



Potential of biologically active plant oils for controlling of *Culex pipiens* (Diptera: Culicidae)

Capacidade de oleos vegetais para controle biológico do *Culex pipiens* (Diptera: Culicidae)

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ABSTRACT

The insecticidal effect of six commercially available plant oils was tested against 4th larval instars of *Culex pipiens*. Larvae were originally collected from Meit El-Attar, Qalyubia Governorate, Egypt, and then reared in the laboratory until F₁ generation. The LC₅₀ values were 32.42, 47.17, 71.37, 83.36, 86.06, and 152.94 ppm for fenugreek (*Trigonella foenum-grecum*), earth almond (*Cyperus esculentus*), mustard (*Brassica compestris*), olibanum (*Boswellia serrata*), rocket (*Eruca sativa*), and parsley (*Carum ptroselinum*), respectively. The tested oils altered some biological aspects of *C. pipiens*, for instance, developmental periods, pupation rates, and adult emergences. The lowest concentrations of olibanum and fenugreek oils caused remarkable prolongation of larval and pupal durations. Data also showed that the increase of concentrations was directly proportional to reduction in pupation rates and adult emergences. Remarkable decrease in pupation rate was achieved by mustard oil at 1000 ppm. Adult emergence was suppressed by earth almond and fenugreek oils at 25 ppm. In addition, the tested plant oils exhibited various morphological abnormalities on larvae, pupae, and adult stages. Consequently, fenugreek was the most potent oil and the major cause of malformation of both larval and pupal stages. Potency of the applied plant oils provided an excellent potential for controlling *C. pipiens*.

Key words: *Culex pipiens*, larvicidal oils, morphological abnormalities, biological aspects, Egypt.

INTRODUCTION

The mosquito *Culex pipiens* is a worldwide insect causing dreadful nuisance and transmitting many dangerous diseases. In Egypt, it is the main vector of filarial worm *Wuchereria bancrofti* [21] as well as Rift valley fever virus [5].

Mosquito control is becoming increasingly difficult in Egypt because of the emergence of resistance of *C. pipiens* to many insecticides [27]. Consequently, there is an urgent need to explore and utilize naturally occurring products for combating such vectors.

Many authors worldwide started large screening activity for using extracts of medicinal and herbaceous plants to control mosquitoes [4,6,10,11,12].

Some plant extracts such as yellow rocket and black mustard [24] and fenugreek [10] expressed insecticidal effect against mosquitoes. We tested earth almond, olibanum, and parsley for the first time.

The botanical insecticides are generally pest-specific and are relatively harmless to non-target organisms including humans. They are biodegradable and harmless to the environment [11] Accordingly, the application of easily degradable plant compounds is considered one of the safest methods of control of insect pests and vectors [20,22,23].

The goal of this study was not only to test the insecticidal effect of six plant oils, readily available in Egypt for domestic use, to control *C. pipiens*, but also to find out the alteration of some biological aspects and the morphological aberrations, following treatment with sublethal concentrations of the tested oils.

MATERIALS AND METHODS

Larvae of *Culex pipiens* were collected from Meit El-Attar, Qalyubia Governorate, Egypt, then reared in the laboratory according to Khater¹² and 4th larval instars from F₁ generation were exposed to the applied oils.

Commercially available plant oils, obtained from El-Kaptain Company, were used. Such oils were fenugreek (*Trigonella foenum-grecum*), earth almond (*Cyperus esculentus*), rocket (*Eruca sativa*), parsley (*Carum ptroselinum*), mustard (*Brassica compestris*), and olibanum (*Boswellia serrata*).

Test procedures were done according to World Health Organization [26]. Early 4th larval instars of *C.*

pipiens were exposed to six plant oils at 5-7 different concentrations, ranged from 7.81 to 2000 ppm. Moreover, tween 80 was used as an emulsifier. Each concentration of the tested oil together with an untreated control group were replicated four times, 15 larvae per replicate were transferred to 500 ml glass beaker containing 250 ml of dechlorinated water. Whereas, tween 80 was used in the control group. In the meantime, all the assays were repeated three times and done at 29±2°C and 80±5% RH.

After starting the experiments, the larval mortality counts were determined daily until pupation. Accordingly, larvae were continuously exposed to the oils in order to determine the LC₅₀ and LC₉₀ values.

In order to determine the latent outcome of the used oils on some biological aspects, the number of developed pupae, for each concentration, was counted and the pupae were placed in a separate cage until the emergence of adults. Consequently, the developmental periods, pupation rates, and adult emergences were determined. During that time, the morphological abnormalities of larvae, pupae, and adults were recorded.

For statistical analysis, the mortality data were subjected to Probit analysis by computer (POLO-PCO following Finney [9]). A Probit transformation was followed by regression analysis to determine the theoretical LC₅₀ and LC₉₀ values; whereas, the biological data were analyzed by Duncan's multiple range test via SPSS program.

RESULTS

All tested oils were effective against 4th larval instars of *C. pipiens*, originally collected from Meit El-Attar, Qalyubia Governorate, Egypt. The calculated LC₅₀ values were 32.42, 47.17, 71.37, 83.36, 86.06, and 152.94 ppm for fenugreek, earth almond, mustard, olibanum, rocket, and parsley oils, respectively, Table 1. Such values indicated that fenugreek was the most effective oil, whereas parsley was the least toxic one. At the level of LC₉₀s, the arrangement of such oils was changed as follows: earth almond, mustard, fenugreek, rocket, olibanum, and parsley.

Based on LC₅₀ values of tested plant oils and that of parsley as a reference substance, the relative potency indicated that fenugreek, earth almond, mustard, olibanum, and rocket were 4.72, 3.24, 2.14, 1.83,

Table 1. Relative efficiency of the tested plant oils against 4th larval instars of *Culex pipiens*, collected from Meit El-Attar, Qalyubia Governorate, Egypt.

Plant oils	LC 50	LC 90	LC90/LC50	Slope Function	Relative Toxicity
Fenugreek	32.42	642.11	19.80	0.99±0.28	4.72
Earth almond	47.17	298.61	6.33	1.59±0.42	3.24
Mustard	71.37	353.84	4.96	1.84±0.26	2.14
Olibanum	83.36	1968.75	23.62	0.93±0.19	1.83
Rocket	86.06	1438.70	16.72	1.05±0.20	1.48
Parsley	152.94	2316.20	15.14	1.09±0.21	1.00

Concentration (ppm, part per million).

and 1.48 times, respectively, more effective than parsley, Table 1.

As a consequence of larval treatments, some biological features were conspicuously varied, such as, developmental periods, pupation rates, and adult emergences, Tables 2&3. The larval and pupal durations were generally dose dependent relationships. Although the developmental periods of larvae and pupae was not affected by the lowest concentration of mustard and rocket oils, they produced significantly different changes of the durations from that of the control groups when higher concentrations were used. Beyond this point, the higher the concentration, the greater the effect of oils on larval developmental periods. Rocket, mustard, and parsley oils tended to shorten the duration of larvae when compared with that of the control group.

The developmental periods of larval and pupal stages were extremely prolonged after treatment with the lowest concentrations of olibanum and fenugreek. The longest larval and pupal periods were 14.9 and 8.2 days, respectively, after treatment of larvae with olibanum oil at 7.81 ppm. On contrary, earth almond and mustard oils decreased pupation periods. The effect was more pronounced with earth almond oil, whereas the pupal duration reached 0.5 days at 125 ppm.

The pupation rates and the adult emergences decreased as the concentration increased. Adult emergences were completely stopped by all applied oils when used at high concentrations. No adults were emerged following treatment with 125 ppm of fenugreek and earth almond oils. Similar observations were recorded after exposure to 500 ppm of mustard, olibanum, and rocket oils.

Several forms of morphological malformations resulted from treatment of larvae with the applied oils. The larval abnormalities were pigmented and twisted larvae, as well as larval- pupal intermediates (pharate pupae). Whereas, the pupal and adult aberrations were albino, elephantoid, pupae with blackish cephalothorax, failure of adult eclosion, and deformed abdomen and legs.

With regard to morphological abnormalities, Table 4, larval aberrations reached the highest values when treated with fenugreek oil (25% at 15 ppm). In the meanwhile, the morphological abnormalities were more pronounced in the pupal stage. It is evident that fenugreek and rocket oils caused the highest effect on the pupal stage, 55.5 and 50% at 62.5 ppm, respectively. At 250 ppm of mustard and parsley, the pupal malformations were 40% and 50%. Olibanum oil caused the highest effect on the adult stage, 21.43% at 31.25 ppm. Accordingly, Fenugreek oil was the foremost cause of deformation in both larval and pupal stages.

DISCUSSION

In Egypt, resistance of *C. pipiens* to insecticides was reported in three filariasis-endemic areas of Egypt, as larval bioassay results showed clear indications of resistance to organophosphate insecticides and adult bioassays also showed widespread resistance to many insecticides related to organochlorine (OC), pyrethroid, organophosphate, and carbamate insecticides [27]. Additionally, larvae of *C. pipiens* were resistant to bacterial agent *Bacillus thuringiensis var. israelensis* in the laboratory [18].

Table 3. The effect of the plant oils on some biological aspects of *Culex pipiens*, Collected form Meit El-Attar, Qalyubia Governorate, Egypt.

Plant oils Conc. (ppm)	Larval Duration (Days ± SE)	Pupal Duration (Days ± SE)	Pupation (% ± SE)	Emergence (% ± SE)
<i>Rocket</i>				
62.5	3.56±0.03 ^a	2.61±0.18 ^a	50.00±0.65 ^b	83.33±0.40 ^b
125	2.79±0.02 ^b	2.59±0.09 ^a	40.00±0.40 ^c	66.67±0.25 ^{b,c}
250	2.72±0.04 ^b	2.47±0.6 ^a	33.33±0.29 ^{c,d}	33.33±0.29 ^{c,d}
500	2.34±0.07 ^c	2.30±0.21 ^a	26.67±0.41 ^d	0.00±0.20 ^{d,e}
1000	1.88±0.02 ^d	1.10±0.04 ^b	6.67±0.41 ^e	0.00±0.00 ^e
Control	3.67±0.04 ^a	2.36±0.06 ^a	95.00±0.63 ^a	100±0.75 ^a
<i>Parsley</i>				
125	2.89±0.24 ^b	1.38±0.12 ^d	43.33±0.65 ^b	80.00±0.65 ^b
250	2.92±0.04 ^b	2.02±0.04 ^c	40.00±0.00 ^c	100.00±0.41 ^c
500	2.76±0.06 ^b	2.14±4.62 ^c	33.33±0.41 ^{b,c}	100±0.29 ^c
1000	2.82±0.04 ^b	3.46±0.20 ^a	23.33±0.65 ^c	0.00±0.00 ^c
2000	1.04±0.02 ^c	3.42±0.20 ^a	3.33±0.29 ^d	0.00±0.00 ^c
Control	3.43±0.08 ^a	2.64±0.11 ^b	93.33±0.71 ^a	100.00±0.65 ^a
<i>Olibanum</i>				
7.81	14.90±0.33 ^a	8.25±0.32 ^a	83.33±0.65 ^b	69.57±0.65 ^b
31.25	11.38±0.24 ^b	5.17±0.15 ^b	53.33±0.41 ^c	64.29±0.41 ^c
62.50	9.13±0.18 ^c	4.18 0.13 ^c	46.67±0.71 ^c	56.52±0.48 ^d
250	8.29±0.33 ^d	3.84±0.14 ^c	30.00±0.65 ^d	62.50±0.41 ^e
500	4.53±0.21 ^e	2.53±0.19 ^d	23.33±0.29 ^{d,e}	0.00±0.25 ^{e,f}
1000	1.79±0.46 ^g	1.55±0.19 ^e	15.00±0.25 ^{e,f}	0.00±0.25 ^{e,f}
2000	1.05±0.08 ^g	0.00±0.00 ^f	8.33±0.25 ^e	0.00±0.00 ^f
Control	3.39±0.14 ^f	2.25±0.09 ^d	91.67±0.65 ^a	94.34±0.48 ^a

Conc. (ppm) means concentration (part per million). Mean within column followed by the same letter are not significantly different. (P>0.05, Duncan's multiple range test).

In the past few years, some plant compounds were investigated by several authors for anti-mosquito potential, including oviposition avoidance [25], larvicidal [10,12,17], adulticidal [4], and repellent activities [4,16].

Fortunately, botanical insecticides are biodegradable and harmless to the environment [11], pest-specific, and relatively harmless to non-target organisms and humans [20,22,23].

In this study, the used oils showed high toxic effect against larvae *C. pipiens*, especially, fenugreek, earth almond, mustard, and olibanum.

Pertaining to the prospective of botanical extracts as mosquito larvicides, fenugreek showed mos-

quito larvicidal activity against *C. pipiens*, LC50 of chloroform extract of fenugreek was 234.05 ppm [10] Fenugreek was also toxic to stored product pests, *Tribolium castaneum* and *Acanthoscelides obtectus* [15] and the cotton leaf worm *Spodoptera littoralis* [10].

Fenugreek seeds induced oviposition avoidance or deterrence to gravid *Aedes aegypti* [25]. Also, mustard repelled vectors of Japanese encephalitis, *C. pseudovishnui* and *C. gelidus* [16]. Furthermore, the herbal repellents, *Zanthoxylum limonella* and *Citrus aurantiifolia* exhibited better protection against bites of *Aedes albopictus* in mustard oil than in coconut oil [6].

Table 2. The effect of the plant oils on some biological aspects of *Culex pipiens*, collected from Meit El-Attar, Qalyubia Governorate, Egypt.

Plant Oils Conc. (ppm)	Larval Duration (Days ± SE)	Pupal Duration (Days ± SE)	Pupation (% ± SE)	Emergence (% ± SE)
<i>Fenugreek</i>				
7.81	9.75±0.32 ^a	5.85±0.27 ^a	66.67±0.41 ^b	87.10±0.63 ^b
15.63	7.38±0.29 ^b	4.95±0.06 ^b	58.33±0.65 ^c	86.36±0.65 ^c
31.25	5.48±0.25 ^c	3.99±0.08 ^c	51.67±0.48 ^c	56.25±0.41 ^d
62.50	5.14±0.12 ^c	3.05±0.13 ^d	33.33±0.41 ^d	42.86±0.48 ^e
125	3.35±0.16 ^d	1.83±0.04 ^f	26.67±0.41 ^d	0.00±0.48 ^e
500	1.0±0.03 ^e	0.83±0.04 ^g	11.67±0.47 ^e	0.00±0.29 ^{e,f}
1000	0.93±0.03 ^e	0.00±0.00 ^h	6.67±0.41 ^e	0.00±0.00 ^f
Control	3.16±0.06 ^d	2.48±0.08 ^e	93.33±0.41 ^a	94.34±0.48 ^a
<i>Earth almond</i>				
15.63	6.71±0.24 ^a	0.75±0.06 ^b	76.67±0.65 ^b	70.00±0.41 ^b
31.25	6.66±0.06 ^a	0.82±0.04 ^b	60.00±0.41 ^c	85.71±0.41 ^c
62.50	5.59±0.05 ^b	0.91±0.04 ^b	41.67±0.25 ^d	84.21±0.48 ^d
125	3.50±0.10 ^c	0.50±0.04 ^c	23.33±0.65 ^e	0.00±0.29 ^e
250	1.12±0.02 ^d	0.00±0.00 ^d	11.67±0.25 ^f	0.00±0.25 ^f
500	1.28±0.16 ^d	0.00±0.00 ^d	5.00±0.25 ^f	0.00±0.00 ^f
Control	4.00±0.41 ^c	2.05±0.13 ^a	98.33±0.48 ^a	96.55±0.65 ^a
<i>Mustard</i>				
62.5	3.66±0.1 ^{a,b}	1.75±0.06 ^{a,b}	56.67±0.65 ^b	55.56±0.65 ^c
125	3.47±0.11 ^b	1.44±0.02 ^{b,c}	26.67±0.42 ^c	66.67±0.65 ^b
250	2.57±0.16 ^c	1.31±0.06 ^c	16.67±0.65 ^{c,d}	50.00±0.41 ^d
500	2.56±0.06 ^c	1.24±0.04 ^c	6.67±0.41 ^{d,e}	0.00±0.25 ^e
1000	2.10±0.04 ^d	0.70±0.20 ^d	1.67±0.25 ^e	0.00±0.00 ^e
Control	3.93±0.15 ^a	2.05±0.13 ^a	98.33±0.63 ^a	98.25±0.75 ^a

Conc. (ppm) means concentration (part per million). Mean within column followed by the same letter are not significantly different. (P>0.05, Duncan's multiple range test).

Sesame, *Sesamum indicum*, nigella, *Nigella sativa*, and onion, *Allium cepa*, oils were highly toxic to *C. pipiens* [12]. On the other hand, sublethal doses of azadirachtin, extracted from neem, inhibited larval growth in *C. tarsalis* and *C. quinquefasciatus* [22]. Besides, ethanolic extract from *Ginkgo biloba* L. *exocarp* from the Chinese ginkgo was highly toxic against three strains of *C. pipiens* [23]. Additionally, crude seed extract of celery (*Apium graveolens*) possessed larvicidal, adulticidal, and repellent activities against *Ae. Aegypti* [4].

Methanolic extracts of the leaves of *Atlantia monophylla* were effective as mosquitocide against immature stages of three mosquito species, *C. quinque-*

fasciatus, *Anopheles stephensi*, and *Ae. aegypti* in the laboratory, such plant exhibit insect regulating activity [20].

Regarding the morphological abnormalities, several authors recorded similar anomalies, when applied different plant extracts at sublethal concentrations. Pigmented larvae and pharate pupae were reported after treatment with sesame oil [12]. Albino pupae were recounted after treatment with neem seed kernel extract [7] and nigella oil [12]. Blackish cephalothoraxes were observed after treatment with fenugreek [10] and onion oil [12]. Finally, failure of adult eclosion as well as deformed abdomen and legs were recorded after

Table 4. Percentage of larval, pupal, and adult morphological abnormalities observed after treatment of 4th larval instars of *Culex pipiens* with plant oils.

Plant Oils Conc. (ppm)	Larvae	Pupae	Adults	Plant Oils Conc. (ppm)	Larvae	Pupae	Adults
<i>Fenugreek</i>				<i>Olibanum</i>			
7.81	12.67	22.5	6.45	7.81	5.00	8.00	19.57
15.63	25.00	37.14	4.54	31.25	8.33	9.38	21.43
31.25	21.67	45.16	6.25	62.5	6.67	10.71	13.04
62.5	16.67	55.00	0.00	250	11.67	55.56	12.50
125	8.33	0.00	0.00	500	0.00	0.00	0.00
500	0.00	0.00	0.00	1000	0.00	0.00	0.00
1000	0.00	0.00	0.00	2000	0.00	0.00	0.00
Control	0.00	0.00	0.00	Control	0.00	0.00	0.00
<i>Earth almond</i>				<i>Rocket</i>			
15.63	8.33	13.04	10	62.5	8.33	50	8.33
31.25	5.00	16.67	3.57	125	3.33	16.67	11.11
62.5	5.00	20.00	0.00	250	3.33	30.00	16.67
125	3.33	0.00	0.00	500	1.67	6.25	0.00
250	0.00	0.00	0.00	1000	1.67	25.00	0.00
500	0.00	0.00	0.00	Control	0.00	0.00	0.00
Control	0.00	0.00	0.00				
<i>Mustard</i>				<i>Parsley</i>			
62.5	1.67	11.77	5.56	125	10	38.46	10.00
125	3.33	31.25	16.67	250	6.67	50.00	0.00
250	6.67	40.00	0.00	500	16.67	60.00	0.00
500	0.00	0.00	0.00	1000	0.00	50.00	0.00
1000	0.00	0.00	0.00	2000	0.00	0.00	0.00
Control	0.00	0.00	0.00	Control	0.00	0.00	0.00

Conc. (ppm) means concentration (part per million).

treatment with fenugreek [10], sesame, nigella and onion oils [12], and garlic [17].

Concerning abnormally percentage, comparable results were recorded against *C. pipiens* following treatment with fenugreek [10] and onion [12].

These results indicated a metamorphosis inhibiting effect of the plant oils, which possibly based on the disturbance of hormonal control [2] as the noticed morphogenetic aberrations suggesting a type of insect growth regulating activity. The most important deformities, larval- pupal intermediates and ecdysal failure, seemed to be the major cause of the mortalities. Likewise, such abnormalities were noted follow-

ing treatment of immature mosquitoes with juvenile hormone (JH) analogues and chitin synthesis inhibitors [8,12,20].

With reference to biological aspects, the data revealed prolongation of the larval developmental periods, following treatment with sublethal concentrations of olibanum and fenugreek, 14.9 and 8.2 days, respectively. Similar results were recorded after treatment of *C. pipiens* with onion, sesame, and nigella oils, 12.6, 9.3, and 8.5 days, respectively [12], and ethanolic extract of fenugreek, 18.02 days [10]. Likewise, white and black mustard lengthened the duration of 2nd larval instars of *Musca domestica* [1].

Comparable prolongations of pupal developmental periods were also recorded [1,17,24]. Although Halawa [10] reported that fenugreek decreased the pupal durations of *C. pipiens* and *S. littoralis*. Such result was similar to that reported by earth almond and mustard oils.

The prolongation of developmental periods occurred because plant extracts had insect growth regulating activity, which may inhibit insect development [12,14,20].

Similar reduction in the pupation rates and adult emergences, following exposure to higher concentrations of several plant extracts were reported after treatment of *C. pipiens* with different plant extracts, such as; neem seed kernel extract [7], fenugreek [10], and nigella, onion, and sesame oils [12].

Likewise, the pupation rates and adult emergence were affected after exposure of *Ae. Aegypti* to yellow rocket and black mustard [24]. and *M. domestica* to white and black mustard [1].

Additionally, topical application of fenugreek was detrimental to stored product pests, *T. castaneum* and *A. obtectus* causing high degree of mortalities, inhi-

bited ovipositing, and larval penetration, together with decreased fecundity, fertility, and longevity [15].

CONCLUSION

The activity of the larvicidal oils extends beyond the larval stages, subsequently, these oils killed *C. pipiens* larvae at higher concentrations, but when diluted, they caused a series of morphological problems that inhibit larvae to complete metamorphosis. Consequently, the applied oils could be classified as insect growth regulators.

Before field application, the safety of the applied oils to human and the environment must be investigated. After that, the readily available plant oils may lead to future development of botanical insecticide that could be integrated into other pest management programs for control of mosquitoes primarily in the developing countries.

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