

LARVICIDE AND OVIPOSITION DETERRENT ACTIVITY OF PLANT EXTRACTS AGAINST *AEDES AEGYPTI*

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Article Received on
24 October 2017,
Revised on 14 Nov. 2017,
Accepted on 04 Dec. 2017

DOI: 10.20959/wjpr201716-10346

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ABSTRACT

Biological control of mosquitoes is an attractive alternative to chemical pesticides, particularly in view of strict environmental legislation and increased resistance to synthetic insecticides there has been a major shift towards use of natural product from plant source. Therefore, continuous efforts are being made to look for safer alternative methods to keep mosquitoes at check. In the present study methanolic leaf extract of two plants *Calotropis gigantea* (family: Asclepiadaceae) and *Datura innoxia* (family: Solanaceae) were evaluated for larvicidal and oviposition deterrent activity against *Aedes aegypti* (Diptera: Culicidae) mosquito. The best way to keep the mosquitoes at check is larviciding. Laboratory reared early fourth instars larvae were screened

using WHO standard protocol. The oviposition deterrent test was performed using gravid female *Aedes aegypti*. *Calotropis gigantea* and *D. innoxia* demonstrated larvicidal activity with LC₅₀ value 579.25 ppm and 375.74 ppm respectively. The oviposition deterrent tests of *D. innoxia* and *C. gigantea* leaves reduced egg laying by 100% and 82.28% at 800 ppm against *Aedes aegypti* respectively. The results thus indicate that both larvicidal and Oviposition deterrent potential of leaves extract could be used as promising mosquito control strategy particularly in view of continuing problem with resistance to synthetic insecticides and environmental pollution.

KEYWORDS: *Aedes aegypti*, Dengue, mosquito larvae, oviposition deterrent.

INTRODUCTION

Mosquito ranks number one among insects' responsible for human health and well-being throughout the world. The problems of mosquito transmitted diseases are quite severe

resulted in high morbidity and mortality.^[1] Mosquitoes are the major vector for the transmission of malaria, filariasis, numerous arboviruses, Japanese encephalitis, dengue, chikungunya and yellow fever.^[2] Control of Mosquitoes in the larval stage are attractive targets for pesticides because they breed in water and, thus, are easy to deal with them in this habitat.^[3] Several plants have demonstrated toxic effects on mosquito larvae.^[4,5,6] Plant extract act as general toxicants like larvicides, oviposition attractants /deterrent, growth regulators, repellents and adulticides.^[7] In recent years due to strict restrictions on pesticide use, habitat management and continuing problems with chemical resistance, there has been a major shift towards biological control of mosquitoes.^[8] Eco-friendly nature and environmental safety are paramount importance for any insecticide or larvicide to be acceptable in nature. Using locally available insecticidal plants will reduce dependence on expensive synthetic compounds.^[9] A variety of synthetic insecticides are used for chemical control of mosquito larvae to prevent proliferation of mosquito borne diseases. Although these insecticides are effective they pose serious public health and environmental hazards.^[10] In most African countries resistance to one or more of the insecticide classes used in vector control, facing a major problem in malaria vector control programs.^[11] Synthetic insecticides has also been restricted because of non-biodegradable nature, biological magnification through ecosystem and adverse effect on human health and non-target species.^[12] Several groups of photochemical such as Chrysanthemum, pyrethrum, Nicotine, Azadirachtin, camphor and many others acts as general toxicants like larvicides, adulticide, repellents and insect growth regulators.^[13,14] Disruption of oviposition activity also play a major role in reducing mosquito population.^[15]

Datura innoxia Mill. (Solanaceae) is the wide spread species of the genus *Datura* and is well known for its use in traditional Indian medicine for centuries.^[16] There are many different species in the *Datura* genus and is commonly known as thorn apple belonging to the family Solanaceae. Its stems and leaves are covered with short and soft greyish hairs, giving the whole plant a greyish appearance. The thorn apple is a bitter narcotic plant that relieves pain and encourages healing. It has a long history of use as herbal medicine. Reports are there in which oviposition deterrent activity was reported against *Aedes aegypti* and *Culex quinquefasciatus* from leaf extract of *Datura stramonium*.^[17]

Calotropis gigantea (Asclepiadaceae) is a plant widely distributed in tropical and subtropical regions of Africa and Asia with a long history of use in traditional medicine.^[18] The plant

from this species are commonly known as milkweeds because of the latex they produce. *Calotropis gigantea* is reported to exhibit mosquito controlling properties against *Culex gelidus* and *Culex tritaeniorhynchus* mosquitoes which serve as vectors for Japanese encephalitis.^[19]

MATERIALS AND METHODS

Aedes mosquitoes

Aedes larvae used in this assay were maintained in the insectary of Haffkine Institute. Cyclic generation of adult mosquitoes were kept in small cages at room temperature 27-30°C and 75-80 R.H., fed with 10% glucose solution and animal blood meal given to the female mosquito for egg laying. Wet filter papers were provided as substrate for egg deposition. The eggs were transferred in enamel trays half-filled with water. The hatching larvae were fed with pedigree dog biscuits and yeast at 3:1 ratio.

Collection and Identification of plants

Plant *Calotropis gigantia* was collected from district Rajsamand located between latitudes 24°46' to 26°01' N and Longitudes 73°28' to 74°18' N of the state Rajasthan, India. *Datura innoxia* was collected from Mumbai (18° 55' N, 72° 54' E) India.

Identification of plants

The plants were identified by Dr. U. C. Bapat, Head, Department of Botany, Director, Blatter Herbarium, St. Xavier's College Mumbai, India. Voucher specimen of both the plants *Calotropis gigantia* (Accession No. NI 1718) and *Datura innoxia* (Accession no. U.P.760) had been deposited for future reference.

Extraction of plant material

The leaves were shade dried and powdered. For each plant 50 g powder of the plant leaves were extracted with methanol in Soxhlet extractor for 72 hours. The quantity of solvent used for each extraction was at least 10 times the quantity of plant material. Solvents were removed under reduced pressure by using rotary vacuum evaporator. The percentage yield for each sample was determined and the crude extracts were stored at 4°C until their use for Larvicidal assay.

Larvicidal activity

Larvicidal activity was determined by following the WHO standard procedure.^[20] 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 a narrower range of 5–6 concentrations were used, to determine the lethal concentration of 50% (LC₅₀).

Larvicidal bioassay

Laboratory reared *Aedes aegypti* were used for larvicidal bioassay under laboratory conditions at 25–30°C and 75–80% relative humidity. Different concentrations were prepared in 500 mL glass beakers containing 250 mL de-chlorinated tap water. Twenty-five early fourth instar larvae (laboratory reared) were released in each beaker for 24 hrs. Doses range was fixed by conducting a non-replicated pilot study. Larvicidal assay was carried out for each extract separately. Three replicates were kept for each concentration including untreated control. No food was added in the beakers as per WHO norms. Mortality was recorded after 24 hrs. of treatment by counting dead and moribund larvae. Moribund larvae were those incapable of rising to the surface or not showing the characteristic diving reaction when water is disturbed. Pupated larvae were discarded. Corrected mortality was calculated by Abbot's formula.^[21]

Oviposition deterrent bioassay

The oviposition deterrent activity was assessed using methods^[22] with slight modifications. Ten blood-fed females of *Aedes aegypti* (2 days after blood feeding) were transferred to separate cages (30 cm × 30 cm × 30 cm) made of mosquito net with a muslin sleeve on the front side for access. In each cage, four plastic bowls holding 200 mL of tap water were placed in opposite corners of each cage with a piece of filter paper with one corner inside the water and other outside; one bowl was treated with the test material (extract), two bowls were used for positive control (temephos and azadirachtin) and the other one served as negative control. The concentrations used were 50 ppm, 100 ppm, 200 ppm, 400 ppm and 800 ppm. Each concentration was replicated four times. Sucrose solution (10%) was provided to the adult as feed throughout the study period. Experiments were carried out at room temperature (28 ± 2°C; RH:75-80%) for a period of 48 hours. After 48 hours, the number of eggs laid on the moistened filter paper in each bowl was counted and recorded. The per cent effective repellency (ER) for each concentration was calculated using the following formula:

$$ER = \frac{NC - NT}{NC} \times 100$$

Where ER is the percent effective repellency; NC is the number of eggs in control cups; and NT is the number of eggs laid in treated cups within 48 h.

RESULTS

Larvicidal activity

The results of larvicidal activity are presented in table-1. Both *calotropis gigantea* and *Datura innoxia* demonstrated promising larvicidal activity with LC₅₀ value 579.25 ppm and 375.74 ppm respectively against *Aedes aegypti* larvae. No mortality was observed in control. All the data showed that the mortality progressively increased with increasing extract concentrations.

Table 1: Larvicidal activity of leaf extracts of two plants against *Aedes aegypti* larvae.

Concentration In ppm	% mortality after 24 hours*	
	<i>Calotropis gignatea</i>	<i>Datura innoxia</i>
50	10.15±1.05	19.20±1.13
100	24.25± 2.41	31.26±1.34
200	43.28± 0.95	49.16±1.08
400	54.98± 1.89	63.25±2.16
800	67.29±1.21	80.44±3.19
1600	83.98±2.01	100
LC50	579.25	375.74
Water control	0.05±0.01	0.00
Abate 0.5ppm	100	100

LC50: lethal concentration required to kill 50% of larvae exposed to test concentration

Values are mean ± SD of three replicates of two separate experiments.

Oviposition deterrent activity

Results of Oviposition deterrence observed against *Aedes aegypti* presented in table 1. Gravid female mosquito preferred to lay eggs in the control cups than in the cups treated with leaves extract of *Calotropis gigantea*, and *Datura innoxia* extracts. Significant reduction in the number of eggs laid was observed in all the test substances. The effective repellency ranged between 100 and 82.28% at highest concentration used for the assay.

Table 2: Per cent oviposition deterrent activity of leaves extract of *Calotropis gigantea* and *Datura innoxia* against gravid female *Aedes aegypti*.

Name of the plant	*Percent Effective Repellency (ER%)				
	% concentration in ppm				
	50	100	200	400	800
<i>Calotropis gigantea</i>	26.57±2.05	63.56±1.98	77.23±3.12	94.55±2.09	100
<i>Datura innoxia</i>	14.81±1.78	43.91±1.23	56.45±2.80	75.13±1.90	82.28±2.02
Azadirachtin (10 ppm)	72.81±0.86				

*Values are mean ±SD of three replicates.

DISCUSSION

Botanical products offer great promise as source of insecticides, may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, bio-degradable and are readily available in many areas of the world.^[23] Several plants have reported to produce phytochemicals which have shown insecticidal property.^[24] Screening of locally available plants for mosquito control may reduce dependence on synthetic chemicals, prevent environmental pollution and health hazards and also help in generating local employment.^[25] *Datura innoxia* demonstrated more promising larvicidal activity as compared to *Calotropis gigantea* whereas oviposition deterrent activity was more pronounced in *Calotropis* as compared to *Datura innoxia*. Therefore, the efficacy of these plants should be scrutinized and determined under field conditions for practical use.

CONCLUSION

Laboratory study demonstrated promising results as larvicide and oviposition deterrent activity. Both the plant *Calotropis gigantea* and *Datura innoxia* are particularly interesting because both are native species and widely distributed in Indian sub-continent. Further studies are needed to determine mode of action, toxicity, stability and their impacts on non-target organisms for field application.

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