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Larvicidal effects of *Calotropis procera* leaf extracts against *Aedes aegypti* (L), vector of dengue fever

Shweta Kaushik[1], Neeta Raj Sharma[2*], Shashank Garg[3], Anu Bansal[4] and T.G. Thomas[5]

[1,5]National Centre for Disease Control, 22 Sham Nath Marg, Delhi 110 054, India.
[2,3,4]School of Bioengineering and Biosciences, Lovely Professional University, Phagwara 144411, Punjab, India.
Email: shweta.lpu111@gmail.com; neeta.raj@ipu.co.in

ABSTRACT: Leaf extracts of *Calotropis procera* were tested against late third instar larvae of *Aedes aegypti* mosquito. Soxhlet extraction of the dried leaves powder with polar and non polar solvents (water, ethanol, hexane and acetone) was carried out. Larvicidal effects of plant extracts were observed after 24h of exposure. The control group showed no mortality. Ethanolic extract was found more toxic with LC_{50} 1.923 ppm and LC_{90} 8.83 ppm followed by aqueous extract (LC_{50} 2.607 ppm and LC_{90} 11.903 ppm), acetone extract (LC_{50} 4.1 ppm and LC_{90} 16.471 ppm) and hexane extract (LC_{50} 5.364 ppm and LC_{90} 31.759 ppm). As the ethanolic extract of *C. procera* leaves showed significant larvicidal properties, it can be used as an eco-friendly alternative for the control of *Ae. aegypti* vector. © 2022 Association for Advancement of Entomology

KEY WORDS: Ethanolic extract, probit analysis, toxicity, biopesticide

Mosquitoes transmit a myriad of harmful diseases like dengue, malaria, chikungunya, lymphatic filariasis and Japanese encephalitis. Approximately 700 million people suffer from such mosquito borne diseases each year that gradually results in about 1 million deaths annually (Taubes, 1997). The distribution of vector borne diseases is determined by complex demographic factors including environmental and social factors as well. Annual dengue incidences are estimated to be in the order of 100 million symptomatic and 300 million asymptomatic. The greatest burden is seen in Asia (75%) followed by Latin America (14%) and Africa. India suffers from three vector-borne diseases, malaria, lymphatic filariasis and visceral leishmaniasis (WHO, 2017). *Aedes aegypti* (Diptera, Culicidae) is the main vector of dengue and chikungunya (WHO, 2022). To control the proliferation of vector species of mosquitoes so many synthetic insecticides have been used worldwide. However, none of the formulations are promising due to its high cost, less environmental friendly, harmful effect on public health and increasing incidence of insecticide resistance. Because of these harmful effects on the public health and environment, herbal eco friendly formulations are in demand (Nerio *et al.,* 2010; Sritabutra *et al.,* 2011 and Reegan *et al.,* 2013). Further, as an alternative, the chemicals derived from the different parts of the plants can be used as a repellent, larvicide, ovipositional attractant and insect growth regulator (Babu and Murugan, 1998; Demirak and Canpolat, 2022).

Calotropis procera (Aiton) Dryand belongs to the family Asclepiadaceae and is mostly found in

* Author for correspondence

Bangladesh, India, Burma, Pakistan and the Sub-Himalayan tract. Indian traditional system of medicine, various parts of the plant are used for the treatment of various diseases like tumors, liver and abdomen diseases, piles, leprosy (CSIR, 1992; Kritikar and Basu, 1999). Moursy (1997) indicated its insecticidal and Markouk *et al.* (2000) larvicidal properties with their various solvents. Considering, the existing preliminary research (Sivagnaname and Kalyanasundaram, 2004; Thomas *et al.,* 2004; Cetin *et al.,* 2004; Ahmed and Hamshary, 2005; Shaleen *et al.,* 2005; Sharma *et al.,* 2006), the present study was focused on the potential of various solvent extracts of *C. procera* leaves against *Ae. aegypti* larvae.

Fresh leaves of *C. procera* were collected and washed with tap water and shaded dried at room temperature at 27±2°C for 15 days. Dried leaves were powdered with the help of an electrical grinder and then 30 g of the powder was extracted with 250 ml of polar and non polar solvents (water, ethanol, hexane and acetone) for 8 h using Soxhlet apparatus with boiling point ranging from 60–80°C followed by filtration through a Buchner funnel with Whatman number 1 filter paper (Vogel, 1978). The crude leaf materials were evaporated in a rotary vacuum evaporator. For the preparation of one per cent stock solution, one gram residue was taken and dissolved in 100 ml of solvent (same solvent that was used in the extraction process). Finally, concentrations ranging from 0.25 ppm to 20 ppm were used to carry out the experiments.

The larvae of *Ae. aegypti* were reared and colonized continuously in the National Centre for Disease Control laboratory. The temperature was kept 27 ± 2°C and maintained the humidity at 45 ±10 per cent and photoperiod 12:12 (light: dark). Larvae were kept in a water tray and the water was cleaned or changed every day to avoid toxic scum formation. Larvae were fed on yeast tablets. Late 3rd instar female larvae were kept in cages (30×30×30 cm) till the pupae were converted into adult mosquitoes. The adult mosquitoes were fed by rabbit blood meal and male mosquito was fed with 2 per cent glucose solution.

WHO (2005) guidelines were used to evaluate the larvicidal activity of extract of *C. procera*. Twenty-five late third instar larvae of *Ae. aegypti* were collected from the larval rearing bowl and moved in a 500 ml glass beaker (having 249 ml dechlorinated water and one ml of desired concentrations). Five replicates of each concentration and two replicates of controls were tested for each dilution under the laboratory conditions (ambient temperature 27 ±1°C and RH 75 – 80%). The control was prepared with 249 ml dechlorinated water and one ml of individual solvent. Larvae were exposed in dechlorinated water only (without solvent) prepared as a control. The larval percentage mortality was recorded for each test and controls after 24 h. LC_{50}, LC_{90} and other statistics like limits of upper and lower confidence limit (UCL and LCL) at 95 per cent confidence and chi-square values were calculated by probit analysis (Finney, 1971) and SPSS 16.0 version was used to find out the regression analysis.

In the larvicidal toxicity effects of *C. procera* leaves at various concentrations in different solvents against the dengue vector, *Ae. aegypti*, ethanol extracts showed the highest mortality rate with LC_{50} and LC_{90} values corresponding to 1.923 and 8.83 ppm respectively, followed by aqueous (LC_{50} and LC_{90} values 2.607 and 11.903 ppm respectively), acetone (LC_{50} and LC_{90} values 4.1 and 16.471ppm respectively), hexane (LC_{50} and LC_{90} values 5.364 and 31.759 ppm) respectively (Table 1). The larval mortality rate of *Ae. aegypti* increased with the increase in concentration of extracts. Ethanol extract of leaves of *C. procera* was found to be the most effective as compared to the other solvent extracts (Figs. 1, 2, 3 and 4).

The study established the usefulness of ethanolic leaf extract of *C. procera* plant against the late third or early forth instar larvae of *Ae. aegypti,* with LC_{50} and LC_{90} values at 1.923 and 8.83 ppm respectively, which shows relevance with the study conducted by Ramos *et al*. (2006) and Jazem *et al*. (2014) indicated medicinal properties of *C. procera* (leaves, roots and bark) against *Ae. aegypti*. Singh *et al*. (2005) showed the moderate larvicidal activity of the latex of *C. procera* against *Ae. aegypti, Anopheles stephensi*

Table 1. Larval toxicity of different solvents of *Calotropis procera* leaves against *Aedes aegypti*

Solvents	LC_{50} (ppm)	LC_{90} (ppm)	Regression equation	95% confidence limit LCL LC50 (LC90) UCL LC50 (LC90)		χ^2
Water	2.607	11.903	Y=1.943X-0.809	2.15(8.83)	3.14(18.17)	10.20*
Ethanol	1.923	8.83	Y=1.936X-0.549	1.56(6.58)	2.33(13.39)	8.49*
Acetone	4.1	16.471	Y=2.122X-1.3	3.49(13.27)	4.74(21.87)	8.19*
Hexane	5.364	31.759	Y=1.659X-1.21	4.52(24.35)	6.27(45.30)	21.92*

*Control – nil mortality; within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT; LCL - lower confidence limit, UCL - upper confidence limit, *P<0.05 level*

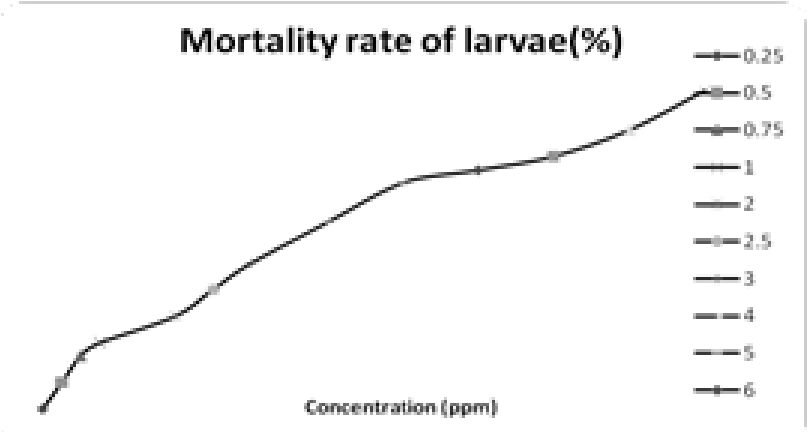

Fig. 1 Toxicity of aqueous extract of *Calotropis procera* against *Ae. aegypti*

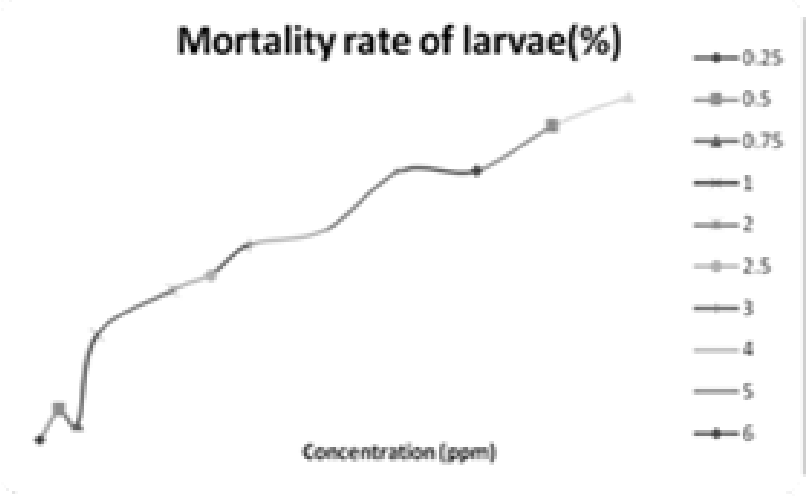

Fig. 2 Larval toxicity of ethanol extract of *Calotropis procera* against *Ae. aegypti*

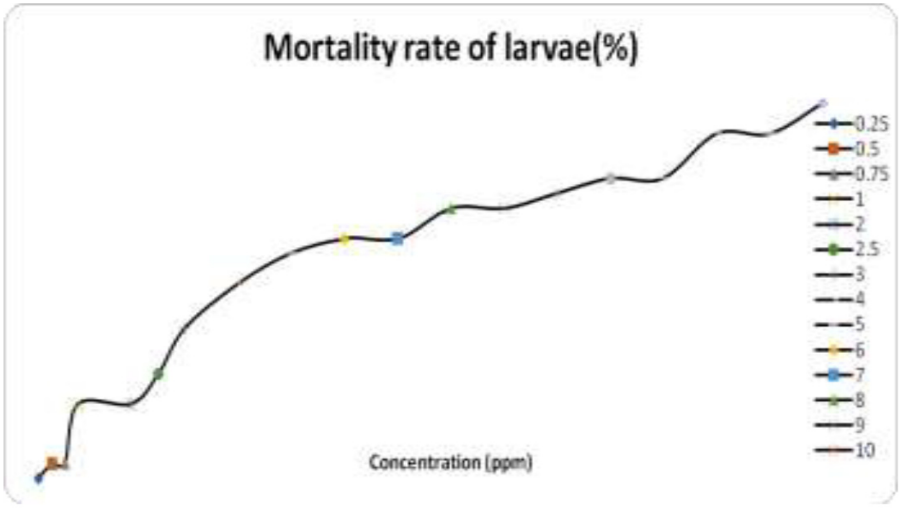

Fig. 3 Larval toxicity of acetone extract of *Calotropis procera* against *Ae. aegypti*

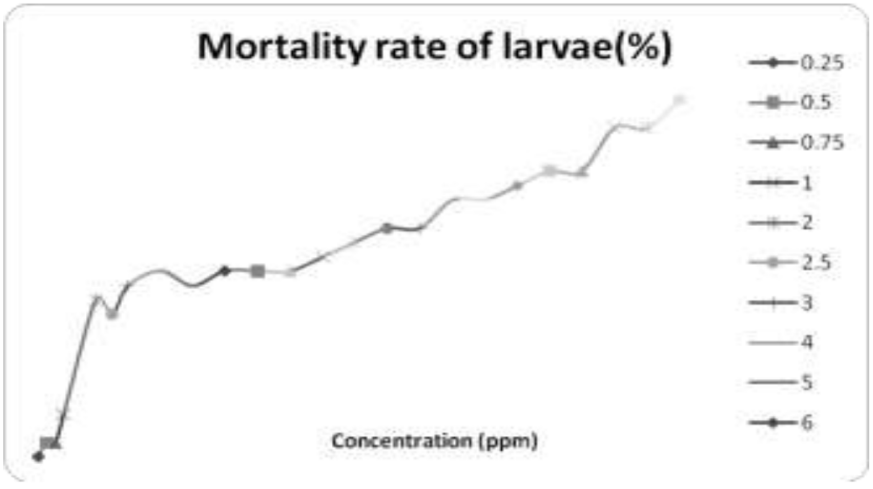

Fig. 4 Larval toxicity of hexane extract of *Calotropis procera* against *Ae. aegypti*

and *Culex quinquefasciatus*. Shreya *et al.* (2012) concluded the LD_{50} value of the ethanolic leaves extract of *Calotropis* spp. against *Ae. aegypti* as 351.43 (95% CI: 345.64-345.51) which shows the resemblance with the present study. The toxicity of different parts of the *C. procera* plant has also been reported earlier against mosquitoes by Staples and Herbst in 2005. *Calotropis* plant has been in use for the prevention of so many diseases for a long time due to its medicinal properties (Dewan, 2000; Van *et al.,* 2005; Chitme *et al*., 2005; Argal and Pathak, 2006). Application of 3 ml *C. procera* leaves extract per 100 ml solvent recorded 100 percent mortality against *Ae. aegypti* (Singh *et al.*, 2005).

Yakubu *et al.* (2021) reported LC_{50} of *C. procera* leaves extract against *Ae. aegypti* and *Cx. quinquefasciatus* at 0.116mg/ml and 0.249mg/ml respectively. The present study indicates that the leaves of *C. procera* have larvicidal properties against dengue vector *Ae. Aegypti.* As *C. procera* is an easily available medicinal plant, its phytochemicals may be less expensive and relatively safe for environment. Hence the ethanolic extract of *C. procera* leaves could be an effective

alternative to synthetic insecticides for the control of *Ae. Aegypti.*

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