Insecticidal, Oxidative, and Genotoxic Activities of *Syzygium aromaticum* and *Eucalyptus globulus* on *Culex pipiens* Adults and Larvae

Karanfil (*Syzygium aromaticum*) ve Okaliptusun (*Eucalyptus globulus*) *Culex pipiens* Yetişkinleri ve Larvaları Üzerindeki insektisidal, Oksidatif ve Genotoksik Aktiviteleri

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ABSTRACT

Objective: The wide-reaching *Culex pipiens* has long been a public apprehension. Combating serious vector-borne diseases requires the use of insecticides effective against both humans and the ecosystem. The wide variation of botanicals that nature has to offer tempts researchers to study their interactions with the insects. Environment-friendly insecticides light up hope for maintaining ecological balance and pollution mitigation. This study aimed at evaluating the insecticidal, oxidative, and genotoxic activities of eucalyptus and clove oils on *C. pipiens* adults and larvae.
Methods: The chemical composition of essential oils was determined via gas chromatography/mass spectrometry. The bioassay was performed, with eucalyptus oil showing the highest toxicity index (LC_{50} of 0.108% after 24 h in adults and LC_{50} of 0.014% after 48 h in larvae).
Results: Fumigation effects showed *Eucalyptus* to have higher toxicity than clove oil, with an LC_{50} of 0.108% and 0.014% after 24 h and 48 h, respectively, in adults and larvae. The effect of tested oils on the activities of glutathione peroxidase, catalase, and superoxide dismutase varied with increasing oil concentrations. The genotoxic effects of the tested oils were dose-dependent, with an increase of all comet parameters compared with those in the control.
Conclusion: The tested oils showed encouraging potentiality as green insecticides in combating *C. pipiens*.
Keywords: *Culex pipiens*, eucalyptus oil, clove oil, GPx, Catalase, SOD, comet assay
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ÖZ

Amaç: Geniş alana yayılan *Culex pipiens* uzun süredir bir toplum sorunudur. Vektör aracılığıyla bulaşan ciddi hastalıklarla mücadelede, insanlara ve ekosisteme karşı etkili insektisitlerin kullanımı gerekmektedir. Doğanın sunduğu geniş botanik çeşitlilik, araştırmacıları böceklerle etkileşimlerini çalışmaya teşvik etmektedir. Çevreyle dost insektisitler ekolojik dengenin sürdürülmesi ve kirliliğin azaltılması için umut vaat etmektedirler. Bu çalışmada okaliptus ve karanfil yağlarının *C. Pipiens* yetişkinleri ve larvaları üzerindeki insektisidal, oksidatif ve genotoksik etkilerinin değerlendirilmesi amaçlandı.
Yöntemler: Esansiyel yağların kimyasal kompozisyonu gaz kromatografisi/kitle spektrometresi ile belirlendi. En yüksek toksisite indeksini gösteren okaliptüs yağı ile biyoanaliz yapıldı (yetişkinlerde 24 saatten sonra %0,108 LC_{50}, ve larvalarda 48 saat sonrasında %0,014 LC_{50}).
Bulgular: Fümigasyon etkileri okaliptüsün karanfil yağına kıyasla daha yüksek toksisitesinin olduğunu gösterdi (yetişkin ve larvalarda sırasıyla 24 ve 48 saat sonrasında, %0,108 ve %0,014 LC_{50}). Test edilen yağların glutatyon peroksidaz, katalaz ve süperoksit dismutaz aktiviteleri üzerindeki etkisi artan yağ konsantrasyonlarıyla birlikte değişiklik gösterdi. Kontrollerle kıyaslandığında, tüm komet parametrelerindeki bir artışla, test edilen yağların genotoksik etkileri doza bağımlı bulundu.
Sonuç: Test edilen yağlar *C. pipiens* ile mücadelede yeşil insektisitler olarak umut verici bir potansiyele sahiptir.
Anahtar Kelimeler: *Culex pipiens*, okaliptüs yağı, karanfil yağı, GPx, Katalaz, SOD, komet analizi
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INTRODUCTION

Culex pipiens mosquito is an eminent vector for several diseases of public health apprehension (1). With rush in using chemical insecticides, several serious health and environmental issues evolved, along with an increase in the resistance to insecticides. Resistance was reported against different modes of insecticidal action, adding more to the control bill (2-4).

Consequently, thought-provoking plant-based products became inspirational to researchers to study the potential insecticidal actions of these products. Among numerous plant species, the aromatics urged the attention by their characteristic odor and flavor (5). Overtime, essential oils were extracted and used for several medicinal and spiritual purposes (6). They also exhibited insecticidal properties (7).

Mediterranean people has long ago been familiar with both tested plants; as the *Eucalyptus* species were brought to Anatolia in 1885, and since then, they have become one of the main forest trees in Turkey (8). Later on, clove being introduced to the Mediterranean part of Turkey (9). Studies highlighting the role of the tested oils were conducted (10) with several applications in industry (11), in conjunction with medicine, giving special focus on their dental phytotherapeutic roles (12). The repellency and larvicidal activity of clove oil against *C. pipiens* were reported by Chaieb et al. (13); Radwan et al. (14); Kang et al. (15) and those of eucalyptus oil were reported by Choi et al. (16); Traboulsi et al. (17); Elbanna (18); Erler et al. (19); Kang et al. (15). However, according to our knowledge, there are no reports on the role of these oils in fumigation toxicity against *C. pipiens* adults. Seeking a replacement of chemicals, the present study aimed to meet this need using environmentally benign essential oils for controlling *C. pipiens*.

Oxidative stress occurs in response to several stress effectors, among which is insecticidal exposure (20). The ability of the insect to detoxify reactive oxygen species (ROS) and maintain the normal physiological state is well regulated by detoxifying enzymes such as superoxide dismutase (SOD) by converting superoxide anions to hydrogen peroxide (21). Also, catalase and glutathione peroxidases (GPx) cause the detoxification of hydrogen peroxide to water molecule (22). With no previous records on the antioxidant status in *C. pipiens* on applying the tested oils, measuring three antioxidant enzymes was done.

The comet assay, also known as single-cell gel electrophoresis, is a simple method for measuring the DNA strand breaks. This assay is used for the estimation of genotoxin exposure and Post hazardous exposure effects (23). A comet shape is generated by the increased migration of damaged DNA fragments from the nucleus (24). This study aimed at evaluating the insecticidal activities of two members of the *Myrtaceae* family, namely clove oil *(Syzygium aromaticum, (L.) Merr and Perry)* and eucalyptus oil (*Eucalyptus globulus, Labill)* on *C. pipiens* adults and larvae by monitoring the biochemical and genotoxic effects.

METHODS

This study was approved by the ethical committee of Faculty of medicine Ain Shams University.

Isolation of Clove and Eucalyptus Oils

Essential oils of clove buds and eucalyptus leaves were purchased from the local market and identified by members of the Botany Department, Faculty of Science, Ain Shams University. The oils were extracted by steam distillation at Unit of Squeezing and extraction of natural oils at The National Research Center, Dokki, Giza. The oils were extracted from *E. globulus* leaves and from *S. aromaticum* buds using the Clevenger apparatus as described by Gunther (25).

E. globulus leaves and from *S. aromaticum* buds were air dried in the shade, and 25 g of dried leaves or flowers were separately mixed with 500 mL of water in a 1-l flask and subjected for hydrodistillation for 3 h. The resulting volatile oils were dried over anhydrous sodium sulfate and stored in dark bottles in the refrigerator (4°C) until used (26, 27).

Gas Chromatography/Mass Spectrometry (GS-MS) of Essential Oils

The GC-MS analysis was performed by injecting 1 µL of the clove and *Eucalyptus* essential oils using a Shimadzu 2010 Plus GC-MS (Germany) equipped with a Quadrupole (QP-5050) detector at the Experiments and Advanced Research Unit, Faculty of Pharmacy, Ain Shams University. Using CP-Wax 52 CB capillary column, the injector, detector, and oven temperature were 240°C, 250°C, and 60°C 220°C. The flow speed was 10 pounds per square inch (psi). 70 electron volt (eV) as ionization detector. With helium as the carrier gas.

The constituents were identified by comparing with the retention times of standard substances with data from the WILEY, NIST, and TUTOR libraries (28).

Mosquito Rearing

The laboratory reared strain of *C. pipiens* mosquitoes was obtained from the Research Institute of Medical Entomology, Mosquitoes Research Department, Dokki, Giza. The larvae were reared in plastic cups measuring 30 × 15 cm, containing dechlorinated water. Under the standard conditions of 25°C±2°C and 70%±5% R.H and 12-h light:dark cycle. Larvae were daily fed on dried bread and dried yeast with a ratio of 1:1. Adults were reared in 0.5 × 0.5 × 0.5-m cages and maintained on a 10% sugar solution. Females were allowed to feed on guinea pigs for 2-3 h every 2 days to obtain protein needed for egg production (29).

Biological Assays

Regarding adults, bioassay was performed using the fumigation method according to Palacios et al. (30) and Rossi and Palacios (31) in a fumigation chamber jar (5 × 5 × 3.5 cm), with exposure for 1 h using replicates of 350 mosquitoes in each replicate. Serial dilutions of the tested oils were prepared using absolute ethanol (El Gomhoureya). Several trials were conducted to determine the concentrations to be tested in this study. Using different concentrations (0.05%, 0.1%, 0.5%, 1%, 2%, and 3%) of each tested oil, mortality was recorded up to 1 h, 6 h, and 24 h after treatment according to Rossi and Palacios (31). Controls were set up using 95% ethanol only.

The larvicidal bioassay was performed on third instar larvae by applying different concentrations of each oil as follows: 0.005%, 0.01%, 0.5%, 1%, and 2%. Mortality was recorded up to 48 h after

treatment (32). Three controls were prepared using 95% ethanol. LC_{50} and LC_{90}, with their 95% confidence limits, were determined using probit analysis.

Biochemical Assay

The *C. pipiens* third instar larvae and adults were exposed to the following concentrations of each tested substance: 0.05% and 2%. Exposure time for larvae was 24 h, whereas that for adults was 1 h. The exposed insects were then subjected to weighing and counting and were mechanically homogenized in special buffers using a Dounce tissue grinder (33). The homogenate was centrifuged at 4000 rpm for 15 min at 4°C to obtain the supernatant to be used for catalase, GPx, and SOD enzymes assay. Double beam ultraviolet/visible spectrophotometer (Sectronic 1201, Milton Roy Co., USA) was used according to the kit in use (Biodiagnostic, Egypt); based on the catalase reaction to hydrogen peroxide - a chromophore forms that is inversely proportionate to the amount of tested enzyme- also the decrease in NADPH absorbance during its oxidation catalyzed by GPx and finally the SOD inhibitory effect on phenazine methosulphate reduction of nitro blue tetrazolium dye. All done at Biochemistry Unit, Faculty of Medicine, Ain Shams University.

Table 1. Chemical constituents of clove essential oil and their percentages

Peak	Retention time	Name	%
1	3.02	Hexane, 2,4-dimethyl-	0.12
2	3.071	Cyclopentane, 1,2,3-trimethyl-	0.31
3	3.187	b-Caryophyllene	3.24
4	3.267	Propylene glycol	0.19
5	3.454	Octacosyl trifluoroacetate	0.10
6	3.543	Decane, 3,3,4-trimethyl-	0.86
7	3.627	Eugenolacetate	3.40
8	3.687	Heptane, 2-methyl-	0.36
9	3.804	Heptane, 3-methyl-	0.72
10	3.964	Cyclohexane, 1,1-dimethyl-	0.09
11	4.066	Octane	0.08
12	4.211	a-Caryophyllene	0.75
13	4.353	Octacosyl trifluoroacetate	0.08
14	4.845	Cyclohexane, ethyl	0.09
15	4.998	Toluene	0.12
16	5.071	Cyclohexane, 1,4-dimethyl	0.03
17	5.434	Cyclohexane	0.07
18	5.524	Butanoic acid	0.22
19	5.731	Ethylbenzene	0.09
20	5.938	Benzene, 1,3-dimethyl-	0.26
21	6.393	3,5-Dimethyl-3-heptene	0.04
22	6.586	o-Xylene	0.03
23	6.73	Nonane	0.10
24	9.229	Cyclohexane, 1,3,5-trimethyl-	0.04
25	9.759	Caryophyllene oxide	0.24
26	11.498	Decane	0.05
27	12.903	Undecane	0.16
28	15.998	Dodecane	0.07
29	17.788	Phenol, 4-(2-propenyl)-	0.06
30	20.973	Eugenol	88.08
31	21.09	Phenol, 2-methoxy-4-propyl-	0.10
32	22.003	Methyleugenol	0.04

Genotoxicity Testing by Comet Assay

DNA damage studies were conducted using the comet assay at the Immunobiology and Immunopharmacology Unit, Animal Reproduction Research Institute, Giza. Applying the protocol described by Singh et al. (34). The aforementioned homogenate was centrifuged at 1000 rpm for 10 min, and the pellet was gently suspended in 1 mL of homogenizing buffer for cell isolation. Preparation and visualization of comet slides were done in accordance with Dua et al. (35). Applying layers of low-melting point agarose with the cells tested in between was done, and slides were then covered and placed at 4°C to solidify, after which cell lysis and DNA unwinding occurred, followed by horizontal gel electrophoresis. The slides stained by ethidium bromide were then observed and evaluated under the Axio fluorescence microscope (CD75V1A Zeiss, Germany) at 400× magnification with a digital color camera. Damaged cells were visualized by the comet appearance with a brightly fluorescent head and a tail to one side formed by the DNA containing strand breaks that were drawn away during electrophoresis.

Statistical Analysis

IBM Statistical Packages for the Social Sciences statistics (V. 23.0, IBM SPSS Corp.; Armonk, NY, USA) was used for data analysis. Probit analysis was used to calculate LC_{50} and LC_{90} related to the log of different concentrations versus response. Chi-square goodness of fitness test was conducted to study the suitability of the tested models. The toxicity index was calculated according to Rawi et al. (36). Results of enzymes activities and genotoxicity were expressed as mean ± SE. Data were analyzed using Student's t-test, and $p>0.05$ was considered as non-significant and $p<0.05$ as significant.

RESULTS

Insecticidal properties were evaluated using different concentrations of clove and eucalyptus oils on *C. pipiens* adults and larvae. Results of chemical compositions of essential oils by GC-MS are shown in Tables 1 and 2 and Figures 1 and 2. The major constituents of clove oil were eugenol (88.08%), eugenol acetate (3.40%), and b-caryophyllene (3.24%) and those of eucalyptus oil were 1,8-eucalyptol (46.76%), D-limonene (9.61%), and o-Cymene (6.49%). The results of *C. pipiens* adulticidal activity of tested oils using the fumigation method after 1 h, 6 h, and 24 h are shown in Table 3, and those of *C. pipiens* larvicidal activity of tested oils after 24 h and 48 h are shown in Table 4. The dose-response relationship of the tested oils showed an increase in the mortality rate with an increase in the tested oil concentrations, with no significant difference ($p>0.05$) between the observed values of mortality and the predicted ones. The residual indicates the appropriateness of the used models. All results of the present study showed signifi-

Table 2. Chemical constituents of eucalyptus essential oil and their percentages

Peak	Retention time	Name	%
1	3.021	Hexane, 2,4-dimethyl	0.31
2	3.072	Cyclopentane, ethyl-	0.75
3	3.147	Cyclopentane, 1,2,4-trimethyl	0.58
4	3.269	Epiglobulol	0.44
5	3.455	2-Methylene-5-(1-methylethenyl) cyclohexanol	0.26
6	3.544	Geranyl acetate	1.70
7	3.627	Toluene	8.61
8	3.688	Heptane, 3-methyl-	0.88
9	3.804	α-Terpineol acetate	1.77
10	4.212	Octane	1.83
11	4.999	α-Eudesmol	0.26
12	5.941	trans-Carveol	0.61
13	6.734	α-Pinene	0.25
14	7.757	Fenchene	6.25
15	8.151	β-Pinene	0.47
16	8.203	Camphene	2.11
17	9.524	β-Sabinene	0.65
18	9.762	L-Pinocarveo	0.75
19	10.283	cis-β-ocimene	5.43
20	10.591	o-Cymene	6.49
21	10.728	D-Limonene	9.61
22	10.821	1,8-Eucalyptol	46.76
23	11.665	γ-Terpinene	0.45
24	12.603	Linalool	1.67
25	12.905	Terpinen-4-ol	0.37
26	15.684	α-Terpineol	0.74

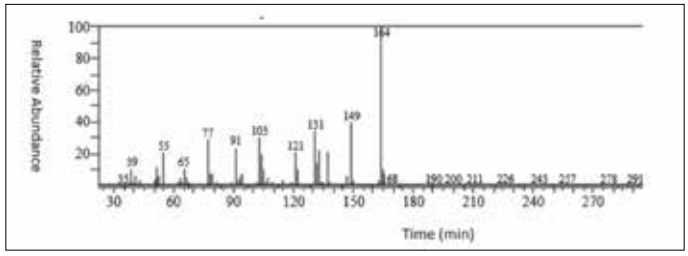

Figure 1. Mass spectrum of the essential oil isolated from clove oil

cant linear predictions ($p<0.05$) from the resultant probit models, as shown by the Z-scores. For *C. pipiens* adults, the fumigation method was performed and the results were recorded after 1 h, 6 h, and 24 h. Eucalyptus oil had a higher toxicity (LC_{50}: 0.108%) than clove oil (LC_{50}: 0.374%) after 24 h. For *C. pipiens* larvae, the biological assay was performed, and the results recorded after 24 h and 48 h showed that eucalyptus oil had a higher toxicity (LC_{50}: 0.014%) than clove oil (LC_{50}: 0.036%) after 48 h.

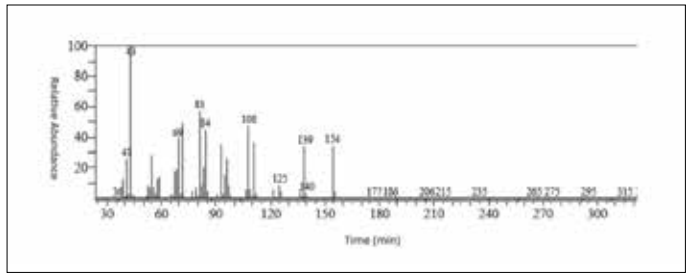

Figure 2. Mass spectrum of the essential oil isolated from eucalyptus oil

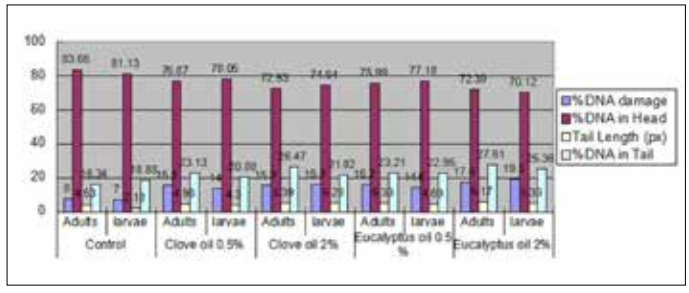

Figure 3. Values of DNA damage detected using the comet assay after exposure to tested oils in *Culex pipiens* adult after 1 h and in larvae after 24 h

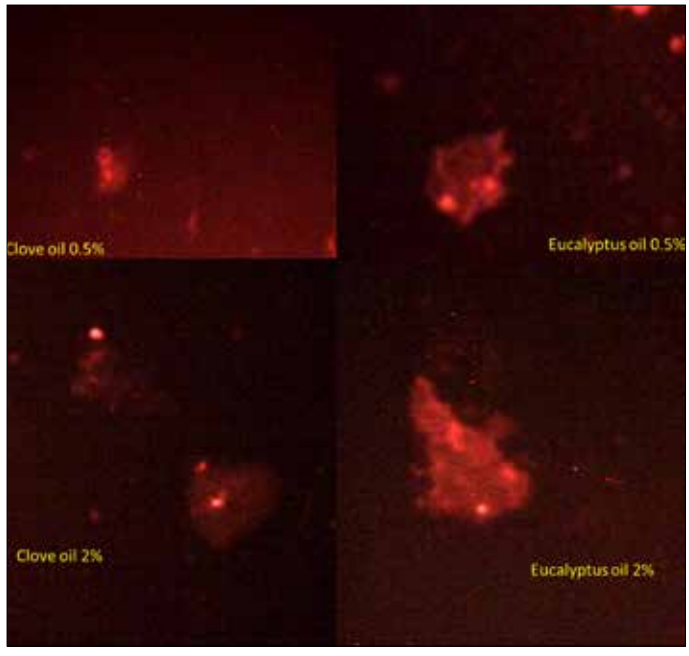

Figure 4. DNA comet assay on *Culex pipiens* adults after exposure to tested oils

Results of the effects of the tested oils on the activity of concerned antioxidant enzymes in *C. pipiens* adults and larvae are shown in Table 5. Catalase activities were significantly increased to approximately two folds compared with those in controls in low concentrations of the tested oils. However, there were no significant differences in high concentrations in both stages. The increase observed could be related to the detoxification role of free radicals generated by the tested oils. Regarding the activities of GPx enzymes, they were significantly increased com-

Table 3. *Culex pipiens* adulticidal activity of tested oils using the fumigation method after 1 h, 6 h, and 24 h

Duration	Tested substance	Response	Concentration (%)						LC50 95% confidence limit	LC90 95% confidence limit	Toxicity index	Z-score*	
			0.05	0.1	0.5	1	2	3				Slope	Intercept
1 h	Eucalyptus oil	Observed	2	3	6	8	10	12	1.837	29.315	100	0.84	–0.222
		Expected	1.89	2.885	6.351	8.245	10.25	11.42	0.925–6.766	9.524–623.219			
		Residual	0.11	0.115	–0.351	–0.245	–0.247	0.581					
	Clove oil	Observed	1	2	5	6	9	12	2.357	45.583	77.94	0.996	–0.371
		Expected	0.96	1.716	5.024	7.107	9.434	10.83	1.261–7.83	11.663–1548.576			
		Residual	0.05	0.284	–0.024	–1.107	–0.434	1.169					
6 h	Eucalyptus oil	Observed	4	8	13	16	17	18	0.214	2.88	100	1.136	0.76
		Expected	4.73	7.071	13.241	15.527	17.29	18.07	0.107–0.359	1.45–10.058			
		Residual	–0.73	0.929	–0.241	0.473	–0.294	–0.07					
	Clove oil	Observed	2	3	6	12	16	18	0.607	4.798	35.25	1.427	0.309
		Expected	1.22	2.636	9.043	12.43	15.4	16.78	0.39–0.942	2.588–14.007			
		Residual	0.78	0.364	–3.043	–0.43	0.598	1.219					
24 h	Eucalyptus oil	Observed	6	10	17	18	19	20	0.108	0.82	100	1.454	1.407
		Expected	6.28	9.623	16.675	18.405	19.35	19.64	0.005–0.173	0.485–1.921			
		Residual	–0.28	0.377	0.325	–0.405	–0.349	0.357					
	Clove oil	Observed	3	4	8	15	18	19	0.374	2.706	28.87	1.491	0.637
		Expected	1.93	3.933	11.494	14.759	17.23	18.23	0.236–0.565	1.569–6.569			
		Residual	1.07	0.067	–3.494	0.241	0.775	0.775					

*Both slope and intercept of the tested materials showed a highly significant linear prediction of the model tested ($p<0.05$). Dose-response relationship of the tested materials showed an increase in the mortality rate with increased concentrations of the tested materials, but with no significant difference ($p>0.05$) between the observed values of mortality and the expected values. Chi-square test was used to calculate the p value.

pared with those in controls, except in eucalyptus high concentration in larvae. Then all dropped with higher concentrations of oils in both stages. As for the SOD enzyme, a significant decrease after exposure to a high concentration of eucalyptus oil and a significant increase that to a low concentration of clove oil were recorded for *C. pipiens* adults.

The values of DNA damages detected by the comet assay after exposure to the tested oils (Figure 3-5, and Table 6, in relation to the control in Figures 6 and 7) show a dose-dependent increase as evidenced by a change in the comet parameters, i.e., tail length (Px), tail DNA (%), % DNA damage and %DNA in the head, compared with those of the control group. The values of tail length (Px), tail DNA (%), and %DNA damage were significant, whereas a significant decrease in %DNA in the head with increased concentrations of tested oils was detected.

DISCUSSION

The incrimination chains of insecticides in human diseases continue to tighten. The potentiality of environmental exposure risk draws the attention of several research works related to asthma (37); diabetes (38); cancer (39); several nervous system diseases sich as autism (40), Parkinson's (41), and Alzheimer's diseases (42); and reproductive problems (43). Moreover, these insecticides are expensive and leave toxic residues in the environment because they are not easily biodegradable, beside the evolving concern of growing insecticidal resistance. Consequently, attention is ever-changing to alternative insect management approaches (44).

The resistance of *C. pipiens* to chemical insecticides in both laboratory bioassay and in field work studies has been addressed by several studies worldwide (44-47). Furthermore, *C. pipiens* larvae have also been shown to be resistant to *Bacillus thuringiensis var. israelensis* in the laboratory (48). Natural insecticides can function as good substitutes to chemical insecticides (49), being target-specific, biodegradable, and environmentally friendly (50).

Table 4. *Culex pipiens* larvicidal activity of tested oils after 24 h and 48 h

Duration	Tested substance	Response	Concentration (%)						LC50 95% confidence limit	LC90 95% confidence limit	Toxicity index	Z-score*	
			0.005	0.01	0.1	0.5	1	2				Slope	Intercept
24 h	Eucalyptus oil	Observed	10	25	44	60	68	72	0.066	2.165	100	0.844	0.998
		Expected	13.813	19.617	44.909	61.74	67.28	71.586	0.045–0.093	1.277–4.303			
		Residual	−3.813	5.383	−0.909	−1.735	0.722	0.414					
	Clove oil	Observed	4	12	32	52	56	68	0.194	4.903	34.02	0.914	0.65
		Expected	5.852	9.559	31.686	51.7	59.39	65.815	0.14–0.27	2.855–9.989			
		Residual	−1.852	2.441	0.314	0.296	−3.385	2.185					
48 h	Eucalyptus oil	Observed	19	44	64	72	76	78					1.682
		Expected	26.905	35.32	62.291	73.62	76.3	77.99	0.014	0.365	100	0.915	
		Residual	−7.905	8.68	1.709	−1.623	−0.3	0.01	0.005–0.03	0.16–1.43			
	Clove oil	Observed	12	29	52	70	73	76					
		Expected	16.219	23.533	53.142	69.13	73.41	76.284	0.036	0.773	38.88	0.965	1.39
		Residual	−4.219	5.467	−1.142	0.871	−0.414	−0.284	0.025–0.051	0.5–1.336			

*Both slope and intercept of the tested materials showed a highly significant linear prediction of the model tested ($p<0.05$). Dose-response relationship of the tested materials showed an increase in the mortality rate with increased concentrations of the tested materials, but with no significant difference ($p>0.05$) between the observed values of mortality and the expected values. Chi-square test was used to calculate p value.

Table 5. Effects of tested oils on the activities of selected antioxidant enzymes in Culex pipiens adult after 1 h and in larvae after 24 h

Tested enzyme (concentration)	Tested concentrations									
	Control		Eucalyptus oil, 0.5%		Eucalyptus oil, 2%		Clove oil, 0.5%		Clove oil, 2%	
	Adult	Larvae	Adult	Larvae	Adult	Larvae	Adult	Larvae	Adult	Larvae
Superoxide dismutase (μ/mL)	0.61±0.028	0.76±0.084	0.62±0.02	0.93±0.05	0.33±0.04*	0.8±0.03	0.93±0.04*	0.93±0.014	0.7±0.01	0.88±0.03
Glutathione peroxidase (μ/L)	63.6±4.99	966.685±8.5	115.9±6.9*	113.2±3.69*	62.5±6.8	89.3±11.88*	160.12±6.1*	477.05±16.9*	134.6±6.8*	352.9±3.52*
Catalase activity (μ/L)	0.45±0.07	0.35±0.07	0.95±0.07*	0.99±0.14*	0.41±0.12	0.41±0.12	1.05±0.07*	1.2±0.08*	0.45±0.07	0.5±0.14

Results are presented as mean ± standard deviation; $p>0.05$ non-significant; *$p<0.05$ significant. Results were analyzed using Student's t-test. Statistical comparisons were conducted between control and exposure data.

Table 6. Comet parameters of *Culex pipiens* after exposure to tested oils

Comet parameters	Tested concentrations									
	Control		Eucalyptus oil, 0.5%		Eucalyptus oil, 2%		Clove oil. 0.5%		Clove oil, 2%	
	Adult	Larvae	Adult	Larvae	Adult	Larvae	Adult	Larvae	Adult	Larvae
% DNA damage	8	7	16.2	19.5	17.6	14.5	15.8	16.5	15.9	14
%DNA in head	83.65	81.12	75.985	70.12309	72.38	77.17	76.86	74.637	72.525	78.04
Tail length (Px)	4.52	2.176	5.33	5.333333	6.16	4.695	4.957	5.2758	5.394	4.3
% DNA in tail	16.34	18.87	23.214	25.36247	27.61	22.95	23.133	21.823	26.474	20.876

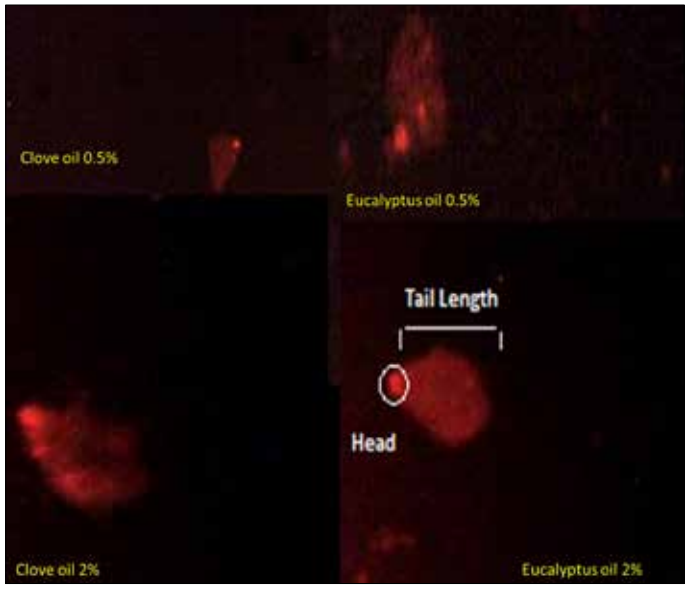

Figure 5. DNA comet assay on *Culex pipiens* larvae after exposure to tested oils

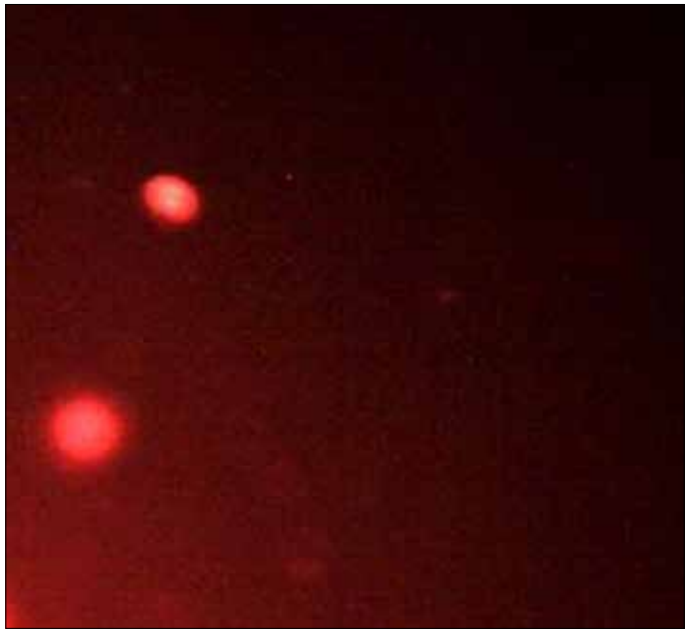

Figure 6. Control cell for the comet assay of *Culex pipiens* adults

Essential oil from clove buds was obtained by hydrodistillation, and its chemical constituents were 31 as detected by GC-MS. (Table 1 and Figure 1). Other studies have addressed similar results for the main constituents but with different concentrations, as in 2007 (13) with eugenol (88.58%), eugenyl acetate (5.62%), and b-caryophyllene (1.39%). Nassar et al. (51) have reported finding different concentrations of eugenol (71.56 %) and eugenol acetate (8.99 %). Also, Prashar et al. (52) indicated that clove oil mainly contains eugenol (78.00%) and b-caryophyllene (13.00%). The variation in the concentrations may be attributed to the variation in the growing seasons and the location of origin (53-55).

The present study revealed 26 chemical constituents of essential oils from *E. globulus* leaves detected by GC-MS (Table 2 and Figure 2). The yield of essential oils and the content of 1,8-eucalyptol was within the values reported in the literature (56), whereas the contents of the main constituents are similar to those reported in the literature (57, 58). Other studies from Egypt have reported results consistent with those of Makhlouf et al. (59) indicating that 1,8-eucalyptol was 55.6%. Contrariwise; Said et al. (60) indicated that the oil contains 1,8-eucalyptol (19.8%).

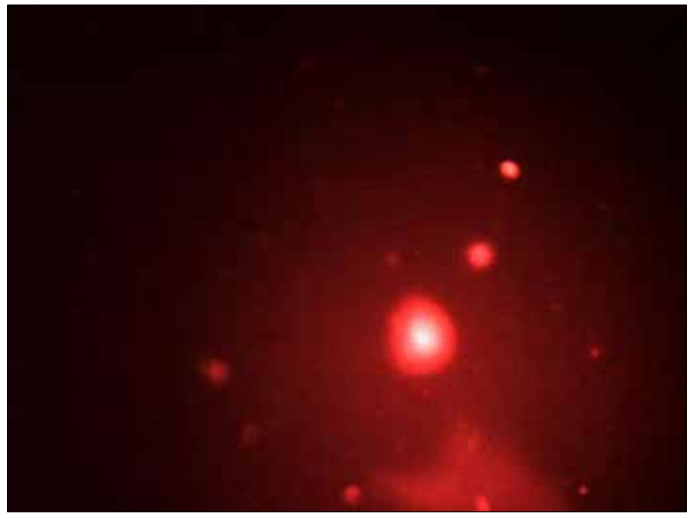

Figure 7. Control cell for the comet assay of *Culex pipiens* larvae

Results of the present study showed that eucalyptus oil was more potent against both adults and larvae (Table 3 and 4). Several studies from Turkey focusing on the use of botanical products in combating C. *pipiens* have been conducted as the larvicidal effect of AkseBio2 in 2004 (61) and *labiatae (lamiaceae*) in 2006 (62). Also, *Chrysanthemum coronarium L., Hypericum scabrum L., Pistacia terebinthus L. subsp. palaestina (Boiss.) Engler, and Vitex agnus castus L* in 2006 (19). And resting the repellency effect of anise, fruits of eucalyptus, mint, basil, and laurel in 2011 (63).

Normal physiological and biochemical activities may be significantly altered in response to insecticidal stress. The antioxidant defense systems, which protect insects against poisons, will have to adapt to maintain insects' life (64). The protective enzymes such as GPx, catalase, and SOD are the natural fences for defending the damage to insect tissues by any exotic toxicant. These protective enzymes have been shown to be involved in the detoxification of insecticides and in developing resistance (65-67). The present study showed the effect of 0.5% and 2% concentrations of tested oils on selected antioxidant enzymes activities in *C. pipiens* adults and larvae (Table 5) for the first time in literature. Variations in the activities of tested enzymes were observed, which could be related to the detoxification role of free radicals generated by tested oils. The detoxification effect of enzymes is considered the backbone for surviving any unfavorable hazard. Homeostatic metabolic mechanisms to defend the oxidative stress caused by tested oils may be responsible for changes detected in the concentrations of the tested enzymes via the upregulation of detoxification enzyme genes, thereby providing a very interesting field of further research.

For the genotoxicity aspect of *C. pipiens*, the alkaline comet assay was used as a measure of DNA strand-break damage because the technique is sensitive and simple and can detect very low levels of damage (68). Changing comet and DNA parameters are shown in Figure 3 and Table 6, and DNA damage of cells of *C. pipiens* adults and larvae exposed to low and high concentrations of the tested oils are shown in Figure 4 and 5, respectively, in relation to the cells of the control (Figure 6, 7). During mitochondrial oxidative phosphorylation, ROS are produced in small amounts, which are important in regulating several cellular functions. However, a failure in the redox potential leads to the accumulation of ROS, causing nucleic acid damage (69). The genotoxic effects of the tested oils reported in the present study were mostly due to the probable inhibitory effects of these oils on the DNA repair systems because they either directly react with DNA or supporting oxidative stress, thereby leading to DNA damage. Literature contains no reports on the genotoxic activity of clove and eucalyptus oils against *C. pipiens*; thus, this study for the first time showed the genotoxic activities of these oils on *C. pipiens* adults and larvae.

CONCLUSION

Finally, eucalyptus oil overpowers clove oil in effective mosquito control and should be particularly well suited for use in insect control. Field trials and the domestic application of these botanical materials in deterrence and intimidation of mosquitoes require further studies. The integrated usage of the tested oils against *C. pipiens* is a future target to achieve. The ubiquitous genetic studying of detoxifying enzymes is required to clarify the homeostasis mechanism that helps mosquitoes to cope with oxidative damage. Antioxidant enzyme pathways are potential targets for insecticides that need future research. Studying DNA repair mechanisms beside DNA damage after exposure to insecticides using the comet assay is mandatory. Further, studying and identifying nucleotides polymorphisms in genes involved in DNA repair mechanisms after exposure to insecticides is also recommended.

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