PUPICIDAL ACTIVITY AND MORPHOLOGICAL DEFORMITIES OF
JUSTICIA ADHATODA LEAF EXTRACTS AGAINST FILARIAL
VECTOR, CULEX QUINQUEFASCIATUS AND DENGUE VECTOR,
AEDES AEGYPTI

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ABSTRACT

The present study deals with the investigation of pupicidal activities of petroleum ether, chloroform, ethyl acetate and methanol leaf extract of Justicia adhatoda against Culex quinquefasciatus and Aedes aegypti. 25 pupae of C. quinquefasciatus and A. aegypti was exposed to various concentrations and was assayed in the laboratory by using the protocol of WHO (1996); the assay was carried out for 24h and 48h; LC₅₀ and LC₉₀ values of the J. adhatoda leaf extract was determined by Probit analysis. The LC₅₀ and LC₉₀ values of methanol leaf extract of J. adhatoda against pupal stages of C. quinquefasciatus were 153.73, 97.04 and 427.40 ppm and A. aegypti were 161.30, 106.79 and 425.20, 431.53 ppm respectively at 24h and 48h. Maximum pupicidal activity was observed in the methanol extract. No mortality was observed in control. Among four solvent tested the methanol and petroleum ether extracts were most effective against C. quinquefasciatus and A. aegypti. From the results it can be concluded the crude extract of J. adhatoda was an excellent potential for controlling C. quinquefasciatus and A. aegypti mosquitoes.

KEYWORDS: Pupicidal, Justicia adhatoda, Culex quinquefasciatus, Aedes aegypti, Morphological Deformities.
1. INTRODUCTION

Mosquitoes serve as obligate intermediate vector for numerous diseases of worldwide. There are a total of 34 genera and 3100 species of mosquitoes out of which three genera, Anopheles, Aedes and Culex are the primary vectors mainly include the malaria parasite (Plasmodium) filaria (Wuchereria and Brugia) and arboviruses. The classification of mosquitoes into the sub families Anophelinae, Culicinae and Toxorhynchitinae is based on their oviposition, morphology of larvae and pupae, breathing trumpet shape and size, resting angle of adults and shapes of proboscis.\[1,2\]

*Culex quinquefasciatus* is an obligatory ectoparasitic vector since it plays a major role in the transmission of the nocturnal periodic form of Bancroftian filariasis all over the world \[3\]. In tropical and subtropical countries dengue is the most important arboviral disease in terms of morbidity and mortality of worldwide. In 2010, there were a total of 28,292 cases and 110 deaths\[4\]. In 2012 a total of 9,000 cases and 50 deaths were reported in Tamilnadu.\[5\]

The problem of mosquitoes is a worldwide concern because of their inherent ability to act as intermediate host or vectors for parasitic and viral diseases of human being and domestic animals. In Tamil Nadu state most of the coastal districts are highly affected by mosquito-borne diseases.\[6\]

Synthetic insecticides and harmful chemicals in vector control has resulted into environmental hazards through persistence and accumulation of non-biodegradable toxic components in the ecosystem, development of insecticide resistance among mosquito species, biological magnification in the food chain and toxic effect on human health and non-target organisms.\[7\]

Medicinal plants have curative properties due to the presence of various complex chemical substances of different compositions, which are found as secondary plant metabolites in one or more parts of these plants.\[8\] Plant based products has been revived because of the development of resistance, cross-resistance and possible toxicity hazards associated with synthetic insecticides, bioaccumulation and pollution. Phytochemicals obtained from huge diversity of plant species are the major sources for safe and biodegradable chemicals, which can be screened for mosquito repellent and insecticidal activities.\[9\]
Acanthaceae, one of the 24 families in the mint order (Limiales) of flowering plants, containing approximately 220 genera and nearly 4,000 species distributed predominantly in tropical and subtropical regions of the world. The greater part of the Acanthaceae family are herbs or shrubs, but vines and trees occur as well. *Justicia adhatoda* is part of the Acanthaceae plant family. The different parts of the plant is used in the Indian traditional medicine for the treatment of various diseases like asthma, joint pain, lumber pain and sprains, cough, eczema, malaria, rheumatism, swellings, venereal diseases. In homeopathy, *Justicia Adhatoda* or *Adhatoda vasica* has been used in the treatment of cold, cough, pneumonia, spitting of blood, fever, jaundice, catarrh, whooping cough and asthma. The present study was carried out to assess the role of pupicidal activities of the leaf extracts of Acanthaceae family plant against *Culex quinquefasciatus* and *Aedes aegypti* at laboratory conditions.

2. MATERIALS AND METHODS

2.1 Plant Collection and authentication

Leaves of *Justicia Adhatoda* (Linn.) of Acanthaceae family were collected from the local areas of Vellore, Tamilnadu, India authenticated by professor P. Jayaraman, Botanist, Director, Plant anatomy research centre, Tambaram, Chennai, India in the month of May 2014 and registered Number of the Specimen is PARC/2014/2074.

2.2 Preparation of plant extracts

The leaves were washed with tap water, shade dried at room temperature (28 ± 2 ºC) for 5-8 days. The air dried materials were powdered separately using electrical blender. The finely ground plant material (500 g/solvent) was loaded in Soxhlet apparatus and was extracted with four different solvents namely petroleum ether, chloroform, ethyl acetate and methanol individually. The solvent from the extract was removed using a rotary vacuum evaporator to collect the crude extract. The crude residue of this plant varies with the solvents used. Standard stock solutions were prepared at 1% by dissolving the residues the universal solvents DMSO (dimethyl sulphoxide). From this stock solution, different concentrations (60-300 ppm) were prepared and these solutions were used for pupicidal activity.

2.3 Culture of test organism

The eggs of *Culex quinquefasciatus* and *Aedes aegypti* were collected from zonal entomological team, Vellore, Tamil Nadu, India using an “O”- type brush. These eggs were brought to the laboratory and transferred to (18×13×4 cm) enamel trays containing 500 ml of
water for hatching. The mosquito larvae were fed with pedigree dog biscuits and yeast at 3:1 ratio. The feeding was continued until the larvae transformed into the pupal stage. The pupae were collected from the culture trays and transferred to plastic containers (12×12 cm) containing 500ml of water with the help of a dipper.

2.4 Pupicidal Bio-assay

Laboratory colonies of *Culex quinquefasciatus* and *Aedes aegypti* pupae were used for the pupicidal activity. Twenty five numbers of pupae were introduced into 500 ml glass beaker containing 249 ml of de-chlorinated water and 1 ml of desired concentrations of plant extracts was added. Each tested concentration was five replicated. The control experiments were also run parallel with each replicate. The larval mortality was calculated after 24 hrs and 48 hrs of the exposure period. The control mortalities were corrected by using Abbott’s formula. The LC$_{50}$ and LC$_{90}$ were calculated from toxicity data by using probit analysis.

2.5 Deformities

During the course of lethal experiments, the morphological features of pupal stages from treated and control media were compared. Any notable difference in appearance between treated and control was recorded as deformity. The deformities were designated according to their similarity to those previously exhibited by.

2.6 Statistical analysis

The average pupal mortality data were subjected to Probit analysis for calculating LC$_{50}$, LC$_{90}$ and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and chi-square values were calculated using the SPSS 20.0 version software. Results with $P \leq 0.05$ were considered to be statistically significant.

3. RESULTS AND DISCUSSION

The pupicidal activity of Petroleum ether, Chloroform, Ethyl acetate and Methanol extracts of *Justicia adhatoda* leaf against *Culex quinquefasciatus* pupae reveals that the Methanol extract indicates the higher mortality rates compared to the other solvent extracts. Table 1 indicates the lethal toxicity of pupal mortality of *Culex quinquefasciatus*. The highest pupicidal potency with LC$_{50}$ and LC$_{90}$ was depicted after 24 hours was 153.73 ppm and 427.40 ppm. While, after 48 hours the LC$_{50}$ and LC$_{90}$ indicated 97.04 ppm and 410.36 ppm. The Chi-square values were 1.46 at 24h and 1.50 at 48h which indicates significant at $p \leq 0.05$ level.
each test included a control group with for each individual concentration. Moderate susceptibility was recorded in Petroleum ether followed by ethyl acetate and chloroform.

The pupicidal activity of petroleum ether, chloroform, ethyl acetate and methanol extracts of Justicia adhatoda leaf against Aedes aegypti pupae revealed that the Petroleum ether extract indicates the higher mortality rates compared to the other solvent extracts. The highest larvicidal potency with LC$_{50}$ and LC$_{90}$ was depicted after 24 hours was 157.41 ppm and 417.76 ppm. While, after 48 hours the LC$_{50}$ and LC$_{90}$ values indicated 101.28 ppm and 415.59 ppm. The 95% confidence limits LC$_{50}$ and LC$_{90}$ (LFL – UFL) were also calculated the results of pupicidal activity clearly indicate that the percentage of mortality being directly proportional to the concentration of the extract. This proves that concentration plays important role in pupicidal activity. The Chi-square values were 1.22 at 24h and 1.26 at 48h which showed significant at p≤0.05 level each test included a control group with for each individual concentration. Methanol followed by ethyl acetate and chloroform extracts shows moderate pupicidal activity in Aedes aegypti.

The morphological deformities occurred among the pupae exposed to lethal concentrations of Petroleum ether, Chloroform, Ethyl acetate and Methanol leaf extract of Justicia adhatoda. In the present study, the Aedes aegypti and Culex quinquefasciatus pupae exposed in different concentration of leaf extracts of Justicia adhatoda, in addition to changes in the indices of development, also exhibited, in common, a variety of metamorphic aberrations. Slightly demelanized pupa with straight abdomen, dwarf pupa with retarded abdomen, pupa with some melanization (black pupa) and partly emerged adult with attached pupal case.

Similar deformities were found to occur during the development of Aedes aegypti, Culex quinquefasciatus and Anopheles stephensi in media treated with hexane, diethyl ether, dichloromethane and ethyl acetate extracts of Murraya koenigii leaf: the hexane, diethyl ether, dichloromethane and ethyl acetate extracts of Abutilon indicum leaf were evaluated for their pupal deformities against A. aegypti, C. quinquefasciatus and A. Stephensi.[24, 25] In a review paper, Sukumar et al., (1991) summarized a list of 104 plant species for 49 families that possessed either larvicidal or pupicidal and/or adulticidal activity on Aedes aegypti.[25]
Table 1: Lethal concentration values of *Justicia adhatoda* leaf extracts against *Culex quinquefasciatus* pupae

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Period (hrs)</th>
<th>LC$_{50}$, ppm (LFL - UFL)</th>
<th>LC$_{90}$, ppm (LFL - UFL)</th>
<th>$X^2$ (df=4)</th>
<th>Regression Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum Ether</td>
<td>24</td>
<td>159.24 (131.20 - 193.27)</td>
<td>426.66 (149.81-1215.15)</td>
<td>1.61</td>
<td>Y = 2.99X - 1.59</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>103.95 (67.41 - 160.28)</td>
<td>421.63 (147.87 - 1202.21)</td>
<td>1.47</td>
<td>Y = 2.11X + 0.74</td>
</tr>
<tr>
<td>Chloroform</td>
<td>24</td>
<td>163.49 (136.09 - 196.40)</td>
<td>429.52 (149.86 - 1231.03)</td>
<td>1.33</td>
<td>Y = 3.06X - 1.76</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>118.21 (83.13 - 168.08)</td>
<td>444.14 (151.41 - 1302.87)</td>
<td>0.86</td>
<td>Y = 2.23X - 0.38</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>24</td>
<td>162.46 (135.03 - 195.46)</td>
<td>416.36 (148.40 - 1231.03)</td>
<td>5.46</td>
<td>Y = 3.15X - 1.93</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>133.67 (102.04 - 175.09)</td>
<td>427.00 (151.41 - 1204.20)</td>
<td>1.23</td>
<td>Y = 2.54X - 0.40</td>
</tr>
<tr>
<td>Methanol</td>
<td>24</td>
<td>153.73 (125.25 - 188.70)</td>
<td>427.40 (150.46 - 1168.14)</td>
<td>5.46</td>
<td>Y = 3.02X - 1.64</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>97.04 (59.80 - 157.46)</td>
<td>410.36 (147.83 - 1139.09)</td>
<td>1.50</td>
<td>Y = 2.05X + 0.93</td>
</tr>
</tbody>
</table>

*Control* nil mortality significant at P≤0.05 level, *LFL* lower fiducial limit, *UFL* upper fiducial limit, $X^2$ chi-square value (df = 4), df degree of freedom.

Table 2: Lethal concentration values of *Justicia adhatoda* leaf extracts against *Aedes aegypti* pupae

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Period (hrs)</th>
<th>LC$_{50}$, ppm (LFL - UFL)</th>
<th>LC$_{90}$, ppm (LFL - UFL)</th>
<th>$X^2$ (df=4)</th>
<th>Regression Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum Ether</td>
<td>24</td>
<td>157.41 (129.43 - 191.44)</td>
<td>417.76 (149.26 - 1169.26)</td>
<td>1.22</td>
<td>Y = 3.02X - 1.64</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>101.28 (64.22 - 159.72)</td>
<td>415.59 (147.71 - 1169.34)</td>
<td>1.25</td>
<td>Y = 2.09X + 0.81</td>
</tr>
<tr>
<td>Chloroform</td>
<td>24</td>
<td>164.21 (137.32 - 196.37)</td>
<td>413.81 (148.60 - 1152.42)</td>
<td>5.31</td>
<td>Y = 3.19X - 2.07</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>135.88 (104.73 - 176.30)</td>
<td>434.85 (151.42 - 1248.80)</td>
<td>1.31</td>
<td>Y = 2.54X - 0.41</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>24</td>
<td>167.99 (140.57 - 200.75)</td>
<td>428.14 (148.08 - 1237.93)</td>
<td>1.41</td>
<td>Y = 3.15X - 2.02</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>114.59 (79.28 - 165.61)</td>
<td>443.21 (152.04 - 1092.05)</td>
<td>1.00</td>
<td>Y = 2.18X + 0.51</td>
</tr>
<tr>
<td>Methanol</td>
<td>24</td>
<td>161.30 (133.80 - 194.44)</td>
<td>425.20 (149.98 - 1205.50)</td>
<td>1.45</td>
<td>Y = 3.04X - 1.72</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>106.79 (70.39 - 162.01)</td>
<td>431.53 (151.91 - 1225.86)</td>
<td>1.36</td>
<td>Y = 2.11X + 0.71</td>
</tr>
</tbody>
</table>

*Control* nil mortality significant at P≤0.05 level, *LFL* lower fiducial limit, *UFL* upper fiducial limit, $X^2$ chi-square value (df = 4), df degree of freedom.

Fig 1:1a. Normal Pupae (*C. quinquefasciatus*); 1b. Slightly demelanized pupa with straight abdomen.
Fig 2: 2a. Normal Pupae (A. aegypti); 2b. Pupa with some melanization (black pupa) and dwarf pupa with retarded abdomen.

4. CONCLUSION
The screening of the results suggest that the petroleum ether and methanol extracts of Justicia adhatoda are most effective in Aedes aegypti and Culex quinquefasciatus control and this plant leaf is an eco-friendly alternative to chemical insecticide which is promising in mosquito control.

5. Competing Interest
The authors declare that they have no competing interests.

6. ACKNOWLEDGEMENT
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7. REFERENCES


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