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Evaluation of different combinations of plant products on different developmental stages of *Anopheles stephensi* (Diptera culicidae)

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Abstract
Acetogenins isolated from the seeds of *Annona squamosa* shows a number of biological activities. In the present study acetogenins in binary combinations with other plant products like *Azadirachta indica* and *Allium sativum* have shown larvicidal and growth regulating activities at a concentration of 1 mg against *Anopheles stephensi*. Mortality in the larvae, pupae and adults produced 79-86% fall in population with respect to control. There was also a decrease in total developmental period as compared to control.

Keywords: *Annona squamosa*, *Anopheles stephensi*, mosquito vector, acetogenins

Introduction
The mosquitoes are potential vectors of many diseases affecting human beings and other animals [1, 2]. One of the approaches for control of these mosquito borne diseases is the interruption of disease transmission by either killing, preventing mosquitoes from biting human beings or by causing larval mortality in a large scale at the breeding centres of the vector.
Resistance to certain conventional pesticides such as malathion, DDT and pyrethroids that are generally used for mosquito control have been reported by certain mosquitoes such as *Culex pipiens*, *Culex quinquefasciatus* say. *Aedes aegypti*, *Anopheles culicifacies* and *Anopheles pseudopunctipennis* in certain areas [3, 4, 5]. Several plant products have been screened to identify the insecticidal and other biological activities against the mosquito larvae [6, 7, 8, 9, 10, 11].
Annona squamosa, commonly known as custard apple is used as an antitumour, anti -helminth and wound healing. Unripe and dried fruits are used for diarrhoea and dysentery [12] leaves of *Annona squamosa* also show growth suppressant activity in *Anopheles stephensi* [13]. The bioactivities of acetogenins (active component of *Annona squamosa* isolated from the seed) as potential antitumor, immunosuppressive, pesticidal, molluscicidal, antimicrobial, anti-protozoal, antifeedant activity have attracted worldwide [14, 15, 16, 17, 18].
The objective of present study is to evaluate the use of acetogenins isolated from the seed of *Annona squamosa* (custard apple) and its binary combinations with other plant products *viz Azadirachta indica* and *Allium sativum* on different developmental stages of *Anopheles stephensi.*

Materials and Methods
Acetogenins were extracted from the seeds of *Annona squamosa* (purchased in the local market of Lucknow in the month of June) by the method of Li *et al*. [19] as modified by Singh and Singh [18].
Dried pulverized seeds of *A. squamosa* (120g) were powdered and extracted by macerating the tissue three times in excess 95% ethanol. The ethanol residues were partitioned in a chloroform and water mixture (1:1 mixture v: v). The chloroform solution was subsequently extracted with 3% HCl to remove alkaloids and then the chloroform solution was dried and the silica residues (12.54g) was partitioned between hexane- 10% H_2O in MeOH (1:1) to afford the aqueous MeOH residues (5.60g) was loaded in a column of silica gel 60 (230-400 mesh) and eluded in 5ml units of chloroform and MeOH mixture (100:2 V.V). The eluant (numbered 70-90) containing the acetogenins were collected, dried and evaporated to yield a total of 479 mg of acetogenins. The essential oil of neem (*Azadirachta indica)* was supplied by the Indian Herbs Co. Pvt. Ltd. Saharanpur, India.

The powdered bulb of garlic *(Allium sativum)* which was collected locally was prepared by cutting the tissue slices, drying at 45ºC overnight and pulverizing the dried tissue with the mortar and pestle. Combinations of the plant materials were prepared in the non- ionic emulsifier Snehakshar (Indian Herbs Research and Supply Co. Pvt. Ltd. Saharanpur).

Colonies of *Anopheles stephensi* 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 *Anopheles stephensi* were released in a 250 ml beaker containing different concentrations of acetogenins and its binary combinations (1:1) dissolved in 100 ml distilled water followed by vigorous stirrings. 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 *Anopheles stephensi* were observed.

Results and Discussions

In the present study acetogenins alone and in combination with other plant products showed larvicidal activity against second instar larvae of *Anopheles stephensi*. Abnormal behaviour was shown by the treated larvae. The changes were observed within 1 hour of exposure. The most obvious sign of behavioural change was that the larva aggregated on the water surface and moved in circles at periphery of the beaker whereas in untreated (control) zig-zag movement was observed. The observation (Table-1) demonstrates that there was a significant difference ($P<0.005$) in average larval, pupal and developmental periods of treated and control groups. Mortality of larvae and pupae in different concentrations of binary combinations during the experiment resulted in 79% to 96% fall in population as compared to control. The growth index of the treated mosquitoes was observed shorter than the control groups. The average larval, pupal and developmental period increases in the binary combinations as compared to acetogenins. This may be due to the interface in normal hormonal activity it is reported that in addition to acute toxicity, compounds from different plants significantly lengthened the larval period in mosquitoes [20]. It has also been reported that in addition to acute toxicity, compounds from different plants significantly lengthened the larval periods of mosquitoes [21].

In the present study the binary mixture of the plant products killed mosquitoes at sublethal doses. Acetogenins in combination with *Azadirachta indica* were most effective. There was 81% larval mortality in the mosquitoes treated with acetogenins and *A. indica* as compared to mosquitoes treated with acetogenins and *A. Sativum* which was 75%.

The combined action of the binary mixture resulting in a substantially increased level of lethality that either of the two components used, represent a form of joint action.

There was a prolongation of larval and pupal periods which may be due to the moulting process caused by an increased titer of juvenile hormone in the insect body due to the exposure of acetogenins and its binary combinations. The phytochemicals interfered with proper functioning of mitochondria more specifically at proton transferring sites and affected midgut and malpighian tubules [22, 23, 24].

Thus in conclusion, it can be said that acetogenins along with other plant products have the potential to disrupt the growth and development and cause mortality in *Anopheles stephensi*. Synergistic approaches will provide better effect in reducing the vector population and the magnitude of epidemiology. Further studies are needed to recommend the above plant combinations for mosquito control and mortality.

Table 1: Effect of acetogenins isolated from seeds of *Annona squamosa* in combination with *A. Indica* and *A. Sativum* on *Anopheles stephensi*

Concentration of acetogenins and combinations (1:1) 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	15.54±0.03	0	4.79±0.01	0	100	19.02±0.06	5.25
Acetogenins (1mg)	20.84±0.03 $P<0.005$	70	7.16±0.01 $P<0.005$	9	21	26.15±0.04 $P<0.005$	0.80
Acetogenins + *Azadirechta indica* (1mg)	24.53±0.01 $P<0.005$	81	9.27±0.01 $P<0.005$	15	4	28.74±0.11 $P<0.005$	0.13
Acetogenins+ *Allium sativum* (1mg)	22.39±0.01	75	10.29±0.01	12	13	27.84±0.03 $P<0.005$	0.41

* 100 second instar larvae were treated at each concentration.

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