Chemical Composition and Bioefficacy of Essential Oil from Bay Laurel Shrub (Laurales: Lauraceae) against *Culiseta longiareolata* (Macquart) (Diptera: Culicidae) Larvae[1]

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Abstract An essential oil extracted from bay laurel, *Laurus nobilis* (L.), was chemically characterized and tested against fourth-instar *Culiseta longiareolata* (Macquart) (Diptera: Culicidae). Percentage composition of the oil in *L. nobilis* was 0.96, and gas chromatography–mass spectrometry analysis identified 56 components with eucalyptol (25.62%), linalool (11.83%), methyl eugenol (10.07%), and camphene (10.18%) as the major constituents. Laboratory bioassays demonstrated significant larvicidal activity of the oil extract with cumulative median lethal concentrations (i.e., LC_{50}) of 203.7 parts per million (ppm) at Day 1, 171.9 ppm at Day 3, and 85.1 ppm at Day 7 after exposure. The essential oil also affected egg hatch and sex ratio of the progeny. These results suggest that the essential oil extracted from *L. nobilis* has potential for development as a management tactic directed against *Cs. longiareolata*.

Key Words *Culiseta longiareolata*, *Laurus nobilis*, essential oil

Essential oils extracted from plants have been evaluated and used as substitutes for synthetic insecticides (Liu 2015) and as repellents (Lee 2018; Mihajilov-Krstev et al. 2014) in mosquito vector management programs. Ovicidal and larvicidal activities of plant extracts that mimic insect growth regulators or disruptors have been the subject of a number of studies with mosquitoes (Djeghader et al. 2018; Dris et al. 2017a,b; Hamaidia et al. 2018; Hamaidia and Soltani 2014). Furthermore, the traditional use of local plants as insect repellents has been investigated in several ethno-botanical studies (Tisgratog et al. 2016). Recently, botanical products such as essential oils and aqueous extracts of plants have been suggested as viable management products (Al-Mekhlafi 2018; Aouinty et al. 2018; Dris et al. 2017a,b). There are no reported impacts on nontarget organisms (Ilahi et al. 2019; Ponsankar et al. 2016) and no development of resistance has been reported (Yuan et al. 2019). Moreover, essential oils have limited persistence under field conditions because of their volatility and biodegradability (Echeverría and Albuquerque 2019).

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Essential oils from *Ocimum basilicum* (L.) (Dris 2017a) and *Thymus vulgaris* (L.) (Bouguerra et al. 2017) have been chemically identified and show larvicidal activity against *Culex pipiens* (L.) larvae. Likewise, an essential oil extracted from *Lavandula dentata* (L.) possesses larvicidal activity against *Culiseta longiareolata* (Macquart) and *Cx. pipiens* (Dris 2017b). Yet the laurel bay, *Laurus nobilis* (L.), is well known for its various culinary and chemical properties. It is an evergreen shrub native to the Mediterranean region (Said and Hussein 2014) and has many applications in the culinary and food industry due to its antioxidant (Casamassima et al. 2017), antimicrobial (Fıçıcılar et al. 2018; Vilela et al. 2016), and antifungal (Peixoto et al. 2017) properties as well as for contributing to the photo-oxidative stability of olive oil (Taoudiat et al. 2018). Bay leaf oil has shown insecticidal activity against selected stored-product pests such as *Tribolium castaneum* (Herbst) (Chahal et al. 2016), *Rhyzopertha dominica* (F.) (Mediouni-Ben Jemâa et al. 2012), and *Trogoderma granarium* (Everts) (Tayoub et al. 2012), as well as against the mosquitoes *Aedes aegypti* (L.) (Tabanca et al. 2013), *Anopheles stephensi* (Liston), and *Cx. pipiens* (Verdian-Rizi 2009).

Our objectives in the present study were to chemically characterize the essential oil extracted from *L. nobilis* growing in Algeria and assay its larvicidal and ovicidal activity as well as the delayed effects (progeny sex ratio) on *Cs. longiareolata*, which is the most abundant mosquito species in the Tebessa region of Northeast Algeria (Bouabida et al. 2012). The results are directed to the continued development of efficacious mosquito vector management in that region.

Materials and Methods

Essential oil source and extraction. Naturally occurring *L. nobilis* plants were collected in March, April, May, June, and July of 2016 in Tebessa (Northeast Algeria). Plants were transported to the laboratory, washed in tap water, and then rinsed with distilled water. The shade-dried leaves were cut into small pieces. According to the methods of Dris et al. (2017a) and Bouguerra et al. (2018), the essential oil was extracted from leaves by hydrodistillation in a Clevenger-type apparatus for 3 h using 100 g of the plant part and 750 ml distilled water. The resulting decoction was dried over anhydrous sodium sulfate to remove residual water. The extract was placed in amber bottles and stored under refrigeration until used in analyses and bioassays.

Gas chromatography–mass spectrometry (GC–MS). This analysis was performed according to the procedure of Dris et al. (2017a). Briefly, 0.2 μl of essential oil was injected as the GC oven temperature was maintained at 60°C for 8 min and then gradually increased to 250°C for 10 min at a rate of 2°C/min. The injector temperature was set at 250°C. An HP-5MS (5% phenyl; 95% dimethyl-polysiloxane) column (30 m × 0.25 mm, 0.25-μm film thickness) was used with helium as the carrier gas. The components were identified based on their retention times (RT), determined with reference to a homologous series of normal alkanes and by comparison of their mass spectral fragmentation patterns with those obtained from the authentic samples and/or the MS library.

Mosquitoes. *Culiseta longiareolata* eggs and larvae were collected in 2016 from areas in Tebessa (Northeast Algeria) and reared as described by the World Health Organization (WHO 1996) in the laboratory (Faculty of Science, University of

Tebessa). Twenty larvae were maintained in 150 ml of water in a Pyrex container, at 25–27°C, with a photoperiod of 14:10 h (L:D). They were fed with a mixture of biscuit–dried yeast (75:25 by weight) as recommended by Rehimi and Soltani (1999). Water was replaced in each container every 3 d.

Bioassays. Whole oil rather than its main components was tested according to Khater (2013). Concentration–mortality response of *Cs. longiareolata* larvae to the essential oil was determined as per the standard procedures recommended by the WHO (1996). The plant extract was initially mixed in 1 ml of ethanol and then diluted in 150 ml of filtered water to final concentrations of 25, 50, 100, 150, and 200 parts per million (ppm). Twenty, fourth-instar *Cs. longiareolata* larvae (<8 h after molting) were placed in each container of solution, and each concentration was replicated six times. The positive control consisted of 1 ml of ethanol in 150 ml of water. After the 24 h of exposure, all larvae within each container were removed, rinsed with untreated water, and placed in clean water. Mortality was recorded after 24, 48, and 72 h.

Once the lethal concentration (LC) levels were established in the larval bioassays, the LC_{50} and the LC_{25} concentrations of the essential oil were used in assessing ovicidal activity according to the method of Su and Mulla (1998). Egg rafts obtained from the laboratory colony were placed in 100 ml of each solution and maintained at laboratory conditions in plastic containers. The number and percentage of eggs hatching from the egg rafts was recorded after 24 h. Control egg mortality was corrected according to Abbott (1925).

Sex ratio determination. As in the bioassays, fourth-instar larvae were placed in 150 ml of an appropriate concentration of the essential oil (25, 50, 100, and 150 ppm) and maintained under bioassay conditions until adult emergence, when the sex ratio was determined (Tabadkani et al. 2013). Each treatment was replicated three times with 20 larvae per replicate.

Statistical analyses. In each of the bioassays, control mortality was corrected by Abbott (1925). Concentration–mortality responses were determined using probit analysis (Finney 1971). The significance between different series was tested using one-way analysis of variance (ANOVA) followed by a post hoc Tukey's honestly significant difference test. All statistical analyses were performed using GraphPad Prism, version 6.01 (GraphPadSoftware, San Diego, CA, USA).

Results and Discussion

Essential oil yield and composition. Based on the total dry matter weight of the *L. nobilis* plant material, the hydrodistillation extraction demonstrated that the oil composition of the plants from Tebessa (Northeast Algeria) was 0.96 ± 0.45%. This yield was higher than the yield extracted from *L. nobilis* in Montecorice, Italy (0.57%) (Caputo 2017) and from Tunisia (0.58%), other areas of Algeria (0.46%), and Morocco (0.65%) (Mediouni-Ben Jemâa et al. 2012).

GC–MS analysis of the oil identified 56 compounds as homologous series of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes (Table 1). The major components were eucalyptol (25.62%) and linalool (11.83%). Mediouni-Ben Jemâa et al. (2012) identified 51, 55, and 40 compounds in the essential oils extracted from *L. nobilis* from Tunisia, Algeria, and Morocco, respectively. The main compounds identified

Table 1. Chemical composition of essential oil extracted from *L. nobilis*.

No.	RT (min)*	Constituents	Concentration (%)
Monoterpene			
Hydrocarbon monoterpene			
1	7.603	α -Thujene	0.54
2	7.988	α-Pinene	3.87
3	8.697	Camphene	0.31
4	10.283	Sabinene	7.34
5	10.393	2-β.Pinene	1.93
6	11.271	β-Myrcene	0.46
7	12.027	α-Phelandrene	0.53
8	12.374	Δ. 3 Carene	0.85
9	14.148	α-Terpinene	0.29
10	16.355	γ.Terpinene	0.61
11	17.647	α.Terpinolene	0.26
12	16.355	Trans-sabinene hydrate	0.32
13	33.546	Pseudolimonene	0.71
Oxygenated monoterpene			
14	15.685	Eucalyptol	25.62
15	17.647	Trans-Sabinene	0.32
16	19.358	Linalool	11.83
17	23.276	Borneol L	0.2
18	24.124	4-Carvomenthenol	1.43
19	27.796	Nerol	0.24
20	29.556	Linalyacetate	0.22
21	36.009	α-Terpinenylacetate	10.18
Sesquiterpenes			
Hydrocarbon sesquiterpene			
22	38.346	β.Elemene	0.73
23	39.248	α-Gurjunene	0.09
24	42.043	α-Humulene	0.11
25	43.759	Germacrene-D	0.24
26	44.038	β.-Selinene	0.12

Table 1. Continued.

No.	RT (min)*	Constituents	Concentration (%)
27	44.689	γ.-Cadinene	0.41
28	44.906	α-Bergamotene	0.12
29	45.778	α-Amorphene	0.19
30	45.951	Δ.-Cadinene	0.22
31	38.346	β.Elemene	0.73
32	45.778	α-Amorphene	0.19
33	46.838	γ-Murolene	0.18
34	55.933	Shyobunol	1.45
35	55.055	Calamenene	0.31
36	53.291	γ-Cadinene	0.43
37	54.01	Murolol	1
38	49.633	(+)Spathulenol	0.73
Oxygenated sesquiterpene			
39	46.376	Naphthalene	0.41
40	49.633	(+)Spathulenol	0.73
41	49.744	(-)-Caryophylleneoxide	0.32
42	50.371	Veridiflorol	0.56
43	50.94	(+)-Ledol	0.38
44	51.711	Torreyol	0.27
45	45.19	NaphthaleneTrans –γ Bisabolene	0.26
46	53.701	β.-Eudesmol	0.16
47	55.055	calamenene	0.31
Secondary alcohol			
48	47.474	α-compaen-11-ol	0.16
Phenylpropenes			
49	48.78	Elemicin	0.76
50	36.626	Eugenol	1.88
54	39.922	Methyleugenol	11.07
Others			
52	52.593	Isobutylcinnammate	0.61
53	53.065	3-chlorohomoadamantane	0.17

Table 1. Continued.

No.	RT (min)*	Constituents	Concentration (%)
54	47.176	(-)-Dehydroaromadendrane	0.23
55	40.38	Veratrolemethyl	2.73
56	22.302	Isobutylcaproate	0.09

* RT = retention time (min).

were 1,8-cineole, linalool, and isovaler aldehyde, although in variable proportions from each locale. The major component in the oil of Iranian *L. nobilis* was 1,8-cineole together with a-terpinyl acetate, terpinene-4-ol, a-pinene, p-pinene, p-cymene, linalool, and terpinene-4-yl-acetate (Mohammadreza 2010). Such variations in chemical composition of oils from *L. nobilis* from different locales may be due to geographic origin, genetic variability, growing conditions, plant part from which the oil was extracted, or any combination of these factors (Da Silveira et al. 2014; Fidan et al. 2019; Mediouni-Ben Jemâa et al. 2012). Da Silveira et al. (2014) also found that the relative composition of 1,8-cineole in the oils varied with whether the plant material was microwave-dried (58.80%), air-dried (35.62%), or from fresh leaves (42.90%). They also found (E)-β-cymene, β-longipinene, cadinene, α-terpinyl acetate, α-bulnesene, terpinene-4-ol (4.25%), and sabinene in the oils and reported that 1,8-cineol was the main component of oils extracted from *L. nobilis* from Iran, India, Lebanon, Turkey, and Italy (Da Silveira et al. 2014).

Larval bioassays. Our results demonstrate that the essential oil from *L. nobilis* has insecticidal activity against fourth-instar *Cs. longiareolata* larvae. The median lethal concentration (LC_{50}) decreased over time from the initial exposure with 203.7 ppm after 1 d, 171.0 ppm after 3 d, 85.1 ppm after 5 d, and 23.45 ppm after 7 d (Table 2). The larvicidal activity was not unexpected in that the essential oil from the Iranian *L. nobilis* has been identified as a natural larvicide against *An. stephensi* and *Cx. pipiens* (Mohammadreza 2010), and essential oils from plants of the genus *Laurus* have been defined as insecticides and acaricides (Kotan et al. 2008; Rozman et al. 2007). Tayoub et al. (2012) suggested that the mode of action may reside in the nervous system, but Sharifi-Rad et al. (2017) noted that there is likely a diversity of modes of action due to the different molecular targets of the various chemicals identified in the oils.

Ovicidal activity. Exposure of *Cs. longiareolata* egg rafts to the LC_{50} of the *L. nobilis* essential oil resulted in a mean of 75.9% of the eggs not hatching. This ovicidal activity was significantly higher ($F = 9.48$; df = 1, 18; $P = 0.006$) than that observed following exposure to the LC_{25} and the untreated control (Fig. 1). Essential oils from a variety of plants have exhibited oviposition deterrence and ovicidal activities against *Ae. aegypti*, *Anopheles dirus* (Peyton and Harrison), *Anopheles coluzzii* (L.), *Culex quinquefasciatus, Culex restuans*, and *Cx. pipiens* (Al-Mekhlafi 2018; FokoDadji et al. 2018; Mirza and Zehra 2018; Muturi et al. 2018; Rajasekar et al. 2016; Ramar et al. 2014; Siriporn and Mayura 2012).

Table 2. Concentration–mortality response of fourth-instar *Culiseta longiareolata* larvae to essential oil from *Laurus nobilis* essential oil against fourth-instar larvae of (LC = lethal concentration; ppm = parts per million; FL = fiducial limit).

Time (days)	Slope	R^2	LC_{25} (ppm) (95% FL)*	LC_{50} (ppm) (95% FL)*
1	0.34	0.98	132.80 (105.6–167.1)	203.70 (168.10–246.90)
3	0.38	0.92	81.96 (38.30–175.4)	171.90 (86.93–339.90)
5	0.23	0.94	31.25 (11.07–88.19)	85.10 (45.42–159.40)
7	0.24	0.93	8.21 (1.33–50.44)	23.45 (9.58–57.38)

* 95% fiducial limits.

Sex ratio effects. According to the ANOVA, the observed sex ratio of *Cs. longiareolata* adults that emerged from larvae treated with the *L. nobilis* essential oil was biased toward females (males versus females: $P < 0.0001$) in all concentrations of the essential oil tested (Table 3). We are not certain that these effects are the result of exposure to the essential oil alone because various other factors (e.g., differential mortality between sexes) may affect sex ratios in natural populations of insects (Tabadkani et al. 2013).

Conclusion. Our results demonstrate that essential oils from *L. nobilis* have potential for development as plant-derived insecticides to control *Cs. longiareolata* populations when directed to the larval stage. Additional research must be conducted on the mode of action of the oils, methods of application or exposure,

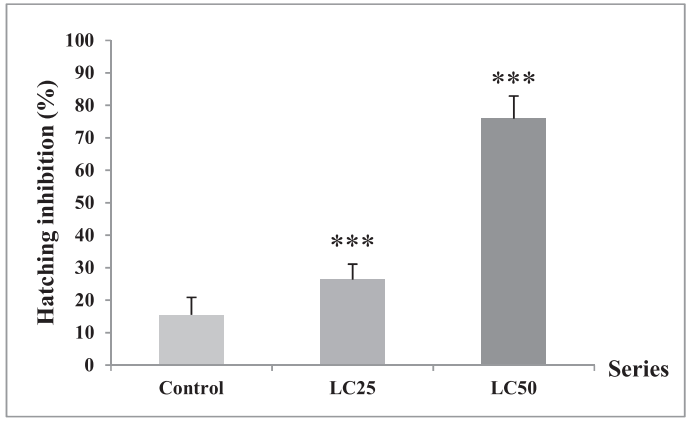

Fig. 1. Inhibition of egg hatch of *Culiseta longiareolata* following exposure to essential oil of *Laurus nobilis* (indicates significant difference from untreated control; $n = 10$ egg rafts).**

Table 3. Mean (± standard deviation [SD]) proportions of female and male sex ratio of *Culiseta longiareolata* following exposure of fourth-instar larvae to essential oil from *Laurus nobilis* essential oil ($n = 10$ rafts). For each concentration (parts per million [ppm]), mean values followed by the different lowercase letters are significantly different at $P < 0.0001$.

Concentration	Sex	Proportion (%)*
25 ppm	Female	63.36 ± 7.45a
	Male	36.64 ± 7.45b
50 ppm	Female	66.51 ± 7.17a
	Male	33.48 ± 7.17b
100 ppm	Female	72.97 ± 8.69a
	Male	27.02 ± 8.69b
150 ppm	Female	73.27 ± 11.56a
	Male	26.72 ± 11.56b

* Means (±SD) followed by the same lowercase letter are not significantly ($P < 0.0001$) different ($n = 10$ egg rafts).

efficacy and residual activity in natural environments, and impact on nontarget organisms including micro- and macroinvertebrates.

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