect growth regulating capacity, repellent and adulticidal activities[4,5]. The *Zanthoxylum armatum*, *Zanthoxylum alatum* (Rutaceae), *Azadirachta indica* (Meliaceae), and *Curcuma aromatica* (Zingiberaceae) were possessing repellent properties against mosquitoes[6]; the repellent activity of active compound Octacosane from *Moschosma polystachyum* against the vector *Cx. quinquefasciatus*[7]; and the essential oil of *Zingiber officinale* as a mosquito larvicidal and repellent agent against the filarial vector *Cx. quinquefasciatus*[8]. In this paper, we tested the larvicidal activity of methanolic extract of four weed plants of different families and their repellent activity of aqueous extracts against *Culex quinquefasciatus*.

2. Materials and methods

2.1 Test plants

Weed plants of four different families viz., *Uvaria narum* A. DC. (Annonaceae), *Morinda pubescens* Sm. (Rubiaceae), *Caesalpinia pulcherrima* (Linn.) Sw.

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(Leguminosae) and *Leea indica* (Burm. f.) Merr. (Vitaceae) were collected from the Calicut University campus, washed thoroughly using tap water and shade dried at room temperature for one week.

2.2 Test organism

The larvae/pupae of *Cx. quinquefasciatus* were collected from the fields and brought to the laboratory, transferred to small sized plastic trays containing ¾ part of 0.08% saline water and kept in the mosquito emergence cage. Freshly emerged adult females were fed by blood meal from an immobilized hen and adult males were fed subsequently by sucrose solution. The freshly laid eggs were collected and allowed to hatch. Larvae of I to IV instars were selected for the bioassay.

2.3 Preparation of crude extract

The shade dried leaves of selected plants were powdered using a mixer grinder and 20 grams of the powder were used for Soxhlet extraction in 250ml analytical grade methanol. The yield of the material was calculated by weighing the dried extract. A part of the extract is taken and 1% stock solution was prepared and used for bioassay.

2.4 Estimation of LC_{50} and LC_{90}

Methanol extracts of selected plants were tested against I–IV instar larvae of *Cx. quinquefasciatus*. The appropriate volumes of 1% stalk solution were diluted into 100ml. ten larvae each were released to each concentration of test medium and also in the control set. Triplicates were also maintained for each set. Observations were recorded after 24 hours and the mortality rate of larvae were recorded at the end of 24-hour exposure to different concentrations. LC_{50} and LC_{90} were calculated using a probit analysis[9].

2.5 Adult repellency

Fresh and clean leaves were selected and grinded with water in order to make decoction of the selected plants. Smeared the water decoction on hand, exposed to female mosquitoes to bite and the number of mosquito incidence were recorded for 1 hour. In control, washed hands without water decoction were exposed to female mosquitoes for 1 hour.

The protection percentage was calculated according to the standard formula,

$$\textbf{Protection percentage} = \mathbf{(C-T)/C \times 100}$$

Where, C is the number of mosquito bites in control and T is the number of a mosquito bite in the test.

3. Results and Discussion

3.1 Percentage yield of selected plants

Uvaria narum (24.75%) is found to be the best yielded plants in the present study followed by *M. pubescens* (24.6%). Among the plants selected *L. indica* exhibited the least yield percentage (10.55%) (Fig. 1).

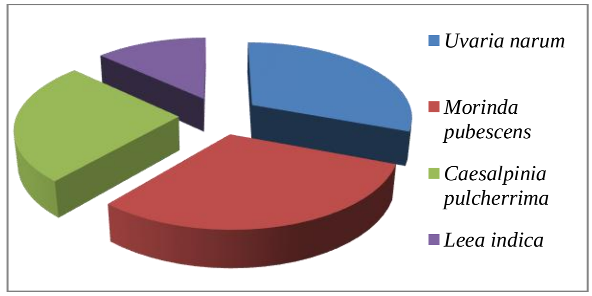

Fig. 1. Percentage yield of the selected plant extracts.

3.2 Critical lethal concentrations

Methanol extracts of the selected plants were tested for larvicidal activity on I–IV instar larvae of *Cx. quinquefasciatus*. The methanol extracts of *U. narum* is found to be very active and potential, as the 24 hours LC_{50} is 10.426 on IV instar larvae. It is also found that a stable trend in the LC_{50} and LC_{90} values against I–II (0.997–1.355) and III–IV (10.062–10.426) instar larvae of *Cx. quinquefasciatus* (Fig. 2). Similar trend was also observed in the case of *L. indica* as there is only one stable result in the LC_{50} value against II–III (334.806–338.301) instar larvae (Fig. 3).

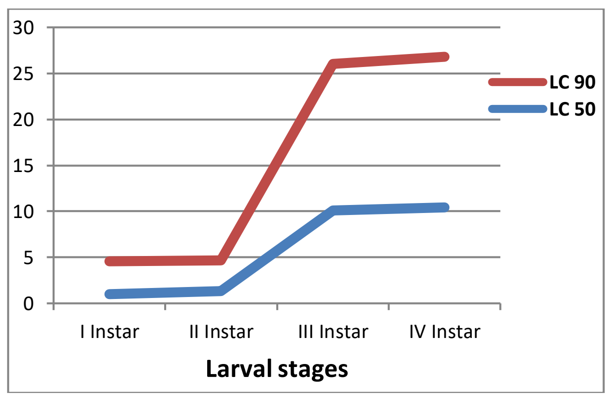

Fig. 2. Effect of methanolic extracts of *Uvaria narum* against different larval stages of *Cx. quinquefasciatus* in 24 hours.

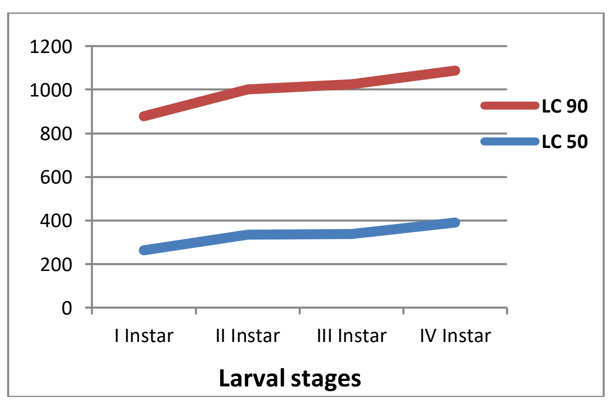

Fig. 3. Effect of methanolic extracts of *Leea indica* against different larval stages of *Cx. quinquefasciatus* in 24 hours.

Larvicidal activity (LC_{50}) of *M. pubescens* is found to be the highest in I instar larvae, but later there is a great activity based jump was observed (Fig. 4). This is supposed to be the fact that *M. pubescens* is highly active against only I instar larvae and later it gradually lost the larvicidal activity. This may be due to the differential response of the larval stages of *Cx. quinquefasciatus* towards the botanicals. It was not observed in the LC_{90} values.

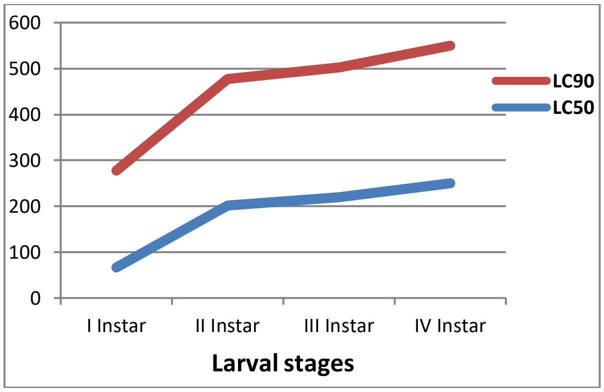

Fig. 4. Effect of methanolic extracts of *Morinda pubescens* against different larval stages of *Cx. quinquefasciatus* in 24 hours.

Larvicidal activity of *Caesalpinia pulcherrima* was found to be similar to that of *L. indica* but it is more active than latter against I and II instar larvae (Figs. 3 & 5). It is different in case of LC_{90} values (Figs. 3 & 5). *Caesalpinia pulcherrima* exhibited the lease larvicidal activity against IV instar larvae of *Cx. quinquefasciatus.*

Among the four plants tested *U. narum* exhibited the highest larvicidal activity against I–IV instars of *Cx. quinquefasciatus* followed by *L. indica*, *M. pubescens* and *C. pulcherrima* (Fig. 6).

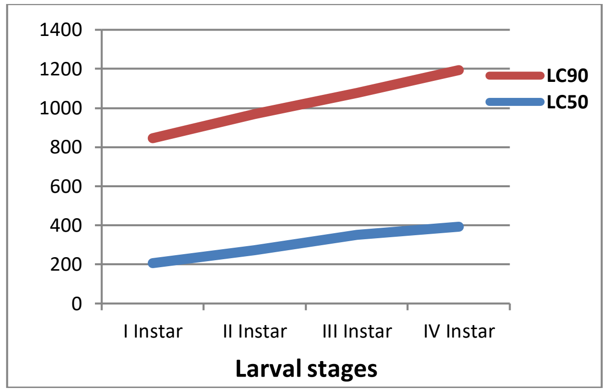

Fig. 5. Effect of methanolic extracts of *Caesalpinia pulcherrima* against different larval stages of *Cx. quinquefasciatus* in 24 hours.

Table 1. Data observed on the repellent activity conducted with water decoction from *Uvaria narum* tested against mosquitoes.

Time period	Number of mosquito bites		Protection percentage
	Control	Treated	(%)
6–7 pm	4	0	100
7–8 pm	8	0	100
8–9 pm	12	0	100
9–10 pm	13	0	100
10–11 pm	11	0	100
11–12 pm	12	0	100

Table 2. Data observed on the repellent activity conducted with water decoction from *C. pulcherrima* tested against mosquitoes.

Time period	Number of mosquito bites		Protection percentage
	Control	Treated	(%)
6–7 pm	5	0	100
7–8 pm	9	2	77.7
8–9 pm	12	3	75
9–10 pm	13	5	61.53
10–11 pm	11	8	27.27
11–12 pm	10	8	20

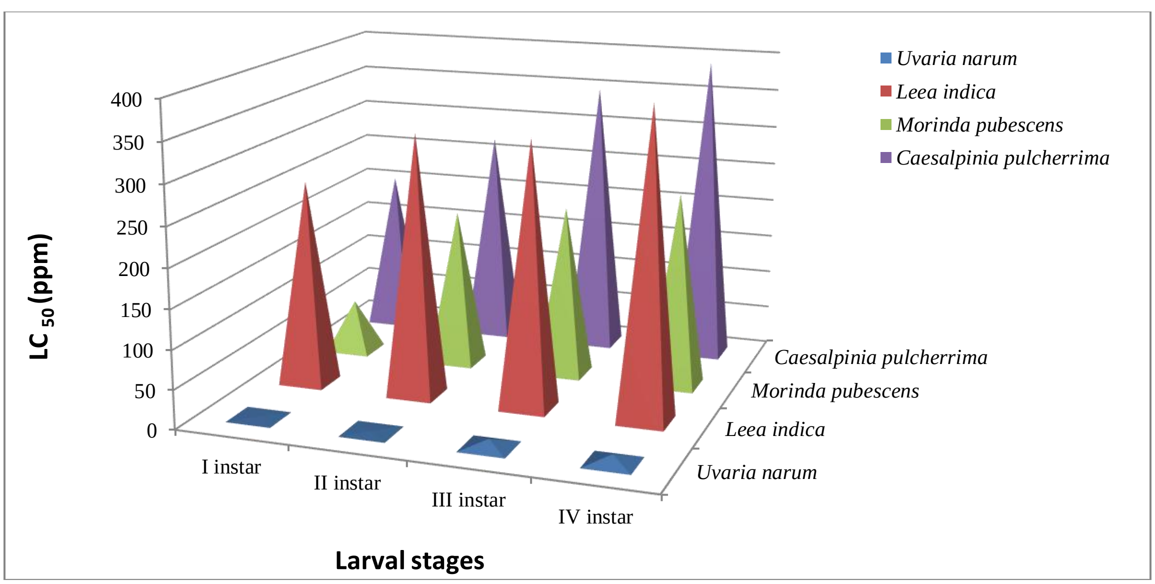

Fig. 6. 24 hrs LC_{50} (ppm) of methanol extracts of the studied plants against different larval stages of *Cx. Quinquefasciatus.*

3.3 Repellency Test

Repellency test using water decoction of the selected plants exhibits great variability in results. Water decoctions of *Uvaria narum* showed 100% protection and *Caesalpinia pulcherrima* showed an average of 60.25% protection against mosquito bites. *M. pubescens* and *L. indica* extract showed repellent activity for 1 hour and the percentage of protection decreased by an increase in time of exposure.

Mosquito control is chiefly focused against larvae and only against adults when necessary. Larval control is considered to be the best method due to the low mobility of larval stages, especially the principal breeding sites are manmade and easy to identify[10]. It remains as a fact that synthetic insecticides of chemical origin may induce resistance in mosquitoes over generation by generation causing difficulty in the management of these vectors. In addition to this, the use of synthetic insecticides causes hazardous effects on ecosystem, to the non-target organisms in particular. Botanical insecticides are found to be more eco-friendly as they don't induce any harmful effects in the ecosystem.

Plants may be a source of alternative agents for control of mosquitoes because they are rich in bioactive chemicals, active against a limited number of species including specific target insects, and are biodegradable. They are potentially suitable for use in integrated pest management programs[11] like the mosquito larvicidal properties of leaf and seed extract of plant *Agave americana*[12]; the mosquito larvicidal activity in the extract of *Tagetes minuta* flowers against *Aedes aegypti*[13]; the methanolic fraction of leaves of *Mentha piperita*, *Phyllanthus niruri*, *Leucas aspera*, and *Vitex negundo* against larvae of *Cx. quinquefasciatus*[14]; the methanolic extracts of *Solanum surattense*, *Azadirachta indica*, and *Hydrocotyle javanica* exhibited larvicidal activity against *Cx. quinquefasciatus*[15].

In the present study, *U. narum* shows the highest percentage of yield (24.75%) than other plants and found to be more active. The methanolic extracts of *U. narum* found to be more active than *Azadirachta indica* based on the higher LC_{50}[16] values. But the Petroleum Ether extracts of *Ocimum basilicum* shows higher activity than *U. narum*[17]. Chakraborty *et al*.,[18] observed cent percent mortality at 50mg/L concentration of distilled water extracts for 24 hours exposure against *Cx. quinquefasciatus*. This result supports the activity of methanolic extracts of *L. indica*. Some of the plants in the family Vitaceae show differential larvicidal activity against *Cx. quinquefasciatus* as Azokou *et al*.,[19] observed the lacked activity of *Vitex grandifolia*, but other species of the genus *Vitex* viz., *V. trifolia*, *V. peduncularis* and *V. altissima* exhibited activity on IV instar larvae of *Cx. quinquefasciatus*[20].

In the present study methanolic extracts of *M. pubescens* is found to be less active than *U. narum* and exhibits LC_{50} at 249.943 ppm. This larvicidal activity is in line with the previous observation by Bagavan *et al*.,[21] as the methanolic leaf extracts of *Morinda tinctoria* found to me effective against the early IV instar larvae of *Aedes aegypti* and *Cx. quinquefasciatus*. Govindarajan *et al*.,[22] studied the larvicidal activity of *Caesalpinia pulcherrima* against *Anopheles subpictus* and *Cx. Tritaeniorhynchus* and found that there is an increased activity of *C. pulcherrima* against *Culex* spp. which is comparatively better than the results of the present study. This may be due to interspecific difference in the response towards *C. pulcherrima* extracts.

Methanolic extracts of *Curcuma longa* (19.07 ppm)[23], *Mentha longifolia* (78.28 ppm)[24], and *Salvia pomifera* (79.46 ppm)[24] against *C. pipiens* show lesser activity than *U. narum*. In addition, the activity of *U. narum* extract is on par with the activity of methanolic extracts of *Rhinacanthus nasutus* against *Aedes aegypti*[25] and the Carbon Tetrachloride extracts of *Momordica charantia* against *Anopheles stephensi*[26].

Uvaria narum exhibited the highest repellent activity against *Cx. quinquefasciatus* as the average protection is 100% in all tested time periods. This result is in corroboration with the following studies. Amer and Mehlhorn[27] have reported that the five most effective oils were those of Litsea (*Litsea cubeba*), Cajeput (*Melaleuca leucadendron*), Niaouli (*Melaleuca quinquenervia*), Violet (*Viola odorata*), and Catnip (*Nepeta cataria*), which induced a protection time of 8 hours at the maximum and a 100% repellency against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. The essential oil of *T. minuta* providing a repellency of 90% protection for 2 h against *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti* was observed by Tyagi *et al*.,[28].

Similarly, the benzene and ethyl acetate extracts of leaves of *Ervatamia coronaria* and *Caesalpinia pulcherrima* show significant repellent activity against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* and the two plant crude extracts gave protection against mosquito bites without any allergic reaction to the test person[29].

4. Conclusion

Vector Control is facing a threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides. Botanical insecticides may serve as suitable alternative to synthetic insecticides in future as they are relatively safer to use. To find new model of action and to develop active agents based on natural plant products, efforts are being made to test selected botanicals possessing insecticidal activity. Observations of the study could encourage the search for new active natural compounds offering an alternative to synthetic repellents and insecticides even from weed plants. The findings of the present study suggest that further purification and isolation of active principle compounds could lead to the development of more potent and eco-friendly biocontrol agents against *Cx. quinquefasciatus* and other related mosquito vectors.

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