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RESEARCH

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Secondary plant products against *Culex pipiens* (Linn.), with reference to some changes detected by scanning electron microscope

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Abstract

To find environment-friendly and low pest resistance natural mosquitocide, the bio-effects of smokes and oils obtained from leaves of *Eucalyptus globulus* and *Ocimum basilicum* against *Culex pipiens* (Linn.) were investigated. An influence of smokes and oils was tested on the loss ratio of eggs, larvae, pupae, and adult mortality of 1st and 2nd generations of *Culex pipiens*. Morphological changes of the 2nd generation, including antennae and mouthparts of larvae and adults, were examined by using the scanning electron microscopy (SEM). The smokes of *O. basilicum* and *E. globulus* reduced the ratio value of the insect, especially the eggs' number and adults significantly within 2 generations that lasted 82 days. The impact of *O. basilicum* smokes on the larval molting and mortality rate was 83.15 and 95.05%, respectively, compared to 29.85 and 82.43 % for *E. globulus*. The lethal effect of *O. basilicum* smoke on adults had a loss rate of 100% in both generations than 75.86% by *E. globulus*. Essential oils from *O. basilicum* and *E. globulus* showed a severe and rapid killing effect of *C. pipiens* within 8 days with a loss ratio of 99.47 and 81.38% of larvae from eggs, respectively. Scanning electron microscopy showed that both smokes could cause prominent malformation to the larval antennae, siphon, and adult mouthparts. In conclusion, either *E. globulus* or *O. basilicum* smokes in the long term or either of their oils in the short term could be used as natural repellent agents against *Culex pipiens*.

Keywords: *Culex pipiens*, Secondary metabolites, *Eucalyptus*, *Ocicum*, Essential oils, Scanning electron microscopy

Background

Plant natural products have been historically used in many parts of the world to repel and kill mosquitoes. Many plant species had been screened for their mosquito repellent and insecticide property (Shaalan et al., 2005; Ghosh et al., 2012; Ravindran et al., 2012). Repellent activities and their effects on the mosquitoes were reported for *Eucalyptus maculate* against *Anopheles gambiae* and *Anopheles funestus* (Trigg, 1996); *Ocicum selloi* against *Anopheles braziliensis* (Paula et al., 2003); neem plant extract against *Aedes*, *Culex*, *Anopheles* (Sharma et al., 1993; Dua and Sharma, 1995); and *O. mericanum* against *Anopheles dirus*, *Aedes aegypti*, and *Culex quinquefasciatus* (Tawatsin et al., 2001). The action of smoke from smoldering mosquito coils against *Aedes aegypti*, *Anopheles stephensi*, and *Culex pipiens fatigans* were studied by Chadwick (1975). Mosquito coil prepared from *Mesue ferra* leaf powder was investigated for its smoke effect against *Cx. quiquefasciatus* adults (Singha et al., 2011). They concluded that the smoke from *M. ferra* could play a crucial role in the pause of transmission of many diseases, whereas the mosquitoes act as vector at the individual level. Smoke toxicity test

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of *Ocimum canum* against *Cx. quinquefasciatus*, *Anopheles stephensi*, and *Ae. aegypti* was tested by Ramkumar et al. (2015) who found that smoke of the leaf powder could be used as an efficient toxicity agent against many species of mosquitoes.

Murugan et al. (2016) reported that *O. canum* leaf extract and powder have toxicity effects on *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephinsi* adults. Furthermore, Asiamah and Botchey (2019) stated that there were repellent and mosquitocidal properties of smoke from oil and the non-polar extracts of *O. viride* leaf against *Ae. aegyptii*.

Essential oils from plants play a major role in protecting plants from pests (Isman and Machial 2006; Bakkali et al., 2008). On the other hand, Sujatha et al. (1988) found that *Acorus calamus* extract induced malformations to *An. stephensi*, *Cx. quinquefasciatus*, *Ae. aegypti*, *Madhuca longifolia*, and *Cx. quinquefasciatus.*

The present study aimed to evaluate the effect of the smokes and oils from *E. globulus* and *O. basilicum* on morphological malformation and biological behavioral activities of the mosquito species, *Cx. pipiens*.

Materials and methods

Insects rearing

Culex pipiens egg batches were obtained in year 2019 from the laboratory of Zoology Department, Faculty of Science, South Valley University (SVU), Egypt. The insects were reared in glass cages (50 mm in diameter and 100 mm high) with a little bit of bread crumbs and covered with cloth. Every day, water and bread crumbs of all jars were removed in order to maintain a population of young larvae (1st, 2nd, 3rd, and 4th instars) ready to be used in the experiments. The treatments were kept under the laboratory conditions of 25 ± 5 °C, 25 ± 10% (RH), and a photoperiod of 16 h of light and 8 h of darkness.

Bioassay tests

Effect of Eucalyptus globulus and Ocimum basilicum smokes on Cx. pipiens

This experiment was conducted to determine the dose-dependent toxicity smokes of *Eucalyptus globulus* and *Ocimum basilicum* against different stages of *Cx. pipiens*. Exposing such stages to the tested smokes was conducted daily by burning 10 dried grams of the leaves from each plant. Smoking process was continued for about 15 min for each plant in 4 rooms (length 5.40 m, width 2.15 m, and height 3 m). After treatments, daily monitoring was carried out for 82 days (two generations of the insect) for each dose of smokes, to estimate the mortality rate of *Cx. pipiens* larvae and adults by picking and separating the dead individuals.

Effect of the essential oils of O. basilicum and E. globulus on Cx. pipiens

O. basilicum and *E. globulus* essential oils were purchased from the Captain Company accredited and registered to the Ministry of Health No. 2978/2002, Qena (Upper Egypt). For the treatments' application, the total volume used in each spray was 1 ml, applied on glass flasks (50 mm in diameter and 100 mm high) layered with a sterilized filter paper, to be sure that the treatments were retained, at 40 kPa. Three doses (6, 12, and 24 l/Petri dish) of *O. basilicum* and *E. globulus* were sprayed and 3 replicates were done. A treatment without essential oils was used as a control. After the application of treatments, daily monitoring, by separating and counting the dead larvae individuals, was carried out during the following 8 days after the application of each dose of essential oil.

Scanning electron microscope (SEM)

Chaudhary and Gupta (2004) protocol for SEM studies, was followed. Egg batches were collected from water drains by taking surface water included egg batches with a small bucket. The developed larvae out of the collected eggs were stored remaining in 70% ethanol, besides, some continued to be reared in the laboratory. The specific identification was made from the reared adult mosquitoes. For SEM studies, both larvae and adults were passed through the essential dry point and placed on SEM stubs with only a thin double-sided adhesive tape strip (Kirti and Kaur, 2011). Under the smokes of *E. globulus* and *O. basilicum*, larvae and adults of *Cx. pipiens* were scanned by JSM- 6100 SEM. The effects on the larval antennae and siphons and adult antennae and mouthparts were investigated.

Statistical analysis

Data were analyzed by SPSS program using one-way analysis of variance (ANOVA). Mean differences were compared using multiple comparison range test by Excel program for three replicates

Results and discussion

The effect of the smoke was continued for 2 generations (82 days), intervals of 15 min smoke every day. Extracted oils from *E. globulus* and *O. basilicum* had been investigated for their effect on the biological behavioral activities of *Cx. pipiens.* The data recorded about the eggs, alive larvae, larval molting, larval mortality, alive pupae, alive adults, and adult mortality of *Cx. pipiens* under the effect of *E. globulus* smoke and in control treatment conditions are presented in Table 1. It was found that in the 1st generation, the *Cx. pipiens* under normal conditions showed very low of loss rates in all stages, while in the 2nd generation, it was found only one adult had

Table 1 *Culex pipiens* individual numbers under the effect of smokes of *Eucalyptus globulus* and *Ocimum basilicum* leaves for two generations

Treatment	Generation	Eggs	Alive larvae	Larval molting	Larval mortality	Pupal no.	Alive adults	Adult mortality
Control	1st	7.05 (289.0)	7.09 (289 ± 9.61)	6.020 (247 ± 2.46)	0.51 (21 ± 0.44)	5.51 (226 ± 2.16)	5.51 (226 ± 2.46)	0.00
	Loss%		0.00%	14.53%	8.500%	000.00%	0.00%	0.00%
	2nd	6.80 (279.0)	6.44 (264 ± 10.41)	5.51 (226 ± 3.21)	0.46 (19.0 ± 0.75)	5.050 (207 ± 3.00)	5.05 (207 ± 3.21)	1.00 (41.0 ± 1.00)
	Loss%		11.83%	14.39%	8.41%	8.41%	0.00%	19.81
	Df	10.0	25.0	21.0	2.00	21.0	21.0	41.0
	1st/2nd	3.46%	8.65%	8.50%	8.46%	8.40%	8.40%	9.47%
E. globulus	1st	19.67 (810)	6.02 (726 ± 8.74)	3.27 (134 ± 1.21)	14.44 (592 ± 18.13)	3.27 (134 ± 1.21)	3.27 (134 ± 1.21)	2.24 (92 ± 4.00)
	Loss%		10.37%	81.54%	81.51%	77.36%	0.00%	68.66
	2nd	4.49 (184.0)	3.51 (144 ± 5.44**)	0.98 (40 ± 0.60**)	2.54 (104 ± 3.14**)	0.98 (40.0 ± 0.60)	0.98 (40.0 ± 0.60)	0.98 (40 ± 0.60**)
	Loss%		21.74%	72.23%	72.23%	72.23%	0.00%	100%
	Df	626.0	582.0	94.00	488.0	94.00	94.00	52.00
	1st/2nd	77.28%	80.17%	29.85%	82.43%	29.85%	29.85%	75.86%
O. basilicum	1st	11.66 (478)	11.39 (467 ± 15.27)	0.56 (23 ± 0.58)	10.83 (444 ± 11.0)	00.56 (23 ± 0.58)	00.56 (23 ± 0.58)	00.56 (23 ± 0.58)
	Loss%		2.31%	95.08%	95.08%	0.00%	0.00%	100%
	2nd	5.51 (22.06)	2.22 (91.0 ± 4.18**)	1.68 (69.0 ± 0.93)	0.54 (22.0 ± 0.60)	1.68 (69.0 ± 0.93*)	1.68 (69.0 ± 0.93*)	1.68 (69.0 ± 0.93)
	Loss%		59.73%	24.18%	31.88%	0.00%	0.00%	100%
	Df	252.0	376	46.0	422	46.0	46.0	46.0
	1st/2nd	52.72%	80.51%	83.15%	95.05%	0.00%	0.00%	100%

Df, difference in the numbers between each stage of two generations; number of individuals of each stage between brackets ± SD of three replicates
*p value significant (< 0.05)
**p value significant (< 0.01)

been lost represented (9.47%). It showed also the lowest loss rate (3.46%) in eggs, and it ranged between 8.40 and 8.65% in alive larvae, larval molting, larval mortality, alive pupae, and alive adults.

Effect of *E. globulus* and *O. bacilicum* smokes on *Cx. pipiens*

As shown in Table 1, the effect of *E. globulus* smoke on the first generation of *Cx. pipiens* exhibited loss rates of 81.5, 81.51, and 77.36% for larval molting, larval mortality, and pupal individual number, respectively. No loss was recorded in the alive adults whereas mortality of the adults showed loss rates of 68.66% under *E. globulus* smoke. On the second generation, the impact of *E. globulus* smoke had a loss ratio on the larval molting, larval mortality, and pupal individual number by 72.23% and for adult mortality by 100%. In contrast, alive adults of *Cx. pipiens* from pupae showed no loss at all. The effect of *E. globulus* smoke on all stages of *Cx. pipiens* upon the two generations is shown in Table 1. *E. globulus* smoke severely affected all stages of *Cx. pipiens*, whereas the highest mortality rate was found in larva by 82.43%. *E. globulus* smoke equally affected larval molting, alive adults, and alive pupa by 29.85%. The loss rate in the eggs, alive larvae, and adult *Cx. pipiens* were found to be in the range between 77.28 and 80.17%. *O. basilicum* smoke affected the first generation of *Cx. pipiens* by a loss rate of 95.08% for larval molting and mortality and 100% for adult mortality. In contrast, no changes were observed in the hatched pupal and alive adults under *O. basilicum* smoke. The second generation of *Cx. pipiens* had been affected by 59.73% for alive larvae, by 24.18% for larval molting, and by 31.88% for larval mortality, whereas the adults had a loss ratio of 100%. Upon the two generations, it showed a sever effect on eggs, alive larvae, larval mortality, molting, and adult mortality whereas the highest mortality rate found to be in larva and adults by 95.05 and 100%, respectively. In contrast, *O. basilicum* smoke did not show any effect on the alive pupal stage. The differences between 2nd and 1st generations of hatched eggs under no smokes were found to be 3.46% less than those subjected to the smokes of *E. globulus* (77.28%) and *O. basilicum* (52.72) (Figs. 1 and 2; Table 1) which proved the toxic effect of both smokes

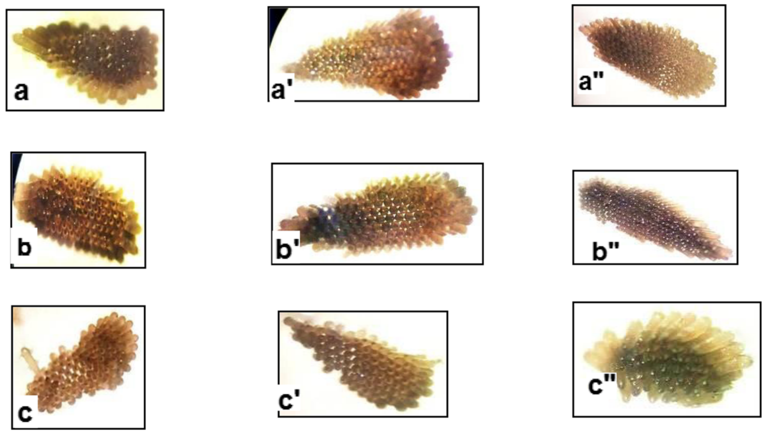

Fig. 1 *Culex pipiens* egg numbers under no smoke (**a**, **b**, and **c**), smokes of *Ocimum basilicum* (**a'**, **b'**, and **c'**), and smokes of *Eucalyptus globulus* (**a"**, **b"**, and **c"**) of the first culicid generation using light microscopy

generated from *E. globulus* and *O. basilicum*. The differences between *E. globulus* smoke and *O. basilicum* smoke are probably due to the oily layer from the smoke of *E. globulus* covering the container surface.

These findings are in agreement with the other researches that showed that the smoke possesses toxicity to various pests. As plant products contain multiple chemical constituents, smoke burning from it also contains some of those active principles. Several plant smokes showed a toxic effect against mosquitoes. For example, smoke from *Albizza amara* reported to have a more toxic effect on *Ae. aegypti* than *O. bascilicum* smoke (Murugan et al., 2007). It was reported that smokes of *Ficus krishnae* had a toxic efficacy against *Anopheles stephensi* Liston and *Cx. vishnui* group of mosquitoes (Haldar et al., 2014). The smoke obtained from various solvent extracts from *Lantana camara* leaves showed different levels of repellence against female anopheles mosquitos (Akumu et al., 2014). Volatiles found in the smoke resulted either from burning or from fresh leaves of *Corymbia citriodora* and *Ocimum suave* and showed remarkable repellent activities against host-seeking *Ae. aegypti* and *An. arabiensis* mosquitoes (Dube et al., 2011).

Obtained results showed that the complete mortality of adults of *Cx. pipiens* was observed by *E. globulus* smoke treatment under room conditions during the 2nd generation. Moreover, the current results showed the

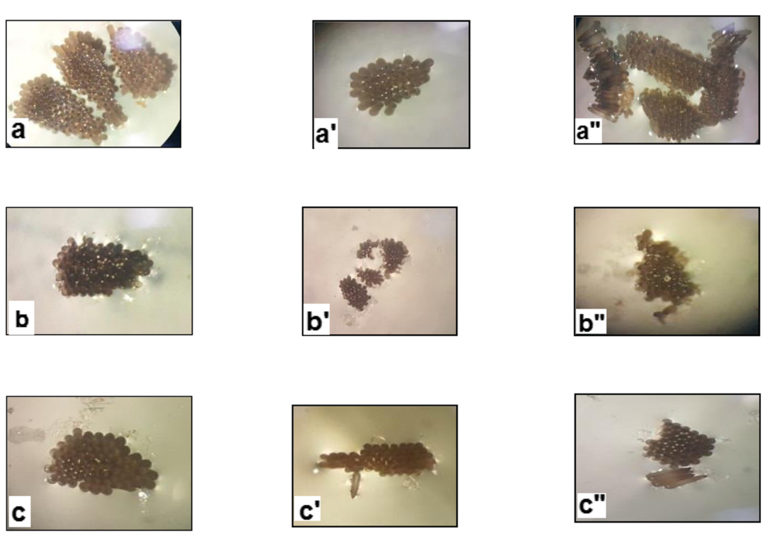

Fig. 2 *Culex pipiens* eggs numbers under no smoke (**a**, **b**, and **c**), smokes of *Ocimum basilicum* (**a'**, **b'**, **c'**, and **d'**), and smokes of *Eucalyptus globulus* (**a"**, **b"**, and **c"**) of the second culicid generation using light microscopy

Table 2 *Culex pipiens* individual numbers under the effect of oils of *Eucalyptus globulus* and *Ocimum basilicum* for eight days

Treatment	Generation	Eggs	Alive larvae	Larval molting	Larval mortality
Control	1st	32.63 (261)	32.63 (200 ± 70.71)	25 (200 ± 2.46*)	7.63 (61 ± 2.46*)
	Loss%		2.338%		
E. globulus	1st	19.67 (190)	0.125 (1 ± 0.92)	0.125 (1.00 ± 1.21**)	14.44 (189 ± 1.77*)
	Loss%		99.47%		
O. basilicum	1st	13.88 (111)	18.92 (21.0 ± 5.04)	18.92 (21.0 ± 0.58*)	81.08 (21.0 ± 4.59)
	Loss%		81.38%		

Number of individuals of each stage between brackets ± SD of three replicates
**p* value significant (< 0.05)
***p* value significant (< 0.01)

severe mortality appeared only after 82 days of continuous smoking. Thus, it could be interpreted that we smoked the *Cx. pipiens* in few minutes and other researchers smoked very heavily in closed areas. Furthermore, the present results found that the toxicity efficacy between smokes of *E. globulus* and *O. basilicum* either for the 1st or 2nd generations varied greatly, as for example that there was an increase in the loss rate of larval molting and larval mortality under *O. basilicum* smoke than that of *E. globules*. However, the loss rate in the number of egg production and adult mortality caused by *E. globulus* was higher than that caused by smoke of *O. basilicum* and could be due to the nature of the chemicals present in each plant (Brooker and Kleinig, 2006). 1, 8-Cineole has been reported to be the most important chemical in the genus of *Eucalyptus* and is largely attributed to various pesticide characteristics (Duke, 2004). On the other hand, *O. basilicum* essential oil was mainly composed of aromatic ether estragol (74.0%) and the terpene with alcohol group linalool (17.8%), which is known to be a repellent and had toxic activities against insect species (Regnault-Roger et al., 1993).

The current results could be due to the layer thickness of oils covering the water surface. Meanwhile, these larvae breathe by their syphon of the terminal body end. Moreover, the number of released larvae of *E. globulus* was fewer than that was treated by *O. basilicum*, and this may be due to the thickness of the *E. globulus* oil that was more than that of the *O. basilicum* oil.

Effect of the essential oils on *Cx. pipiens*

According to Table 2, the results showed an acute effect on hatched eggs for those treated by oils of *E. globulus* and *O. bacilicum* than the control across 8 days. In the case of control, the loss ratio of larvae resulted from hatched egg was 2.338% and those treated by oils of *O. basilicum* and *E. globulus* were 81.38% and 99.47%, respectively. Again, this support that both smokes from

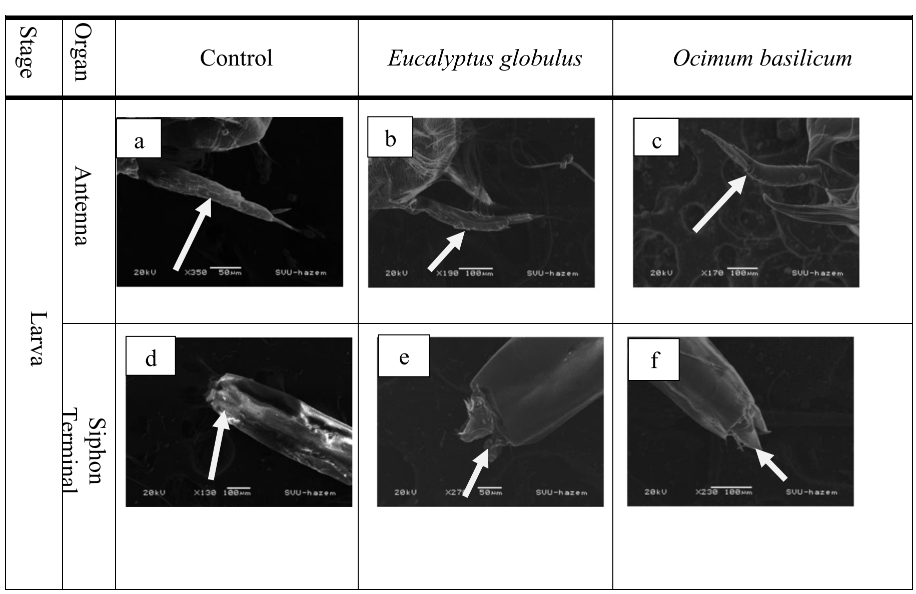

Fig. 3 Scanning electron microscopic (SEM) of the larval antennae and siphons of *Culex pipiens* under no smoke, smoke of *Eucalyptus globulus*, and smoke of *Ocimum basilicum*

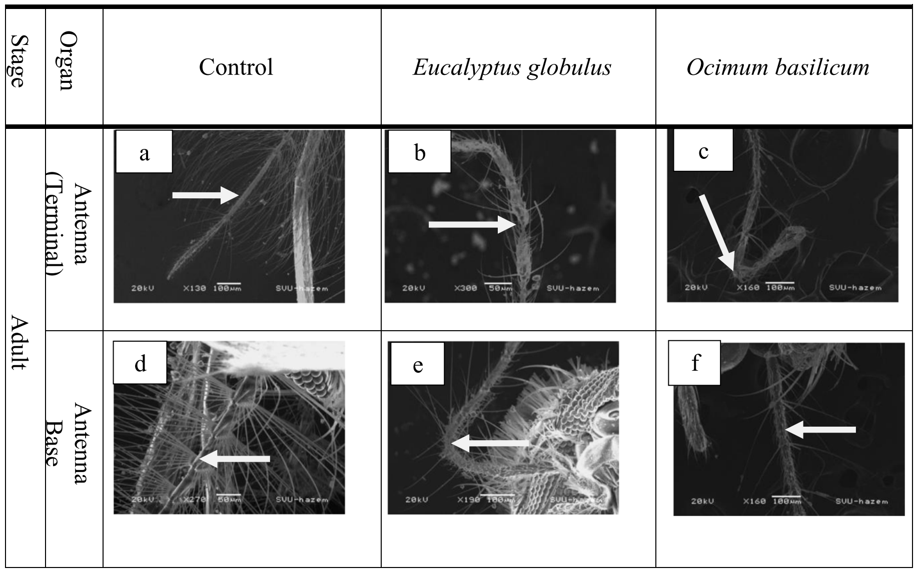

Fig. 4 Scanning electron microscopic (SEM) of adult antennae and mouth parts of *Culex pipiens* under no smoke and smoke of *Eucalyptus globulus* and smoke of *Ocimum basilicum*

leaves of *E. globulus* and *O. bacilicum* had a toxic effect probably due to specific toxic chemicals against *Cx. pipiens.* It was found that *E. globulus* oil possesses a wide spectrum of biological activity including anti-insecticidal repellent (Batish et al., 2008). Previous researches showed that oils of many plants had various activities against eggs hatching, adults, developmental traits, and larva/pupa susceptibilities (Regnault-Roger and Hamraoui, 1994; Papachristos and Stamopoulos, 2002).

SEM observations

SEM of the larval antennae, siphons, adult antennae, and mouth parts of *Cx. pipiens* subjected to the *E. globulus* and *O. basilicum* smokes were found to be morphologically abnormal compared to control (Figs. 3 and 4). It was evident that the bending of *Cx. pipiens* under *E. globulus* smoke (Fig. 3b) was more than that *O. basilicum* smoke (Fig. 3c). Also, the number of sensory hairs on the larval antennae of non-exposed *Cx. pipiens* to smoke was more than those exposed to *E. globulus* and *O. basilicum* smokes. The variation in the number of sensory hairs is probably due to the oily layer formed from *E. globulus* and *O. basilicum* smokes and why larvae underwent abnormal growth with little bed sensory hairs. As shown in Fig. 3d, there are no active processes in the terminal ends of the siphon of the control sample, while complete active processes in the siphons were observed under smokes of *E. globulus* and *O. basilicum* (Fig. 3e, f). Also, it was observed that there was abnormal growth of the antenna as there was a cut in some segments under *E. globulus* smoke (Fig. 4b) and prominent curvature under the effect of both smokes of *O. basilicum* and *E. globulus* (Fig. 4e). A curvature in the antennae may be a result from an increasing surface area provided with more sensory hairs and plates to have a benefit from any amount of oxygen that is contaminated by smokes. These results were confirmed by Sujatha et al. (1988) who estimated the effect of *Acorus calamus* extract on *Cx. quinquefasciatus* and to *Cx. quinquefasciatus* and found morphological malformations to the *Culex* spp.

Conclusion

Smokes and oils of *E. globulus* and *O. basilicum* leaves affected the life cycles of *Cx. pipiens* with varying degrees of mortality rate. Therefore, both smokes and oils of *E. globulus* and *O. basilicum* can be applied as natural products against mosquitoes.

Abbreviations

ANOVA: Analysis of variance; Eos: Essential oils; PM: Post meridiem; RH: Relative humidity; SEM: Scanning electron microscope; SPSS: Statistical Package for the Social Sciences

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Authors' contributions

KO and MM performed the idea of this article, achieved the laboratory performance, and wrote the manuscript. AA participated in writing the manuscript and mathematical analysis. The authors read and approved the final manuscript.

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Availability of data and materials
All data of the study have been presented in the manuscript, and high quality and grade materials were used in this study.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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