COMPONENTS AND TOXICOLOGICAL EFFECTS OF *MYRTUS COMMUNIS* L. (MYRTALES: MYRTACEAE) ESSENTIAL OIL AGAINST MOSQUITO *CULEX PIPIENS* L. (DIPTERA: CULICIDAE)

YEZLI, A.[1*] – BOUDJELIDA, H.[1] – ARROUSSI, D. E. R.[1,2]

[1]*Laboratory of Applied Animal Biology, Department of Biology, Faculty of Sciences, Badji Mokhtar University, Annaba 23000, Algeria*
(e-mail: boudjelidahamid@yahoo.fr; phone: +213-780-387-606)

[2]*National University of Forests, Khanchela 40000, Algeria*
(e-mail: arroussi.rachid@yahoo.fr; phone: +213-667-830-807)

**Corresponding author*
e-mail: yezliamina@gmail.com; phone: +213-699-034-984

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Abstract. The purpose of the present study was to determine the chemical composition of *Myrtus communis* L. essential oil (EO) and to assess its effectiveness against newly exuviated fourth instar larvae and adults of *Culex pipiens*. The EO of the plant was extracted by hydrodistillation and its chemical profile has been analyzed by gas chromatography coupled with mass spectrometry (GC-MS) method. During this analysis, 36 Compounds were identified and the main components were α-Pinene (50.81%) and 1.8-Cineole (18.98%). The EO toxicity was tested using several concentrations (100, 200, 400, 600, 700 µl/ml) and lethal concentrations (LC_{50} and LC_{90}) were estimated at 329.5 µl/ml and 737.6 µl/ml against fourth-instar larvae. The adulticidal effect of this EO was tested using different concentrations ranging between 0.15 and 1.25 µl/ml air and their lethal concentration values were estimated. The EO of *M. communis* L. applied at its LC_{50} and LC_{90} against fourth-instar larvae and the effect action pathway was studied using the acetylcholinesterase (AChE) and glutathione S-transferase (GSTs) biomarkers analyzes. These Biomarkers analyzes have confirmed the neurotoxic activity and activation of the detoxification system. It was concluded that *M. communis* EO exhibits insecticidal effects against *Cx. pipiens* and could be considered as a potential alternative to conventional insecticides.
Keywords: *phytochemical screening, plant essential oil, insecticidal activities, biomarkers, medicinal plants*

Introduction

Mosquitoes are a major public health concern worldwide as they transmit many pathogens to humans and other vertebrate animals (Krishnappa et al., 2019). They are recognized as the primary vectors of various significant diseases, such as malaria, filariasis, dengue fever, yellow fever, chikungunya, West Nile virus, Zika virus and other arboviruses; that can lead to encephalitis, bacteriosis, and helminthiasis (Amraoui et al., 2019). These pathogens are among the most significant health concerns globally (Hamama et al., 2022). The domestic mosquito, *Culex pipiens* is considered as the most dominant with a large distribution in urban areas in Algeria. A culicidian systematic study, in North-East of Algeria (Annaba region) revealed an abundance of 55.83% of this species and a their presence was recorded through the all year because of the temperature rising due the climate changes (Arroussi et al., 2021). Mosquito-borne diseases are a growing global health challenge, threatening more than 40% of the

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world's population, by 2050 and half of them will be exposed to arbovirus transmission (Jone et al., 2021). Due to the lack of vaccines and no treatment for these diseases, prevention strategies focus on controlling larval and adult mosquito populations with insecticides and repellents that were considered the most effective approach for reducing the mosquito proliferation (Jone et al., 2021).

Synthetic and neurotoxic insecticides are commonly used to control mosquitoes (Ioannou et al., 2021). However, in recent decades, the development of insect resistance and adverse effects on non-target organisms, soil, water, and air have increased significantly (Johnson et al., 2023) and became a real health and environmental problems. Scientists are looking forwards to develop and to propose new alternatives, instead of the used conventional pesticides, such as a natural plant extracts, called botanical insecticides or bioinsecticides (Bendjedid et al., 2021; Aïssaoui et al., 2022). Many bioassays using plant extracts and essential oils (EOs), from different plant parts are tested against different insect orders (Yang et al., 2020). According to these positive results, insecticides of botanical origin are increasingly being used as safe alternatives to the conventional chemical and synthetic insecticides for pest control, in both agriculture and public health sector, a sustainable and ecologically friendly manner (Zahoor et al., 2020; Ma et al., 2023).

EOs are mainly odorless volatile compounds produced spontaneously by plants as secondary metabolites for purposes other than feeding (i.e., protection or attraction) (Nenaah et al., 2022). EOs extracted from plants can act as insect growth disruptive (Abdel Haleem et al., 2022), repellents, synergists (El-Wakeil, 2013) or as phagodeterrents (Bibiano et al., 2022). Due to their lipophilic properties, they can enter the interior of insects and interfere with their biological, physiological, and neurological systems, resulting in metabolic disorders and death (Taffar et al., 2021).

Myrtus communis is a medicinal and aromatic species belonging to the Myrtaceae family that is endemic to the Mediterranean region (Tuberoso et al., 2010). Previous studies have confirmed that *M. communis* has anti-inflammatory (Amira et al., 2012), antiseptic (Amensour et al., 2010), antibacterial and antifungal properties (Bouzabataa et al., 2014). Also some plants exhibit other activities, such as an anti-protozoal (Belmimoun et al., 2016), antioxidant (Aidi et al., 2010), hypoglycemic (Onal et al., 2005) and other medicinal uses (Hennia et al., 2018).

The current study was aimed to determine the chemical composition of the EO of *M. Communis* and to evaluate its larvicidal and adulticidal effects towards a disease vector *Culex pipiens*. In order to give additional information on its mode of action, the activities of the biomarker of neurotoxicity (AChE) and the detoxifying enzyme (GSTs) were measured and interpreted.

Materials and methods

Plant collection

Fresh leaves of *Myrtus communis* (Myrtales: Myrtaceae) were collected from Seraidi area; (Northeast Algeria, Annaba: 36° 55' 53" North 7° 43' 26" E), and identified according to the voucher specimen (N° PH009-03) which is deposited in the herbarium of the Department of Botany (Faculty of Science, University of Badji Mokhtar Annaba, Algeria). The leaves of this plant remain green during the all year, but the spring period remain the best time for sampling in order the get better oil yield.

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The essential oil extraction

The essential oil extraction is carried out using hydrodistillation process, using a Clevenger 19 type apparatus. It consists by immersing the cut fresh leaves of *M. communis* (100 g) directly into the bottle filled with distilled water (1500 ml). The whole is boiled for 3 h, then the essential oil is separated from the aqueous phase in the presence of anhydrous sodium sulfate, in order to remove all traces of water and then it was stored in a small opaque bottle at 4°C in refrigerator. The used plant yield is calculated according to the French standardization association (AFNOR) formula (AFNOR, 2000): Y % oil (w/w) = weight of essential oil (g) / weight of plant material (g) × 100.

Gas chromatography-mass spectrometry analysis

The Essential Oil (EO) Analysis was performed using Gas chromatography-mass spectrometry (GC/MS) with an HP (Hewlett Packard) Agilent 6890 plus equipped with a HP- 5MS column (a length of 30 m × internal diameter of 0.25 mm, and 0.25 µm film thickness). The column oven temperature was set at 60°C for 8 min and then increased to 250°C at the rate of 2°C/min. The injector and detector temperatures carrier gas was helium, which flows through the column at 0.5 ml/min and the split ratio was set to 50:1 with injection of 0.2 µl of oil sample. The component quantification was done by an external standard method using calibration curves generated by running GC analysis of representative compounds. The mass spectrometry (MS) analysis was performed with a HP spectrometer (Hewlett Packard Agilent 5973) that operated at 70 eV.

The constituent's identification was based on a comparison of retention times with those of corresponding reference standards using the NIST 02 and by comparison of the retention index to n-alkanes of the components with published data (Adams, 2007).

Mosquito rearing

The mosquito colonies of *Culex pipiens* were kept in the Animal Biology Laboratory inscterium. The rearing was carried under laboratory conditions with a temperature of 25 ± 2°C, the relative humidity of 70 ± 5% and with a photoperiod of 14 h light and 10 h darkness as previously described (Rehimi and Soltani, 1999).

Larvicidal test

The effects of this essential oil were tested in a jars containing 100 ml of dechlorinated stored water. Three repetitions were carried out for each concentration, each consisting of 25 newly exuviated fourth-stage larvae. A stock solution of the selected concentrations was prepared by dissolving 0.01 g of *Myrtus communis* essential oil in 1 ml of ethanol solvent. After a screening test, newly exuviated 4th instar larvae of *Cx. pipiens* were exposed to different selected concentrations for 24 h, following the World Health Organization (WHO) standard procedure (WHO, 2005). The used concentrations were 100, 200, 400, 600, and 700 µl/ml. A parallel control series was conducted using ethanol. Lethal concentrations (LC_{50} and LC_{90}), 95% confidence limits LC, and the Hill slope of the concentration-mortality curve were calculated (*Table 2*).

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Adulticidal test

After a preliminary screening, *Myrtus communis* L EO was applied (by fumigation) at different concentrations: 0.15; 0.25; 0.5; 0.75, 1 and 1.25 µl/ml of air on a filter paper disk of 2.5 cm in diameter (Khani and Besayand, 2012). Female adults of *Cx. pipiens* were introduced into glass bottles of a 250 ml capacity. Three replicates of 10 individuals were made for each concentration. A control series was conducted in parallel. Mortalities were recorded at 1 h after treatment and observed mortalities were corrected according to the formula of Abbott (1925). Lethal concentrations and their confidence limits (95% LC) were calculated with GRAPH PAD PRISM 6 software.

Biomarkers activities

The fourth-instar larvae from the control and treated series, with LC_{50} and LC_{90}, were used to determine the enzyme activity of acetylcholinesteratse (AChE) and glutathione-S-transferases (GSTs). The tests were performed on the treated and control larvae sampled at 24, 48, 72, and 96 h after treatment with two previously determined lethal concentrations (LC_{50} = 329.5 µl/ml, LC_{90} = 737.6 µl/ml) of *M. communis* EO. The assays were performed with four replicates, each comprising 15 individuals and the same for the control series.

AChE activity was assessed in accordance with the method of Ellman et al. (1961), using acetylthiocholine as a substrate, The samples were homogenized in 1 ml of detergent solution (1 mM EGTA / 1% triton X / 1 M NaCl / 0.01 M Tris, pH 7). After centrifugation (5,000 rpm for 5 min), the activity of AChE was measured on an aliquot of 100 µl of supernatant to which 100 µl of 5,5'-dithiobisnitrobenzene (DTNB) acid and 1 ml of Tris buffer (0.1 M, pH 7) were added. After 5 min of reaction, 100 µl of substrate (acetylthiocholine) was added. Optical density was measured at a wavelength of 412 nm every 4 min for 20 min. The specific activity of GSTs and AChE are expressed in µM/min/mg of protein. The assay of GSTs was carried out according to Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as an artificial substrate, as previously described (Afnor, 2000). The samples were homogenized individually in 1 ml of phosphate buffer (0.1 M, pH 6). After centrifugation (14.000 rpm for 30 min), an aliquot of 200 µl of the supernatant was added to 1.2 ml of a mixture of CDNB (1 mM)/GSH (5 mM) substrate in phosphate buffer (0.1 M, Ph 6). The absorbance readings were taken, every minute, for 5 min at a wavelength of 340 nm.

Data analysis

Statistical analyses were performed using Prism version 6 for Windows (Graph Pad Software, La Jolla, CA, USA, www Graphpad.com), and $p < 0.05$ was considered to be a statistically significant difference. Data have been expressed by the mean ± standard deviation (mean ± SD). All data were verified by the Brown-Forsythe test and analyzed by one-way ANOVA and two-way analysis of variance (ANOVA). A Tukey post-hoc analysis HSD test was used to evaluate differences between the control and treated series.

Results

Yield and chemical composition of essential oil

The estimated average essential oil yield from the hydrodistillation was 0.63 g from 100 g of the plant *M. communis* and the percentage of essential oil yield was 0.62%

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(w/w). The isolated essential oil, from *M. communis*, was analyzed by GC/MS. A total of 36 chemical constituents were identified in the essential oil constituting 99.034% of the total content (*Table 1*). The essential oil profile is characterized by a high amount of α-Pinene (50.817%), followed by 1,8-Cineole (18.982%), then D-Limonene (8.135%), other compounds like Linalool (4.871%), α-Terpineol (2.620%), and Geranyl acetate (1.870%) were identified as minor components.

Table 1. *Chemical composition of Myrtus communis leaves essential oil analyzed using GC/MS*

No.	Compounds	Concentration (%)	RT (Min)	RI ^{lit}	RI^{exp}
1	n-Butyl isobutyrate	0.190	8.56	911	912
2	α-Thujene	0.135	9.23	930	924
3	α-Pinene	50.817	9.84	939	934
4	Sabinene	0.213	12.04	975	972
5	β-Pinene	0.062	13.05	979	989
6	α-Phellandrene	0.287	13.84	1002	1002
7	δ-3-Carene	0.332	14.2	1011	1007
8	α-Terpinene	0.088	14.67	1017	1014
9	para- cymene	0.425	15.3	1024	1023
10	D-Limonene	8.135	15.75	1029	1029
11	1,8-Cineole	18.982	15.93	1031	1032
12	(E)-β-Ocimene	0.161	16.95	1050	1046
13	γ-Terpinene	0.505	17.59	1059	1056
14	Terpinolene	0.620	19.65	1088	1085
15	Linalool	4.871	20.86	1096	1102
16	Hotrienol	0.158	21.05	-	1105
17	Endo-Fenchol	0.032	21.52	1116	1111
18	Trans-Pinocarveol	0.189	23.25	1139	1136
19	Trans-Sabinol	0.058	23.86	1188	1190
20	Terpin-4-ol	0.336	26.02	1117	1175
21	α -Terpineol	2.620	27.12	1188	1190
22	Methyl Chavicol	0.082	27.58	1188	1190
23	Trans-Sabinene acetate	0.490	31.68	1256	1256
24	α -Terpinyl acetate	0.337	37.81	1349	1347
25	Geranyl acetate	1.870	40.26	1381	1385
26	Methyl eugenol	1.571	41.55	1403	1405
27	(E)-Caryophyllene	1.714	42.09	1419	1414
28	α -Humulene	0.387	44.17	1454	1448
29	Neryl propanoate	0.030	48.16	1454	1458
30	Durohydroquinone	1.117	48.53	-	1520
31	Flavesone	0.093	49.83	1547	1542
32	Germacrene B	0.697	50.35	1561	1551
33	Geranyl butanoate	0.128	51.17	1564	1565
34	Caryophyllene oxide	0.630	51.92	1583	1578
35	Humulene epoxide II	0.136	53.41	1608	1604
36	Tasmanone	0.538	60.52	1727	1734
	Total identified	**99.034**			

RT: retention time index (min); RIlit: retention index from literature (Adams, 2007); RIexp: retention index reported in the present investigation

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Larvicidal bioassay

The *M. communis* essential oil applied to newly exuviate fourth instar mosquito larvae of *Cx. pipiens*, the mortality (%) recorded in control series was insignificant with $0.33 \pm 0.05\%$. Indeed, our data revealed that, the observed mortality of larvae values varied from $13.04 \pm 2.30\%$ for the lowest concentration (100 µl/ml) to $89.29 \pm 6.14\%$ for the highest tested one (700 µl/ml). This EO caused a mortality of larvae with a concentration-response relationship (*Fig. 1*). The mortality concentrations (LC) and the Hill slope recorded with their fiducial limits (95%) were listed in *Table 2*.

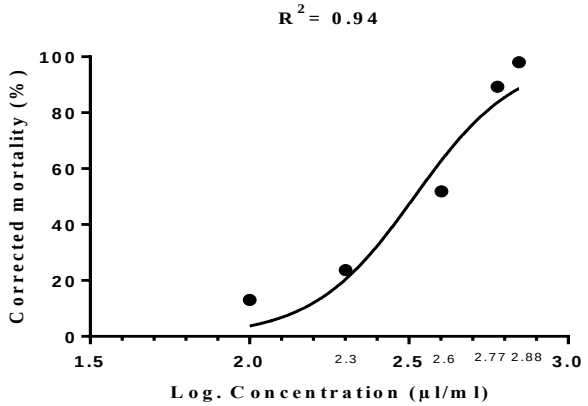

Figure 1. *Effect of Myrtus communis on Cx. pipiens larvae at different concentration (µl/ml), Mortality observed in (%) of newly exuviated stage 4 larvae: curve concentration-reponse expressed the corrected mortality and the logarithm of Myrtus communis oil concentrations (µl/ml)*

Table 2. *Concentration (µl/ml) of Myrtus communis essential oil against newly exuviated fourth instar larvae of Cx. pipiens. Determination of lethal concentrations (µl/ml) and their 95% confidence intervals*

Concentrations	Values (µl/ml)	Fiducial limits (95%)	R^2
LC_{50}	329.5	223.1 - 486.5	
LC_{90}	737.6	370.9 - 1467	
Hill slope	0.734	0.388 - 5.064	**0.94**

Statistical analysis revealed a significant concentration effect ($F_{4, 10} = 432.8$; $P < 0.0001$) and Tukey's HSD test showed a significant increase in mortality with increasing concentration. Also a significant difference was noticed between concentrations (*Fig. 2*).

Adulticidal bioassay

Results of the effect of *Myrtus communis* EO applied by fumigation for 60 min against *Cx. pipiens* mosquitoes, our data revealed that the corrected mortality of adults values varied from 10% for the (0.15 µl/ml area) concentration, and 100% for the highest concentration (1.25 µl/ml area). This EO caused a mortality of larvae with a concentration-response relationship (*Fig 3*). *Table 3* presents the mortality

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concentrations (LC) along with their corresponding Hill slope values, both accompanied by their fiducial limits (95%).

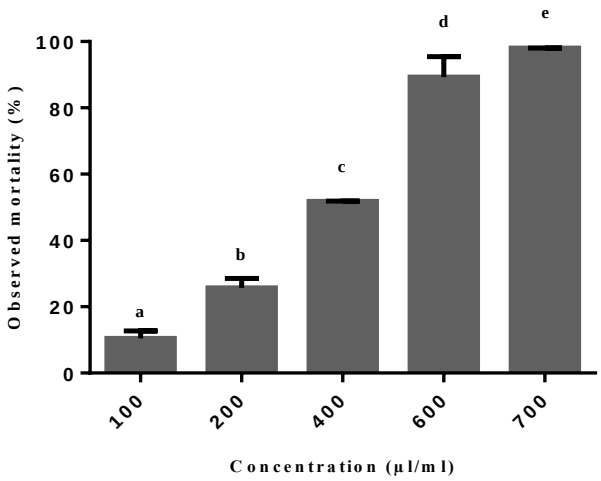

Figure 2. *Effect of Myrtus communis on Cx. pipiens larvae at different concentrations (µl/ml), Mortality observed in (%) of newly exuviated 4th instar larvae of Cx. pipiens (Mean ± SD; n = 3 replicates containing each 25 larvae; values indicated by different letters are significantly different by HSD test at P < 0.001)*

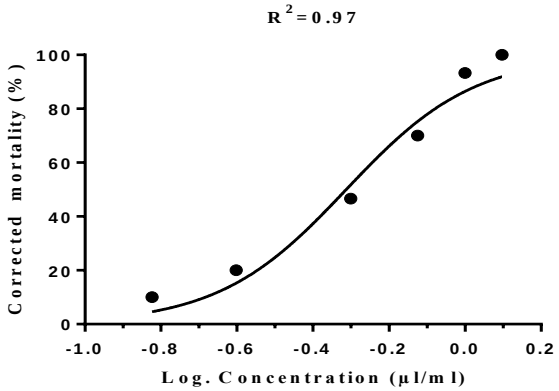

Figure 3. *Effect of Myrtus communis at different concentrations (µl/ml of air), applied by fumigation, in adult female Culex pipiens Mortality corrected in (%): Curve concentrations-response expresses the corrected mortality for the logarithm of Myrtus communis oil concentrations (µl/ml of air)*

Statistical analysis revealed a significant concentrations effect ($F_{5, 12} = 379.2$; $P < 0.0001$). They showed a significant concentrations-dependent increase in adult mortality. Ranking the concentrations by Tukey's HSD test allows to classify the concentrations according to their toxicities (*Fig. 4*), the results reveal the existence of 6 groups with different effects of the essential oil on the percentages of mortalities.

Effects on the enzymatic activities

The letal concentrations (LC_{50} and LC_{90}) were used to evaluate their effects on two of the common enzymes targeted by conventional insecticides, Acetylcholinesterase

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(AChE) and Glutathione S-transferase (GSTs). *Figures 5* and *6*, respectively, showed the results of the essential oil effects on ACHE and GSTs isolated from fourth-instar larvae of *Cx. Pipiens* larvae (mean ± SD, n = 6).

Table 3. *Concentrations (µl/ml air) of Myrtus communis EO applied by fumigation for 60 min in female Culex pipiens mosquitoes. Determination of lethal concentrations (µl/ml air) and their 95% confidence intervals*

Concentrations	Values (µl/ml air)	Fiducial limits (95%)	R^2
LC_{50}	0.47	0.38 - 0.42	
LC_{90}	1.13	0.76 - 1.67	
Hill slope	2.515	1.38 - 3.64	**0.97**

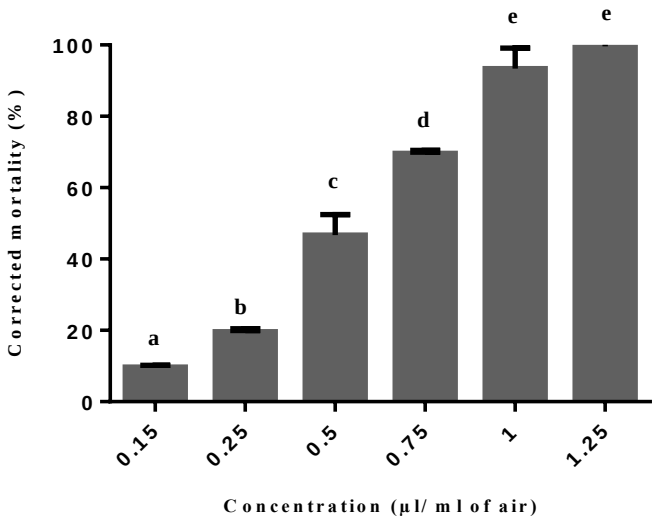

Figure 4. *Effect of Myrtus communis at different concentrations (µl/ml air), applied by fumigation, in adult female Culex pipiens Mortality observed in (%): values indicated by different letters are significantly different by HSD test at P < 0.001)*

For AChE, the activity was determined in the control and treatment series. The oil induced a significant AChE inhibition from 24 h of treatment, with a maximum inhibition occurring at 96 h of treatment. The two-way ANOVA indicated a significant effect of time ($F(3, 24) = 683.6$; $p < 0.0001$), treatment ($F(2, 24) = 265$; $p < 0.0001$) and time-treatment interaction $F(6, 24) = 39.78$; $p < 0.0001$).

Different lowercase letters above the same exposure time indicated a significant difference and different uppercase letters above the same exposure treatment indicated a significant difference ($p < 0.05$). It can be observed that, the *M. communis* essential oil caused a significant increase in GST enzyme activity compared to the control series at 24 h (0.139609 ± 0.0026 for LC_{50} and 0.252121 ± 0.007 µg/min/mg protein for LC_{90}). With a clear increase at 96 h (0.28965 ± 0.011 for LC_{50} and 0.441818 ± 0.001 µg/min/mg protein for LC_{90}) after treatment as compared to controls (*Fig. 6*). Two-way ANOVA confirms these results and indicated a significant effect of concentrations ($F\ 2, 24 = 6890$; $P < 0.0001$), time ($F\ 3, 24 = 1703$; $P < 0.0001$) and interaction concentrations time ($F\ 3, 24 = 74.58$; $P < 0.0001$).

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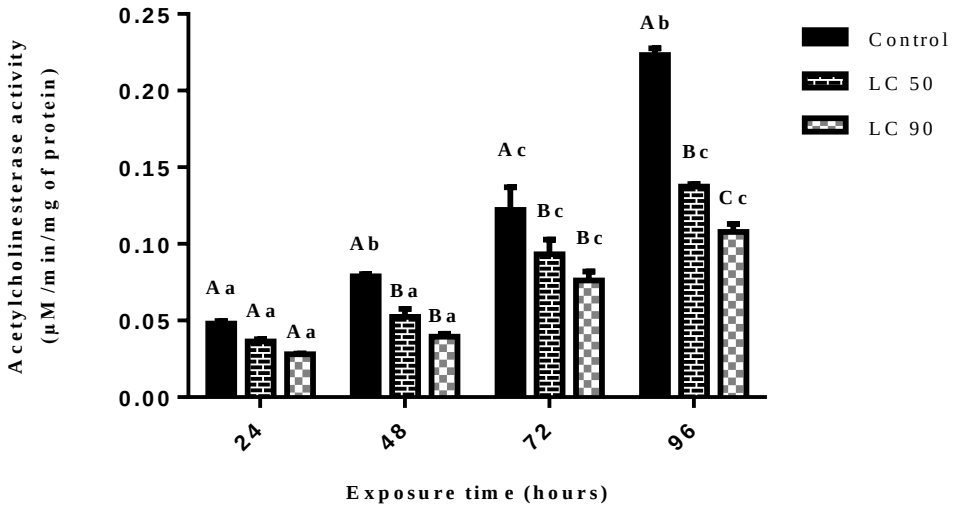

Figure 5. *Effect of Myrtus communis on AChE activity (M/mn/mg of protein) in Culex pipiens larvae (mean ± SD, n = 6). Different lowercase letters above the same exposure time indicated a significant difference and different uppercase letters above the same exposure treatment indicated a significant difference (p < 0.05)*

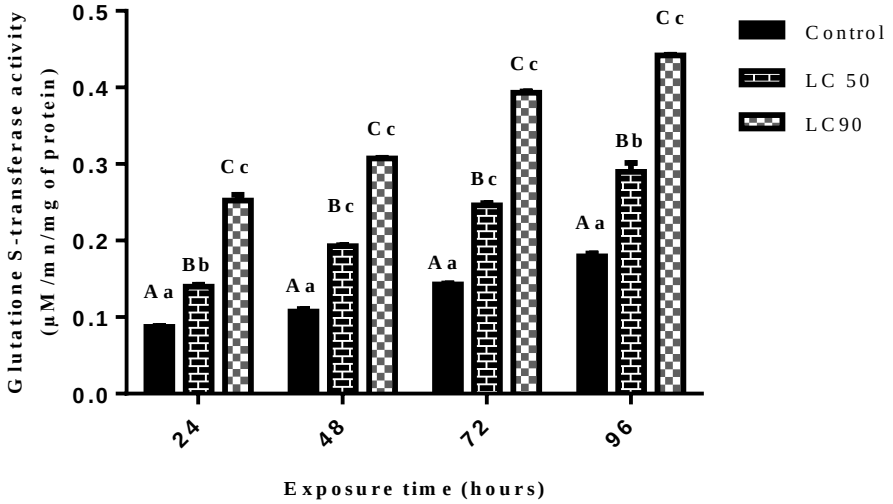

Figure 6. *Effect of Myrtus communis on GSTs activity (µM/mn/mg of protein) in Culex pipiens larvae (mean ± SD, n = 6). Different lowercase letters above the same exposure time indicated a significant difference and different uppercase letters above the same exposure treatment indicated a significant difference (p < 0.05)*

Discussion

Yield and chemical composition of the essential oil

The yield of extracted essential oil of *M. communis* cultivated in Annaba (Algeria), by hydro-distillation was 0.62% ± 0.05% (w/w). This result is relatively similar to the estimated yield of the same collected plant, from different regions (Dadazadeh and Nourafcan, 2021; Mohamadi et al., 2021). Myrtle studies from other countries showed the same yields. In Tunisia, Aidi et al. (2010) obtained an average of 0.61%. In

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Morocco, 0.2% (w/w) essential oil was obtained on a dry weight basis (Brahimi et al., 2022). The yield of Iranian myrtle varies from 0.8 to 2.2% (w/w) (Salimi-Beni et al., 2017). Essential oil of *M. communis* is directly related to altitude (Mohamadi et al., 2021), also Several factors can directly impact EO yield, such as harvest time and season, climate, geographic area and drying time, and extraction methods (Dadazadeh and Nourafcan, 2021).

The essential oil of *M. communis* analysis revealed the presence of 36 compounds. The major compounds in this essential oil were α-Pinene (50.817%), followed by 1,8-Cineole (18.982%). In Tunisia it was reported that the essential oil of *M. communis* contained 58.1% α-pinene (Aidi et al., 2010). Also Corsican myrtle (France) oils have high levels of α-pinene (Barhouchi et al., 2016). In contrast to these results, in Italy the two majority compounds are Acetate Geranyl (16.36%) and 1,8-Cineole (16.36%) (Barac et al., 2018). In the essential oil of *M. communis* collected from different regions of Greece, the main compounds were identified as α-pinene, 1,8-Cineole, linalool and limonene (Koutsavitia et al., 2015). Environmental factors such as the relative humidity, temperature, duration of sunstroke, altitude, and other factors directly influence the proportion of the different constituents of essential oil. Indeed, the cultivation conditions (nature of the soil, fertilizer supply) (Joshi et al., 2016), the Isolation process, the Isolation time and other factors such as preliminary treatments (transport conditions, drying and storage time of plant material, etc.) can cause great variability in the composition of essential oil, following enzymatic degradation (Bendif et al., 2017).

Larvicidal bioassay

The toxicity bioassay results show a larvicidal activity, of applied EO of *Myrtus communis* with a concentrations-response relationship. The insecticidal efficacy of EOs is given not only by their specific chemical profile and total content of major compounds, but also by the mutual ratios of the major compounds, which may lead to both synergistic and antagonistic effect (Pavela, 2015). Previous studies on the toxicity of EO have been conducted notably the study of Bouguerra et al. (2019) tested the effect of *Origanum vulgare* on *Cx. pipiens* larvae; the results indicate a very high toxicity with an LC_{50} value of 12.41 ppm. Recently, bioassays showed the larvicidal activities of *Lavandula angustifolia*, *Mentha x piperita*, *Rosmarinus officinalis* essential oils against *Culex pipiens*, of which rosemary oil acquired the most powerful larvicidal activity (Abo El-kasem Bosly, 2022).

Adulticidal bioassay

The results of the present study indicated that the tested oil displayed fumigant toxicity towards adults of *Cx. pipiens*. The toxicity of essential oil increased significantly with increasing the time of exposure and the concentrations of EO *M. communis*. Zahran et al. (2017) reported a high adulticidal activity for *Origanum vulg*are oils against adults of *Culex pipiens*, with LC_{50} values from 0.06 to 12.84 mg/ml. Moreover, various authors have reported that adulticidal activity against another mosquito species on essential oils from different plant species, such as *Eucalyptus maculata* (Myrtaceae), *Callistemon linearis* (Myrtaceae), *Cymbopogon citratus* (Poaceae), *Eucalyptus globulus* (Myrtaceae), and *Zanthoxylum limonella* (Rutaceae), with LC_{50} ranging from 23 to 85.5 mg/ml (Sarma et al., 2019; Soonwera and Sittichok, 2020). Even if the compound is of a natural origin, the selectivity is a key issue. The

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fumigant toxicity of OE may be attributed to their major monoterpenes. It is been reported some major compounds of the tested oils, such as α-pinene, limonene, α-terpineol, β-pinene, 1,8-cineole, camphor β-citronellol, geraniol, linalool and α-citral had fumigant toxicity against the adults of *Cx. Pipiens* (Ma et al., 2014). In addition, this is the first report showing the adulticidal activity of *M. communis* essential oils against *Cx pipiens*. The difference of sensitivity may be is attributed mainly to the phenotypic resistance (modifications in the target site), metabolic resistance (ability to detoxify insecticides) or behavioral modification. Behavioral changes that minimize contact between insect and the insecticide may cause a severe impact in the insecticide application efficacy, especially if physiological features (Martins et al., 2012) select resistance.

Biomarkers responses

A biomarker is defined as a measurable change in a biological or biochemical response (Joshi et al., 2016) and measures the interaction between a biological system and an environmental agent, they can be chemical, physical, or biological (Winfield et al., 2012). The inhibition or induction of biomarkers depends on the assessment of the level of exposure and the toxic effects of xenobiotics on the organism (Varo et al., 2002).

Acetylcholine esterase (AChE) is one of the most important hydrolytic enzymes in insect nervous system that equilibrate neural signal transduction by rapid hydrolyzing of acetylcholine signal in the synaptic cleft (Jacob and Mason, 2005). The monoterpenes abundantly present in essential oils (EOs) are lipophilic in nature and can interfere with the metabolic, biochemical, physiological and behavioral functions of insects (Mann and Kaufman, 2012). Due to their bioactive chemical constituents, aromatic plant EOs act on the nervous system of insects (Abdullah et al., 2015). The high level of esterases in various insect species, including mosquitoes, has been primarily associated with the phenomenon of resistance against insecticide compounds (Polson et al., 2011). The application of certain EOs or their purified constituents has induced symptoms such as hyperactivity, convulsions, and tremors followed by paralysis, which demonstrates their neurotoxic mode of action. The observed symptoms are similar to those produced by organophosphates and carbamates (Chintalchere et al., 2020). Several monoterpenes found in essential oils act as inhibitors of acetylcholinesterase (Ryan and Byrne, 1988).

The results demonstrate that *M. communis* EO significantly reduced the activity of AChE in the treated series compared to the control ones. This decrease in activity is likely due to the essential oil's inhibition of the enzyme. These findings are consistent with those of Dris et al. (2017), who showed that the essential oil of *Ocimum basilicum* inhibited AChE activity in fourth-stage larvae of *Cx. pipiens*, and with the results presented by Kharoubi et al. (2021), who found that EO of *Mentha x piperita* significantly decreased AChE activity in the treated group compared to the control.

Glutathione S-transferases (GSTs, EC 2.5.1.18) are multifunctional enzymes involved in many cellular physiological activities, such as detoxification of endogenous and xenobiotic compounds or biosynthesis of hormones and protection against oxidative stress (Adeyi et al., 2015). They have been recognized with their important role in xenobiotic detoxification (Tang et al., 2020). The results obtained with *Cx. pipiens* after treatment with *M. communis* EO showed a very significant increase in enzyme levels in the treated series compared to the control series at 24, 48, 72 and 96 h, respectively. This result could be explained by induction of the process of detoxification, a reaction

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of the organism against the entry of the essential oils. The previous studies have demonstrated an increase in GSTs activity after treatment of *Cx. pipiens* larvae with EO of *Rosmarinus officinalis* (Zeghib et al., 2020). Similar observations were also reported regarding the essential oil derived from *Piper betle* against *Ae. Aegypti* (Vasantha-Srinivasan et al., 2017). Moreover, Shahriari et al. (2018), recorded significant-high activities of glutathione *S*-transferases in the treated larvae of *Ephestia kuehniella* with α-pinene, trans-anethole, and thymol. The significant specific activity of these enzymes are indicative of their high capacity for detoxification, which is attributed to several physiological mechanisms. These mechanisms include the reduced sensitivity of the target site and the increased production of detoxifying enzymes; that is functioning to safeguard the biosynthetic pathways from any inhibition by toxic substances (Shahat et al., 2020). The molecular mechanism responsible for elevated GSTs activity is mostly due to regulatory changes associated with increased GSTs mRNA levels indicating de novo synthesis of the enzyme (Zahran et al., 2017).

Conclusion

Essential oil extracted from *M. communis* belongs to the Myrtaceae family contains many important chemical compounds which were analyzed using GC/MS indicating that α-Pinene is the major compound of the EO. The obtained results have showed of EO of *M. communis* exhibited toxic effects against larvae and adult of the domestic mosquito *Cx. pipiens*. It is concluded that the present EO could presented as a good alternative for neurotoxic products against mosquito control programs.

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