PHYTOCHEMICAL SCREENING AND BIOLOGICAL EFFECTS OF *LAURUS NOBILIS* (LAURACEAE) ESSENTIAL OIL AGAINST MOSQUITO LARVAE, *CULEX PIPIENS* (LINNEAUS, 1758) (DIPTERA: CULICIDAE) SPECIES

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Abstract. The present study aimed to determine the phytochemical compounds and to evaluate the activities of the essential oil (EO) of *Laurus nobilis* against mosquito larvae, *Culex pipiens*. The EO was extracted by hydro-distillation from *L. nobilis* leaves and subsequently, their chemical profile was identified by gas chromatography coupled with mass spectrometry (GC-MS) method. 39 compounds, representing 99.98% of the crude oil were identified; namely 1.8-cineole (22.42%), isolongifolene (10.22%), 3-carene (7.74%), Alpha-zingiberene (6.69%), Eremophilene (4.87%), Aristola-1 (10), 8-diene (3.09%) icyclo, sabinene (4.13%), α-pinene (1.94%) and Beta-caryophyllene (0.36%). Overall, 21 of these components, out of 39, were over 1%. The *L. nobilis* EO toxicity was tested at different concentrations, ranging between 5 and 35 µl/l, against third and fourth-instar of *Cx. pipiens* larvae. Mortality was recorded for both stages and their lethal concentrations (LC_{50} and LC_{90}) were estimated at 3.74 µl/l and 14.47 µl/l for the third-instar larvae and 18 µl/l and 39.08 µl/l for the fourth-instar larvae, respectively. The bioassay results showed a larvicidal effect with a concentration response–relationship. Furthermore, the compound effects, applied at LC_{50} and LC_{90} against the fourth-instars larvae showed an increase in the development duration and a disturbance in reproduction. The investigation confirmed the toxicological effects of *L. nobilis* EO against mosquito larvae; consequently, it could be considered as a potent source for producing natural larvicidal agents and bio-insecticides for pest an insect vector control.
Keywords: *toxicity, plant essential oil, larvicidal activity, medicinal plants, arthropods*

Introduction

Mosquitoes (Diptera: Culicidae) are the most widely distributed arthropods in the world and, in addition to causing a great annoyance, are considered the most significant arthropod vectors of diseases. The role of several mosquito species of the Culicidae family, in bites and pruritus of humans has doubled their medical importance (Salavati et al., 2021). Furthermore, the involvement of these vectors in the transmission of some

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pathogens among domesticated and wild birds and animals has put this family at the forefront of important medical and veterinary insects. It is known that several mosquito species belonging to the genera *Anopheles*, *Culex* and *Aedes* are the main vectors for pathogens of various diseases causing serious health problems with increasing mortality rate to human beings (Raja et al., 2018; Mouhamadou et al., 2020). *Culex pipiens* is recognized as the primary vector especially of arboviruses (arthropod-borne viruses), such as St. Louis Encephalitis (SLE), and it was the main force in spreading the West Nile virus (Lounibos, 2002). The domestic mosquito, *Cx. pipiens* is considered among the most abundant species in Algeria (Aïssaoui and Boudjelida, 2017). It is widely distributed in the urban and suburban areas, due to the presence of many artificial breeding sites practically throughout the year (Arroussi et al., 2021).

Pest populations control around the world is essentially dependent upon chemical pesticides and fumigants. Repeated uses of these expensive synthetic products have disrupted natural biological control systems and led to the resurgence of these pests, the development of insect resistance, and undesirable effects on the environment, non-target organisms, and human health (Minetti et al., 2020; Richards et al., 2020; Smith and Perfetti, 2020). Also, the increasing concern; over the pesticide residue level in different environmental sites and especially in food has encouraged the scientists to propose a new alternatives of conventional pesticides (Boudjelida et al., 2005). Although there has been much research on the potential of natural plants to control insect pests, the interest in herbal based products was subsequently reduced due to the advent of synthetic chemicals or insecticides. However, the interest in anti-mosquito products derived from natural origin is being revived with considerable attention because the continued applications of synthetic compounds have some drawbacks, including the widespread development of insecticide resistance among vector population and biological magnification through food chain (Rawani et al., 2010; Flores and O'Neill., 2018; Wilson et al., 2020). Scientists, whose investigating in the control of vector-borne diseases, thought mosquitoes can be easily targeted in their larvae stages, since they breed in water (Bouaziz et al., 2017; Djeddar et al., 2021; Dar and Jamal, 2021). Global scientific efforts were initiated to discover and to develop safe, effective, and sustainable alternatives to synthetic chemical insecticides (Muturi et al., 2017). Phytochemicals have a promising major role in mosquito control programs owed to their efficiency, biodegradability and lack of secondary effects (Amalraj et al., 2000; Gunasekaran et al., 2004).

In the last decades, human has extensively used various medicinal plant extracts, in particular essential oils, that therapeutic properties have tangled global attention (Dadalioğlu and Evrendilek, 2004). Furthermore, the larvicidal activity of plant essential oils against mosquitoes has been tested and showed toxic effects (Govindarajan et al., 2012). Many studies, using plant extracts (Nabti and Bounechada, 2019; da Costa et al., 2020), in order to explain their mode of action, have reported physiological changes that occurred in neuro-endocrine system function controlling insect behaviour, growth, molting process and metamorphosis (Djeghader et al., 2018; Corzo et al., 2020).

The aromatic plant *Laurus nobilis* belongs to the Laurel family, Lauraceae. It is one of the evergreen trees, cultivated in many warm regions of the world and its native territories are the southern Mediterranean countries (Marzouki et al., 2011). The bioactive plant ingredient(s) can be obtained from the whole plant or from a specific part. The leaf essential oil of the woody plant *L. nobilis* (Derwich et al., 2009), has been

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traditionally used as an effective remedy to treat some health problems related to epigastric bloating, flatulence and fungal diseases (Patrakar et al., 2012; Caputo et al., 2020). In Algeria, it is cultivated in different regions and its leaves and fruits are used in food, cosmetics and pharmaceutical industries. The plant is widely used by local people as a source of spice and for its medicinal properties. In addition, laurel essential oil is currently used in folk medicines for the treatment of different health problems, such as rheumatism and dermatitis (Kilic et al., 2004; Georgiev and Stoyanova, 2006). Its name is dedicated to Apollo, the ancient Greek sun god, that symbolizes peace and victory (Dmitry and Naida, 2019).

The purpose of the present study was to determine the chemical composition of essential oil of the local plant, *L. nobilis.* and to examine its toxicity against larval stages of *Cx. pipiens* mosquito and to evaluate some developmental and physiological effects in order to understand its mode of action.

Materials and methods

Rearing of mosquito larvae of Culex pipiens

The toxicological essays were conducted in the laboratory following the standard methods for testing larval susceptibility, according to World Health Organization (WHO, 1991). The mosquito larvae of *Cx. pipiens* were obtained from the laboratory colonies. They are reared under controlled conditions (temperature at 25 ± 2 °C and photoperiod cycle of 12 light/12 dark). Mosquito larvae are reared in Pyrex storage jars containing 1000 ml of stored tap water. Larvae were daily fed with fresh food composed of a mixture of Biscuit-dried yeast (75:25 by weight), till the pupal stage was reached. Subsequently, the resulting pupae were transferred, with the help of a dipper, to jars containing water and placed in a cubic cage (30 × 30 × 30 cm) covered with a net until adult emergence. The adults were fed with 10% sugar solution for a period of three days before they were provided by mobilized shaved mouse for blood feeding.

Plant material and essential oil extraction

Fresh aerial parts of *L. nobilis* were randomly harvested, during the study period from a high-plains region (Setif, Northeast Algeria; 36°03’N 5°31’E) and were transported to the laboratory for chemical analysis. After collection, the plant leaves were initially rinsed with distilled water, soaked in paper towel and air-dried for 24 h. A sample of 100 g of plant powder was hydrodistilled for 3 h using a Clevenger-type apparatus (Ildam, Ankara), and the obtained essential oil was stored at 4 °C in an amber bottle. The essential oil yield was estimated according to dry leaves mass which is estimated by the ratio between the weight of extracted oil and that of the treated plant, that is expressed as a percentage; using the following equation: Y (%) = (m/MO) × 100, where m represents the essential oil mass (g), MO represents the dry leaves mass (g) and Y is the essential oil yield (%).

Gas chromatography-mass spectrometry analysis

The plant *L. nobilis* EO was analyzed by gas chromatography-mass spectrophotometry (GC-MS) equipped with a 5% phenyl-methylpolysiloxane DB-5MS column (30 m length × 0.25 mm LD × 0.25 µm) and Agilent automatic injection system

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(Agilent GC 7890A and Agilent MS5975C – MSD. The chromatogram was produced by keeping oven temperature at 60 °C for 5 min initially, then the temperature was increased to 90 °C, at a rate of 2 °C/min and then to 90 °C at 5 °C/m for 40 min (Dadalioğlu and Evrendilek, 2004). As a result, MSD conditions were as follows: capillary direct interface temperature (280°C), ionization energy (70 eV), mass range (33-330 AMU), EM voltage (Atune + 200) and scan rate (5 scan/s). Herein, helium (He) was used as the gas carrier at a flow rate of 1.0 ml/min with injection of 0.2 µl of oil sample. Quantification was done by an external standard method using calibration curves generated by running GC analysis of representative compounds (*Fig. 1*). The essential oil components were identified by using the WILEY-NIST MS data library.

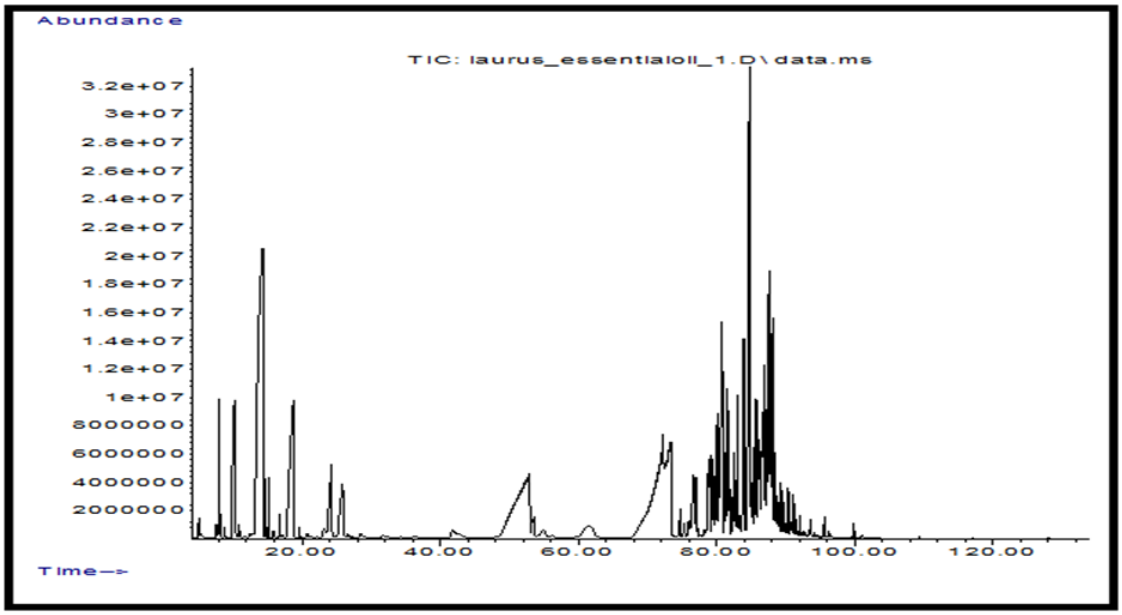

Figure 1. *Typical gas-chromatogram trace of the essential oil profile extracted from the leaves of the L. nobilis L*

Concentration–response larvicidal bioassay

Larvicidal bioassays, following the WHO standard protocols instructions (WHO, 2005) for determining the susceptibility or resistance of mosquito larvae to insecticides with suitable modifications, were carried out against the mosquito larvae, treated with the EO extracted from *L. nobilis*. Selected different concentrations of this EO (5, 10, 25 and 35 µl/l) from preliminary toxicological tests, were dissolved in 1 ml acetone and were added separately to the jars, containing 1000 ml of breeding water and 25 larvae of the third or the fourth instar of *Cx. pipiens*. For each larval stage, the experiments were done by using 3 repetitions per concentration with 24 h exposure times (WHO, 2005). Three other jars were left untreated with 1 ml of acetone as negative control replicates. After the exposure time, larvae were removed and placed in clean water. The observed mortality rates were recorded after 24, 48 and 72 h of post-exposure. The observed mortality, in 48 and 72 h, were expressed by the addition of the mortality at 24 h and 48 h, respectively. Dead larvae were identified when they stopped moving after probing the body with a needle.

Statistical analysis

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All statistical analyses were performed using Minitab and Prism 7.0 for Windows (Graph Pad Software Inc, USA) with a significance level of $p < 0.05$. The observed percentage mortality was corrected during the assays of the EO larvicidal potentiality. Data have been expressed by the mean ± standard deviation (M ± SD). All data were analyzed by one-way ANOVA analysis of variance; after angular transformation of observed mortality (%), in order to evaluate the differences between the control and treated series. The mortality (%) of each concentration were analyzed using a Probit model and the lethal concentrations (LC_{50} and LC_{90}), the upper and lower confidential limits and the slope were estimated as described elsewhere (Abbott, 1925; Swaroop et al., 1966).

Reproduction tests

Fecundity experiments were carried out on the eggs of *Cx. pipiens* females emerged from fourth larval stage, survived from treatment with the lethal concentrations estimated after 72 h of delayed mortality (LC_{50} = 7.18 µl/l and the LC_{90} = 39.08 µl/l) of the *L. nobilis* EO. For each lethal concentration only 10 females and 10 males were kept in separate breeding cages, and the longevity was recorded until the death of all adults. The laying eggs for each series were collected, counted and transferred to a new jar containing 1000 ml of breeding water for larval hatching. Different parameters of reproduction; the number of laid eggs, hatching rate and the fecundity were studied. The fecundity was calculated by the number of eggs laid in ovitrap divided by number of females let to mate. The significance of *L. nobilis* EO effect on reproduction activity was estimated using Student's t-test.

Results

Yield and chemical composition of L. nobilis essential oil

The essential oil extracted by hydrodistillation from leaves of *L. nobilis* exhibited a liquid color change from a dark green to yellow with herbaceous and a woody odor. The result of the obtained leaf-oil yield of the present study was 0.79% (w/w). The chemical composition of the essential oil was analyzed by GC-MS. 39 different components were characterized, during an elution time of 1 h 20 min (*Fig. 1*), accounting for 99.98% of the total EO composition and these were identified and their quantity were estimated (*Table 1*). The major EO components were mainly 1.8-cineole (22.41%), Isolongifolene (10.22%), 3-carene (7.74%), Alpha-zingiberene (6.64%), Eremophilene (4.87%), sabinene (4.13%), Euasarone (3.27%), Aristola-1(10), 8-diene (3.09), Gamma-terpinene (2.85%), Delta-selinene (2.36%), 2-carene (2.34%), α-pinene (1.94%), Beta-vatirenene (1.78%) and Beta-caryophyllene (0.36%). Other components existed in traces with less than 1%.

Toxicity assays

The toxicity effects of *L. nobilis* essential oil against the third and fourth instar larvae of *Cx. pipiens* was expressed by the corrected observed mortality, which was recorded at different periods during the treated developmental stage. The lowest used concentration of 5 µl/l of the EO caused mortality of 32% for the third stage (L3) and 25% for the fourth stage larvae (L4), after 24 h of exposure time. This observed mortality (%) increased in function of time and doses (*Fig. 2*). After 72 h, it was noticed that all treated third larvae were dead (100%) with the highest concentration of 35 µl/l of the EO, whereas for the fourth ones the mortality for the same dose was 85%. The

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data variance analysis showed a significant ($p < 0.001$) insecticidal activity with a concentration-response relationship (*Fig. 2*). With probit analysis, for the cumulated mortality after 72 h, the lethal concentrations (LC_{50} and LC_{90}) were estimated with their fiducial limits (95%) from the linear regression curves (*Fig. 3*) and are listed in *Table 2*. The non-linear regression curves (*Fig. 3*), expressed by the mortality probits and the logarithm of *L. nobilis* EO concentrations, reveal a very strong relationship between the corrected mortality (%) and the concentration logarithm for both treated stages. The results of regression analysis of used EO revealed that the mortality rate (Y) is positively correlated with the exposure dose (X) having a regression coefficient (R^2) close to 1 in each stage (*Table 2*). The lethal concentrations were estimated at, $LC_{50} = 3.74$ µl/l and the $LC_{90} = 14.47$ µl/l for the third larval stage and LC_{50} was 7.18 µl/l and the LC_{90} was at 39.08 µl/l, for the fourth larval stage (*Table 2*).

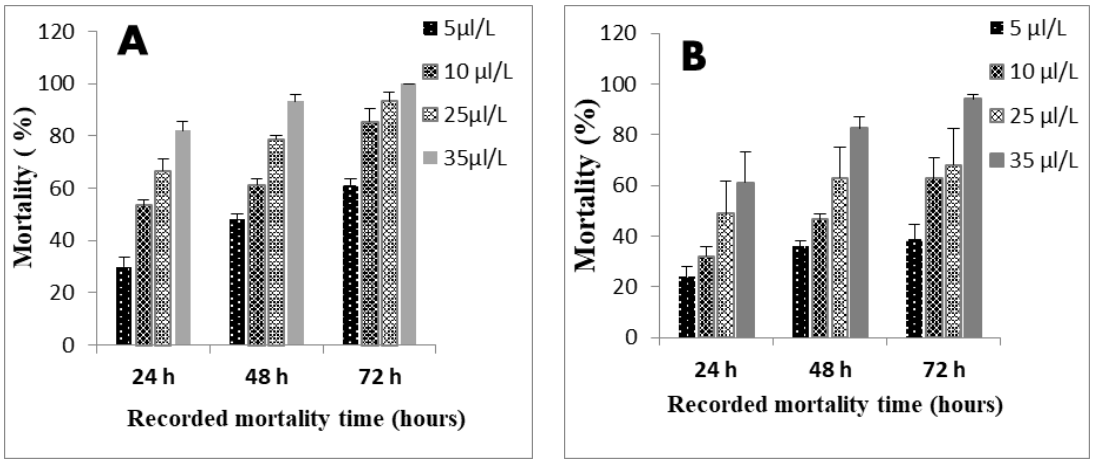

Figure 2. *Recorded mortality (%) with a concentration-response relationship of the treatment with the L. nobilis EO applied to newly exuviated larvae of Cx. pipiens. (Means ± SD, n = 75) (A = Third instar Larvae, B = fourth instar larvae)*

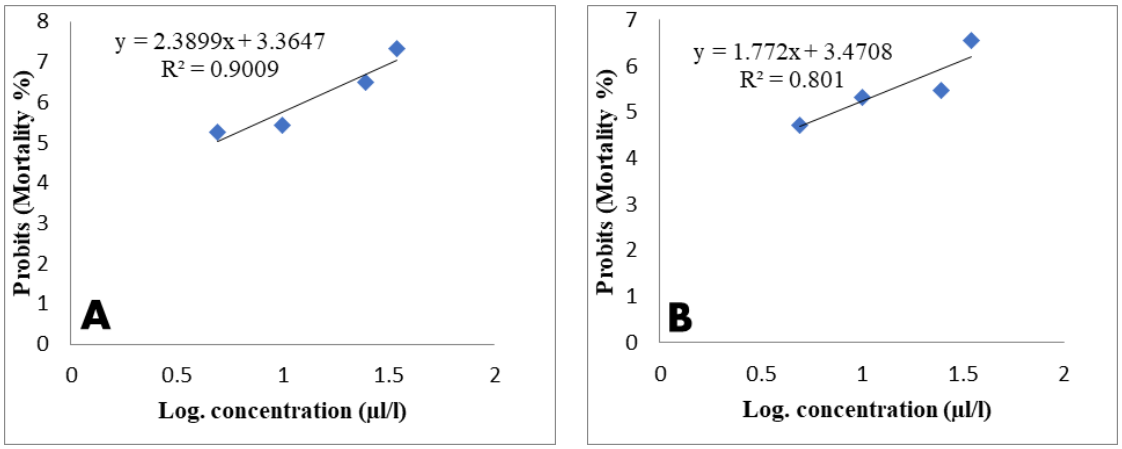

Figure 3. *L. nobilis essential oil applied to newly exuviated larvae of Cx. pipiens at different doses (µl/l): Linear regression dose-response expresses by the probits mortality (%) and the decimal logarithm of L. nobilis EO concentrations (µl/l). (A, B = Third and fourth larval stage of Cx. pipiens toxicity respectively)*

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Table 1. *Abundance (%) of L. nobilis essential oil components identified, using gas chromatography-electron impact mass spectrometry (GC-MS) and their retention time (RT: min)*

No.	Compound	Abundance (%)	RT (min)
1	α-pinene	1.943%	8.104
2	sabinene	4.137%	10.199
3	2-β-pinene	1.576%	10.398
4	1,8-cineole	22.417%	14.326
5	b-ocimene	0.482%	14.611
6	γ -terpinene	0.595%	15.217
7	3-carene	7.745%	18.746
8	Gamma.-terpinene	2.857%	25.911
9	2-carene	2.342%	76.703
10	α-caryophyllene	1.289%	76.703
11	α-gurginene	0.697%	76.977
12	Ethyl (e)-cinnamate	1.052%	78.835
13	(e)-germacrene d	1.648%	79.083
14	Aromadendrene	1.630%	79.545
15	Delta.-selinene	2.364%	80.125
16	Alpha-zingiberene	6.694%	80.753
17	Beta-vaterene	0.545%	81.197
18	Alpha-amorphene	1.512%	81. 448
19	Gamma-murolene	0.612%	81.551
20	Delta-cadenene	1.149%	81.784
21	Beta-humulene	1.040%	82.468
22	Cis-alpha-bisabolene	2.302%	83.015
23	Euasarone	3.270%	83.953
24	Isolongifolene	10.223%	84.763
25	Neoisolongifolene	0.752%	84.907
26	Bicyclogermacrene	0.752%	85.313
27	Junipene	1.010%	85.742
28	1.1.4.4. Tetrametryl-2-tetralone	0.653%	86.034
29	Valencene 1	0.920%	86.159
30	Caryophyllene	0.815%	86.529
31	Trans-caryophyllene	1.312%	86.669
32	Isoamylcinnamate	0.719%	86.751
33	Ethoxymethyl	1.582%	86.873
34	Caryophylladienol i	0.935%	86.932
35	Beta-vateranene	1.786%	87.238
36	Eremophilene	4.879%	87.582
37	Aristola-1(10),8-diene	3.079%	88.096
38	Dehydroaromadendrene	0.326%	88.469
39	Beta-caryophyllene	0.360%	89.348
Total		**99. 98%**	

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Table 2. *Toxicity of L. nobilis essential oil against Third (L3) and fourth (L4) instar larvae of Culex pipiens. Lethal concentrations (LC_{50} and LC_{90}, Fiducial limits (FL), µl/l)*

Larval stage	Regression equation	Slope	LC_{50} (µl/l) FL (95%)	LC_{90} (µl/l) FL (95%)	R^2	*P*
L3	y = 2.3899x + 3.3647	0.44	3.33 < **3.74** > 4.33	12.47 < **14.47** > 16.78	0.9009	0.0001
L4	y = 1.772x + 3.4708	0.46	5.93 < **7.18** > 8.68	32.34 < **39.08** > 47.19	0.801	0.0001

Effect of L. nobilis essential oil on the longevity of mosquito developmental stages

The result of *L. nobilis* essential EO effect on the longevity of the different developmental stages of *Cx. pipiens* after treatment of the third and fourth instar larvae, with their lethal concentrations (LC_{50} and LC_{90}) respectively are mentioned in *Table 3*. The exposure of the new exuviated larvae to the EO caused significant difference ($P < 0.05$) in the duration of the treated third and fourth larval stage, with both lethal doses compared to control. During the following pupal stage, no difference was recorded in the duration, after treatment with the used lethal concentrations. However, the adult longevity of mosquito species *Cx. pipiens* was considerably reduced ($P < 0.005$) by the *L. nobilis* EO treatment**.** The longevity of adults of C*x. pipiens* emerged from the treated fourth-instar larvae series, was reduced up to 19 days with LC_{90} and to 21 days for LC_{50}, whereas it was recorded 25 days for the control ones.

Table 3. *Effect of the L. nobilis EO on the longevity of developmental stages (days); after treatment with the lethal concentrations (LC_{50} & LC_{90}), of 3rd and 4th instar larvae of Cx. pipiens*

Stage	Duration of the developmental stages (day) of *Cx. pipiens* after treatment with lethal concentrations (LC_{50} & LC_{90}).				
	Third instar larvae (L3)			Fourth instar larvae (L4)	
	Control	LC_{50} = 3.74 µl/l	LC_{90} = 14.47 µl/l	LC_{50} = 7.18 µl/l	LC_{90} = 39.08 µl/l
L3	4.60 ± 1.33	4.60 ± 2.36	5.16 ± 2.5		
L4	6.15 ± 1.88[a]	6.80 ± 1.19	7.33 ± 1.13[a]	7.76 ± 1.33[a]	8.50 ± 2.66[a]
Pupae	3.13 ± 1.66	3.43 ± 1.43	3.43 ± 1.33	3.23 ± 1.33	3.23 ± 1.33
Adult	25 ± 1.23[a,b]	21.53 ± 2.33[a]	20.5 ± 2.23[b]	21 ± 1.33[a,b]	19.53 ± 3.66[a,b]

The duration (means ± SD) followed by the same letter indicates a significant difference ($P < 0.05$). (n = 10-70)

Effect of the L. nobilis essential oil on reproduction

The effect of *L. nobilis* EO on the female reproduction emerged from the treated fourth instar larvae of *Cx. pipiens*, was evaluated using different parameters (*Table 4*). Fecundity was highly reduced ($P < 0.005$) after the treatment with the *L. nobilis* EO. The number of eggs laid was inversely proportional to treatment concentrations. In control females, the number was 672 eggs, whereas it was 583 and 299 eggs, in treated females treated with the two lethal concentrations (LC_{50} and LC_{90}) respectively (*Table 4*). The same effect was observed, in the hatching process, with a reduction of the hatching rates. This showed a significant reduction ($P < 0.005$) according to the increase in the concentration (*Table 4*). The percentage

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of hatching eggs laid by the females emerged from the treated larvae of *Cx. pipiens* was significantly reduced ($p \leq 0.001$) comparatively to control, with a rate of 73.33% with LC_{50} and 55.2% with CL_{90}, moreover the calculated fecundity percentage showed a decrease at 59.4% and 44.8% for the treated series, with LC_{50} and LC_{90} respectively (*Table 4*).

Table 4. *Effect of L. nobilis EO on reproduction of the females emerged from the treated fourth instar larvae of Cx. pipiens (n = 10 females)*

Treatment	No. of eggs laid	Hatching rate (%)	Fecundity (%)
Control	672[a]	98.66[a]	96.6[a]
LC_{50} = **7.18** µl/l	583[a,b]	73.33[a,b]	59.4[a,b]
LC_{90} = **39.08** µl/l	299[a,b]	55.02[a,b]	44.8[a,b]

Numbers followed by the same letter indicate a significant difference ($P < 0.005$)

Discussion

Yield and chemical composition of the essential oil

The essential oil extraction from dry *L. nobilis* leaves plant, collected from the study area (high-plains region, Setif, Algeria), demonstrated that the essential oil yield was 0.79% (w/w) which confirm the result previously reported (Elharas et al., 2013). Whereas the EO yield of the same plant from Tebessa (Northeast Algeria) was 0.96% (Bouzidi et al., 2020). These EO yields from the Algerian local plants of *L. nobilis*, were higher than the yield extracted from *L. nobilis* from Montecorice, Italy (0.57%) (Caputo et al., 2017), Tunisia (0.58%) and Morocco (0.65%) (Mediouni-BenJemaâ et al., 2012). While this finding showed a lower yield than those reported elsewhere, with the value of 1.13% (de Oliveira et al., 2021) and 1.3% (Ouibrahim et al., 2013). The varied essential oil yield is based on the quality of the used plant material, combined with other factors, such as the growth stage, soil quality, climate conditions, time of harvest and drying period (Mohammedi et al., 2020). Indeed, methods of drying aromatic and medicinal plants can affect the yield and the chemical composition percentages, mainly in the quantitative aspect of essential oils (Sellami et al., 2011; Abdelmajeed et al., 2013). The non-dried plant under good conditions may be degraded, and consequently can lose some of its entire essential oils (Sarić-Krsmanović et al., 2020). As such, reports on the chemical compositions of *L. nobilis* EOs indicated variations both quantitatively and qualitatively. This study, for instance, identified 39 EO components, or 99.98% of the total oil content, whereas a previous study (Caputo et al., 2020) has identified 55 compounds accounting for 91.6% of the total essential oil; including 1.8 cineole (31.9%), sabinene (12.2%), linalool (10.2%), α-terpinylacetate (5.9%), α-pinene (5.8%), α-terpineol (3.3%), methyl-eugenol (3.3%), neoiso-isopulegol (2.5%), eugenol (1.6%), β-pinene (1.4%) and γ-terpinene (1.0%). This mixture of chemicals is almost similar qualitatively to the one reported previously (Marzouki et al., 2009) but still incompatible with our sample.

Based on the recent available literature, the comparison of the chemical composition of *L. nobilis* essential oil under investigation and that of other areas showed substantial similarities and differences. The percentage of 1,8-cineole was found as 22.41%, which is closely related to EOs observed in the laurel plants from Algeria (24.65%) (Miliani et al., 2017), Greek (30.08%) and Gorgia (29.2%) (Galina et al., 2020). Moreover, higher

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percentages of 1.8 cineole were recorded from some Mediterranean countries, Turkey (44.97%), Tunisia (56.0%) (Nabila et al., 2020) and Morocco (52.43%) (Nafis et al., 2020). In the present work the amount of other constituents, such as the sabinene was found 4.137% which is higher than that found in the essential oils yield previously reported and lower than that reported elsewhere (Miliani et al., 2017) with 6.13%. Also, it is important to notice that the linalool component that was found with different percentages in previous studies (Miliani et al., 2017), was absent in our analysis. This result is in accordance with other analysis where linalool has not been found (Derwich et al., 2009). The content of 1.8-cineole (22.41%) is by far the most dominant component of *L. nobilis* EO from Setif area (Northeast Algeria) besides that of Tizi Ouzou (Northeast Algeria) exhibiting 35.5% of the total essential oil composition (Mohammedi et al., 2020). Beside the difference of the plants geographical origins (Sangun et al., 2007), the reported differences in the EO composition and yield, in this study, might be attributed to environmental conditions, harvest and postharvest processing factors (Galina et al., 2020).

Insecticidal activity of L. nobilis L. essential oil

Use of natural plant origin products, with insecticidal properties have been encouraged in the control of a variety of insect pests and vectors. Mosquitoes in the larval stage are the main attractive targets for control because they breed in water, and thus, it is easy to deal with them in this habitat. Many studies have reported the plant extract larvicidal effect against mosquito larvae (Prabhu et al., 2011). The crude extract obtained from the *Leucas aspera* leaf showed a larvicidal activity against *Culex quinquefasciatus*, *Aedes aegypti* larvae (Maheswaran et al., 2008; Govindarajan, 2009) and *Anopheles stephensi* larvae (Govindarajan, 2011). Other works have reported the *Cassia fistula* extracts of the larvicidal toxicity and ovicidal and repellent activities against *Aedes aegypti* (Govindarajan, 2009). The *Acalypha indica and Citrullus vulgaris* leaf extract effectiveness was tested too on larvicidal, ovicidal activity against *Anopheles stephensi* (Mullai et al., 2008). The bioassays using the *L. nobilis* essential oil, against the larval stages of the mosquito species *Cx. pipiens* showed a larvicidal activity expressed by a high larval mortality of the treated series compared to the control ones. The difference in sensitivity to plant extracts appears within the mosquito genus (genera: *Culex*, *Aedes*, *Ochlerotatus*, or *Anopheles*) that would be caused by behavioral and physiological variations of different species (Saxena and Tikku, 1990). The *L. nobilis* EO exhibits also high effective larvicidal activity in the newly exuviated third and fourth stage larvae of *Cx. pipiens*, along with mortality rates range with a dose-response relationship. the same efficacy was reported using the same plant extract against *Culiseta longiareolata* (Elharas et al., 2013).

In the present study**,** the *L. nobilis* essential oil treatment reduced the larval duration, of the third and fourth larval stages. Therefore the developmental time of pupal and the emerged adult were not affected. Also the emerged adults, showed a significant reduction in their longevity. The adult which emerged from treated larvae were morphologically normal but showed a great reduction in fecundity. The same results were mentioned against *Aedes aegypti*, using EO plants (Warikoo, et al., 2011) and *Cx. pipiens* (Djeghader, et al., 2018; Djeddar, et al., 2021) using plant extracts. From the present study it was concluded that the *L. nobilis* EO showed a promising larvicidal agent against mosquito larvae and also reduced the longevity of different developmental stages, egg productions and fecundity.

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Conclusion

The essential oil extracted from *L. nobilis* with an interesting yield contains many important components. The bioassay investigation confirm that this EO exhibited potent larvicidal activity, a development disturbance and reduction in fecundity against *Cx. pipiens.* This plant essential oil proves to be a promising alternative as an efficient bio-insecticidal agent. The potential effect of this essential oil is reasonable with the presence of beneficial phytochemicals like 1.8-cineole, isolongifolene, 3-carene and sabinene as well as some minor constituents, which may play a disruptive role.

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