Egypt. Acad. J. biolog. Sci., 4 (1): 33 - 48 (2011) A. Entomology
Email: egyptianacademic@yahoo.com ISSN: 1687–8809
Received: 11/2/2011 www.eajbs.eg.net

Evaluation of the biological activity of some *Cupressus sempervirens* (Cupressaceae) extracts against the mosquito vector *Culex pipiens* L. (Diptera: Culicidae)

Tarek M.Y. EL-Sheikh[1], Mostafa I. Hassan[1], Walaa A. Moselhy [2], Mouneer S. Amer[1] and Ahmed Z. Shehata[1]

1- Department of Zoology, Faculty of Science (boys), Al-Azhar University, Nasr City, Cairo, Egypt.

2- Department of Zoology, Faculty of Science(girls), Al-Azhar University, Nasr City, Cairo, Egypt.

E-mail: Tarek_elshekh2000@yahoo.com

ABSTRACT

Ethanolic, acetone and petroleum ether extracts of leaves from the Egyptian plant *Cupressus sempervirens* (Cupressaceae) were tested against 3rd instar larvae of the mosquito *Culex pipiens* L. The obtained results indicated that petroleum ether extracts were more efficient than ethanolic and acetone extracts. The toxicity, based on Lc_{50} values, are arranged in a descending order as follows: ethanolic (Lc_{50} 263.6ppm) > acetone extract (Lc_{50} 104.3ppm) > petroleum ether extracts (Lc_{50} 37.8 ppm). As shown by the present results, a remarkable reduction in both the pupation percent and adult emergence was obtained. Moreover, all plant extracts exerted a delayed toxic effect on the pupae and adults after treatment of larvae. Also, various degrees of morphogenic abnormalities were observed in the immature and adult stages. Thus, these results may provide an opportunity to develop alternatives to costly organic pesticides and environmentally hazardous chemicals with some available cheap plants which are uaually environmentally safe to different living organisms.

Key words: Ethanolic extract, Acetone extract, Petroleum ether extract, Toxicity, *Cupressus sempervirens*, *Culex pipiens*, growth, mortality, pupation, emergence, malformation.

INTRODUCTION

Mosquitoes are vectors of many vertebrate blood parasites. In Egypt, *Culex pipiens* has a wide distribution and 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). Recently, Hassan *et al*. (2003) studied the possibility of *C. pipiens* for the transmission of Hepatitis C virus (HCV). However, such possibility is still under investigation.

Insecticide applications although highly efficacious against the target species, vector control is facing a threat due to the development of resistance to chemical insecticides resulting in rebounding vectorial capacity (Liu *et al*., 2006). Furthermore, they are responsible for substantial hazards to a variety of non-target organisms and environment in the form of biomagnification (Gold *et al*., 2001).

Therefore, researchers have diverted their attention since few decades ago towards the plant world, which are ecofriendly and cost effective. Many studies on plant extracts against mosquito vectors have been conducted around the world, but most of them are restricted to preliminary screening (Prajapati *et al*., 2005; Shaalan *et*

al., 2005; Amer and Mehlhorn, 2006; Chaiyasit *et al*., 2006; Promsiri *et al*., 2006; Pavela, 2007).

Bioactive organic compounds produced by plants can act as repellent, oviposition or food deterrents, growth inhibitors, and toxins (Ezeonu *et al*., 2001; Carlini and Grossi-de-Sá, 2002). Thus, crude plant extracts have been screened as natural and biodegradable forms to control pests and vectors of infectious diseases (Omena *et al*., 2007). Sukumar *et al*., (1991) reviewed the bioactivity of 344 plant species against mosquitoes. They showed that some phytochemicals act as general toxicants to all life stages of mosquitoes, whereas others interfere with growth and reproduction or act on the olfactory receptors eliciting responses of attractancy or repellency. The present study aimed to evaluate the biological activity of leaf extracts of *C. sempervirens* (Cupressaceae) against the third instar larvae of the mosquito vector, *C. pipiens*.

MATERIALS AND METHODS

***1. Mosquito culture*:**

The mosquito *C. pipiens L.*, was obtained from Medical Entomology Research Center, Doqqi, Giza. The sample was reared for several generations in the Department of Zoology, Faculty of science Al-Azhar University, Madenit Nasr, Cairo under controlled conditions (27±2°C, RH 70±10% 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. Plastic cup oviposition (15x15cm) containing dechlorinaed 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 (Kasap and Demirhan, 1992).

***2. Collection and extraction of plant materials*:**

Freshly leaves of *C. sempervirens* (Family: Cupressaceae) were collected in the month of March 2010 from the Sadat city (Cairo- Alexandria desert road) and the taxonomic identification was made by Dr. Abdo Marey Ass. Prof. of Botany and Microbiology Department, Faculty of science, Al-Azhar University. The leaves were washed and dried in the shade at room temperature (27–31 °C) for 7 days till they become brittle, then pulverized to powder in a hammer mill. The extraction was performed using 70% ethanol, acetone and petroleum ether solvents. One hundred grams of powder for each solvent separately were extracted five times with 300 ml of aqueous 70% ethanol, acetone and petroleum ether at room temperature. After 24 h., the supernatants were decanted, filtrated through Whatman filter paper No. 5. and dried in a rotary evaporator at 40 ºC for (2– 3) h to ethanol and (40 – 60) minutes to other solvents to obtain 150.0 g/kg (ethanol), 142.0 g/kg (acetone) and 49.0 g/kg (petroleum ether) of a semi solid crude extract. The dry extracts were kept in deep freezer (- 4ºC) till used for experiments.

3. Larval treatment:

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 and petroleum extracts was dissolved in 2 drop of Tween. 80 as emulsifier to facillate the dissolving of tested material in water. Different concentrations of each extract were prepared in order to detect mortalities. All tested

materials were performed in 100ml. of dechloronated tap water contained in 200ml plastic cups. Then, third 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 (27±2°C, RH 70±10% and 12-12 light-dark regime). Control larvae received only 0.1 ml of 70% ethanol or 2 drop of Tween.80 in 100ml water. Mortality was recorded daily and the dead larvae and pupae were removed until adult emergence. Abnormally formed pupae were removed daily and placed in were labeled glass vials containing 70% ethanol and one drop of glycerine for the photography under binocular microscope.

***4. Criteria studied*:**

The larvae were observed daily until pupation and adult emergence to estimate the following parameters:

Larval mortality was indicated by a failure to respond to mechanical stimulation (Williams *et al*. 1986). Larval mortality percent was estimated using the following equation (Briggs, 1960): larval mortality % = A – B / A × 100 (where: A = number of tested larvae, B = number of tested pupa).

Larval duration was calculated as the intervals between the commencement of first instar larvae and the commencement of pupation. It was calculated for each larva and then the mean value was taken.

Pupation rate was estimated using the following equation: Pupation % = A / B × 100 (where: A = number of pupae, B = number of tested larvae).

Pupal mortality was indicated by a failure to respond to mechanical stimulation or failure to metamorphose into the adult stage. The pupal mortality percent was estimated using the following equation: Pupal mortality % = A – B / A × 100 (where: A = number of produced pupae, B = number of observed adults).

Pupal duration was calculated as the interval between the commencement of pupation and the commencement of adult emergence. It was calculated for each one and then the mean value was taken.

Adult emergence of males and females were counted and calculated using the following equation: Adult emergence % = A / B × 100 (where: A = number of emerged adults, B = number of tested pupae).

Growth index was calculated according to Saxena and Sumithra (1985): Growth index = a / b (where: a = % of adult emergence, b = mean developmental period (days).

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

5. Statistical analysis:

Statistical analysis of the data was carried out according to the method of Lentner *et al*., (1982). Lc_{50} was calculated using the multiple linear regression (Finney, 1971).

RESULTS

The biological activity of ethanolic, acetone and petroleum ether extracts of leaves against the 3rd instar larvae of *C. pipiens* has been studied. The biological activity included the larvicidal activity, larval duration, pupal rate, pupal mortality,

pupal duration, total larval and pupal mortality, adult emergence and growth index. The present results can be arranged as follows:

Ethanolic extract:

Data given in table (1) indicated the biological activity of ethanolic extract of *C. sempervirens* against the 3rd instar larvae of *C. pipiens*. Complete larval mortality percent (100.0%) was caused at the highest concentrations (1500 ppm). Meanwhile, the larval mortality % decreased to 23.3 % at the lowest concentrations (150 ppm) (compared to 6.7% for the untreated larvae). The larval duration was affected because the mean duration significantly (P<0.01) increased to 9.65±0.9, 10.1±1.6 and 11.3± 0.6 days at the higher concentrations 150, 325 and 750 ppm, respectively (compared to 7.04±1.3 days for the untreated larvae). A negative correlation between the pupation % and the concentration was observed, where the pupation % was 0.0% at the highest concentration (1500ppm) compared to 93.3% for the untreated group.

Table 1: Effect of ethanolic extract of *Cupressus sempervirens* (leaves) on mortality percent, development and growth index of different stages of *Culex pipiens*.

Conc. ppm	Larval mort. %	Mean Larval Period (days)±SD	Pupation %	Pupal Mort. %	Malf. pupae %	Mean Pupal Period (days)±SD	Larval and pupal Mort. %	Adult Emergence % (a)	Adult Mort. %	Mean Development (days) (b)±SD	Growth Index (a/b)
1500	100.0	—	—	—	—	—	—	—	—	—	—
750	90.0	11.3±0.56***	10.0	33.3	33.3	2.0±1.03ns	93.3	66.7	50.0	13.3±1.59***	5.02
325	66.7	10.1±1.63***	33.3	30.0	30.0	2.7±0.45*	76.7	70.0	42.9	12.8±2.08***	5.47
150	23.3	9.65±0.89***	76.7	21.7	21.7	2.14±0.88ns	40.0	78.26	16.7	11.79±1.77***	6.64
Control	6.7	7.04±1.34	93.3	0.0	0.0	2.07±1.06	6.67	100.0	3.6	9.11±2.40	10.98

No. of tested larvae = 30; Conc. = Concentration; ppm = particle per million; SD = standard deviation; mort = mortality; inter = intermediate; malf. = malformed.

The lethal effect of ethanolic extract from *C. sempervirens* was extended to the pupal stage, scince the highest pupal mortality percent (33.3%) was induced at the concentrations 750 ppm vs. 0.0% for the control groups. The mean pupal duration was insignificantly affected (P>0.01) at all concentrations used except at the moderate concentration (325ppm) where the mean duration significantly (P<0.01) was prolonged to 2.7±0.45 days (compared to 2.07±1.06 days for the controls).

Results of table (1) showed that the highest total larval and pupal mortality percent was 93.3% at 750 ppm (compared to 6.6% for the controls). In table (1) a remarkable reduction in the adult emergence percent was observed. The lowest emergence percent was 66.7% at the concentration 750 ppm , (compared to 100 % for the controls). The lethal effect of leaves extract was extended to the adult stage at all concentrations used because the adult mortality percent was calculated as 50.0, 42.9 and 16.7% at the concentrations: 750, 325 and 125 ppm, respectively (compared to 3.6% for the controls).

The ethanolic extract of *C. sempervirens* exhibiteded some malformation effects on the pupae resulted from treated larvae, since the highest pupal malformation percent was 33.3% at the highest concentrations, compared to 0.0% for the controls (for more details see Plate 1 & 2). The growth index for larvae and pupae was greatly affected by ethanolic extract of *C. sempervirens* (leaves), where it recorded 5.2, 5.47

and 6.64 at the concentrations 750, 325 and 150 ppm, in comparison of 10.98 for the control congeners.

Acetone extract:

Arranged data in table (2) indicated a biological activity of acetone extract from *C. sempervirens* (Leaves) against the 3rd instar larvae of *C. pipiens*. As shown in table (2), the highest mortality percent (100%) was recorded at the concentration (500ppm) and the lowest mortality percent (23.3%) was observed at the lowest concentrations (50ppm) (compared to 6.7% for the controls). Acetone extract from leaves significantly shortened ($P<0.05$) the mean larval duration (6.0±0.81 days at 250 ppm vs. 7.5±0.9 days for the controls). The pupation % of treated larvae decreased as the concentration increased as shown in table 2 (76.7% at 50 ppm; respectively compared to 93.3% for the controls).

Table 2: Effect of acetone extract of *Cupressus sempervirens* (leaves) on mortality percent, development and growth index of different stages of *Culex pipiens*.

Conc. ppm	Larval mort. %	Mean Larval Period (days)±SD	Pupation %	Pupal Mort. %	Malf. pupae %	Mean Pupal Period (days)±SD	Larval and pupal Mort. %	Adult Emergence % (a)	Adult Mort. %	Mean Development (days) (b)±SD	Growth Index (a/b)
500	100.0	—	—	—	—	—	—	—	—	—	—
250	83.3	6.0±0.81***	16.7	60.0	40.0	2.6±0.96 ns	93.3	40.0	100.0	8.6±1.77 ns	4.7
125	63.3	7.18±1.19ns	36.7	36.4	36.4	2.29±0.41 ns	76.7	63.6	57.1	9.5±4.6 ns	6.7
100	53.3	7.36±0.74 ns	46.7	28.6	28.6	1.7±1.05 ns	66.7	71.4	30.0	9.06±1.79 ns	7.9
50	23.3	7.3±1.21ns	76.7	26.1	26.1	2.45±0.98 ns	43.3	73.9	35.3	9.75±2.19 ns	7.6
Control	6.6	7.5±0.9	93.3	3.5	0.0	1.91±1.53	10.0	96.5	0.0	9.41±2.43	10.3

No. of tested larvae, Conc., ppm, SD, mort., inter., malf.: see footnote of table (1)

The acetone extract from leaves exhibited a toxic effect against the pupae resulted from the treated larvae at all the concentrations (250,125, 100 and 50ppm) (the pupal mortality percents were 60.0, 36.4, 28.6 and 26.1%, respectively, compared to 3.5% at the controls). On the hand, the mean pupal duration was insignificantly affected ($P>0.01$) with acetone extract from leaves at all concentrations. Also, no pupal duration or adult emergence was observed by acetone extract from Stems because the extract induced 100% pupal mortality at all concentrations.

The total larval and pupal mortality percents were 93.3, 76.7, 76.7and 43.3% at 250, 125, 100 and 50 ppm, respectively, compared to 10.0% for the untreated group. A remarkable reduction in the adult emergence was observed (see Table 2). At 250 ppm the adult emergence % was 40.0%, this percent increased to 63.6% at the next concentration (125 ppm) and gradually increased reaching to the highest value (73.9%) at 50 ppm while the adult emergence percent for the untreated group was 96.5%. The highest percent of adult mortality (100%) was recorded at 250 ppm. The mortality percent decreased to 57.1, 30.0 and 35.3% at 125, 100 and 50 ppm, respectively, compared to 0.0% for the untreated insects (Table 2).

According to the results of table (2), the acetone extract from leaves caused some malformations among the pupae at all concentrations where it recorded as 40.0, 36.4, 28.6 and 26.1%, respectively, vs. 0.0% for the untreated group (see Plat 1 & 2). The growth index for *C. pipiens* was affected by acetone extract from leaves (4.7, 6.7,

7.9 and 7.6 reductions at 250, 125, 100 and 50 ppm, respectively, compared to 10.3 for the controls.

Petrolum ether extract:

As seen in table (3), data indicated the biological activity of petroleum ether extract from leaves against the 3rd instar larvae of *C. pipiens*. The highest concentrations (100 ppm) caused complete mortality, meanwhile the lowest mortality (16.7%) was caused by the lowest concentration (5 ppm), compared to 3.3% for the untreated insects. The larval duration was shortened where the mean duration significantly decreased ($P<0.01$) to 5.6±0.88, 5.6±0.91 and 5.4±0.82 days at 50, 20 and 10 ppm, respectively, compared to 6.4±0.8 days for the untreated larvae. The pupation percent was 83.0% at the lowest concentration (5 ppm). Furthermore, no pupation was observed at concentration 100 ppm, (compared to 96.0% for the controls).

Table 3: Effect of Petrolum ether extract of *Cupressus sempervirens* (leaves) on mortality percent, development and growth index of different stages of *Culex pipiens*.

Conc. ppm	Larval mort. %	Mean Larval Period (days)±SD	Pupation %	Pupal Mort. %	Malf. pupae %	Mean Pupal Period (days)±SD	Larval and pupal Mort. %	Adult Emergence % (a)	Adult Mort. %	Mean Development (days) (b)±SD	Growth Index (a/b)
100	100.0	—	—	—	—	—	—	—	—	—	—
50	63.3	5.64±0.88*	36.7	9.1	9.1	2.7±1.20^{ns}	66.7	90.9	60.0	8.34±2.08^{ns}	10.9
20	36.7	5.55±0.91***	63.3	10.5	10.5	2.0±0.33^{ns}	43.3	89.5	47.1	7.55±1.34^{ns}	11.8
10	30.0	5.38±0.82***	70.0	14.3	14.3	2.42±1.16^{ns}	40.0	85.7	38.9	7.8±1.98^{ns}	10.9
5	16.7	6.48±0.90^{ns}	83.3	8.0	8.0	1.43±1.11^{ns}	23.3	92.0	13.0	7.91±2.01^{ns}	11.6
Control	3.3	6.4٠±0.80	96.7	0.0	0.0	2.02±1.22	3.3	100.0	0.0	8.42±2.02	11.9

No. of tested larvae, Conc., ppm, SD, mort., inter., malf.: see footnote of table (1).

The pupal mortality was slightly affected by petroleum ether extract and the mean duration of pupae produced by treated larvae was insignificantly affected ($P>0.01$) at all concentrations. The total mortality of larvae and pupae were calculated as 66.7% at 50 ppm, compared to 3.3% for the untreated insects.

The adult emergence was not severely affected by all concentrations. The petroleum ether extract had extended toxic activity on the survival adults resulted from treated larvae at the all concentrations because the adult mortality was recorded as 60.0, 47.1, 38.9 and 13.0%, respectively, compared to 0.0% for the controls.

In addition, the petroleum ether extract exhibited a malformative effect on the pupae. Also, the growth index for larvae and pupae was slightly affected by petroleum ether extract (Plate 1 & 2).

Recalling to the aforementioned results, the toxicity %s of tested extracts of *C. sempervirens* (leaves) based on Lc_{50} values (Tables 4) can be arranged in a descending order as follows: petroleum ether extracts > acetone extracts > ethanolic extracts.

Table 4: Lc_{50} values (ppm) of ethanolic, acetone and petroleum ether extract of *Cupressus sempervirens* leaves against *C. pipiens* larvae.

Extract	LC_{50} (ppm)	Slope (b)	Correlation coefficient (r)
Ethanol	263.6	0.0479	0.7121
Acetone	104.3	0.1454	0.8027
Petroleum ether	37.8	0.8341	0.986

DISCUSSION

Synthetic insecticides are today at the forefront of mosquito controlling agents. Nevertheless, controlling the mosquitoes has become complicated because of their resistance to these chemicals, 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. Plant extracts have been suggested as alternative for insect control because some are selective, biodegrade to nontoxic products, and have few effects on non-target organisms and the environment (Singh and Upadhyay, 1993; Isman, 2006; Pavela, 2007).

Antifeedant and development inhibiting activity reduces pest damage to products even without killing the pest. Further, in the long run, populations are reduced through disrupted metamorphosis or reduced fecundity (Schmutterrer, 1995). The yield and activity of the most active fractions determine the suitability of plant products for mosquito control (Pushpalatha and Muthukrishnan, 1999). Sukumar *et al*. (1991) suggested the existence of variations in toxicities of phytochemical compounds on target species depending on the plant part from which they are extracted. In addition, (Jeyabalan *et al*. 2003; Maurya et *al.* 2009) noted that other variations were due to responses by species and developmental stages of species to the specified extract, solvent of extraction, geographical origin of the plant, photosensitivity of compounds in the extract, effect on growth and reproduction, and other factors.

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). The present study showed high bioactivity of the different extracts from *C. sempervirens* which are grown widely in Egypt. Such results may offer an opportunity for developing alternatives to rather expensive and environmentally hazardous organic insecticides. For controlling the mosquito *C. pipiens* as discussed along the following paragraphs.

Ethanol, petroleum ether and acetone extracts from *C. sempervirens* and their application against the larval stage of *C. pipiens* clearly affected the various biological aspects as follows:

1. Larvicidal activity:

Toxicity of the tested plant extracts against the 3rd larval instar varied according to plant part used and the extract concentration. The larval mortality percent increased as extract concentration increased for all plant extracts. The toxicity values of tested extracts from leaves of *C. sempervirens* based on Lc_{50} values may be arranged in a descending order as follows: petroleum ether extracts > acetone extracts>ethanolic extracts. These results agree, to some extent, with the previously mentioned suggestions of Sukumar *et al*. (1991) and Maurya *et al.* (2009).

Extracts from several other plant species were tested on different species of mosquitoes by many authors worldwide. The activity of the plant extracts on larval mortality of *C. pipiens,* in the present study, were in agreement with the results obtained by Hamouda *et al*. (1996), Shalaby *et al*. (1998), Al- Dakhil and morsy (1999), Masoud *et al.* (2001), Ahmed *et al.* (2001), Pelah *et al*. (2002), Jeyabalan *et al* (2003), Nathan *et al*. (2005), Nathan *et al*. (2006), Sharma et al. (2006b), Coria *et al.* (2008), Maurya *et al.* (2009). The efficacy of extracts from rhizomes of *Curcuma*

aromatica were tested against the larvae of filariasis vector mosquitoes, *Culex quinquefasciatus* employing standard WHO procedure at Mysore by Madhua *et al.* (2010),The soxhlet extraction was carried out using non-polar organic solvent, petroleum ether. The efficacy of petroleum ether extract seemed to be effective with LC_{50} and LC_{90} values of 11.42 and 18.00ppm respectively. In the present study, Also, the petroleum ether extracts seemed to be more effective than other extracts with LC_{50} values of 37.8ppm.

2. Larval and pupal durations:

Exposure of *C. pipiens* 3rd instar larvae to sub-lethal dose of all extracts from the present plant resulted in variable effects according to solvent and concentration of the extract. Acetone and petroleum ether extract significantly shortened the duration of larvae. Larvae were observed to pupate faster as their environment increased in toxicity. Consequently, the average development period (a factor in the Growth Index formula) was consistently negatively correlated with insecticide concentrations and the duration of exposure. Meanwhile, the larval duration significantly increased by ethanolic extract. Moreover all plant extracts insignificantly affected the pupal duration. This effect can vary, however, with some researchers showing no effect on the larval and pupal developmental periods (Saxena et al. 1993) and other researchers showing prolongation of the larval and pupal developmental periods (Jeybalan *et al* 2003 using methanol extract of *Pelargonium citrosa* leaf against *An. stephensi* larvae, Nathan *et al*. 2005 using the neem, *Azadirachta indica* extract against *An. stephensi* larvae, Nathan *et al*. 2006 using methanolic extracts of leaves and seeds from chinaberry tree, *Melia azedarach* against *An. stephensi* larvae, Sharma *et al*. 2006 a & b using petroleum ether extract of *Artemisia annua* against *An. stephensi* and *C. quinquefasciatus* larvae, Coria *et al.* 2008 using ethanolic extract of *M. azedarach* leaves on *Aedes. aegypti* larvae and Juliene *et al*. 2009 using *Moringa oleifera* lectin against *Ae. aegypti* larvae). In another study, *Melia volkensii* was observed to prolong the lifespan of *An. arabiensis* larvae but not the pupal period (Mwangi and Mukiama 1988). Conversely, Supavarn *et al*. (1974) reported on 11 of 36 botanicals that significantly inhibited pupal development while only a few botanicals affected larval development.

3. Pupation, pupal mortality and adult emergence:

A remarkable decrease in the pupation percent was induced by all plant extracts in the present study. The pupation% decreased as the concentration of the plant extract increased. Moreover, the pupation rate depended on the solvent used in extraction.

The present study showed that the toxic effects of plant extracts tested had been extended to the pupae. In addition, all plant extracts induced some reductions of the adult emergence. The reduction was found as a concentration–dependent. These results are comparable to earlier results of Abou El-Ela *et al*.(1994) using *Cardiospermum halicacabum* extracts against *C. pipiens* larvae, Shalaby *et al*. (1998) using peel oils of lemon, grapefruit and naval orange against *C. pipiens* larvae, Khalaf (1999) using the two plant volatile oils of *Lantana camara* and *Conyza dioscaridis* against *C. pipiens* larvae, Al-Dakhil and Morsy (1999) using ethanol extracts of peel oils of lemon, grapefruit and naval orange against *C. pipiens* larvae, Assar and El–Sobky (2003) using water extracts of *Eichhornia crassipes* and *Artemisia Monosperma* 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 *A. Monosperma* against *C. pipiens* larvae and Nathan *et al*. (2006) using methanolic extracts of leaves and seeds of *M. azedarach*

against *An. stephensi* larvae, Sharma *et al.* (2006 a & b) using petroleum ether extract of *A. annua* against *An. stephensi* and *C. quinquefasciatus* larvae, respectively, Wiesman and Chapagain (2006) using one fraction obtained from the silica gel column chromatography of the methanol extract against *Ae. aegypti* mosquito larvae and Pavela (2009) using essential oils obtained from *Thymus vulgaris*, *Satureja hortensis* and *Thymus satureioides* plants.

4. Adult survival:

Results obtained in the present study indicated that the toxicity of plant extracts against the 3rd instar larvae of *C. pipiens* was extended to the 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 *An. stephensi*, Nathan *et al*. (2005) using the neem *A. indica* extract against *An. stephensi* and Nathan *et al*. (2006) using methanolic extracts of leaves and seeds from the chinaberry tree *Melia azedarach* against *An. stephensi*.

The growth index (G.I.) of *C. pipiens* was remarkably affected by the present plant extracts tested. It decreased as the concentration of the extract increased. Retardation in growth was induced by acetone extract tested. Such results are in agreement with earlier studies using different plant extracts against different mosquito species (Saxena *et al*., 1993; Jeyabalan *et al.*; 2003; Shaalan *et al.* 2005; Nathan *et al*. 2006 ; Sharma *et al.* 2006 a&b).

5. Morphogenic effects:

In the present study, almost all extracts, against the 3rd instar larvae of *C. pipiens,* induced some morphological abnormalities in pupae and adults. The malformed pupae were not able to develop normally and then died. Also, the present results showed that the percent and degree of malformation were concentrations dependent. Similar observations were obtained by different plant extracts against different mosquito species as cited by Abahussain (1999) using *Calotropis procera* extracts against *C. pipiens* and *A. multicolor* observed morphological abnormalities among pupae. El–Bokl (2003) recorded varying degrees of morphogenic abnormalities in immatures and adult stages of *C. pipiens* when larvae were treated with the neem, *Azadirachta indica* extract. These morphogenetic abnormalities are commonly caused by botanical extracts and are thought to result from a disturbance to growth regulating hormones (Saxena *et al*. 1993).

In general, it could be concluded that almost the plant extracts used in the present study act as larvicidal, and possess growth and emergence inhibiting against 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. Further studies on the tested plants including mode of action, synergism with the biocides under field condition are needed.

REFERENCES

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.

Abou El – Ela, R.G.; Hilmy, N.M.; El – Monairy, O.M. and El – Barky, N.M. (1994): Susceptibility of *C. pipiens* Linn. to *Cardiospermum halicacabum* extract. Bull. Ent. Soc. Egypt. Econ. Ser., 21: 205 – 212.

Ahmed, A.H.; Kamal, I.H. and Ramzy, R.M. (2001): Studies on the molluscicidal and larvicidal properties of *Solanum nigrum* L. leaves ethanol extract. J. Egypt. Soc. Parasitol., 31 (3) 843 – 852.

Al-Dakhil, M.A. and Morsy, T.A. (1999): The larvicidal activities of the peel oils of three citrus fruits against *C. pipiens*. J. Egypt. Soc. Parasitol., 29 (2) 347 – 352.

Alkofahi, A.; Rupprecht, J.K.; Anderson, J.E.; Mclaughlin, J.L.; Mikolajczak, K.L. and Scott, B.A. (1989): Insecticides of plant orign, American chemical society, Washington, DC, 25 – 43.

Amer, A. and Mehlhorn, H., (2006): Larvicidal effects of various essential oils against *Aedes, Anopheles* and *Culex* larvae (Diptera, Culicidae). Parasitol. Res. 99, 466–472.

Assar, A.A. and El- Sobky, M.M. (2003): Biological and histopathlogical studies of some plant extracts on larvae of *C. pipiens* (Diptera : Culicidae). J. Egypt. Soc. Parasitol., 33 (1) 189 – 200.

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.

Carlini, C.R., Grossi-de-Sá, M.F., (2002): Plant toxic proteins with insecticidal properties. A review on their potential as bioinsecticides. Toxicon 40, 1515–1539.

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– 544.

Chaiyasit, D., Choochote, W., Rattanachanpichai, E., Chaithong, U., Chaiwong, P., Jitpakdi, A., Tippawangkosol, P., Riyong, D., Pitasawat, B., (2006): Essential oils as potential adulticides against two populations of *Ae. aegypti*, the laboratory and natural field strains, in Chain Mai Province, northern Thailand. Parasitol. Res. 99:715–721.

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 *Ae. aegypti* (L.) (Diptera: Culicidae). Bioresource Technology 99: 3066–3070.

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.

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.

Ezeonu, F.C., Chidume, G.I., Udedi, S.C., (2001): Insecticidal properties of volatile extracts of orange peels. Bioresour. Technol. 76, 273–274.

Finney, D.J. (1971): Probit analysis Third edition. Cambridge Univ. Press., 333 p.

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.

Gold, L.S., Slone, T.H., Ames, B.N., Manely, N.B., (2001): Pesticide residues in food and cancer risk; a critical analysis. In: Krieger, R. (Ed.), Hand book of Pesticide Toxicology, 2nd ed. Academic Press, San Diego, C.A., pp.799-843.

Hamouda, L.S.; Elyassaki, W.M. and Hamed, M.S. (1996): Toxicity and hisopathological effects of *Artemisia judaica* and *Anagallis arvensis* extracts on *C. pipiens* larvae. J. Egypt. Ger. Soc. Zoll., 20(E): 43 – 60.

Hassan, M.I.; Mangoud, A.M.; Etewa, S.; Amin, I.; Morsy, T.A.; El hady, G.; El Besher, Z.M. and Hammad, k. (2003): Experimental demonstration of Hepatitis C virus (HCV) in an Egyptian strain of *C. pipiens* complex. J. Egypt. Soc. Parasitol., 33(2): 373 – 384.

Isman, M.B., (2006): Botanical insecticides, deterrent, and repellent in modern agriculture and increasingly regulated world. Annu. Rev. Entomol. 51:45-66.

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.

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.

Juliene, S.C.; Nataly D.L.S.; Thiago, H.N.; Francis, S.G.; Rodrigo, S.F.; Russolina, B.Z.; Luana, C.B.B.C.; Sônia, P.L.; Daniela, M.A.F.N. and Patricia, M.G.P. (2009): Effect of *Moringa oleifera* lectin on development and mortality of *Ae. aegypti* larvae. Chemosphere 77: 934-938.

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.

Khalaf, A.A. (1999): Evaluation of toxicity of two plant volatile oils against laboratory and field strains of *C. pipiens* larvae. J. Egypt. Ger. Soc. Zoll., 28(E): 61 – 71.

Lentner, C.; Lentner, C. and Wink, A. (1982): Studentis t- distribution tables. In Geigy scientific Tables Vol. 2. International Medical and Pharmaceutical information, Ciba – Geigy Limited, Basal, Switzerland.

Liu, N., Xu, Q., Zhu, F., Zhang, L., (2006): Pyrethroid resistance in mosquitoes. Insect Sci. 13:159–166.

Madhu, S.K.; Shaukath, A.K. and Vijayan, V.A. (2010): Efficacy of bioactive compounds from *Curcuma aromatica* against mosquito larvae. Acta Tropica 113: 7–11.

Masoud, A.M.; Labib, I.M. and Rady, M. (2001): Biochemical changes of *C. pipiens* larvae treated with oil and oleo- resin extracts of Myrrh *commiphora molmol*. J. Egypt. Soc. Parasitol., 31(2): 517 – 529.

Maurya, P. ; Sharma, p.; Mohan, L. ; Batabyal L. and Srivastava, C.N. (2009): Evaluation of the toxicity of different phytoextracts of *Ocimum basilicum* against *An. stephensi* and *Culex quinquefasciatus*. Journal of Asia-Pacific Entomology 12: 113–115.

Murugan, T.S.; Babu, R.; Jeyabalan, D.; Senthil Kumar, N. and Sivaramkrishnan, S. (1996): Antipupational effect of neem oil and neem seed kernel extract against mosquito larvae of *An. stephensi* (Liston). J. Entomol. Res., 20:137-139.

Muthukrishnan, J.; Pushalatha, E. and Kasthuribai, P. (1997): Biological effect of four plant extracts on *C. quinquefasciatus* (Say) larval stages. Insect. Sci. Appl. 17: 389 – 394.

Mwangi, R.W and T.K. Mukiama. (1988): Evaluation of *Melia volkensii* extract fractions as mosquito larvicides. J. Am. Mosq. Contr. Assoc. 4: 442-447.

Nathan, S.S.; Kalaivani, K. and Murugan, k. (2005): Effects of neem limonoids on the malaria vector *An. stephensi* Liston (Diptera: Culicidae). Acta Trop. 96(1): 47-55

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 *An. stephensi* Liston (Diptera: Culicidae). Bioresour Technol 79: 1316 – 1323.

Omena, M.C. ; Navarro, D.M.A.F. ; Paula, J.E. ; Luna, J.S. ; Lima, M.R. and Sant'Ana, A.E.G. (2007): Larvicidal activities against *Ae. aegypti* of some Brazilian medicinal plants. Bioresource Technology, 98: 2549–2556.

Pavela, R., (2007): Larvicidal effects of various Euro-Asiatic plants against *C. quinquefasciatus* say larvae (Diptera: Culicidae). Parasitol. Res. 36:821–823.

Pavela, R. (2009): Larvicidal property of essential oils against *C. quinquefasciatus* Say (Diptera: Culicidae). Industrial Crops and Products 30: 311–315.

Pelah, D.; Abramovich, Z.; Markus, A. and Wiesman, Z. (2002): The use of commercial saponin from *Quillaje saponaria* bark as a natural larvicidal agent against *Ae. aegypti* and *C. pipiens*. J. Ethnopharmacol., 81(3):407-9.

Prajapati, V., Tripathi, A.K., Aggarwal, K.K., Khanuja, S.P.S., (2005): Insecticidal, repellent and oviposition deterrent activity of selected essential oils against *An. stephensi*, Aedes aegypti and *C. quinquefasciatus*. Biores. Technol. 96, 1749–57.

Promsiri, S., Naksathit, A., Kruatrachue, M., Thavara, V., (2006): Evaluations of larvicidal activity of medicinal plants extracts to *Ae. aegypti* (Diptera: Culicidae) and other effects on a non target fish. Insect Sci. 13:179–188.

Pushpalatha, E. and Muthukrishnan, J. (1999): Efficacy of two tropical plant extracts for the control of mosquitoes. J. Appl. Ent., 123: 369 – 373.

Rohani, A., Chu,W.L., Saadiyah, I., Lee, H.L., Phang, S.M., (2001): Insecticide resistance status of *Ae. albopictus* and *Ae. aegypti* collected from urban and rural in major towns of Malaysia. Trop. Biomed. 18 (1): 29–39.

Saxena, S.C. and L. Sumithra. (1985): Laboratory evaluation of leaf extract of a new plant to suppress the population of malaria vector *An. stephensi* Liston (Diptera: Culicidae). Curr. Sci. 54: 201-202.

Saxena, R.C.; Harshan, V.; Saxena, A.; Sukumaran, P.; Sharma, M.C. and Lakshamana, K.M. (1993): Larvicidal and chemosterillant activity of *Annona squamosa* alkaloids against *An. stephensi*. J. Am. Mosq. Control. Assoc., 9(1): 84 – 87.

Schmutterrer, H. (1995): The neem tree *Azadirachta indica*, *A. juss* and other Meliaceous plants., VCH Publishers, Weinheim, Germany, 696 pp.

Shaalan, E.A., Canyon, D.V., Younges, M.W.F., Abdel-Wahab, H., Mansour, A., (2005): A review of botanical phytochemicals with mosquitocidal potential. Environ. Int. 31:1149–1166.

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.

Sharma, p.; Mohan, L. and Srivastava, C.N. (2006 a): Phytoextract-induced developmental deformities in malaria vector. Bioresource Technology 97: 1599–1604.

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.

Singh, G., Upadhyay, R.K., (1993): Essential oils a potent source of natural pesticides. J. Sci. Ind. Res. 52: 676–683.

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.

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.

Supavarn, P., R.W. Knapp, and R. Sigafus. (1974): Biologically active plant extracts for control of mosquito larvae. Mosq. News. 34: 398-402.

Wattanachai, P. and Tintanon, B., (1999): Resistance of *Ae. aegypti* to chemical compounds in aerosol insecticide products in different areas of Bangkok, Thailand. Commun. Dis. J. 25: 188–191.

Wiesman, Z. and Chapagain, P. (2006): Larvicidal activity of saponin containing extracts and fractions of fruit mesocarp of *Balanites aegyptiaca*. Fitoterapia 77: 420–424.

Williams, K.A.; Green, D.W.J.; Pascoe, D. and Gower, D.E. (1986): The acute toxicity of cadmium to different larval stages of *Chrionomus riparius* (Diptera : Chironomidae) and its ecological significance for pollution regulation. J. Sci. Unvi. Tokushima. 22: 25 - 31.

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 plates (1&2) and can be summarized as follows:

Plate: 1 (Fig. A–C) shows some morphological abnormalities among pupae resulted from larvae treated with plant extracts tested.

A – Deformed decolorized and partially exuviated attached to the moulting skin as induced by petroleum ether and acetone extract (5, 10 and 250ppm), respectively.

B – pupal- adult intermediate resulted from larvae treated with ethanolic and acetone extracts (325 and 125pm), respectively.

C – Deformed decolorized pupal- adult intermediate resulted from larvae treated with petroleum ether extract (5 and10ppm) and acetone extract (all concentrations used).

Plate: 2 (Fig. A–C) shows some morphological abnoramalities among adults resulted from larvae treated with the plant extracts tested.

A – Half- ecdysed adult resulted from the treatment of the larvae with petroleum ether extract (20 and 50ppm).

B – Incompletely emerged adult with mouthparts, thoracic appendages, and tip of abdomen attached to the pupal skin. This feature was induced by ethanolic extract (150 and 750ppm).

C – Incompletely emerged adult with thoracic appendages and abdomen attached with the pupal skin. This feature was induced by acetone extracts (50 and 100ppm), petroleum ether extract (20ppm).

Plate 1

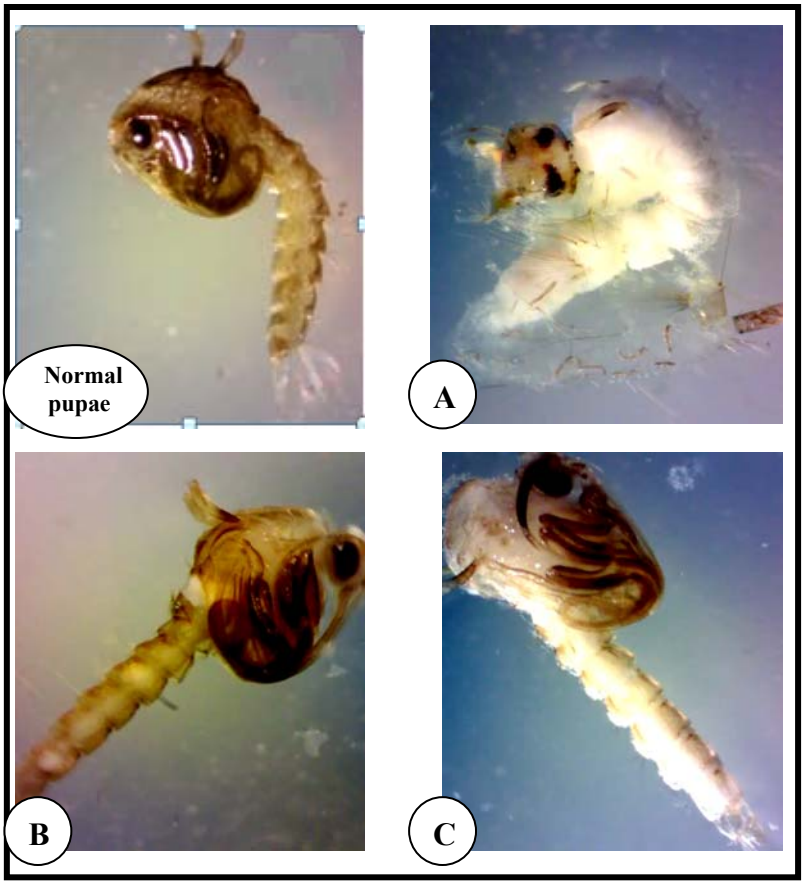
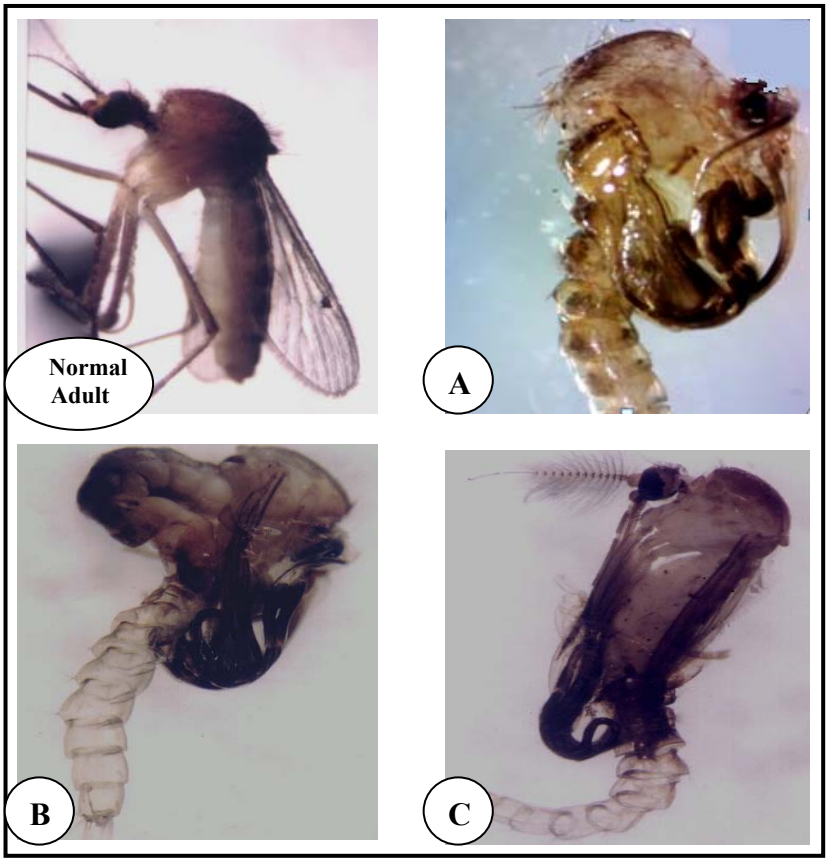


Plate 2



ARABIC SUMMARY

تقييم النشاط البيولوجي لبعض مستخلصات نبات السرو ضد البعوضة الناقلة للأمراض *كيولكس بيبينز* (ثنائية الأجنحة : كيولسيدى)

طارق محمد يسرى الشيخ[١]، مصطفى إبراهيم حسن ، ولاء أحمد مصيلحى[٢]، منير صالح عامر[١]
أحمد زينهم شحاتة[١]
١- قسم علم الحيوان- كلية العلوم- جامعة الأزهر- القاهرة (بنين)
٢- قسم علم الحيوان- كلية العلوم- جامعة الأزهر-القاهرة (بنات)

أجريت الدراسة الحالية بهدف الكشف عن النشاط البيولوجي لثلاثة مستخلصات نباتية (الإيثانول، الأسيتون والاثير البترولي) من أوراق أحد النباتات الصحراوية المحلية في مصر، وهو السرو *كوبرسوس سمبرفيرينز*، ضد بعوضة *كيولكس بيبينز*. *وقد تم* تقويم النشاط البيولوجي لهذه المستخلصات ضد الدور اليرقى الثالث، وكذلك العذارى و الطور اليافع الناتجة منه.

ودلت نتائج الدراسة على أن مستخلص الإثير البترولى كان أشد تأثيرا ضد الدور اليرقى الثالث، والأطوار اليافعة مضاهاة بمستخلصات الايثانول والأسيتون. كما تم حساب التركيز نصف المميت (ت ن م ٥٠) لهذه المستخلصات المختلفة ضد الطور اليرقى للبعوضة وترتيبها ترتيبا تنازليا، كما يلى : مستخلص الايثانول (ت ن م ٥٠: ٢٦٣.٧ ج ف م) > مستخلص الأسيتون (ت ن م ٥٠:١٠٤.٣ ج ف م) > مستخلص الاثير البترولى (ت ن م ٥٠: ٣٧.٨ ج ف م).

كما أوضحت نتائج الدراسة حدوث نقص واضح في معدل التعذر، ومعدل حياة العذارى الناتجة من اليرقات المعاملة، وكذلك نقص ملحوظ في نسبة تحول العذارى إلى يافعات بعد إستعمال معظم المستخلصات النباتية. ودلت النتائج على أن سمية بعض المستخلصات النباتية المختبرة على الدوراليرقى الثالث قد امتدت إلى الطور اليافع الناتج عن المعاملة. وظهر بعض التأثيرات التشوهية في العذارى، وكذلك أشكال وسطية بين العذارى و الطور اليافع، وذلك بعد إستعمال معظم المستخلصات النباتية على يرقات الدور الثالث.

وقد تمنح نتائجِ البحث الحالي فرصةً لتَطوير البدائلِ النباتية زهيدة الأثمان للمبيداتِ الحشرية العضويةِ المكلفة، والخطرةِ بيئياً، وهي البدائل المتوفرة بيئيا وغير الخطيرة على الكائنات الحية في مجال مكافحة البعوض الناقل للأمراض.