

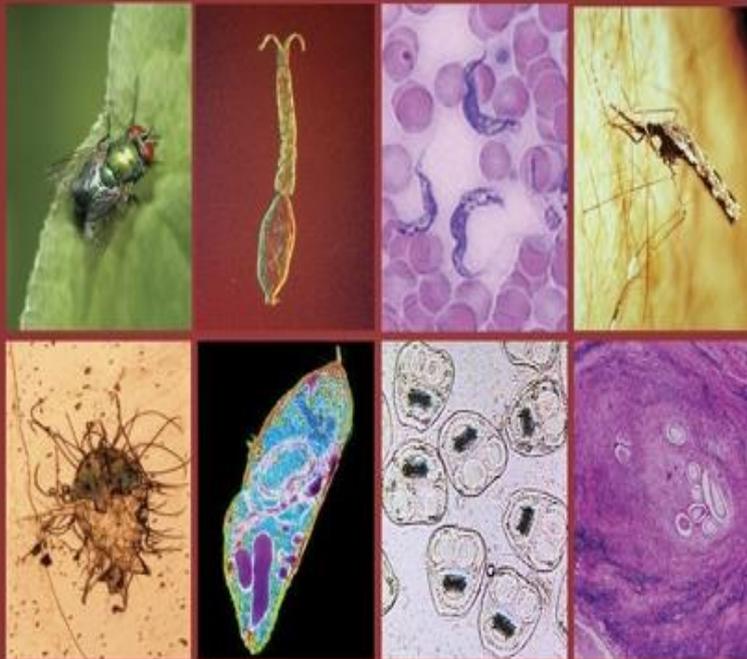


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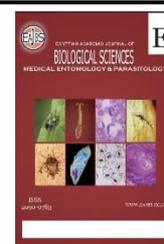
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Larvicidal Activity of *Menthe longifolia* (Lamiaceae) Different Extracts against *Culex pipiens* L. and *Culex antennatus* Becker (Diptera: Culicidae)

Sayed A. Sayed, Kotb M. Hammad, Ahmed Z.I. Shehata and Mostafa M. Mokhtar
Department of Zoology, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt
E-mail : drmosta80@azhar.edu.eg

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ABSTRACT

Larvicidal activity of *Menthe longifolia* different extracts against lymphatic filariasis vector, *Culex pipiens* and Rift Valley Fever vector, *C. antennatus* was tested. The extraction was carried out using methanol, acetone, chloroform and petroleum ether solvents. Obtained results revealed that petroleum ether extraction from leaves of *M. longifolia* was more effective against 3rd instar larvae of *C. pipiens* and *C. antennatus* than those of chloroform, acetone and methanol extractions. Methanol, acetone, chloroform and petroleum ether extracts from leaves of *M. longifolia* recorded LC₅₀ values of 1741.5, 1322.0, 702.4 and 379.5 ppm against *C. pipiens* third larval instar and 1064.6, 850.3, 459.9 and 250.4 ppm against *C. antennatus* third larval instar, respectively. In addition, LC₉₀ values recorded 2282.0, 1897.7, 1300.3 and 511.9 ppm by methanol, acetone, chloroform and petroleum ether extract from leaves of *M. longifolia* against *C. pipiens* third larval instar and 1596.5, 1388.6, 979.4 and 388.2 ppm against *C. antennatus* third larval instar, respectively. Also, both *C. pipiens* and *C. antennatus* larval and pupal periods were prolonged by all tested extracts at all concentrations used as compared with the untreated groups. *Menthe longifolia* tested extracts are considered as new promising controlling agents against *C. pipiens* and *C. antennatus*, however, more studies are necessary to reach the active ingredient in the tested extracts.

INTRODUCTION

Mosquitoes stand out most among the numerous species of blood-sucking arthropods that annoy humans. But what is more important, is their role in the transmission of many serious diseases (Morsy *et al.*, 1990). About 3500 species of mosquitoes are known all over the world and transmit diseases, such as malaria, filariasis and dengue causing millions of deaths every year (Stone, 1975; Shehata, 2018). *Culicine*

mosquitoes in Egypt mainly, *Culex pipiens* has a wide distribution and it is the main vector of Rift Valley fever virus (Meagan *et al.*, 1980; Darwish and Hoogastrall, 1981), *Wuchereria bancrofti* (Khalil *et al.*, 1930; Gad *et al.*, 1996) and Western Nile virus (Pelah *et al.*, 2002). Another *Culicine* mosquito in Egypt, *Culex antennatus* is the main vector of Rift Valley Fever virus during an outbreak in the Nile Delta of Egypt (Hanafi *et al.*, 2011).

Immature stages of different mosquito species are usually targeted using synthetic chemical insecticides and microbial agents. Continues usage of synthetic chemical insecticides in mosquito control has resulted in lower efficacy of these insecticides and enhancing resistance in mosquito populations (Pates and Curtis, 2005; Nathan *et al.*, 2005) and thus increasing the demand for new products which are eco-friendly, target-specific and degradable.

Plants are a rich source of control agents against mosquitoes because they possess bioactive chemicals, which act as insecticides and growth inhibitors, (Murugan *et al.*, 1996; Koul, 2005). In addition, botanical pesticides offer an advantage over synthetic pesticides as they can be much less toxic, less prone to the development of resistance and more easily degradable.

The wild mint, *Menthe longifolia* used in the present study is used in the pharmaceutical and food industries, as well as in cosmetology. Also, *M. longifolia* various parts, leaves, flower, stem, and seeds have been also used in traditional folk medicine as antimicrobial, carminative, stimulant, antispasmodic and for the treatment of various diseases such as headaches and digestive disorders (Mikaili *et al.*, 2013).

The current study aimed at investigating the activity of different extracts from leaves of *Menthe longifolia* against *Culex pipiens* and *Culex antennatus* larvae.

MATERIALS AND METHODS

Colonization of Tested Mosquitoes:

Tested mosquitoes' larvae, *Culex pipiens* and *C. antennatus* were collected from Tamia Centre, Al-Fayyum Governorate, Egypt (29° 29' 26.097" N, 30° 58' 36.945" E) and reared for six generations in Mosquito insectary, Animal House, Faculty of Science, Al-Azhar University. The rearing procedure described by Shehata *et al.*, (2020) was applied to provide larvae needed for the assay.

Preparation of *Menthe longifolia* Tested Extracts:

Leaves of Horsemint, *M. longifolia* (Lamiaceae) were collected from Dahab,

South Sinai Governorate, Egypt (28° 30' 32.8824" N, 34° 30' 49.0824" E) and allowed to dry at room temperature (25±3°C). The extraction was performed using a procedure of Hassan *et al.*, (2014). The extraction was carried out using methanol, acetone, chloroform and petroleum ether solvents. Dry *M. longifolia* extracts were stored at -4°C until using.

Larvicidal Activity:

A standard larvicidal assay described by Hassanain *et al.*, (2019) was applied to study the larvicidal activity of the tested *M. longifolia* extracts with minor modifications. Briefly, tested material of the methanolic extracts was dissolved in 0.1ml of methanol, while tested materials of other extracts were dissolved in 2 drops of Tween₈₀.

Statistical Analysis:

All the statistical analyses were carried out using Statistical Package Social Science (SPSS) software version 11.5 (SPSS, 2007). Statistical analyses were conducted according to the method recorded by lentner *et al.*, (1982). LC₅₀ and LC₉₀ were calculated using multiple linear regressions (Finney, 1971). All data were recorded as Mean±SD.

RESULTS

The biological activity of methanol and acetone extracts from leaves of *Menthe longifolia* against the 3rd instar larvae of *C. pipiens* is recorded in table 1. Complete larval mortality (100.0%) was achieved at the highest concentrations (2500 and 2000 ppm), meanwhile, the lowest mortality values (10.7 and 12.0%) occurred at the lowest concentrations (1300 and 800 ppm) for both methanol and acetone extracts, respectively, compared with 2.7 and 2.3% for the control groups. The pupal mortality recorded 33.1, 23.7, 16.7 and 16.5% at 2300, 2100, 1900 and 1700 ppm of methanol extract, respectively, compared to 0.0% for the control group.

Generally, both mean larval and pupal periods were significantly prolonged at all concentrations used from two tested extracts as compared with control congeners. A retarded effect on growth was observed, especially at the highest concentrations of methanol and

acetone extracts from leaves of *M. longifolia* recorded 6.4 and 7.9, compared with 11.9 and (2300 and 1800 ppm), where the growth index 11.8 for the untreated groups (Table 1).

Table 1: Effect of methanol and acetone extracts from leaves of *Menthe longifolia* on some biological aspects of *Culex pipiens*.

Extract	Conc. (ppm)	Larval Mort. (%)	Pupal Mort. (%)	Adult Emergence (%)	Larval Period	Pupal Period	Developmental Period	Growth Index
Methanol	2500	100.0±0.0	---	---	---	---	---	---
	2300	93.3±2.3	33.1±14.4	66.9±14.4	6.9±0.2 ^d	3.5±0.1 ^c	10.4±0.3 ^d	6.4
	2100	80.0±6.9	23.7±6.4	76.3±6.4	6.7±0.2 ^c	3.4±0.2 ^b	10.1±0.4 ^c	7.6
	1900	62.7±2.3	16.7±7.3	83.3±7.3	6.3±0.3 ^a	3.1±0.2 ^a	9.4±0.5 ^a	8.9
	1700	50.7±4.6	16.5±2.1	83.5±2.1	6.0±0.1 ^a	3.0±0.4 ^a	9.0±0.5 ^a	9.3
	1500	34.7±6.1	0.0	100.0±0.0	5.9±0.4 ^a	2.9±0.1 ^a	8.8±0.5 ^a	11.4
	1300	10.7±2.3	0.0	100.0±0.0	5.7±0.3 ^a	2.8±0.2 ^a	8.5±0.5 ^a	11.8
	Control	2.7±2.3	0.0	100.0±0.0	5.7±0.2 ^a	2.7±0.1 ^a	8.4±0.3 ^a	11.9
Acetone	2000	100.0±0.0	---	---	---	---	---	---
	1800	84.0±4.0	12.0±2.0	88.0±2.0	7.4±0.1 ^d	3.7±0.4 ^d	11.1±0.5 ^d	7.9
	1600	62.7±2.3	9.0±3.5	91.0±3.5	7.2±0.3 ^d	3.6±0.1 ^d	10.8±0.4 ^d	8.4
	1400	54.7±3.5	0.0±0.0	100.0±0.0	7.1±0.3 ^d	3.3±0.1 ^b	10.4±0.4 ^d	9.6
	1200	44.0±8.0	0.0±0.0	100.0±0.0	6.8±0.2 ^d	3.1±0.2 ^a	9.9±0.4 ^d	10.1
	1000	30.7±2.3	0.0±0.0	100.0±0.0	6.3±0.1 ^d	3.0±0.1 ^a	9.3±0.2 ^c	10.8
	800	12.0±4.0	0.0±0.0	100.0±0.0	6.0±0.1 ^d	2.8±0.3 ^a	8.8±0.4 ^a	11.4
	Control	2.3±1.3	0.0±0.0	100.0±0.0	5.1±0.1 ^a	2.8±0.2 ^a	7.9±0.3 ^a	11.8

No. of tested larvae = 25 per one replicate; Conc. = Concentration; ppm = particle per million; SD = standard deviation; mort. = mortality. Means followed by the same letter in the same column are not statistically significant (P>0.05)

In addition, complete larval mortality (100.0%) attained by chloroform and petroleum ether extracts from leaves of *M. longifolia* at the highest concentrations (1500 and 550 ppm), meanwhile, the lowest values (20.0 and 12.0 %) was occurred at the lowest concentrations (300 and 250 ppm) compared with 20.0% for the control group. Also, there was no effect of petroleum ether

extract from leaves of *M. longifolia* on *C. pipiens* pupae resulted from treated larvae. Both chloroform and petroleum ether extracts from leaves of *M. longifolia* induced a prolongation in larval and pupal periods as compared with untreated groups. The growth index recorded the lowest value (5.8) by 1300 ppm of chloroform extract from leaves of *M. longifolia* (Table 2).

Table 2: Effect of chloroform and petroleum ether extracts from leaves of *Menthe longifolia* on some biological aspects of *Culex pipiens*.

Extract	Conc. (ppm)	Larval Mort. (%)	Pupal Mort. (%)	Adult Emergence (%)	Larval Period	Pupal Period	Developmental Period	Growth Index
Chloroform	1500	100.0±0.0	---	---	---	---	---	---
	1300	90.7±2.3	35.5±3.9	64.5±3.9	7.2±0.4 ^d	3.9±0.2 ^c	11.1±0.6 ^d	5.8
	1100	80.0±2.3	23.6±4.5	76.4±4.5	7.0±0.1 ^d	3.8±0.3 ^b	10.8±0.4 ^d	7.1
	900	62.7±4.6	17.8±3.2	82.2±3.2	6.9±0.2 ^d	3.6±0.1 ^a	10.5±0.3 ^d	7.8
	700	52.0±4.0	14.2±2.2	85.7±2.2	6.7±0.2 ^d	3.5±0.1 ^a	10.2±0.3 ^c	8.4
	500	37.3±2.3	0.0±0.0	100.0±0.0	6.6±0.2 ^d	3.2±0.3 ^a	9.8±0.5 ^b	10.2
	300	20.0±0.0	0.0±0.0	100.0±0.0	6.4±0.2 ^d	3.1±0.3 ^a	9.5±0.5 ^a	10.5
	Control	9.2±5.3	0.0±0.0	100.0±0.0	5.3±0.2 ^a	3.0±0.2 ^a	8.3±0.4 ^a	12.0
Pet. ether	550	100.0±0.0	---	---	---	---	---	---
	500	82.7±4.6	0.0±0.0	100.0±0.0	7.8±0.1 ^d	4.3±0.2 ^d	12.1±0.3 ^d	8.3
	450	74.7±2.3	0.0±0.0	100.0±0.0	7.7±0.3 ^d	4.1±0.3 ^d	11.8±0.6 ^d	8.5
	400	64.0±4.0	0.0±0.0	100.0±0.0	7.6±0.3 ^d	4.0±0.1 ^c	11.6±0.4 ^d	8.6
	350	38.7±2.3	0.0±0.0	100.0±0.0	7.3±0.2 ^d	3.9±0.1 ^c	11.2±0.3 ^d	8.9
	300	21.3±2.3	0.0±0.0	100.0±0.0	7.1±0.1 ^d	3.7±0.3 ^b	10.8±0.4 ^d	9.3
	250	12.0±4.0	0.0±0.0	100.0±0.0	6.9±0.1 ^d	3.5±0.3 ^a	10.4±0.4 ^d	9.6
	Control	7.2±2.4	0.0±0.0	100.0±0.0	5.3±0.1 ^a	3.0±0.3 ^a	8.3±0.4 ^a	12.0

See the footnote of table 1.

On the other hand, Data presented in table 3 showed the biological activity of methanol and acetone extracts from leaves of *M. longifolia* against *C. antennatus* third larval instar. Complete larval mortality (100.0%) was caused at 1800 and 1600 ppm, meanwhile, the lowest values (10.7 and 9.3%) was occurred at the lowest concentrations (600 and 400 ppm), respectively, compared with 12.0 and 4.0 % for the control groups.

A prolongation in both larval and pupal periods as the result of tested extracts

was recorded. The growth index recorded 10.8, 11.1, 11.2, 11.6, 12.3 and 12.8 at 1600, 1400, 1200, 1000, 800 and 600 ppm of methanol extract from leaves of *M. longifolia*, respectively vs. 13.0 for the control group. Meanwhile, the growth index for *C. antennatus* larvae and pupae slightly affected by acetone extract of *M. longifolia* (leaves) at all concentrations used as compared with the control group (Table 3).

Table 3: Effect of methanol and acetone extracts from leaves of *Menthe longifolia* on some biological aspects of *Culex antennatus*.

Extract	Conc. (ppm)	Larval Mort. (%)	Pupal Mort. (%)	Adult Emergence (%)	Larval Period	Pupal Period	Developmental Period	Growth Index
Methanol	1800	100.0±0.0	---	---	---	---	---	---
	1600	92.0±4.0	0.0±0.0	100.0±0.0	6.0±0.2 ^d	3.3±0.1 ^b	9.3±0.3 ^d	10.8
	1400	78.7±2.3	0.0±0.0	100.0±0.0	5.8±0.1 ^d	3.2±0.3 ^a	9.0±0.4 ^c	11.1
	1200	61.3±2.3	0.0±0.0	100.0±0.0	5.7±0.1 ^d	3.2±0.2 ^a	8.9±0.3 ^c	11.2
	1000	48.0±4.0	0.0±0.0	100.0±0.0	5.5±0.3 ^b	3.1±0.1 ^a	8.6±0.4 ^a	11.6
	800	30.7±2.3	0.0±0.0	100.0±0.0	5.1±0.1 ^a	3.0±0.1 ^a	8.1±0.2 ^a	12.3
	600	10.7±6.1	0.0±0.0	100.0±0.0	5.0±0.2 ^a	2.8±0.2 ^a	7.8±0.4 ^a	12.8
	Control	12.0±4.0	0.0±0.0	100.0±0.0	4.9±0.1 ^a	2.8±0.2 ^a	7.7±0.3 ^a	13.0
Acetone	1600	100.0±0.0	---	---	---	---	---	---
	1400	93.3±2.3	0.0±0.0	100.0±0.0	6.3±0.3 ^d	3.9±0.4 ^a	10.2±0.7 ^c	9.8
	1200	76.0±4.0	0.0±0.0	100.0±0.0	6.2±0.2 ^d	3.7±0.3 ^a	9.9±0.5 ^c	10.1
	1000	64.0±4.0	0.0±0.0	100.0±0.0	6.0±0.1 ^d	3.6±0.1 ^a	9.6±0.2 ^b	10.4
	800	52.0±4.0	0.0±0.0	100.0±0.0	5.8±0.2 ^c	3.6±0.2 ^a	9.4±0.4 ^a	10.6
	600	33.3±4.6	0.0±0.0	100.0±0.0	5.7±0.1 ^b	3.5±0.1 ^a	9.2±0.2 ^a	10.9
	400	9.3±2.3	0.0±0.0	100.0±0.0	5.4±0.1 ^a	3.3±0.3 ^a	8.7±0.4 ^a	11.5
	Control	4.0±0.0	0.0±0.0	100.0±0.0	5.1±0.1 ^a	3.2±0.2 ^a	8.3±0.3 ^a	12.0

See the footnote of table 1.

Data presented in table 4 recorded the biological activity of chloroform and petroleum ether extracts from leaves of *M. longifolia* against the 3rd instar larvae of *C. antennatus*. Obtained data revealed that the highest concentrations (1200 and 450 ppm) caused complete larval mortality; meanwhile, the lowest concentration (100 and 150 ppm) caused larval mortality percentages of 17.3 and 14.7, respectively, compared with 10.7 and 8.0% for the control groups. In addition, booth larval and pupal periods were prolonged as compared with the untreated groups.

The chloroform extract from leaves of *M. longifolia* exhibited a very slight effect on the pupae resulted from treated larvae at 1000 and 800 ppm, where, the pupal mortality was 24.4 and 21.0%, respectively, compared with 0.0% for the control group. Also, the growth index recorded 7.0, 7.5, 9.7, 10.1, 10.3 and 11.2 at 1000, 800, 600, 400, 200 and 100 ppm of chloroform extract from leaves of *M. longifolia*, respectively, vs. 12.5 for the untreated group (Table 4).

Table 4: Effect of chloroform and petroleum ether extracts from leaves of *Menthe longifolia* on some biological aspects of *Culex antennatus*.

Extract	Conc. (ppm)	Larval Mort. (%)	Pupal Mort. (%)	Adult Emergence (%)	Larval Period	Pupal Period	Developmental Period	Growth Index
Chloroform	1200	100.0±0.0	---	---	---	---	---	---
	1000	94.7±2.3	24.4±13.3	75.6±13.3	6.8±0.2 ^d	4.0±0.4 ^c	10.8±0.6 ^d	7.0
	800	80.0±4.0	21.0±11.6	79.0±11.6	6.7±0.4 ^d	3.9±0.3 ^b	10.6±0.7 ^d	7.5
	600	66.7±2.3	0.0±0.0	100.0±0.0	6.4±0.4 ^d	3.9±0.1 ^b	10.3±0.5 ^d	9.7
	400	42.7±2.3	0.0±0.0	100.0±0.0	6.1±0.1 ^c	3.8±0.2 ^b	9.9±0.3 ^c	10.1
	200	32.0±4.0	0.0±0.0	100.0±0.0	6.0±0.3 ^c	3.7±0.1 ^a	9.7±0.4 ^b	10.3
	100	17.3±2.3	0.0±0.0	100.0±0.0	5.5±0.2 ^a	3.4±0.3 ^a	8.9±0.5 ^a	11.2
	Control	10.7±5.3	0.0±0.0	100.0±0.0	4.9±0.1 ^a	3.1±0.2 ^a	8.0±0.3 ^a	12.5
Pet. ether	450	100.0±0.0	---	---	---	---	---	---
	400	93.3±4.6	0.0±0.0	100.0±0.0	7.2±0.1 ^d	4.2±0.2 ^c	11.4±0.3 ^d	8.8
	350	84.0±4.0	0.0±0.0	100.0±0.0	7.0±0.1 ^d	4.1±0.3 ^c	11.1±0.4 ^d	9.0
	300	73.3±2.3	0.0±0.0	100.0±0.0	6.9±0.1 ^d	3.9±0.3 ^b	10.8±0.4 ^d	9.3
	250	50.7±2.3	0.0±0.0	100.0±0.0	6.7±0.3 ^d	3.7±0.3 ^a	10.4±0.6 ^c	9.6
	200	34.7±4.6	0.0±0.0	100.0±0.0	6.5±0.4 ^d	3.6±0.4 ^a	10.1±0.8 ^c	9.9
	150	14.7±2.3	0.0±0.0	100.0±0.0	6.2±0.2 ^c	3.2±0.1 ^a	9.4±0.3 ^a	10.6
	Control	8.0±0.0	0.0±0.0	100.0±0.0	5.2±0.3 ^a	3.1±0.2 ^a	8.3±0.5 ^a	12.0

See the footnote of table 1.

Based on LC₅₀ and LC₉₀ values, petroleum ether extraction from leaves of *M. longifolia* was more effective against 3rd instar larvae of

C. pipiens and *C. antennatus* than those of chloroform, acetone and methanol extractions (Table 5).

Table 5: Lethal concentrations (LC₅₀ and LC₉₀) of *Menthe longifolia* (leaves) tested extracts against *Culex pipiens* and *Culex antennatus* larvae.

Extract	Mosquito Specie	LC ₅₀ (LC ₉₀) ppm	Slope	95% Confidence Limits LC ₅₀ (LC ₉₀)		χ^2
				Lower	Upper	
Methanol	<i>Culex pipiens</i>	1741.5 (2282.0)	0.074	1719.0 (2264.6)	1763.8 (2299.4)	2.6 ^{Ns}
Acetone		1322.0 (1897.7)	0.0695	1303.2 (1883.2)	1340.8 (1912.2)	1.1 ^{Ns}
Chloroform		702.4 (1300.3)	0.0669	677.2 (1291.4)	727.7 (1309.3)	1.5 ^{Ns}
Pet. ether		379.5 (511.9)	0.302	368.3 (501.2)	390.8 (522.6)	2.9 ^{Ns}
Methanol	<i>Culex antennatus</i>	1064.6 (1596.5)	0.0752	1046.4 (1578.9)	1082.9 (1614.1)	1.8 ^{Ns}
Acetone		850.3 (1388.6)	0.0743	830.7 (1373.5)	869.9 (1403.7)	2.9 ^{Ns}
Chloroform		459.9 (979.4)	0.077	442.9 (971.1)	477.0 (987.8)	2.4 ^{Ns}
Pet. ether		250.4 (388.2)	0.2903	236.2 (372.5)	264.6 (403.8)	1.8 ^{Ns}

χ^2 Chi square value; Ns non-significant (P>0.05)

DISCUSSION

Lymphatic filariasis vector, *Culex pipiens* L. infects more than a hundred million people (Sayed *et al*, 2018; Shehata 2019). In addition, *C. antennatus* considered the main vector of Rift Valley Fever virus in the Nile Delta of Egypt. So, controlling *C. pipiens* and *C. antennatus* is a paramount technique for barring the propagation of diseases (Elango *et al*, 2009; Shahat *et al*, 2020).

The new materials of natural origin (plant extracts) revoke the hazards of synthetic chemical insecticides used in mosquito control, as some are selective, biodegrade to

nontoxic products, and safe for non-target organisms, as well as the environment (Singh and Upadhyay, 1993; Isman, 2006; Pavela, 2007).

In the present study, biological aspects of *C. pipiens* and *C. antennatus* were clearly affected by methanol, acetone, chloroform and petroleum ether extracts from leaves of *Menthe longifolia*. The larvicidal activity of the tested plant extracts was varied according to the solvent used in the extraction and concentration of the extract. Generally, the obtained results indicated that petroleum ether extract from leaves of *M. longifolia* was the

most effective extract against larvae of *C. pipiens* and *C. antennatus* as compared with those of chloroform, acetone and methanol. These results are inconsistent with the previous suggestions of Prabakar and jebanesan, (2004) where, *Momordica charantia*, *Trichosanthes anguina*, *Luffa acutangula*, *Benincasa cerifera* and *Citrullus vulgaris* extracts recorded LC₅₀ values after 24 h against *C. quinquefasciatus* third larval instar equal to 465.85, 567.81, 839.81, 1189.30 and 1636.04 ppm and Coria *et al.*, (2008) using extracts from *Melia azedarach* against *Aedes aegypti*. Similar results were also recorded by Ullah *et al.*, (2018) where, LC₅₀ and LC₉₀ values of *Cassia fistula* and *Nicotiana tabacum* extracts recorded 50.27, 203.99 and 17.77 and 206.49 ppm against larvae of *C. quinquefasciatus*, Hassanain *et al.*, (2019) who used petroleum ether extract from leaves of *Lantana camara* against larvae of *Anopheles Multicolor*, where, the highest larval mortality (100.0%) achieved at 140 ppm, Shehata, (2019) where, petroleum ether extract from leaves of *Prunus domestica* and *Rhamnus cathartica* was more effective against *C. pipiens* (LC₅₀ 33.3 and 63.4 ppm) than chloroform (LC₅₀ 70.8 and 192.1 ppm) and methanolic extracts (LC₅₀ 132.7 and 273.5 ppm) and Shahat *et al.*, (2020) where, hexane extract from leaves of *Otostegia fruticosa* was the most effective extract against *C. pipiens* larvae with LC₅₀ and LC₉₀ values equal to 126.27 and 236.84 ppm, respectively followed by chlorobenzene (242.14 and 501.17 ppm), ethyl acetate (578.07 and 856.29 ppm) and methanol (653.00 and 1127.10 ppm), respectively.

Also, tested extracts from leaves of *M. longifolia* induced a prolongation in both larval and pupal periods of both *C. pipiens* and *C. antennatus* depending on the solvent used in extraction and concentration of the extract. Generally, the highest concentrations from all tested extracts significantly (P<0.001) prolonged larval and pupal durations. These results are inconsistent with results recorded by Sharma *et al.*, (2006a&b) using petroleum ether extract of *Artemisia annua* against *An. stephensi* and *C. quinquefasciatus* larvae,

Coria *et al.*, (2008) using ethanolic extract of *Melia azedarach* leaves on *Ae. aegypti* larvae, Juliene *et al.*, (2009) using *Moringa oleifera* lectin against *Aedes aegypti* larvae, Hassanain *et al.*, (2019) using petroleum ether extract from leaves of *L. camara* against larvae of *An. Multicolor*, Shehata, (2019) using methanol, chloroform and petroleum ether extracts from leaves of *Pr. domestica* and *R. cathartica* against *C. pipiens* and Shahat *et al.*, (2020) using hexane extract from leaves of *O. fruticosa* against *C. pipiens* larvae.

On the other hand, a decrease in the percentage of *C. pipiens* and *C. antennatus* adult emergence due to treatment with tested extracts especially at the highest concentrations were recorded except petroleum ether extract from leaves of *M. longifolia* against *C. pipiens*, methanol extract from leaves of *M. longifolia* against *C. antennatus* and acetone extract from leaves of *M. longifolia* against *C. antennatus*. The reduction in the adult emergence percentages was similar to that recorded previously by Nathan *et al.*, (2006) using methanolic extracts of leaves and seeds of *Melia azedarach* against *A. stephensi* larvae, Sharma *et al.*, (2006a) using petroleum ether extract of *Ar. annua* against *An. stephensi* and *C. quinquefasciatus* larvae, Asiry *et al.*, (2017) using ethanolic leaf extracts of *Citrullus colocynthis*, *Artemisia annua*, *Pergularia tomentosa* and *Rhanterium epapposum* against the larval stages of *Ae. Aegypti*, and Shehata, (2019) using methanol, chloroform and petroleum ether extracts from leaves of *Pr. domestica* and *R. cathartica* against *C. pipiens* larvae.

The growth index of *C. pipiens* and *C. antennatus* was decreased as the concentration of the tested extract increased. Such results are in agreement with earlier studies using different plant extracts against some dipteran species by Sharma *et al.*, (2006b) using *Artemisia annua* extract against *C. autnauetesctetus*, Bream *et al.*, (2010) using *Echinochloa stagninum* extracts against *C. pipiens*, Hassanain *et al.*, (2019) using petroleum ether extract from leaves of *L. camara* against larvae of *An. Multicolor* and

Shahat *et al*, (2020) using hexane extract from leaves of *O. fruticososa* against *C. pipiens*.

Conclusion:

All tested extracts from leaves of *Menthe longifolia* showed larvicidal activity against *Culex pipiens* and *C. antennatus* and are considered as new promising controlling agents against *C. pipiens* and *C. antennatus*, however, more studies are necessary to reach the active ingredient in the tested extracts.

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