Calotropis procera : Larvicidal Effect against *Culex quinquefasciatus*

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ABSTRACT
In present studty larvicidal activities of leaf extract of *Calotropis procera* against *Culex quinquefasciatus* investigated.This was monitored against 2nd ,3rd and 4th instar larvae of the mosquitoes species 24h post- treatment.the results shows that the leaf extract of *C.propera* possess remarkable larvicidal effect against *Cx. Quinquefasciatus* and might be used as natural biocides for mosquito control.

INTRODUCTION

The transmission of most important vector-borne diseases like malaria, lymphatic filariasis, Japanese encephalitis, and dengue as well as yellow fever and other forms of encephalitis is done by mosquitoes (WHO, 2006a). Malaria and filariasis rank amongst the world most prevalent tropical infectious diseases. According to the latest available data, about 3 .2 billion people were at risk of the disease in 97 countries, territories and areas in 2013, and an estimated 198 million cases occurred (range: 124 million–283 million) . In the same year, the disease killed about 584 000 people (range: 367 000–755 000), mostly children aged under 5 years in sub-Saharan Africa(WHO 2015) Lymphatic filariasis impairs the lymphatic system and can lead to the abnormal enlargement of body parts, causing pain, severe disability and social stigma. 893 million people in 49 countries worldwide remain threatened by lymphatic filariasis and require preventive chemotherapy to stop the spread of this parasitic infection. In 2010 over 500 million people were infected, with about 60 million disfigured and incapacitated by the disease. Lymphatic filariasis is a significant public health and economic problem in many tropical and subtropical regions of the world, including Sudan, (El setouhy and Ramzy, 2003) and (Aiah et al., 2005). One of the effective methods to control these diseases is to target the vectors for the interrupting disease transmission. The control effort can target all stages of the mosquito life cycle, but has focused almost on adult stage by using conventional insecticides based on indoor residual house spraying (Manzava et al., 1993) and (Curtis, 1994) or more recently, the use of insecticide treated bed nets or curtains. The control of mosquito at the larval stage is necessary and efficient in integrated mosquitos' management. During the immature stage, mosquitoes are relatively immobile; remaining more concentrated than they are in the adult stage (Rutledge et al., 2003). Larval control strategies against malaria vectors in sub-Saharan Africa could be highly effective, complementary to adult control interventions, and should be prioritized for further development, evaluation and implementation as an integral part of rolling back malaria (Killeen et al., 2002). Since the discovery of DDT, mosquito control approach has been almost completely based on synthetic organic insecticides, but the extensive use of synthetic organic insecticides resulted in environmental pollution and also in the development of physiological resistance in major vector species in addition to the increased costs of insecticides. This has necessitated the need for search and development of environmentally safer, low cost, indigenous methods for vector control. During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides (Mittal and Subbarao, 2003), (Rajkumar and Jebanesan, 2005a), (Rajkumar and Jebanesan, 2005b) and (Promsiri et al., 2006). In addition to application as general toxicant against mosquito larvae, botanical insecticides also have potential uses as growth and reproduction inhibitors, repellents, ovicidal and oviposition deterrents (Prajapati et al., 2005), (Rajkumar and Jebanesan, 2005a), (Rajkumar and and Jebanesan, 2005b) and (Pushpanathan et al., 2006).

Calotropis procera R. Br. (Asclepiadaceae) is a plant widely distributed in tropical and subtropical regions of Africa and Asia with a long history of use in traditional medicine. A wide range of chemical compounds including cardiac glycosides, flavonoids, phenolic compounds, terpenoides have been isolated from this species (Mueen Ahmed et al., 2005). The bioactive constituents of these plants could be either a single substance or a mixture of substances. The separation of the mixture is neither practical nor advantageous in the insect economic control strategies. The aim of the current study is to investigate the activity of aqueous leaves extract of *C. procera* against the larval stages of *Culex quinquefasciatus*, the vector of filariasis (WHO, 2006a).

The larvicidal activity was monitored against 2nd, 3rd and 4th instar larvae 24h post-treatment.

It was found that, LC50-LC90 values calculated were 273.53 783.43, 366.44-1018.59 and 454.99–1224.62 ppm for 2nd, 3rd and 4th larval instars, respectively.. These results suggest that the Leave extract of *C. procera* possess remarkable larvicidal effect against *Cx. quinquefasciatus,* and might be used as natural biocides for mosquito control.

MATERIAL AND METHODS

Area of study, Pali is situated on the bank of river Bandi and is around 70 km south east of jodhpur. The city lies between 25° 77′, N. latitude and 73°33′, E. longitude. Pali is the industrial dyeing and printing hub of Rajasthan state.

COLLECTION AND REARING OF MOSQUITOES

Larvae of the mosquito were collected from breeding sites within the study area, and reared under laboratory condition at 25-28 °C. The larvae were fed by adding finely ground powdered yeast on the surface of the water. Water was changed every day to avoid scum formation; which might create toxicity.

EXTRACTION OF PHYTOCHEMICALS USING DIFFERENT SOLVENTS

Leaves of the plant C. procera were collected, washed thoroughly in water, air dried in shade and powdered using a pulverizer and stored in plastic containers. The powdered material was weighed and extracted in crude methanol (40-60 %) as solvent in the ratio of 1:10 w/v using Soxhlet apparatus at 55°C. The crude methanol extract was filtered through a funnel using glass filter and evaporated using a rotary evaporator. The residue was re-dissolved in methanol and defatted in equal volume of petroleum ether in a separating funnel. The fractions were separated, dried in a rotary evaporator. The methanol fraction was further dissolved in ethyl acetate and insoluble derbies were removed by filtration. Water soluble materials from the ethyl acetate fraction were removed in a separating funnel using double distilled water. The fractions were collected separately and dried. Yields in relation to the initial weight of the powder of the different fractions were determined. One percent stock solutions of all the fractions in methanol were prepared from the residues obtained at each stage of the purification process and the fractions were tested at different concentrations.

PREPARATION OF EXTRACT

Leaves of the plant C. procera , (Family: Ascelpiadaceae), were collected from plants within the study area, during the flowering season, dried under shade and finely ground to powder. Five grams from leaves powder was soaked in separate bottle (500 ml) containing 250 ml distilled water. The solution was allowed to stand for 24 h with vigorous occasional shaking, the suspension was filtered with filter paper. The marc was washed several times with distilled water and filtered. The final volume was adjusted to 500 ml by adding distilled water to prepare stock solution of 1%. The stock solution was then serially diluted by add water to prepare the test concentrations required.

LARVICIDAL ACTIVITIES

Larvicidal activities of the extract were determined by following the WHO standard procedure (WHO, 2005b). Initially, mosquito larvae were exposed to a wide range of test concentrations and a control to find out the activity range of the aqueous extract of plant under test. After determining the mortality of larvae in this wide range of concentrations, a narrower range of 5-6 concentrations was used, to determine the lethal concentration of 50% (LC50) and the lethal concentration of 90% (LC90) values. Twenty-five laboratory reared 2nd, 3rd and 4th instars larvae of each mosquito species were transferred by means of dropper to the small test cups (250 ml), each containing 100 ml of de-chlorinated tap Water to which the required concentration were added. Four replicates were setup for each test concentration. In each replicate 25 larvae larvae were used, with four replicate of control. The experiment was performed under laboratory conditions at 25-28 °C.

Mortality in larvae was recorded 24 h post Treatment. If more than 10% of the control larvae pupate in the course of the experiment, the test is discarded and repeated. If the control mortality is between 5% and 20%, the mortalities of treated groups should be corrected according to Abbott (1925) formula.

RESULTS

The aqueous leaf extract of *C. procera* showed high level of toxicity against the larvae of mosquitoes *Cx. quinquefasciatus.* The results are presented in Table 1 . The 50% mortality (LC50 values) was shown at 187.93, 218.27 and 264.85 ppm for 2nd, 3rd and 4th instar larvae of *Cx. quinquefasciatus.*

Table

Larvicidal activity of leaves extract of C.*procera* against
2nd ,3rd and 4th instar larvae of *Cx. quinquefasciatus*

Mosquito Species	Larval instar	LC50 (ppm)	LC90 (ppm)	Regression equation	FL with 95%CL	r^2
Cx. quinquefasciatus	2nd	187.93	433.51	Y = 3.528X — 3.24	+2.372	O.973
	3rd	218.27	538.27	Y = 3.261X — 2.626	+2.675	O.984
	4th	264.85	769.13	Y = 2.77X — 1.713	+2.675	0.996

The LC90 values (90% mortality) were shown at 433.51, 538.27 and 769.13 ppm for 2nd, 3rd and 4th instar larvae, respectively of *Cx. quinquefasciatus.* From LC90 values it was evident that 2nd instars were more susceptible than 3rd instar and the later was more susceptible than 4th instar. Also the two species of selected mosquito larvae showed different susceptibility to the leaf extract of *C. procera. Cx. quinquefasciatus* was found more susceptible.

DISCUSSION

In this study it was observed that leave extract of *C. procera* has showed larvicidal activity against the mosquitoes *Cx. quinquefasciatus* The biological activity of this plant extract may be due to various compounds, including phenolics, terpenoides, flavonoids and alkaloids existing in plant, these compounds may jointly or independently contribute to produce larvicidal activity against mosquitoes. The obtained results agree with some previous studies. One plant species may possess substances with a wide range of activities, e.g. Neem (*Azadirachta indica*) products showed antifeedant, oviposition deterrence, repellency, growth disruption, sterility and larviedal action against insects ((Schmutterer, 1990) and Mulla and Su, 1999). The leaf extract of five species of Cucurbitaceous plants, *Momordica charnia, Trichosanthes anguina, Luffa acutangula, Benincasa cerifera and Crithis vulgaris* showed larvicidal activity " LC50 of 465.85, 567.81, 839.8I, 1189 30 and 1636.04 ppm, respectively after 24 h treatment) against the 3rd instar larvae of *Cx. quinquefasciatus* (Prabakar and Jebanesan, 2004). The leaf extract of *Pavonia zeylanica* and *Acacia ferruginea* showed larval mortality at LC50 of 2214.7 and 5362.6 ppm, respectively against the third larval instar of *Cx. quinquefasciatus* after 24 h treatment (Vahitha et al. 2002), also the results agree with the finding of Pushpanathan et al. (2006) who had reported that 2nd instar larvae of *Cx. Quinquefasciatus* was more susceptible than 3rd instar and the later more susceptible than 4th instar larvae to the essential oils extracted from *Cymbopogan citratus* plant, with 1090 1890 of 54-284.27 ppm, 165.70-31848 ppm and 184.18-35901 ppm for 2nd, 3rd and 4th larval instar, respectively. Also it was fond that *Cr. quinquefasciatus* was more susceptible to the Stem extract of *C. procera.* The varying susceptibility of the species of mosquitoes is probably due to differences in the physiological characteristics of mosquito. This agree with (Thekkevilayil et al., 2004) who had reported that the four mosquitoes *Cx tritaeniorhynchus*, *Aedes aegypti* and *C. quinquefasciatus* larvae showed different susceptibility to the oils extract of Ipomoea cairica Linn., higher concentration was required for *Cx. quinquefasciatus* followed by *Aedes. aegypti*, *Anopheles stphensi* and lower concentration for *Culex tritaeniorhynchus*, with the LC50-LC90 of 58.9-1616 ppm for *Cx. quinquefasciatus*, 22.3-92.7 ppm for *Ae. aegypti*, 14.9-109.9 ppm for *Anopheles stphensi*, and 14.8-78.3 ppm for *Culex tritaeniorhynchus*. The leaf extract of *C. procera* did not show any pupal mortality till higher concentration of (5000 ppm) against the two species of mosquitoes, suggesting that the effects of the extract on the pupal Stage appear after more than 24h exposure.

The whole latex of *C. procera* was shown to cause 100% mortality of 3rd instar larvae of *Ae, aegypti* within five minutes, and most of individual growing under experimental conditions died before reaching 2nd instars or stayed in 1st instars (Marcio et al., 2006). The effect of alkaloid extracts of *C. procera* leaves at the vegetative stageon the survival of 5th instar larvae and on ovarian growth of
Shistocerca gregaria have revealed that a mortality rate of 100% was reached in the hoppers on the 15th day after the beginning of the treatment. In the adult the arrest of ovarian growth in females and the absence of sexual maturity in males have been observed (Abbassi et al., 2004). In laboratory the leaf extract of *Solanum trilobatum* greatly reduced the number of eggs laid by gravid *Anopheles stephensi* at several concentrations. At the highest concentrations (1-

0,075%) the extract reduced eggs laying by 90 99%. Lower concentrations (0.01%) also had deterrent activity of 18.4% (Rajkumar and Jebanesan, 2005b). These findings prove that, mosquitoes are known to perceive visual, thermal and olfactory stimuli which enable them to detect light source, odour and several other volatile chemicals emanating from the skin, breath and waste products of their hosts (Takken, 1991) and (Davis and Bowen, 1994).

In conclusion, leave extract of *C. procera* can be suggested as a natural larvicidal for controlling mosquitoes in Rajasthan, Since it is considered environmentally safe, less expensive and economical, a well as practical in application with minimum care by individual and communities.

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