

## Larvicidal Efficacies of Certain Plant Extracts against Fourth Instar Larvae of *Aedes albopictus* Skuse

O.G. Indusree<sup>1\*</sup>, J.S. Chandana<sup>2</sup>, V.S. Ajitha<sup>3</sup>

<sup>1,2,3</sup>PG and Research Department of Zoology, University College, Thiruvananthapuram, Kerala, India

\*Corresponding Author: [indhusreeparvana@gmail.com](mailto:indhusreeparvana@gmail.com), Tel.: +91-9495629496

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**Abstract**— Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae) is becoming a competent vector for dengue, chikungunya and other viruses. Most common insecticides and repellents may exert toxic effect on non-target organisms including man. Hence alternative vector management strategies need to be explored. Biopesticides including plant products may be employed as mosquito control agents. In this study, an attempt has been made to evaluate the insecticidal potential of aqueous leaf extracts of *Clerodendrum infortunatum*, *Ailanthus excelsa*, *Aloe vera* and *Sesbania grandiflora* on mortality of fourth instar larvae of *Aedes albopictus*. Larvicidal effect was studied at doses ranging from 100 to 600 ppm. The results indicate that aqueous leaf extracts of *C. infortunatum* (LC<sub>50</sub>- 614.6ppm) showed a higher mortality rate than *A. excelsa*, *A. vera* and *S. grandiflora*. Biochemical analyses revealed an increase in protein, glycogen and lipid concentration in plant extract treated larvae, when compared to control ones, whereas amount of amino acid decreased in treated larvae. This clearly indicates a physiological as well as biochemical imbalance in larvae, induced by plant extracts. Presence of secondary metabolites such as alkaloids, flavonoids, tannins, glycosides, phenols was detected by qualitative analyses of plant extract which may contribute for its insecticidal effect.

**Keywords**— Asian tiger mosquito, *Aedes albopictus*, *Clerodendrum infortunatum*, *Ailanthus excelsa*, *Aloe vera*, *Sesbania grandiflora*, Larvicidal.

### I. INTRODUCTION

Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae) is becoming a competent vector for dengue, chikungunya and at least 22 other viruses including yellow fever virus, Japanese encephalitis virus etc. Climate change predictions suggest *Aedes albopictus* will continue to be a successful invasive species that will spread beyond its current geographical boundaries [1]. *Aedes albopictus* feeds on a wide range of hosts and this generalized feeding behaviour contributes to its vector potential.

Vector control has been practiced since the early 20<sup>th</sup> century. From the early 1950s, DDT and other synthetic insecticides were extensively used to interrupt transmission of vector borne diseases. In the mid-1970s, the resurgence of vector borne diseases, along with development of insecticide resistance in vector population led to a rethinking in vector control strategies.

Application of alternative methods in mosquito control as part of the Integrated Mosquito Management (IMM) has been gaining importance [2]. One of the main approaches of IMM include application of insect growth regulators,

phytochemicals etc. In the field of IMM, larviciding approach is more target specific and safer approach than controlling adult mosquitoes [3]. Repeated use of synthetic insecticides for mosquito control has already disrupted natural ecological balance and led to resurgences in mosquito populations. Globally greater awareness has been created on plant derived substances as they are eco-friendly and possess more insecticidal properties through multifarious functions.

Generally, the active toxic ingredients of plant extracts are secondary metabolites that are evolved to protect them from herbivores. The insects feed on these secondary metabolites potentially encountering toxic substances with relatively non-specific effects on a wide range of molecular targets such as enzymes, receptors, signaling molecules, ion-channels, structural proteins and bio membranes [4].

Considering these facts and findings, this study has been undertaken to evaluate the larvicidal efficacy of four different plants (*Clerodendrum infortunatum*, *Ailanthus excelsa*, *Aloe vera* and *Sesbania grandiflora*) on fourth instar larvae of *Aedes albopictus*. Biochemical analyses were also conducted in both treated and control larvae to find out

whether any metabolic imbalances were induced as a result of plant extract treatment. The results obtained in this study may pave way for the development of a comparatively safer vector management strategy utilizing natural plant resources especially in the current scenario of chemical pesticides imposing serious health hazards as well as environmental problems.

## II. METHODOLOGY

### Collection, Selection and Culture of Mosquito Species

Eggs of *Aedes* were collected from controlled breeding sites around households of Thiruvananthapuram district. The larvae were pooled in the laboratory and subjected to species level identification using standard manual [5, 6, 7 and 8]. The screened larvae were reared and kept in a 30cm × 30cm × 30cm cloth cage, for the establishment of colony in the laboratory. They were maintained at room temperature. Adults (Figure 1) were fed with cotton pads soaked in 1% glucose solution- and sliced apples /oranges as source of energy. For egg laying, the females were blood fed on rabbit blood. The gravid females oviposited (Figure 1) on the water surface, in small plastic containers lined with filter paper. The larvae reared from these eggs were used for the experimental study. This procedure also helped to maintain the uniform age of larval instar. For further experiments, all larval instars were reared in tap water and fed with crushed dog biscuits and yeast in the ratio 3:1.

### Collection and identification of plants

Leaves of plants namely *Clerodendrum infortunatum*, *Ailanthus excelsa*, *Aloe vera* and *Sesbania grandiflora* (Figure 2) were collected from local areas of Thiruvananthapuram and identified in the Department of Botany, University College, Thiruvananthapuram. Their aqueous extracts were used for the present investigation.

### Preparation of plant extract

The leaves were washed thoroughly with water, shade dried and powdered in domestic grinder. The extraction procedure was done in Soxhlet apparatus [9], using distilled water as solvent. The extract obtained was further evaporated to get a dry residue.

### Preparation of stock solutions

The dry extracts were made up to 10% stock solution using the solvent, distilled water. From this stock solution, different concentrations ranging from 100 ppm to 600 ppm were selected for testing the larvicidal effect.

### Larvicidal Bioassay

Larvicidal effect was studied using aqueous extracts of all

four plants (*Clerodendrum infortunatum*, *Ailanthus excelsa*, *Aloe vera* and *Sesbania grandiflora*) with fourth instar larvae of *Aedes albopictus* which were randomly selected from the stock culture. 100 larvae were used for each test. Larvicidal effect was studied at doses 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm and 600 ppm which were made up to 100 ml using standard protocols [10]. Equal numbers of controls were also set up. At each tested concentration, two trials were made, and each trial consisted of four replicates and mortality was recorded after 24 hours. LC<sub>50</sub> was calculated using probit analysis using the software IBMSPSS 20 for Windows. Data analysis was done by ANOVA followed by Tukey's test. Results with  $P < 0.05$  were statistically significant.

### Biochemical Analyses

Biochemical analyses were done using standard protocols. Estimation of total body protein [11]; free amino acids [12]; glycogen [13]; lipids [14] were done. Statistical analysis was performed using the software IBMSPSS 20 for Windows. Data analysis was done by ANOVA followed by Tukey's test. Results with  $P < 0.05$  were statistically significant.

### Qualitative Estimation of Phytochemicals

The phytochemical screening of aqueous leaf extract of *Clerodendrum infortunatum* and *Ailanthus excelsa* were done as these two extracts exhibited significant larvicidal effect. Alkaloids (Wagners Test), Flavonoids (Alkaline Reagent Test), Tannins (Gelatin Test), Saponins (Froth Test), Terpenoids (Salkowski Test), Glycosides (Keller killani Test), Quinones (H<sub>2</sub>SO<sub>4</sub> Test), Phenols (Ferric Chloride Test) were done using standard protocols [9].

## III. RESULTS AND DISCUSSION

### Mortality

The percentage mortality observed for aqueous extract of *Clerodendrum infortunatum* was 0.5%, 1%, 2%, 28.5% and 46% at doses 200 ppm, 300 ppm, 400 ppm, 500 ppm and 600 ppm respectively.

For *Ailanthus excelsa*, percentage mortality was 0.5%, 1%, 2%, and 4% at doses 300 ppm, 400 ppm, 500 ppm and 600 ppm respectively.

Mortality could not be observed in larvae treated with *C. infortunatum* at a dose of 100 ppm and for *A. excelsa* at a dose of 100 ppm and 200 ppm.

For *Aloe vera*, the mortality was shown at 600 ppm only (5.5%) and for *Sesbania grandiflora*, the mortality was shown

at doses 500 ppm and 600 ppm (0.5% and 2.5%) respectively (Figure 3).

No mortality was observed in larvae treated with *S. grandiflora* at doses 100 ppm, 200 ppm, 300 ppm and 400 ppm; and for *A. vera* besides this, at a dose of 500 ppm also. In all control sets, larvae emerged normally without any mortality.

Treated larvae showed 50% mortality at a concentration 614.59 ppm (Table 1) and 9769.79 ppm (Table 1) in aqueous extracts of *C. infortunatum* and *Ailanthus excelsa* respectively.

From the results, it is evident that *C. infortunatum* showed more larvicidal potential than other three plant extracts against the larvae of Asian tiger mosquito, *Aedes albopictus*.

### Biochemical Analyses

Estimation of important biochemicals - total Protein, Amino acid, Glycogen and Lipid were also conducted in order to get information on the influence of the plant extracts on major metabolic events. Fourth instar larvae of *Aedes albopictus* were treated with LC<sub>25</sub> and LC<sub>10</sub> dose of *C. infortunatum* using standard protocols. The results showed significant increase at LC<sub>10</sub> and LC<sub>25</sub> treated larvae in total body protein ( $86 \pm 10.583$  [control],  $156 \pm 12.49$  [LC<sub>10</sub>],  $218 \pm 22.5388$  [LC<sub>25</sub>]); glycogen ( $4.5 \pm 0.05774$  [control],  $6.5 \pm 0.05774$  [LC<sub>10</sub>],  $8.0 \pm 0.05774$  [LC<sub>25</sub>]) and lipid ( $54.0 \pm 0.73205$  [control],  $99.0 \pm 24.9799$  [LC<sub>10</sub>],  $180 \pm 15.5885$  [LC<sub>25</sub>]). The amount of free amino acid in the treated larvae ( $11.667 \pm 0.02848$  [LC<sub>10</sub>],  $8.92 \pm 0.52577$  [LC<sub>25</sub>]) showed decreased concentration when compared to control ones ( $18.9433 \pm 0.5429$ ) (Figure 4).

### Qualitative Analyses of Phytochemicals

Larvicidal activity of *C. infortunatum* is supported by the presence of certain phytochemicals such as alkaloids, flavonoids, tannins, glycosides, phenols (Table 2), which may contribute to their insecticidal effect as these secondary metabolites show variation among plants.

### Discussion

The medicinal plant *Clerodendrum* spp. possesses insecticidal activity against various insect pests [15]. In this study, *Clerodendrum* aqueous extract caused 50% mortality in fourth instar larvae of *Aedes albopictus* at 614.59 ppm. The toxic components of *Clerodendrum inerme* disrupt the process of digestion and absorption in *Aedes aegypti* larvae [16]. Furthermore, the impact of *Clerodendrum infortunatum* against *Oryctes rhinoceros* [17], and *Helopeltis theivora* Waterhouse [18] were also available in the literature. The leaf powder of *C. inerme* reported to have insecticidal and growth inhibitory activities against *Ae. aegypti* larvae [19].

Proteins are the major biological factors that play an important role in insect growth, development and various other physiological processes. This study showed a significant increase in total body protein concentration after treatment. The protein level increased after treatment with the phenol extracts of *Ziziphus jujube* in *Aedes aegypti* larvae and pupae [20]. The increase may be due to the production of detoxifying enzymes or defense proteins. It is reported that, animal under toxic stress activates a compensatory mechanism, increases whole body protein content or may synthesize set of conserved polypeptides, collectively referred to as heat shock proteins to combat with the toxic stress and to nullify the toxic effect [21, 22].

Qualitative analyses showed a significant increase in both glycogen as well as lipid when treated with aqueous extract of *Clerodendrum infortunatum*. The carbohydrate level also increased in anopheline larval tissue after treatment with *Artemisia annua* and *Azadirachta indica* extract. However, after treatment with *Azadirachta indica* extract, lipid profile increased in anopheline and culicine larval tissue. All types of insecticides have some negative impact on the growth and development of the insect and affect the metabolic and biochemical processes. The larvae, thus, were unable to assimilate the food resulting in increase of carbohydrate content. Anopheles larvae treated with methanol extract of *Azadirachta indica* displayed an increase in lipid content. *Culex* larvae also showed elevations over control in lipid level after treatment with the same extract [23]. The increase in lipid content may be due to alteration in lipid peroxidation rate during the detoxification, induced by the insecticidal stress [24].

The decrease in free amino acids can be attributed to increased neuromuscular activity of treated larvae which resulted in higher demands for energy. As a result of this, high amount of free amino acids may enter into the TCA cycle and oxidized amino acids are reported to have a role in accelerating moulting in insects [25]. Depletion of amino acids showed a definite impact in physiology and moulting process producing morphological abnormalities in treated insects [26]. The increase in biochemical profile demonstrates the physiological stress induced by the extract and the disturbed metabolic activity of the larvae. The effect of extracts on the metabolism of treated larvae depends on the nature and action of different phytochemicals present in these extracts.

Plants synthesize several secondary metabolites; among them some compounds are recognized as insecticidal molecules. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the body [27]. These chemicals may kill, retard or accelerate development or interfere with the life cycle of the insect in some other ways [28]. These chemicals can disrupt major metabolic pathways leading to rapid death.

The botanical insecticides are generally target-specific, readily biodegradable and usually lack toxicity to higher animals. According to Bowers *et al.* [29], the biological activity of the plant extract is due to various compounds like alkaloids, terpenoids etc. which are synthesized within plants in varying proportions. These compounds, either independently or jointly contribute to larvicidal activity of mosquitoes. Phytochemicals can thus be used as promising alternatives to synthetic insecticides as they are relatively safe, inexpensive and are readily available [30].

**IV. CONCLUSION AND FUTURE SCOPE**

The present study highlights the effect of aqueous extract of *Clerodendrum infortunatum* as potent larvicide against the mosquito vector *Aedes albopictus*. Result of this preliminary screening study and subsequent biochemical analyses clearly demonstrates the larvicidal effect as well as metabolic imbalance induced by this plant extract on mosquito larvae. In the field of IMM, this preliminary study opens the possibility of further investigations on evaluation, identification and isolation of major bioactive components of this plant extract. This can be incorporated into integrated mosquito control strategies. Further research in this area would eventually facilitate the application of this extract as an effective eco-friendly larvicidal agent in breeding sites around human dwellings.

**Figures and Tables**

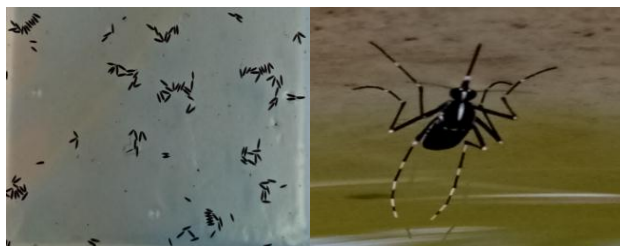


Figure 1: Eggs and Adult of *Aedes albopictus*

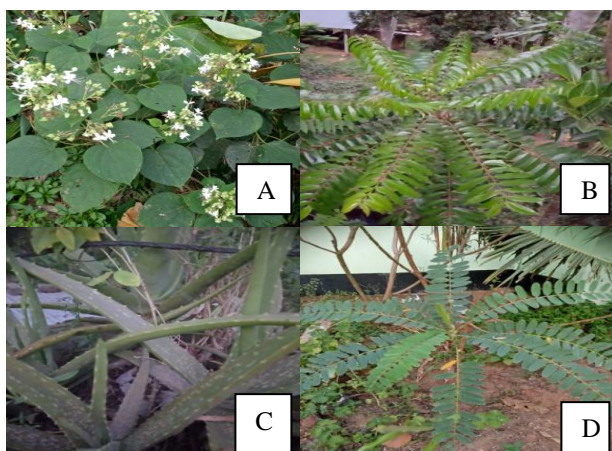
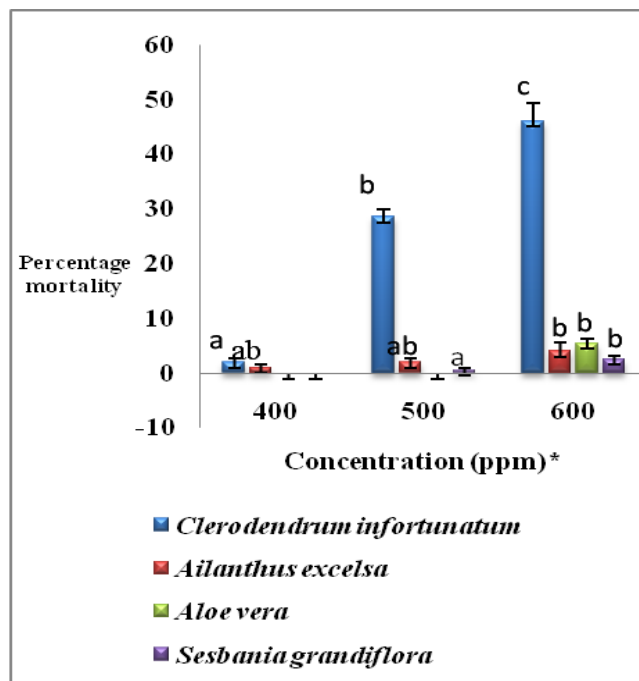


Figure 2: A: *Clerodendrum infortunatum*; B: *Ailanthus excelsa*; C: *Aloe vera*; D: *Sesbania grandiflora*



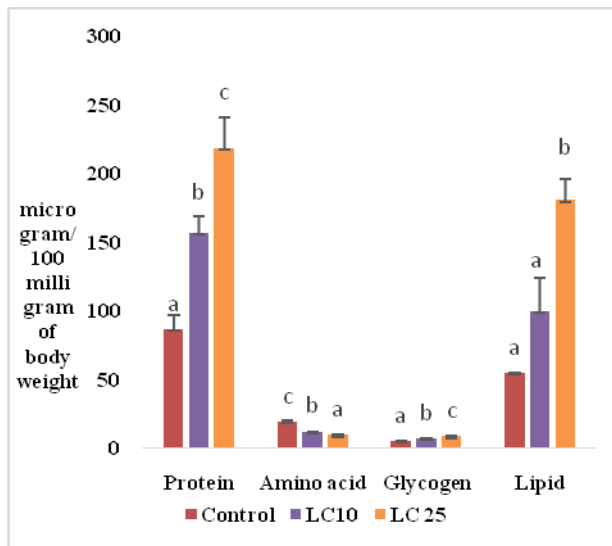
\*ppm - parts per million  
 Mean ± SE followed by the same letter in each plant do not differ significantly

Figure 3: Mean Values of Percentage Mortality after treating fourth instar larvae of *Aedes albopictus* with *C. infortunatum* and *A. excelsa*

Table 1: Lethal Concentration Values of Aqueous extracts on fourth instar larvae of *Aedes albopictus*

Aqueous Extracts	LC <sub>50</sub> (ppm)	LCL	UCL
<i>C. infortunatum</i>	641.6	596.942	714.134
<i>A. excelsa</i>	9769.8	2695.529	1,30,19,301.447
<i>A. vera</i>	12,200.2	3103.613	2,53,86,391.630
<i>S. grandiflora</i>	24,665.6	3371.092	**

\*\*not determined  
 LC<sub>50</sub>: lethal concentration that kills 50% of the exposed larvae  
 UCL: upper confidence limit (95% fiducial limit)  
 LCL: lower confidence limit (95% fiducial limit)



**Lc10:** lethal concentration that kills 10% of the exposed larvae.  
**Lc25:** lethal concentration that kills 25% of the exposed larvae.  
 Mean ± SE followed by the same letter in each group do not differ significantly

**Figure 4:** Mean Values of biochemical analyses of fourth instar larvae of *Aedes albopictus* after treating with *C. infortunatum*

**Table 2:** Qualitative Analyses of phytochemicals on aqueous extracts

Phytochemicals	<i>Clerodendrum infortunatum</i>	<i>Ailanthus excelsa</i>
Alkaloids	+	-
Flavonoids	+	-
Tannins	+	-
Saponins	-	+
Terpenoids	-	-
Glycosides	+	+
Quinones	-	-
Phenols	+	-

‘+’ Present, ‘-’ Absent

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**AUTHORS PROFILE**


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Indusree O.G, currently doing her Ph.D. programme in Vector Biology, Department of Zoology, University College, Thiruvananthapuram, Kerala, India.



Chandana J.S, now doing her Ph.D. programme in Pest Management, Department of Zoology, University College, Thiruvananthapuram, Kerala, India.



Dr. Ajitha V.S. awarded her M.Phil., Ph.D. and Post Doctorate in Zoology. She is currently working as Assistant Professor of Zoology, University College, Thiruvananthapuram; Kerala. She joined teaching profession in 2010. She published more than 13 research papers in reputed International and National journals and now doing research on insect pests and vectors. She has 13 years of research experience and has four research scholars working under her supervision.

