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Insecticidal activity of *Eucalyptus globulus* (Labill) essential oil against *Culiseta longiareolata* (M., 1838) (Diptera: Culicidae)

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ABSTRACT

This study was done to determine the chemical composition of essential oil of *Eucalyptus globulus* (Labill.) (Myrtaceae) leaves cultivated in Tebessa (Algeria) and to assess their potential larvicidal activity against the most abundant mosquito specie, *Culiseta longiareolata* (M., 1838) (Diptera, Culicidae). Under standard laboratory conditions the larvicidal activity of essential oil extracted from *E. globulus* was tested at 6-concentrations (0,5,10,20,30 and 40 ppm) on newly molted fourth-instar *Culiseta longiareolata* larvae. The effects were examined on the mortality, morphometric measurements and biochemical composition of larvae, pupae and adults body. The steam distillation of *E. globulus* dry flowers yielded 2.89 $\pm$ 0.64 % essential oil. Its chemical composition by GC/MS, identified compounds. The major compounds were : Sabinene (35.38 %) and α-Phellandrene (12.64 %). Bioassay test revealed that *E. globulus* essential oil exhibited larvicidal activity against fourth instar larvae of *C. longiareolata* at 24 h (LC_{50} and LC_{90} values were 24.23 and 46.13 ppm, respectively). The morphometric study showed that the essential oil decreased the growth of different developmental stages of *C. longiareolata*. Moreover, it reduced significantly the body contents of carbohydrates and lipids in larvae, pupae, adult male and female and increased the protein content. Due to its mosquitocidal efficacy, *E. globulus* essential oil may be used as an attractive candidate for further study to monitor resistance of mosquito vectors.

Keywords: Biochemical composition, *Culiseta longiareolata*, essential oil, *Eucalyptus globulus*, GC/MS, larvicidal activity, morphometry. *C. longiareolata*

INTRODUCTION

Mosquitoes are the most important group of vectors of pathogens and parasites (1) widely distributed worldwide and are a global public concern (2). Mosquito-borne diseases (Malaria, yellow fever, filariasis, Dengue, chikungunya, hemorrhagic fever and encephalitis), annually cause extensive injuries and many deaths worldwide (3). To improve quality of environment and public health, mosquito control is essential. However, the intensive use of synthetic insecticides has caused adverse effects on the environment and lead to the development of resistance in vectors (4). Hence, essential oils draw larger attention as potentially useful bioactive compounds against pest and vectors and as an alternative source for mosquito larval control agent (5). They are ecofriendly, biodegradable, target specific, lower bioaccumulation and low or sometimes non-toxic to higher animals (6).

Essential oils have bioactivities [ovicidal, larvicidal, pupicidal (7) to adulticidal activities (8) that include oviposition deterrence and repellent actions (9)] against mosquito spp. Insecticidal activity of essential oils is highly related to its chemical composition (10).

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Their chemical profile depends on plant and its environment, origin, climatic conditions, method and period of extraction and part of the plant extracted (11).

Eucalyptus is an evergreen and widely cultivated tree worldwide and used for various purposes viz., medicinal, phytochemical, pharmaceutical, mosquito repellent, antioxidant and antibacterial properties (12). This study aimed to determine (i). are there any toxic effects of this essential oil tested on the different stages of development of *Culiseta longiareolata* ? and (ii). if so, what are the mechanisms of action of this oil ?

MATERIALS AND METHODS

(i). Study site: The study was done during March-June, 2021 in semi-arid Tébessa, Eastern Algeria (35°24′15″N, 8°07′27″E; elevation: 867 m a.s.l.), Total Annual Precipitation: 470.6 mm, Highest maximum temperature: 27.05°C and minimum temperature: 5.9 °C.

(ii). Plant material and extraction of the essential oil

Leaves of *Eucalyptus globulus* (Labill.) (Fig. 1) were collected in March-June, 2021 in Tebessa (Northeast Algeria) and transported to laboratory. Dried flowers (50 g) were hydrodistilled in a Clevenger type apparatus for 3 h according to the method of British Pharmacopoeia (13). The volatile oil was dried over anhydrous sodium sulphate and stored in sealed vials at 4 ºC until analysis. The oils yield was calculated based on dried weight of plant materials.

Figure 1. *Eucalyptus globulus* leaves

This study was conducted under laboratory conditions on *Culiseta longiareolata* mosquito species in Tebessa area Northeast algeria). We assessed the efficacy of *Eucalyptus globulus* essential oil against fourth-instar larvae by determining the lethality parameters. In addition, its effects on morphometric measurements and on main biochemical components (carbohydrates, proteins and lipids) in whole body of the different instars (fourth instar larvae, pupae and adults) were investigated.

(iii). Gas chromatography-mass spectrometry analysis

The essential oil of *E. globulus* was subjected to GC-MS analysis using Trace GC ULTRA/Polaris Q (GC-MS, Thermo Electron). A VB-5 (5 % phenyl/95 % dimethyl-polysiloxane) column (length 30 m, internal diameter 0.25 mm, film thickness 0.25 µm) was used. The GC oven temperature was kept at 60 °C for 8 min and then increased to 250 ºC at

rate of 2 ºC /min. The injector and detector temperatures were kept respectively at 250 and 280 ºC. Carrier gas was helium, the flow through the column was 1 ml/min, and the split ratio was set to 50:1 with injection of 0.2 µl of oil sample. The GC-mass spectrometry (MS) analysis was performed with a Quadrupole mass spectrometer that operated at 70 V. Constituent's identification was based on comparison of retention times with those of corresponding reference standards using the NIST and WILEY libraries. Essential oils (%) compositions were calculated based on the area of chromatographic peaks.

(iv). Mosquito rearing

The larvae of *Culiseta longiareolata* were obtained from a reared stock colony, laboratory of Applied Animal Biology (14). Each 25 larvae were kept in pyrex storage jar containing 150 ml of tap water and maintained at temperature between 25-27 °C. Larvae were daily fed with fresh food mixture of Biscuit Petit Regal-dried yeast (75:25 by weight). The water was replaced every two days.

(v). Larvicidal bioassays

Larval bioassays were done as per Bouguerra *et al*. (15). The essential oil of *E. globulus* was dissolved in 1 ml ethanol and then diluted in 150 ml filtered tap water to obtain the desired concentrations (5, 10, 20, 30 and 40 ppm). Newly molted fourth-instar larvae of *C. longiareolata* were exposed to EO for 24 h as per World Health Organization standard procedure (16). The controls were prepared using 1 ml of ethanol in 150 ml of water for positive controls and no additive with negative control. After the exposure time of 24 h, larvae were removed, washed and placed in clean water. The treatments were replicated 4-times in complete Randomised Design. There were 25 larvae per concentration. Mortality was recorded 24, 48 and 72 h after treatment. The mortality obtained was corrected according to Abbott (17) and lethal concentrations with their 95 % confidence limits (95 % CL) were calculated.

(vi). Morphometric measurements

Newly fourth instar larvae, pupae, male and female adult of *C. longiareolata* were treated with *E. globulus* essential oil at its LC_{25} and LC_{50} (13.42 and 20.23 ppm respectively) as determined before. The body size was recorded by measuring under a dissecting microscope, the width of thorax in larvae, the cephalothorax in pupae and the wing length in adults (male and female) (18) as previously reported (19) on 3-replicates of 20 individuals. The body weight of individuals from different instars was also determined.

(vii). Biochemical composition of body

The main biochemical constituents (proteins, carbohydrates and lipids) were extracted following the procedure of Shibko *et al.* (20) and quantified as per Bouabida *et al*. (21). Pooled samples (10 individuals per pool) from each stage were weighed and extracted in 1 ml of trichloracetic acid (20 %). The quantification of proteins was done following the Coomassie Brilliant Blue G-250 dye-binding method (22) with bovine serum albumin as a standard. The absorbance was measured at 595 nm. Carbohydrates were determined following the method of Duchateau and Florkin (23) using glucose as standard and anthrone as reagent and the absorbance was measured at 620 nm. Lipids were estimated as per Goldsworthy *et al*. (1972) (24) using sunflower oil as standard and vanillin as reagent with an absorbance at 530 nm. Each assay was conducted with 3 replicates per treatment.

(viii). Statistical analysis

The number of individuals tested in each series is given with the results. Data are presented as mean ± standard deviation (SD). The significance between different series was tested using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. All statistical analyses were performed using Prism 7.0 for Windows (GraphPad Software Inc., www.graphpad with a significant level $p < 0.05$).

RESULTS AND DISCUSSION

Oil extraction yield

The yield of *Eucalyptus globulus* essential oil extracted from dry flowers by steam distillation was 2.89 ± 0.64 %. This yield is higher compared to those reported in other regions in Algeria. It was 0.96 % in Constantine (25) and 1.87 % in Ain Defla (26), but was lower than in Brazil (3.1 %) (27). The relatively good essential yield in our study may be due to the drought in Tebessa region. It is known that the maximum yields are achieved in dry weather. Several reports also show similar yields of *E. globulus* essential oil: 0.95 to 1.32 % in Ethiopia (28), 0.08-3.5 % in Bangladesh (29), 1.05 - 1.1 % in India (30) and 2.68 % in Argentina (31). Several factors (tree age, leaf age, altitude, season, harvest time and fertilizer) affects the yields of Eucalyptus oils (32). In terms of age, young leaves contain more oil than old leaves, while leaves from older trees gives higher yield (32).

Chemical composition of essential oil

Chemical constituents of the essential oil extracted from *E. globulus* leaves, their percentages and the retention times are presented in Table 1 and Figure 2. Hundred compounds are found by GC/MS analysis, the major constituents identified were: Sabinene (35.38 %) and α-Phellandrene (12.64 %) followed by Spathulenol (6.88 %) and Ledene (5.68 %).

Table 1. Chemical composition of *Eucalyptus globulus* flowers essential oil : retention time (RT) and most abundant constituents (%).

Constituents	RT	Concentration (%)
α-Thujene	9.739	1.643
β-Myrcene	13.778	1.125
α- Phellandrene	**14.848**	**12.646**
Sabinene	**16.886**	**35.384**
γ-Terpinene	18.506	1.529
Linalool	21.812	1.086
p-Menth-1-en-8-ol	23.200	1.395
4-Terpineol	27.345	4.368
Cryptone	27.880	2.326
Spatulenol	44.454	6.882
Bicyclogermacrene	48.102	4.560
Ledene	53.673	5.688

Letters in bold indicate the major constituents

Boukhatem *et al.* (26) found that in the essential oil of *E. globulus* collected from the region Ain Defla (Center Algeria) Eucalyptol (85.80 %) was a major component followed by α-Pinene (7.2 %) and β-Myrcene (1.5 %). These results differ from those obtained by Atmani-Merabet *et al.* (25) in Constantine region (Algeria), which showed that 1,8-cineole

(78.45 %), o-cymene (2.18 %), isopinocarveole (1.74 %) were the major components of *E. globulus* essential oil. Studies from Algerian Eucalyptus essential oil collected from different sites revealed variability in the composition from 47.05 to 53.3 % (33). These variations in Algeria *E. globulus* essential oil and those of elsewhere denote the existence of several chemotypes, as previously reported (34). Also, Sebei *et al.* (35) found that the main compound of essential oils extracted from 7-Tunisian species of *Eucalyptus* (*E. maideni; E. astrengens; E. cinerea; E. leucoxylon; E. lehmani; E. sideroxylon; E. bicostata*) was Eucalyptol.

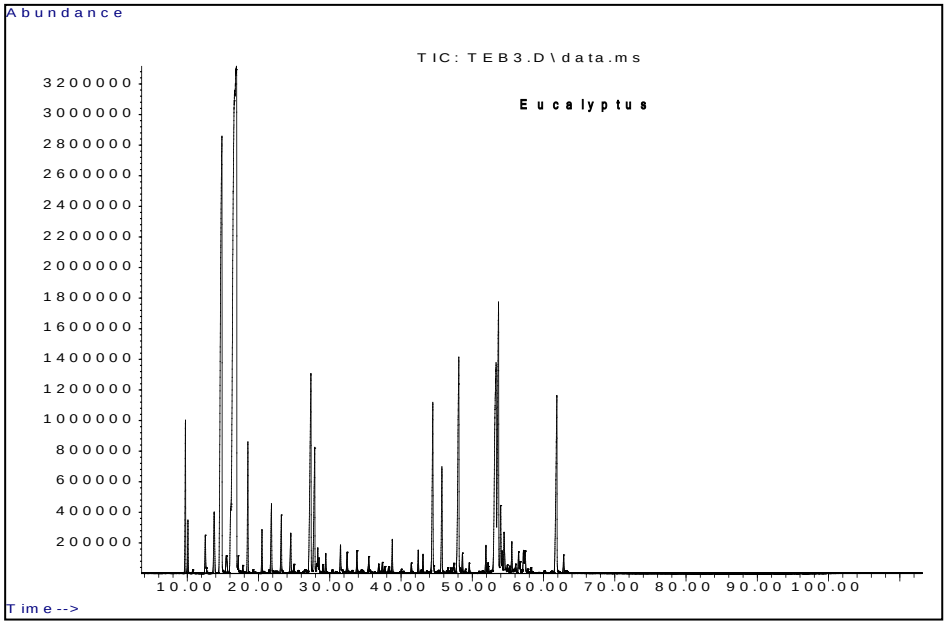

Figure 2. GC-MS chromatogram for essential oil obtained from *Eucalyptus globulus* (Abundance as function the time in min).

The *Eucalyptus* oil is a complex mixture of variety of monoterpenes and sesquiterpenes and aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketones; however, the exact composition and proportion varies with species and parts of plants (36). Akin *et al.* (37) reported that environmental factors Played a key role in the chemical composition of essential oil. Differences in the volatile composition of plants could be attributed to genetic, chemotype, distinct environmental and climatic conditions (38).

Larvicidal activity of essential oil

Dose-response relationship was determined for *E. globulus* essential oil applied on newly ecdysed fourth-instar larvae of *C. longiareolata*. The mortality was scored 24, 48 and 72 h after treatment. The positive controls showed no effects of ethanol against *C. longiareolata* compared to untreated series. The calculated corrected mortality showed that the mortality rates ranged for newly ecdysed fourth-instar larvae from 9.00 % (5 ppm)

to 97.00 % (40 ppm) at 24 h, from 15.00 % (5 ppm) to 97.00 % (40 ppm) at 48 h and from 19.00 % (5 ppm) to 100 % (40 ppm) at 72 h (Fig. 3).

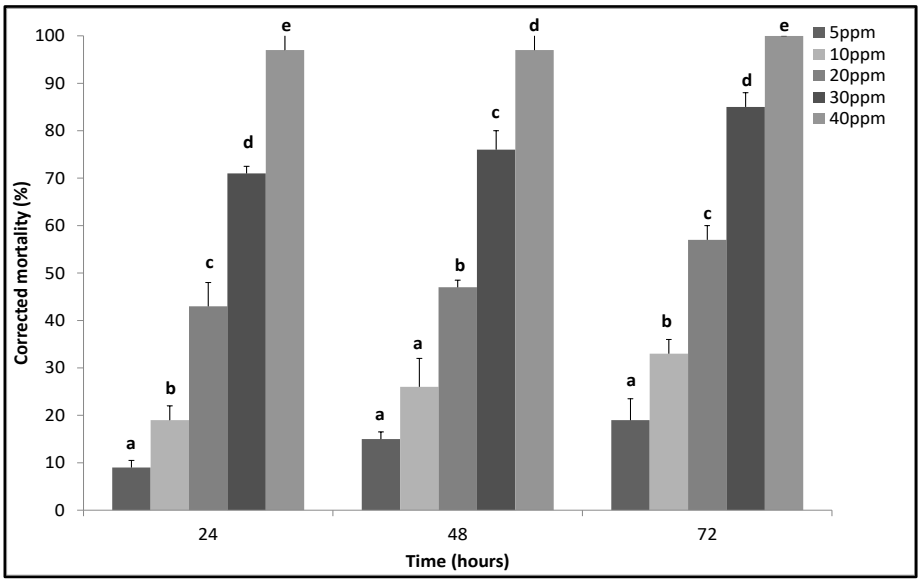

Figure 3. Efficacy of *E. globulus* EO applied on fourth instar larvae of *Culiseta longiareolata:* corrected mortality (m ± SD, n :4 repeats each containing 25 individuals): Tukey HSD test.

The lethal concentrations showed variations according to the periods after treatment (Table 2). After treatment, intoxicated larvae showed a change in their behaviour by sinking to the bottom of the jar and remaining immobile until they died.

Table 2. Toxicity of *Eucalyptus globulus* essential oil applied on fourth instar larvae of *Culiseta longiareolata* : Determination of lethal concentrations and their confidence intervals (95 %).

Time (h)	Hill slope	Lethal concentrations (ppm)	
		LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)
24	0.66	**24.23** [14.79 – 27.73]	**46.13** [24.21 – 87.70]
48	0.54	**17.45** [11.53 – 26.48]	**49.31** [20.94 – 115.87]
72	0.46	**14.06** [9.16 – 21.57]	**42.46**[18.15 – 99.31]

(LCL: Lower confidence Limit, UCL: Upper confidence Limit)

Eucalyptus essential oils possess insecticidal properties against mosquitoes (39,40) and stored product pests (41). Lalthazuali (42) demonstrated that the essential oils from *Ocimum sanctum, Mentha piperita, E. globulus* and *Plectranthus amboinicus* and their blend has potential as mosquito repellents against *Aedes aegypti*. The eucalyptus essential oil had larvicidal activity against *Culex* with an LC_{50} of 64.64 ppm and LC_{90} of 294 ppm (39). Three Iranian Eucalyptus species essential oils tested against *Rhyzopertha dominica* (Coleoptera) showed that the LC_{50} achieved was 41.69, 34.39 and 27.98 ppm for *Eucalyptus dundasii, E. floribunda* and *E. kruseana* respectively (41).

The insecticidal activity of *Eucalyptus* oils is due to their components [1,8-Cineole, Citronellal, Citronellol, Citronellyl acetate, p-Cymene, Eucamalol, Limonene, Linalool, Pinene, γ-Terpinene, Terpineol (36) and Terpinene (43)]. Phellandrene LD_{50} was equal to 16.6 µg/ml and 39.9µg/ml against fourth-instar larvae of *Aedes aegypti* (Diptera) and *Aedes albopictus* (Diptera) respectively (44). These volatile and lipophilic compounds rapidly penetrates into insects and interferes with their physiological functions (45). The different solvents used for leaf extracts of *E. globulus* were investigated against the third instar larvae of *Aedes aegypti* and *Anopleles stephensi* (Diptera) and the LC_{50} values were 225.2 and 118.8 ppm respectively (40).

The bioactivity of the essential oil depends on the type and nature of the constituents and their concentration. It further varies with species, location, season, soil type, climate, age of the leaves, fertility regime, the method used for drying the plant material and the method of oil extraction (36). The various constituents of *Eucalyptus* essential oil act synergistically for the overall pesticidal activity (36).

Effects on weight and volume

Measurements of the whole body of *C. longiareolata* larvae, pupae, male and female adults showed that the body weight was affected under treatment of *Eucalyptus globulus* essential oil at its LC_{25} and LC_{50} (Table 3). The treatments decreased significantly the weight of larvae ($F_{2.6}$= 24.49 ; p=0.0013), pupae ($F_{2.6}$= 39.24 ; p =0.0004), and female adult ($F_{2.6}$= 13.81 ; p =0.0057). However, treatment with *E. globulus* EO significantly decreased the body volume in All tested stages: larvae ($F_{2.6}$= 247.4 ; p < 0.0001), pupae ($F_{2.6}$= 15.92 ; p =0.004), male ($F_{2.6}$= 42.85 ; p =0.0003) and female ($F_{2.6}$= 18.12 ; p =0.0029) adults.

Table 3. Effects of *Eucalyptus globulus* essential oil (LC_{25} and LC_{50}) on the fresh body weight (mg) and on the body volume (mm^3) in different stages of development of *Culiseta longiareolata*.

Stages	**Morphometric parameters**	**Control**	**LC_{25}**	**LC_{50}**
Larvae	Body volume (mm^3)	4.73 ± 0.16 a	2.77 (-41.4)b	2.09 (-55.8)c
	Body weight (mg)	4.81 ± 0.16 a	3.48 (-27.7)b	3.24 (-32.6)b
Pupae	Body volume (mm^3)	11.38 ± 0.82 a	8.96 (-21.3)b	8.80 (-22.1)b
	Body weight (mg)	7.01 ± 0.08 a	5.61 (-20.0)b	5.14 (-26.7)b
Male adult	Body volume (mm^3)	78.31 ± 4.89 a	62.97 (-19.6)b	55.37 (-29.3)b
	Body weight (mg)	2.62 ± 0.51 a	2.15 (-17.9)a	1.78 (-32.1)a
Female adult	Body volume (mm^3)	143.89 ± 5.21 a	107.48 (-25.3)b	92.65 (-35.6)b
	Body weight (mg)	4.24 ± 0.57 a	2.90 (-31.6)b	2.70 (-36.3)b

m ± SD, n: 3 pools each containing 20 individuals.
For each stage, mean values followed by the same letter are not significantly different at p = 0.05.

The body size is a pivotal trait for mosquitoes, because it influences their blood-feeding ability, host attack rate and fecundity. All these traits are important determinants of their potential to transmit diseases (46).

In previous studies, authors have reported similar observations using other plant essential oils such as *Ocimum basilicum*, *Lavandula dentata*, *Mentha piperita* (14,47), *Laurus nobilis* (48) and *Petroselinum crispum* ((49) against *Culex pipiens* and *Culiseta longiareolata*. Moreover, *Laurus nobilis* and *Mentha pulegium* significantly reduces the body weight and body volume of fourth instar larvae of *Culiseta longiareolata, Culex pipiens*

and *Aedes caspius* (50). In addition, *Rosmarinus officinalis* essential oil (LC_{25} and LC_{50}) significantly decreases the weight and the body volume of larvae of *Culex pipiens* than controls in all tested periods (51).

The decrease in larval weight and body size may be due to an impaired absorption process caused due to the effects of essential oil on larval digestive cells (52). Also, several studies have demonstrated that the botanical insecticides inhibits the activity of several digestive enzymes, which converts the complex food materials in to micromolecules necessary to provide energy and metabolites for growth and development (53).

Effects on biochemical composition of bodies

The amounts of carbohydrates, lipids and proteins were estimated in the whole body at different developmental stages of *Culex pipiens* using LC_{25} and LC_{50} of *E. globulus* essential oil (Table 4).

Table 4. Effects of *Eucalyptus globulus* essential oil (LC_{25} and LC_{50}) applied on amounts of proteins, carbohydrates and lipids (µg/mg) from different stages of development of *Culiseta longiareolata*.

Stages	**Components**	**Control**	**LC_{25}**	**LC_{50}**
Larvae 4	Protein	56.05 ± 3.01 a	70.03 (+25.0)b	88.67 (+58.2)c
	Lipid	70.65 ± 1.10 a	65.60 (-7.1)a	36.35 (-48.5)b
	Carbohydrates	179.80 ± 20.12 a	148.45 (-17.4)a	44.83 (-75.1)b
Pupae	Protein	77.99 ± 3.59 a	91.70 (+17.6)b	100.28 (+28.6)c
	Lipid	119.56 ± 4.03 a	110.49 (-7.6)a	50.88 (-57.4)b
	Carbohydrates	239.64 ± 22.49 a	214.49 (-10.5)a	219.06 (-8.6)a
Male adult	Protein	61.96 ± 2.98 a	62.22(+0.4) a	75.31 (+21.5)b
	Lipid	147.24 ± 6.68 a	124.85 (+15.2)b	107.54 (-26.7)b
	Carbohydrates	127.24 ± 19.68 a	136.25 (+7.1)a	233.10 (+183.2)b
Female adult	Protein	65.67 ± 2.02 a	68.84 (+4.8)a	70.63 (+7.6)a
	Lipid	161.67 ± 7.61 a	140.11 (-13.3)a	133.00 (-17.7)a
	Carbohydrates	126.85 ± 17.18 a	173.85 (+3.7)b	136.74 (-10.8)b

m ± SD, n : 3 pools each containing 20 individuals.
For the same component, the different letters indicate significant differences based on Tukey's HSD test ($p < 0.05$).

(i). Proteins : The comparison of mean values showed a significant increase in the protein amounts in larvae ($F_{2.6}$= 113.4 ; $p < 0.0001$), pupae ($F_{2.6}$= 56.58 ; $p = 0.0041$) and male adult ($F_{2.6}$= 24.9 ; $p = 0.0012$).

(ii). Carbohydrates : The carbohydrate levels were significantly reduced in larvae ($F_{2.6}$= 60.89 ; $p = 0.0001$). However, in female adult, a significant increase ($F_{2.6}$= 13.75 ; $p = 0.0057$) was reported at the LC_{25} followed by a decrease in the treated series with the highest concentration (LC_{50}). Moreover, a significant increase ($F_{2.6}$= 23.75 ; $p = 0.0014$) was observed after treatment with the two concentrations (LC_{25} and LC_{50}) in male adult than controls.

(iii). Lipids: The lipid content was decreased in treated series in all stages tested: larvae ($F_{2.6}$= 45.03 ; p= 0.0002), pupae ($F_{2.6}$= 112.70 ; $p < 0.0001$) and male adult ($F_{2.6}$= 8.993 ; $p = 0.0151$) as compared to control.

The exposure of an organism to xenobiotic products can modify the synthesis of some metabolites and disturb its functionality (54). Biochemical analysis revealed a decrease in the level of lipids and an increase in the proteins amount in the whole body of larvae, pupae and adults treated by *E. globulus* essential oil compared to control series.

Proteins help in various reactions such as the hormonal regulation and they are integrated in the cell as a structural element with the carbohydrates and the lipids (55). Our results are in agreement with those of Hazarika *et al.* (56) who recorded increase in proteins after application of the essential oils of *Cympobogon nardus* (Poaceae) and *Pogostemon cablin* (Lamiaceae) against the larvae of *Ae. aegypti*. Moreover, an increase in proteins was observed in *Culex pipiens* treated by *Rosmarinus officinalis* essential oil (51). The raised protein level may be due to an increase in the synthesis of proteins and their accumulation by the fat in body, haemolymph and other tissues under the stress of insecticides (57). Whereas, Sugumar *et al.* (58) showed that the total protein level of larvae *Culex* was reduced after exposure to *Eucalyptus* oil nanoemulsions. Similar results were obtained in *Culex pipiens* and *C. longiareolata* after treatment with *Petroselinum crispum* essential oil (49).

In insects, lipids act as hormones and form important energy reserves for greater metabolic activity such as flight and egg production (59). Our data corroborate with those obtained by the treatment of *Culex pipiens* larvae with *Ocimum basilicum* (47) and *Thymus vulgaris* (15) essential oils. Sharma *et al.* (2011) suggested that lipids content reduction in insects occurs because of a change in lipids metabolism due to stress caused by the plant extract. The same observations were also reported with *Artemisia annua* extract in Anopheline and Culicine larvae (60). Zeghib *et al.* (51) and Seghier *et al.* (2020) (49) have reported a decrease in the total lipid content in *Culex pipiens* mosquito treated with *Rosmarinus officinalis* and *Petroselinum crispum* essential oils respectively. The decline of lipid levels might be due to the effects of these oils on the mobilization of lipid reserves for energy production as a result of induced stress (61). Reduction of lipid levels in the larvae treated with plant essential oils may be due to their effects on the lipid metabolism, and use of lipid reserves for energy generation as a result of induced stress (62).

The carbohydrates are important energy elements playing a crucial role in the physiology of the insects, such as the molt and the reproduction (63). This component was reduced in all tested stage of *Culex pipiens* after treatment with essential oil of *Eucalyptus globulus*. This is in accord with the observations of Dris *et al.* (47) and Seghier *et al.* (49) where they found a significant reduction in the levels of lipids in all stages of development of *Culex pipiens* treated by *Ocimum basilicum* and *Petroselinum crispum* essential oil and in the fourth larval stage of *Culex pipiens* treated by *Rosmarinus officinalis* essential oil (51).

CONCLUSIONS

The *E. globulus* essential oil major constituents viz., Thujene and α- Phellandrene possess potent larvicidal activity against *Culex pipiens* larvae. Furthermore, this essential oil disrupts the main biochemical components, decreased the weight and the volume at all tested stages of *Culex pipiens*. Due to its mosquitocidal efficacy, *E. globulus* essential oil could be considered as an attractive candidate for further study in monitoring resistance of mosquito vectors.

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DECLARATION

We declare that all authors of this manuscript made a significant contribution, and we have not excluded any author that substantially contributed. We have followed the ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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