Chemical Composition and Insecticidal Activity of *Laurus nobilis* Essential Oil on *Culiseta longiareolata* (Diptera : Culicidae) larvae

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***Abstract*—**The current study was undertaken in order to determine larvicidal activity of Laurus nobilis essential oil and its effects at LC25 and LC50 on biochemical composition and on the body weight of the fourth instar larvae of Culiseta longiareolata (Diptera: Culicidae), the most common and abundant species in Tebessa area (Northeast Algeria). The obtained percent yield of the hydrodistilled oil from aerial parts of Laurus nobilis was 0.96 ± 0.045%. The GC/MS analysis of Laurus nobilis essential oil has led to the identification of 56 components. Eucalyptol (25.62 %), Linalool (11.83 %), Methyl Eugenol (11.07%) and Camphene (10.18%), were the major constituents of which. Bioassay test done following the World Health Organization standard protocol revealed that this essential oil exhibited larvicidal activity with dose-response relationship. The morphometric study shows that the essential oil tested was found to decrease the growth of larvae. Moreover, it reduces significantly the body contents of proteins, carbohydrates and lipids of treated individuals. Overall, our results indicate that L. nobilis essential oil has potential for the development of new and safe control products against mosquitoes.

***Keywords*—** Culiseta longiareolata, Essential oil, Laurus nobilis, Toxicity, Chemical composition

I. Introduction

Insects are a very important part of the biodiversity terrestrial and aquatic ecosystems. Hematophagous insects like mosquitoes play an important role in global disease transmission such as Zika, dengue fever, yellow fever, chikungunya, malaria and Japanese encephalitis [1]. Mosquito controls, using synthetic chemical insecticides have adverse effects on the environment and also cause growing of insecticide resistance in arthropods [2-3]. However, the search for new insect control agents from natural products which are selective, biodegradable and of low environmental toxicity is crucial [4]. Mosquito larvae can be controlled by natural enemies [5] such as larvivorous fish [6], bacteria [7] and by insect growth disruptors (IGDs) [8-9]. The phytochemicals derived from plant resources can act as larvicides, insect growth regulators, repellents, and ovipositional attractants, having deterrent activities observed by different researchers [10-12].

In general, essential oils have been considered as important natural resources to act as insecticides [13-14], with low mammalian toxicity and rapidly degradable in the environment [15]. They show a various bioactivities against mosquito species with ovicidal, larvicidal, pupicidal [16-17] and adulticidal potentials [18-19].

Approximately, 2000 plant species have been known to produce secondary metabolites of value in biological pest control programs and only 344 plant species showed insecticidal activity against mosquitoes [20-23]. Essential oils can be synthesized by all plant organs (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and root) and can therefore be extracted from these parts [24]. They are mainly formed by mixtures of monoterpenes, sesquiterpenes, phenylpropanoids and metabolites that confer the mixtures with organoleptic characteristics and biological activities [25]. Laurus nobilis L. is evergreen shrub, belonging to family Lauraceae comprises 32 genera and about 2000-2500 species. Some studies have shown that the Laurel essential oil used as antioxidant [26], antifungal [27], antibacterial [28] and insecticidal agent [29]. Culiseta longiareolata M. represent the most representative mosquito species in the Tebessa area [30]. The present study was designed to determine the chemical composition of the L. nobilis essential oil and its larvicidal activity against fourth instar larvae of C. longiareolata. In addition, its effects on morphometric measurements and on main biochemical components (carbohydrates, proteins and lipids) in whole body were investigated.

II. Details Experimental

A. *Plant materials and extraction of the essential oil*

The fresh aerial parts of L. *nobilis* samples were collected in Tébessa area (Northeast Algeria) in march-july 2016. Dried leaves of the plants (about 100 g) were cut into small pieces and hydrodistilled in a clevenger type apparatus for 3 h according to the method recommended in the British Pharmacopoeia (1988). The volatile oils were dried over anhydrous sodium sulfate and stored in sealed vials at 4 °C until analysis. The yield of the oils was calculated based on dried weight of plant.

B. *Gas chromatography-mass spectrometry*

The GC/MS analysis were performed with an HP Agilent 2890 plus gas chromatograph (GC) equipped with a HP-5MS column (a length of 30 m × internal diameter of 0.25 mm, and 0.25 mm film thickness). The helium was used as transporter gas. The GC oven temperature was kept at 60°C for 8 min and programmed to 250°C for 10 min at rate of 2°C/min. The

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injector temperature was set at 250 °C. The split flow was adjusted at 50ml/min. MS were taken at 70 eV. The sample was dissolved in pure hexane. A volume of 0.2µl was injected for GC-MS analysis. Constituent's identification was found on comparison of retention times with those of corresponding reference standards using the NIST 02 and WILEY 7N libraries [31; 32]. Percentage compositions of essential oil were calculated according to the area of the chromatographic peaks.

C. Mosquito rearing

The larvae of *C. longiareolata* (Diptera: Culicidae) were obtained from a stock colony of the laboratory. Each 20 larvae were kept in Pyrex storage jar containing 150 ml of stored tap water and they were daily fed with fresh food consisting of a mixture of Biscuit Petit Regal-dried yeast (75:25 by weight). The water was replaced every three days.

D. Larvicidal test

Newly ecdysed fourth-instar larvae of *C. longiareolata* were exposed to the different concentrations (25, 50, 100, and 200 ppm) for 24 h. According to the World Health Organization, the larvae were removed, and placed in clean water. The test was realized with three repetitions containing 20 larvae each. Mortality was registered at 1st day, 3rd day, 5th day and 7th day following treatment. The control mortality was corrected by Abbott's formula. Sub-lethal and lethal concentrations (LC_{25}, LC_{50} and LC_{90}) and 95% confidence limits (95% CL) were calculated.

E. Morphometric measurments

As above, newly molted fourth instar larvae were treated with *L. nobilis* EO at its LC_{25} and LC_{50} as determined before. The body size was recorded by measuring the width of the thorax in larvae [33] on 3 replicates of 20 individuals and the body weight was also determined.

F. Biochemical procedure

Carbohydrates, Proteins and lipids were extracted following the procedure of Shibko et al. (1966) [34]. Pooled samples (20 individuals per pool) were weighed and extracted in 1 ml of trichloracetic acid (20%). In brief, quantification of proteins was carried following the Coomassie Brilliant Blue G-250 dye-binding method [35] with bovine serum albumin as a standard. The absorbance was measured at 595 nm. Carbohydrates were determined following the anthrone method [36] using glucose as standard. Lipids were measured by the vanillin method [37]. Data were expressed in µg per individual.

G. Statistical analysis

Results are presented as mean ± standard deviation (SD).The significance between different series was tested using Student's t tests at 5 % level. Data were subjected to one-way analysis of variance (ANOVA) followed by a post-hoc honestly significant difference (HSD) Tukey's test.

III. Results And Discussion

A. Extraction yield and chemical composition of essential oil

The results of the steam distillation show that the yield of *L. nobilis* EO was 0.45± 0.96% (dry leaves of the plant). The percentage and the retention time of the identified compound of this essential oil were given in Table 1 and figure 1. Gas chromatography and mass spectrometry analysis resulted in the identification of fifty-six compounds, with Eucalyptol (25.62 %) and Linalool (11.83 %) as the major components. The variations of the chemical components of *L. nobilis* essential oil related to the geographic origins, genetic variability, growing conditions, organ development, seasonal, methods of extraction and the plant part from which it was extracted such as seed, leaf, flower and fruits [38]. Compared to the published data, the chemical profile obtained presented differences, but also some similarities.

This difference is confirmed by the chemical composition of essential oil of fresh bay leaves from Turkey with 1,8 cineol (64.61%) as a major component. It has been found that the *L. nobilis* oil from Algeria contains major components, 1-8 cineol (39.69%) and camphene (14.21%) [39]; from Brazil; 1-8 cineol ((35.50 g/100 g) and linalool (14.10 g/100 g) [40], from Turkey; 1,8-cineole (51.73-68.48%) and α-terpinyl acetate (4.04-9.87%) [41] and from Tunisia ; 1,8-cineole (56%) and α-terpinyl acetate (9.0%). Mediouni et al. (2012)[42] mentioned that three *L. nobilis* EO from Algeria, Tunisia and Morocco showed quantitative, rather than qualitative, differences in their chemical composition that depended on their cultivation locations.

TABLE I: CHEMICAL COMPOSITION OF *L. NOBILIS* OIL: RETENTION TIME (RT) AND AREA (%) OF 10 MAJOR CONSTITUENTS

N°	RT	Compounds	Area %
1	14.14	Eucalyptol	25.62
2	19.35	Linalool	11.83
3	39.92	Methyl Eugenol	11.07
4	36.00	α-terpinyl acetate	10.18
5	10.28	Sabinene	7.34
6	7.98	α-Pinene	3.87
7	25.35	1-α-Terpineol	3.45
8	40.03	Veratrole methyl	2.73
9	36.62	Eugenol	1.88
10	55.93	Shyobunol	1.45

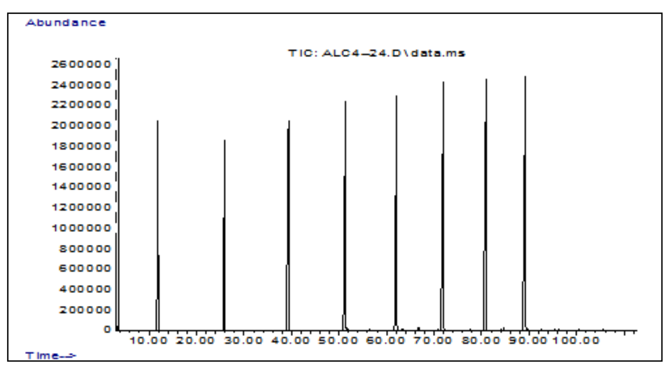

Fig. 1. GC-MS chromatogram for essential oil obtained from *L. nobilis* (abundance as function the time in min).

B. Insecticidal activity

Essential-oil extracted from plants deed as promising alternative natural Products for the control of many insect pests particularly mosquitoes [43]. Dose-response relationship was determined for *L. nobilis* essential oil applied to newly molted fourth instar larvae of *C. longiareolata* (Table 2 and Fig. 2). Preliminary tests showed that the solvent (ethanol) had no significant effect on larvae compared to untreated series. The mortality was scored at different periods after treatment (1st, 3rd, 5th and 7th day). After the treatment, the intoxicated larvae showed a change in their behaviour by sinking to the bottom of the jar and remain there motionless until they died. Our results indicate that the *L. nobilis* essential oil and its active components could be developed as control agents against the mosquito larvae. Diverse studies reported the larvicidal activity of monoterpenes against various species of mosquitoes [44-45].

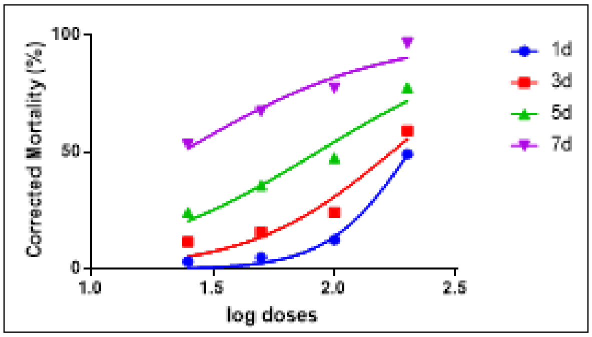

Fig. 2. Efficacy of L. *nobilis* EO against fourth instar larvae of C. *longiareolata*

TABLE II. LARVICIDAL ACTIVITY OF L. *NOBILIS* EO AGAINST C. LONGIAREOLATA AT DIFFERENT PERIODS AFTER TREATMENT.

Time (Day)	Slope	R^2	CL_{25} (ppm)	CL_{50} (ppm)	CL_{90} (ppm)
			95% FL	95% FL	95% FL
1	0.34	0.98	**132.80** 105.6-167.1	**203.70** 168.1-246.9	**479.10** 260.8-879.9
3	0.38	0.92	**81.96** 38.30-175.4	**171.9** 86.93-339.9	**756** 94.44-6052
5	0.23	0.94	**31.25** 11.07-88.19	**85.1** 45.42-159.4	**631** 85.22-4672
7	0.24	0.93	**8.21** 1.33-50.44	**23.45** 9.58 -57.38	**191.12** 37.85-965.6

C. Effect on weight and volume of body

Measurements of the whole body weight of *C. longiareolata* larvae showed that the weight was affected under treatment of *L. nobilis* EO at its LC_{25} and LC_{50} (Table 3). The treatment decreased significantly the weight of larvae on the 3rd (p = 0.0041) and 7th day (p= 0.0096) after treatment with the highest dose.

However, treatment with *L. nobilis* essential oil caused a significant decrease in the body volume in larvae treated with the two concentrations (LC_{25} and LC_{50} respectively) at 3rd (p=0.0023 and p=0.0031) and 7th day (p=0.0328 and 0.0094) after treatment. The body size is a pivotal trait for mosquitoes, because it can influence their blood-feeding ability, host attack rate and fecundity. All of these traits are important determinants of their potential to transmit diseases [46].

Dris et al. (2017) and Dris (2018) [25, 47] found that the morphometric parameters were decrease at different developmental stages of *C. pipiens* treated with *Ocimum basilicum* and *Mentha piperita* and *Lavandula dentata* respectively.

TABLE III. EFFECT OF L. *NOBILIS* EO (LC25 AND LC50) ON THE FRESH BODY WEIGHT (MG) AND BODY VOLUME (MM3) IN THE FOURTH INSTAR LARVAE OF C. *LONGIAREOLATA* (M ± SD, N=3 POOLS EACH CONTAINING 20 INDIVIDUALS)

Parameters	Time (Days)	Control	LC_{25}	LC_{50}
Volume (mm^3)	1	22.19 ± 4.45 A	16.48 ± 2.32 A	14.63 ± 1.91 A
	3	19.94 ± 5.44 A	14.14 ± 2.12 A	14.19 ± 2.45 B
	5	16.6 ± 2.21 A	14.14 ± 2.12 A	12.92 ± 2.16 A
	7	13.64 ± 2.13 A	12.83 ± 1.87 B	12.83 ± 1.87 C
Weight (mg)	1	6.055 ± 1.22 A	5.89 ± 0.76 B	4.79 ± 0.07 C
	3	5.96 ± 0.95 A	5.79 ± 0.27 B	4.69 ± 0.41 C
	5	5.94 ± 1.72 A	3.96 ± 1.30 B	2.91 ± 0.08 C
	7	5.04 ± 0.42 A	3.86 ± 0.06 A	2.82 ± 0.14 A

The different letters indicate significant differences among concentration treatments based on student test (p <0.05).

D. Effect on biochemical composition

The amounts of carbohydrates, lipids and proteins were estimated in the whole body from different periods of fourth instar larvae of *C. pipiens* using LC_{25} and LC_{50} of *L. nobilis* essential oil (Table 4).

The comparison of mean values shows that a significant increase in the protein amounts was recorded in larvae at 1st (p = 0.0007 and p=0.0002), 3rd (p = 0.0004 and p=0.0003), 5th (p = 0.0002 and p<0.001) and 7th day (p<0.001). Concerning the carbohydrate levels, a significant reduction was observed at all tested periods after treatment with the highest dose (p=0.0392, p=0.0435, p=0.0078 and p=0.00215 respectively). Finally, the lipid content was decreased in treated series (LC_{25} and LC_{50}) at 3rd day (p= 0.020 and 0.019), and only with the highest dose at 1st day (p=0.0095) as compared to control series.

Several researches indicated that the exposure of an organism to xenobiotic product can modify the synthesis of certain proteins (enzymes of biotransformation, proteins of stress) [48]. Protein synthesis is necessary for the maintenance of body growth and reproduction [25]. Proteins enter in various reactions such as the hormonal regulation and they integrated in the cell as a structural element at the same time as the carbohydrates and the lipids [49; 50]. In the present investigation, after treatment of fourth instar larvae of *C. longiareolata* with *L. nobilis* EO, a stimulating action on proteins was generally exhibited during the tested periods. This stimulation might be due to the induction of protein synthesis such as some enzymatic and non-enzymatic biomarkers.

The reduction of the lipid levels after treatment with *L. nobilis* EO may be due to their effect on the lipid metabolism, and due to the utilization of lipid reserves for energy generation as a result of induced stress [51].

Carbohydrates play a crucial role in the physiology of the insects; the rates of glycogen in tissues are related to the physiological events such as the reproduction, the moult and the flight [52]. *Agastache foeniculum* EO applied in *Tribolium castaneum* reduced the energy reserves [53]. Askar et al. (2016) [54] reported that the application of clove oil in adults of three *Sitophilus* species increased lipid and protein levels, while anise oil induce a reduction in the protein content in *S. granarius* and an increased in *S. oryzae*.

Many researchers have reported the depletion of larval energy reserves when exposed to several factors of stress (environmental, chemical and nutritional) [55- 56].

TABLE IV. EFFECT OF L. NOBILIS EO (LC25 AND LC50) ON AMOUNTS OF PROTEINS, CARBOHYDRATES AND LIPIDS (MG/INDIVIDUAL) FROM THE FOURTH INSTAR LARVAE OF C. LONGIAREOLATA AT DIFFERENT PERIODS (M ± SD, N = 3 POOLS EACH CONTAINING 20 INDIVIDUALS).

Content (µg/individual)	Time (Day)	Control	LC_{25}	LC_{50}
Proteines	1	139.11 ± 9.20 A	212.6 ± 0.75 B	239.6 ± 0.31 C
	3	133.77 ± 11.44 A	209.08 ± 3.67 B	217.1 ± 3.55 C
	5	128.8 ± 1.05 A	172.9 ± 3.80 B	220.8 ± 4.22 C
	7	116.16 ± 4.66 A	157.5 ± 2.17 B	219.68 ± 1.92 C
Lipids	1	68.05 ± 6.65 A	59.35 ± 9.15 A	43.21 ± 2.01 B
	3	67.35 ± 7.46 A	45.46 ± 2.04 B	43.14 ± 3.85 C
	5	52.82 ± 9.64 A	45.09 ± 2.14 A	40.26 ± 2.84 A
	7	44.36 ± 7.11 A	39.63 ± 0.76 A	38.42 ± 4.21 A
Carbohydrates	1	152.55 ± 12.08 A	126.56 ± 25.72 A	99.03 ± 19.54 B
	3	113.04 ± 13.71 A	107.01 ± 8.08 A	76.58 ± 6.81 B
	5	108.16 ± 7.19 A	93.43 ± 8.61 A	70.62 ± 7.13 B
	7	92.64 ± 5.92 A	82.80 ± 10.56 A	64.01 ± 8.02 B

IV. CONCLUSION

Phytoproducts possess different bioactive components that can be used as general toxicants against various larval stages of mosquitoes [57].

In the present study, it can be concluded that the essential oil of *L. nobilis* with Eucalyptol and Linalool as major compounds was found to exhibit potent larvicidal activity against *C. longiareolata* larvae. Moreover, the *L. nobilis* essential oil caused modification in morphometric parameters and biochemical composition.

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