Laboratory study on larvicidal activity of different plant extracts against *Aedes aegypti*

Muhammad Uzair Mukhtar, Shumaila Mushtaq, Ali Arslan, Arkam Bakhtyar Zaki, Muhammad Hammad, Adil Bhatti

Abstract

**Background:** Mosquitoes transmit serious human diseases causing millions of deaths every year. Use of synthetic insecticides to control vector mosquitoes has caused physiological resistance and adverse environmental effects in addition to high operational cost. Insecticides of botanical origin have been reported as useful for control of mosquitoes.

**Methodology:** WHO methodology was adopted for larvicidal bioassay. Thirty late 3rd and early 4th instar larvae were subjected to four different concentrations i.e. 1%, 2%, 3% and 4% against *Cucurbita moschata* (seed), *Sesamum indicum* (seed), *Azadirachta indica* (Leaves) and *Pinus roxburghii* (Bark) oils in acetone as solvent. Mortality counts were made every 24 and 48 hours in each treatment. The LC50, LC99, standard error, fiducial limits at 95% confidence and regression equations were calculated.

**Results:** The results showed neem and pine oil extracts are best in terms of LC50 and LC99, with 100% mortality at 3% and 4% concentration after 24 hours. The trend with respect to LC50 and LC99 after 48 hours was Pine > Neem > Til > Kadu respectively.

**Conclusion:** The results suggested these plant extract were found effective in controlling *Aedes aegypti* larvae under lab conditions. As these trees are widely distributed in Pakistan, their formulation might prove to be an effective and eco-friendly larvicide, which could be used as an alternative for dengue control.

**Keywords:** Plant extracts, *Aedes aegypti*, Larvicidal, Dengue.

1. Introduction

Dengue is one of the most important viral diseases transmitted by *Aedes aegypti* because it afflicts humans worldwide whose symptoms ranging from mild fever to severe and potentially life threatening hemorrhagic disease. *Aedes aegypti* is of supreme concern because of its wide distribution and close association with humans [1]. *Aedes aegypti* is present in heavy polluted areas like Asia, America and some Pacific islands and about 2/3 of the world’s population are infected [2].

One recent estimate indicates 390 million dengue infections per year and prevalence of dengue, estimates that 3900 million people, in 128 countries, are at risk of infection with dengue viruses globally during year 2015 [3].

In Asia, the first outbreak of DHF began in the 1950s in the Philippines and Thailand. However, in the next 20 years, the disease spread throughout South East Asia and by the mid-1970s. Dengue fever epidemics were common in Asia and Pacific throughout the twentieth century [4]. In Pakistan, 40987 cases of dengue reported with 490 deaths during year 2006-11 [5]. In August 2013, dengue outbreak occurred in Kyber Pakhtun khaw province affecting more than 7000 people with 26 deaths [6].

Since there is no particular treatment and vaccination available, emphasis should be on control of dengue vector. Different mosquito control methods are being used including chemical method by targeting the adult mosquito through spraying chemical insecticides or by killing the mosquito larvae by using synthetic larvicides [7]. Insecticides though work good in terms of vector control but poses threats not only to human health but also to the ecosystem [8]. Other than the detrimental effect on human health, the significant increase in insecticide-based vector control in the past decade has resulted in increasing resistance among vectors. Resistance to pyrethroids had been identified in 64 countries [9]. Resistance to temephos has been recorded in *Aedes aegypti* in Asia including Cambodia [10], Thailand [11, 12], and Malaysia [13]. Emergence of resistance among vector mosquitoes is a recent problem. Safe and ecofriendly agents from biological origin are need of the hour [14]. The attempt of the present study was to screen and
identify those plants, getting their extracts and evaluating their 
efficacy against larvae to control the dengue vector \textit{Aedes} 
aegypti. 

2. Material and Methods 

2.1. Collection of Plants 

Following plants were collected from different cultivators in 
Rawalpindi and Islamabad. These are: 

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Botanical Name</th>
<th>Part used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kadu</td>
<td>\textit{Cucurbita moschata}</td>
<td>Seed</td>
</tr>
<tr>
<td>Til</td>
<td>\textit{Sesamum indicum}</td>
<td>Seed</td>
</tr>
<tr>
<td>Neem</td>
<td>\textit{Azadirachta indica}</td>
<td>Leaves</td>
</tr>
<tr>
<td>Chir pine</td>
<td>\textit{Pinus roxburghii}</td>
<td>Stem Bark</td>
</tr>
</tbody>
</table>

2.2. Extraction of oil 

The seeds were washed with tap water to remove the pulp and then 
dried in oven for 48 hours at 60 °C and later would be 
ground in an electric grinder (Anex Germany). While the other 
parts like leaves and bark were also dried. The grounded 
material was put in soxhelt apparatus for the extraction of oil 
by steam distillation method (Vogel, 1978) 

2.3. Preparation of Stock Solution 

These oils were collected in small vials and the quantity was 
measured. Stock solutions were prepared by adding 1 ml of oil 
from each plant in 99ml of acetone and were considered as 1% 
stock solution from which series of 4 concentrations (%) were 
prepared \cite{15}. 

2.4. Mosquito Rearing 

Adult susceptible colonies of \textit{Aedes aegypti} were maintained 
in an insectary of Medical Entomology and Disease Vector 
Control department of Health Services Academy Islamabad on 
10% sugar solution and females were blood fed on live white 
rats. Larvae were reared in steel trays (24 x 36 x 6 cm) and fed 
on sterilized broiler chicken liver diet. 

2.5. Larvicidal Bioassay 

The extracted oils were used in four different concentrations 
(1%, 2%, 3% and 4%) and their efficacy was evaluated by 
standard WHO method \cite{16}. Each replicate contained 200ml of 
the oil solution were placed in 500ml glass beakers. Batches of 
thirty late 3rd and early 4th instar larvae were exposed in each 
beaker containing the crude oil solution. A total of three 
replicates were conducted for each concentration \cite{17} and 
against each replicate, a control was present. The numbers of 
dead larvae were counted after 24 and 48 hours interval. The 
experiment was conducted under lab conditions at 27± 2 °C 
and 80± 5% relative humidity. 

2.6. Data analysis 

The data obtained was subjected to probit analysis and LC\textsubscript{50} & 
LC\textsubscript{99} values were calculated using MINITAB-16 software. Chi 
square analysis was also calculated to check the homogeneity 
of the tested population. 

3. Results 

Larvae of \textit{Aedes aegypti} were subjected against crude plant 
oils of \textit{Cucurbita moschata} (Kadu), \textit{Sesamum indicum} (Til), 
\textit{Azadirachta indica} (Neem) and \textit{Pinus roxburghii} (Chir pine). 
Four different concentrations of crude plant oils were tested. 
The results on the use of different concentration of plant 
extracts were recorded in terms of mortality against larvae of 
\textit{Aedes aegypti} under laboratory condition. Table 1 shows that 
Neem and Pine oil was considered best with LC\textsubscript{50} values 0.052 
and 0.089 respectively with 100% mortality at 3% and 4% 
concentration after 24 hours, followed by Kadu and Til with 
LC\textsubscript{50} values 0.71 and 1.41 with 45% and 18.33% mortality at 
4% concentration respectively after 24 hours. The mortality 
percentage of \textit{Aedes aegypti} larvae at each concentration after 
24 hours are shown in figure 1. 

<table>
<thead>
<tr>
<th>Crude plant oil</th>
<th>Lethal concentration</th>
<th>LFL</th>
<th>UFL</th>
<th>Slope ±S.E.</th>
<th>$\chi^2$</th>
<th>P value</th>
<th>Regression equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neem</td>
<td>LC\textsubscript{50}</td>
<td>0.05</td>
<td>0.004</td>
<td>0.09</td>
<td>6.77±0.97</td>
<td>0.15</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>LC\textsubscript{99}</td>
<td>0.39</td>
<td>0.31</td>
<td>0.52</td>
<td>6.77±0.97</td>
<td>0.15</td>
<td>0.92</td>
</tr>
<tr>
<td>Pine</td>
<td>LC\textsubscript{50}</td>
<td>0.08</td>
<td>0.41</td>
<td>0.13</td>
<td>6.17±0.76</td>
<td>0.88</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>LC\textsubscript{99}</td>
<td>0.46</td>
<td>0.39</td>
<td>0.58</td>
<td>6.17±0.76</td>
<td>0.88</td>
<td>0.64</td>
</tr>
<tr>
<td>Kud</td>
<td>LC\textsubscript{50}</td>
<td>0.71</td>
<td>0.614</td>
<td>0.92</td>
<td>2.67±0.54</td>
<td>4.09</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>LC\textsubscript{99}</td>
<td>1.58</td>
<td>1.25</td>
<td>2.34</td>
<td>2.67±0.54</td>
<td>4.09</td>
<td>0.12</td>
</tr>
<tr>
<td>Til</td>
<td>LC\textsubscript{50}</td>
<td>1.41</td>
<td>0.91</td>
<td>10.85</td>
<td>1.1±0.51</td>
<td>0.58</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>LC\textsubscript{99}</td>
<td>3.51</td>
<td>2.02</td>
<td>32.3</td>
<td>1.1±0.51</td>
<td>0.58</td>
<td>0.74</td>
</tr>
</tbody>
</table>

LC\textsubscript{50} = Lethal concentration 50 at which 50% of target population died.  
LC\textsubscript{99} = Lethal concentration 99 at which 99% of target population died.  
LFL = Lower fiducial limit  UFC = Upper fiducial limit  
SE = Standard error  
$\chi^2$ = Chi-square.  
p-value = Level of significance $p \leq 0.05$, $p \geq 0.05$ non-significant 

Fig 1: Total Larval mortality of \textit{Aedes aegypti} against different crude plant oils after 24 hours
Table 2 indicates Neem and Pine oil presented excellent results with LC₅₀ values 0.10 and 0.18 respectively with 100% mortality at 3% and 4% concentration, after 48 hours exposure followed by Til and Kadu with LC₅₀ values 0.21 and 0.27 with 98.3% and 96.6% mortality at 4% concentration respectively. The mortality percentage of Aedes aegypti larvae at each concentration after 48 hours are shown in figure 2.

<table>
<thead>
<tr>
<th>Plant Extract</th>
<th>Lethal Concentration</th>
<th>LC₅₀</th>
<th>LC₉₀</th>
<th>UFL</th>
<th>LFL</th>
<th>Slope ±SE</th>
<th>χ²</th>
<th>P value</th>
<th>Regression equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neem</td>
<td>LC₅₀</td>
<td>0.10</td>
<td>-0.26</td>
<td>-0.03</td>
<td>4.72±1.01</td>
<td>0.30</td>
<td>0.85</td>
<td>Y = 0.51+4.72x</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LC₉₀</td>
<td>0.38</td>
<td>0.27</td>
<td>0.61</td>
<td>4.72±1.01</td>
<td>0.30</td>
<td>0.85</td>
<td>Y = 0.51+4.72x</td>
<td></td>
</tr>
<tr>
<td>Pine</td>
<td>LC₅₀</td>
<td>0.18</td>
<td>-0.49</td>
<td>-0.07</td>
<td>4.56±1.28</td>
<td>0.13</td>
<td>0.93</td>
<td>Y = 0.83+4.56x</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LC₉₀</td>
<td>0.32</td>
<td>0.21</td>
<td>0.67</td>
<td>4.56±1.28</td>
<td>0.13</td>
<td>0.93</td>
<td>Y = 0.83+4.56x</td>
<td></td>
</tr>
<tr>
<td>Til</td>
<td>LC₅₀</td>
<td>0.21</td>
<td>-0.56</td>
<td>-0.06</td>
<td>2.32±0.49</td>
<td>0.53</td>
<td>0.76</td>
<td>Y = 0.50+2.32x</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LC₉₀</td>
<td>0.78</td>
<td>0.60</td>
<td>1.19</td>
<td>2.32±0.49</td>
<td>0.53</td>
<td>0.76</td>
<td>Y = 0.50+2.32x</td>
<td></td>
</tr>
<tr>
<td>Kadu</td>
<td>LC₅₀</td>
<td>0.27</td>
<td>-0.75</td>
<td>-0.08</td>
<td>1.98±0.47</td>
<td>0.40</td>
<td>0.81</td>
<td>Y = 0.53+1.98x</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LC₉₀</td>
<td>0.89</td>
<td>0.67</td>
<td>1.47</td>
<td>1.98±0.47</td>
<td>0.40</td>
<td>0.81</td>
<td>Y = 0.53+1.98x</td>
<td></td>
</tr>
</tbody>
</table>

LC₅₀ = Lethal concentration 50 at which 50% of target population died.
LC₉₀ = Lethal concentration 99 at which 99% of target population died.
LFL = Lower fiducial limit
UFL = Upper fiducial limit
SE = Standard error
χ² = Chi-square.
p value = Level of significance ≤ 0.05, ≥ 0.05 non-significant.

**4. Discussion and conclusion**

Many plant based products are widely used for their insecticidal properties for the control of mosquitoes [18]. In recent years interest in plant origin products has been revived because of the development of resistance, cross resistance and toxicity hazards associated with synthetic insecticides [19]. A large number of pant products have been reported to have mosquito larvicidal activity [20]. The results of the present study evaluated the larvicidal activity of Cucurbita moschata (Kadu), Sesamum indicum (Til), Azadirachta indica (Neem) and Pinus roxburghii (Chir pine) oils which were comparable with findings of other researchers like Vatandoost et al. (2004) who tested 400 larvae against neem oil and the mortality rate of larvae was 15.8%. Examination of larvicidal activities of pine concluded that pine oil has varying degree of larvicidal activity with LC₅₀ value ranging between 82 and 112 ppm (Ansari et al. 2005). Larvicidal activity of five species of Cucurbitaceae plants showed extremely effective against the larvae of Aedes aegypti with values (LC₅₀=74.57, 309.46, 492.73, 199.14, and 554.20 ppm) respectively (Rahuman et al. 2008). Larvicidal activities of 100 Indian coastal plant extracts were examined against Aedes aegypti among which Til oil showed 34% to 100% mortality at different concentrations (Nazar et al. 2009). From the results of the present study it was concluded that Neem and Pine oil were found to have larvicidal activity under lab conditions with best efficacy in terms of LC₅₀, LC₉₀ and percentage mortality after 24 and 48 hours respectively. In search of alternative and safe methods of controlling dengue vector mosquito products, essential oils might prove to be a good vector control tool which might be more safe to use and cost effective.

**5. Acknowledgment**

The facilities provided by Health Services Academy to carry out this research and technical guidance by respected faculty of department of MEDVC and our insectary staff for the rearing and collection of larvae are highly acknowledged.

**6. References**


