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Larvicidal, Biological and Histological Activities, and Chemical Composition of Plant Essential Oils Against *Culex pipiens*

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ABSTRACT

Despite their diminutive size, mosquitoes serve as significant vectors for numerous pathogenic organisms. In this research, five commercial essential oils (EOs) were screened (lemon, camphor, sandalwood, orange, and black pepper oil)against larvae of the filarial vector *Culex pipiens* to evaluate their larvicidal activity, their effects on different biological aspects and midgut histological architecture. Moreover, the chemical profile of the tested EOs was conducted employing gas chromatography-mass spectrometry (GC-MS) analysis. Results revealed that the most toxic oil was orange oil, with LC_{50} of 117.69 ppm and LC_{90} of 442.64 ppm. With LC_{50} and LC_{90} values of 1439.63 and 22489.03 ppm, respectively, lemon oil exhibited the least larvicidal activity. Larval treatments with the five EOs decreased pupation, pupal duration, adult female longevity, fecundity, and egg hatchability, with complete inhibition of egg deposition due to orange and black pepper oil treatments. The effects of EOs revealed destructive changes in midgut epithelial cells and peritrophic membranes. GC-MS analysis revealed that monoterpenes and fatty acids were the major constituents of the studied EOs. In conclusion, EOs can be used as effective alternatives to conventional insecticides for disease vector *Cx. pipiens* control.

INTRODUCTION

Significant pathogens transmitted by mosquitoes include Zika, yellow fever, dengue fever, the West Nile, chikungunya, and the Japanese encephalitis viruses; protozoa, including *Plasmodium* spp. in addition to the filarial nematodes, including *Brugia malayi*, *Wuchereria bancrofti*, and *Brugia timori* **(Tolle, 2009; Du *et al.*, 2019)**. These filarial nematodes are accountable for numerous diseases and fatalities on a global scale **(Mirzaian *et al.*, 2010; Franklinos *et al.*, 2019)**. Epidemics and disease transmission to humans are facilitated by many mosquito species, numbering over 23 **(Turell, 2012)**. Mosquitoes are temporary ectoparasites, and females are obligate bloodsuckers on animals or humans in order to develop eggs **(Killick-Kendrick, 1996)**. Females' propensity for bloodsucking is a major contributor to the transmission of pathogenic microbes to humans **(Chaves *et al.*, 2010)**.

According to **Kady *et al.* (2008),** *Culex pipiens* L. (Diptera: Culicidae) is a common species in Egypt that is responsible for numerous human diseases. *Cx. pipiens* is the vector of the Rift Valley fever (**Abdel-Hamid *et al.*, 2009**), *Wuchereria bancrofti*, and *Bancroftian filariasis*, which can affect up to 100 million people annually (**Sayed *et al.*, 2018**). *Bancroftian filariasis* is one of the fastest spreading insect-borne diseases for men in the tropics **(Badawy *et al.*, 2015).**

The eradication of these diseases primarily relies on interrupting the disease transmission cycle. This can be achieved by targeting the mosquito larvae in their breeding sites or eliminating adult mosquitoes with the use of pesticides **(Joseph *et al.*, 2004)**.

The primary method employed for mosquito control was the persistent application of traditional insecticides, including organophosphates and pyrethroids. For larvicides, organophosphate insecticides are frequently employed in mosquito reproductive sites; however, pyrethroids are the most widely used insecticides for controlling adult mosquitoes indoors **(Liu *et al.*, 2012)**. Nevertheless, the persistent rise in mosquito resistance to these insecticides represents a significant barrier to the success of control programs. Consequently, there is an urgent need to develop novel modes of action for safer, more environmentally friendly, and more effective alternatives to synthetic pesticides **(Tapondjou *et al.*, 2005)**. Thus, plants have attracted interest as a potential source of novel chemical compounds, particularly botanical insecticides that do not disrupt the ecosystem **(Lundberg, 2002)**.

Plant essential oils (EOs) are potential alternatives to synthetic insecticides against mosquito vectors **(Vera *et al.*, 2014; Pavela, 2015)**. In addition, EOs comprising blends of active constituents exert their effects on insects via distinct mechanisms of action or at various target locations, potentially mitigating resistance among mosquito populations **(Feng & Isman, 1995)**. EOs, which are produced through secondary plant metabolism, have demonstrated larvicidal properties. The chemical components of these oils have the ability to repel insects, impede enzyme activity, and induce deformities and mortality in a wide range of insect larval phases **(Pavela, 2015; Senthil-Nathan, 2020)**. The complex interplay between these plants' physiological and behavioral impacts poses a challenge for insects seeking to develop resistance **(Rice, 1993; Charleston *et al.*, 2005)**.

The objective of this investigation was to assess the larvicidal effects of five commercially available plant EOs against *Cx. pipiens* larvae. In addition, their biological and histological effects as well as their chemical profile were evaluated.

MATERIALS AND METHODS

1. Rearing of *Culex pipiens*

In Qena Governorate, Egypt, mosquito larvae were collected from their breeding habitats. They were cultivated and established in the insectary of the Department of Zoology, Faculty of Science, South Valley University. Morphological identification of

adults was accomplished by employing the taxonomic criteria of **Harbach (1985)**. The stock colony was kept at 27 ± 2°C and 60-70% RH **(Adham *et al.*, 2003)**.

2. Essential oils (EOs)

Five commercial EOs were selected: lemon oil (*Citrus limon*) (Rutaceae), camphor oil (*Cinnamomum camphora*) (Lauraceae), sandalwood oil (*Santalum album*) (Santalaceae), orange oil (*Citrus sinensis*) (Rutaceae) and black pepper oil (*Piper nigrum*) (Piperaceae). They were obtained from El-Kaptain Co., Cairo, Egypt.

3. Preparation of EO solutions

A stock solution of 1000ppm of each oil was prepared in distilled water using 0.6ml of 100% acetone **(Manzoor *et al.*, 2013)**. This stock was used to prepare six serial dilutions (1000, 500, 250, 125, 62.5, and 31.25 ppm) through dilution with acetone. The concentrations were kept in a refrigerator at 4°C till use.

4. Larvicidal bioassays

A 250-ml plastic cup was filled with 100ml of each concentration. Twenty late 3rd instar larvae of *Cx. pipiens* were transferred into each cup according to WHO protocol **(WHO, 2005)**. Four replicates of each concentration were established. A parallel control of 20 3rd instar larvae was conducted. Control larvae were reared in distilled water plus acetone without EOs. Each control was replicated four times. Following a 24-hour period, the count of deceased larvae was recorded for all concentrations.

5. Biological studies

Late third- instar larvae of *Cx. pipiens* were treated with LC_{50} of each EO for 24 hours. Experiments were continued until pupation. Pupae were then collected in 250-ml beakers filled with distilled water and left to the emergence of adults. Thus, pupal duration, pupation, and adult emergence were estimated. The resulting adults were released into cages that were supplied with a 10% sugar solution. Females were fed on blood. Each virgin blood-fed female was isolated in a rearing cage containing an unmated male to determine fecundity (total no. of eggs laid/female). The cage was supplied with a cup containing water as an oviposition site and a 10% sugar solution. The total number of eggs was collected until the hatch to determine fertility (% egg hatchability). Adult longevity (females) was determined. Each treatment was replicated four times. Additionally, a parallel control was conducted and replicated four times.

6. Histopathological studies

For the histological studies, the late 3rd instar larvae of *Cx. pipiens* were treated with the LC_{50} of each EO for 24 hours. Then, following a twenty-four-hour fixation in aqueous Bouin's solution, the specimens underwent three 15-minute washes in 100% ethyl alcohol, dehydration in a progressively increasing series of ethyl alcohol (70%, 80%, 90%, and 99%), xylene clearing, and embedding in paraffin wax. Using a rotatory microtome, 6 µm-thickness sections were cut. Hematoxylin and eosin staining were applied, and an Olympus BX51 microscope was used for analysis **(de Souza *et al.*, 2022)**.

7. Gas chromatography-mass spectrometry (GC-MS) analysis

EOs were analyzed for their chemical composition employing a mass spectrometer (GC-TSQ, Thermo Scientific, Austin, TX, USA). A fused silica capillary column was utilized with the following dimensions: 30m in length, 0.25µm in diameter, and 0.25µm film in thickness. Ionization energy was established at 70eV for GC-MS detection, while the injector and mass transfer line temperatures were set at 260 and 270°C, respectively. The carrier gas utilized was pure helium (99.995%), operating at a 1ml/ min rate. A volume of 0.5µl of the prepared EO was utilized for injection. The temperature of the column was progressively raised from 50 to 250°C for 2 minutes at a rate of 5°C per minute and finally to 300°C for an additional 2 minutes at a rate of 30°C per minute. A comparison of the mass spectra of the components with those of the Wiley Registry® 12th Edition/NIST 2020 Mass Spectral Library was utilized to identify them **(de Souza *et al.*, 2022)**.

8. Statistical analysis

Mortality counts in bioassays were corrected against those of the controls, using Abbott's formula **(Abbott, 1925)**. Data were then subjected to probit analysis **(Finney, 1971)** to estimate each oil treatment's LC_{50} and LC_{90} values. Utilizing one-way analysis of variance (ANOVA), the data of biological effects were analyzed. $P \leq 0.05$ was established as the level of significance. SIBOS PANEL V.25 (IBM, Armonk, New York, NY, USA) was utilized to perform every statistical calculation. Duncan's test (**Duncan, 1955**) was employed to ascertain significant differences among the treatments.

RESULTS

1. Toxicological effects

The results of acute toxicity of the five EOs against *Cx. pipiens* larvae after 24 hours of treatment are presented in Table (1). Data showed that all the tested EOs were highly toxic. The most toxic oil was the orange oil, with LC_{50} of 117.69 ppm and LC_{90} of 442.64 ppm. Lemon oil showed the most minor larvicidal activity with LC_{50} and LC_{90} values of 1439.63 and 22489.03 ppm, respectively.

2. Biological effects

The number of resultant pupae after larval treatments with different EOs was significantly decreased ($P< 0.05$) compared to the control groups, as the percentages of pupation in lemon oil, camphor oil, sandalwood oil, orange oil, and black pepper oil treatments were 22.28, 41.13, 35.19, 69.25, and 55.72%, respectively. Orange oil treatment produced the highest percentage of pupation (69.25%) (Table 2).

The mean pupal duration was significantly reduced in all treatments with the EOs; it reached 2.25, 3.03, 2.57, 3.02, and 2.97 days for treatments with lemon oil, camphor oil, sandalwood oil, orange oil, and black pepper oil, respectively (Table 2). The number of emerged adults after larval treatments with different EOs did not significantly differ compared to the controls. The emergence percentages in lemon oil, camphor oil,

sandalwood oil, orange oil, and black pepper oil were 33.33, 94.87, 38.62, 100 and 71.73%, respectively (Table 2).

Table (2) shows a significant reduction in female adult longevity. The female adult longevity was 11, 14.73, 15, 7.8, and 9.4 days at lemon oil, camphor oil, sandalwood oil, orange oil, and black pepper oil, respectively.

As demonstrated in Table (2), the treatment of larvae significantly reduced the number of eggs deposited by female mosquitoes in comparison to the control group, while in case of orange oil and black pepper oil, the female adult mosquitoes could not lay any eggs. Besides, the egg-hatch percentage was significantly decreased in all treatments.

Table 1. Susceptibility of *Cx. pipiens* larvae to plant essential oils

Essential oil	LC_{50} (ppm)	95% confidence limit (ppm) LCl-UCL	LC_{90} (ppm)	95% confidence limit (ppm) LCl-UCL	Slope ± SE	$\chi^2_{(df)}$	P
Lemon oil	1439.6	925.10-2918.90	22489.0	8462.9-120676.7	1.1 ± 0.1	$4.2_{(4)}$	<0.90
Camphor oil	184.6	148.90-229.30	1721.8	1140.4-3084.8	1.3 ± 0.1	$6.4_{(4)}$	<0.90
Sandalwood oil	628.5	492.90- 860.71	4805.4	2851.0-10391.2	1. 5 ± 0.2	$4.5_{(4)}$	<0.90
Orange oil	117.7	66.60-197.50	442.6	340.4-1395.8	2.2 ± 0.2	$14.8^*_{(4)}$	0.005
Black pepper oil	195.8	110.80-349.90	559.5	457.8-1788.8	2.8 ± 0.2	$22.2^*_{(4)}$	<0.005

LCL: Lower confidence limit, UCL: Upper confidence limit. * Significant heterogeneity at $P<0.05$.

Table 2. Biological effects of different plant essential oils on *Cx. pipiens*

Essential oil	Pupation (%)	Pupal duration (days)	Adult emergence (%)	Female longevity (days)	No. of deposited eggs/female	Egg hatchability (%)
Control	95.25 ± 1.75^{a}	3.64 ± 0.06^{a}	85.50 ± 4.26^{a}	22.92 ± 0.39^{a}	159.30 ± 10.14^{a}	82.69 ± 5.93^{a}
Lemon oil	22.28 ± 5.04^{b}	2.25 ± 0.38^{b}	33.33 ± 22.05^{a}	11.0 ± 1.47^{bc}	100.70 ± 42.68^{ab}	93.44 ± 3.28^{a}
Camphor oil	41.13 ± 7.78^{bc}	3.03 ± 0.15^{b}	94.87 ± 2.56^{a}	14.73 ± 0.94^{b}	91.8 ± 15.14^{ab}	85.55 ± 6.28^{a}
Sandalwood oil	35.19 ± 6.55^{bd}	2.57 ± 0.32^{b}	38.62 ± 12.71^{a}	15.0 ± 0.31^{b}	68.0 ± 0.0^{b}	69.12 ± 0.0^{a}
Orange oil	69.25 ± 4.08^{ac}	3.02 ± 0.07^{b}	100.0 ± 0.0^{a}	7.8 ± 1.11^{c}	–	–
Black pepper oil	55.72 ± 6.07^{cd}	2.97 ± 0.11^{b}	71.73 ± 19.44^{a}	9.4 ± 0.86^{c}	–	–

Means within columns with the same letter are not significantly different ($P> 0.05$), using Duncan's test.

3. Histopathological effects

The midgut of control mosquito larvae consists of columnar epithelial cells with rounded nuclei in the middle of the cells and rest on a basal membrane (Fig. 1A). The regenerative cells lie in the cleft between cells as shown in Fig. (1A). The peritrophic membrane lines the epithelial cells from the inside toward the lumen (Fig. 1A).

In larvae treated with lemon oil for 24 hours, the midgut showed rupture and degeneration of epithelial cells and nucleus deformation , as shown in Fig. (1B). In larvae treated with camphor oil for 24 hours, the midgut showed degeneration and exudation of epithelial cells, where the cells discharged their contents of cytoplasm (Fig. $1C_1$, C_2, C_3). The nuclei were degenerated and sloughed off the cells, as shown in Fig. ($1C_2$, C_3).

In larvae treated with sandalwood oil for 24 hours, the midgut showed some epithelial cells devoid of nuclei and expelled their contents of the cytoplasm outside the cell, and the rupture of peritrophic membrane (Fig. $1D_1$, D_2). In larvae treated with orange oil for 24 hours, the midgut showed ruptured epithelial cells with nuclei devoid of chromatin materials (Fig. $1E_1$, E_2).

In larvae treated with black pepper oil for 24 hours, the midgut showed degeneration and rupture of epithelial cells and basal cell membrane, deformation of the nuclei, and rupture of the peritrophic membrane, as shown in Fig. (1F).

4. Chemical composition of Eos

Tables (3- 7) exhibit the GC-MS analysis of the tested EOs. Lemon oil has three compounds; one monoterpene (D-limonene, 64.77%), one unsaturated fatty acid (linoleic acid, 15.10%) and one unsaturated fatty acid ester (methyl linoleate, 20.13%) (Table 3). Camphor oil has 12 compounds (Table 4) – three monoterpenes (56.25%), two unsaturated fatty acids (12.08%), two unsaturated fatty acid esters (8.33%), one saturated fatty acid (2.53%), one fatty acid ester (4.31%), one cyclic hydrocarbon (2.52%), one sesquiterpene (1.95%), and one shikimate (11.89%). Sandalwood oil contains 12 compounds (Table 5) – five unsaturated fatty acids (36.45%), four unsaturated fatty acid esters (38.39%), one saturated fatty acid (10.69%), one cyclic hydrocarbon (10.70%), and one sesquiterpene (3.77%). Orange oil had only one compound– the monoterpene D-limonene (Table 6). Black pepper oil showed 18 compounds (Table 7) – six unsaturated fatty acids (19.11%), three unsaturated fatty acid methyl esters (22.48%), two saturated fatty acid methyl esters (6.08%), one saturated fatty acid (1.09%), one fatty acid ester (7.16%), three fatty acids (5.2%), one sterol (8.41%), and one sesquiterpene (0.6%).

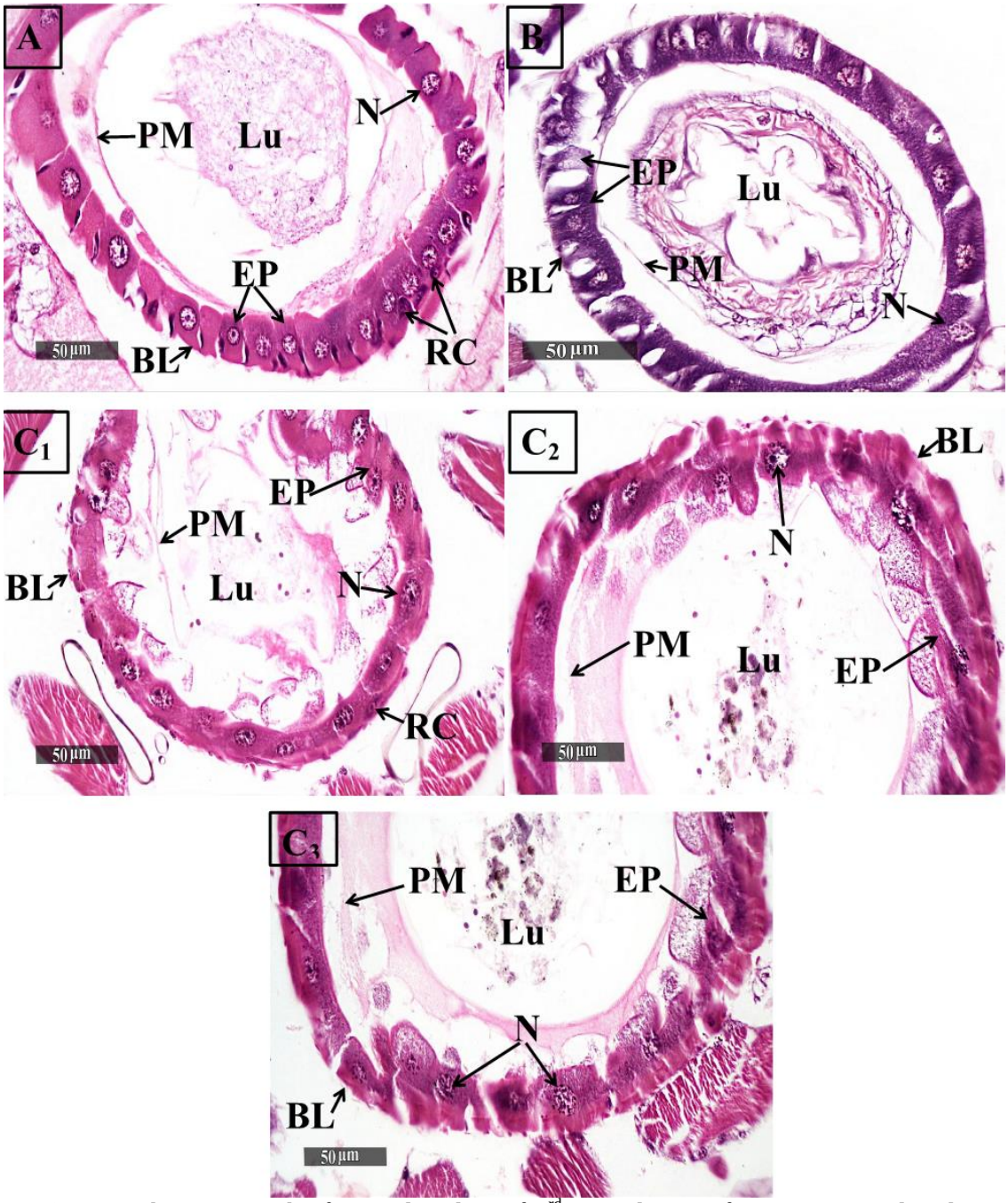

Fig. 1. Light micrograph of normal midgut of 3rd instar larvae of *Cx. pipiens* and midgut of the same instar larvae treated for 24 h with plant essential oils. (**A**) Control midgut showing columnar epithelial cells (EP), with nucleus (N), regenerative cells (RC), basal lamina (BL), peritrophic membrane (PM), and lumen (Lu). (**B**) Midgut treated with lemon essential oil showing rupture of epithelial cells (Ep), deformed nucleus (N), basal lamina (BL), peritrophic membrane (PM), and lumen (Lu). (**C_1, C_2, C_3**) Midgut treated with camphor essential oil showing rupture of epithelial cells (Ep), deformed nucleus (N), basal lamina (BL), regenerative cells (RC), degenerated peritrophic membrane (PM), and lumen (Lu).

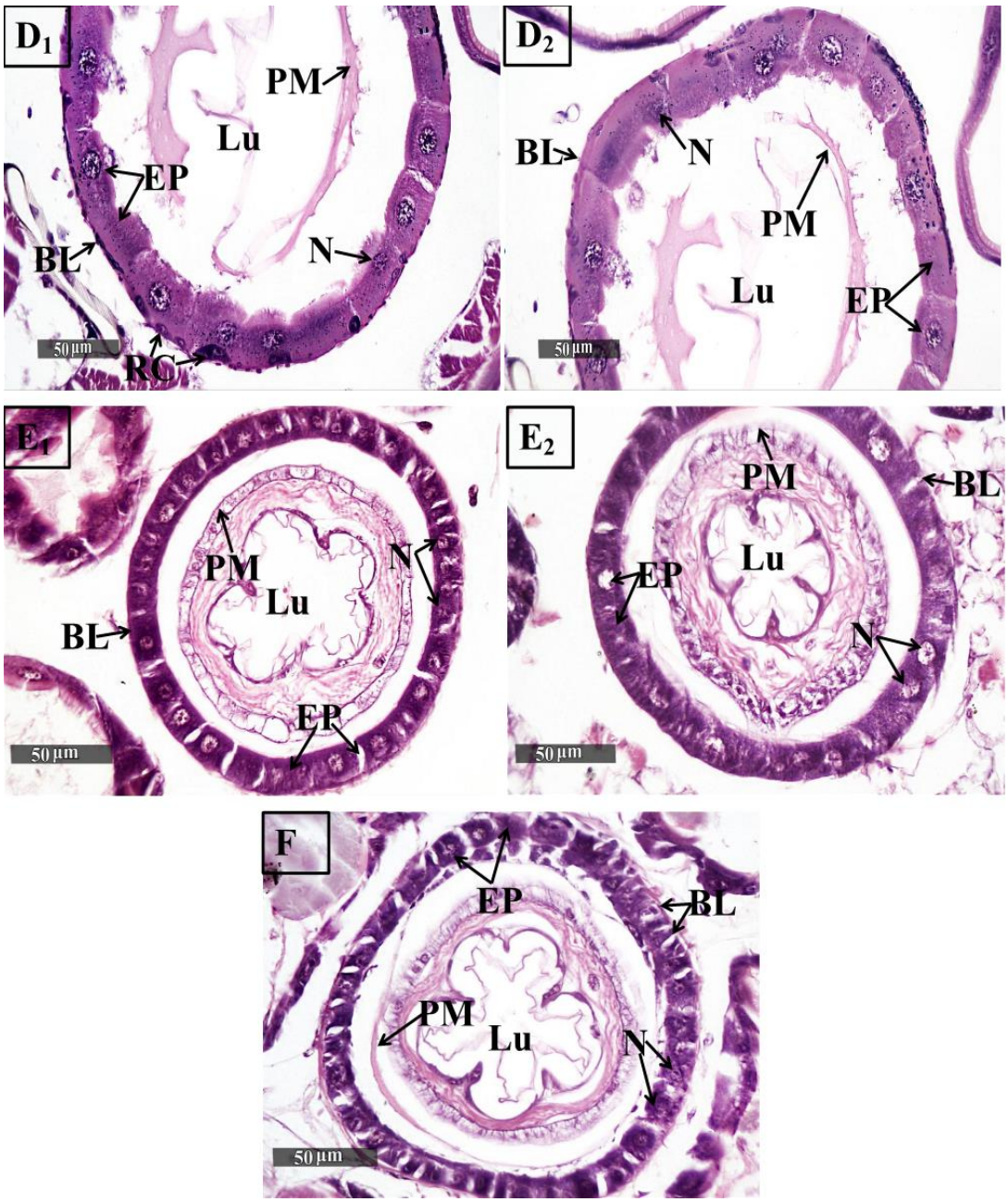

Fig. 1. (Continued). Light micrograph of midgut of 3rd instar larvae of *Cx. pipiens* treated for 24 h with plant essential oils. (**D_1, D_2**) Midgut treated with sandalwood essential oil showing rupture of epithelial cells (Ep) and some epithelial cells devoid of nuclei, degenerated nuclei (N), basal lamina (BL), regenerative cells (RC), degenerated peritrophic membrane (PM), and lumen (Lu). (**E_1, E_2**) Midgut treated with orange essential oil showing rupture of epithelial cells (Ep) and some epithelial cells devoid of nuclei, deformed nucleus (N), basal lamina (BL), degenerated peritrophic membrane (PM), and lumen (Lu). (**F**) Midgut treated with black pepper essential oil showing rupture of epithelial cells (Ep) and some epithelial cells devoid of nuclei, deformed nucleus (N), basal lamina (BL), degenerated peritrophic membrane (PM), and lumen (Lu).

Table 3. Chemical constituents identified by GC/MS from lemon oil (*Citrus limon*)

No. of peaks	Chemical name	Classification	Retention time (min)	Molecular weight	Molecular formula	Composition (%)
1	D-Limonene	Monoterpene	6.55	136	$C_{10}H_{16}$	64.77
2	Linoleic acid	Unsaturated fatty acid	30.88	280	$C_{18}H_{32}O_2$	15.10
3	Methyl linoleate	Unsaturated fatty acid ester	31.42	294	$C_{19}H_{34}O_2$	20.13

Table 4. Chemical constituents identified by GC/MS from camphor oil (*Cinnamomum camphora*)

No. of peaks	Chemical name	Classification	Retention time (min)	Molecular weight	Molecular formula	Composition (%)
1	Eucalyptol	Monoterpene	6.54	154	$C_{10}H_{18}O$	6.66
2	(+)-2-Bornanone	Monoterpene	8.98	152	$C_{10}H_{16}O$	45.12
3	Levomenthol	Monoterpene	10.10	156	$C_{10}H_{20}O$	4.47
4	2-Hydroxybenzoic acid methyl ester	Shikimates	10.50	152	$C_8H_{16}O_3$	11.89
5	Furoscrobiculin B	Sesquiterpene	23.63	232	$C_{15}H_{20}O_2$	1.95
6	Isopropyl myristate	Fatty acid ester	25.75	270	$C_{17}H_{34}O_2$	4.31
7	Palmitoleic acid	Unsaturated fatty acid	27.69	254	$C_{16}H_{30}O_2$	2.63
8	palmitic acid	Saturated fatty acid	28.30	256	$C_{16}H_{32}O_2$	2.53
9	Octadecahydro-benzo[cd]pyrene	Cyclic hydrocarbon	28.74	258	$C_{19}H_{30}$	2.52
10	Methyl palmitoleate	Unsaturated fatty acid ester	28.60	268	$C_{17}H_{32}O_2$	1.63
11	methyl (9Z,11E,13E)-octadeca-9,11,13-trienoate	Unsaturated fatty acid ester	30.15	292	$C_{19}H_{32}O_2$	6.70
12	cis-13-Eicosenoic acid	Unsaturated fatty acid	30.87	310	$C_{20}H_{38}O_2$	9.45

Table 5. Chemical constituents identified by GC/MS from sandalwood oil (*Santalum album*)

No. of peaks	Chemical name	Classification	Retention time (min)	Molecular weight	Molecular formula	Composition (%)
1	Critonilide	Sesquiterpene	23.64	232	$C_{15}H_{20}O_2$	3.77
2	Palmitoleic acid	Unsaturated fatty acid	28.31	254	$C_{16}H_{30}O_2$	9.04
3	Methyl palmitoleate	Unsaturated fatty acid ester	28.60	268	$C_{17}H_{32}O_2$	5.58
4	Octadecahydro-benzo[cd]pyrene	Cyclic hydrocarbon	28.74	258	$C_{19}H_{30}$	10.70
5	Linoleic acid	Unsaturated fatty acid	29.49	280	$C_{18}H_{32}O_2$	4.88
6	Oleic acid	Unsaturated fatty acid	29.60	282	$C_{18}H_{34}O_2$	8.09
7	methyl oleate	Unsaturated fatty acid ester	30.16	296	$C_{19}H_{36}O_2$	15.25
8	Methyl linoleate	Unsaturated fatty acid ester	30.72	294	$C_{19}H_{34}O_2$	7.94
9	cis-Vaccenic acid	Unsaturated fatty acid	30.88	282	$C_{18}H_{34}O_2$	9.75
10	Gadoleic acid	Unsaturated fatty acid	31.30	310	$C_{20}H_{38}O_2$	4.69
11	Nonadecanoic acid	saturated fatty acid	31.42	298	$C_{19}H_{38}O_2$	10.69
12	methyl (9Z,11Z)-nonadeca-9,11-dienoate	Unsaturated fatty acid ester	31.94	308	$C_{20}H_{36}O_2$	9.62

Table 6. Chemical constituents identified by GC/MS from orange oil (*Citrus sinensis*)

No. of peaks	Chemical name	Classification	Retention time (min)	Molecular weight	Molecular formula	Composition (%)
1	D-Limonene	Monoterpene	6.55	136	$C_{10}H_{16}$	100%

Table 7. Chemical constituents identified by GC/MS from black pepper oil (*Piper nigrum*)

No. of peaks	Chemical name	Classification	Retention (min)	Molecular weight	Molecular formula	Composition (%)
1	β-Caryophyllene	Sesquiterpene	16.54	204	$C_{15}H_{24}$	0.60
2	Linolelaidic acid, methyl ester	Unsaturated fatty acid methyl ester	25.14	294	$C_{19}H_{34}O_2$	12.50
3	Methyl-11-octadecenoate	Unsaturated fatty acid methyl ester	25.77	296	$C_{19}H_{36}O_2$	6.16
4	Methyl 10-octadecenoate	Unsaturated fatty acid methyl ester	25.87	296	$C_{19}H_{36}O_2$	3.82
5	Oleic acid	Unsaturated fatty acid	26.97	282	$C_{18}H_{34}O_2$	7.09
6	Methyl octadecenoate	saturated fatty acid methyl ester	27.37	298	$C_{19}H_{38}O_2$	4.26
7	cis-Methyl 11-(3-pentyl-2-oxiranyl)undecanoate	saturated fatty acid methyl ester	27.69	312	$C_{19}H_{36}O_2$	1.82
8	2,3-Dihydroxypropyl palmitate	Fatty acid derivatives	28.00	330	$C_{19}H_{38}O_4$	1.29
9	stearic acid	Fatty acid	29.36	284	$C_{18}H_{36}O_2$	2.10
10	Nonadecanoic acid	saturated fatty acid	29.78	298	$C_{19}H_{38}O_2$	1.09
11	cis-13-Eicosenoic acid	Unsaturated fatty acid	30.17	310	$C_{20}H_{38}O_2$	1.89
12	(8E,11E)-icosa-8,11-dienoic acid	Unsaturated fatty acid	30.34	308	$C_{20}H_{36}O_2$	1.50
13	(E)-icos-11-enoic acid	Unsaturated fatty acid	30.52	310	$C_{20}H_{38}O_2$	3.60
14	methyl heptadecanoate	Fatty acid ester	32.14	284	$C_{18}H_{36}O_2$	7.16
15	cis-12-Heneicosenoic acid	Unsaturated fatty acid	33.15	324	$C_{21}H_{40}O_2$	1.44
16	cis-9-docosenoic acid	Unsaturated fatty acid	35.31	338	$C_{22}H_{42}O_2$	3.59
17	docosanoic acid	Fatty acid	35.76	340	$C_{22}H_{44}O_2$	1.81
18	Cholesta-3,5-diene	Sterol	42.31	368	$C_{27}H_{44}$	8.41

DISCUSSION

In this study, the tested EOs showed that a highly toxic effect against *Cx. pipiens* larvae, with orange oil was the most potent one. Several studies focusing on the use of botanical products in combating mosquitoes exhibited larvicidal effects of 13 oils from

41 plants (thyme, camphor, amyris, cedarwood, lemon, frankincense, juniper, dill, myrtle, helichrysum, black pepper, verbena, and sandalwood) toward the third-instar larvae after a short period **(Amer & Mehlhorn, 2006)**.

Xu *et al.* (2020) studied the larvicidal activities of EO of *Cinnamomum camphora* leaf against *Anopheles stephensi*. **Aljameeli (2023)** studied the larvicidal effects of lemon oil against *Aedes aegypti*. Besides, **Kurniasih *et al.* (2021)** elucidated that the toxicity of EO from orangeon *Ae. aegypti*. *Cx. quinquefasciatus* was found to be entirely susceptible to larvicidal and suppression activities of cinnamon oil, calamus oil, mentha oil, citronella oil, lemon oil, clove oil, eucalyptus oil, and orange oil according to **Manimaran *et al.* (2012)**.

Based on the findings of the present investigation, it is evident that the development of the treated larvae was aberrated. Our findings align with those of **Jacobson (1990)** who reported that, applying active compounds derived from the neem plant to mosquito larvae resulted in hormonal disruptions that hinder the larval development and reproductive capabilities. The current results are consistent with those of **Amusan *et al.* (2005)**, who demonstrated that applying a methanolic extract derived from orange peels resulted in a higher mortality rate among *Ae. aegypti* larvae.

The impact of 5% concentration of neem oil and neem seed extract against *An. stephensi* was investigated by **Murugan *et al.* (1996)**. According to the study, neem oil was found to be more efficacious against mosquito larvae than seed kernel extract. However, both extracts were capable of impeding the development of adult mosquitoes. On the other hand, **El-Banoby (2005)** discovered that the unprocessed alkaloids derived from *Solanum nigrum* exhibited a genocidal efficacy against *Cx pipiens* larvae. The inhibitory effects on adult emergence ranged from 28% to 90% at effective concentrations ranging from 200 to 900ppm. Our findings indicate a reduction in the adult female lifespan. These results are consistent with those of **Ascher (2005)** who observed that, using an extract derived from neem scales reduced the adult lifespan of numerous insects. In their investigation, **Jayakumar *et al.* (2016)** examined the erosive properties of various plant oils, including lemon oil and lavender oil, against the larvae of *Cx. quinquefaciatus* mosquitoes. The efficacy of these oils against larvae was validated through tests, and their influence was observed to persist in pupae and adults, aligning with the present findings. According to another study by **Jayarama and Pushpalatha (2008)**, the growth of mosquito larvae from the genera *Culex*, *Aedes*, and *Anopheles* in water treated with plant leaf seed extracts of *Solanum surattense* and *Samadera indica* reduced female fertility by 62.4% to 87.4%. Additionally, the hatching rate of eggs in the current investigation was impacted. This result aligns with that of **Devi and Bora (2017)**, who postulated that, the phenolic extract of *Ziziphus jujube* reduced the hatchability rate when applied to larvae of *Ae. aegypti*, in addition to impeding the growth rate.

The present study demonstrated midgut cell degeneration in the treated third-instar larvae of *Cx. pipiens*. In accord with our findings, **Oliveira *et al.* (2021)** showed that after 24 hours of treatment of third-instar larvae of *Ae. aegypti* with the LC_{50} of R-limonene, a complete disorganization of the epithelium was observed. Similar results

were also obtained in the late third-instar larvae of *Ae. aegypti* after 24 hours of treatment with niosomes encapsulated with neem oil **(Jeno & Nakkeeran, 2022).** In addition, the treatment of the third-instar larvae of *Cx. pipiens pallens* for 24 hours with the LC_{50} of *Perilla frutescens* EO revealed a rupture of peritrophic membrane and brush border of the midgut cells, with swollen nuclei, damaged cytoplasm and deformed epithelial cells extending into the lumen **(Zhang *et al*., 2023).** Moreover, histopathological abnormalities in *Ae. aegypti* larvae subjected to nanoparticle treatment using *Artemisia vulgaris* leaf extract were emphasized in the study of **Sundararajan and Kumari (2017).** The anomalies were predominantly observed in the adipose tissue, musculature, and midgut epithelia.

The majority of the compounds in the examined EOs were monoterpenes and fatty acids, according to GC-MS analysis. **Tripathi *et al.* (2009)** reported that most monoterpenes are cytotoxic to insect and animal tissues, resulting in a significant decrease in Golgi bodies and mitochondria, impaired respiration, and reduced cell membrane permeability. In addition, monoterpenes have the potential to exert their effects on a multitude of insect targets, particularly those associated with the nervous system. These targets include acetylcholinesterase (AChE), nicotinic acetylcholine receptor (nAChR), sodium channels, and gamma-aminobutyric acid (GABA)-gated chloride channels, among others **(Priestley *et al*., 2003; Tripathi *et al.*, 2009; López & Pascual-Villalobos, 2010; Mossa, 2016)**.

The target sites of fatty acids are AChE and/or octopaminergic receptors **(Perumalsamy *et al.*, 2015)**. Insecticidal activity of fatty acids has been reported in several studies **(Ramos-López *et al.*, 2012; Yousef *et al.*, 2013; Perumalsamy *et al.*, 2015; Moustafa *et al.*, 2018)**. Besides, exposure to fatty acids prolonged the developmental times of larvae and pupae and decreased their weight **(Ramos-López *et al.*, 2012; Moustafa *et al.*, 2018)**.

CONCLUSION

In conclusion, EOs can be effective alternatives to conventional insecticides in managing the disease vector *Cx. pipiens* within a frame of integrated pest management (IPM). The larvicidal activity of these oils in addition to their inhibitory effects on several biological aspects, such as pupation, fecundity and egg hatchability would ultimately reduce the population dynamics of this pest. Furthermore, the reduction in the longevity of females that survived following larval treatments with studied oils might be expected to reduce their ability to transmit pathogens.

ETHICAL APPROVAL

All experiments in this research were approved by the Research Ethics Committee of the Faculty of Science, South Valley University, Qena Governorate, Egypt (Code No. 006/10/23).

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