

Effect of acetogenins of *Annona squamosa* on different developmental stages of *Culex quinquefasciatus* Say.

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Abstract

A large number of biological activities are shown by acetogenins, isolated from the seeds of *Annona squamosa*. In the present study, acetogenins have shown larvicidal and growth regulating activities against *Culex quinquefasciatus* at a concentration of 1 and 2 mg. Mortality in the larvae, pupae and adult produced about 88-98% fall in population as compared to control. The total developmental period also reduced from the control group.

Keywords :- Mosquitoes, Acetogenins, *Annona squamosa*, Larvicidal

Introduction

Mosquitoes present an immense threat to millions for many diseases including malaria, filariasis, yellow fever, dengue, Chikunguniya and Zika virus disease^{1,2}. *Culex* mosquito is probably the most abundant house mosquitoes in town and cities of tropical countries. *Culex* mosquitoes develop in standing water such as polluted ponds, marshes, tanks, street gutters, barrels, tin cans, creeks, ditches, and ornamental ponds³. This mosquito possesses the necessary potential to initiate and facilitate the disease transmission by establishing the effective vector-host transmission cycle for diverse pathogens in different environments⁴. *Culex quinquefasciatus* is the principal vector of bancroftian filariasis and a potential vector of *Dirofilaria immitis*⁵⁻⁶. At present the need of the hour is to check and control the pest population and to reduce the vector borne disease, caused by them. The field of bio discovery and plant based pesticides steadily progresses and the development of herbal insecticides have become more rigorous in recent years⁷. Certain plants are known to contain some toxic properties and can play a very significant role in suppression of pest population.

Use of bio-products in the control of vector mosquitoes has gained momentum due to immense hazards caused by synthetic insecticides. Resistance to certain synthetic pesticides by certain mosquitoes such as *Culex pipiens*, *Culex quinquefasciatus*, *Aedes aegypti*, *Anopheles culifacies* and *Anopheles pseudopuntipennis* have been reported in certain areas⁸⁻¹². At present the need of the hour is to check and control the pest population and to reduce the vector borne diseases, caused by them. Some plants are known to contain certain toxic principles and can play a useful role in suppression of pest population. Use of bio-products in the control of vector mosquitoes has gained momentum due to immense hazards caused by synthetic insecticides., control of disease causing mosquito species has been almost completely based on synthetic organic insecticides following DDT, conventional pesticides such as malathion and pyrethroids are

generally used for mosquito control. But the extensive use of synthetic organic insecticides during the last five decades has resulted in environmental hazards. Besides this, there was development of environmental hazards. Since the discovery of DDT, this has the need for search and development of environmentally safe, biodegradable, low cost and indigenous methods of vector control¹³. The control of mosquito-borne diseases can be achieved by killing, preventing mosquito bite or by causing larval mortality in a large scale at the breeding centers of the vectors in the environment. A survey of literature on the control of different species of mosquito revealed that the assessment of efficacy of different phytochemicals obtained from various plants.

Several plant products were screened to identify the insecticidal and other biological activity against the mosquito larvae.¹⁴⁻²⁴ It is reported that crude extracts of *Premina integifolia* and *Ageratum conyzoides*, were toxic and caused 100% mortality to second instar larvae at 500ppm conc.²¹⁻²⁵ The larvicidal activity of various plant extract such as *Cleome icosandra*, *Dictyosa dichotoma* and *Pendalium murax* were found to be lethal against *Culex quinquefasciatus* Say.¹⁷ The bioactivities of acetogenins (active component of *Annona squamosa* isolated from the seed) as potential anti-tumour, immunosuppressive, pesticidal, molluscicidal, antiprotozoal, antifeedant, antihelminthic and antimicrobial agents have attracted interest world wide.²⁶⁻³⁰ So, we deemed it appropriate to study the larvicidal activity of acetogenins against *Culex quinquefasciatus* an important vector of many diseases in India.

Materials and Methods

Seeds were collected from the fruit of *Annona squamosa* plant which were collected locally from the Botanical Garden of Lucknow University. The seeds were kept in an incubator at 45°C for 24h. Dried seed were pulverized in a grinder. Acetogenins were extracted and isolated from the seeds of *A. squamosa* by the method of Li et al.,³¹ as modified by Singh and Singh³⁰. The dried pulverized powder of *A. squamosa* (120g) was extracted by macerating three times in excess 95% ethanol. The ethanol residue was partitioned between chloroform-H₂O (1:1) aqueous extracts. The chloroform solution was extracted with 3% HCl to remove alkaloids and then the chloroform solution was dried and the residue (12.54g) was partitioned between hexane-10% H₂O in MeOH (1:1) to afford the aqueous MeOH residue (5.60g) packed in a column of silica gel 60 (230-400 mesh) and eluted 5ml gradually with chloroform- MeOH (100:2). The eluant number 70-90 were then dried and evaporated. A total 479 mg of acetogenins was pooled which was used for the toxicity testing against the mosquitoes.

Colonies of *Culex quinquefasciatus* were reared in the laboratory in clean water and yeast tablets were given as food. Females were allowed to blood feed by placing a rat inside the cages 3 days after emergence. 100 second instar larvae of *Culex quinquefasciatus* were released in 250 ml beaker containing different test concentration of acetogenins (1 & 2mg) dissolved in 100ml distilled water followed by vigorous stirring. The treatments were replicated three times. Each replicate set contained one control which was untreated. The effects of the above treatments on development moulting and metamorphosis of larvae and pupae of *Culex quinquefasciatus* were observed.

Results and Discussion

Acetogenins in the present study showed larvicidal activity against second instar larvae of *Culex quinquefasciatus*. Abnormal behaviour was shown by the treated larvae. The larva aggregated on the water surface and moved in circles in periphery of the beaker whereas in control (untreated) zig zag motion was observed. The observations (Table-1) demonstrate that there was a significant difference ($P < 0.005$) in average larval, pupal and developmental periods of treated and control mosquitoes. The growth index of the treated mosquitoes was observed shorter than control groups. Mortality of larvae and pupae in different concentration during the experiment resulted in 88% to 98% fall in population as compared to control.

The average larval, pupal and developmental periods increase with the increase in the concentration of acetogenins in treated *Culex quinquefasciatus*. It is reported that in addition to acute toxicity, different compounds from different plants significantly lengthened the larval period in mosquitoes.³² This may be due to the interference in normal hormonal activity. It may be possible that imbalance between growth stimulating and growth inhibiting hormones caused by the acetogenins may be the cause of increase in larval, pupal and developmental periods.³³ Prolongation of larval and pupal periods may be due to the moulting process caused by an increase titer of juvenile hormone in the insect body due to acetogenin exposure. Juvenile hormone promotes the larval development maintains the status quo and so prevents metamorphosis.³⁴⁻³⁶ It may be possible that the synthesis or release of ecdysone hormone which is responsible for insect moulting, may be affected by the acetogenins and the insect lapses into a state of developmental standstill and results into ecdysal failure. Hence, the larval and pupal mortality can be attributed either to presence of toxic materials or due to the imbalance between growth stimulating and growth inhibiting hormones. Chitin plays an important role integument development in mosquitoes.²⁵ Mortality during moulting in larval stages and emergence may be the consequence of insufficient availability of chitin during metamorphosis

Thus, acetogenins have the potential to disrupt the growth, development and Cause mortality of *Culex quinquefasciatus*. Although current study would serve as an initial step towards replacement of synthetic insecticides to plant based bio-pesticides against *Culex* mosquitoes in future.

Plant extracts have been reported as AChE inhibitors.³⁷ The death in insects due to treatment with plant extracts suggested that the molecules present there in possibly interferes at the cholinergic synapse and destroying the communication network from one end to another, there by blocking the nerve impulse transmission. Thus the lethal effect may also be due to the accumulation of acetylcholine (ACh) a neurotransmitter at synaptic junctions which interrupts the coordination between the nervous and muscular functions (Neurotoxic).³⁷

Table 1: Effect of acetogenins isolated from the seed of *Annona squamosa* on *Culex quinquefasciatus*

Concentration of acetogenins (mg)*	Av. larval pd±SE (days)	% larval mortality	Av. Pupal period	% pupal mortality	% adult emergence (a)	Av. Dev. Pd. ±SE (days) (b)	Growth index (a/b)
Control	15.59±0.03	0	4.89±0.01	0	100	19.79±0.06	5.05
1mg	21.75±0.03 P<0.005	67	7.39±0.01 P<0.005	8	22	27.05±0.04 P<0.005	0.81
2 mg	25.45±0.01 P<0.005	79	9.98±0.01 P<0.005	14	6	29.96±0.11 P<0.005	0.20

* 100 second instars were treated in each concentrations.

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