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Larvicidal Effects of Plant Extract and *Bacillus thuringiensis.var israelensis* on *Aedes aegypti* (Diptera: Culicidae)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Mosquitoes are the most important group of arthropods that transmits disease causing pathogens. Several plants are used in traditional medicines for the mosquito larvicidal activities in many parts of the world. The use of bacterial toxin in plant extract has a remarkable effect on the larvae of the mosquito. The larvicidal efficacy of plant extract *Annona squamosa* with *B. thuringiensis* was tested against third-instar larvae of *Aedes aegypti*. The mean mortality and percentage mortality of larval populations were noted at different concentrations after 24 and 48 hours period of exposure. Bti and extracts of *Annona squamosa* showed high insecticidal activity with LC 50 and LC 90. The values of LC 50 at 24 and 48hours exposure are 17.34 and 15.70 respectively. Values of LC 90 at 24 and 48 hours exposure are 31.48 and 28.38 respectively.

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1. INTRODUCTION

"Mosquitoes are responsible for several infectious disease like malaria, filariasis, Japanese Encephalitis, yellow fever, dengue and chikungunya" [1]. Mosquitoes are an important vector of etiological agents of diseases in humans and animals [2]. According to the World Health Organization [3], contemporary mosquito-borne diseases are still chief concerns for global public health as there has been a rise in dengue virus infections worldwide during the last two decades.

Aedes is a common household vector species of mosquitoes that lives in close association with the human community. Dengue spreads throughout the tropical and subtropical regions [4]. *Aedes aegypti* has tremendous biting ability compared to *Aedes albopictus*; both species are well adapted to oviposit in smaller containers that have water in it [5,6]. "The high density of *Aedes* mosquitoes determines the high risk of dengue in that area. It is one of the world's most widely distributed mosquitoes and is the most efficient vector for arbovirus because it is highly anthropophilic, frequent bites and thrives in close proximity to humans" [3]. "*Ae. aegypti*, often referred to as a container breeder mosquito, typically breeds in domestic and peri-domestic artificial containers such as plastic drums, overhead tanks, cans, and buckets, as well as natural containers containing some organic matter, like tree holes" [7]. *Aedes* mosquitoes breed both indoor and outdoor [8,9].

"The mosquito control operations were lacking due to poor infrastructure and knowledge about the specific breeding habits of the dengue vector. Prevention of Dengue hemorrhagic fever outbreaks in endemic areas is based on long-term anti-mosquito control measures, particularly household and environmental sanitation, with an emphasis on larval source reduction. The microbial control agent *Bacillus thuringiensis var-israelensis* (Bti) has been successfully used worldwide to reduce the emergence of adult mosquitoes" [10,11]. Early mosquito control methods included source reduction. Chemicals have a significant negative impact on the environment therefore, plant extracts or phytochemicals have been focused on as potential sources of control agents. Neem components show multiple effects against different insects such as mosquitoes. "Therefore, natural herbal larvicide is the key strategy that highlights the action against mosquito larvae and their breeding sites. Present study focuses the new control ecofriendly methodologies for eradication of dengue. *Bacillus thuringiensis var-israelensis (Bti)* is one of these insect bio-insecticides" [12]. "Control of mosquitoes using Bti has a particularly strong potential to affect ground-based and soil-based food webs" [13].

Current investigations have also emphasized on microbial mosquito larvicides like *Bacillus thuringiensis var israelensisi (Bti)* and *Bacillus sphaericcus* [14] with larvicidal oviposition inhibitory, repellence, and insect growth regulatory effects [15]. Along with Bti, the *Annona squamosa* leaf extract acts as an alternative to chemical applications to fight resistance problems in mosquitoes.

2. MATERIALS AND METHODS

Fresh leaves of *Annona squamosa* were collected. They were shade dried, powdered and 10 g was extracted with 100 ml of methanol solvent and kept overnight in a shaker. The extract was filtered using whatman No.1 filter paper, and the solvent residue was obtained.

2.1 Microbial Bioassay

Bacillus thuringiensis was obtained from the Inbiotics Lab, Nagercoil. The required quantity of *B. thuringiensis* was thoroughly mixed with distilled water and prepared to various concentrations, ranging from 10 to 50 g/L, respectively.

2.2 Preparation of Phytoextracts

The 10 g of dried powdered plant sample was dissolved in 100 ml of methanol. The content was left for 3 days at room temperature to extract the plant secondary metabolites [16-18]. The extract was then filtered through Whatmann No. 1 filter paper, and the filtrate was collected in a separate container. After that, the extract was concentrated by evaporating the solvent using a rotary evaporator at 40^{o}C to 60°C for 30 minutes until all the solvent evaporated separately. Finally, we got the extracts in semisolid form. The dried content was weighted to calculate the yield of the dried extract. The extract was kept and stored in the refrigerator at 4 ºC for further use.

The percentage of yield is calculated by dividing the dry weight of extract (g) by the dry weight of plant biomass and multiplying by 100.

2.3 Larvicidal Activity

A larvicidal activity trial was carried out with the different concentration of the test plant extract (10, 20, 30, 40, 50mg) and *Bacillus thuringiensis* in a separate beaker containing 100ml of dechlorinated water. To this, 20 given larvae were introduced in to each test concentration, which also supplied 0.1 mg of larval food and the control was maintained. The trial was duplicated. The number of dead larvae was recorded at the end of two intervals of 24 and 48 hours.

The mortality percentage was calculated by using the following Abbott formula and the lethal concentrations (LC50 and LC90) were calculated from toxicity data by using probit analysis.

Corrected mortality = (Observed mortality in treatment –Observed mortality in Control)/ (100 – Control Mortality) x 100

Percentage of larval mortality = (Number of dead larvae or pupae / Number of larvae or pupae introduced) x 100

3. RESULTS

The results of the larvicidal activity of *Annona squamosa* L. extract against *Ae. aegypti* were noted and presented in Tables 1 to 3 and Figs. 1 and 2.

The LC 50 value of *A. squamosa* extract with *Bti* after 24 hours and 48 hours at different concentration was found to be 17.34 and 15.70 respectively (Table 2) (Fig. 4).

The LC 90 value of *A. squamosa* extract with *Bti* after 24 hours and 48 hours at different concentrations was found to be 31.48 and 28.38, respectively. As the concentration increases the percentage mortality also increases (Table 2) (Fig. 4).

4. DISCUSSION

Mosquitoes in the larval stages are attractive targets for pesticides because mosquitoes breed in water, which makes it easy to deal with them in this habitat. Vector control is facing a serious threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides. Tikar et al. [19] have reported that “the development of insecticides resistance in populations of *Aedes aegypti* indicates the need for the search for safe and effective alternative measures. Natural pesticides, especially those derived from plants, are more promising in this aspect”.

Nowadays, the control of mosquitoes at the larval stage is focused on plant extracts [20-22]. Nayak [23] reported “the larvicidal activity of *A. reticulate* leaf crude extract at different concentrations, showing a 100% mortality rate of larvae was observed at 5, 10,25, 50, 100 and 200 ppm concentrations of crude extract. The present results are in agreement with these findings, as nearly 100% mortality was observed after 48 hours of treatment”.

Wandscheer et al*.* [24] reported that “naturally occurring insecticides may play a more prominent role in mosquito control programs in the future”. A similar result was observed by Mathew et al*.* [25] in “the leaf extract of *Nycthanthes arbortristis* which showed lethal values (LC50 = 526.3 and 780.6 ppm (24 hrs) and LC50 = 303.2 and 518.2 ppm (48 hrs) against *Aedes aegypti* and *Anopheles stephensi*, respectively”.

The larvicidal activity depends on the presence of several bioactive chemicals in different parts of the plant [26]. The presence of the cytotoxic compound saponin was observed, and the presence of saponin along with other phytoconstituents may be the reason for the 100 percent mortality observed with reference to the extracts of the tested plant.

Table 1. Mean and percentage mortality of IIIrd instar *Ae. aegypti* mosquito larva treated with the various concentration of leaf plant of *A. squomosa* and Bti after 24 hours

Concentration of sample	Mean mortality % at 24 hours	Mortality rate (%) at 24 hours	Mean mortality % at 48 hours	Mortality rate (%) at 48 hours
10 mg	5±0.06	12.82051282	6.5+0.02	7.894736842
20 mg	9.5±1.02	35.8974359	12±0.18	36.84210526
30 mg	12±1.2	48.71794872	15±0.24	52.63157895
40 mg	14.5±0.8	61.53846154	18.5±1.5	71.05263158
50 mg	19±2.6	84.61538462	20±0.06	78.94736842

Table 2. Determination of LC 50 and LC 90 after 24 and 48 hours

SI.No	Plant Species	Duration	LC 50	LC 90
1		24 hrs	17.34	31.48
2	*A.squomosa*	48 hrs	15.70	28.38

Table 3. Probit Analysis of % mortality at 24 hours and 48 hours

S.No	Log C	Probit Analysis 24 hrs	Probit Analysis 48 hrs
1	1	3.87	3.59
2	1.301029996	4.64	4.67
3	1.477121255	4.97	5.08
4	1.602059991	5.31	5.55
5	1.698970004	6.04	5.81

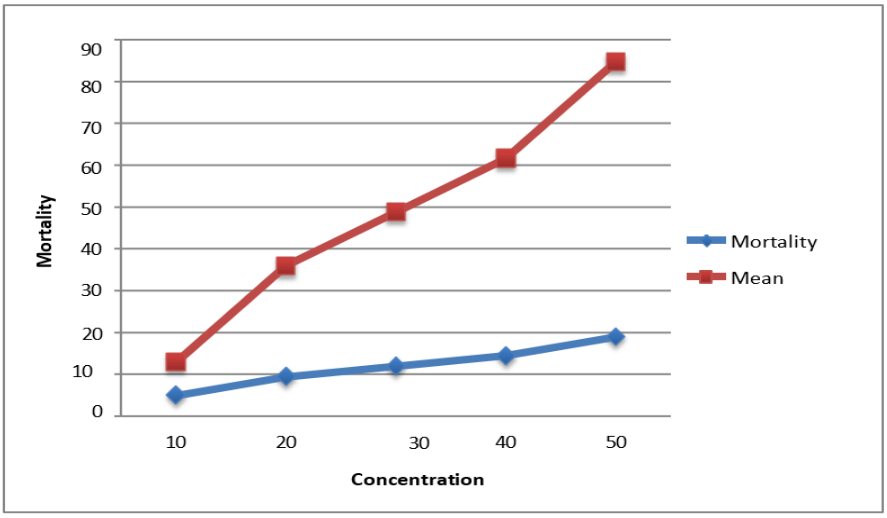

Fig. 1. Mean and percentage mortality of *A.aegypti* larva treated with various concentration of *A.squamosa* leaf extract and *Bti* after 24 hours

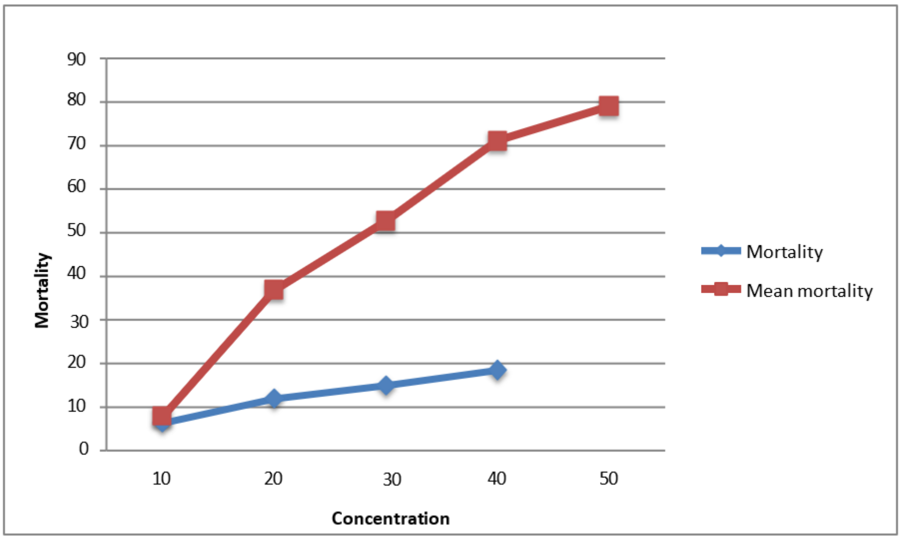

Fig. 2. Mean and percentage mortality of *Ae. aegypti* larva treated with various concentration of *A. squamosa* leaf extract and *Bti* after 48 hours

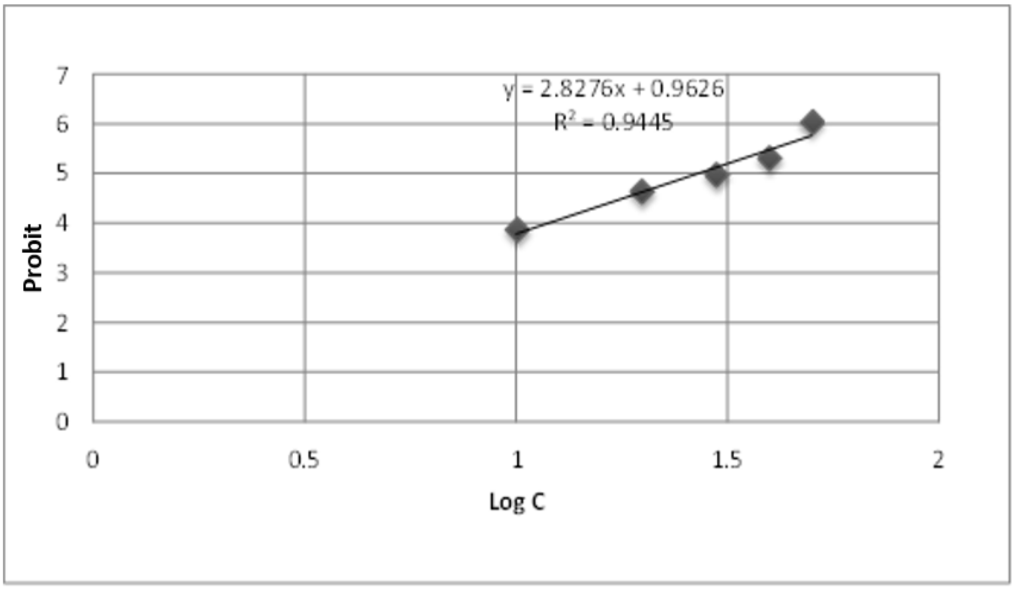

Fig. 3. Probit Analysis of % Mortality at 24 hours

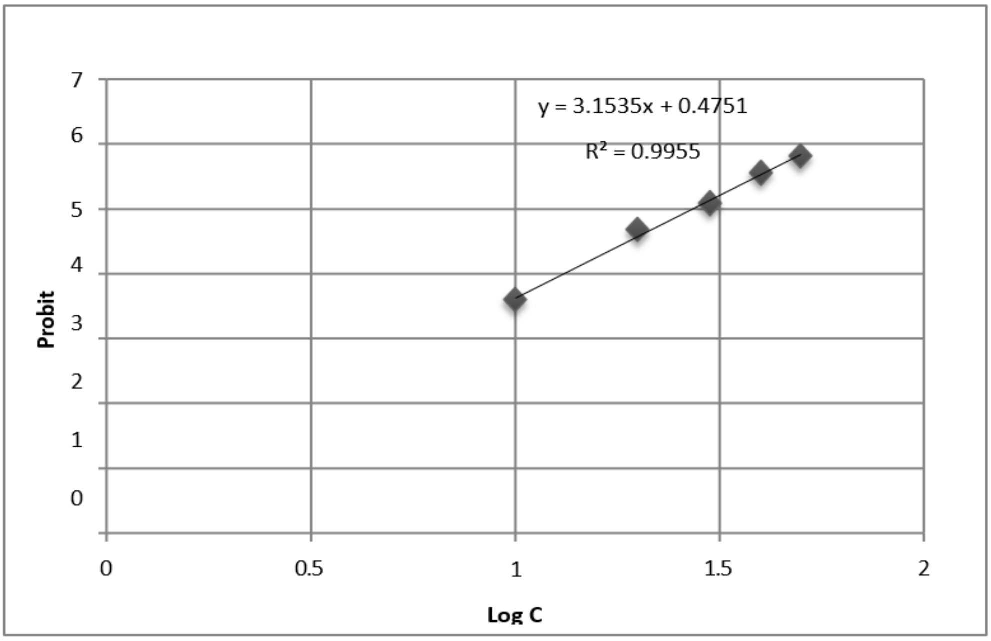

Fig. 4. Probit Analysis of % Mortality at 48 hours

The synergistic larvicidal action of *Citrus limon* and *Bacillus thuringiensis* on the *Aedes aegypti* larvae was studied by Grace et al*.* [27]. The *C. limon* leaf extract with *Bti* were tested separately on the third-instar larvae of *Ae. aegypti* mosquitoes at different concentrations. The larvicidal action showed that the increasing concentrations had a significant effect on *Ae. aegypti* larvae. This larvicidal action on *Ae. aegypti larvae* was due to the toxins from *Bacillus thuringiensis* and phytocompounds of *Citrus limon*.

Murugan et al*.* [28] studied the effect of bacterial toxin *(B. sphaericus)* with neem seed kernel extract against the filarial vector (*Culex quinquefasciatus*). The result shows that the plant extract of *A. squamosa* and *Bti* can be used to control the dengue vector population. The maximum mortality is obtained at the concentration of 50gm/ml after 48 hours in *Annona squamosa* extract with *Bti* [29,30].

5. CONCLUSION

The results of the study indicate that plants are more advisable to use for the control of mosquito- borne diseases. These plants along with *B. thuringiensis,* are remarkably economical and eco-friendly with more larvicidal properties. Hence, *Annona squamosa* with *B. thuringiensis* can be considered as a potential resource for mosquito larvae. Such practice would not only reduce the disadvantages of insecticides on the environment but also promote sustainable utilization of locally available bioresources by rural communities.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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