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LARVICIDE AND GROWTH RETARDING ACTIVITY OF ALKALOIDAL EXTRACT OF TWO PLANTS TOWARDS AEDES AEGYPTI (DIPETRA: CULICIDAE)

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ABSTRACT

The crude alkaloidal extract from leaves of *Ricinus cummunis* L. (family: Euphorbiaceae) and *Argemone Mexicana* L. (family: Papaveraceae) were evaluated for larvicidal and growth inhibitory activity against *Aedes aegypti* mosquito larvae. The extracts of both the plants demonstrated promising larvicidal activity and prolonged larval and pupal period with an overall increase in the development period of mosquito life cycle. *A. Mexicana* affected moulting of larva into pupa and reduced adult emergence as compared to *R. Communis* extract. Growth index was considerably reduced with extract concentrations (500 ppm and 1000 ppm) as compared to control signifying its anti-juvenile activity. The results thus indicates that both larvicidal and growth inhibiting potential of alkaloidal extracts from leaves 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*, *Ricinus communis*, *Argemone Mexicana*, Temphose.

INTRODUCTION

Mosquitoes are important pests that affect human health and well-being throughout the world. The problems of mosquito transmitted diseases are quite severe worldwide resulted in high morbidity and mortality.[1] Mosquitoes can transmit more diseases than any other group of arthropod transmitting 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] Plant extract act as general toxicants like larvicides, oviposition attractents/deterrent, growth regulators, repellents and adulticides.[5] In recent years due to strict environmental legislation and increased resistance to synthetic insecticides there has been a major shift towards use of natural product from plant source.[6] 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.[7] 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.[8] 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 in these countries.[9] In recent years due to dramatic increase in resistance to standard larvicides, the use of synthetic insecticides has been restricted because of non-biodegradable nature, biological magnification through ecosystem and adverse effect on human health and non-target species. There has been a major shift towards use of natural product from laboratory to the field use.[10] Several groups of phytochemicals such as Chrysanthemum, pyrethrum, Nicotine, Azadirachtin, camphor and many others acts as general toxicants like larvicides, adulticides, repellents and insect growth regulators.[11] Many botanicals especially secondary metabolites, reported to have insecticidal property.[12]

The main objectives of present study was to evaluate the larvicidal efficacy and growth inhibitory effect of alkaloidal extract from the leaves of *Ricinus communis* L. and *Argemone mexicana*, widely distributed in India against larvae of dengue vector *Aedes aegypti*.

MATERIALS AND METHODS

Aedes aegypti 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 27-30^0 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. The feeding was continued until the larvae transformed into the pupal stage.

Plant collection

Mature leaves of castor bean plant (*Ricinus communis*) were obtained from the campus of Haffkine Institute Mumbai (18º 55´ N, 72º 54´ E) India during May -June 2014 and the plant *Argemone Mexicana* was collected from district Rajsamand located between latitudes 24˚46′ to 26˚0 1′ N and Longitudes 73˚28′ to 74˚18′ N of the state Rajasthan, India between March-May, 2014.

Identification of plant

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 *Ricinus communis Linn* (Accession No.117) and *Argemone mexicana* (Accession no. NYD 2882) had been deposited for future reference.

Extraction of plant material

The leaves were washed twice in distilled water, shad dried powdered and processed for alkaloid extraction in methanol and ammonia (9:1) using standard protocol.[13,14] Solvents were removed under reduced pressure by using rotary vacuum evaporator. The per cent yield of crude alkaloid extract was determined and stored at 4ºC prior to larvicidal assay. TLC characterization was done for crude alkaloid extract and the presence of alkaloids was identified by Dragendroffs reagent. Further characterization was done by HPTLC.

Larvicide susceptibility bioassay

All the experiments were carried out in 500 mL glass beakers containing 250 mL water using standard WHO procedure with slight modifications. [15] Late 3rd or early 4th instar larvae of laboratory strain of *Aedes aegypti* in three replicates (25 larvae/beaker) were introduced into beakers containing graded concentration (250 ppm to 2500 ppm) in 250 mL de-chlorinated water. Two controls were setup, one with Standard larvicide Temephos 50% EC at 0.05 ppm and other with distilled water (250 mL). Beakers were covered with muslin cloth to avoid the entry of any foreign material. No food was offered to the larvae during exposure period of 24 hrs. Larval mortality was recorded by counting dead and moribund larvae after 24 hrs. Dead larvae were those, which could not be induced to move when they were probed with needle in the siphon or cervical region. 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 because larvae that have pupated during the test period will negate the test. Corrected mortality was calculated by Abbots formula.[16]

Growth inhibitory assay

Growth inhibitory effect was evaluated according to per cent retardation of larvae into pupa and pupa into adult. Total developmental time from egg to adult was recorded to check the growth index.

Different concentrations of alkaloidal extract from leaves of *Argrmone Mexicana* and *Ricinus communis* (500 ppm and 1000 ppm) were prepared in beakers containing 250 mL of de-chlorinated water. Eggs approximately 100 in number on filter paper were exposed to 500 and 1000 ppm concentration along with distilled water control for 15 hrs. After 15 hrs the larvae were transferred in clean de-chlorinated water for 72 hrs. larval food (dog biscuits and yeast) was provided during this period.

Pupae developed within 72 hrs were removed from the experiment. The number of adults that emerge from the pupa was counted in order to calculate per cent inhibition.

RESULTS

Larvicidal activity

The results of larvicidal activity are presented in table-1. Both *R. communis* and *A. Mexicana* demonstrated promising larvicidal activity with LC_{50} value 802.12 ppm and 997.4 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 and there was a significant difference between treatment and control.

Growth inhibition activity

In this assay per cent larval and pupal mortality, total duration of lifecycle from egg to adult and per cent transformation of larvae into pupa and adult emergence from pupa were recorded. The results showed comparatively higher mortality of larvae and pupa with *R. communis* extract as compared *to A. mexicana*. Whereas, moulting of larva into pupa and emergence of adult was greatly reduced with *A. Mexicana* as compared to *R*. communis. at both 500 and 1000 ppm concentrations. Results are presented in table-2.

Table: Larvicidal activity of alkaloidal leaf extract of two plants against *Aedes aegypti* mosquito larvae

Concentration (ppm)	%larval mortality after 24 hrs.	
	Ricinus communis	*Argemone mexicana*
250	27.78±2.31	22.96± 1.06
500	42.34 ±3.16	39.21±1.79
1000	56.21 ±2.54	50.51±0.98
1500	77.89 ±4.12	67.43± 2.06
2000	100	78.45±1.76
2500	100	98.62±3.01
LC_{50}	802.12	997.54
LC_{90}	1960.76	2265.94
Water control	0.00	0.05±0.01
Temephos (0.05ppm)	100	

LC_{50}: lethal concentration required to kill 50% of larvae exposed to test concentration

LC_{90}: lethal concentration required to kill 90 % of larvae exposed to extract concentratios

Table-2 Effect of alkaloidal extract from leaves of *Argemone mexicana* and *Ricinus communis* on growth and metamorphosis of *Aedes* mosquito.

S. No.	Name of the plant	Concentration (ppm)	% larval mortality	%development of pupa	% pupal mortality	%adult emergence (a)	Total dev. Period in days (b)	Development index a/b
1	*Ricinus communis*	500	17.28 ±1.82	81.41±4.15	6.53 ± 4.65	54.22±4.43	15	03.61
		1000	31.17 ±3.16	56.35±1.99	12.75±4.05	34.91±2.36	13	02.68
		Control	04.47 ±0.98	95.12±2.03	01.9±4.87	92.12±1.29	09	10.23
2.	*Argemone mexicana*	500	21.67 ±1.54	63.52±1.67	3.19±4.11	28.90±3.47	14	02.06
		1000	33.59 ±1.87	29.31±2.37	7.91±3.16	16.84±2.16	12	01.40
		control	04.18 ±2.09	93.24±2.88	2.30 ± 1.25	90. 11±1.99	08	11.26

DISCUSSION

Extensive use of synthetic insecticides during the last six decades resulted in environmental hazards and development of physiological resistance in major mosquito vector species. This has necessitated the need for search of eco-friendly, biodegradable products from indigenous plants. Botanical 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.[17] Several plants have reported to produce phytochemicals which have shown insecticidal property.[18 & 19] 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.[20 & 21] Results of present study demonstrated promising larvicidal activity. The growth inhibitory effect was observed in larval inability to moult to pupa and adult suggesting inhibition of moulting process resulting in reduced adult emergence and an overall increase in life cycle period as compared to control. The high mortality at larval and pupal stage also reduced adult emergence. The development index was considerably abridged in test concentrations as compared to control signifying its anti-juvenile effect. Therefore, the efficacy of alkaloid extract of both these plants should be scrutinized and determined under field conditions for practical use.

CONCLUSION

Phytochemicals isolated from plant sources offer great potential as insecticides. The results of alkaloid extract from plant source are particularly interesting because both *R. communis* and *A. mexicana* are native species widely distributed in Indian sub-continent. Laboratory study demonstrated promising results both as larvicide and growth inhibitory activity. Further studies are needed to determine mode of action, toxicity, stability and their impacts on human health and non-target organisms for field application.

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