Evaluation of mosquitocidal activity of *Annona squamosa* leaves against filarial vector mosquito, *Culex quinquefasciatus* Say

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Methanolic extract of leaves of *A.squamosa* was tested for mosquitocidal effect against *C.quinquefasciatus*. A liquid mosquito insecticide formulation was prepared with the extract (1, 3 and 5 % w/w) using deodorized kerosene as solvent and investigated for its knock-down and 24 hr mortality. The extract formulation produced dose dependent activity, exhibited significantly shorter knock down KD50 and KD90 values and produced significant mortality. The results suggest the potential mosquitocidal effect of *A.squamosa* on *C.quinquefasciatus*.

Mosquitoes are still the world’s number one vector of human and animal diseases and are conspicuous nuisance pests as well. They are potential vectors of malaria, filariasis, yellow fever, brain fever, dengue fever etc. There is a crying need to check the proliferation of population of vector mosquitoes in order to reduce vector-borne diseases by using appropriate control methods. Pyrethrin-based mosquito liquid formulations are widely used in many countries, especially in the household of rural population, to protect people from mosquito bites through their repellent effects, knocking-down and killing effects. Synthetic pyrethrins such as D-allethrin and D-trans allethrin possessing potent insecticidal activity with low mammalian toxicity have increasingly been used as substitutes for natural pyrethrins in many mosquito coil and liquid formulations. However, prolonged exposure to these chemicals may lead to local irritation, severe allergic dermatitis and systemic allergic reactions. Large amounts may cause nausea, vomiting, tinnitus, headache and other CNS disturbances.

*Annona squamosa* (Annonaceae) is a large, evergreen straggling shrub or small tree of 7 m height found throughout India. The leaves possess insecticidal and pesticidal effects against bugs and lice. Leaves, seeds and unripe fruits have vermifugal and insecticidal properties and are used to kill lice on cattle. Therefore, the present study has been designed to test the mosquitocidal effect of the methanolic extract of *Annona squamosa* and the knocking down, killing effects of the liquid mosquito insecticide formulation prepared with the extract.

**Plant extract**—The leaves of *Annona squamosa* were collected in the fruiting season of September 2000 at Tiruchirappalli, Tamilnadu, India. The leaves were shade dried, pulverized by a mechanical grinder, sieved through 40 mesh. The powdered leaves (200 g) were extracted with methanol using a soxhlet extraction apparatus. This methanol extract was then concentrated and dried under reduced pressure. The methanol free semi-solid mass (yield 16%) thus obtained was used for the experiment.

**Liquid mosquito insecticide extract formulation (LMIEF)**—The methanolic extract of *A.squamosa* (5% w/w) was dissolved in the deodorized kerosene (94.0% w/w, perfume 1% w/w). The contents were filtered through Whatman filter paper No.1 and stored in a well-closed container. An empty container of the commercial liquid mosquito insecticide formulation (CLMIF) was rinsed with kerosene and dried. The LMIEF was filled in the container and fitted in the destroyer equipment supplied with the CLMIF and used.

**Organism**—*Culex quinquefasciatus* Say (Diptera : Culicidae) a filarial vector mosquito has been selected for this study. A colony of *Culex quinquefasciatus* Say was established in the laboratory from egg rafts obtained from Research Department of Zoology, American College, Madurai. The larvae were reared in clean tap water and yeast tablets were given as food.

**Mosquito chamber**—The mosquito chamber (50×50×30 cm; made up of wood) was designed in the laboratory. The top was partly covered with wire mesh and the remaining with a wooden lid. The front
portion was fitted with a PVC pipe measuring 30 x 15 cm. The PVC pipe was closed with a cloth tied in front. The chamber was securely sealed with rubber beading in the edges of the lid and PVC pipe so that the mosquitoes after emergence might not escape out of the chamber. Inside the chamber, a metallic weld mesh cage measuring 25 x 15 x 15 cm with a lid in front having a padlock was fixed in the mid bottom. The cage can be easily handled one handed through the PVC pipe and it can be opened and closed. The mesh size may be a square of 5 x 5 cm, so as to enable the mosquitoes to fly freely inside and come out of the metallic cage (Fig. 1).

The pupae of C. quinquefasciatus taken in clean tap water in a plastic cup were placed in the chamber. Adult mosquitoes emerging out were provided with 2% glucose solution. The female mosquitoes were provided with avian blood during the night by placing a domestic fowl inside the metallic cage. While handling the cage through the PVC pipe the opening was closed with the cloth to prevent mosquitoes coming out.

Bioassay for knocking-down and killing effects —

The bioassay for knocking-down and killing effects was conducted by modifying the method of Jantan et al. It was performed in a chamber measuring 120 x 120 x 60 cm with glass at front and sides and wooden lamination with white coloured interior at top, bottom, and backside. Sliding glass doors (60 x 30 cm) were provided at both sides of the chamber. An electric plug point was provided at the interior back of the chamber (Fig. 2). Mosquitoes (30) from the mosquito chamber were introduced into this chamber through the sliding window at one side. The liquid mosquito insecticide extract formulation (LMIEF) was allowed to vapourise for 2 min by using the vapouriser equipment provided with CLMIF. Knocked-down mosquitoes (i.e. those that no longer maintained normal posture and were unable to fly or were on their backs) were recorded at 1 min intervals up to 3 hr or until total knock-down was achieved. Knocked-down mosquitoes were placed in a clean container containing cotton wool soaked with 5% sucrose solution and the mortality of the mosquitoes was observed after 24 hr. Four replicates of the experiment were carried out for each liquid mosquito formulation. Control 1 was performed by exposing the mosquitoes to the vapour of the solvent (deodorized kerosene). A test carried out without liquid mosquito insecticide formulation served as control 2. A commercial reference standard liquid mosquito insecticide formulation (allethrin 3.6% w/w) was included for comparison. Knock-down times (KD50 and KD90, as the minutes needed to knock-down 50% and 90% of mosquitoes, respectively) were determined by the probit analysis (Finney). In bioassay for knocking-down and killing effects, LMIEF produced significant knock down (KD50 and KD90) and mortality at 5% (W/W) concentration, when compared with the control (Table 1). LMIEF (5%, w/w) showed significantly shorter KD50 and KD90 values and also produced significant mortality (93.6±4.1%) when compared with the control. The results were also comparable to the reference CLMIF.

Use of natural products in the control of mosquitoes has gained high priority due to hazards caused by insecticides. Multiple resistance to organo-chlorines, organophosphorus insecticides, carbamates and certain pyrethroids in the same population of mosquito species has been developed in certain areas by Aedes aegypti, Culex pipiens, Culex quinquefasciatus Say, Anopheles albimanus, An. culicifacies, An. Pseudo-
Several plant products were screened to identify the larvicidal action against the mosquito larvae\textsuperscript{11,12}. Management of vector mosquitoes seems to be very difficult because on one hand the vectors develop resistance to pesticides and larvicides and on the other hand abundant usage of chemicals enhances the health hazards of humans. Finding suitable alternatives to check the population is still in progress. The extract of \textit{Annona squamosa} in mosquito coil formulation and isolation of the insecticide component is underway in our laboratory, which will be reported in the near future.

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### References