LARVICIDAL AND REPELLENT EFFECT OF LEAF AND STEM EXTRACTS FROM CESTRUM NOCTURNUM (Solanaceae) AGAINST CULEX PIPIENS L. (DIPTERA: CULICIDAE)

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Abstract

The larvicidal and repellent effects of the crude ethanol and acetone leaf and stem extracts of the widely grown plant, Cestrum nocturnum, against Culex pipiens 3rd instar larvae and adults were evaluated. Ethanolic leaves extract was found to be the most effective against the larvae, with LC50 value of 377.1ppm followed by acetone leaves and stems extracts with LC50 values of 484.2 and 994.0ppm; respectively and finally ethanolic leaves extract with LC50 value of 1043.8ppm. The plant extracts tested reduced the percentages of pupation and adult emergence. Also, varying degrees of morphogenic abnormalities in immature and adult stages were observed. Moreover, the acetone stem extract showed a highly delayed toxic effect on pupae resulted from the treated larvae, where the pupal mortality % was 100% at all concentrations used. All concentrations of plant extracts used in the present study exhibited a repellent activity against the adult mosquitoes. The repellent action of the plant extracts tested was varied depending on plant part, solvent and the dose of the extract. Because of the high larvicidal and pupicidal potential of plant extracts tested, they can be used as effective alternatives to the existing synthetic pesticides for the control of Culex pipiens.

Key words: Ethanolic extract, Acetone extract, Petroleum ether extract, Toxicity, Cupressus sempervirens, Culex pipiens.

Introduction

Insect-transmitted disease remains a major cause of illness and death worldwide. Mosquitoes alone transmit disease to more than 700 million people annually (Taubes, 2000). Therefore, the control of mosquitoes is an important public health concern around the world. For example, Culex pipiens is the main vector of Rift valley fever virus (Darwish and Hoogastall, 1981), Wuchereria bancrofti (Gad et al., 1996) and Western Nile virus (Pelah et al., 2002). The only efficacious approach to minimizing the incidence of these diseases is to eradicate and control mosquito
vectors, mainly by applying insecticides to larval habitants, and educating the public (Corbel et al., 2004).

Chemical control is an effective strategy used extensively in daily life. Synthetic insecticides are today at the forefront of mosquito controlling agents. Nevertheless, controlling the mosquitoes has become complicated because of their resistance to synthetic insecticides, as well as the toxicity of insecticides to fish and other non-target organisms (Wattanachai and Tintanon, 1999; Rohani et al., 2001). There is an urgent need to develop new materials for controlling mosquitoes in an environmentally safe way, using biodegradable and target-specific insecticides against them.

Due to environmental concern on use of existing synthetic insecticides for vector control and further risk of development of widespread insecticides resistance in disease vector; interest on possible use of environment friendly natural products such as extracts of plants or plant parts increased for vector control Jawale et al., (2010).

Sukumar et al. (1991) listed 346 plant species of 276 genera and 99 families which have been tested against mosquitoes for various effects such as toxicity, growth inhibition, ovipositional determinacy and repellency. This list includes many species from Solanaceae family. Recently, Ghosh and Chandra (2006) and Ghosh et al. (2008) evaluated phytosteroidal compound of mature leaves of day jasmine, Cestrum diurnum (Solanaceae: Solanales) against larvae of Culex quinquefasciatus and Anopheles stephensi. The plant extracts used in the present study have been pointed as a promising alternative to combat this vector. In this work we evaluate the potential of extract from C. nocturnum as larvicide and repellent against 3rd instar larvae and adult of Culex pipiens.

Materials and Methods

1. Mosquito as colony:

Culex pipiens used in this study were obtained from Medical Entomology Research Center, Doki, Cairo, Egypt. They were reared for several generations, in the insectary of medical entomology at the Department of Zoology faculty of science, Al-Azhar University under controlled conditions at temperature of 27±2°C, relative 70±10% R.H. and 12-12 light-dark regime. Adult mosquitoes were kept in (30 x 30 x 30 cm) wooden cages and daily provided with sponge pieces soaked in
10% sucrose solution for a period of 3-4 days after emergence. After this period the females were allowed to take a blood meal from a pigeon host, which is necessary for laying eggs (anautogeny). Plastic oviposition cup (15x15cm) containing dechlorinated tap water was placed in the cage. The resulting egg rafts picked up from the plastic dish and transferred into plastic pans (25 x 30 x 15 cm) containing 3 liters of tap water left for 24 h. The hatching larvae were provided daily with fish food as a diet. This diet was found to be the most preferable food for the larval development and a well female fecundity, (Kasap and Demirhan, 1992).

2. Plant tested:

The plants selected for this study are listed in table (1) which included common name, scientific name, family, habitat, collection site.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>family</th>
<th>Habitat</th>
<th>collection site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Night jessamen</td>
<td>Cestrum nocturnum</td>
<td>Solanaceae</td>
<td>Desert</td>
<td>Sadat city (Cairo-Alexandria desert road)</td>
</tr>
</tbody>
</table>

3. Extraction of plant materials:

The plant parts after they collected from natural habitat, were left to dry at room temperature (27-31°C) and pulverized to powder separately in a hammer mill. The extraction was performed using 70% ethanol, and acetone solvents. One hundred grams of powder from each part of the plant for each solvent separately were extracted five times with 300 ml of aqueous 70% ethanol, and acetone at room temperature. After 24 h., the supernatants were decanted, filtrated through whatman filter paper No. 5. and dried in a rotary evaporator. The dry extracts were weighed and kept in deep freezer (- 4°C) till used for experiments.

4. Experimental bioassay:

4.1. Larvicidal activity:

In order to study the toxicity of the concerned plant extracts, the tested material of the ethanolic extracts was dissolved in 0.1ml of 70% ethanol, while the tested material of acetone extracts was dissolved in 2 drop of Tween. 80 as emulsifier to facilitate the dissolving of tested material in water. Different range of concentrations of each concerned extract was prepared in order to detect mortalities. All tested
materials were performed in 100ml of dechloronated tap water contained in 200ml plastic cups. Then, third 3rd instar larvae were put immediately into plastic cups contained different concentrations of extracts. At least three replicates were usually used for each tested concentration. All plastic cups were incubated under controlled conditions of 27±2°C, 70±10% relative humidity and 12-12 light-dark regime. Control larvae received 0.1 ml of 70% ethanol or 2 drop of Tween.80 in 100ml water. Mortality was recorded daily and dead larvae and pupae removed until adult emergence. Abnormal pupae were removed daily and placed in labeled glass vials containing 70% ethanol and one drop of glycerine then photographed under binocular microscope.

4.2. Repellent /antifeedant action:

Standard cages (20×20×20cm) were used to test the repellent activity of plant extracts. Different weights from each extract was dissolved in 2ml (70% ethanol or water + drop of Tween) in glass 4×4cm to prepare different concentrations. One ml from each concentration was directly applied onto 5×6cm of ventral surface of pigeon after removing feathers from the abdomen to evaluate the repellency against C. pipiens, compared with commercial repellent (Off!) 15 % Deet (N. N. diethyl toulamid) (Johnson Wax Egypt) as a positive control. After 10 minutes of treatment, the treated pigeons were placed in the cages containing at least 20 Culex pipiens starved females 5-7 d-old for 4h. Control tests were carried out alongside with the treatments using ethanol or water. Each test was repeated three times to get a mean value of repellent.

5. Criteria studied:

5.1. Biological activity of plant extracts against the larval stages: The larvae were observed daily until pupation and adult emergence to estimate the following parameters:

5.1.1. Larvicidal activity: Larval mortality percent was estimated by using the following equation (Briggs, 1960): \[ \text{larval mortality} \% = \frac{A - B}{A} \times 100 \] where: A = number of tested larvae, B = number of tested pupa.

5.1.2. Pupation rate: The pupation percent was estimated by using the following equation: \[ \text{pupation} \% = \frac{A}{B} \times 100 \] where: A = number of pupae, B = number of tested larvae.
5.1.3. Pupal mortality: The pupal mortality percent was estimated by using the following equation: pupal mortality % = A – B / A × 100 where: A = number of produced pupae, B = number of observed adults.

5.1.4. Adult emergence: The emerged males and females adults were counted and the adult emergence percent was calculated by using the following equation: Adult emergence % = A / B × 100 where: A = number of emerged adults, B = number of tested pupae.

5.1.5. Malformative effects: Pupal malformation was estimated by any change in color, size, shape or failure to develop to adult stage (pupal-adult intermediate). All malformed pupae were counted and removed immediately. The pupal malformation percent was calculated by using the following equation: pupal malformation % = C / A × 100 where: C = number of malformed pupae, A = number of tested pupae.

6. Repellent activity of plant against adult stage:

After treatments, the number of fed and unfed females were counted and calculated according to Abbott, (1925). Repellency % = [% A - % B / 100 - % B] × 100 Where: A = percent of unfed females in treatment, B = percent of unfed females in control

7. Statistical analysis:

Statistical analysis of the data was carried out according to the method of lentner et al., (1982). Lc50 was calculated using multiple linear regression (Finney, 1971).

Results

1. Plant extract tested:

Data given in table (2) indicated the amounts of dry 70% ethanol and acetone soluble material from 100 gm of different plant parts. As shown from the results, these amounts were varied from one part to another. In addition, ethanolic plant extracts produced higher weights followed by acetone extracts generally. The highest weight (8.2 gm) was obtained from the ethanolic leaves extract, while the lowest weight of the extracts was obtained from the acetone stems extract (3.1 gm).

Table (2): The weight of dry 70% ethanol and acetone soluble material from 100 gm of C. nocturnum and their parts.
2. Biological activity of plant extracts against the larval stage of *Culex pipiens*:

The biological activity (larvicidal activity, pupal rate, pupal mortality, total larval and pupal mortality, adult emergence) of ethanolic and acetone extracts against the 3rd instar larvae of *C. pipiens* has been studied. The results may be arranged as follows:

### 2.1. Ethanolic extract of leaves and stems:

Data given in tables (3&4) indicated the biological activity of ethanolic extract of *C. nocturnum* (leaves and stems), respectively against the 3rd instar larvae of *C. pipiens*.

The highest larval mortality percent (100%) occurred at the highest concentrations (2500 and 3500ppm), while the lowest mortality percent (23.3 and 26.7%) occurred at the lowest concentrations (200 and 250ppm), respectively compared to 13.3% for the control.

The pupation percent decreased as the concentration level of ethanolic extract of *C. nocturnum* (leaves and stems) increased. The pupation percent recorded 0.0 % at 2500 and 3500ppm and 76.7 and 73.3% at the lowest concentrations (200 and 250ppm), respectively compared to 86.7% of the control.

Data given in table (3) revealed that there is no effect of ethanolic extract of *C. nocturnum* (leaves) on the mortality percent of pupae developed from treated larvae. Also, it is cleared from table (4), that ethanolic extract of *C. nocturnum* (stems) has low toxic effect against the pupae resulted from the treated larvae especially at the concentrations (2000 and 500 ppm), where the pupal mortality percent was 14.3 and 10.0 %; respectively compared to 3.8% for the control group.

As shown from the results in table (4) the total mortality percent of larvae and pupae were: 93.3, 80.0, 53.3, 50.0 and 30.0% at the concentrations 2500, 2000, 1000, 500 and 250 ppm, respectively compared to 16.7 for control group.
The adult emergence percent was not affected at all concentrations used by ethanolic extract of *C. nocturnum* (leaves) as compared with the control. On the other hand, the adult emergence percent was affected only by ethanolic extract of *C. nocturnum* (stems) at the concentration (2000ppm) where it reduced to 85.7%, compared to 96.2% for the untreated larvae.

The lethal effect of the ethanolic extract of *C. nocturnum* (leaves and stems) did not extend to the adult stage because no adult mortality percent had been observed.

The ethanolic extract of *C. nocturnum* (leaves and stems) did not induce malformation effects on pupae resulted from treated larvae.

From the aforementioned results it is obvious that the toxicity values of the tested ethanolic extracts of different plant parts of *C. nocturnum* based on Lc50 values (Table 5 and Fig 1) may be arranged in a descending order as follows: leaves > stems.

### Table (3): Effect of ethanolic extract of *Cestrum nocturnum* (leaves) on mortality percent of different stages of *Culex pipiens*.

<table>
<thead>
<tr>
<th>Conc. ppm</th>
<th>Larval Mortality %</th>
<th>Pupation %</th>
<th>Pupal Mortality %</th>
<th>Larval and pupal Mortality %</th>
<th>Adult Emergence %</th>
<th>Adult Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2500</td>
<td>100.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2000</td>
<td>93.3</td>
<td>6.7</td>
<td>0.0</td>
<td>93.3</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1000</td>
<td>90.0</td>
<td>10.0</td>
<td>0.0</td>
<td>90.0</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>500</td>
<td>76.7</td>
<td>23.3</td>
<td>0.0</td>
<td>76.7</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>250</td>
<td>33.3</td>
<td>66.7</td>
<td>0.0</td>
<td>33.3</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>200</td>
<td>23.3</td>
<td>76.7</td>
<td>0.0</td>
<td>23.3</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Control</td>
<td>13.3</td>
<td>86.7</td>
<td>0.0</td>
<td>13.3</td>
<td>100.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

No. of tested larvae = 30; Conc. = Concentration; ppm = particle per million

### Table (4): Effect of ethanolic extract of *Cestrum nocturnum* (stems) on mortality percent of different stages of *Culex pipiens*.

<table>
<thead>
<tr>
<th>Conc. ppm</th>
<th>Larval Mortality %</th>
<th>Pupation %</th>
<th>Pupal Mortality %</th>
<th>Malformed pupae %</th>
<th>Larval and pupal Mortality %</th>
<th>Adult Emergence %</th>
<th>Adult Mortality %</th>
</tr>
</thead>
</table>
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<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>3500</th>
<th>2500</th>
<th>2000</th>
<th>1000</th>
<th>500</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC50 (ppm)</td>
<td>100.0</td>
<td>93.3</td>
<td>76.7</td>
<td>53.3</td>
<td>33.3</td>
<td>26.7</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.0</td>
<td>6.7</td>
<td>23.3</td>
<td>46.7</td>
<td>66.7</td>
<td>73.3</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.0</td>
<td>93.3</td>
<td>80.0</td>
<td>85.7</td>
<td>53.3</td>
<td>80.0</td>
</tr>
</tbody>
</table>

Table (5): Relative efficiency of ethanolic extract of different Parts of *Cestrum nocturnum* against *C. pipiens* larvae.

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>LC50 (ppm)</th>
<th>Slope (b)</th>
<th>Correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>377.1</td>
<td>0.0279</td>
<td>0.6696</td>
</tr>
<tr>
<td>Stems</td>
<td>1043.8</td>
<td>0.0239</td>
<td>0.9551</td>
</tr>
</tbody>
</table>

Fig. (1): Regression line of larval mortality of *C. pipiens* treated with different concentrations from ethanolic extracts of *C. nocturnum*.

2.2. Acetone extract of leaves and stems:

Data given in table (6&7) indicated the biological activity of acetone extract of *C. sempervirens* (Leaves and stems), respectively against the 3rd instar larvae of *C. pipiens*.

Leaves extract caused Complete larval mortality (100%) at the highest concentration (2000ppm), meanwhile the lowest value (26.7%) was occurred at the lowest concentration (250ppm) compared to 10.0% for the control group. The highest mortality percent (100%) caused by stem extract was at the concentration (3000ppm) and the lowest mortality percent (10.0%) was at the concentration (125ppm). Compared to 6.7% for the control.
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At the highest and lowest concentrations: (2000 and 250ppm) of leaves extract the pupation percent was 0.0 and 73.3%; respectively vs. 90% for the untreated group (Table 6). The pupation % recorded 10.0 and 90.0% at the highest and lowest concentration (2000 and 125ppm) of stem extract; respectively compared to 93.3% for the control group.

The lethal effect of acetone extract (leaves) was extended to the pupal stage at the all concentrations used: 1500, 1000, 500 and 250ppm, where the pupal mortality percent was 100.0, 87.5, 93.3 and 90.9%; respectively, vs. 0.0% for the control. Data given in table (7) revealed that there was a very highly toxic effect of acetone extract (stems) on the survivorship of pupae developed from the treated larvae, where the mortality % was 100.0% at all concentrations used, while it was 3.6 for the control group.

The total larval and pupal mortality were found to be highly affected by acetone extract (leaves). The highest mortality (100.0%) was noticed at the concentration; 1500ppm and the lowest mortality (93.3%) was noticed at the concentration (250ppm); respectively compared to 10.0% at the control group.

A remarkable reduction in the percentage of adult emergence from pupae produced by treated larvae with the acetone extract (leaves). The adult emergence percent (0.0%) was occurred at the concentration 1500ppm, meanwhile the percent increased to 12.5, 6.7 and 9.1% at the concentrations 1000, 500 and 250ppm, respectively compared to 100.0% of the control group. On the other hand, the adult emergence was not observed by acetone extract (stems) because this extract induced 100% pupal mortality at all concentrations used.

As shown from the results (table 7) the toxicity of acetone extract (leaves) extended to the adult stage, where the adult mortality percent was 100% at 1000ppm, while at the two lowest concentration (500 and 250ppm) the mortality percent was 0.0% as in the control group.

The results recorded in table (7&8) showed that the acetone extract (leaves and stems) induced high % of malformation on the pupae developed from the treated larvae. The pupal malformation percent was 100.0% at the all concentrations used compared to 0.0% for the control group (Table 8).

From the aforementioned results it is obvious that the toxicity values of the tested acetone extracts of different plant parts of *C. nocturnum* based on *Lc*<sub>50</sub> values (Table 8 and fig. 2) may be arranged in a descending order as follows : leaves > stems.
In general, the toxicity values of tested extracts of the different parts of *C. nocturnum* based on LC\textsubscript{50} values (Tables 5 and 8) may be arranged in a descending order as follows: Ethanolic extract (leaves) > acetone extract (leaves) > acetone extract (stems) > ethanolic extract (stems).

**Table (6): Effect of acetone extract of *Cestrum nocturnum* (leaves) on mortality percent of different stages of *Culex pipiens*.**

<table>
<thead>
<tr>
<th>Conc. ppm</th>
<th>Larval mortality %</th>
<th>Pupation %</th>
<th>Pupal Mortality %</th>
<th>Malformed pupae %</th>
<th>Larval and pupal Mortality %</th>
<th>Adult Emergence %</th>
<th>Adult Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>100.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1500</td>
<td>93.3</td>
<td>6.7</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>1000</td>
<td>73.3</td>
<td>26.7</td>
<td>87.5</td>
<td>87.5</td>
<td>96.7</td>
<td>12.5</td>
<td>100.0</td>
</tr>
<tr>
<td>500</td>
<td>50.0</td>
<td>50.0</td>
<td>93.3</td>
<td>93.3</td>
<td>96.7</td>
<td>6.7</td>
<td>0.0</td>
</tr>
<tr>
<td>250</td>
<td>26.7</td>
<td>73.3</td>
<td>90.9</td>
<td>90.9</td>
<td>93.3</td>
<td>9.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Control</td>
<td>10.0</td>
<td>90.0</td>
<td>0.0</td>
<td>0.0</td>
<td>10.0</td>
<td>100.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

No. of tested larvae, Conc., ppm,: see footnote of table (3).

**Table (7): Effect of acetone extract of *Cestrum nocturnum* (stems) on mortality percent of different stages of *Culex pipiens*.**

<table>
<thead>
<tr>
<th>Conc. ppm</th>
<th>Larval mortality %</th>
<th>Pupation %</th>
<th>Pupal Mortality %</th>
<th>Malformed pupae %</th>
<th>Larval and pupal Mortality %</th>
<th>Adult Emergence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>3000</td>
<td>100.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2000</td>
<td>90.0</td>
<td>10.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1000</td>
<td>53.3</td>
<td>46.7</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>500</td>
<td>43.3</td>
<td>56.7</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>250</td>
<td>30.0</td>
<td>70.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>125</td>
<td>10.0</td>
<td>90.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Control</td>
<td>6.7</td>
<td>93.3</td>
<td>3.6</td>
<td>0.0</td>
<td>10.0</td>
<td>96.4</td>
</tr>
</tbody>
</table>

No. of tested larvae, Conc., ppm : see footnote of table (3).

**Table (8): Relative efficiency of acetone extract of different parts of *Cestrum nocturnum* against *C. pipiens* larvae.**

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>LC\textsubscript{50} (ppm)</th>
<th>Slope (b)</th>
<th>Correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>484.2</td>
<td>0.0244</td>
<td>0.759</td>
</tr>
<tr>
<td>Stems</td>
<td>993.98</td>
<td>0.0294</td>
<td>0.9217</td>
</tr>
</tbody>
</table>
Fig. (2): Regression line of larval mortality of *C. pipiens* treated with different concentrations from acetone extracts of *C. nocturnum*. 
3. Repellency / antifeedant action:

3.1. Ethanolic extract of leaves and stems:

Table (9) indicated that the leaves extract had a more repellent activity against *C. pipiens* females than stems extract. The repellency action for leaves and stems extracts were 81.25 and 79.0% at the dose 3.6 mg/cm², while; it recorded 67.7 and 54.7% at the dose 1.8 mg/cm²; respectively compared to 100% repellency for Off! at the dose 1.8 mg/cm².

3.2. Acetone extract of Leaves and stems:

Table (10) showed that acetone extract of leaves and stems caused 86.8 and 83.1%, respectively at the dose 3.6 mg/cm². However, at the lowest dose (1.8 mg/cm²) the two extracts caused 72.4 and 69.7%; respectively compared to 100% repellency for Off! at the dose 1.8 mg/cm².

4. Morphogenetic effects:

The different forms of morphogenetic effects as induced by the different plant extracts tested against the 3rd instar larvae of *C. pipiens* are illustrated in Fig.(3) from A to D and can be summarized as follows:

A – pupal- adult intermediate resulted from larvae treated with acetone leaves and stems extracts (All concentrations used).

B – Deformed decolorized pupal- adult intermediate resulted from larvae treated with acetone extract of stems (500, 250 and 125 ppm).

C – Half- ecdysed adult resulted from the treatment of the larvae with acetone leaves extract (250 ppm).

D – Incompletely emerged adult with legs attached to the pupal skin, wings unequal and abdomen not completely segmented. This abnormality feature was obtained when larvae treated with ethanolic and acetone extracts (1000, 500 and 1000 ppm), respectively.

Table (9): Repellency / antifeedant effect of ethanolic extract of *Cestrum nocturnum* on *Culex pipiens*.

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Dose (mg/cm²)</th>
<th>No. of tested females</th>
<th>No. of fed</th>
<th>%</th>
<th>No. of unfed</th>
<th>%</th>
<th>Repellency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>3.6</td>
<td>28</td>
<td>5</td>
<td>17.9</td>
<td>23</td>
<td>82.1</td>
<td>81.3</td>
</tr>
<tr>
<td></td>
<td>1.8</td>
<td>26</td>
<td>8</td>
<td>30.8</td>
<td>18</td>
<td>69.2</td>
<td>67.7</td>
</tr>
<tr>
<td>Stem</td>
<td>3.6</td>
<td>20</td>
<td>4</td>
<td>20.0</td>
<td>16</td>
<td>80.0</td>
<td>79.0</td>
</tr>
<tr>
<td></td>
<td>1.8</td>
<td>21</td>
<td>9</td>
<td>42.9</td>
<td>12</td>
<td>57.1</td>
<td>54.9</td>
</tr>
<tr>
<td>Off</td>
<td>1.8</td>
<td>25</td>
<td>0.0</td>
<td>0.0</td>
<td>25</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Table (10): Repellency / antifeedant effect of acetone extract of *Cestrum nocturnum* on *Culex pipiens*.

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Dose (mg/cm²)</th>
<th>No. of tested females</th>
<th>No. of fed</th>
<th>%</th>
<th>No. of unfed</th>
<th>%</th>
<th>Repellency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>3.6</td>
<td>24</td>
<td>3</td>
<td>12.5</td>
<td>21</td>
<td>87.5</td>
<td>86.8</td>
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<tr>
<td></td>
<td>1.8</td>
<td>23</td>
<td>6</td>
<td>26.1</td>
<td>17</td>
<td>73.9</td>
<td>72.4</td>
</tr>
<tr>
<td></td>
<td>3.6</td>
<td>25</td>
<td>4</td>
<td>16.0</td>
<td>21</td>
<td>84.0</td>
<td>83.1</td>
</tr>
<tr>
<td>Stem</td>
<td>1.8</td>
<td>28</td>
<td>8</td>
<td>28.6</td>
<td>20</td>
<td>71.4</td>
<td>69.7</td>
</tr>
<tr>
<td></td>
<td>1.8</td>
<td>25</td>
<td>0.0</td>
<td>0.0</td>
<td>25</td>
<td>100.0</td>
<td>100.0</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-----</td>
<td>18</td>
<td>17</td>
<td>94.4</td>
<td>1</td>
<td>5.6</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Normal pupae

Normal adult

A

B

C

D
Discussion

The plants tested in the present study are known to be eco – friendly and are not toxic to vertebrates. Moreover, it is clearly proved that crude or partially purified plant extracts are less expensive and highly efficacious for the control of mosquitoes rather than the purified compounds or extracts (Jang et al., 2002; Cavalcanti et al., 2004 and Maurya et al. 2009). The present study showed high bioactivity of the different extracts from plants which are grown widely in Egypt. Such results may offer an opportunity for developing alternatives to rather expensive and environmentally hazardous organic insecticides.

Ethanolic and acetone extracts of the different parts of the C. nocturnum plant against the larval and adult stages of C. pipiens clearly affected the various biological and repellency aspects as follows:

1. Biological activity of plant extracts against the larval stage:

1.1. Larvicidal activity:

The present study showed that, the toxicity of the tested plant extracts against 3rd larval instar was varied according to plant part used and concentration of the extract. The larval mortality percent was increased by increasing extract concentration for all plant extracts tested. The toxicity of ethanolic and acetone extracts based on LC$_{50}$ was leaves > stems. These results are in consistent with the previously mentioned suggestions of Sukumar et al. (1991) and Maurya et al. (2009). In all larvicidal assays, the methanol extract of C. nocturnum leaves extracted with percolation and its fractions presented higher larvicidal activity (Jawale et al. 2010).

Several plant extracts other than those used in the present study had been tested against different species of mosquitoes by many authors worldwide. The tested plant extracts on larval mortality of C. pipiens were in agreement with the results obtained by Shalaby et al. (1998), Pelah et al. (2002), Jeyabalan et al (2003), Nathan et al. (2005 & 2006), Sharma et al. (2006b), Coria et al. (2008), Maurya et al. (2009). The hexane, ethyl acetate and methanol using soxhlet and percolation extraction separately of C. nocturnum were tested against the 3rd instar larvae of Aedes aegypti at different concentrations by Jawale et al. (2010); they recorded that among the
three extracts of *C. nocturnum*, percolation method extracts showed effective larvicidal activity over the soxhlet method. Methanol extract exhibit significant larvicidal activity causing 100% mortality in a concentration of 100μg/mL. However, the present study showed that the acetone extract of this plant (leaves and stems) caused 100% larval mortality of *C. pipiens* at concentration 500ppm, respectively.

1.2. Pupation percent, pupal mortality and adult emergence:

In the present study, a remarkable decrease in the pupation percent was induced by all plant extracts tested. The pupation% was decreased as the concentration of the plant extract increased. Moreover, the pupation rate was found to be plant part - and solvent used in extraction – dependent.

The present study showed that the toxicity of plant extracts tested has been extended to the pupae, where 100% pupal mortality was induced by acetone stems extract. In addition, the acetone leaves and stems extracts tested induced a remarkable reduction in the % of adult emerged from the pupae produced from treated larvae. The reduction was concentration– dependent. These results are comparable with earlier results of Shalaby *et al.* (1998) using peel oils of lemon, grapefruit and naval orange against *C. pipiens* larvae, El – Bokl (2003) using the neem, *Azadirachta indica* extract against *C. pipiens* larvae, Jeyabalan *et al.* (2003) using water extracts of *E. crassipes* and *Ar. Monosperma* against *C. pipiens* larvae, Nathan *et al.* (2006) using methanolic extracts of leaves and seeds of *Melia azedarach* against *A. stephensi* larvae, Sharma *et al.* (2006 a & b) using petroleum ether extract of *Artemisia annua* against *An. stephensi* and *Culex quinquefasciatus* larvae, respectively and Pavela (2009) using essential oils from 22 aromatic plant species against *Culex quinquefasciatus* Say (Diptera: Culicidae).

1.3. Survivorship of the resulted adults:

Results obtained in the present study indicated that the toxicity of acetone extracts tested against the 3rd instar larvae of *C. pipiens* was extended to the produced adults causing mortality reached to 100% for acetone leaves extract. Similar results were obtained by Shalaby *et al.* (1998) using peel oils of lemon, grapefruit and naval orange against *C. pipiens* larvae, Jeyabalan *et al.* (2003) using methanol extract of *Pelargonium citrosa* leaf against *A. stephensi*, Nathan *et al.* (2005) using the neem *Azadirachta indica* extract against *A. stephensi* and Nathan
et al. (2006) using methanolic extracts of leaves and seeds from the chinaberry tree Melia azedarach against A. stephensi.

1.4. Morphogenetic effects:

In the present study almost all extracts of plant parts tested against the 3rd instar larvae of C. pipiens induced some morphological abnormalities in pupae. The malformed pupae were not able to develop normally and died. Also, the present results showed that the percent and degree of malformation among pupae were dependent on the conc. of the plant extract and solvent used in extraction. Similar observations were obtained by different plant extracts against different mosquito species in earlier studies. Similary, Abahussain (1999) using Calotropis procera extracts against C. pipiens and A. multicolor observed morphological abnormalities among pupae. El-Bokl (2003) recorded varying degrees of morphogenetic abnormalities in immature and adult stages of C. pipiens when larvae were treated with the neem, Azadirachta indica extract.

2. Repellency/antifeeding activity:

All the concentrations of plant extracts used in the present study exhibited repellency activity against the starved female adults of C. pipiens. The repellent action of the plant extracts tested was varied depending on plant part, solvent used in extraction and the dose of the extract. The present study indicated that the acetone extraction of the plant used was more effective in exhibiting the repellent action against the mosquito tested as compared with the ethanol extraction and showed less repellency percent than a commercial formulation, N,N-diethyl-m-methylbenzamide (DEET).

using extracts of the neem *Azadirachta indica* and methanolic extracts of leaves and seeds from the chinaberry tree, *Melia azedarach* against *A. stephensi*, *Choochote et al. (2007)* using repellent activity of selected essential oils from ten plant species against *Aedes aegypti* and *Chio and Yang (2008)* using neem tree (*Azadirachta indica*) oil against the Asian tiger mosquito (*Aedes albopictus*).

In general, it could be concluded that almost the plant extracts used in the present study act as larvicidal, and inhibited growth and emergence of the mosquito vector, *C. pipiens*. Furthermore, the results of the present study may contribute to a reduction in the application of synthetic insecticides, which in turn increases the opportunity for natural control of various medically important pests by botanical pesticides. These botanical pesticides are often active against specific target insects, less expensive, easily biodegradable to non-toxic products and potentially suitable for use in mosquito control program (*Alkofahi et al., 1989* and *Su and Mulla, 1999*).

Further studies on the tested plants including mode of action, synergism with the biocides under field condition are needed.

References


The larvicidal and repellent effect of leaf and stem extracts from Cestrum nocturnum (nightshade).

A.M. Hussein, M.H. Sairi, M. Salih, A. Elsheikh, and A. Zineh Shagha (Faculty of Science, Cairo University, Cairo, Egypt)

The current study was conducted to determine the larvicidal and repellent effectiveness of four extracts (ethanol and citronella) of leaves and stems from local Cestrum nocturnum, against the pupal stage of the mosquito, Aedes aegypti. Also, studies were conducted to investigate the repellent or deterrent effect of feeding in different larval stages, and the deformations of the plant extracts on different larval stages.

The results of the current study showed that the ethanol extract of the leaf had the highest effect against the pupal stage (377.1 LC50) followed by the ethanol extract of the stem (484.2 LC50), then the citronella extract of the stem (994.0 LC50), and finally, the ethanol extract of the leaf (1043.8 LC50). The results of the current study showed a significant decrease in the incidence of emergence of the larval stage, especially with the use of citronella extracts of the stem and leaf.

The results of the current study showed that the citronella extracts of the stem and leaf against the pupal stage of the mosquito, Aedes aegypti, have extended to the cases resulting from the treated pupae where a 100% death rate was recorded with all concentrations used. It was noted that these plant extracts could be used as a substitute for industrial pesticides that are harmful to the environment in the control of mosquito vectors that carry diseases in Egypt.