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Volatile oils of *Thymus serpyllum* and *Artemisia absinthium*: GS-MS analysis and insecticidal activity against *Culiseta longiareolata*

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Culiseta longiareolata (Macquart, 1838) (Diptera, Culicidae) is one of the important mosquito species distributed in Algeria. It acts as a vector of avian malaria, tularemia, Malta fever (brucellosis), and several arboviruses, such as West Nile fever and western encephalitis virus. To eliminate undesirable insect vectors of pathogens, botanical-based insecticides are increasingly recommended. This research was focused on the analysis of the chemical composition of essential oils from *Artemisia absinthium* (Asteraceae) and *Thymus serpyllum* (Lamiaceae) obtained by hydrodistillation using gas chromatography – mass spectroscopy (GC-MS). Furthermore, these oils were tested for the first time for their larvicidal activity against fourth-instar larvae of *C. longiareolata*. In the *Th. serpyllum* essential oil, linalool (25.40%) was the major constituent, followed by camphor (19.75%) and α-pinene (11.25%). In the *A. absinthium* essential oil, the major compound was camphor (39.01%), followed by chamazulene (14.51%). Our study found significant larvicidal effects against the fourth-instar larvae, with a direct correlation among the larval mortality rate, applied concentrations, and duration of exposure to the treatment, where the mortality increased over time. Also, the results indicated a significant difference in the mortality rate between the two samples of essential oils. The essential oil extracted from *A. absinthium* was highly effective against mosquito larvae, achieving the LC50 values of 10.42, 6.19 and 3.82 ppm at 24, 48, and 72 h, respectively, while the essential oil from *Th. serpyllum* achieved the LC50 values of 12.16, 7.79, and 6.88 ppm. Based on the current results, both the plants could be used as new alternatives for mosquito control.

Keywords: essential oil; Asteraceae; Lamiaceae; Culicidae; larvicidal activity; mosquitoes; Algeria.

Introduction

Most of the increasingly widespread epidemics or pandemics around the world, whether affecting the human or animal population, are caused by arthropods, which are considered important vectors of many parasites and viruses (Govindarajan & Benelli, 2016). These vector-borne diseases cause significant morbidity and mortality, with over 700,000 deaths per year (Benhissen et al., 2024). Mosquitoes (Diptera), a large family comprised of 3.490 species, are widespread and can adapt to an array of climates: temperate, hot, humid, and tropical, and can even be found in several regions of the Arctic. *Culiseta* has been at the forefront of entomological studies and research worldwide, serving as a vector for many pathogenic microorganisms to humans and animals and causing serious diseases such as malaria, filariasis, yellow fever, West Nile fever, dengue, Japanese encephalitis, yellow fever, Zika virus, Chikungunya fever, lymphatic filariasis, and other arboviruses. Among this extensive list, just malaria, dengue fever, and yellow fever are responsible for millions of fatalities and hundreds of millions of illnesses each year (WHO, 2019). This makes these mosquitoes some of the most dangerous insects for both public health and the economy (Alami et al., 2024; Chaouch et al., 2024).

According to Merabti et al. (2021), there are five species belonging to the genus *Culiseta*: *C.* (*Allotheobaldia*) *longiareolata* (Macquart, 1838), *C.* (*Culicella*) *fumipennis* (Stephens, 1825), *C.* (*C.*) *litorea* (Shute, 1928), *C.* (*C.*) *annulata* (Schrank, 1776), and *C.* (*C.*) *subochrea* (Edwards, 1921).

Culiseta longiareolata (Macquart, 1838), is one of important mosquito species distributed in the south of the Palearctic and Mediterranean regions, as well as in Europe, Africa, and Asia (Soltanbeiglu et al., 2022). This species is ornithophilic, acts as a vector of avian malaria, tularemia, Malta fever (brucellosis), and several arboviruses, such as West Nile fever and western encephalitis virus (Merabti et al., 2021; Soltanbeiglu et al., 2022). *Culiseta longiareolata* is considered a primary vector of *Plasmodium circumflexum*, *P. relictum*, *P. polare,* and *P. relictum* in birds (Nabti & Bounechada, 2019). In Algeria (North Africa), it was first reported at the beginning of the 20th century. It is found in ditches, lagoon depressions, valleys, fountains, freshwater rocks, pools, plastic containers, tire basins, and casks (Merabti, 2016).

Mosquito control is essential to prevent the spread of mosquito-borne diseases and to improve environmental quality and public health. Chemical insecticides have been widely utilized since the Second World War, entailing the emergence of resistance among species, undesirable effects on the environment, pollution, non-biodegradability, impacts on non-target organisms, and high concentration of residues in humans, as well as becoming highly expensive (Bouaziz et al., 2023). These negative effects made it clear that new, environmentally and human health-friendly methods of insect control are urgently needed, encouraging the search for new alternatives like bioinsecticides (Kharoubi et al., 2021; Bouaziz et al., 2023).

The development of safer alternatives to replace chemicals in pest control is growing, and the use of plant extracts is one of the most desirable methods of pest control. Plants' secondary metabolites are recognized for their crucial role in the control of harmful insects due to the selective, biodegradable, and non-toxic nature of their products, as well as only a few harmful side-effects on non-target organisms and the environment. Indeed, insecticidal properties have been reported for compounds of 250,000 species of plants (Bounadji et al., 2024).

Essential oils (EOs) – secondary metabolites extracted from different parts (flowers, buds, seeds, leaves, twigs, bark) of aromatic plants – have strong odor and represent complex mixtures of volatile constituents, including monoterpenes, sesquiterpenes, and their oxygenated derivatives. They play significant roles in the plant resistance against pests, herbivores, fungi, and bacteria (Uniyal et al., 2014). Since ancient times, EOs have been widely used for antibacterial, antifungal, insecticidal, and cosmetic applications. Recently, essential oils and their main compounds have garnered extensive interest as natural alternatives for controlling mosquitoes because they are biodegradable, have minimal side effects on non-target organisms and the environment, pose no risk of developing resistance, and are inexpensive (Kharoubi et al., 2021).

The genus *Thymus* (Lamiaceae) comprises about 214 species throughout the world, native to North Africa, Europe, and Asia. Eleven species grow in Algeria (Bouguerra et al., 2018; Li et al., 2019). *Thymus* species exert strong spasmolytic, antioxidant, antiparasitic, antifungal, antiviral, antibacterial (Bouguerra et al., 2018), and mosquitocidal activities (larvicidal, adulticidal, and oviposition-deterrent activities) (Damtie & Mekonnen, 2021). *Thymus serpyllum* L., or wild thyme (Lamiaceae), is an up to 5–7 cm high shrub with blue-green leaves and deep pink-purple flowers (Salaria et al., 2022). Due to its metabolites, such as vegetable oils, tannins, saponins, polysaccharides, vitamins, triterpene, essential oils, and inorganic elements (one of the best sources of iron, potassium, manganese, calcium, selenium and magnesium), it is beneficial for therapeutic purposes as antioxidant, anti-inflammatory, and antimicrobial drug (Mazulin et al., 2023).

The genus *Artemisia* (Asteraceae) includes over 500 species, principally found in North America, Asia, Europe, and South Africa (Govindarajan & Benelli, 2016). A notable representative of the genus is a perennial shrub *A. absinthium* L (wormwood). Its flowering season typically lasts from May to October. The wormwood has been utilized for centuries as hepatoprotective, neuroprotective, antibacterial, antifungal, anticancer, antidepressant, and antimalarial drug, and also as insecticide (Lakhdari et al., 2024).

To our knowledge, this is the first report on essential oils from *A. absinthium* and *Th. serpyllum* potentially being effective as larvicides against *C. longiareolata*. In addition, both wild thyme and wormwood plants growing in the Setif region have not been studied yet. Thus, objectives of this study were analyzing their volatile constituents, and evaluating their insecticidal activity against *C. longiareolata*.

Materials and methods

The aerial parts of *Th. serpyllum* and *A. absinthium* used in this study were collected during the flowering phase, in El Hamma (35°44'21.1" N 5°19'47.9" E), southwest of Setif in Algeria (Table 1). Species identification was carried out using the work of Baba Aissa (1991), and Quezel and Santa (1962, 1963). The identification was confirmed by Professor Zarrouk Salim at the Biological Department, Laghouat University, Algeria. The harvested plants were cleaned, washed with distilled water, and dried in the shade in a dry and ventilated place for 21 days. This technique allows for good preservation of chemical and physical characteristics of the plants, while also preventing the growth of germs (Wichtel & Anton, 1999). Then, the material was stored in paper bags.

Table 1
Presentation of the plants

Plant species	Botanical family	Origin	Harvest date
Artemisia absinthium (AA)	Asteraceae	cultivated	May 2022
Thymus serpyllum (TS)	Lamiaceae	wild	April 2022

The essential oils (EOs) from the *Th. serpyllum* and *A. absinthium* aerial parts were obtained by hydrodistillation. This method is recommended for the following reasons: the released steam is simple to manage; oil constituents do not thermally decompose because the temperature does not rise over 100 ºC; in addition, water and oil are completely separated through condensation, thus preserving all of the vital characteristics of the plant portion used for the extraction (Handa, 2008). The extraction was carried out using a clevenger-type apparatus for 2 hours according to the *European Pharmacopoeia*. Hydrodistillation consisted of introducing a quantity of 100 g of dry plants cut into small pieces to a 1 liter flask filled with distilled water up to 2/3 of its capacity. Once essential oil was obtained, it was separated from the water layer, dried with anhydrous sodium sulfate (Na_2SO_4), and stored in hermetically sealed sterile glass bottles, protected from light, and kept at 4 ºC to prevent air-induced oxidation and light-induced polymerization, which can cause the degradation of essential oils (Bruneton, 2002). The yield of each EO was evaluated in triplicate separately by the following formula (Bouabida & Dris, 2020):

$$R (\%) = (EOW / DPW) \times 100$$

where "EOW" is the essential oil weight, determined using a precision balance (0.0001 g), and "DPW" is the dried plant weight.

The analysis of the samples was conducted at the Technical Platform of Physico-Chemical Analysis (PTAPC-CRAPC)-Laghouat-Algeria, using a Shimadzu GCMS-QP2020 Instrument, programmed with well-defined operating conditions: equipped with a 5 ms-type capillary column, with dimensions of 30 m × 0.25 mm and a film thickness of 0.25 µm. The carrier gas used was helium (99.995% purity) with a flow rate of 1 mL/min. A sample volume of one microliter was injected in split mode (1:50). The injector and detector temperatures were maintained at 250 and 310 °C, respectively. The column temperature was programmed to start at 50 °C, held for 2 minutes, increased to 310 °C at a rate of 3 °C/min, and then maintained at 310 °C for an additional 2 minutes. The mass spectrometer conditions were as follows: ionization voltage 70 eV, ion source temperature 200 °C, and electron ionization mass spectra acquired over the mass range of 45–600 m/z.

The components of the mixture are separated based on their polarity. Their distinct physicochemical properties result in varying elution rates, allowing for separation according to retention time (Lamarti et al., 1993). The identification of the constituents from the GC/MS spectra of TS and AA was carried out by calculating the retention indices (Kovats index). The linear retention indices (LRIs) were calculated for separate compounds relative to a homologous n-alkanes serial (n-C_7-C_{32}). The components were identified by comparison of their calculated LRIs with such mentioned in the literature (Adams, 2007; Babushok et al., 2011), as well as their mass spectra with such recorded by the Wiley libraries "NIST17.lib", "W11N17MAI" and "FFNSC1.2.lib" and NIST (National Institute of Standards and Technology).

The eggs and larvae of *C. longiareolata* were collected using the dipping technique (Silver, 2008) from the natural and artificial breeding sites in the Laghouat city (Algerian Northern Sahara). The samples were transferred to the Laboratory of Biological and Agricultural Sciences at the Amar Telidji University, where the eggs were separated from the larvae in plastic containers containing non-chlorinated water to be reared under the controlled conditions of temperature (25 ± 2 ºC), humidity (75 ± 5%), and a photoperiodic cycle of 12 hours. The larvae were fed with a mixture of crushed biscuits, yeast, and algae. To keep everything clean, the water was changed every two days, as previously indicated (Alayat et al., 2023). Once the larvae reached the fourth-larval instar (L_4), they were identified and used for the larvicidal bioassays. The morphological identification of the larvae was carried out using identification software: the Moskey tool (The mosquitoes of the Euro-Mediterraean area) and the identification software of Culicidae of Mediterranean Africa (Brunhes et al., 2000). Only L_4 were subject to the toxicity tests of TS and AA essential oils.

The larvicidal activity of the *Th. serpyllum* and *A. absinthium* essential oils was evaluated using the standard protocol recommended by the World Health Organization (2005). Since essential oils are insoluble in water, they were first dissolved in 1 mL of ethanol 99.0% (as a co-solvent), and then diluted in 99 mL of distilled water to obtain the final needed concentrations. The preliminary experiments with different doses were performed on the newly ecdysed fourth-instar larvae of *C. longiareolata* (25 larvae per concentration) in goblets over a 24-hour period to test the toxicity of the essential oils and select the range of concentrations that would result in larval mortality between 5% and 100%. Six concentrations (6.25, 12.5, 25, 50, 100, and 200 ppm) of the EOs were chosen. The positive control consisted of ethanol and distilled water (1 mL/99 mL), while the negative control consisted of distilled water only (100 mL). The tests and controls were performed in three replicates simultaneously and under the same laboratory conditions comprising 600 larvae per EO. The mortality rates were noted at 24, 48, and 72 hours.

During the entire exposure period (72 h), no control mortality was recorded in any test; therefore, mortality was not adjusted by the Abbott's formula. The sublethal (LC_{10}, LC_{25}) and lethal concentrations (LC_{50}, LC_{90}), as well as their 95% confidence limits (upper and lower), were calculated using the probit analysis (Finney, 1971) and confirmed by the Lichfield-Wilcoxon test (Lichfield & Wilcoxon, 1949). Every extraction and measurement was carried out in triplicate. Results are presented as mean ± standard deviation (SD). The significance of the larvicidal activity of different concentrations of the essential oils was tested at $P \leq 0.05$ using one-way and two-way analysis of variance (ANOVA), followed by a classification using the post-hoc Tukey test (HSD) that enabled the grouping of the mortality rates into similar categories. All the statistical analyses were performed using the IBM SPSS 27 software program.

Results

The yields of the EOs obtained by hydrodistillation from the dried aerial parts of *Th. serpyllum* and *A. absinthium* were 0.69 ± 0.04% and 0.53 ± 0.03%, respectively. The essential oils were yellow and dark blue.

The results obtained from the GC-MS chemical analysis of the EOs of wild thyme and wormwood are grouped in Table 2. A total of 88 compounds belonging to different EO families were identified. Of these, the oxygenated monoterpenes were the major components (28 constituents), with the highest content detected in *Th. serpyllum* (65.84%), followed by oxygenated sesquiterpenes (20 constituents). The table shows that the plants were found to contain 63 and 49 components, representing 99.22% and 98.59% of the total oils of *Th. serpyllum* and *A. absinthium*, respectively. In the *Th. serpyllum* EO, the main constituent was linalool (25.40%), followed by camphor (19.75%) and α-pinene (11.25%). In the *A. absinthium* EO, the major compound was camphor (39.01%), followed by chamazulene (14.51%).

Table 2
Chemical composition of the *Th. serpyllum* and *A. absinthium* essential oils

No.	Compounds	RI	Area% in *Thymus serpyllum*	Area% in *Artemisia absinthium*
1	Tricyclene	920	0.29	0.10
2	α-Thujene	925	0.13	0.18
3	α-Pinene	935	11.25	5.44
4	Verbenene	946	0.55	–
5	Camphene	947	6.36	2.91
6	Sabinene	971	0.37	0.09
7	β-Pinene	974	1.90	0.17
8	Myrcene	983	0.84	2.77
9	α-Phellandrene	1003	–	0.42
10	1,5,8-p-menthadriene	1004	0.14	–
11	δ-3-Carene	1009	–	0.13
12	α-Terpinene	1011	0.31	1.36
13	P-Cymene	1015	–	0.60
14	o-Cymene	1023	0.94	–
15	Limonene	1027	–	0.59
16	Linalool	1029	25.40	0.18
17	E-β-Ocimene	1047	0.17	–
18	γ-Terpinene	1057	0.52	2.30
19	cis-Sabinene hydrate	1065	0.14	0.76
20	cis-Linalool oxide	1070	0.29	–
21	Camphenilone	1081	0.25	–
22	Terpinolene	1086	–	0.49
23	trans-Linalool oxide	1087	0.50	–
24	Camphenone	1088	0.27	–
25	trans-Sabinene hydrate	1097	0.17	0.19
26	Eucalyptol	1099	2.22	4.23
27	α-Pinene oxide	1104	0.33	–
28	trans-Thujone	1114	0.04	–
29	cis-p-Menth-2-en-1-ol	1120	0.13	0.23
30	α-Campholenal	1124	1.38	–
31	Nopinone	1135	0.24	–
32	trans-Pinocarveol	1138	1.43	–
33	Camphor	1144	19.75	39.01
34	p-Mentha-1,5-en-8-ol	1147	0.40	–
35	Pinocarvone	1161	0.69	–
36	Borneol	1163	3.23	0.62
37	Isopulegol	1165	1.72	–
38	Terpinen-4-ol	1176	1.40	5.96
39	m-Cymen-8-ol	1184	0.34	–
40	(-)-cis-Myrtanol	1185	–	0.32
41	α-Terpineol	1188	0.97	0.60
42	Myrtenal	1192	1.83	–
43	Verbenone	1206	1.92	–
44	trans-Carveol	1217	0.83	–
45	Thymol methyl ether	1234	0.56	–
46	Carvone	1242	0.38	–
47	cis-Chrysanthenyl acetate	1251	0.1	–
48	Perillaldehyde	1272	–	0.04
49	Bornyl acetate	1285	1.96	–
50	Thymol	1292	0.08	–
51	Perillyl alcohol	1297	–	0.06
52	α-Terpinyl acetate	1348	0.06	–
53	α-Copaene	1376	–	0.14
54	β -Bourbonene	1384	–	0.10
55	Caryophyllene	1419	0.26	0.64
56	α-Humulene	1453	–	0.06
57	Alloaromadendrene	1461	0.12	–
58	(-)-Germacrene D	1482	–	1.88
59	Bicyclogermacrene	1497	0.03	–
60	γ-Cadinene	1512	0.06	–
61	α-Dehydro-ar-himachalene	1514	–	1.32
62	3,6-Dihydrochamazulene	1519	–	0.25
63	δ -Cadinene	1523	0.12	0.08
64	α-Elemol	1549	0.41	0.59
65	Caryophyllene alcohol	1561	0.18	–
66	(-)-Spathulenol	1577	0.61	0.09
67	Neryl isovalerate	1583	–	0.43
68	Caryophyllene oxide	1584	2.76	0.02
69	Viridiflorol	1590	0.16	–
70	Geranyl 2-methylbutanoate	1602	–	0.20
71	Ledol	1604	0.18	–
72	Geranyl isovalerate	1608	–	0.70
73	Humulene oxide II	1609	0.12	–
74	1-epi-Cubenol	1614	–	1.47
75	γ-Dehydro-ar-himachalene	1617	–	2.00
76	α-Cadinol	1640	0.19	–
77	T-Cadinol	1641	0.11	–
78	β-Acorenol	1642	–	0.25
79	Methyl jasmonate	1648	–	0.07
80	β-Eudesmol	1651	0.36	0.25
81	dihydro-Eudesmol	1655	–	0.99
82	Eudesma-4(15),7-dien-1-β-ol	1686	0.22	–
83	Shyobunol	1690	0.37	–
84	α-Isonootkatol	1704	0.11	–
85	Chamazulene	1731	–	14.51
86	Arborescin	1976	–	0.34
87	Pentylcurcumene	2012	–	2.46
88	Epi-13-Manool	2057	0.07	–
Total detected by GC-MS (%)/number			100% (78)	100% (66)
Total identified (%)/number			99.22% (63)	98.59% (49)
Monotepene hydrocarbons (%)/number			23.77% (13)	17.55% (14)
Oxygenated monoterpenes (%)/number			65.84% (25)	52.20% (12)
Sesquiterpene hydrocarbons (%)/number			0.59% (5)	6.22% (08)
Oxygenated sesquiterpenes (%)/number			05.78% (13)	5.33% (11)
Diterpene hydrocarbons (%)/number			–	2.46% (1)
Oxygenated diterpenes (%)/number			0.07% (1)	–
Others (%)/number			3.17% (6)	14.83% (3)

Note: RI – retention indices, "–" – not detected.

In our work, the toxicity towards the newly ecdysed fourth-instar larvae of *C. longiareolata* was assessed by recording the mortality rate over the 72 hours of treatment, based on concentration gradient (6.25, 12.5, 25, 50, 100, 200 ppm). Our findings revealed a direct correlation among the larval mortality rate, applied concentrations, and duration of exposure to treatment, with the mortality progressively increasing from the first to the final hour. The volatile EO from *A. absinthium* induced 100 % mortality at 50 ppm in 24 h. However, a dosage of 100 ppm of EO from *Th. serpyllum* was required to reach 100% larval mortality (Table 3). The regression parameters of the probit analysis and LC_{10}, LC_{25}, LC_{50}, and LC_{90} for the larval mortality after 24 h show that the oils from both the plants produced a highly significant (<0.001) larvicidal effect against *C. longiareolata* (Table 4). The *A. absinthium* EO was the most effective one, showing the best sublethal and lethal concentration values throughout the treatment. The values of LC_{10}, LC_{25}, LC_{50}, and LC_{90} decreased after 72 hours of exposure and inversely correlated with time. The two-way ANOVA indicated a significant effect of the treatment ($F_5 = 1050.39$; $P < 0.001$), time ($F_2 = 369.869$; $P < 0.001$), and treatment-time interaction ($F_{10} = 81.48$; $P < 0.001$) for *A. absinthium*. The same was observed for *Th. serpyllum*: treatment ($F_5 = 633.121$; $P < 0.001$), time ($F_2 = 47.91$; $P < 0.001$), and treatment time interaction ($F_{10} = 10.35$; $P < 0.001$). The ANOVA indicated a statistically significant difference in the mortality rate between the two samples of essential oils.

Discussion

The yields and chemical compositions of the essential oils from both the plants varied from one region to another within the same country, between countries (Table 5), and even between species of the same genus.

Table 3
Mortality rate observed in fourth-instar larvae of *Culiseta longiareolata* at 24, 48, and 72 hours of treatment with the essential oils from *Artimesia absithium* (AA) and *Thymus serpyllum* (TS) (mean ± SD, n = 3 replicates each containing 25 larvae)

EO	Time, hours	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm	200 ppm
Artimesia absithium	24	43.33 ± 3.33 A (a)	50.00 ± 3.71 A (a)	60.00 ± 3.33 A (b)	100.00 ± 0.00 A (c)	100.00 ± 0.00 A (c)	100.00 ± 0.00 A (c)
	48	56.66 ± 0.29 B (a)	70.00 ± 3.33 B (b)	73.33 ± 0.67 B (b)	100.00 ± 0.00 A (c)	100.00 ± 0.00 A (c)	100.00 ± 0.00 A (c)
	72	66.66 ± 1.44 C (a)	90.00 ± 2.03 C (b)	93.33 ± 1.74 C (b)	100.00 ± 0.00 A (c)	100.00 ± 0.00 A (c)	100.00 ± 0.00 A (c)
Thymus serpyllum	24	33.33 ± 2.95 A (a)	40.00 ± 4.58 A (a)	80.00 ± 4.15 A (b)	86.66 ± 1.00 A (b)	100.00 ± 0.00 A (c)	100.00 ± 0.00 A (c)
	48	43.33 ± 4.58 B (a)	63.33 ± 4.40 B (b)	86.66 ± 1.00 B (c)	90.00 ± 0.67 B(c)	100.00 ± 0.00 A (d)	100.00 ± 0.00 A (d)
	72	50.00 ± 2.00 C(a)	63.33 ± 4.40 B (b)	86.66 ± 1.00 B (c)	93.11 ± 5.97 B (cd)	100.00 ± 0.00 A (d)	100.00 ± 0.00 A (d)

Note: for the same exposure time, mean values followed by the same lowercase letter are not significantly different at P < 0.05; while for the same concentration, mean values followed by the same capital letter are not significantly different at P < 0.05.

Table 4
Toxicological parameters (ppm, 95 % confidence limits) of *Artimesia absithium* (AA) and *Thymus serpyllum* (TS) essential oils against fourth-instar larvae of *Culiseta longiareolata* during the entire exposure period

EO	Time, hours	LC_{10}/95% (LCL-UCL)	LC_{25}/95% (LCL-UCL)	LC_{50}/95% (LCL-UCL)	LC_{90}/95% (LCL-UCL)	Regression equation	R^2	X^2 (df)	P-value
Artimesia absithium	24	2.55 (0–6.54)	4.97 (0.09–10.28)	10.42 (1.61–20.09)	42.46 (21.67–1122)	y = 0.7x–0.74	0.98	38.40 (4)	<0.001
	48	1.24 (0–3.89)	2.66 (0.02–6.30)	6.19 (0.47–11.56)	30.76 (16.78–285.75)	y = 0.76x–0.39	0.90	23.34 (4)	<0.001
	72	1 (0.37–1.74)	1.89 (0.9–2.88)	3.82 (2.39–5.08)	14.55 (12.07–18.57)	y = 0.78x–0.87	0.89	4.12 (4)	0.380
Thymus serpyllum	24	3.15 (0.83–5.72)	5.98 (2.39–9.37)	12.16 (7.21–17.45)	46.88 (30.54–109.14)	y = 1.9x–2.05	0.91	13.62 (4)	0.009
	48	1.74 (1.01–2.54)	3.54 (2.4–4.68)	7.79 (6.15–9.39)	34.83 (28.57–45.08)	y = 1.71x–1.47	0.95	5.87 (4)	0.208
	72	1.36 (0 .71–2.10)	2.93 (1.85–4.04)	6.88 (5.19–8.51)	34.83 (28.31–45.81)	y = 1.54x–1.22	0.94	6.67 (4)	0.154

Note: EO – plant's essential oil, LC_{10}/95% CI – sublethal concentration causing 10% mortality and its 95% confidence interval; LC_{25}/95% CI – sublethal concentration causing 25% mortality and its 95% confidence interval; LC_{50}/95% CI – lethal concentration causing 50% mortality and its 95% confidence interval; LC_{90}/95% CI – lethal concentration causing 90% mortality and its 95% confidence interval; LCL – lower confidence limit, UCL – upper confidence limit; X_2 – heterogeneity around the regression line; df – degree of freedom; P-value – represent heterogeneity in the tested population, y – probits of mortality rates; x – the logarithm of the concentrations.

Table 5
Comparison of yield and chemical composition of wormwood and wild thyme around the world

Region	Part used	Extraction method	Yield	Total identified	Major volatile constituents	References
			A. absinthium			
Algeria (Tipaza)	dried aerial part	hydrodistillation (3 h)	0.50%	22	ß-thujone (60.82%), chamazulene (16.65%), γ-terpinène (3.71%)	Bouchenak et al. (2018)
Algeria (M'sila)	aerial flowering part	hydrodistillation (UD)	0.29%	75	camphor (47.59%), chamazulene (10.34%)	Benkhaled et al. (2020)
Saudi Arabia (Jizan)	fresh aerial part	hydrodistillation (4 h)	0.83%	57	cis-davanone (34.7%), camphor (16.2%), chamazulene (8.2%)	Aati et al. (2020)
Saudi Arabia (Al-Baha)	leaves	UD	0.83%	23	cis-davanone (58.5%), camphor (16.0%), terpinene-4-ol (5.39%)	Al-Ghamdi (2020)
Saudi Arabia (Qassim)	dried aerial part	hydrodistillation (3 h)	0.94%	34	cis-davanone (52.51%), α-gurjunene (7.15%), chamazulene (3.38%)	Mohammed (2022)
Tunisia (Kasserine)	dried leaves	hydrodistillation (3 h)	1.24–2.22%	40	Camphor (33.29%), chamazulene (24.68%), linalool (12.96%)	Riahi et al. (2015)
Tunisia (El Kef)				39	Camphor (28.94%), chamazulene (18.98%), bornyl acetate (15.50%)	
Tunisia (Bou Salem)	fresh aerial part	hydrodistillation (1.5 h)	1.10–1.46%	55	Chamazulene (39.93%), α-thujone (16.17%), trans-sabinene hydrate (12.07%)	Msaada et al. (2015)

Region	Part used	Extraction method	Yield	Total identified	Major volatile constituents	References
Tunisia (Kairouan)				53	Chamazulene (34.81%), trans-sabinene hydrate (21.78%), α-thujone (6.72%)	
Turkey (Erzurum)	dried aerial part	hydrodistillation (4 h)	UD	111	chamazulene (17.8%), nuciferol butanoate (8.2%), nuciferol propionate (5.1%)	Kordali et al. (2005)
Iran (Namin)	dried leaves	hydrodistillation (3 h)	1.05%	19	1,8-Cineole (36.46%), borneol (25.99%), camphor (10.20%)	Taherkhani et al. (2013)
Iran (Alborz)	aerial part	hydrodistillation (2.5 h)	1.30%	UD	α-Pinene (23.8%), β-thujone (18.6%)	Rezaeinodehi et al. (2008)
Iran (Khorasan)	dried aerial part	hydrodistillation (3 h)	0.685%	44	Chamazulene (4.18–13.88%), β-pinene (12.29%, 16.81%), α-phellandrene (9.76–16.4%)	Mohammadi et al. (2015)
Hungary (Budapest)	leaves and flowers	hydrodistillation (UD)	0.13–1.77 mL/10 mg	25	ß-Thujone (63.41%), α-hhujone (28.15%)	Nguyen et al. (2018)
Tajikistan (Mumino-bod & Yovon)	dried aerial part	hydrodistillation (3 h)	0.5–0.8%	49	Myrcene (22.7%), cis-chrysanthenyl acetate (15.5%), dihydrochamazulene-1 (10.5%)	Sharopov et al. (2012)
India (Karnataka)	fresh leaves	hydrodistillation (5 h)	0.03%	64	borneol (18.7%), methyl hinokiate (12.9%), isobornyl acetate (4.0%)	Joshi (2013)
Lithuania (Vilnius)	dried aerial part	hydrodistillation (2 h)	1.3–3.6%	49	trans-Sabinyl acetate (23.0%), trans-thujone (15.9%), myrcene (9.2%)	Judzentiene et al. (2009)
Lithuania (25 different locations)	dried aerial part	hydrodistillation (2 h)	1.2–2.9%	20	trans-Sabinyl acetate (21.8–1.3%), β-pinene (7.2–12.9%), sabinene (≤10.9%)	Judzentiene & Budiene (2010)
Europe (15 geogra-phical areas)	dried aerial part	hydrodistillation (3 h)	0.1–0.9%	107	β-Thujone (0.1–64.6%), myrcene (0.1–38.9%), sabinene (0.9–30.1%)	Orav et al. (2006)
Serbia (Bela Palanka)	dried aerial part	hydrodistillation (2.5 h)	0.08–0.29%	UD	α-Thujone (20.2%), 1,8-cineole (16.3%), trans-sabinyl acetate (15.5 %)	Blagojević et al. (2006)
Ethiopia (Butajira + Addis Ababa)	dried aerial part	hydrodistillation (3 h)	0.2%	34	β-Thujone (42.3–66.4%), camphor (21.2–28.3%), davanone (21.3–26.5%)	Asfaw & Demissew (2015)
			Th. serpyllum			
Kazakhstan	dried aerial part	hydrodistillation (2.0–2.5 h)	2.0%	41	Thymol (41.8%), γ-terpinene (15.3%), o-cymene (10.9%)	Tazabayeva & Sylibaeva (2018)
Pakistan	whole shrub	steam distillation (UD)	0.48%	20	Thymol (53.33%), carvacol (10.4%)	Ahmad et al. (2003)
Italy	whole shrub	hydrodistillation (4 h)	UD	22	Thymol (32.57%), α-terpinene (22.83%), γ-terpinene (9.15%)	De Lisi et al. (2011)
Italy	aerial part	hydrodistillation (3 h)	1.9%	26	Carvacrol + carvacrol methyl ether (44.9%)	Porto & Decorti (2012)
Serbia	whole shrub	hydrodistillation (5 h)	0.08%	65	trans-Nerolidol (24.2%), germacrene D (16.0%), thymol (7.3%)	Petrovic et al. (2014)
Serbia	UD	UD	UD	29	Thymol (56.02%), carvacrol (14.00%), p-cymene (6.2%)	Nikolic et al. (2014)
Lithuania (Vilnius)	dried aerial part	hydrodistillation (2 h)	0.12–0.2%	36	1,8-Cineole (8.9–13.9%), caryophyllene oxide (6.5–12.5%), borneol (7.4–10.5%)	Mockute & Bernotiene (2004)
Egypt	fresh leaves	hydrodistillation (2 h)	1.23%	18	thymol (62.02%), β-phellandrene (13.50%), cis-sabinene hydroxide (8.09%)	Eweis et al. (2012)
Iran	dried aerial part	steam distillation	0.57%	34	γ-Terpinene (22.7%), p-cymene (21.1%), thymol (18.7%)	Santana-Rios et al. (2001)
Montenegro	UD	industrial capacity extractor	UD	UD	p-Cymene (19.04%), geraniol (11.09%), linalool (9.16%)	Preljevic et al. (2024)
Poland	aerial part	hydrodistillation (2 h)	1.50%	47	Carvacrol (37.49%), γ-terpinene (10.79%), β-caryophyllene (6.51%)	Wesolowska et al. (2015)
Spain	flowers	hydrodistillation (3 h)	UD	52	Thymol (21.5%), carvacrol (18.7%)	Rosello et al. (2024)
India	whole plant	hydrodistillation (UD)	2.21%	48	Thymol (15.79%), phenol,2-(1.1-dimethylethyl (11.55%), o-cymene (10.96%)	Salaria et al. (2022)

Note: UD – undeclared, h – extraction duration (hour).

Compared with the previous studies, the yield of *A. absinthium* EO obtained in this study (0.53%) was much higher than such obtained by Joshi (2013) (0.03%) and Asfaw & Demissew (2015) (0.2%), but lower than such obtained by Judzentiene et al. (2009) (1.3–3.6%). According to Bencheqroun et al. (2012) and Chebbac et al. (2023), *A. mestlantica* and *A. annua* both presented an essential oil yield of 0.5%. Khebri (2011) stated that the essential oil yields from *A. cana* and *A. campestris* were 1.3% and 0.2%, respectively. Chebbac et al. reported 1.2% yield from each *A. negrei* and *A. aragonensis* (2021, 2022), and Aati et al. obtained 0.42% yield from *A. sieberi* and 0.34% yield from *A. scoparia* (2020).

The essential oil yield from our wild thyme (0.69%) was higher compared with such obtained by Ahmad et al. (2006) (0.48%) and lower than such obtained by Eweis et al. (2012) (1.23%). As with some other plants of the *Thymus* genus, the yields measured 0.4% from *Th. algeriensi* (El Ouahdani et al., 2021), 1.58% from *Th. Numidicus* (Messara et al., 2017), 2.64% from *T. vulgaris* (Zahed et al., 2021), and 3.6 % from *Th. pallescens* de Noé (Bertouch et al., 2023).

According to Chebbac et al. (2023), the yield is influenced by factors such as the cultivation location, seed source, harvesting period, and whether the leaves or flowers are used. Furthermore, the type of plant material and the extraction techniques employed significantly impact the volume of EO produced (Khan et al., 2022).

The yellow color of our wild thyme oil is consistent with all scientific publications, while the dark blue EO from our absinthe is consistent with the studies on *A. absinthium* growing in Tunisia (Msaada et al., 2015), Algeria (Benkhaled et al., 2020), China (Jiang et al., 2021), the south of Saudi Arabia (Aati et al., 2020), and Ethiopia (Tariku et al., 2011), due to the high percentage of chamazulene (approximately ≥8%). However, the EOs from Lithuanian absinthe were dark brown in color, with the major constituent being trans-sabinyl acetate, while the chamazulene content did not exceed 1.5% (Judzentiene et al., 2010). A yellow essential oil from *A. absinthium* was obtained from the plants growing in Tajikistan (Sharopov et al., 2012) and the central region of Saudi Arabia (Mohammed, 2022). In the latter example, the concentration of chamazulene was very low (3.38%) compared with cis-davanone (52.51%). Therefore, *A. absin-*

thium EOs from various regions had a variety of colors and appeared to be related to the contents of the main constituents, influenced by the growing conditions, stage of development, and many other factors (Nguyen & Németh, 2016).

The GC-MS results obtained in this study show that wormwood oil belongs to the "camphor chemotype", which is in agreement with the data in the literature; in particular, the studies conducted in Ethiopia (Tariku et al., 2011), Tunisia (Riahi et al., 2015), Algeria (Benkhaled et al., 2020), and Morocco (Alami et al., 2024). However, Khan et al. (2022) indicated that the most abundant compounds in the EOs from *A. absinthium* of Pakistan origin were oleic acid (41.45%) and 5-hydroxymethyl (14.24%). Jiang et al. (2021) stated that the most abundant constituents were eucalyptol (25.59%), linalool (11.99%), and β-myrcene (10.05%). According to Fuzimoto (2021), artemisinic acid and alpha-thujone were the major compounds of *A. absinthium*. In addition, there are different chemotypes, with chamazulene and thujone as the main components (Asfaw & Demissew, 2015; Mohammadi et al., 2015; Bouchenak et al., 2018). Aati et al. (2020), Al-Ghamdi (2020), and Mohammed (2022) found that most of the plants from Saudi Arabia were of "cis-davanone chemotype".

Studies on *Th. serpyllum* in the past have established the existence of secondary metabolites such as phenolic acid, flavonoids, Ɣ-terpinene, thymol, (E)-b-cariophelene, carvacrol, linalool, and p-cymene (Goyal et al., 2020; Vassiliou et al. 2023). Based on the first main compound, Mockute & Bernotiene (2004) categorized EOs from *Th. serpyllum* into the following chemotypes: carvacrol (Italy, India, the former Soviet Union, Hungary), thymol (Pakistan, Italy, India), linalool (Finland), myrcene (Finland), p-cymene (Italy), linalyl acetate (Finland), geranyl acetate (France), geraniol (Russia), and 1,8-cineole (Finland, Lithuania).

Gas Chromatography Mass Spectrometry analysis of EO from our wild thyme revealed that linalool (25.40%) was the major constituent, followed by camphor (19.75%) and α-pinene (11.25%). These findings are consistent with the previously published study by Baj et al. (2020), who found that the major compound was linalool (30.9%). At the same time, Verma et al. (2016), Tazabayeva & Sylibaeva (2018), Pruteanu et al. (2018), Goyal et al. (2020), Xie et al. (2020), Šoji´c et al. (2020), Galovicová et al. (2021), and Roselló et al. (2024) reported thymol as the main constituent, while Kulisic et al. (2007), Males et al. (2022), and Nutrizio et al. (2020) showed that carvacrol was the major component in the EOs. According to D'Auria & Racioppi (2015), the main components of the EO of *T. serpyllum* were p-cymene (30.35%) and c-terpinene (11.85%). Nikoli´c et al. (2019) extracted oils from the air-dried parts of *Th. serpyllum* from the Mountain Zlatar region in Serbia using three different methods: hydrodistillation (HD), simultaneous hydrodistillation and extraction (SDE), and head space (HS). They identified that α-terpinyl acetate (66.2–35.3%) was the main compound in the HD and SDE volatiles and myrcene (74.2%) was the major component of the HS volatiles.

Alami et al. (2024) and Tamma et al. (2024) concluded that several factors are affecting the quality and variety of volatile oils from aromatic plants: the climate, humidity, quality of the soil, the plants' age, time of harvest, sexual polymorphism, and genetic mechanisms. The impacts of these factors on the diversity of chemical composition of essential oils vary from one place to another.

The synthesis of essential oils by plants serves a multitude of functions, such as defense against fungi, bacteria, and insects (due to the terpenoids' ability to repel animals and insects), allelopathic activity, attraction of dispersal and pollination agents by their volatility and strong odor (Nieto, 2017; Feng et al., 2021).

Luz et al. (2020) have set the following standards for evaluating the effectiveness of a natural product as an insecticide: LC_{50} < 50 g/mL = highly active, 50 g/mL < LC_{50} < 100 g/mL = moderately active, 100 g/mL < LC_{50} < 750 g/mL = effective, and LC_{50} > 750 g/mL = inactive. According to these metrics, the essential oils from AA and TS in our study were undoubtedly highly active.

To the best of our knowledge, no studies have been conducted on the larvicidal efficacy of *A. absinthium* and *Th. serpyllum* essential oils against the *C. longiareolata* mosquito. Therefore, we will compare our findings with those concerning other insect species.

Regarding the toxic effect of *A. absinthium* EOs, our result (LC_{50} = 10.42 ppm) is comparatively similar to such observed in Morocco (LC_{50} = 8.4 mg/g) against *Acanthoscelides obtectus* (Coleoptera, Chrysomelidae) (Derwich et al., 2009), and more effective than the one obtained by Govindarajan & Benelli (2016) against the larval populations (L_3) of *Aedes aegypti*, *Anopheles stephensi*, *A. subpictus*, *Culex quinquefasciatus*, *C. tritaeniorhynchus,* and *Aedes albopictus*, with the LC_{50} values of 46.33, 41.85, 52.02, 50.57, 62.16, and 57.5 µg/mL, respectively. Also, the methanolic extract of the *A. absinthium* roots from India showed larvicidal activity against fourth-instar larvae of the mosquitos *C. quinquefasciatus*, *Anopheles stephensi,* and *Aedes aegypti*: the LC_{50} values accounted for 124.42, 59.37, and 88.71 ppm, and the LC_{90} values were 311.23, 233.36, and 315.04 ppm, respectively (Ali et al., 2018). In addition, Alami et al. (2024) reported that the EO from Moroccan *A. absinthium* displayed larvicidal activity against *C. pipiens*, with the LC_{50} value of 16.98 µg/mL and the LC_{90} value of 60.79 µg/mL.

Thymus serpyllum exhibited high larvicidal activity against *C. longiareolata*: in 24 h (LC_{50} = 12.16 ppm), in 48h (LC_{50} = 7.79 ppm), and in 72h (LC_{50} = 6.88 ppm). Compared with other reports on mosquitoes in literature, these results are lower than those obtained by Amer & Mehlhorn (2006) against *C. quinquefasciatus*, *Anopheles stephensi,* and *Aedes aegypti,* with LC_{50} = 10 ppm after 24 h of exposure, and more effective than those found by Uniyal et al. (2014) against *Aedes aegypti,* with LC_{50} = 161.15 µg/mL. Xie et al. (2020) tested the EOs from *Th. serpyllum* for contact and fumigation toxicity, observing toxic effects on the larvae, with LC_{50} = 0.4 µL/cm^2 and 20.9 µL/L for contact toxicity and fumigation toxicity, respectively, and toxic action against the pupae, with LC_{50} of 1.0 µL/cm^2 for contact toxicity and 25 µL/L for fumigation toxicity. Likewise, in Pakistan, Shafique et al. (2020), determined that a 5% concentration of *Th. serpyllum* methanolic extract was enough to inhibit the development of *Curvularia clavata*, S*erohilum rostratum*, and *Cladosporium cladosporioides*.

The essential oils from *A. absinthium* and *Th. serpyllum* exerted strong larvicidal impacts against the *C. longiareolata* larvae. Several natural products have been tested against the same mosquito species, e.g. essential oils from *Lavandula dentata* (LC_{50} = 77.09 ppm and LC_{90} = 104.45 ppm) (Dris et al. 2017), *Ocimum basilicum* (LC_{50} = 122.70 and LC_{90} = 151.80 ppm), *Mentha piperita* (LC_{50} = 41.63 and LC_{90} = 69.2 ppm) (Dris, 2018), *M. piperita* (LC_{50} = 38.81 and 93.78 µL/L for larvae and pupae, respectively) (Djemâa & Hayette, 2023), *Artemisia herba alba* (LC_{50} = 86.6 and LC_{90} = 139.55 ppm), *Thymus vulgaris* (LC_{50} = 25.64 and LC_{90} = 50.53 ppm), *Juniperus phoenicea* (LC_{50} = 59.83 and LC_{90} = 137.68 ppm), *Rosmarinus officinalis* (LC_{50} = 64.18 and LC_{90} = 96.55 ppm), and *Eucalyptus globules* (LC_{50} = 95.83 and LC_{90} = 168.25 ppm) (Nabti & Bounechada, 2019), aqueous extract of *Euphorbia bupleuroides* (LC_{50} = 6.68, 4.95, 8.33 g/L and LC_{90} = 11.58, 6.88 and 4.80 g/L after 3, 7 and 12 days, respetiely) (Bounadji et al., 2024), aqueous extracts of the leaves of *Hyoscyamus albus* (LC_{50} = 121.15, 14.09, 13.02 g/L and LC_{90} = 93.04, 27.70 and 17.97 g/L after 48, 72 and 96 h, respectively), *Solanum elaeagnifolium* (LC_{50} = 135.30, 105.46, 104.04 g/L and LC_{90} = 197.25, 208.30 and 196.09 g/L after 48, 72 and 96 h, respectively), and *Solanum nigrum* (LC_{50} = 20.9, 7.01, 4.67 g/L and LC_{90} = 91.2, 21.8 and 6.45 g/L after 48, 72 and 96 h, respectively) (Benhissen et al., 2024).

In our study, camphor (39.01%) and chamazulene (14.51%) were the main components of the *A. absinthium* EO, while linalool (25.40%), camphor (19.75%), and α-pinene (11.25%) were the major constituents of the *Th. serpyllum* oil. Consequently, we determined that the high levels of larvicidal efficacy of the essential oils from both the plants were probably due to their high contents of camphor, linalool, chamazulene, and α-pinene. However, the biological activity of these essential oils may result from the individual actions of the majority of the compounds or their combinations (Moussa et al., 2020). Additionally, it is likely that other minor constituents influence the main components' activity (Santana-Rios et al., 2001). Luz et al. (2020) reported that camphor, linalool, and α-pinene exhibited larvicidal activities against *Aedes aegypti* with LC_{50} values > 150 µg/mL, 113.47 μg/mL, and 200 ppm, respectively, whereas linalool produced an LC_{50} value of 275.2 μg/mL against *Artemia salina*. Camphor demonstrated repellent effects with the RD_{50} values of 1.4×10^{-3} mg/cm and 1.4 mg/cm against female *Anopheles gambiae* and female *Aedes aegypti*, respectively. Also, linalool and camphor were able to repel female *A. aegypti* with 67% and 47% repellencies, respectively, while α-pinene provided two-hour protection against mosquitoes (Pohlit et al., 2011). According to Wojtunik-Kulesza & Wisniewska (2022), camphor has been employed as an insecticide and repellent that is absorbed through the skin, digestive tract, or by inhalation, causing central nervous system dysfunction, and leading to insect death. Furthermore, monoterpenes inhibit the nicotinic acetylcholine receptors, leading to decreased secretion of catecholamines. Numerous authors have widely documented that the presence of bioactive chemicals in plants can cause various neurological and muscular problems, leading to larval mortality. Additionally, larvicidal products typically operate by either flooding the larva's trachea or disrupting its buoyancy, which prevents it from remaining on the surface and breathing (Deepalakshmi & Jeyabalan, 2017).

Conclusion

In our study, we analyzed the larvicidal effects of the essential oils from *Artimesia absinthium* and *Thymus serpyllum* against the avian malaria vector *C. longiareolata*. The results showed that both the plants displayed highly effective larvicidal activity. Further research is required to fully understand this activity against a variety of mosquito species at all stages. In addition, the bioactive chemical components derived from these oils can be recommended for the use in the preparation of mosquito insecticides instead of chemical larvicides.

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