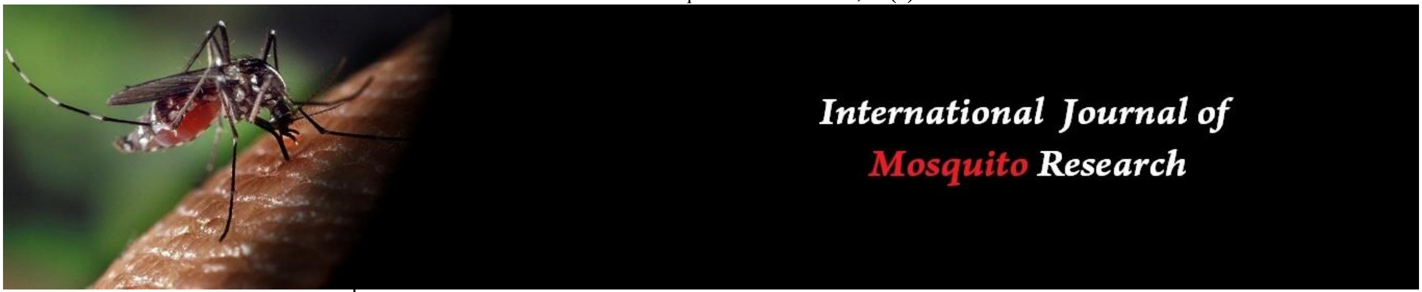

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Larvicidal and repellent efficacy evaluation of *Calotropis procera* against *Aedes aegypti* Linn and *Culex quinquefasciatus* (Diptera: Culicidae) mosquito species under laboratory condition

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Abstract
To evaluate the larvicidal efficacy of methanol extract of *Calotropis procera* the experiments were conducted against larvae of *Aedes aegypti* and *Culex quinquefasciatus* mosquito species. Third instar larvae of selected mosquitos species were exposed to various concentrations (50-200 ppm) and were evaluated in the laboratory. The morality observation of exposed larvae was recorded at 24 hrs. After treatment. LC values of the *Calotropis procera* leaf extract was determined following Probit analysis. The LC_{50} and LC_{90} values of *Calotropis procera* against *Aedes aegypti* and *Culex quinquefasciatus* larvae were recorded as 67.01, 95.92 and 138.49, 213.17 ppm, respectively. The Dhoop candle containg active ingredient *Calotropis procera* exhibited 80.00% and 67.92% repellency against *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes, respectively. From the results it can be concluded the crude extract and Dhoop candle incorporating *Calotropis procera* offers an excellent potential for controlling and as well as repelling *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes.

Keywords: Botanicals, larvicidal, repellent, *Aedes aegypti*, *Culex quinquefasciatus*

Introduction

Mosquito borne diseases are a scourge to mankind and pets. They not only spread deadly like Malaria, dengue and chikungunya which affects the productivity in terms of DALYS but also cause sleepless nights due to bites. New emerging diseases like Zika are posing additional threats to human health. For example, in 2020, there were an estimated 241 million cases of malaria worldwide. The estimated number of malaria deaths stood at 627 000**.** In case of dengue there are 390 million dengue virus infections per year worldwide. In 2022 for chikungunya there were, 229 029 cases and 41 deaths. Currently an estimated 120 million cases of lymphatic filariasis have been reported every year [14, 23].

India alone contributes 0.17 million cases of malaria which is transmitted through *Anopheles sp* with around 90 deaths.

In 2016, India recorded 101388 dengue cases and 210 deaths, including 4337 cases and six deaths in Delhi, whereas Chikungunya outbreaks were reported from several states in 2006, with 1.3 million cases [13]. Cases of filariasis in India from about 257 districts in 21 States/Union Territories. (NVBDCP) which is transmitted through *Culex* mosquito species.

Growing urbanization that create mosquito genic situations, resistance to conventional insecticides leading to lack of efficacy against vector programs are adding to the complexity of vector control program. Currently used vector control products suffer from various drawbacks in terms of poor efficacy because resistance of vectors to conventional insecticides or due to non-environmentally friendly formulations which have lower margin of safety to non-targets. Due to such negative impact of the chemical insecticides, there is a strong requirement of other alternative management strategies which will be effective, sustainable, ecofriendly and affordable. In search of new vector control strategy, science has intensified its focus on herbal extracts which bestows advantages in terms of higher margin of safety to non-targets, biodegradable with efficacy on par with conventional insecticides.

Plants are livings entities, and they work as natural laboratories where a large number of useful chemicals are biosynthesized. Plants are enriched with range of metabolites which some of them confer protective action against Insects, such as alkaloids, non-protic unusual amino acid, steroids, phenol, flavonoids etc. Phytochemicals are beneficial due to their eco-safety, target-specificity, longer efficacy, higher acceptability and suitability, delayed onset of resistance due to blend of metabolites having different mode of action and target sites in insects, easily availability, low cost, ecofriendly for rural areas and biodegradable. Due to such benefits, botanicals can be used as alternative to synthetic insecticides or with other bio-rational insecticides used under integrated vector management programs. Botanicals may be a good source of alternative for the management of mosquitoes, because they are rich in bioactive chemicals and easily biodegradable.

Presently, more than 1,005 plant species are reported to possess insecticidal properties, out of that extracts from 384 plant species contain antifeedants, 297 plant species act as repellents, and 27 plants species contain attractants and possess growth inhibitors[8]. Many studies have been conducted in the field of management of mosquitoes and related diseases in search for efficacious phytochemicals. As India is endowed with varied agroclimatic conditions large number diverse plants having the insecticidal properties are easily available like Neem (*Azadiractin indica*), Rui (*Calotropis procera*), Congress Grass (*Parthenium hysterophorus*), Dhatura (*Dhatura alba, Dhatura metal*), Karanj (*Pongamiyapinnatta*), Nilgiri (E*ucaliptus*sps.), Mint (*Mentha spicata*), Marigold (*Tagetes erecta* L.), Ginger (*Zingiber officinalis*), Garlic (*Allium sativum*) and many more[3]. They are potentially suitable for use in integrated pest management programs [2]. Many plant extracts have shown great promise as mosquito larvicides such as extract of Tagetes minute flowers against *Aedes aegypti* [6]; methanolic fraction of leaves of *Mentha piperita*, *Phyllanthus niruri*, *Leucas aspera*, and *Vitex negundo* against larvae of *Culex quinquefasciatus* [16]; methanolic extracts of *Solanum suratense, Azadirachta indi*ca, and *Hydrocotylejavanica* against *Culex quinquefasciatus* [15].

In view of the current developing interest in developing botanical insecticides as alternative to synthetic insecticides, this study was undertaken to assess the larvicidal potential of the methanolic extracts of the medicinal plant *Calotropis procera*against the medically important mosquito vectors, *Aedes aegypti* and *Culex quinquefasciatus. Calotropis procera* is a wastrel weed commonly known as milkweed. These weeds are abundant in sub-tropics and tropics but rare in temperate zones. The efficacy of this plant extract has been reported against many agricultural pests as well as stored grain pest, but their efficacy against mosquitoes was not demonstrated till now.

Materials and Methods

Plant material

Plant sampling *Calotropis procera*were carried out during the growing season (March– April) of 2022 from different places of Chikhali Village, Tah. Haveli, Dist Pune, Maharashtra, India. Bulk samples were air-dried in the shade for 12-15 days after collection. After dryingsamples were grounded to affine powder. At the time of collection, two pressed voucher herbarium specimens were prepared per species and identified with the help of plant taxonomist. Wherever possible, flowering or fruiting specimens were collected to facilitate taxonomic identification.

Extraction method

The leaves were grounded to fine powder (100 g) mechanically by using commercial electrical stainless-steel blender and extracted sequentially with methanol (500 ml, Ranchem), in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure 22–26 mmHg at 45 °C by 'Rotavapor' and the residue obtained was stored at 4 °C.

Test Organisms

All tests were carried out against laboratory reared vector mosquitoes *viz*., *Aedes aegypti* and *Culex quinquefasciatus* free of exposure to insecticides and pathogens. Cyclic generations of vector mosquitoes were maintained at 27$\pm$ 2 °C and 80-90% relative humidity in the insectariums. Larvae were fed on larval food (rabbit feed) and adult mosquito's on 10% sugar solution. Adult female mosquitoes were periodically blood-fed on live chicken for egg production.

Larvicidal activity

The larvicidal efficacy of botanical extract was assessed by using the standard method as prescribed by World Health Organization [20]. Based on the broad range and narrow range tests, botanical extract was tested at 50-200 ppm and they were tested against the late third instar larvae of selected mosquito species. The plant extract was dissolved in appropriate solvent and then diluted in 249 ml of DE chlorinated water to obtain each of the desired concentrations. Similarly, control was run using 1ml of DMSO in 249 ml of DE chlorinated water. The larvae of test mosquito species at 25 number per replication/concentration were introduced in 500-ml glass beaker containing 250 ml of aqueous medium. The larval mortality was observed and recorded after 24 h of post exposure. For each experiment, five replicates were maintained at a time. The LC_{50} and LC_{90} value was calculated by using probit analysis [4].

Repellent Efficacy

The Dhoop candle were prepared by following the method suggested by Saini, *et al*. (1986) [20] with some minor modifications by using 4 grams of *Calotropis procera* dried powdered plant parts as active ingredient and two grams of saw dust as binding material and two grams of charcoal powder as burning material. All the materials were thoroughly mixed with distilled water to form a semisolid paste and were shade dried. The Control Dhoop candle were prepared without plant parts. The repellent efficacy of *Calotropis procera* were evaluated in 30 cubic meter study chamber which is made up of glass and interconnected by passage having dimension of 2 F length X 4 F height. The chambers having port at the bottom on all 4 glass walls which was used as insect introduction port.

100 numbers 2-5 days old adult female *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes (starved for 24 hours prior to testing) were used released in chamber A. Live chicken with legs tied was kept in small iron cage and placed in center of the chamber B. Dhoop candle was ignited in the chamber B for first 15 minutes to facilitate spreading of volatilesthe chamber which was closed. After 15 minutes, the connecting passage door between the chambers was opened and

stopwatch was started. Observation on numbers of mosquitoes repelled from chamber A to chamber B through interconnected glass passage was recorded at everyone minute interval up to 60 minutes. Dhoop candle were allowed to smolder up to 60 minutes.

Statistical analysis

Larvicidal Activity

Percent mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, slope and regression values were calculated using the SPSS 25.0 version software. Results with $p \leq 0.05$ were considered to be statistically significant.

Repellent Efficacy

The Percent Repellency were calculated by using following formula

$$\text{Percent Repellency (\%)} = \frac{\text{Number of mosquitoes travelled in untreated control} - \text{Number of mosquitoes travelled in in the treated chamber}}{\text{Number of mosquitoes travelled in untreated control}} \times 100$$

Result and Discussion

Larvicidal Efficacy

Data of the larvicidal activity of the of crude methanolic leaf extract of *Calotropis procera* against selected species of mosquitoes are presented in Table 1. The LC_{50} and LC_{90} values of methanolic extract of *Calotropis procera* were recorded as 67.01 ppm and 138.49 ppm against *Aedes aegypti* and 95.92 ppm & 213.174 ppm against *Culex quinquefasciatus*. From the results it can be concluded the crude extract of has excellent potential for controlling *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes.

Larviciding as a measure of vector control is important tool in urban areas wherein surface area of water body is less than the wall area. Also, it is easy to control mosquito larvae wherein they are less mobile than flying adults and present in stagnant water collections which is easier to treat. Botanical larvicide such as methanolic extract of *Calotropis procera* could prove to be a good alternative to conventional insecticides because of obvious advantages described in this paper above.

Many researchers have conducted work on botanicals to check the efficacy against insect pests. Many of them reported efficacy against developmental stages of mosquitoes. The results of present study are compared with earlier reports, alcoholic extract of *C. procera* was reported LC_{50} as 387.93 ppm and LC_{90} as 630.66 ppm which is higher than we had recorded against *Culex quinquefasciatus* mosquito larvae [20]. Whereas, acetone extract of *Nerium indicum* and *Thuja orientelis* had LC_{50} values of 127.53 ppm and 155.97 ppm against III instar larvae of *Culex quinquefasciatus*, respectively [18]. Leaf hexane, chloroform, ethyl acetate, acetone, and methanol extracts of *Acalypha alnifolia* also tested against *Aedes aegypti* mosquito larvae and LC_{50} values was reported as 202.15, 182.58, 160.35, 146.07, and 128.55 ppm, and LC_{90} 476.57, 460.83, 440.78, 415.38, and 381.67 ppm; *Culex quinquefasciatus* the LC_{50} values was reported as 198.79, 172.48, 151.06, 140.69, and 127.98 ppm, and LC_{90} 458.73, 430.66, 418.78, 408.83, and 386.26 ppm, respectively [12]. The field examination of plant derived larvicides is conducted against *Anopheles stephensi* and *Culex quinquefasciatus* by using *Neemarin,* a plant derived larvicide which obtains from *Azadiracta indica* and LC_{50} and LC_{90} values were reported as 0.35 and 1.81 mg/l and 0.69 and 3.18 mg/l respectively [21].

Repellent Efficacy

Calotropis procera was found effective against adult *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes in 30-meter cubic interconnected Glass chamber by showing repellent activity. The 9 and 17 mean numbers of *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes travelled in to the treatment chamber when Dhoop candle evaluated with *Calotropis procera.* Whereas, the 45 and 53 mean numbers of *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes travelled in to the treatment chamber when Dhoop candle evaluated without *Calotropis procera* active. The percent repellency of Dhoop candle (Active- *Calotropis procera*) was exhibited as 80.00% against *Aedes aegypti* and 67.92% against *Culex quin quinquefasciatus* mosquitoes.

Today, the environmental safety of an insecticide is considered to be of supreme importance. Aninsecticide must not cause any mortality in non-target organisms in order to be acceptable [9]. Botanical derivatives may be the future of personal protection measures that would complement public health programs. Objective of household insecticides is to avoid man-mosquito contact thereby protecting individuals from mosquito borne diseases. The Dhoop candle with herbal active ingredient will serve as good alternative mosquito repellent to conventional household repellents containing synthetic insecticides like coils, Liquid vaporizer and aerosols.

Table 1: Larvicidal efficacy of methanol extracts of *Calotropis procera* against mosquito larvae

Mosquito Species	Concentration (PPM)	Mortality %	LC_{50}	95% Fiducial Limit		LC_{90}	95% Fiducial Limit		Regression Equitation (Y)
				LCL	UCL		LCL	UCL	
Aedes aegypti	50	42	67.01	55.218	76.467	138.49	126.981	154.319	Y= -1.201 + 0.018 x
	100	66							
	150	94							
	200	100							
Culex quinquefasciatus	50	32	95.92	81.161	108.276	213.174	192.159	244.819	Y=-1.048 + 0.0110 x
	100	49							
	150	74							
	200	87							

LC_{50}= Lethal Concentration required to kill 50% mortality and LC_{90}= Lethal Concentration required to kill 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit

Table 2: Repellent efficacy of methanol *Calotropis procera* Dhoop against *Aedes aegypti* and *Culex quinquefasciatus* mosquito

Sr. No.	Mosquito Species	Details of the active in Dhoop	Mean Numbers of mosquitoes in treated chamber	Mean Percent Repellency (%)
1	*Aedes aegypti*	Dhoop candle with 4 gm of powder of *Calotropis procera*	9	80.00
	Culex quinquefasciatus		17	67.92
2	*Aedes aegypti*	Without active Dhoop Candle	45	-
	Culex quinquefasciatus		53	-

*Mean of three replicates

Conclusions

The present finding based on experimental results demonstrates that methanolic extract *Calotropis procera* can be used as mosquito larvicide in vector control programs and powdered parts of the plant can be used as mosquito repellent as an household insecticide. Further work would involve identification of metabolites of *Calotropis procera* extract which are responsible for giving specific efficacy against mosquitoes and purification of same.

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