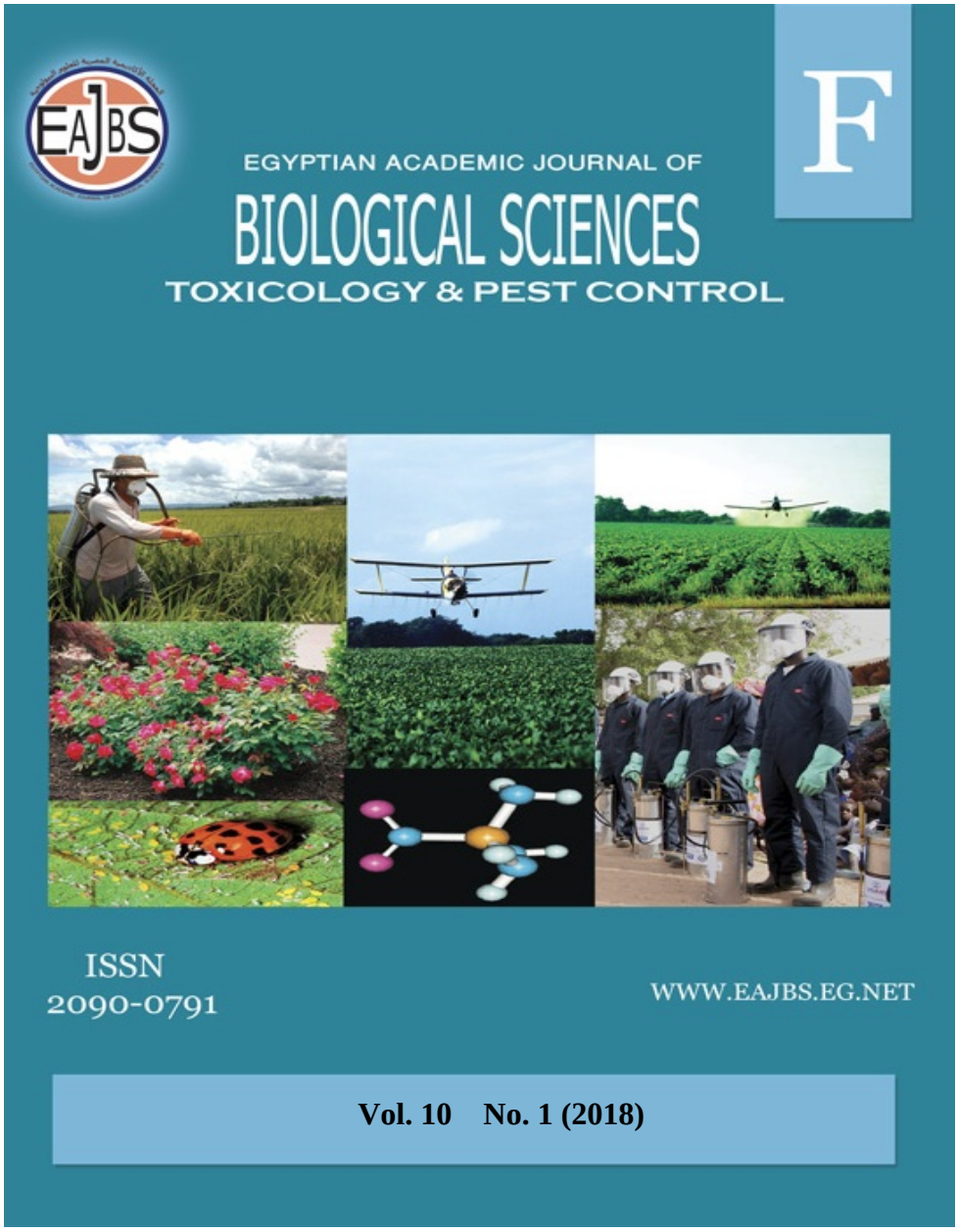

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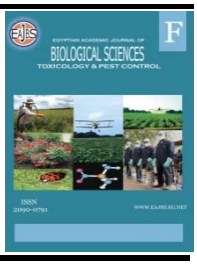

Effect of some natural products on the vector of *Bancroftian filariasis* in Jizan, KSA

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ABSTRACT

Rotenone, limonine and jojoba oil bioactivity was studied on the 3rd instar larvae of *Culex (Cx.) pipiens pallens* as a Bancroftian filariasis vector and the prevalent mosquito in Jizan, KSA. They induced toxic effects on different stages of *Cx. pipiens*, but in different percent. They revealed $Lc_{50}$of 13.6, 658.39 and 5991.5 ppm for rotenone, limonine and jojoba oil respectively.

INTRODUCTION

The house mosquito (*Culex (Cx.) pipiens pallens*) is the major vector of wuchereriasis and epidemic encephalitis B, which cause millions of deaths every year, especially in India and Africa (Wang *et al.*, 2016). It is the prevalent mosquito in Jazan province, Kingdom of Saudi Arabia (KSA) Bakr *et al*., (2014) Three filarial cases were reported from Saudi residences in Armed Forces Hospital, Riyadh in 2002 (Haleem *et al.*, 2002). Omar (1996) reported that local *Cx. pipiens* mosquitoes might act as a potential vector of introduced Bancroftian filariasis in KSA.

Most mosquito control programs target the larval stage at their breeding sites with larvicides since adulticides may reduce the adult population only temporarily. Therefore, a more efficient approach to reduce the population of mosquitoes would be to target the larvae (Chung *et al.*, 2009; Conti *et al.*, 2010).

Vector control programs using synthetic insecticides organophosphates (e.g., temephos, fenthion, and malathion), and insect growth regulators (e.g., diflubenzuron, methoprene) have long been utilized to stop the transmission of these diseases. However, frequent and indiscreet application of these synthetic insecticides has caused the disruption of the natural biological control systems and sometimes resulted in the widespread development of resistance as well as undesirable effects on

non-target organisms, toxic residues in food, workers' safety, and high cost of procurement (Isman, 2006) (Regnault-Roger, *et al.*, 2012). As a result, there is a critical need for the development of alternatives to synthetic insecticides. Essential oils and their constituents have been recommended as alternative sources for insect control, predominantly because some are selective, biodegrade to nontoxic products, and have minimal impacts on non-target organisms and the environment (Isman, 2006). Many essential oils and constituent compounds that come from various essential oils can put forth toxic activity against mosquito species (Chang, *et al.,* 2014).

Limonoids are tetranortriterpenes and secondary metabolites produced in plants of the order Rutales. Within this order, limonoids are most often found in the family Meliaceae and less frequently in the families of Rutaceae and Cneoraceae (Champagne *et al.,* 1989, 1992). They have attracted greater concern due to their growth regulating activity (Champage *et al.,* 1992) besides having anti-carcinogenic effects (Sohail *et al.,* 2005). They have some potential as insecticide (Akram *et al.,* 2010). Alpha-terpinene and Limonene exhibited the best larvicidal effect against the larvae of *Aedes aegypti* and *Aedes albopictus* (Cheng et al., 2009). Rotenone is used to control a wide range of arthropod pests. It is an inhibitor of site I respiration within the electron transport chain of susceptible insects and is a selective, nonsystemic insecticide with contact and stomach action and secondary acaricidal activity. Rotenone has been cleared for use in organic farming when insect pressure is very high. Rotenone, one of the most extensively used natural insecticides, has been reported to be highly active against 4th instar larvae of *Aedes aegypti* L. (Abe *et al.,* 1985). Zubairi *et al.,* (2004) found that rotenone gave optimum mortality of mosquito larvae of 83.33 % at 0.05 mg/ml after 5 hours post treatment.

Yenesew *et al.* (2005) reported that Rotenone LC_{50} on *Aedes agypti* and *Cx. quinquefasciatus* is 0.52 and 0.45 µg/ml respectively but the crude extract of *Derris trifoliate* was less toxic as a larvicidal agent against the two mosquitoes. Extract of *Derris trifoliate* was less toxic as a larvicidal agent against the two mosquitoes. Seed, root and stem parts LC_{50} was 0.56 and 0.63 µg/ml, 0.74 and 0.45 µg/ml and 7.53 and 8.23 µg/ml respectively against *Aedes agypti* and *Cx. quinquefasciatus*.

Jojba oil acts as a pesticide by forming a physical barrier between the insect pest and the leaf surface. Jojoba oil caused an increase in larval and pupal durations of *Agrotis ipsilon* Abdel-Rady and Osman (2005). Shonouda & Mehanney (2000) studied the toxicity jojoba oil against the 4th instar larvae of *Cx. pipiens* leading to LD_{50} of 649.43 ppm but it showed insignificant effect on the 3rd instar larvae of *Musca domestica* and also Tanani, (2001) stated that Jojoba oil exhibits some biological activites against various insect pests. The biological effect of rotenone, limonene and jojoba oil against *Cx. pipiens* will be studied at this work.

MATERIALS AND METHODS

Tested materials:

Rotenone was obtained from Sigma Chemicals Company. Rotenone is the extract of *Lanochocrapus* species or *Derris* species root. Different concentrations (1, 5, 10, 15, 20, 25 and 30 ppm) of rotenone insecticide were prepared using aceton as solvent.

Limonene was obtained from Sigma Chemicals Company. Limonine is the extract of citrus fruit peels. Different concentrations (125, 250, 500, 1000 and 1500 ppm) of limonene insecticide were prepared using water as solvent.

Jojoba is non-volatile oil obtained from the seeds of the Jojoba bean (*Simmondsia chinesis*). It is reported as emulsifiable concentrate 96% obtained from Agricultural Research Centre, Laboratory of Pesticides, Doqqi, Giza. Six conc. levels of Jojoba: 4000, 6000, 8000, 10000 and 12000 ppm were prepared.

Larval treatment:

Laboratory reared 3rd instar larvae of *Cx. pipiens* were tested with different concentrations of selected plant extracts in Biology 1 laboratory, Science faculty, Jazan University during September to December 2013 according to the standard WHO procedure (1981) with some modification. A total of 25 larvae were introduced in 500 ml glass beaker containing various concentrations of different tested materials. The treatments were replicated three times, and each replicate set contained one control. Mortalities were reported after 48 hours of the exposure period. Laboratory room temperature was maintained at 27 ± 2 ^{0}C during the experiment period. The moribund and dead larvae in three replicates were combined and expressed as percentage mortality for each concentration. Dead larvae were acknowledged when they failed to move after probing with a needle. Moribund larvae were those unable of rising to the surface within reasonable period of time. The percentage mortality was calculated and analysis of data was carried out by employing probit analysis (Finney, 1971) and corrections for mortality if needed were done by using Abbott formula (Abbott, 1925).

Criteria studied:

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

Larval mortality, larval duration, pupation rate, pupal mortality percent, pupal duration, Adult emergence, Growth index, pupal malformation and total inhibition percent.

Statistical analysis:

Data is presented as mean ± standard deviation (SD) of mean. Statistical comparisons were performed using Students t-test. $p<0.05$ was considered statistically significant.

RESULTS

Rotenone effect:

Data given in Table (1) indicated that Rotenone had a high toxic effect against the 3rd instar larvae of *Cx. pipiens*. The larval mortality percent were 6.7, 20, 36.7, 56.7, 73.3, 96.7 and 100 % at concentrations 1, 5, 10, 15, 20, 25 and 30ppm, respectively. Larval duration was affected by Rotenone, where the mean duration significantly ($P<0.01$) increased to 9.2±3.5, 12.4±3.4, 12.6±3.6, 12.8±3.7 and 12.1±3.2 days at the concentrations 1, 5, 10, 15 and 20ppm; respectively compared to 6.9±2.7 days for the untreated larvae. Positive correlation between the concentration of Rotenone extract and the percent of pupal mortality was observed. The pupal mortality percent was 10.7, 29.2, 52.6, 77, 87.5 and 100 % at the concentrations 1, 5, 10, 15, 20 and 25ppm respectively, compared to 3.3% at the control. Inverse relationship between the pupation % and the concentration was observed, where the pupation % was 0.0% at the highest concentration (30ppm) and 93.3% at the lowest concentration (1ppm) compared to 100% for the untreated group. It induced some malformation percent among pupae resulted from treated larvae especially at the highest concentrations (25, 20, 15, 10 and 5ppm), where the malformation percent was 100.0, 87.5, 77, 42.1 and 16.7%; respectively compared to 0.0% for the control group. Results showed that, the mean pupal duration was insignificantly ($P>0.01$)

affected at all concentrations used except the highest concentration 15ppm, where the pupal duration significantly (P<0.05) increased to 3.28±0.46 days vs. 2.97±0.24 days for the untreated group. The adult emergence 0.0 % was occurred at the concentration of 25ppm, meanwhile the percent increased to 12.5 and 23.0% at the concentrations 20 and 10ppm, respectively and gradually increased till reaching the highest percent (89.3%) at the lowest concentration 1ppm. The adult emergence percent for the untreated group was 96.7%. The lethal effect was extended to the adult stage at all concentrations, the highest percent of adult mortality (100%) was recorded at 20 ppm, the mortality percent decreased to 66.6, 44.4, 23.5 and 8.0 % at 15, 10, 5 and 1 ppm, respectively, compared to 0.0% for the untreated insects. Rotenone total inhibition on different stages of *Cx. pipiens* reached 23.3, 56.7, 83.3, 96.7 and 100 % at 1, 5, 10, 15 and 20ppm, respectively, compared to 3.3 % for the untreated group. The growth index for larvae and pupae was greatly affected by Rotenone. Growth index was negatively correlated to the concentration, where it recorded 0.8, 1.4, 3.1, 4.6 and 7.4 reductions at 20, 15, 10, 5 and 1 ppm, respectively, compared to 10.1 for the controls.

Table (1): Effect of rotenone on mortality percent, development and growth index of 3rd larval instar of *Cx. pipiens*.

Conc. ppm	Larval mort %	Mean Larval Period (days)±SD	Pupal Mort. %	Malf. pupae %	Pupation %	Mean Pupal Period (days)±SD	Adult Mort. %	Adult Emergence % (a)	Total inhibition	Mean Development (days) (b)±SD	Growth Index (a/b)
Control	0.0	6.9±2.7	3.3	0.0	100	2.97±0.24	-	96.7	3.3	9.87±2.94	10.1
1	6.7	9.2±3.5**	10.7	0.0	93.3	2.93±0.44^{ns}	8.0	89.3	23.3	12.1±3.94**	7.4
5	20	12.4±3.4**	29.2	16.7	80	2.91±0.38^{ns}	23.5	69.8	56.7	15.3±3.78**	4.6
10	36.7	12.6±3.6**	52.6	42.1	63.3	2.94±0.39^{ns}	44.4	47.4	83.3	15.5±3.99**	3.1
15	56.7	12.8±3.7**	77.0	77.0	43.3	3.28±0.46*	66.6	23.0	96.7	16.1±4.16**	1.4
20	73.3	12.1±3.2**	87.5	87.5	26.7	4.0^{B}	100	12.5	100.0	16.1±3.2**	0.8
25	96.7	12.0^{A}	100	100	3.3	-	-	0.0	-	-	-
30	100	-	-	-	0.0	-	-	-	-	-	-

No. of tested larvae = 30; Conc. = Concentration; ppm = particle per million; SD = standard deviation; mort = mortality; malf. = malformed, * = significant (P >.05), **= significant (P >.01), ^{ns} = insignificant

A = one larva has developed into pupa. B =one pupa has developed into adult

Jojoba oil Effect: it induced complete larval mortality percent (100%) at the highest concentration (12000ppm). Meanwhile, the larval mortality % decreased to (26.7%) at the lowest concentration (4000ppm) compared to 3.3% for the untreated larvae. Duration of larvae was insignificantly (P>0.01) affected at all concentrations used as compared with the untreated group. The lethal effect of Jojoba oil was extended to the pupal stage inducing 100, 100, 88.9 and 59.1 % pupal mortality at the concentrations 10000, 8000, 6000 and 4000ppm. Its lethal effect was extended also to the emerged adults inducing 100 % adult mortality at the concentrations 4000 and 6000ppm. The pupation percent of treated larvae decreased as the concentration increased (73.3, 60.0, 33.3, and 10.0% at 4000, 6000, 8000 and 10000ppm; respectively compared to 96.7 % for the control. Pupal malformation percent was 100, 100, 88.9 and 53.8% at the concentrations 10000, 8000, 6000 and 4000ppm; respectively compared to 0.0% for the control group. The adult emergence percent was highly affected at all concentrations: 10000, 8000, 6000 and 4000ppm, where it recorded 0.0, 0.0, 11.1 and 40.9%, respectively compared to 0.0% for the control group. Total inhibition percent were 100 and 100 % at 4000 and 6000 ppm, respectively, compared to 6.6 % for the control group. A very highly retarded effect

on growth index was induced by the Jojoba oil extract. At the concentrations 6000 and 4000ppm, the growth index was 1.2 and 4.8 compared to 11.4 for the untreated group Table (2) .

Table (2): Effect of Jojopa oil on mortality percent, development andgrowth index of 3rd larval instar of *Cx. pipiens*

Conc. ppm	Larval mort. %	Mean Larval Period (days)±SD	Pupal Mort. %	Malf. pupae %	Pupation %	Mean Pupal Period (days)±SD	Adult Mort. %	Adult Emergence % (a)	Total inhibition	Mean Development (days) (b)±SD	Growth Index (a/b)
Control	3.3	6.3±1.1	3.4	0	96.7	2.2±0.27	0	96.6	6.6	8.5±1.37	11.4
4000	26.7	6.5±0.6 ns	59.1	53.8	73.3	2.1±0.29 ns	100	40.9	100	8.6±0.89 ns	4.8
6000	40.0	6.4±0.6 ns	88.9	88.9	60.0	2.5±0.39 ns	100	11.1	100	8.9±0.99 ns	1.2
8000	66.7	6.5±0.8 ns	100	100	33.3	0.0	-	-	-	-	-
10000	90.0	6.7±0.7 ns	100	100	10	0.0	-	-	-	-	-
12000	100	-	-	-	0.0	-	-	-	-	-	-

No. of tested larvae; Conc.; ppm; SD; mort; malf.: see footnote of Table (1).
A = one pupa has developed into adult

Limonine effect: was as the following; larval mortality % was found to increase as the concentration of Limonine extract increased, where it recorded 13.3, 23.3, 46.7, 73.3 and 100.0% at 125, 250, 500, 1000 and 1500ppm; respectively compared to 6.7% for the untreated larvae. Limonine extract significantly prolonged the larval duration at the two highest concentrations (500 and 1000ppm), where the mean duration prolonged to 6.9±0.94 and 7.3±0.57 days; respectively compared to 6.3±1.1 days for control group. pupation rate decreased as the concentration increased. At the highest and lowest concentration: 1500 and 125ppm the pupation percent was 0.0 and 86.7%; respectively vs. 93.3% for the untreated group. The highest pupal malformation percent (87.5%) was induced by the concentration 1000ppm and the lowest pupal malformation percent (19.2%) was occurred at the concentration 125ppm compared to 0.0% for the control group. The highest pupal mortality percent (87.5%) was induced at the highest concentration 1000ppm. This percent decreased to (75.0, 47.8 19.2%) at the concentrations 500, 250 and 125ppm, compared to 3.6% for the control group. Data obtained showed that the mean pupal duration was insignificantly ($P>0.01$) affected at the lowest concentrations (125 and 250ppm), while at the highest concentration (500ppm) the mean duration was significantly ($P<0.05$) prolonged as compared with the control group (2.8±0.47 vs. 2.2±0.27 days). Adult emergence % decreased as the concentration increased, where it recorded 12.5, 25, 42.2 and 81.8% at 1000, 500, 250 and 125ppm compared to the control group (96.4%). Limonine extract had extended toxic action on the survivorship of adults at the all concentrations. The adult mortality percent was 100.0, 75.0, 33.3 and 19.0% at 1000, 500, 250 and 125ppm compared to 0.0% of the control group. The extended toxicity of Limonene on different mosquito stages very obvious when reading the values of the total inhibition percent which were 43.3, 73.3, 96.7 and 100 % at 125, 250, 500 and 1000 ppm, respectively, while untreated group inhibited by 13.4 %. Growth index for larvae and pupae was affected especially at the highest concentrations (1000, 500 and 250ppm) where it recorded 3.4, 3.3 and 4.8 vs. 11.8 for control Table (3) .

Table (3): Effect of Limonene on mortality percent, development and growth index of 3rd larval instar of *Cx. pipiens*.

Conc. ppm	Larval mort. %	Mean Larval Period (days)±SD	Pupal Mort. %	Malf. pupae %	Pupation %	Mean Pupal Period (days)±SD	Adult Mort. %	Adult Emergence % (a)	Total inhibition	Mean Development (days) (b)±SD	Growth Index (a/b)
Control	6.7	6.3±1.1	3.6	0	93.3	2.2±0.27	3.7	96.4	13.4	8.5±1.37	11.8
125	13.3	6.6±0.88^{ns}	19.2	19.2	86.7	2.1±0.24^{ns}	19.0	81.8	43.3	8.7±1.24^{ns}	9.0
250	23.3	6.5±1.8^{ns}	47.8	47.8	76.7	2.3±0.47^{ns}	33.3	42.2	73.3	8.8±2.27^{ns}	4.8
500	46.7	6.9±0.94*	75.0	75.0	53.3	2.8±0.47**	75.0	25.0	96.7	9.7±1.41*	3.3
1000	73.3	7.3±0.57**	87.5	87.5	26.7	3.0^{A}	100.0	12.5	100.0	10.3±0.57**	3.4
1500	100	0.0	-	-	0.0	-	-	-	-	-	-

No. of tested larvae; Conc.; ppm ; SD; mort; malf. : see footnote of Table (1).
A = one pupa has developed into adult

In general, the toxicity values of tested extracts based on Lc_{50} values (Figs 1, 2 and 3) may be ordered in a descending order as follows: Rotenone > Limonine > Jojoba oil (Table 4).

Table (4): LC_{50} of the tested plants' products

Sr	Natural product	LC_{50} in ppm
1	Rotenone	13.6
2	Limonine	658.39
3	Jojoba oil	5991.5

DISCUSSION

The present study showed high bioactivity of the tested plant extracts rotenone, limonene and jojoba oil against the 3rd instar larvae of *Cx. pipiens*, the most prevalent mosquito in Jazan region. Such results may offer an opportunity for developing alternatives to rather expensive and environmentally hazardous organic insecticides for controlling the mosquito *Cx. pipiens* as discussed along the following paragraphs.

Rotenone, limonene and jojoba oil were applied against the larval stage of *Cx. pipiens* and clearly affected the various biological aspects as follows:

Larvicidal activity:

Results were indicating that rotenone, jojoba and limonene had a toxic effect against the 3rd instar larvae of *Cx. pipiens* but in different degrees. Lc_{50} of rotenone, jojoba and limonene were 13.6, 5991.5 and 658.39 ppm respectively which meaning that rotenone larvicidal effect is greater than limonene and both of them are greater than jojoba oil larvicidal activity on *Culex pipiens.* Rotenone larvicidal effect results according to our study is in agreement with the findings of Zubairi *et al.*, (2004), Yenesew *et al.*,(2003), Yenesew *et al.*, (2005) and Abe *et al.*, (1985) that reporting rotenone was highly active against 4th instar larvae of *A. aegypti L.* and *Cx. quinquefasciatus*. The mechanism by which rotenone larvicidal effect take placed is

that mosquito larvae secrete a layer of non-cellular material which is separates the food from the epithelial cells of the gut. This layer is called peritrophic matrix (PM). The PM acts as a protective barrier against various chemical, physical and microbial food components (Beier, 1998). Also our findings go in parallel with that of Kassir and Mohsen (1989) reported that limonene have larvicidal action against larvae of *Cx. quinquefasciatus* and so Cheng *et al.* (2009) who reported toxic effect of limonene against larvae of *A. aegypti* and *A. albopictus* but in the case of that larvicidal effect of jojoba oil on *Cx. pipiens* larvae according to our results are in agreement with Tanani, 2001 who reported that jojoba oil exhibits some biological activities against various insect pests as well as Shonouda & Mehanney 2000 who stated its toxicity, showing LD_{50} of 649.43 ppm, against the last larval instar of *Cx. pipiens* and have insignificant effect on the 3rd instar larvae of *Musca domestica*.

Larval and pupal durations:

Exposure of *Cx. pipiens* 3rd instar larvae to sub-lethal dose of all plant extracts used in this study resulted in variable effects according to the applied extract. The larval duration was affected by rotenone, where the mean duration significantly ($P<0.01$) increased compared to the untreated larvae as well as the mean pupal duration was insignificantly ($P>0.01$) affected at all concentrations used. In parallel to that limonine extract showed significant prolongation at the larval duration at the two highest concentrations (500 and 1000ppm) on the other hand the mean pupal duration was insignificantly ($P>0.01$) affected at the lowest concentrations (125 and 250ppm), while at the highest concentration (500ppm) the mean duration was significantly ($P<0.05$) prolonged as compared with the control group. These effects 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 (Jeyabalan *et al*., (2003) using methanol extract of *Pelargonium citrosa* leaf against *Anopheles(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, 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 that 11 of 36 botanicals that significantly inhibited pupal development while only a few botanicals affected larval development.

Jojoba oil have no effect, as shown from the results insignificant ($P>0.01$), on the mean duration of larvae and pupae at all concentrations used as compared with the untreated group. These results do not agree with results of some researchers as (Abdel-Rady & Osman, 2005) that approved that jojoba oil caused an increase in larval and pupal durations of *Agrotis ipsilon* and Marei *et al.*, (2009) who found that jojoba oil extracts caused pronounced prolongation in both larval and pupal duration of *Spodoptera littoralis* Boisd.

Pupation, pupal mortality and adult emergence:

An observable reduction in the pupation percent was induced by all plant extracts in the present study. The pupation percent decreased as the concentration of the plant extract increased. Our present study showed that the toxic effects of the tested plant extracts had been extended to the pupae. In addition, all plant extracts induced some decrease of the adult emergence. The decrease was found as a concentration–dependent. These results are comparable to previous results of (Assar

& El–Sobky, 2003) using water extracts of *Eichhornia crassipes* and *Artemisia Monosperma* against *Cx. pipiens* larvae, El–Bokl (2003) testing the neem, *Azadirachta indica* extract against *Cx. pipiens* larvae, Nathan *et al.* (2006) evaluating the effect of methanolic extracts of leaves and seeds of *M. azedarach* against the mosquito of *An. stephensi* larvae, Sharma *et al.,* (2006 a & b) using petroleum ether extract of *A. annua* against *An. stephensi* and *Cx. quinquefasciatus* larvae, respectively and (Wiesman & Chapagain, 2006) testing one fraction obtained from the silica gel column chromatography of the methanol extract against *Ae. aegypti* mosquito larvae.

Adult survival:

The present study indicated that the toxicity of the tested plant extracts against the 3rd instar larvae of *Cx. pipiens* was extended to the adults causing mortality reached to 100%. These results are similar to the findings of 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 *Cx. 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 different fractions used. Such results are in agreement with earlier studies using different plant extracts against different mosquito species (Jeyabalan *et al.,* 2003; Shaalan *et al.,* 2005; Nathan *et al.,* 2006; Sharma *et al.,* 2006 a&b).

Morphogenic effects:

In the present study, all used extracts, against the 3rd instar larvae of *Cx. 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 El–Bokl (2003) recorded varying degrees of morphogenic abnormalities in immature and adult stages of *Cx. 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, *Cx. 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 other extracts including mode of action, synergism with the biocides under field condition are needed.

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