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Larvicidal Potential of Some Essential Oils against the Filarial Vector *Culex pipiens* L. (Diptera: Culicidae)

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Abstract: Mosquitoes have significant effect on humans and animals especially in tropical and subtropical regions. *Culex pipiens* L is widely distributed mosquito including North Africa and it is the most common mosquito in Egypt agriculture. This study aimed to evaluate the larvicidal efficacy of ginger (*Zingiber officinale*) and nut grass (*Cyperus rotundus*) oils against third instar larvae of *Cx. pipiens* L and to monitor the damages in treated larvae by scanning electron microscope (SEM). Third instar larvae of *Cx. pipiens* (L.) were exposed to 16, 32, 64, 125, 250, 375 and 500 ppm of ginger and nut grass oils. The mortality percentages and LC_{50} for all treatments were calculated. Larvae treated with 125 ppm of both tested oils and untreated larvae were prepared to photograph by SEM. The mortality percentage of *Cx. pipiens* larvae increased with the increase of the concentration and exposure time. LC_{50} values for oils of *Z. officinale* and *C. rotundus* after 3, 6, 9 and 12 h were (286 & 267 ppm), (76 &151 ppm), (20 & 25 ppm) and (17 & 18 ppm), respectively. SEM revealed cuticular damages in larvae which exposed to either *Z. officinale* oil or *C. rotundus* oil but the level of damage in case of *C. rotundus* treatment was lower. The tested essential oils have larvicidal activity against *Cx pipiens* L. The toxicity of *C. rotundus* oil is relatively lower than that of *Z. officinale* oil. Further study is needed to evaluate the repellent properties of the two essential oils against the adult flies of *Cx. pipiens*.

Key words: *Culex pipiens* L · *Zingiber officinale* · *Cyperus rotundus* · Control · Toxicity

INTRODUCTION

Mosquitoes have significant effects on humans or animals especially in tropical and subtropical regions and transmit fatal diseases such as malaria, filaria, yellow fever, Japanese encephalitis, dengue, West Nile and zika [1, 2]. *Cx. pipiens* is widely distributed mosquito including North Africa and it is the most common mosquito in Egypt [3]. It is found where stagnant water is available near to people who live in urban and sub-urban areas [4] The medical importance of *Cx. pipiens* due to its ability to transmit many diseases to human or animals such as lymphatic filariasis [5] Louis encephalitis [6] Rift Valley fever (RVF) [7] avian malaria [3] and Japanese encephalitis [8].

Different commercial insecticides could be effective in controlling the *Cx pipiens* larvae [9, 10]. The extensive use of insecticides has led to negative effects on public health and agriculture such as the rapid development of resistant mosquito strain, harmful impacts on beneficial organisms, environmental pollution and toxic hazards to mammals [11, 12]. As a result, there has been an increased interest in developing potential alternative or additional control methods/materials that are effective against the target vector species, eco-friendly, biodegradable, with low cost and can be used by individual and communities in specific situations [13].

Currently, there are many natural products have larvicidal activity and lesser toxic to mammals than chemical insecticides. Plant-derived materials were

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evaluated and revealed effective and applicable in use for control in a wide range. To discover new insecticide from plants, many trials should be done to confirm its action and applied in the field [14, 15]. The new natural compounds extracted from plants are preferable in mosquito control because they are safe and have not any side effects [16]. Recently, botanical products including essential oils have been suggested as alternative bio-control against arthropod vectors [17, 18]. The low price of the essential oils, their availability on the market and their global production make them as ideal candidate ingredients to be used in insecticidal formulations [19]. Whole essential oils (EO) have higher effective than their separated compounds [20]. There are many medicinal plants have an essential oils that have insecticidal properties. Several researches proved that essential oil extracted from different plants exhibited larvicidal or repellent against several species of mosquitoes. In Himalaya, EO extracted from eleven plants revealed larvicidal efficacy against *Aedes aegypti* [21]. EO extracted from five *Cinnamomum* species had larvicidal activity against *A. aegypti* and *A. albopictus* [22]. EO extracted from thirteen plants had larvicida properties against *Aedes*, *Anopheles* and *Culex* mosquitoes.

Z. officinale (Family Zingiberaceae), commonly called ginger, is a perennial plant cultivated extensively in almost all tropical and subtropical countries. It is an edible rhizome which is used for a variety of purposes, including culinary and medicinal. For centuries, *Z. officinale* was used in traditional medicine for respiratory disorders, stroke, inflammation, hyper-cholesterolaemia and helminthiasis. Yet, this plant still attracts extensive research attention. Researchers have reported that the *Schistosoma mansoni* infected mice were orally inoculated with *Z. officinale* extract could reduce the deleterious effects of *S. mansoni* [23, 24]. Also, *Z. officinale* offers an opportunity for new compound, which is a cheap alternative for the more costly anthelmintics. Its ovicidal efficacy and structural alterations of the cuticle of adult worms are clear indication of being a promising nematocidal agent [25].

Cyperus rotundus is a weed grown in the fields and on the canal borders. *C. rotundus* L. (Known as Su'd) is used as an antimalarial, anti-inflammatory, antipyretic and insecticidal treatment [26]. It shows cyto-protective effects against ethanol induced gastric ulceration [27]. Certain *Cyperus* species also exhibit insecticidal properties [28].

The aim of the present the study is to evaluate *in vitro* larvicidal efficacy of the essential oils of ginger *Z. officinale* and nut grass *C. rotundus* against third instar larvae of *Cx. pipiens*.

MATERIALS AND METHODS

Insect: Third instar of *Cx. pipiens* larvae (Diptera: Culicidae) were selected as the target insect for *in vitro* assay of the essential oils. A local strain was collected from a lake in Giza Governorate, Egypt during July, 2017. The larvae were placed into plastic dishes (30×20×10 cm) with 1500 ml distilled water at room temperature. Larvae were fed on equal parts of dried yeast, biscuits and dried milk powder, according to the method of Hafez [29].

Essential Oils: Two essential oils, ginger *Z. officinale* and nut grass *C. rotundus* were obtained from El-Captain Company for extracting natural oils, herbs and cosmetics, Al-Obor city, Cairo, Egypt, approved for human use from the Egyptian Ministry of Health.

Toxicity of the Essential Oils: Third instar *Cx. pipiens* larvae were exposed to seven concentrations 16, 32, 64, 125, 250, 375 and 500 ppm of the two tested essential oils. The stock oils were diluted in 10 ml distilled water and few drops of Tween 80 were added as an emulsifier to the essential oils. The procedures were applied five times for each concentration and 20 larvae were used per replicate in each test (i.e., 100 larvae were used for each concentration. Larvae were immersed in a tested concentration during the exposure period. The mortality of larvae in all dishes was observed 3, 6, 9, 12 and 24 h post treatment. Alive and dead larvae were counted, larvae were considered alive if they exhibited normal behavior when breathed upon or physically stimulated with wooden dowels. Larvae which were incapable of movement, maintaining any signs of life, were considered moribund or dead [30].

Scanning Electron Microscopy (SEM): Control larvae and those that exposed to 125 ppm concentration of both tested oils were fixed intact for 12 h in a 3:1 mixture of 4 % (w/v) glutaraldehyde in 0.12 M Millonig'sbuffer, pH 7.4 and 1 % aqueous osmium tetroxide. After this, the specimens were processed for SEM following a method previously described by Shalaby *et al*. [31].

Statistical Analysis: The mortality results of *Cx. pipiens* larvae were statistically analyzed by ANOVA followed by Duncan test using the SPSS computing program. LT_{50} values were also calculated log-concentrations probit model using Ldp line[R] software [32].

RESULTS

Two essential oils extracted from *Z. officinale* and *C. rotundus* were evaluated as eco-friendly bio-control against 3rd instar larvae of *Cx. pipiens* under laboratory conditions. The mortality percentage of *Cx. pipiens* larvae increased with the increase of concentration and exposure time for the two tested essential oils (Tables 1 & 2).

Effect of *Z. officinale* Essential Oil: The larvae treated with 16-125 ppm of *Z. officinale* oil appeared active after 3 h and revealed significant mortalities after 6 and 9h. At 12 h, the mortality percentages of larvae treated with 16 and 32 ppm were 56±5.1 and 60±3.2 %, while, the larvae treated with 63 and 125 ppm dead completely. The complete mortality delayed until 24 h in larvae treated with only 16 and 32 ppm. The concentrations 250 and 375 ppm achieved significant mortality earlier than the lowest concentrations at 3 h recording 28±8.6 and 88±5.8%, respectively. They recorded 100% mortality after 9 and 6 h, respectively. The highest concentration 500 ppm gave a rapid toxic on larvae, it recorded 100 mortality after 3 h (Table 1).

Effect of *C. rotundus* Essential Oil: The larvae treated with 16-125 ppm of *C. rotundus* seem to be active at 3 h like that happened with *Z. officinale* oil. The larvae maintained active until 9 h in 16 ppm and 6 h in 32 and 63 ppm. The significant mortalities were recorded at 12 h in 16 ppm, 9 h in 32 and 63 ppm and 6 h in 125 ppm. The concentrations 250 and 375 ppm caused earlier significant mortalities in larvae 40±3.2 and 90±6.3, respectively. The highest concentration 500 ppm had quick high toxicity against larvae; it recorded 100% mortality after 3 h like that happened with same concentration of *Z. officinale* oil. Complete mortality was obtained after 6 h in larvae treated with 375 ppm and after 24 h in larvae treated with 32 - 250 ppm. The maximum mortality recorded in larvae treated with 16 ppm after 24 h was 58±3.7 (Table 2).

Table 1: Mortality percentages (Mean ± SE) of 3rd instar larvae of *Culex pipiens* treated with different concentrations of the essential oil extracted from ginger *Zingiber officinale*

	Concentration (ppm)						
Hours post treatment	16	32	63	125	250	375	500
3	00±0.0 ^{a}	00±.0.0 ^{a}	00±0.0 ^{a}	00±0.0 ^{a}	28±8.6 ^{a}	88±5.8	100±0.0
6	14±4.1 ^{b}	20±0.0 ^{b}	36±6.8 ^{b}	66±4.0 ^{b}	88±4.9 ^{b}	100±0.0	
9	56±5.1 ^{c}	56±6.0 ^{c}	60±3.2 ^{c}	66±5.1 ^{b}	100±0.0 ^{b}		
12	56±5.1 ^{c}	60±3.2 ^{c}	100±0.0 ^{d}	100±0.0 ^{c}			
24	100±0.0 ^{d}	100±0.0 ^{d}					
F value	114.941	139.815	126.000	166.857	45.551	4.235	
P value	<0.001	<0.001	<0.001	<0.001	<0.001	Insignificant	

No mortality was recorded in the control. Values with different letters (a, b, c and d) are significantly different within treatments (based on the non-overlapping confidencelimits).

Table 2: Mortality percentages (Mean±SE) of 3rd instar larvae of *Culex pipiens* treated with different concentrations of the essential oil extracted from nut grass *Cyperus rotundus*

	Concentrations (ppm)						
Hours post treatment	16	32	63	125	250	375	500
3	00±0.0^{a}	00±0.0^{a}	00±0.0^{a}	00±0.0^{a}	40±3.2^{a}	90±6.3	100±0.0
6	00±0.0^{a}	00±0.0^{a}	00±0.0^{a}	44±7.5^{b}	64±4.0^{b}	100±0.0	
9	00±0.0^{a}	56±6.0^{b}	70±4.5^{b}	70±4.5^{c}	76±2.4^{c}		
12	58±3.7^{b}	56±6.0^{b}	70±4.5^{b}	70±4.5^{c}	76±2.4^{c}		
24	58±3.7^{b}	100±0.0^{c}	100±0.0^{c}	100±0.0^{d}	100±0.0^{d}		
F value	180.214	126.444	258.750	72.979	62.526	2.500	
P value	<0.001	<0.001	<0.001	<0.001	<0.001	Insignificant	

No mortality was recorded in the control. Values with different letters (a, b, c and d) are significantly different within treatments (based on the non-overlapping confidence limits)

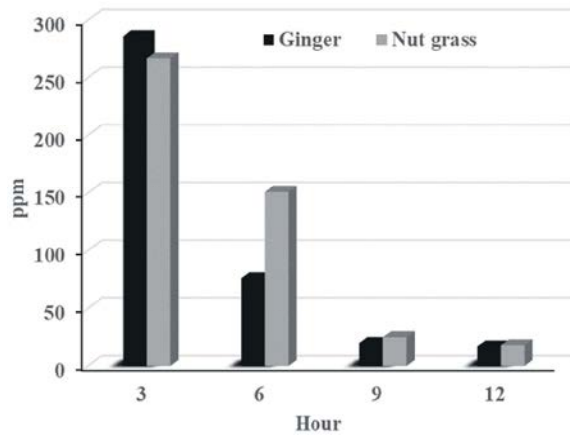

Fig. 1: LC_{50} values of 3rd instar larvae of *Culex pipiens* treated with essential oils of ginger *Zingiber officinale* and nut grass *Cyperus rotundus* at various exposure hours.

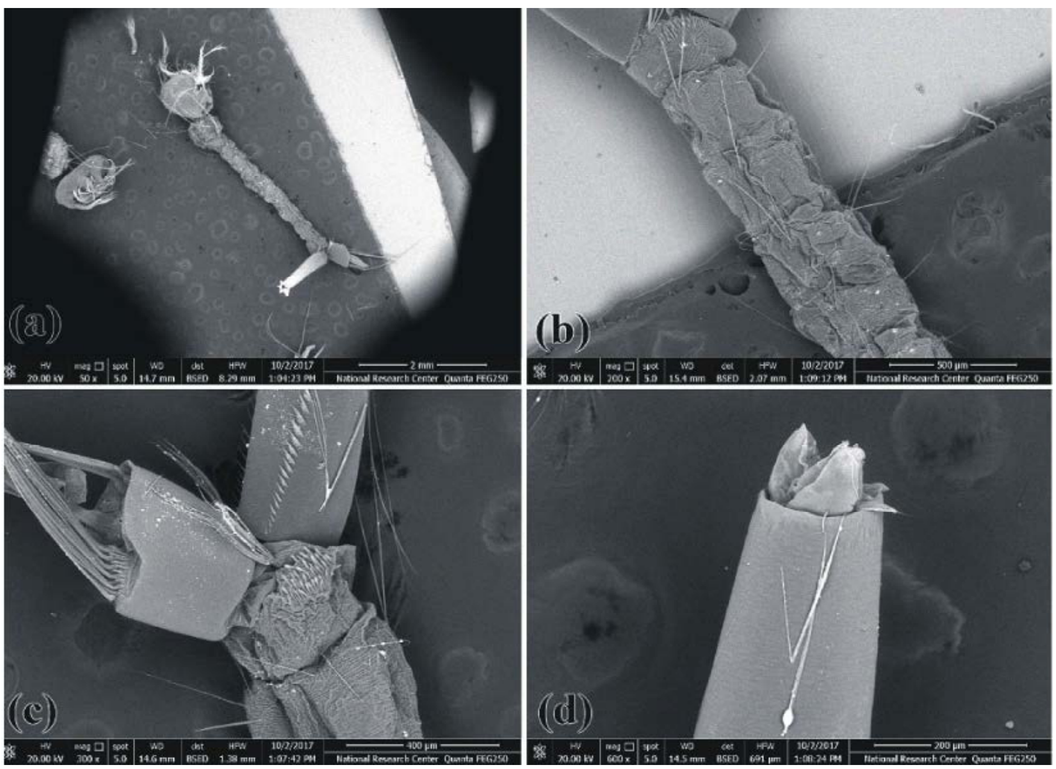

Fig. 2: Scanning electron micrographs of *C. pipiens* larva without treatment (control): (a) whole larvae 50X, (b) abdomen 200X, (c) terminal segment 300X, (d) siphon 600X.

Sub-lethal Concentration (LC_{50}): The LC_{50} values for *Z. officinale* and *C. rotundus* were estimated to compare their toxicity. LC_{50} values for *Z. officinale* and *C. rotundus* after 3, 6, 9 and 12 h were (286 & 267 ppm), (76 &151 ppm), (20 & 25 ppm) and (17 & 18 ppm), respectively. This finding indicates that the *Z. officinale* oil had higher toxicity than *C. rotundus* oil especially at 6 and 9 h, whereas, they were close to each other at 12 h (Fig. 1).

SEM Observations: The control larva that did not expose to any essential oil (Fig. 2). The larva appears healthy with smooth cuticle at the end segment and siphon cuticle. The abdominal segments of larva are well and no damage appears. The larva composed of 10 segments; one cephalic, one thoracic, eight abdominal and the last abdominal segment bears a dorsal siphon with an apical spiracular apparatus, which encompasses the spiracles

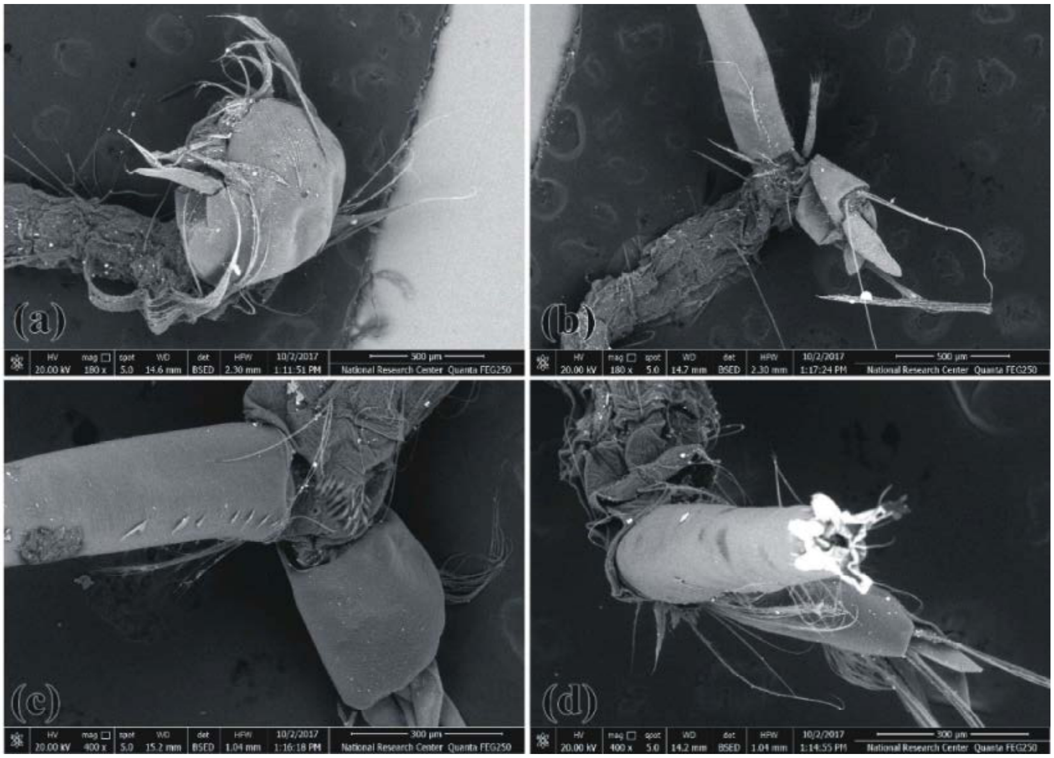

Fig. 3: Scanning electron micrographs of *C. pipiens* larva treated with 125 ppm of *Z. officinale* for 24 h (a) the head 180X, (b) whole body excepting the head 180X, (c) terminal segment 400X, (d) siphon 400X.

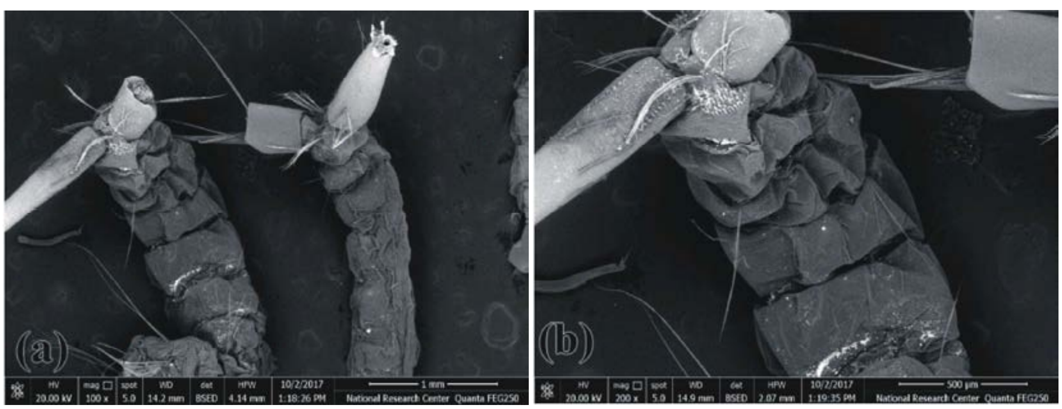

Fig. 4: Scanning electron micrographs of *C. pipiens* larva treated with 125 ppm of *C. rotundus* for 24 h (a) whole body excepting the head 100X, (b) terminal segment 200X.

(Fig. 2 a, b). The anterior end, comprising the cephalic region and the last segment (Fig 2 c) had uniformly comb shaped scales with one or two row of teeth. The siphon had arrow of ten pectin spines (Fig. 2 d). Fig. 3 shows the damages in larvae exposed to 125 ppm of *Z. officinale* oil for 24 h. A cuticular damage had occurred in the majority of examined specimens. The damages are shrinkage in the abdominal segments, cracking of head capsule, intrusion of the last segment, severe damages in siphon and the spiracular apparatus. At the anterior end after exposure to ginger oil the sensorial structures; antennae and papillae, were distorted (Fig. 3a) and was degenerated. The cuticle appeared deformed with wrinkled surface. The posterior end showed broken cuticle (Fig.3b-c). Fig. 4 shows the damages of larvae exposed to 125 ppm of *C. rotundus* oil for 2h h. The cuticular disruption was lower than that observed in larva treated by *Z.officinale* oil and both anterior and posterior ends were little distorted (Fig.4 a,b).

DISCUSION

Few previous studies evaluated *Zingiber officinale* essential oil against mosquito [37, 38]. The *Z. officinale* oil had ovicidal efficacy against *Anopheles stephensi, Aedes aegypti* and *Cx. quinquefasciatus* [33]. The oil exhibited larvicidal activity against *Cx. quinquefasciatus, Cx. tritaeniorhynchus* and *An. subpictus* with LC_{50} 50.78, 98.83 and 57.98 ppm, respectively [34, 35]. Also, it revealed larvicidal activity against *Aedes aegypti* and *Cx. quinquefasciatus* with LC_{50} 154 and 197 ppm, respectively [36]. In Egypt, only a unique study evaluated the essential oil of *C. esculentus* against fourth instar larvae of *Cx. pipiens* recording LC_{50} 47.17 ppm [37].

In the present study, the mortality percentage of *Cx. pipiens* larvae exposed to *Z. officinale* essential oil increased with the increase of concentration and exposure time. The significant mortality was recorded during the period from 12 to 24 h post treatment with all concentrations. The complete mortality was recorded during the period from 3-9 h with the concentrations of 250 ppm and above. However, the concentrations of 125 ppm and below caused 100% mortality during the period 12 - 24 h post treatment. LC_{50} of *Z. officinale* oil was 286, 76, 20 and 17 ppm after 3, 6, 9 and 12 h respectively. In the current study, the toxicity of *Z. officinale* essential oil on 3rd larvae of *Cx. pipiens* was higher than that recorded in previous studies. LC_{50} of *Ae. aegypti* and *An. stephensi* larvae treated with *Ocimum canum* oil was 301 and 234 ppm, respectively [38]. LC_{50} values were 60.0, 120.0, 44.0, 117.0 and 75.0 ppm against *Cx. pipiens* larvae treated with the essential oils of *Citrus sinensis*, *Eucalyptus* spp., *Ferula hermonis*, *Laurus nobilis* and *Pinus pinea*, respectively [39]. The essential oil of *Z. nimmonii* revealed larvicidal activity with LC_{50} = 41.19, 44.46 and 48.26 ppm against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*, respectively [40]. Also, the essential oil of Z. *officinale* exhibited larvicidal activity against different mosquitoes with LC_{50} range 50.78 - 197 ppm [34- 36]. This variation may attribute to the difference in strains of mosquitoes. Whereas, the Egyptian strain of *Cx. pipiens* larva was most susceptible to the essential oil of Z. *officinale* than other mosquito strains exposed to Z. *officinale* oil before. The larvicidal activity of Z. *officinale* oil may due to its main constituents, zingiberene, citral and camphene [37-43].

In the current study, the larvicidal activity of *C. rotundus* essential oil against *Cx. pipiens* is relatively lower than that of *Z. officinale* oil. The LC_{50} and scanning electron graphs of *C. rotundus* oil confirm this finding. The LC_{50} values of *C. rotundus* oil were higher (LC50= 151, 25 and 18 ppm after 6, 9 and 12 h, respectively) than that recorded with Z. *officinale* oil (76, 20 and 17 ppm after 6, 9 and 12 h, respectively). The main constituents which may have a role in the toxicity of *C. rotundus* oil on *Cx. pipiens* larvae were α-cyperone, cyperene and α-selinene [44, 45]. In Egypt, this study is considered the first that evaluated the larvicidal activity of *C. rotundus* essential oil on *Cx. pipiens*. There was a single study conducted in Egypt by Khater and Shalaby [37] who used the essential oil of another species of the plant genus *Cyperus* (*C. esculentus*) against 4th instar larvae of *Cx. pipens*. They found that the *C. esculentus* oil had significant toxicity against *Cx. pipiens* larvae with LC_{50} 47.17 ppm.

In the present study SEM observations could be used to determine the cuticular changes, as the cuticle of larvae was essential for protective and sensorial functions. SEM revealed cuticular damages in 3rd instar *Cx. pipiens* larvae which exposed to either *Z. officinale* oil or *C. rotundus* oil but the level of damage in case of *C. rotundus* treatment was lower. Similar cuticular changes were observed in fourth instar of *Cx. pipiens* larvae exposed to essential oil of *Allium cepa* that showed marked changes in abdominal segments [46]. Comparable distortion of the body cover in *Chrysomyia albiceps* larvae treated with spinosad; a metabolite of actinomycete *Saccharopolyspora spinosa*, ginger roots of *Z. officinale* and garlic fruits of *A. sativum* [47] as well as *C. megacephlala* treated with volatile oils of *Eucalytal eucalyptal* [48] and neem extract [49].

The essential oil of both *Z. officinale* and *C. rotundus* has larvicidal activity against the third larvae of *Cx. pipiens*. The toxicity of *C. rotundus* oil is relatively lower than that of *Z. officinale* oil. Further study is needed to evaluate the repellent properties of the two essential oils against the adult flies of *Cx. pipiens*.

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