

---

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

---

MOSTAFA I. HASSAN<sup>1</sup>, TAREK M. Y. EL-SHEIKH<sup>1</sup>, MOUNEER S. AMER<sup>1</sup>,  
WALAA A. MOSELHY<sup>2</sup> and AHMED Z. SHEHATA<sup>1</sup>

<sup>1</sup>: Department of Zoology, Faculty of Science(boys), Al-Azhar University, Nasr City,  
Cairo, Egypt.

<sup>2</sup>: Department of Zoology, Faculty of Science(girls), Al-Azhar University, Nasr City,  
Cairo, Egypt.

E-mail: mostafa012@gmail.com

---

**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* 3<sup>rd</sup> instar larvae and adults were evaluated. Ethanolic leaves extract was found to be the most effective against the larvae, with LC<sub>50</sub> value of 377.1ppm followed by acetone leaves and stems extracts with LC<sub>50</sub> values of 484.2 and 994.0ppm; respectively and finally ethanolic leaves extract with LC<sub>50</sub> 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 Hoogastrall, 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 habitats, 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 3<sup>rd</sup> 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  $27 \pm 2^\circ\text{C}$ , relative  $70 \pm 10\%$  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 (1): Plant used as ethanolic and acetone extracts against *Culex pipiens* larvae and adult.**

Common name	Scientific name	family	Habitat	collection site
Night jessamen	<i>Cestrum nocturnum</i>	<i>Solanaceae</i>	Desert	Sadat city (Cairo-Alexandria desert road)

## 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 3<sup>rd</sup> 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  $27 \pm 2^\circ\text{C}$ ,  $70 \pm 10\%$  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 toluamide) (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**): larval mortality % =  $(A - B) / A \times 100$  **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: pupation % =  $A / B \times 100$  **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 % =  $(A - B) / A \times 100$  **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 % =  $A / B \times 100$  **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 % =  $C / A \times 100$  **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 % =  $[(\% A - \% B) / 100 - \% B] \times 100$  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)**.  $LC_{50}$  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.**

Plant	Plant part used	Weight of extract (gm)	
		Ethanollic	Acetone
<i>C. nocturnum</i>	Leaves	8.21	6.72
	Stems	6.92	3.13

## 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 3<sup>rd</sup> 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 3<sup>rd</sup> 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  $LC_{50}$  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*.**

Conc. ppm	Larval Mortality %	Pupation %	Pupal Mortalit %	Larval and pupal Mortality %	Adult Emergence %	Adult Mortality %
2500	100.0	—	—	—	—	—
2000	93.3	6.7	0.0	93.3	100.0	0.0
1000	90.0	10.0	0.0	90.0	100.0	0.0
500	76.7	23.3	0.0	76.7	100.0	0.0
250	33.3	66.7	0.0	33.3	100.0	0.0
200	23.3	76.7	0.0	23.3	100.0	0.0
Control	13.3	86.7	0.0	13.3	100.0	0.0

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*.**

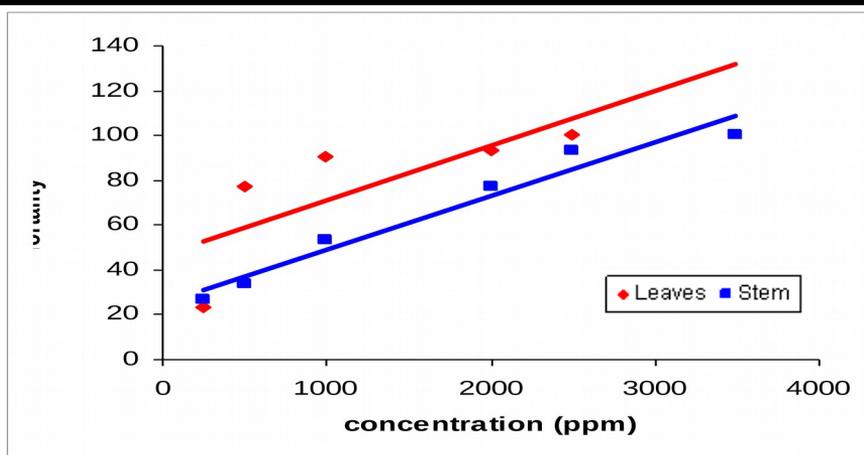
Conc. ppm	Larval Mortality %	Pupation %	Pupal Mortality %	Malformed pupae %	Larval and pupal Mortality %	Adult Emergence %	Adult Mortality %
-----------	--------------------	------------	-------------------	-------------------	------------------------------	-------------------	-------------------

3500	100.0	—	—	—	—	—	—
2500	93.3	6.7	0.0	0.0	93.3	100.0	0.0
2000	76.7	23.3	14.3	14.3	80.0	85.7	0.0
1000	53.3	46.7	0.0	0.0	53.3	100.0	0.0
500	33.3	66.7	10.0	10.0	50.0	90.0	0.0
250	26.7	73.3	4.5	4.5	30.0	95.5	0.0
<b>Control</b>	<b>13.3</b>	<b>86.7</b>	<b>3.8</b>	<b>0.0</b>	<b>16.7</b>	<b>96.2</b>	<b>0.0</b>

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

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

Plant parts	LC <sub>50</sub> (ppm)	Slope (b)	Correlation coefficient (r)
Leaves	377.1	0.0279	0.6696
Stems	1043.8	0.0239	0.9551



**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 3<sup>rd</sup> 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.

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  $LC_{50}$  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  $LC_{50}$  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*.**

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

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*.**

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

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.**

Plant parts	$LC_{50}$ (ppm)	Slope (b)	Correlation coefficient (r)
Leaves	484.2	0.0244	0.759
Stems	993.98	0.0294	0.9217

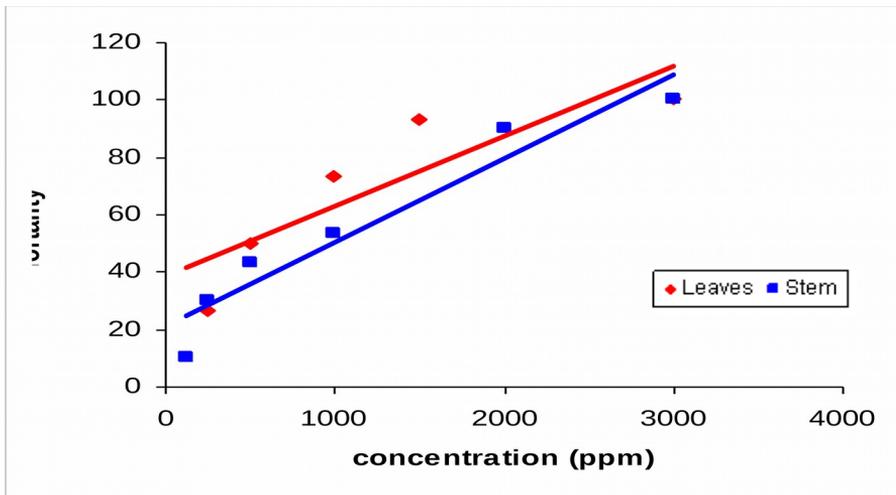


Fig. (2): Regression line of larval mortality of *C. pipiens* treated with different concentrations from acetone extracts of *C. nocturnum*.

### 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/cm<sup>2</sup>, while; it recorded 67.7 and 54.7% at the dose 1.8 mg/cm<sup>2</sup>; respectively compared to 100% repellency for Off! at the dose 1.8 mg/cm<sup>2</sup>.

#### 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/cm<sup>2</sup>. However, at the lowest dose (1.8 mg/cm<sup>2</sup>) the two extracts caused 72.4 and 69.7%; respectively compared to 100% repellency for Off! at the dose 1.8 mg/cm<sup>2</sup>.

### 4. Morphogenetic effects:

The different forms of morphogenetic effects as induced by the different plant extracts tested against the 3<sup>rd</sup> 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.

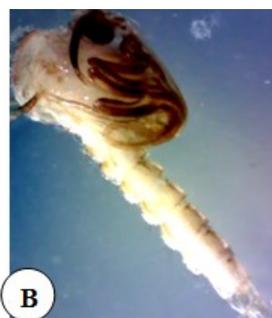
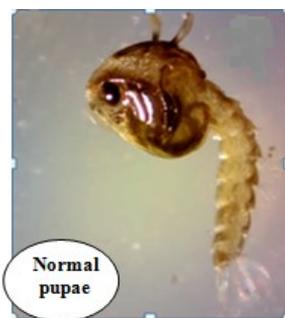
**Table (9): Repellency / antifeedant effect of ethanolic extract of *Cestrum nocturnum* on *Culex pipiens*.**

Plant parts	Dose (mg/cm <sup>2</sup> )	No. of tested females	No. of fed	%	No. of unfed	%	Repellency %
Leaves	3.6	28	5	17.9	23	82.1	81.3
	1.8	26	8	30.8	18	69.2	67.7
Stem	3.6	20	4	20.0	16	80.0	79.0
	1.8	21	9	42.9	12	57.1	54.9
Off	1.8	25	0.0	0.0	25	100.0	100.0

<b>Control</b>	----	<b>21</b>	<b>20</b>	<b>95.2</b>	<b>1</b>	<b>4.8</b>	<b>0.0</b>
----------------	------	-----------	-----------	-------------	----------	------------	------------

**Table (10):** Repellency / antifeedant effect of acetone extract of *Cestrum nocturnum* on *Culex pipiens*.

Plant parts	Dose (mg/cm <sup>2</sup> )	No. of tested females	No. of fed	%	No. of unfed	%	Repellency %
<b>Leaves</b>	3.6	24	3	12.5	21	87.5	86.8
	1.8	23	6	26.1	17	73.9	72.4
<b>Stem</b>	3.6	25	4	16.0	21	84.0	83.1
	1.8	28	8	28.6	20	71.4	69.7
<b>Off</b>	1.8	25	0.0	0.0	25	100.0	100.0
<b>Control</b>	----	<b>18</b>	<b>17</b>	<b>94.4</b>	<b>1</b>	<b>5.6</b>	<b>0.0</b>



**Fig. (3): Malformative effects in *C. pipiens* pupae and adult as induced by the plant extract tested (160 X). Explanation of figures (See result 4).**

## **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.

Ethanollic 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 3<sup>rd</sup> 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<sub>50</sub> 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 3<sup>rd</sup> 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 – Bohl (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 3<sup>rd</sup> 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 3<sup>rd</sup> 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. Similarly, **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).

Many plant extracts and essential oils manifest repellency activity against different mosquito species. The present results are in accordance with such results obtained by **Sharma et al. (1995)** using neem oils against mosquito bites of *Anopheles* spp., *Culex* spp. And *Aedes* spp., **Govere et al. (2000)** using extracts of fever tea (*Lippia javanica*) rose geranium (*Pelargonium reniforme*) and lemon grass (*Cymbopogon excavatus*) against *A. arabiensis*, **Kim et al., (2002)** using ethanol extract of fruits from *Foeniculum vulgare* against hungry *Aedes aegypti* females, **Jeyabalan et al., (2003)** using methanol extracts of *Pelargonium citrosa* against *A. stephensi*, **Yang et al. (2004)** using methanol extracts from 23 aromatic medicinal plant species against female blood – starved *Aedes aegypti*, **Nathan et al. (2006 b)**

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

1. Abahussain, M.O. (1999): Effect of *Sorghum bicolor* and *Nerium oleander* extracts on of the grey flesh fly *Parasarcophaga argyrostoma* (Diptera: sarcophagidae). J. Egypt. Ger. Soc. Zool., 28(2): 233 – 243.
2. Abbott, W.S. (1925): A method for computing the effectiveness of an insecticide. J. Econ. Entomol., 18 : 265 – 277.
3. Alkofahi, A.; Rupprecht, J.K.; Anderson, J.E.; Mclaughlin, J.L.; Mikolajczak, K.L. and Scott, B.A. (1989): Insecticides of plant origin, American chemical society, Washington, DC, 25 – 43.
4. Briggs, J.N.(1960): Reduction of adult house fly emergence by the effective *Bacillus* sp. on the development of immature forms. J. Insect pathology. 2 : 418 – 432.
5. Cavalcanti, E.S.; Morais, S.M.; Lima, M.A. and Santana, E.W. (2004): Larvicidal activity of essential oils from Brazilian plants against *Ae. aegypti* L. Mem. Inst. Oswaldo. Cruz., 99(5) : 541 – 4.
6. Chio, E.H. and Yang, E.C. (2008): A bioassay for natural insect repellents. Journal of Asia-Pacific Entomology 11 : 225–227.
7. Choochote, W.; Chaithong, U.; Kamsuk, K. ; Jitpakdi, A.; Tippawangkosol, P.; Tuetun, B.; Champakaew, D. and Pitasawat, B. (2007): Repellent activity of selected essential oils against *Aedes aegypti*. Fitoterapia 78 : 359–364.

8. Corbel, V., Duchon, S., Zaim, M., Hougard, J.M., (2004): Dinotefuran: a potential neonicotinoid insecticide against resistant mosquitoes. *J. Med. Entomol.* 41 (4), 712–717.
9. Coria, C. ; Almiron, W. ; Valladares, G. ; Carpinella, C. ; Luduen˜a, F. ; Defago, M. and Palacios, S. (2008): Larvicide and oviposition deterrent effects of fruit and leaf extracts from *Melia azedarach* L. on *Aedes aegypti* (L.) (Diptera: Culicidae). *Bioresource Technology* 99 : 3066–3070.
10. Darwish, M. and Hoogstraal, H. (1981): Arboviruses infesting human and lower animals in Egypt., A review of thirty years of research. *J. Egypt. Pub. Hlth. Assoc.*, 56 : 1 – 112.
11. El – Bokl, M.M. (2003): Latent toxicity of azadirachtin treatment on *C. pipiens* (Diptera : Culicidae). *J. Egypt. Acad. Soc. Environ. Develop.*, 3(1): 63 – 74.
12. Finney, D.J. (1971): Probit analysis Third edition. Cambridge Univ. Press., 333 p.
13. Gad, A.M.; Hammad, R.E. and Farid, H.A. (1996): Uptake and development of *Wucheria bancrofti* in *C. pipiens* L. and *Ae. caspius* Pallas. *J. Egypt. Soc. Parasitol.*, 26(2): 305 – 314.
14. Ghosh A, Chandra G.(2006): Biocontrol efficacy of *Cestrum diurnum* L. (Solanaceae: Solanales) against the larval forms of *Anopheles stephensi*. *Nat Prod Res.* 20: 371-79.
15. Ghosh A, Chowdhury N, Chandra G.(2008): Laboratory evaluation of phytosteroid compound of mature leaves of Day Jasmine (Solanaceae: Solanales) against larvae of *Culex quinquefasciatus* (Diptera: Culicidae) and nontarget organisms. *Parasitol Res.* 103: 271-77.
16. Govere, T.A.; Durrheim, D.N.; Du, T.N.; Hunt, R.H. and Coetzee, M. (2000): Local plants as repellents against *A. arabiensis*, in Mpumalanga Province, South Africa. *Cent. Afr. J. Med.*, 46(8): 213 – 6.
17. Jang, Y.S.; Baek, B.R.; Yang, Y.C.; Kim, M.K. and Lee, H.S. (2002): Larvicidal activity of leguminous seeds and grains against *Ae. aegypti* and *C. pipiens* pallens. *J. Am. Mosq. Control. Assoc.*, 18(3): 210 – 3.
18. Jawale, C.; Kirdak, R. and Dama, L. (2010): Larvicidal activity of *Cestrum nocturnum* on *Aedes aegypti*. *Bangladesh J. Pharmacol.* , 5: 39-40
19. Jeyabalan, D.; Arul, N. and Thangamathi, P. (2003): Studies on effects of *Pelargonium citrosa* leaf extracts on malarial vector, *A. stephensi* Liston. *Bioresour. Technol.*, 89(2): 185 – 9.
20. Kasap, M. and Demirhan (1992): The effect of various larval foods on the rate of adult emergence and fecundity of mosquitoes. *Turkiye Parazitologi Dergisi.*, 161 : 87 – 97.
21. Kim, D.H.; Kim, S.I.; Chang, K.S. and Ahn, Y.J. (2002): Repellent activity of constituents identified in *Foeniculum vulgare* fruit against *Ae. aegypti* (Diptera : Culicidae). *J. Agric. Food. Chem.*, 50(24): 6993 – 6.

22. Lentner, C.; Lentner, C. and Wink, A. (1982): Student's t- distribution tables. In Geigy scientific Tables Vol. 2. International Medical and Pharmaceutical information, Ciba – Geigy Limited, Basal, Switzerland.
23. Maurya, P. ; Sharma, p.; Mohan, L. ; Batabyal L. and Srivastava, C.N. (2009): Evaluation of the toxicity of different phytoextracts of *Ocimum basilicum* against *Anopheles stephensi* and *Culex quinquefasciatus*. *Journal of Asia-Pacific Entomology* 12 : 113–115.
24. Nathan, S.S.; Kalaivani, K. and Murugan, k. (2005): Effects of neem limonoids on the malaria vector *A. stephensi* Liston (Diptera: Culicidae). *Acta Trop.* 96(1): 47-55
25. Nathan, S.S.; Savitha, G.; George, D.K.; Narmadha, A.; Suganya, L. and Chung, P.G. (2006): Efficacy of *Melia azedarach* L. extract on the malarial vector *A. stephensi* Liston (Diptera: Culicidae). *Bioresour Technol.* 79: 1316 – 1323.
26. Pavela, R. (2009): Larvicidal property of essential oils against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Industrial Crops and Products* 30 : 311–315.
27. Pelah, D.; Abramovich, Z.; Markus, A. and Wiesman, Z. (2002): The use of commercial saponin from *Quillaja saponaria* bark as a natural larvicidal agent against *Ae. aegypti* and *C. pipiens*. *J. Ethnopharmacol.*, 81(3):407-9.
28. Rohani, A., Chu, W.L., Saadiyah, I., Lee, H.L., Phang, S.M., (2001): Insecticide resistance status of *Aedes albopictus* and *Aedes aegypti* collected from urban and rural in major towns of Malaysia. *Trop. Biomed.* 18 (1), 29–39.
29. Shalaby, A.A.; Allam, K.A.M.; Mostafa, A.A. and Fahmy, S.M.E. (1998): Insecticidal properties of citrus oils against *C. pipiens* and *M. domestica*. *J. Egypt. Soc. Parasitol.*, 28(2): 595 – 606.
30. Sharma, S.K.; Dua, V.K. and Sharma, V.P. (1995): Field studies on the mosquito repellent action of neem oil. *Southeast Asian J. Trop. Med. Public. Health.* 26(1):180-2.
31. Sharma, p.; Mohan, L. and Srivastava, C.N. (2006 a): Phytoextract-induced developmental deformities in malaria vector. *Bioresour Technol.* 97: 1599–1604.
32. Sharma, p.; Mohan, L. and Srivastava, C.N. (2006 b): Growth Inhibitory Nature of *Artemisia annua* Extract against *Culex autnauetesctetus* (Say). *J. Asia-Pacific Entomol.* 9(4): 389-395.
33. Su, T. and Mulla, M.S. (1999): Oviposition bioassay responses of *C. tarsalis* and *C. quinquefasciatus* to neem products containing azadirachtin. *Entomol. Exp. Appl.*, 91: 337 – 345.
34. Sukumar, K.; Perich, M.J. and Boobar, L.R. (1991): Botanical derivatives in mosquito control: A review. *Am. Mosq. Control. Assoc.*, 7(2): 210 – 237.
35. Taubes, G., (2000) Vaccines. Searching for a parasites weak spot. *Science* 290 (5491), 434–437.

36. Wattanachai, P., Tintanon, B., (1999): Resistance of *Aedes aegypti* to chemical compounds in aerosol insecticide products in different areas of Bangkok, Thailand. *Commun. Dis. J.* 25, 188–191.
37. Yang, Y.C.; Lee, E.H.; Lee, H.S.; Lee, D.K. and Ahn, Y.G. (2004): Repellency of aromatic medicinal plant extracts and a steam distillate to *Ae. aegypti*. *J. Am. Mosq. Control. Assoc.*, 20(2): 146 – 9.

## التأثير الطارد و السمي لمستخلصات الورقة و الساق لنبات مسك الليل ضد كيولكس بينيز (ثنائية الأجنحة:كيولسيدي)

<sup>1</sup>مصطفى إبراهيم حسن،<sup>1</sup> طارق محمد يسرى الشيخ ،<sup>1</sup> منير صالح عامر ،<sup>2</sup> ولاء أحمد مصيلحي و<sup>1</sup> أحمد زينهم شحاتة

<sup>1</sup> قسم علم الحيوان- كلية العلوم- جامعة الأزهر-القااهرة (بنين)

<sup>2</sup> قسم علم الحيوان- كلية العلوم- جامعة الأزهر-القااهرة (بنات)

أجريت الدراسة الحالية لتحديد الكفاءة السمية لأربعة مستخلصات نباتية (الايثانول و الأستيون)، لأجزاء (أوراق- سيقان) أحد النباتات الصحراوية المحلية في مصر، مسك الليل (*Cestrum nocturnum*)، ضد العمر اليرقى الثالث لبعوضة كيولكس بينيز . أيضا تم دراسة التأثير الطارد أو التأثير المانع للتغذية في الأطوار اليافعة ، التشوهات للمستخلصات النباتية الحالية على أطوار البعوضة المختلفة.

دلت نتائج الدراسة الحالية أن مستخلص الايثانول الورقي كان أكثر تأثيرا ضد العمر اليرقى الثالث ( $377,1LC_{50}$ ) يليه مستخلص الأستيون الورقي ( $484,2LC_{50}$ ) ثم مستخلص الأستيون الساق ( $994,0LC_{50}$ ) و أخيرا مستخلص الايثانول الورقي ( $1043,8LC_{50}$ ) .

أوضحت نتائج الدراسة الحالية نقضا واضحا فى معدل التعذر و نسبة ظهور الطور اليافع و خاصة مع استخدام مستخلصات الأستيون للساق و الورقة. كذلك دلت نتائج الدراسة الحالية أن سمية مستخلصات الأستيون للساق و الورقة ضد العمر اليرقى الثالث لبعوضة ، كيولكس بينيز قد امتدت إلى العذارى الناتجة من اليرقات المعاملة حيث سجلت نسبة وفيات العذارى 100% مع كل التركيزات المستخدمة. علاوة على ذلك لم تظهر كل المستخلصات النباتية وفيات في الأطوار اليافعة الناتجة من معاملة العمر اليرقى الثالث. بينت التجارب أن معظم المستخلصات النباتية المختبرة ضد العمر اليرقى الثالث للبعوضة قد أحدثت بعض التأثيرات التشوهية للعذارى، الأطوار اليافعة و كذلك ظهور أشكال وسطية بين العذارى و الطور اليافع.

سجلت الدراسة الحالية أن كل المستخلصات النباتية المختبرة قد أظهرت نشاطا طاردا متباينا ضد إناث الطور اليافع لبعوضة، كيولكس بينيز. و قد لوحظ أن التأثير الطارد لهذه المستخلصات المختبرة كان يختلف باختلاف نوع و جزء النبات والمذيب المستخدم فى عملية الاستخلاص و كذلك جرعة المستخلص. المستخلصات النباتية التي تحدث أعلى نسبة (86,8, و 81,3%) طرد أو مانع لتغذية إناث الطور اليافع لبعوضة كيولكس بينيز هي : مستخلص الأستيون و الايثانول الورقي على الترتيب عند جرعة 3.6 مج/سم<sup>2</sup>.

و بما أن هذه المستخلصات النباتية السابقة لها تأثير ملحوظ على التحول و لها تأثير سمي على اليرقات و العذارى بالتالي يمكن أن تستخدم كبديل للمبيد بعوضة كيولكس بينيز الناقلة للأمراض