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Insecticidal, Repellency, and Histopathological Effects of Four Extracts of Clove Oil (*Syzygium aromaticum*) Against *Culex pipiens* (Diptera: Culicidae)

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ABSTRACT

Solvent extraction, a classic method to separate different components, was used in this study. Clove (*Syzygium aromaticum*) was macerated using four solvents, namely, water, petroleum ether, ethyl acetate, and ethanol, to extract the essential oil. The insecticidal and repellent activities on third-instar larvae and adult females of *Culex pipiens* were investigated. Based on LC_{50}, water extract was more active (LC_{50} = 39 ppm) against larvae. The highest efficacy of the repellent solvent of the clove extract against adult females was 28%. The ultrastructural effect was studied on the cuticle, muscles, and midgut of third-instar larvae. The tested larvicide showed histopathological changes in the treated larvae compared with the untreated ones. Thus, the water extract could be a good candidate and promising alternative in control programs of mosquitoes, especially against *Cx. pipiens* in Egypt and other developing countries.

INTRODUCTION

Culex pipiens is the most widespread mosquito species in Egypt **(Hoogstraal *et al*. 1979)**. It causes Filariasis, Rift Valley fever, and other diseases that are deadly to man and animals **(Harb *et al*., 1993; Ghosh *et al*. 2012)**. The mosquito control program is based on synthetic pesticides such as pyrethroids, organophosphates, and carbamates. Continuous application of these pesticides leads to many hazards to the environment and beneficial insects **(Pushpanathan *et al*. 2008; Bayen 2012)**.

Mosquitoes developed a resistance to many insecticides, especially synthetic ones. Thus, the dosage is usually increased to control mosquitoes. The biological method is an eco-friendly and cost-effective approach to mosquito control. Usage of plant oils as pesticides is an old practice. After the Second World War, farmers neglected this practice and used synthetic insecticides. However, the hazards of chemical insecticides for mammals, the ecological needs increase of insecticides with lower toxicity to mammals, and with lower persistence in the environment **(Regnault-Roger *et al*. 1993)**.

Plant extracts exert an insecticidal effect with low toxicity toward non-target organisms **(Isman 1999)**. Secondary metabolites in plants confer the essential oils (EOs) their characterized odors. Many plant oil extracts show a broad spectrum of insecticidal activity, deterrents of oviposition, and growth regulators **(Bakkali *et al*. 2008)**. Many scientists reported the insecticidal activity of EOs against insects, including the cabbage looper and armyworm **(Akhtar *et al*. 2008)**, mosquitoes **(Watanabe *et al*. 1993)**, cockroaches **(Appel *et al*. 2001)**, stored product pests **(Dales 1996)**, and termites **(Zhu *et al.* 2001). Enan (2001)** and **Priestley *et al*. (2003)** reported that EOs exert their insecticidal activity by targeting the nervous system, interfering with GABA-gated sodium channels, and antagonizing octopamine receptors. EOs are generally composed of complex mixtures of sesquiterpenes, monoterpenes, and phenols **(Isman 2008)**. Many techniques employ different solvents to improve the properties of the extracted EOs **(Chemat *et al*. 2004)**. **Nassar *et al*. (2007)** identified 16 volatile compounds from the n-hexane extract of *Syzygium aromaticum* buds through gas chromatography-mass spectroscopy (GC–MS). The major components were eugenol (71.56%) and eugenol acetate (8.99%). **Polatoğlu and Karakoç (2016)** found that eugenol is toxic to many insects under laboratory conditions. Insect repellents have great attention nowadays. Mosquito repellents are available commercially, and the standard one is N, N, -diethyl-3-methylbenzamide (DEET, C12H17NO) **(Leal 2014)**. DEET acts as a contact and spatial repellent **(Syed and Leal 2008; Degennaro *et al*. 2013)**. **Abdel-Rahman *et al.* (2004)** reported that DEET harms people and may cause disturbances in learning ability and memory. High concentrations of DEET are not recommended for children because of its side effects, such as encephalopathy **(Abdel-Rahman *et al*. 2001).** In many areas in the world, DEET is not convenient; many people use natural and handmade repellents, such as alcoholic extract of Indian clove.

The main target of many insecticides is the digestive system of pests **(Chapman 1998)**. Any changes in the gut affect the growth and development of the insect **(Mordue and Nisbet 2000)**. Many authors investigated the ultrastructural effect of different plant extracts on *Cx* sp larvae. **Al-Mekhlafi, (2018)** evaluated the changes in the midgut of *Cx. pipiens* larvae after treatment with *Carum copticum*. **Jiraungkoorskul (2016)** observed histopathological alterations in the larvae of *Cx. quinquefasciatus* after treatment with *Andrographis paniculata*. **Procópio et al. (2015)** studied ultrastructural changes in the larvae of *Ae. aegypti* by treatment of *Schinus terebinthifolius*. Most of the ultrastructural changes occurred in the midgut region and included the destruction of midgut epithelial cells, microvilli, and the formation of cytoplasmic vacuoles.

The current study aimed to evaluate and compare the insecticidal activity on the larvae of *Cx. pipiens* and the repellency effect on adult females and monitor the histopathological changes in the water extract of clove oil to develop an eco-friendly, safe, and bio-degradable natural insecticide.

MATERIALS AND METHODS

Insect rearing

Egg rafts of the mosquito were obtained from the Research and Training Center on Vectors of diseases. The mosquitoes were reared in an insectary of the Entomology Department under controlled conditions, including 25°C ± 2°C and relative humidity (RH) 70% ± 10%, and photoperiod 14:10 light: dark hours. The *Cx. pipiens* larvae were fed on

Tetramin daily. The adult insects were reared in wooden cages and fed with 10% sucrose solution. The females were allowed to take a blood meal from a pigeon host **(Gerberg 1970)**.

Clove oil extraction and preparation of different clove extracts

Clove buds were purchased from the local market (Cairo, Egypt) and identified by the Botany Department, Faculty of Science, Ain Shams University. Buds of the clove plant were dried and ground to a fine powder. Four samples of the fine powder of clove (30 g for each) were macerated for extractions in dark bottles (200 mL for each extraction) with water (WC), petroleum ether (PEC), ethyl acetate (EAC), and ethanol (EC) for 3 days with shaking. The samples were filtered, and the collected extract of light solvents PEC, EAC, and EC were evaporated under reduced pressure using a rotatory evaporator at 40°C. The WC extract was lyophilized. For bioassays, 0.5 mL of each extract (PEC, EAC, and EC) was mixed, except for the WC extract with 0.5 mL of tween 80, and then completed to 10 ml with distilled water. Positive control was prepared by mixing 0.5 mL of surfactant (5% tween 80) with 9.5 mL of distilled water.

Insecticidal activity of *S. aromaticum* on third-instar larvae of *Cx. pipiens*

In a series of experiments, the larvicidal activity of four extractions of *S. aromaticum* was detected against the third-instar larvae of *Cx. pipiens*. Twenty-five third-instar larvae of *Cx. pipiens* were transferred into plastic test cups, each containing 100 mL of water. All the experiments were conducted under laboratory conditions (27°C ± 2°C, RH 70% ± 10%, and 14:10 light: dark regime) according to **WHO (2005)**.

Five concentrations of each plant extract were prepared (10, 30, 50, 100, and 150 µL), as shown in Table 1, with three replicates for each concentration. Lethal concentrations were determined at the 95% confidence level and recorded in probit regression line, and LC_{25}, LC_{50}, and LC_{95} were calculated **(Finney 1971)**. Correction for control mortality was conducted using Abbott's formula **(Abbott 1987)**.

Repellency action of *S. aromaticum* against *Cx. pipiens* adult females

Standard cages were used to detect the repellent activity of the five tested concentrations of *S. aromaticum* (150, 100, 50, 30, and 10 ppm). *S. aromaticum* extracts were dissolved in 2 mL of distilled water with a drop of triton X 100 to prepare different concentrations used. A 1 mL aliquot of each concentration was applied onto the abdomen of the pigeon for 10 min. Pigeons were placed for 3 h in cages containing at least 10 female adults *Cx. pipiens*. Water was used as a negative control. Commercial repellent 15% DEET (Johnson Wax Egypt) was used as a positive control. Each repellency test was repeated three times to calculate the mean repellency value. After treatment, the number of fed and unfed females was counted, and the repellency percentage was recorded statistically by using the Abbott formula **(Abbott 1987)**.

Repellency % = (%Y − %Z / 100 − Z%) × 100,

where

Y is the percentage of unfed females in treatment.

Z is the percentage of unfed females in the control.

Ultrastructure study

Histological and ultrastructural samples were investigated using JEOL transmission electron microscopy (TEM) JEM1011 at the regional center of Mycology and Biotechnology, Al-Azhar University. Untreated and treated third-instar larvae of *Cx. pipiens* with an LC_{50} concentration of *S. aromaticum* after 24 h were subjected to histopathological and ultrastructural studies. The specimens were fixed in 5% glutaraldehyde and then washed in 70% alcohol as previously described **(Sabine 1971)**.

RESULTS

The extract yields obtained by different solvents are listed in Table I. The PEC, EAC, and EC extracts were oily, whereas the WC extract was a semisolid of residues.

Table I. Different solvents with the amount of clove oil *Syzygium aromaticum* recovered.

Solvent	Amount of oil /g
Petroleum ether	3.3
Ethyl acetate	6.2
Ethanol	10.3
Water	4.2

Insecticidal activity

LC values for the different extracts of *S. aromaticum* on the third-instar larvae of *Cx. pipiens* are shown in Table II. Data revealed that the treated larvae were the most susceptible to WC, followed by EC, PEC, and EAC. Based on LC_{50}, the WC extract was more active (LC_{50} = 39 ppm), and the EAC extract was less active (LC_{50} = 60 ppm). LC_{25} and LC_{95} also indicated that the WC extract was more effective (17 and 303.8 ppm, respectively) than the other extracts. The 95% confidence limits between four extracts of *S. aromaticum* showed a substantial overlap.

Table II. Susceptibility of third-instar larvae of *Culex pipiens* to water, ethanol extract, petroleum, and ethyl acetate *of Syzygium aromaticum* at 24 h post-treatment.

Solvents	LC values in ppm (95% C.I.)			
	LC_{25}	LC_{50}	LC_{95}	slope
water extract	17 (13–21)	39 (33.5–45.9)	303.8 (222.7–465.8)	1.8
ethanol extract	20 (15.9–24)	47 (40–54.6)	357.8 (258.5–561)	1.8
petroleum extract	19 (14.7–23)	48 (40–56)	441 (300.7–756)	1.6
ethyl acetate extract	27 (21.7–32)	60 (52–69.9)	420 (301–669.)	1.9

Repellency action of *S. aromaticum* against *Cx. pipiens* adult females

The average numbers of *Cx. pipiens* female mosquitoes of the untreated control, DEET, fed and unfed on pigeon treated with five concentrations (150, 100,50, 30, and 10 ppm) for each extract of *S. aromaticum* were recorded. Data of the WC extract are listed in Table III. The repellency percentage (R%) increased and ranged from 0 to 28% with increasing EO concentration.

For the EC extract, the fed percentage of the treated group decreased with increasing concentration of the EC extract. In specific, it ranged from 40% to 16% with increasing concentration. The unfed percentage on the treated group ranged from 0 to 24%, indicating a significant increase with increasing extract concentration. Moreover, repellency remarkably increased with increasing extract concentration (Table IV).

The results of the PEC extract are recorded in Table V. The average number of mosquitoes fed on the control was 9.3 ± 0.6, whereas that of the unfed group was 0.7 ± 0.6. The repellency percentages of the solvent were 2.7%. The fed percentage of females in the treated group ranged from 36% to 18%, indicating an inverse effect about the extract concentration. A direct relationship was found between the repellency (1.3%–18.6%) and unfed (4%–21.3%) percentages of the treated group with the PEC extract concentration (10–150 ppm).

Data of the EA extract is represented in Table VI. The average numbers of fed and unfed females in the control group were 9.7 ± 0.6 and 0.3 ± 0.6, respectively. The repellency percentage of the solvent was 1.3%. The effect of treatment indicates that the fed percentage of females was inversely proportional to the concentration of the extract; in specific, it ranged from 38.7% to 21.3% for successive concentrations. Meanwhile, the unfed percentage of females on the treated trials was directly proportional to the extract concentration. It ranged from 1.3% to 18.7%. Moreover, the repellency percentage increased from 0% to 17.3% with increasing EO concentration.

Table III. Repellency of water extract of *Syzygium aromaticum* on *Culex pipiens* adult females

Repellency %						
	Fed No.		Unfed No.		Repellency (R) %	Corrected R%
Concentration (ppm)	Avg ± SD	Fed%	Avg ± SD	Unfed %		
150	3.0 ± 0.0	12.0	7.0 ± 0.0	28.0	28.0	28.0
100	5.7 ± 1.2	22.7	4.3 ± 1.2	17.3	17.3	17.3
50	7.3 ± 0.6	29.3	2.70.6	10.7	10.7	10.7
30	9.3 ± 0.6	37.3	0.7 ± 0.6	2.7	2.7	2.7
10.0	10.0 ± 0.0	40.0	0.0 ± 0.0	0.0	0.0	0.0
DEET (10ppm)	0.0 ± 0.0	0.0	10.0 ± 0.0	40.0	40.0	100
Control	10.0 ± 0.0	40.0	0.0 ± 0.0	0.0	0.0	0.0

Table IV. Repellency of ethanol extract of *Syzygium aromaticum* on *Culex pipiens* adult females.

Repellency %						
	Fed No.		Unfed No.		Repellency (R) %	Corrected R%
Concentration (ppm)	Avg ± SD	Fed%	Avg ± SD	Unfed %		
150	4.0 ± 1.0	16.0	6.0 ± 1.0	24.0	24.0	24.0
100	5.7 ± 0.6	22.7	4.3 ± 0.6	17.3	17.3	17.3
50	7.7 ± 0.6	30.7	2.3 ± 0.6	9.3	9.3	9.3
30	8.3 ± 0.6	33.3	1.7 ± 0.6	6.7	6.7	6.7
10.0	10.0 ± 0.0	40.0	0.0 ± 0.0	0.0	0.0	0.0
DEET (10ppm)	0.0 ± 0.0	0.0	10.0 ± 0.0	40.0	40.0	100
Control	10.0 ± 0.0	40.0	0.0 ± 0.0	0.0	0.0	0.0

Table V. Repellency of petroleum ether extract of *Syzygium aromaticum* on *Culex pipiens* adult females.

Repellency %						
	Fed No.		Unfed No.		Repellency (R) %	Corrected R%
Concentration (ppm)	Avg ± SD	Fed%	Avg ± SD	Unfed %		
150	4.7 ± 0.6	18.7	5.3 ± 0.6	21.3	21.3	18.6
100	6.7 ± 0.6	26.7	3.3 ± 0.6	13.3	13.3	10.6
50	7.7 ± 0.6	30.7	2.3 ± 0.6	9.3	9.3	6.6
30	8.3 ± 0.6	33.3	1.7 ± 0.6	6.7	6.7	4.0
10.0	9.0 ± 0.0	36.0	1.0 ± 0.0	4.0	4.0	1.3
DEET (10ppm)	0.0 ± 0.0	0.0	10.0 ± 0.0	40.0	40.0	100.0
Control	9.3 ± 0.6	37.3	0.7 ± 0.6	2.7	2.7	2.7

Table VI. Repellency of ethyl acetate extract of *Syzygium aromaticum* on *Culex pipiens* adult females.

Repellency %						
	Fed No.		Unfed No.		Repellency (R) %	Corrected R%
Concentration (ppm)	Avg ± SD	Fed%	Avg ± SD	Unfed %		
150	5.3 ± 0.6	21.3	4.7 ± 0.6	18.7	18.7	17.3
100	6.7 ± 0.6	26.7	3.3 ± 0.6	13.3	13.3	12.0
50	8.3 ± 0.6	33.3	1.7 ± 0.6	6.7	6.7	5.3
30	9.0 ± 0.6	36.0	1.0 ± 0.6	4.0	4.0	2.7
10.0	9.7 ± 0.6	38.7	0.3 ± 0.6	1.3	1.3	0.0
DEET (10ppm)	0.0 ± 0.0	0.0	10.0 ± 0.0	40.0	40.0	100.0
Control	9.7 ± 0.6	38.7	0.3 ± 0.6	1.3	1.3	1.3

Histological and ultrastructure studies

Integument changes

The normal cuticle of untreated third-instar larvae of *Cx. pipiens* L. is illustrated in Fig. (Ia). The epicuticle, exocuticle, and striated endocuticle were characterized by a group of lamellae. The cuticle of the third-instar larvae treated with LC_{50} of *S. aromaticum* water extract showed loose papillae projection and vacuolization in the epidermal layer (I and II), as demonstrated in Figs. (Ib) and (Ic).

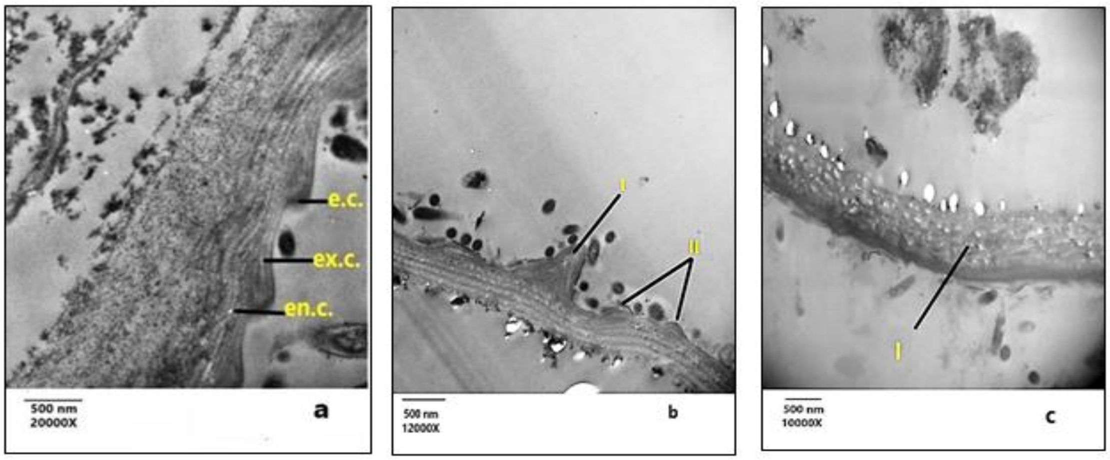

Fig. I. TEM microphotograph of the cuticle layers of larvae of *Culex pipiens*: (a) control larvae (20000×); (b & c) larvae treated with the LC_{50} of *Syzygium aromaticum* water extract (12000× and 10000×).

Skeletal muscles changes

The normal skeletal muscle of the control larvae is highlighted in Fig. (IIa). The treated larvae showed remarkable variations characterized by shrinkage and disorganized fibrils, as depicted in Figs. (IIb) and (IIc).

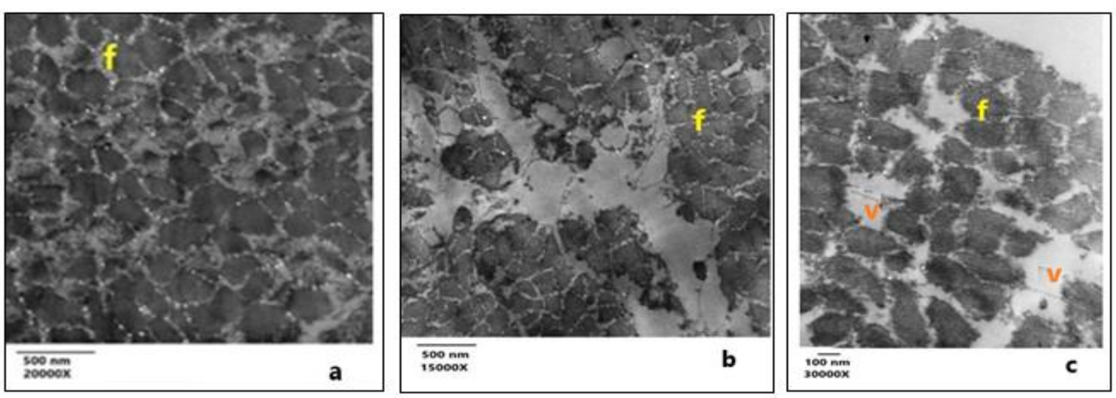

Fig. II. TEM microphotograph of cross-sectioned skeletal muscle fibrils of larvae of *Culex pipiens*: (a) control larvae (20000×); (b) larvae treated with LC_{50} of *Syzygium aromaticum* water extract (15000× and 20000×). (f) fibril, (v) vacuole.

Midgut changes

The larval midgut of untreated *Cx. pipiens* third-instar larvae is displayed in Figs. (IIIa) and (IVa). It was composed of one layer of columnar epithelial cells with normal microvilli and situated on an intact basement membrane. Well-developed longitudinal and circular muscle layers were surrounded externally by a basal lamina. The epithelial cells included moderately centric rounded nuclei. The peritrophic membrane (pm) was distinctly surrounded the food callus (fc). The gut lumen showed a regular adhesive brush border membrane. Distinct histopathological variations were induced in the treated *Cx. pipiens* larval midgut, as shown in Figs. (IIIb) and (IIIc) and Figs. (IVb) and (IVc). In specific, the midgut was characterized by disruption in the epithelial cell layer, elongated microvilli, and vacuolization.

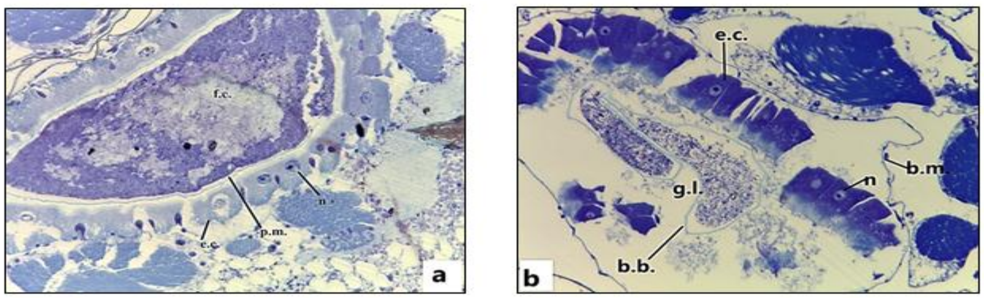

Fig.III: Semi-thin section of third-instar larvae of *Culex pipiens* L. (x = 40); a: control larvae, b. treated larvae with LC_{50} of *Syzygium aromaticum* water extract. (e.c.) epithelial cell, (g.l.) gut lumen, (b.b.) brush border, (b.m.) basement membrane, (n) nucleus, (p.m.) peritrophic membrane, (f.c.) food callus.

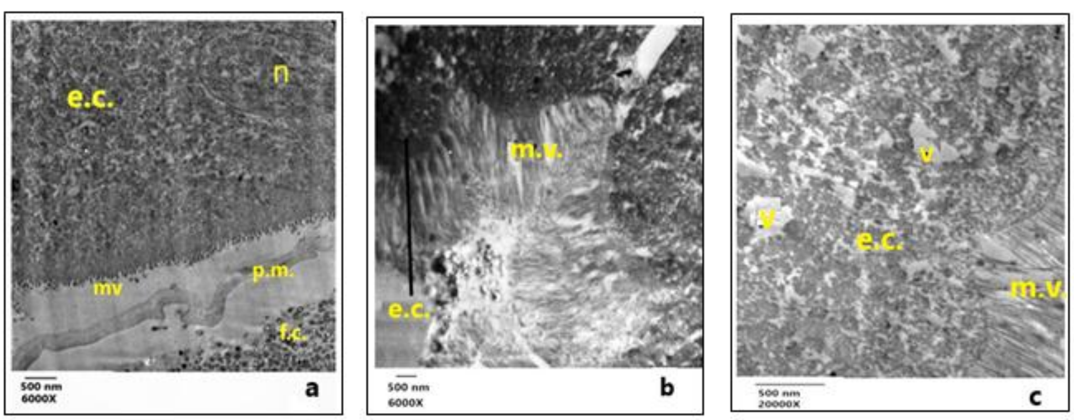

Fig. IV. TEM microphotograph of cross-sectioned midgut of *Culex pipiens* L. larvae:
(a) control larvae (6000×); (b, c) larvae treated with LC_{50} of *Syzygium aromaticum* water extract (60000× and 20000×). (fc) food callus, (mv) microvilli, (pm) peritrophic membrane, (e.c.) epithelial cell.

DISCUSSION

Many plant extracts exert insecticidal activity (**Chaiyasit *et al.* 2006; Azmy *et al*. 2019; El Gohary *et al.* 2021)** and mosquito repellent activity **(Trongtokit *et al.* 2005; Phukerd and Soonwera 2014**). Active compounds in *S. aromaticum* include eugenol, β-caryophyllene, and eugenol acetate. Eugenol shows larval mortality (**Huang *et al*. 2015)** through its lipophilicity, which facilitates the penetration through the insect cuticle and exhibits insecticide activity similar to deltamethrin (**Jumbo *et al.* 2018**). In addition, it can be easily biodegraded by microorganisms **(Mishra *et al.* 2013; Kadakol and Kamanavalli 2010)**. Plant extracts and the effect of using different extraction solvents have been previously determined (**Ghosh *et al*. 2012**). Our study compared four extracts of *S. aromaticum* oil (water, ethanol, petroleum ether, and ethyl acetate) against the third larval stage of *Cx. pipiens*. The larvicidal activity was reported in terms of LC_{50}. The larval mortality among *Cx. pipiens* increased with increasing concentration of *S. aromaticum* EO. A comparison among the four plant extracts revealed that the WC extract was the active one, followed by the EC, PEC, and EAC extracts. **Chore *et al.* (2014**) and **Kovendan *et al.* (2012)** studied the influence of extraction solvent on the toxicity of EO and found that the type of solvents and the solvent polarities used for the extraction of essential oils influence larval mortality. Different solvents provide different polarity gradients in dissolving the toxic components in the plant materials **(Umezawa, 2003; Zarnowski** and **Suzuki, 2004**). The effect of extractions of different solvents on the toxicity of the EOs was recorded in this study, and the findings are consistent with those obtained by **Kovendan *et al.* (2012)**, who used *Morinda citrifolia* leaf extract as a larvicide against *Aedes aegypti*, *Anopheles stephensi*, *and Culex quinquefasciatus.* They found that hexane, chloroform, acetone, and water solvents cause moderate mortality. **Al Dawsari (2020)** used chloroform and petroleum ether to extract clove extract oil and found that the chloroform extract is more active than the petroleum ether extract on adult weevil *Rhynchophorus ferrugineus*.

Eugenol has been isolated from the essential oil *Monarda bradburiana* and used as a mosquito repellent (**Tabanca *et al*., 2013)**. Eugenol is a volatile and lipophilic compound (monoterpenes) that can penetrate and interfere with the physiological functions of insects (**Saad, *et al.* 2018**). The mode of action of monoterpenes has been studied by various authors, but it was not well understood (**Soujanya *et al*. 2016**). The main entrance of eugenol and other monoterpenes into the insect body is through the respiratory system. Eugenol shows strong inhibition of acetylcholinesterase and adenosine triphosphatase activities in *Sitophilus oryzae* **(Athanassiou *et al*. 2014; Saad *et al.* 2018)**. Our results showed differences in the repellency of clove oil extraction by using different solvents. The repellency effect on adult female mosquitoes is consistent with the results of the bioassay test of the four clove extracts on *Cx pipiens* larvae. A comparison between four extracts revealed that the more effective extract was the WC extract, followed by the EC and PEC extracts; the least effective one was the EAC extract. The repellency effect of these four extracts was still weaker than that of DEET. The obtained result of repellency agreed with the results by **Tan *et al.* (2019)**, who found that the homemade extract of Indian clove-based ethanolic extract shows significantly lower protection than 1% DEET.

In the present work, the histopathological effect of the WC extract of *S. aromaticum* was assessed because it was the most active one. The obtained results showed histological alterations on *Cx. pipiens* cuticle, muscles, and midgut after treatment with clove oil *S. aromaticum*. The obtained results showed vacuolization in the epidermal layer and midgut epithelial cells. According to **Wigglesworth (1972**), these vacuoles form autotrophic vacuoles. The insect midgut is the main organ that is susceptible to the toxic effect of plant extract, where it is the main organ responsible for digestion and absorption (**Correia *et al*. 2009**). Plant extracts can affect the epithelial cells of the insect midgut, which is the main reason for the reduction of enzyme activity (**Selin-Rani *et al*. 2016**).

Selin-Rani *et al.* (2016) found that plant extracts can damage the midgut of insects. Based on the results of this study, clove oil *S. aromaticum* may be considered as an eco-friendly, safe, inexpensive, and available pesticide and may act as a suitable alternative to synthetic pesticides.

CONCLUSION

The present study revealed that, using four solvents; water, petroleum ether, ethyl acetate, and ethanol of *S. aromaticum* have a larvicidal effect on the 3rd larval instar of *Cx pipiens*. Additionally, based on LC values *S. aromaticum* water extract has the highest toxicity on *Cx pipiens* larvae and repellency efficiency against adult females. Also, it induced histopathological alterations in the tested insects. So, *S. aromaticum* water extract could be a good candidate and promising alternative in control programs of mosquitoes, especially against *Cx. pipiens* in Egypt and other developing countries.

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الملخص العربى

دراسة التأثيرات السمية و الطاردة والتغيرات النسيجية المرضية لأربعه مستخلصات من زيت القرنفل *Syzygium aromaticum*على بعوض *Culex pipiens* L (رتبة: ثنائية الاجنحة. Culicidae)

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استُخدمت طريقة الاستخلاص بالمذيبات، وهي طريقة كلاسيكية لفصل المكونات المختلفة، في هذه الدراسة. تم نقع القرنفل (*Syzygium aromaticum*) باستخدام أربعة مذيبات ، وهي الماء ، وإيثر البترول ، وخلات الإيثيل ، والإيثانول ، لاستخراج الزيت العطري . تم دراسة فعالية هذه المستخلصات كمبيدات حشرية وطارده للحشرات على يرقات الطور الثالث والإناث البالغة من *Culex pipiens*. بناءً على التركيز المميت النصفي ، كان المستخلص المائي أكثر نشاطًا (LC $_{50}$ =٣٩ جزء في المليون) ضد اليرقات. وايضا الاعلى فاعليه كطارد للاناث البالغة بنسبه بلغت ٢٨٪. تمت دراسة تأثير هذه المستخلصات على الجلد والعضلات والمعي المتوسط ليرقات الطور الثالث من بعوض *Cx. pipiens*. أظهرت المستخلصات المختبرة تغيرات نسيجية مرضية في اليرقات المعاملة مقارنة باليرقات غير المعالجة. وبالتالي ، يمكن أن يكون مستخلص الماء مرشحًا جيدًا وبديلًا واعدًا في برامج مكافحة البعوض ، خاصةً ضد *Cx. pipiens* في مصر والدول النامية الأخرى.