The Effect of Neem (*Azadirachta indica*) Leaves Extract on the Ecdysis and Mortality of Immature Stages of Common House Mosquito *Culex pipiens fatigans*

*MISBAH RASHID* & **AFTAB AHMAD**

**Department of Zoology, Government College University Lahore**

**ABSTRACT**

Larvicidal effect of *Azadirachta indica* on mosquitoes has been studied in the present work. The water extracts of air dried leaves and fresh leaves separately have been used for the study. Different concentrations were made of all these extracts and twenty second instar larvae of *Culex pipiens fatigans* were placed in each concentration separately. Control was set up for each experiment. Mortality and ecdysis inhibition effect were used as effective parameters. Observations on mortality were carried out after each 24 hours. The 25% stock solution of dry leaves extract showed greatest larvicidal activity i.e. 100% mortality within first 24 hours as compared to the mortality rate of fresh leaves and simple dry leaves extracts. The LC$_{50}$ of 25% stock solution extract, fresh leaves and dry leaves extracts was found to be 19%, 33%, 40% respectively. Percentage mortality per day, percentage pupation and percentage emergence from pupae are calculated. In lower concentrations 1%, 5%, 10%, pupation was delayed by two or three days. In 40%, 50% and 60% concentration of all these extracts, pupation did not occur. All the larvae died at larval stage but the larval period was extended to 12-14 days. Conclusively it can be said that simple aqueous extract of neem leaves have some biologically active components which show insecticidal activity. So they can be applied easily for the biological control of mosquitoes.

**Key words:** Neem, Leaf extract, *Culex pipiens fatigans*

---

**INTRODUCTION**

*Culex pipiens fatigans* (Diptera: Culicidae) is a common house mosquito. It is a major vector of filariasis, Eastern equine encephalitis and St. Louis encephalitis. It may also be involved in the transmission of bird malaria, heart worm of dogs and fowl pox (William & Maurice, 1961). It is prevalent in urban areas and is one of the most important biting nuisance mosquitoes, having high densities in nearly all residential areas of the cities (Youdeowei & Service, 1983; Curtis, 1994; Collins & Paskewitz, 1995). It breeds in highly polluted, stagnant waters. Different insecticides are used for the chemical control of mosquitoes. But these insecticides and larvicides are very expensive. They also cause pollution and toxicity to man, crop, plants, domestic animals, wild life and also kill the other desirable fauna by introducing the toxicant in food chain. The mosquitoes are becoming resistant to a wide range of pesticides (Rathore et al., 1986). This makes room to consider some other larvicides or bio insecticides which must be cheap and appropriate and could safely be used for vector control.

Every part of *Azadirachta indica* (neem) has been advocated to possess medicinal properties. Pruthi (1937) first proved scientifically the insecticidal effect of neem. “Azadirachtin a microcrystalline compound isolated from neem kernel extract is a promising larvicide against *Culex pipiens*. Naturally occurring bio pesticides could be an alternative to chemical pesticides” (Abdelouaheb et al., 2009). It has been reported that it possesses many substances which interfere with insect molting, food uptake, reproduction and provides a nontoxic insect controlling agent for use in agriculture. These cause growth inhibition, abnormal development, elongation of larval period and no pupation (Ascher, 1984; Isman, 1993; Ladd, 1984; Mari, 1989; Naqvi et al., 1991; Naqvi et al., 1994).

Aqueous neem kernel extracts were used for warding off insect attack on crops. Neem leaf juice is used for expelling worm and curing jaundice and skin diseases. Oil from nuts and leaves is a stimulant insecticide and antiseptic. It inhibits feeding in a variety of insects and also inhibits ecdysis at much lower concentrations (Mari & Watanabe, 1989). This prevents the insect larvae from developing into mature insects which could further multiply and produce new generations. It blocks receptor of ecdysteroids which are needed for larval development (Govindachari, 1992). Azadirachtin also increased residence time in the feeding and nonfeeding immature stages, larva treated with 1.6µg of azadirachtin for example, had
significant longer larval periods than did untreated larvae; length of prepupal and pupal stages was extended (Ladd, 1984). Lin & Liu (2006) studied properties and efficacy of pesticides from neem tree, and found them effective antifeedants for pest control. Azadirachtin were growth inhibitors. They interfere with neuroendocrine regulation of juvenile and molting hormone titers (Rembold, 1988).

Toxicity and abnormalities caused by neem fractions, RBU-9, RB-b and Margosan-OTM were determined against fourth instar larvae of Aedes aegypti, partially emerged adults were found with crumpled and entangled legs in puparium (Naqvi et al., 1994).

Simple formulations of neem derivatives, such as leaf or kernel powder or extracts are safe to non-target organisms including humans (Saxena, 1988). Recent studies encouraged the investigation of insecticidal properties of plant-derived extracts; and concluded that they are environmentally safe, degradable, and target specific (Senthil et al., 2006).

Neem is a natural insecticide and is nonhazardous to man and other mammals (Oudegans, 1991). Therefore simple nonhazardous and inexpensive methods of extraction should be developed to enable practical use of neem (Feuerhake, 1984).

The aims and objectives of the present work is to develop a simple inexpensive and nontoxic method for the control of mosquito larvae by A. indica (neem), which can be applied easily by the ordinary man without use of costly spraying equipment.

MATERIALS AND METHODS

Culex pipiens fatigans larvae were chosen as experimental insect for this study, because it is the major vector for filariasis and is one of the most important biting nuisance mosquitoes, having high densities in nearly all residential areas of the cities. Larvae of Culex pipiens fatigans were reared in the insectary and second star larvae were selected for experimental work.

Extracts were prepared from leaves of neem in water and no organic solvent was used. The extracts of air dried and fresh leaves were prepared separately. For fresh leaves extract, 1000 gms of fresh leaves were crushed in 1000 ml of water and kept for 24 hours. This mixture was then filtered with the help of muslin cloth and the filtrate was used for the experiment.

For dry leaves extract, 1000 gms of fresh leaves were dried in shade for 15 days and then powdered. 500 gms of dry powder of leaves was mixed in 1000 ml of water and kept for 48 hours. This mixture was then filtered with the help of muslin cloth and the filtrate was used for the experiment.

Another method was adopted for the preparation of neem leaves extract. For this, 500 gms powder of dry leaves was mixed in 1500 ml of water and kept for 48 hrs. This mixture was then filtered with muslin cloth and the filtrate was heated on water bath, till all the water was evaporated, 25 gm. dry neem extract was dissolved in 75ml of water to make the total volume 100ml so that 25% stock solution was prepared. This 25% stock solution was used for the preparation of further dilutions.

All the above extracts were used for the preparations of different concentrations i.e. 1%, 5%, 10%, 20%, 30%, 40%, 50% and 60%. Second instar larvae of about same age were treated with such concentrations according to WHO method. Three replicates were taken for each concentration. In control only 250 ml water was added. Then 20 second instar larvae were placed in each beaker. A pinch of liver powder was sprinkled on each beaker, which serves as larvae’s food. The beakers were covered with nets to avoid egg laying of mosquitoes. The room temperature and beaker’s temperature were noted. The survival and mortality of these were checked after each 24 hours and the results were recorded in tabular form.

Statistical analysis

The data was analyzed statistically. % mean mortality per day was calculated. Regression lines were plotted on graph papers to determine the LC₅₀ values. And the time taken by the larvae to pupate and for emergence was also noted. %age pupation, %age emergence were calculated.

RESULTS

Effects of neem leaf extracts on the larvae mortality of C. pipiens: Larval mortality was noted after every 24 hrs. for 8 days. In the first set of experiments using 25% stock solution of dry leaves extracts, the 100% mortality was observed in 30%, 40%, 50% and 60% concentrations (Table 1). In the second set of experiments using dry leaves extracts, the 100% mortality was observed in 50% and 60% concentrations (Table 2). In the third set of experiments using fresh leaves extracts, the 100% mortality was observed in 40%, 50% and 60% concentrations (Table 3). Average percentage mortalities of eight concentrations were calculated and regression line was plotted on graphs papers (fig 1, 2, 3). The LC₅₀ value of 25% stock solution, dry and fresh leaves were 19% (fig 1), 40% (fig 2) and 33% (fig 3).
<table>
<thead>
<tr>
<th>Conc.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>% Pupation</th>
<th>% Emergence from pupae</th>
<th>Pupation Period in days</th>
<th>Emergence Period in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>8</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>15</td>
<td>20</td>
<td>21</td>
<td>_</td>
<td>86</td>
<td>90</td>
<td>3.5</td>
<td>5.7</td>
</tr>
<tr>
<td>5%</td>
<td>13</td>
<td>16</td>
<td>20</td>
<td>21</td>
<td>23</td>
<td>33</td>
<td>40</td>
<td>_</td>
<td>78</td>
<td>76</td>
<td>3.5</td>
<td>5.7</td>
</tr>
<tr>
<td>10%</td>
<td>53</td>
<td>56</td>
<td>58</td>
<td>60</td>
<td>61</td>
<td>71</td>
<td>78</td>
<td>81</td>
<td>40</td>
<td>40</td>
<td>4.6</td>
<td>5.8</td>
</tr>
<tr>
<td>20%</td>
<td>61</td>
<td>73</td>
<td>76</td>
<td>80</td>
<td>81</td>
<td>90</td>
<td>_</td>
<td>_</td>
<td>0</td>
<td>0</td>
<td>No pupation</td>
<td>_</td>
</tr>
<tr>
<td>30%</td>
<td>70</td>
<td>90</td>
<td>100</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>0</td>
<td>0</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>40%</td>
<td>86</td>
<td>100</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>0</td>
<td>0</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>50%</td>
<td>100</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>0</td>
<td>0</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>60%</td>
<td>100</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>0</td>
<td>0</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>_</td>
<td>_</td>
<td>5</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>100</td>
<td>95</td>
<td>3.5</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Table 2: Effect of dry leaves extract of *Azadirachta indica* on larvae/pupae of mosquito

<table>
<thead>
<tr>
<th>Conc.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>% Pupation</th>
<th>% Emergence from pupae</th>
<th>Pupation Period in days</th>
<th>Emergence Period in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>11</td>
<td>13</td>
<td>16</td>
<td>18</td>
<td>20</td>
<td>25</td>
<td>30</td>
<td>_</td>
<td>80</td>
<td>89</td>
<td>3.5</td>
<td>5.7</td>
</tr>
<tr>
<td>5%</td>
<td>16</td>
<td>21</td>
<td>25</td>
<td>26</td>
<td>30</td>
<td>40</td>
<td>73</td>
<td>_</td>
<td>71</td>
<td>79</td>
<td>3.6</td>
<td>5.8</td>
</tr>
<tr>
<td>10%</td>
<td>26</td>
<td>31</td>
<td>35</td>
<td>36</td>
<td>40</td>
<td>56</td>
<td>63</td>
<td>_</td>
<td>60</td>
<td>38</td>
<td>4.6</td>
<td>5.8</td>
</tr>
<tr>
<td>20%</td>
<td>36</td>
<td>41</td>
<td>45</td>
<td>50</td>
<td>53</td>
<td>58</td>
<td>65</td>
<td>70</td>
<td>0</td>
<td>No</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>30%</td>
<td>43</td>
<td>51</td>
<td>56</td>
<td>61</td>
<td>66</td>
<td>70</td>
<td>75</td>
<td>80</td>
<td>0</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>40%</td>
<td>48</td>
<td>58</td>
<td>63</td>
<td>68</td>
<td>73</td>
<td>75</td>
<td>80</td>
<td>85</td>
<td>0</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>50%</td>
<td>65</td>
<td>75</td>
<td>81</td>
<td>86</td>
<td>91</td>
<td>95</td>
<td>100</td>
<td>_</td>
<td>0</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>60%</td>
<td>68</td>
<td>86</td>
<td>98</td>
<td>100</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>0</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>0</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>95</td>
<td>100</td>
<td>3.5</td>
<td>5.6</td>
</tr>
</tbody>
</table>
Table 3: Effect of fresh leaves extract of *Azadirachta indica* on larvae/pupae of mosquito

<table>
<thead>
<tr>
<th>Conc.</th>
<th>% Mean Mortality per day (Approx.)</th>
<th>% Pupation</th>
<th>% Emergence from pupae</th>
<th>Pupation Period in days</th>
<th>Emergence Period in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>3 6 8 11 16 21 – –</td>
<td>90</td>
<td>87</td>
<td>3.5</td>
<td>5.7</td>
</tr>
<tr>
<td>5%</td>
<td>15 20 21 26 31 38 – –</td>
<td>75</td>
<td>80</td>
<td>3.6</td>
<td>5.7</td>
</tr>
<tr>
<td>10%</td>
<td>31 38 43 48 56 65 70 –</td>
<td>50</td>
<td>60</td>
<td>3.7</td>
<td>5.8</td>
</tr>
<tr>
<td>20%</td>
<td>41 48 55 60 66 80 85 –</td>
<td>40</td>
<td>37</td>
<td>4.7</td>
<td>5.8</td>
</tr>
<tr>
<td>30%</td>
<td>51 61 70 75 80 88 91 93</td>
<td>18</td>
<td>18</td>
<td>5.8</td>
<td>7</td>
</tr>
<tr>
<td>40%</td>
<td>63 75 86 88 91 95 96 98</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>50%</td>
<td>68 86 100 – – – – – – – – – – –</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>60%</td>
<td>81 100 – – – – – – – – – – – –</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Control</td>
<td>0 0 0 0 0 – – – – – – – – – – – –</td>
<td>100</td>
<td>100</td>
<td>3.5</td>
<td>5.6</td>
</tr>
</tbody>
</table>

**Effect of neem leaf extracts on the ecdysis of C. pipiens:**

The %age pupation, %age emergence and % morality were drawn in the form of graphs, which showed the difference in different types of extracts (Fig. 4, 5, 6). In lower concentrations 1%, 5%, 10%, pupation was delayed by two or three days. In 40%, 50% and 60% concentration of all these extracts, pupation was nil. All the larvae died at larval stage but the larval period was extended to 12-14 days on the average and the ecdysis of larvae was inhibited. In fresh leaves extracts, only 40% and 18% larvae could pupate in 20% and 30% (Fig., 6).

**Fig., 1:** Toxicity curve showing % mortality and LC<sub>50</sub> value of *Culex pipiens fatigans* larvae in 25% stock solution extracts.

**Fig., 2:** Toxicity curve showing % mortality and LC<sub>50</sub> value of *Culex pipiens fatigans* larvae in dry leaves extracts.

**Fig., 3:** Toxicity curve showing % mortality and LC<sub>50</sub> value of *Culex pipiens fatigans* larvae in fresh leaves extracts.
The effect of Neem leaves extract on mosquito

Fig., 4: Percentage larval mortality, pupation and adult emergence of *Culex pipiens fatigans* larvae in 25% stock solution of dry leaves extracts.

Fig., 5: Percentage larval mortality, pupation and adult emergence of *Culex pipiens fatigans* larvae in dry leaves extracts.

Fig., 6: Percentage larval mortality, pupation and adult emergence of *Culex pipiens fatigans* larvae in fresh leaves extracts.

**DISCUSSION**

Simple aqueous extract method was used, so that an ordinary man could prepare it at home, without using expensive organic solvent and scientific equipment. The 25% stock solution was most effective as compared to fresh leaves and simple dried leaves extract. The molting period of larvae was delayed. Most of the larvae could not pupate and remained alive in larval stage till 12 days. Similar results was also achieved by Abdelouaheb *et al.* (2009) that Azadirachtin treatment prolonged the duration of the larval stage of *Culex pipiens*. And the results of study indicate that plant-based compounds such as Azadirachtin may be an effective alternative to conventional synthetic insecticides for the control of *Culex pipiens*.

Larvicidal activity of *Azadirachta indica* against various species of mosquitoes has been observed by various researchers (Wandscheer, 2004; Chavan, 1984; Virendra *et al.*, 2009; Aliero *et al.*, 2003; Vatandoost & Vaziri, 2004; Abdelouaheb *et al.*, 2009; Senthil *et al.*, 2006).

The extracts produced some abnormalities in larvae. Larval pupal intermediates were observed. Partially emerged adults showed crumpled legs and entangled in pupation. All these abnormalities were also reported by Naqvi (1987).

The development of insects' growth regulators (IGR) has gained considerable attention for selective control of insect of medical and veterinary importance and has produced mortality due to their high neurotoxic effects (Wandscheer *et al.*, 2004; Senthil *et al.*, 2006). Lucantoni *et al.*, (2006) results indicated that the neem, revealed a delay in oocyte development in the vitellogenesis of female mosquito, *Anopheles stephensi*. This disruption of reproductive capability could lead to significant population decline over time.

In addition to azadirachtin, a number of other active ingredients have also been isolated and identified from different parts of the neem tree, such as salannin, melantriol and nimbin (Mulla & Su, 1999; Ruskin, 1992). Two new triterpenoids (22, 23-dihydrorimocinol and des-furano-6-alpha-hydroxyazadiradione) were isolated from a methanolic extract of the fresh leaves of *Azadiracta indica* along with a known meliacin, 7-alpha-senecioyl-(7-deacetyl)-23-O-methylnimocinolide (Siddique, 2002). Neem components show multiple effects against different insects such as mosquitoes, flies, triatomine bugs, cock-roaches, fleas, lice and ticks (Mulla & Su, 1999; Ruskin, 1992). Neem leaf and seed extracts also showed efficacy against stored grain pests (Sharif *et al.*, 2007). Neemarin, at the recommended concentrations in field studies of
1 and 2 L/hectare, significantly reduces the frequency of larvae and the estimated residual effect was 7 days (Vatandoost & Vaziri, 2004).

The extracts of neem leaves in different solvents (petroleum ether, ether and EtoH) were evaluated for mosquito (Culex pipiens fatigans) larvicidal activity according to W.H.O. method. The 1% petroleum ether extract showed 100% mosquito larvicidal activity, it also had good residual activity (for 144hr) at 0.2% (Chavan, 1984).

Conclusively it can be said that neem has some biologically active components which show insecticidal activity. This conclusion is supported by the previous investigations of various workers. So neem products may be used as mosquito population controlling agent, which is a vector of many diseases. They are cheaper and biodegradable and can be used easily by an ordinary man without hazardous effects. Moreover resistance does not develop in insects against them, due to their multiple mode of action on insects (Vollinger, 1987).

REFERENCES


