Check for updates
Full scientific article

Revista Actualidades Biológicas
Actual. Biol. 45(118): 1-12, 2023 | DOI:10.17533/udea.acbi.v45n118a01

Chemical composition of *Artemesia herba-alba* essential oil and its larvicidal and pupicidal effects against *Culex pipiens* (Diptera; Culicidae)

Composición química del aceite esencial de *Artemesia herba-alba* y sus efectos larvicida y pupicida contra *Culex pipiens* (Diptera; Culicidae)

Salma Kaoutar Abdelali[1*], Karim Souttou[2], Mohamed Kacimi-Elhassani[3], Lynda Aissaoui[1], Hamid Bendachou[2]

Abstract

Artemisia herba-alba Asso *(A. herba-alba)* (Asteraceae) is widely used in herbal medicine as it is a mine of natural molecules such as davanone, which is an interesting product on the international market. The present research proposes a method for controlling the late larval (L4) and pupal stages of *Culex pipiens* based on the essential oil of *A. herba-alba*. The aerial part of this plant was extracted by hydrodistillation and then analyzed by gas chromatography coupled with mass spectrometry (CPG/SM) to determine its chemical composition. Three concentrations (1, 5 and 10 μl/ml) were prepared and directly tested on larvae (L4) and pupae under laboratory conditions to measure LC_{50}, LC_{90}, LT_{50}, and LT_{90} values. The yield obtained in this study was 1.5%. Further, the analysis showed that the oil of *A. herba-alba* is a davanone chemotype that consists mainly of davanone (48.8%). The efficiency of this essential oil for toxicological parameters (LC_{50} and LC_{90}) were 3.278 μl/ml and 7.573 μl/ml for larvae, and 1.213 μl/ml and 2.288 μl/ml for pupae. This study indicates that the essential oil of *A. herba-alba* has toxic properties for *Cx. pipiens* larvae and pupae. These results are encouraging and open up exciting and promising horizons for its application in the production of bioinsecticides.

Keywords: bioinsecticides, biological control, extraction, natural molecules, toxicological parameters

Resumen

Artemisia herba-alba (A. herba-alba) (Asteraceae) es ampliamente utilizada en fitoterapia ya que tiene una verdadera mina de moléculas naturales como la davanona, que es un producto muy interesante en el mercado internacional. La presente investigación propone un método para el control de los estadios preimaginarios de *Culex pipiens* (L4 y pupas) a base de aceite esencial de *A. herba-alba*. La parte aérea de esta planta fue extraída por hidrodestilación, luego fue analizada por cromatografía de gases acoplada a la espectrometría de masas (CPG/SM) para la determinación de su composición química. Se prepararon tres concentraciones (1, 5 y 10 μl/ml) y se probaron directamente en larvas (L4) y pupas en condiciones de laboratorio para medir los valores LC_{50}, LC_{90}, LT_{50} y LT_{90}. El rendimiento obtenido en este estudio fue del 1,5 %, además el análisis mostró que el aceite de *A. herba-alba* es un quimiotipo de davanona que consiste principalmente en davanona (48,8 %). La eficiencia de este aceite esencial se expresa por los parámetros toxicológicos calculados en la LC_{50} y LC_{90}, para larvas de 3,278 μl/ml y 7,573 μl/ml, y para pupas 1,213 μl/ml y 2,288 μl/ml, respectivamente. Este estudio muestra que el aceite esencial de *A. herba-alba* tiene propiedades tóxicas sobre larvas y pupas

1. Laboratory of Research on the Improvement and Development of Animal and Plant Production, University of Ferhat Abbas, Setif, Algeria.
2. Laboratory for Exploration and Valorization of Steppe Ecosystems, University of Ziane Achour, Djelfa, Algeria.
3. Laboratory of Organic Chemistry and Natural Substances, University of Ziane Achour, Djelfa, Algeria.

* Corresponding author: <abdelali.selmakaoutar@gmail.com>

Received: May 2022; accepted: July 2022.

CC BY NC SA

Revista Actualidades Biológicas
Actual. Biol. 45(118): 1-12, 2023 | DOI:10.17533/udea.acbi.v45n118a01

de *Culex pipiens*. Estos resultados son alentadores y abren interesantes y prometedores horizontes para su aplicación en la producción de bioinsecticidas.

Palabras clave: bioinsecticidas, extracción, control biológico, moléculas naturales, parámetros toxicológicos

INTRODUCTION

The control of immature mosquitoes is considered advantageous for preventing the transmission of vector because the larvae are usually concentrated, relatively immobile, and occupy minimal habitat compared to adults (Imbahale et al., 2011). The widespread use of chemical insecticides has developed disadvantages due to their persistent nature and residues in various environments and food (Airparif, 2016). Today, to preserve the health of non-target populations, it is necessary to focus on natural compounds from plants (Habbachi et al., 2013) by exploiting their capacity to produce secondary metabolites which can be used as new bioinsecticides (Acheuk et al., 2017). *Artemisia herba-alba* is a silvery perennial dwarf shrub that grows in arid and semi-arid climates, and displays rapid growth under dry and hot temperatures and in muddy areas (Tilaoui et al., 2015). In Algeria, *A. herba-alba* represents an important fodder resource (Belhattab et al., 2014). The essential oil of this herb has antioxidant, disinfectant, antibacterial, antileishmanial, anthelmintic, nematicide, and antispasmodic properties (Abu-Darwish et al., 2015).

In Algeria, studies on the insecticidal activity of plant extracts against the mosquito larva are limited (Benhissen et al., 2018), but in recent years has started to develop through a multitude of recent works (Acheuk et al., 2017; Belhattab et al., 2014; Benhissen et al., 2018; Habbachi et al., 2013; Matoug et al., 2017; Merabti et al., 2015, 2016). Therefore, this study is oriented toward biological control by using non-polluting, natural substances that are less harmful to develop an extract that is less expensive and effective. We examined the essential oil of *A. herba-alba* to evaluate its toxic activities on *Cx. pipiens* larvae and pupae.

MATERIALS AND METHODS

Insect

Culex pipiens are holometabolous insects that pass successively through very different stages: egg, larva, pupa, then adult (imago) (Delaunay et al., 2001). *Culex* females lay their eggs in the form of rafts (Michaelakis et al., 2005). The cycle breaks down into two phases: an aquatic phase for the first three stages, and an aerial phase for the last stage. Under optimal conditions, the cycle lasts from 10 to 14 days (Resseguier, 2011). *Cx. pipiens* larvae are found in diverse urban and peri-urban environments, especially those rich in organic matter (Jiafeng et al., 2011).

Mosquito Rearing

In the laboratory, captured larvae were sorted by larval stage and then transferred to containers for rearing in cages (20 x 20 x 20 cm) at a temperature of 25 ± 2 °C and humidity of 75 ± 10%, and a scotophase of 12 hours. A mixture of biscuit and dry yeast ensured the nutrition of the larvae (Rehimi and Soltani, 2002). Only larvae that have reached the fourth stage are the subject of a reliable identification with the help of the identification software of the *Culicidae* of Mediterranean Africa (Brunhes et al., 1999). Adults were fed on raspberry and cotton swabs soaked in sugar water. The blood meal, essential for egg laying, was provided by the introduction of a Petri dish containing about 5 ml of horse blood mixed with the anticoagulant heparin (Couzin, 2006).

Plant material

The plant material used in this study consisted of the aerial part of *A. herba-alba*, determined by comparison to a sample from the New York garden herbarium; voucher number (02708325). The identification was confirmed by Mr. Brague A., Principal

Forest Inspector at the National Institute of Forest Research of the province of Djelfa. The plant was harvested in May from the Medjbara (34° 30' N, 3° 28' E) region in Djelfa (figure 1). After recovery of the plant, the aerial part was cleaned. Drying was carried out naturally, protected from light and humidity, at room temperature (around 24 °C) for 15 days to preserve the molecules integrity as much as possible.

Extraction

The essential oil was obtained after 4 main stages: hydrodistillation, liquid-liquid extraction, water elimination, and solvent elimination. Hydrodistillation: A quantity of 50 g of the dried plant was introduced into a balloon of 1000 ml. 500 ml of distilled water was transferred and the whole is stirred. The balloon was then placed into a hydro-distillation assembly using a Clevenger type device (Clevenger, 1928) according to the recommendations of the Hellenic Pharmacopoeia (Hellenic Pharmacopoeia, 2002). Liquid-liquid extraction: The distillate was put into a separatory funnel, the solvent added, and the funnel is closed. Vigorous stirring was practiced for a time necessary to establish a concentration equilibrium between the two phases and degassed after it was fixed on a support with the removal of the cover. Each step was collected in an appropriate container (Abe et al., 2010). Water Removal: To remove all traces of water, the organic phase was dried by adding a few grams of anhydrous magnesium sulfate $MgSO_4$, then filtered using filter paper (Feknous et al., 2014). Solvent Removal: The liquid obtained in the previous step was poured into an appropriate flask, then fixed to a rotary evaporator to carry out a simple distillation under reduced pressure at a temperature of 37 °C (Mecquenem et al., 2018). The obtained oil was stored in hermetically sealed sterile glass bottles, protected from light, and stored at a temperature of 4 °C.

Extraction efficiency of essential oil

The extraction yield was calculated using the following formula (Falleh et al., 2008):

R (%) = (Mext / Méch.) * 100

$R = 3/200 = 1.5\%$

Figure 1. *Artemisia herba-alba.*

Revista Actualidades Biológicas
Actual. Biol. 45(118): 1-12, 2023 | DOI:10.17533/udea.acbi.v45n118a01

R is the yield in %.

Mext is the mass of the extract (in g) after evaporation of the solvent.

Méch is the dry mass of the plant sample (in g).

Chemical analysis

The chemical composition of the essential oil was analyzed by gas chromatography coupled with mass spectrometry (CG/MS), which allows both a qualitative and quantitative determination of the majority of compounds of the sample (2-5 μl). The essential oil was transferred to a GC vial, diluted in hexane (1-2 ml), and sealed with a high-performance septum (Delazar et al., 2004).

The identification of the constituents was carried out by coupling a Chromatograph in the gas phase of the Clarus 680 Perkin Elmer type coupled to the Clarus SQ 8 mass spectrometer. The Rtx-5MS in fused silica (30 mx 0.25 mm ID, 0.25 pm df, RESTEK, USA) was directly coupled to the mass spectrometer (Delazar et al., 2004). The carrier gas was helium (1 ml/min). The program used was 2 min isothermal at 60 °C, then 3 °C/min at 160 °C, then 6 °C/min at 240 °C for 2 min. The temperature of the injection port was 250 °C, and the detector temperature was 240 °C. The ionization of components of the sample was performed in EI mode (70 eV). The MS scan ranged from 30 to 300 amu (Delazar et al., 2004). The individual constituents were identified by comparing their mass spectra to spectra stored in the NIST/EPA/NIH mass spectral database (Version 2.0 g from May 19, 2011).

Treatment

Sensitivity tests were carried out using the recommended protocol of the World Health Organization to test the sensitivity of the larvae towards insecticides used in control campaigns (World Health Organization, 2005). This test was carried out on 2 stages, the 4th instar larvae and pupae of *Cx. pipiens*. Preliminary tests with different doses were carried out to select a range of concentrations before starting the toxicity test. Three dilutions of 10% = 1 μl/ml, 50% = 5μl/ml and 100% = 10μl/ml were prepared from the initial extract (1% stock solution). A total of 15 individuals (larvae/pupae) were sampled using a Pasteur pipette and placed in goblets, each containing 99 ml of water. A milliliter of each solution was then added to each previously prepared goblet. The same number of individuals were placed in a control cup containing 100 ml of water. Three repetitions were performed for each dilution as well as for the control. Mortality rates were assessed after 24, 48, and 72 hours.

Statistical analysis

The mortality values obtained for the two stages in various concentrations were considered as means. These results were subjected to a probit analysis to calculate the lethal concentrations and lethal times (LC_{50}% LC_{90}%, LT_{50}%, and LT_{90}%). This analysis was performed using the IBM SPSS Statistics program23 in Windows.

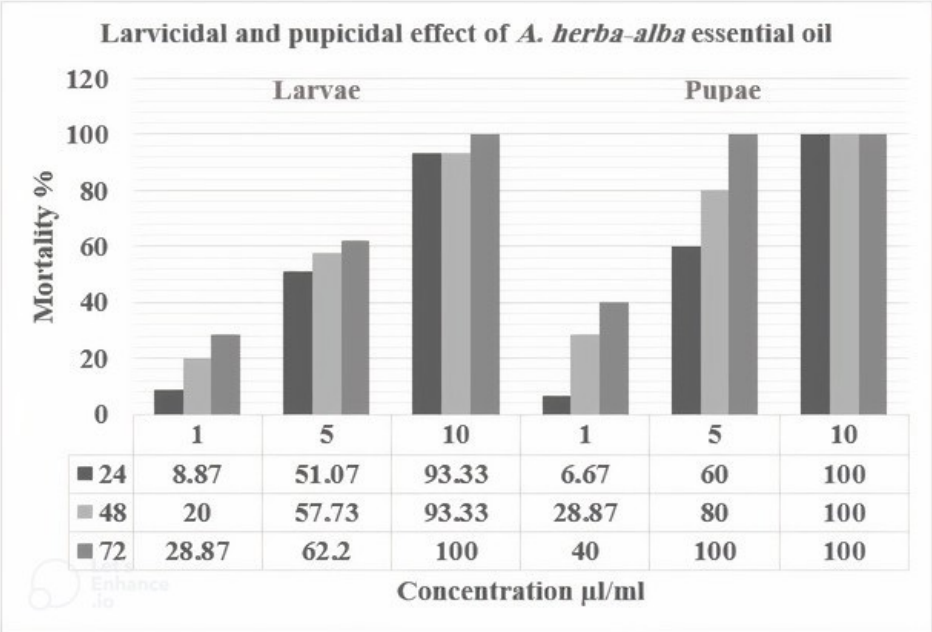
RESULTS

The effect of *A. herba-alba* on the mortality of *Cx. pipiens*

The two stages of *Cx. pipiens* are sensitive to *A. herba-alba*. This sensitivity is reflected by higher or lower mortality rates depending on the concentrations used, especially according to the time of exposure to the extract (figure 2). In the fourth larval stage, the mortality rate ranged between 8.87% and 28.87% for the lowest concentration (1 μl/ml), while reaching 100% after 48 h when the larvae were exposed to the highest concentration (10 μl/ml). In the pupae, the mortality rate ranged between 6.67% and 40% for the lowest concentration (1 μl/ml), while reaching 100% after 72 h when pupae were exposed to the medium concentration (5 μl/ml).

Toxicological parameters of *A. herba-alba*

The results show a strong positive correlation between recorded mortality rates and the exposure time and/or the concentration of the extract used against mosquitoes (tables 1 and 2). To ensure a 50% mortality of the fourth larval stage after 24 h, the concentration of *A. herba-alba* must be equal to 5.081



	1	5	10	1	5	10
24	8.87	51.07	93.33	6.67	60	100
48	20	57.73	93.33	28.87	80	100
72	28.87	62.2	100	40	100	100

Figure 2. Evolution of mortality rate % in the larvae and pupae of *Culex pipiens* treated with the different doses of *A. herba-alba* essential oil.

μl/ml. On the contrary, 9.128 μl/ml of *A. herba-alba* ensures the mortality of 90% (table 1). After 48 h, the calculations show that the LC_{50}% is 4.241 μl/ml, while the LC_{90}% is 9.166 μl/ml. After 72 h of treatment, the LC_{50}% is 3.278 μl/ml and the LC_{90}% is 7.573 μl/ml.

On the lethal times, the concentration of 1 μl/ml of *A. herba-alba* eliminated 50% of the population of *Cx. pipiens* in 4.37 days and 90% during 7.70 days of treatment (table 1). When 5 μl/ml of *A. herba-alba* extract is applied, LT_{50}% was 0.75 days, while the LT_{90}% was 9.77 days. To ensure a 50% mortality of the pupae after 24 h, the concentration of *A. herba-alba* must be equal to 4.356 μl/ml. On the contrary, 7.110 μl/ml of *A. herba-alba* ensures the mortality of 90% (table 2). After 48 h, the calculations show that the LC_{50}% is 2.579μl/ml, while the LC_{90}% is of 6.075 μl/ml. After 72 h of treatment, the LC_{50}% is 1.213 μl/ml and the LC_{90}% is 2.288 μl/ml.

On the lethal times, the concentration of 1 μl of *A. herba-alba* eliminated 50% of the population of *Cx. pipiens* in the 3.3 days and 90% during 5.52 days of treatment (table 2). When 5 μl/ml of *A. herba-alba* extract is applied, LT_{50}% is 0.82 days, while the LT_{90}% is 0.99 days.

Average yield of AEO and its chemical characterization

The yield of *A. herba-alba* essential oil obtained in this study was 1.5%. Further, twenty-nine main molecules were extracted within forty minutes. We note that a large proportion was monopolized by Davanone (48.84%), which accounted for approximately half, followed by chrysanthenone (15.97%), camphor (14.84%), with the remaining proportions ranging from 0.04% to 5.69% (table 3, figure 3).

DISCUSSION

More than 2,000 plant species with insecticidal activity have been identified (Jacobson, 1989). Some plants have evolved a wide range of physical conditions and chemical defenses against a variety of insects through substances such as phenols, polyphenols, terpenoids, and alkaloids that can be isolated using various extraction methods (Dubey, 2010).

Revista Actualidades Biológicas
Actual. Biol. 45(118): 1-12, 2023 | DOI:10.17533/udea.acbi.v45n118a01

Table 1. Toxicological parameters of *A. herba-alba* essential oil in larvae treated with *Cx.pipiens*

EXPOSED TIME			
Time (hours)	**24**	**48**	**72**
Regression line	Y = -1.62 + 0.32x	Y = -1.1 + 0.26x	Y = -0.77 + 0.22x
LC 50% (μl/ml)	5.081	4.241	3.278
LC 90% (μl/ml)	9.128	9.166	7.573
95% Confidence Interval	[0.169 0.465]	[0.126 0.394]	[0.134 0.463]
Chi square value	0.055	0	0.867
P value	0.815	0.992	0.352
R	0.99	1	1
CONCENTRATION USED			
Concentration (μl/ml)	**1**	**5**	**10**
Regression line	Y = -1.71 + 0.02x	Y = -0.11 + 5.92E-3x	*
LT 50% (hours)	104.910	18.025	*
LT 90% (hours)	184.869	234.389	*
95% Confidence Interval	[-0.007 0.039]	[-0.013 0.025]	[-0.022 0.056]
Chi square value	0.058	0.004	0.662
P value	0.81	0.947	0.416
R	0.99	0.99	*

Table 2. Toxicological parameters of *A. herba-alba* essential oil in pupae treated with *Cx. pipiens*

EXPOSED TIME			
Time (hours)	**24**	**48**	**72**
Regression line	Y = -1.94 + 0.44x	Y = -0.91 + 0.35x	*
LC 50% (μl/ml)	4.356	2.579	1.213
LC 90% (μl/ml)	7.110	6.075	2.288
95% Confidence Interval	[-5.332 7.499]	[0.061 0.413]	[-11.275 13.056]
Chi square value	0	0.49	0
P value	0.983	0.484	0.993
R	1	1	*
CONCENTRATION USED			
Concentration (μl/ml)	**1**	**5**	**10**
Regression line	Y = -2.02 + 0.03x	Y = -0.33 + 0.02x	*
LT 50% (hours)	79.077	19.693	*
LT 90% (hours)	132.479	53.257	*
95% Confidence Interval	[0.029 0.083]	[0.009 0.067]	*
Chi square value	2.848	0.876	*
P value	0.091	0.349	*
R	0.96	1	*

Pranati et al. (2018) have shown the larvicidal and pupicidal effect of extracts of *Clerodendrum philippinum* leaves against *Aedes aegypti* and *Anopheles stephensi* with considerable mortality rates. In addition, the study by Kaura et al. (2019) reveals the larvicidal and pupicidal effect of *Eucalyptus globulus* essential oil, which acts quickly on *Ae. aegypti* and *Ae. albopictus* larvae and pupae, with an LC_{50} of 93.3 and 144.5 ppm, and an LC_{90} of 707.9 and 741.3 ppm,

Table 3. Main chemical compounds (%) of *A. herba-alba* essential oil analyzed by the CG/SM

Ret. Time	Compound Name	%
13.604	α-Pinene	0.04
16.65	Camphene	1.34
17.575	2(5H)-Furanone, 5,5-dimethyl-	0.42
9.691	β-Myrcene	0.16
10.031	o-Cymene	0.10
11.196	Cyclohexene, 1-methyl-5-(1-methylethenyl)-, (R)-	0.28
11.591	Eucalyptol	5.69
12.112	2(3H)-Furanone, 5-ethenyldihydro-5-methyl-	0.21
13.647	1,5-Heptadien-4-ol, 3,3,6-trimethyl-	0.20
14.743	Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-	0.71
15.173	Thujone	0.47
15.643	Chrysanthenone	15.97
16.083	Cyclohexane, 2-ethenyl-1,1-dimethyl-3-methylene-	0.37
16.253	Isopinocarveol	0.70
16.568	Camphor	14.84
16.868	cis-p-mentha-1(7),8-dien-2-ol	0.63
17.329	Pinocarvone	0.42
17.449	endo-Borneol	1.61
17.899	Terpinen-4-ol	0.91
18.264	Tricyclo[4.3.0.0(3,8)]nonan-2-ol,2-(aminomethyl) stereoisomer	0.06
18.544	α-Terpineol	0.41
19.344	2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-, cis-	0.55
19.84	Ethanol, 2-(3,3-dimethylbicyclo[2.2.1]hept-2-ylidene)-	0.77
23.081	Thymol	0.19
25.612	3-Cyclohexene-1-methanol, α,α,4-trimethyl-, acetate	1.51
27.728	3,5-Heptadienal, 2-ethylidene-6-methyl-	1.00
28.138	3-Methyl-2-pent-2-enyl-cyclopent-2-enone	0.80
35.621	(-)-Spathulenol	0.82
36.086	**Davanone**	**48.84**

respectively.

The results of the present study reveal a considerable and variable sensitivity of *Cx. pipiens* to *A. herba-alba* essential oil, translated by rates of low to very high mortality, which correlates with the extension of time from one concentration to the other. The same observation was made by Aksorn and Mayura (2018) on the larvae of *Ae. aegypti*, which showed that mortality is correlated with the doses used and is increased as the exposure of larvae to insecticides is extended over time. The activity of *A. herba-alba* essential oil can be expressed by the diversification of the bioactive molecules which compose the essential oil. The effects of the *A. herba-alba* essential oil may be due to a singular action of one of the major components, dominated by Davanone (48.8%), or by a synergistic effect between several compounds towards the larvae and pupae of the mosquitos which are exposed to it.

The oil yield recorded in the present study was relatively higher than those extracted from the same species collected in Spain with 0.8% (Salido et al., 2001) and Tunisia 0.7% (Haouari and Ferchichi, 2009). While it equal to those extracted in Tunisia by Zouari et al. (2010) and by Boutemak et al. (2009) in Algeria. Also, it is lower than the one extracted

Revista Actualidades Biológicas
Actual. Biol. 45(118): 1-12, 2023 | DOI:10.17533/udea.acbi.v45n118a01

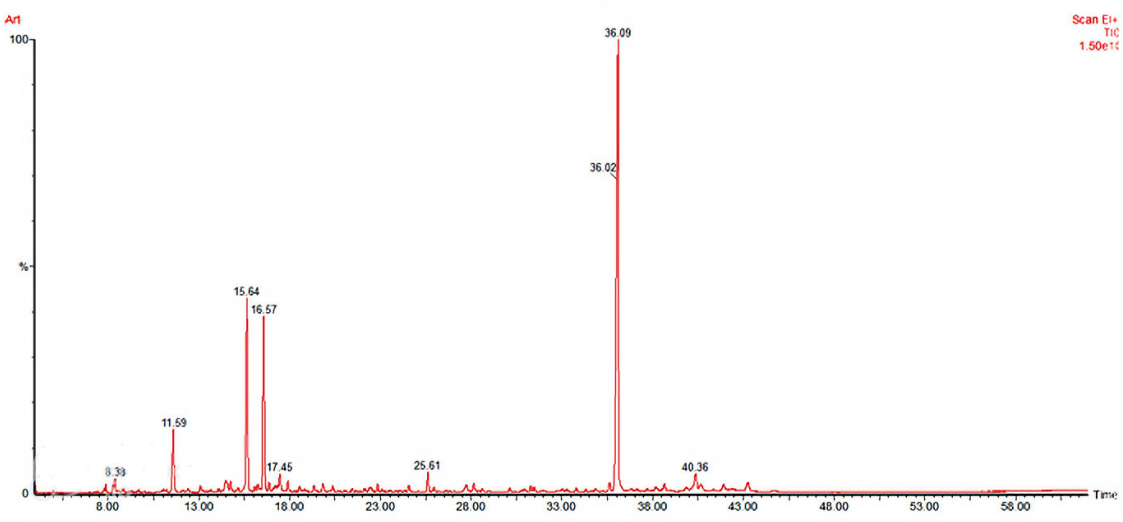


Figure 3. Chromatographic profile of *A. herba-alba* essential oil analyzed by CG/SM.

in Morocco: 3.3% by Paolini et al. (2010). This difference in yield can be explained by the impact of several factors such as the nature of the species, the effect of the vegetative stage of the plant, and the edaphic conditions of the region (Ghanmi et al., 2010).

Regarding the chemical composition of this oil a variability of volatile constituents was observed in many countries from previous studies as such in Moroco [Camphor (40–70%), α-or β-Thujone (32–82% and 43–93%, respectively), Chrysanthenone (51.4%), Chrysanthenyl acetate (32–71%), or Davanone (20–70%)] were the major components from that of Paolini et al. (2010). Whereas [Davanone (0.5–39.1%), 1,8-Cineole (0.8–25.8%), Chrysanthenone (0.1–36.4%), Cis-Chrysanthenol (0.2–27.8%), Cis-Chrysanthenyl acetate (0.2–18.4%), p-Cymene (0.6–20.6%), α-Pinene (0.2–17.2%)] were reported as dominant in Spain (Salido et al., 2002). In Tunisia the major components were [Cineole (1.5–26.99%), Thujones (1–64.67%), Chrysanthenone (1–17.37%), Camphor (0.56–16.73%), Borneol (0.72–10.75%), Chrysanthenyl acetate (0.52–7.37%), Sabinyl acetate (0.53–22.46%), Davana ethers (0.65–6.23%) and Davanone (2.37–20.14%)] as referred by Haouari and Ferchichi (2009).

On the other hand, with the exception of Davanone, which is the main compound of the present work, it was not detected in the study of Abu-Darwish et al. (2015) in Jordan [β-Thujones (25.1%), α-Thujones (22.9%), Eucalyptol (20.1%) and Camphre (10%)] neither in that of Abou El-Hamd et al. (2010) in Egypt [1,8-Cineole (50%), Thujone (27%), Terpinen-4-ol (3.3%), Camphor (3%) and Borneol (3%)], as well in Iran, where Sharifian et al. (2012) reported [β-Thujone (35.66%), Camphor (34.94%), 1,8-Cineole (7.42%), α-Thujone (4.12%)] as the main components. In addition, even within Algeria, different chemical compositions of the essential oil of *A. herba-alba* have been recorded. For example, in the region of Djelfa, Touil and Benrebiha (2014) found Davanone (62.20%), Carvacrol (4.88%), Davana ether (3.62%), Camphor (3.48%) as major components. In Msila region, the main components announced by Dob and Benabdelkader (2006) were the Camphor (19%), trans-pinocarveol (17%), chrysanthenone (16%), b-thujone (15%), b-Thujone (3241%), camphor (16–25%), cineol (0.1–10%). However, Boutekedjiret et al. (1992) suggest that there is a variation of the volatile component of *A. herba-alba* under the seasonal change factor within the same region.

Overall, this wide chemical variability may be a result of the genetic characteristics of the plant com-

bined with the influences of geographical locations and climatic conditions, as well as the difference in the developmental stages of the plant and the method used to obtain the essential oil (Belhattab et al., 2014; Lakehal et al., 2016). Indeed, previous studies have revealed the different bioactivities of the components of *A. herba-alba* extracts against many pests, such as an insecticidal activity against tobacco whitefly *Bemisia tabaci* (Gennadius), cotton aphid *Aphis gossypii* (Glover), thrips of tobacco and onion *Thrips tabaci* (Lindman) (Soliman, 2007). The study by Tani et al. (2008) on bean leaf beetle *Acanthoscelides obtectus* (Say) and of Hifnawy et al. (2001) on Cotton Worm *Spodoptera littoralis* (Boisduval) also revealed a toxic effect against insects. Moreover, acaricidal activity was reported against carmine spider mite *Tetranychus cinnabarinus* (Boisduval) per Azaizeh et al. (2007). Further Hifnawy et al. (2001) proved the ability of this essential oil to control white mice *Mus musculus* (Linnaeus) by provoking a rodenticidal activity.

The mechanism of action of the essential oil on insects is mainly due to neurotoxic effects involving several modes of action, including acetylcholinesterase (AChE) inhibition (Mills et al., 2004), disruption of gamma-aminobutyric acid (GABA) receptor functionality (Priestley et al., 2003) and agonist of the octopamine system (Enan, 2005). According to Pavela (2016) the most important neurotoxic mode of action are hyperactivity followed by hyperarousal leading to rapid reversal and immobilization, as well as the insects' mouthparts becoming paralyzed which stops feeding and leads to starvation. In addition, Rattan (2010) confirms that essential oils and their constituents affect biochemical processes, which specifically disturb the endocrinological balance of insects. They can be neurotoxic or act as insect growth regulators, disrupting the normal process of morphogenesis. In insects, the result of this nerve poisoning can be immediate death or several days of paralysis before death.

In the same context, Jun-Hyung and Murray (2015) note that insecticidal activity results from a series of complex actions and contractions between a toxic tissue and an insect tissue. This mechanism of toxicity can be expressed in three steps: penetration, activation (target site interaction), and detoxification. Plant extracts act in two possible ways: a larvicidal action that can cause appreciable mortality of larvae in 1 to 12 days, or a juvenile hormone mimetic action, with an extension of the larval life span that can inhibit pupation (Rageau and Delaveau, 1979). Taking into account the toxic effect of these essential oils, a study was carried out to ensure the therapeutic safety; therefore Boukhennoufa et al. (2021) confirmed the indemnity of the toxic effect of the essential oil of *A. herba-alba* on the proper functioning and survival of the organism after a cutaneous exposure.

CONCLUSION

This study indicates that the essential oil of *A. herba-alba* has toxic properties on the larvae and pupae of *Cx. pipiens*. These results are encouraging and open interesting and promising horizons for its application as a bioinsecticide. These are readily available, and the cost constraint can be overcome by the low value of the LC_{50}. However, another deep chemical study would be necessary to precisely isolate the molecule responsible for the toxic effect. In addition, a histological study is desirable to know the mode of action of this oil on the tissues of *Cx. pipiens* larvae and pupae.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

RESEARCH INVOLVING HUMAN PARTICIPANTS AND/OR ANIMALS

Not applicable.

INFORMED CONSENT

Not applicable.

REFERENCES

Abe, E., Delyle, S. G., & Alvarez, J. C. (2010). Extraction liquide-liquide: Théorie, applications, diffi-

cultés. *Annales de Toxicologie Analytique, 22*(2), 51-59. DOI:10.1051/ata/2010018

Abou El-Hamd, H. M., Magdi, A. E., Mohamed, E. H., Soleiman, E. H., Abeer M. E., & Naglaa S. M. (2010). Chemical Constituents and Biological Activities of *Artemisia herba-alba. Rec. Nat. Prod., 4*(1), 1−25. DOI:10.13140/RG.2.1.2544.8806

Abu-Darwish, M. S., Cabral, C., Gonçalves, M. J., Cavaleiro, C., Cruz, M. T., Efferth, T., & Salgueiro, L. (2015). *Artemisia herba-alba* essential oil from Buseirah (South Jordan): Chemical characterization and assessment of safe antifungal and anti-inflammatory doses. *Journal of Ethnopharmacology, 174*, 153-160. DOI:10.1016/j.jep.2015.08.005

Acheuk, F., Abdellaoui, K., Lakhdari, W., Dehliz, A., Ramdani, M., Barika, F., & Alouen, R. (2017). Potentiel bio-insecticide de l'extrait brut de la plante saharienne *Artemisia judaica* en lutte anti-vectorielle: Cas du moustique commun *Culiseta longiareolata. Journal Algérien des Régions Arides (JARA), 14*, 109−116.

Airparif. (2016). *Les pesticides dans l'air francilien Partie I: État des connaissances.* L'observatoire de l'air en Île-de-France, 29.

Aksorn, C., & Mayura, S. (2018). Efficacies of four plant essential oils as larvicide, pupicide and oviposition deterrent agents against dengue fever mosquito, *Aedes aegypti* Linn. (Diptera: Culicidae). *Asian Pacific Journal of Tropical Biomedicine, 8*(4), 217−225. DOI:10.4103/2221-1691.231284

Azaizeh, H., Kobaisy, M., Dakwar, S., Saad, B., Shaqir, I., & Said, O. (2007). Botanical pesticides as a source of safe bioacaricides for the control of *Tetranychus cinnabarinus. Acta Phytopathologica et Entomologica Hungarica, 42*(1), 143-152. DOI:10.1556/aphyt.42.2007.1.8

Belhattab, R., Amor, L., Barroso, J., Pedro, L., & Figueiredo, A. (2014). Essential oil from *Artemisia herba-alba* Asso grown wild in Algeria: Variability assessment and comparison with an updated literature survey. *Arabian Journal of Chemistry,* 7, 243-251. DOI:10.1016/j.arabjc.2012.04.042

Benhissen, S., Rebbas, K., Habbachi, W., & Masna, F. (2018). Bioactivity of *Nicotiana glauca graham*(solanaceae) and its toxic effects on *Culiseta longiareolata* (diptera; culicidae). *International Journal of Research in Ayurved Pharmacy, 9*, 123-126. DOI:10.7897/2277-4343.09124

Boukhennoufa, A., Boumediene, M., & Aicha, T. T. M. (2021). A study of acute dermal toxicity of *Artemisia herba-alba* Asso essential oils. *Indian Journal of Natural Products and Resources (IJNPR) [Formerly Natural Product Radiance (NPR)], 12*(2), 225-229. http://op.niscair.res.in/index.php/IJNPR/article/view/38811

Boutekedjiret, C., Charchari, S., Belabbes, R., & Bessière, J.M. (1992). Contribution à l'étude de la composition chimique de l'huile essentielle d'*Artemisia herba-alba* Asso. *Rivista Italiana EPPOS, 3,* 39-42.

Boutemak, K., Bezzina, M., Périno-Issartier, S., & Chemat, F. (2009). Extraction by Steam Distillation of *Artemisia herba-alba* Essential Oil from Algeria: Kinetic Study and Optimization of the Operating Conditions. *Journal of Essential Oil Bearing Plants, 12*(6), 640-650. DOI:10.1080/0972060X.2009.10643768

Brunhes, J., Rhaim, A., Geoffroy, B., Angel, G., & Hervy, J.P. (1999). *Les Moustiques de l'Afrique méditerranéen, logiciel d'identification et d'enseignement.* IRD edition.

Clevenger, J. F. (1928). Apparatus for the Determination of Volatile Oil*. *The Journal of the American Pharmaceutical Association (1912), 17*(4), 345-349. DOI:10.1002/jps.3080170407

Couzin, J. (2006). Biomedical research. Bone disease gene finally found. *Science, 312*(5773), 514-515. DOI:10.1126/science.312.5773.514b

Delaunay, P., Fauran, P., & Marty, P. (2001). Les moustiques d'intérêt médical. *Revue Française des Laboratoires, 2001*(338), 27-36. DOI:10.1016/S0338-9898(01)80348-6

Delazar, A., Reid, R. G., & Sarker, S. D. (2004). GC-MS Analysis of the Essential Oil from the Oleoresin of *Pistacia atlantica* var. Mutica. *Chemistry of Natural Compounds, 1*(40), 24-27. DOI:10.1023/B:CONC.0000025459.72590.9e

Dob, T., & Benabdelkader, T. (2006). Chemical Composition of the Essential Oil of *Artemisia herba-alba* Asso Grown in Algeria. *Journal of Essential Oil Research, 18*(6), 685-690. DOI:10.1080/10412905.2006.9699206

Dubey, N. K. (2010). *Natural products in plant pest management* (p. 293).

Enan, E. E. (2005). Molecular response of *Drosophila melanogaster* tyramine receptor cascade to plant essential oils. *Insect Biochemistry and Molecular Biology, 35*(4), 309-321. DOI:10.1016/j.ibmb.2004.12.007

Falleh, H., Ksouri, R., Chaieb, K., Karray-Bouraoui, N., Trabelsi, N., Boulaaba, M., & Abdelly, C. (2008). Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities. *Comptes Rendus Biologies, 331*(5), 372-379. DOI:10.1016/j.crvi.2008.02.008

Feknous, S., Saidi, F., & Mohamed Said, R. (2014). Extraction, caractérisation et identification de quelques métabolites secondaires actifs de la mélisse (*Melissa officinalis* L.). *Revue Nature et Technologie, 6*(2), 07-13. https://www.asjp.cerist.dz/en/article/43397

Ghanmi, M., Satrani, B., Aafi, A., Isamili, M. R., Houti, H., El Monfalouti, H., Benchakroun, K. H., Aberchane, M., Harki, L., Boukir, A., Chaouch, A., & Charrouf, Z. (2010). Effet de la date de récolte sur le rendement, la composition chimique et la bioactivité des huiles essentielles de l'armoise blanche (*Artemisia herba-alba*) de la région de Guerçif (Maroc oriental). *Phytothérapie, 8*(5), 295-301. DOI:10.1007/s10298-010-0578-1

Habbachi, W., Benhissen, S., Ouakid, M. L. & Farine, J. P. (2013). Effets Biologiques d'extraits Aqueux de *Peganum Harmala* (L.) (Zygophyllaceae) sur la Mortalité et le Développement Larvaire de *Drosophila Melanogaster* (Diptera-Drosophilidae). *Algerian Journal of Arid Environment, 3*(1), 82-88. DOI:10.12816/0008892

Haouari, M., & Ferchichi, A. (2009). Essential Oil Composition of *Artemisia herba-alba* from Southern Tunisia. *Molecules, 14*(4), 1585. DOI:10.3390/molecules14041585

Hellenic Pharmacopoeia (2002). *National Organization for Medicines of Greece* (5th ed, Vol. 12).

Hifnawy, O., Rashwan, A., & Rabeh, M. (2001). Comparative chemical and biological investigations of certain essential

oils belonging to families Asteraceae, Lamiaceae and Graminae. *Bulletin of the Faculty of Pharmacy, 39*(2), 35-53.

Imbahale, S. S., Mweresa, C. K., Takken, W., & Mukabana, W. R. (2011). Development of environmental tools for anopheline larval control. *Parasites Vectors, 4*(1), 130. DOI:10.1186/1756-3305-4-130

Jacobson, M. (1989). Botanical Pesticides Past, Present, and Future. *Insecticides of Plant Origin. 387*, 10. DOI:10.1021/bk-1989-0387

Jiafeng, W., Nick, H.O., & Huaiping, Z. (2011). The impact of weather conditions on *Culex pipiens* and *Culex restuans* (Diptera: Culicidae) abundance: a case study in Peel Region. *Journal of Medical Entomology, 48*(2), 468-475. DOI:10.1603/me10117

Jun-Hyung, T., & Murray, B. I. (2015). Enhanced cuticular penetration as the mechanism for synergy of insecticidal constituents of rosemary essential oil in *Trichoplusia ni. Scientific Reports, 5*, 10. DOI:10.1038/srep12690

Kaura, T., Mewara, A., Zaman, K., Sharma, A., Agrawal, S. K., Thakur, V., Garg, A., & Sehgal, R. (2019). Utilizing larvicidal and pupicidal efficacy of Eucalyptus and neem oil against *Aedes* mosquito: An approach for mosquito control. *Tropical Parasitology, 9*(1), 12-17. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6542315/

Lakehal, S., Meliani, A., Benmimoune, S., Bensouna, S. N., Benrebiha, F. Z., & Chaouia, C. (2016). Essential Oil Composition and Antimicrobial Activity of *Artemisia herba–alba* Asso Grown in Algeria. *Medicinal Chemistry, 6*(6), 5. https://doi.org/10.4172/2161-0444.1000382

Matoug, H., Merabti, B., Tadjer, W., Djamila, E., & Ouakid, M. L. (2017). Biological Control Test of Ethanol Extracts Of *Peganum Harmala* (L.) on The Mortality And Development of *Culex Pipiens* (Diptera). *World Journal of Environmental Biosciences, 6*(6), 15−19.

Mecquenem, C., Drommi, M., & Topart, C. (2018). Utilisation de l'évaporateur rotatif. https://culturesciences.chimie.ens.fr/thematiques/chimie-organique/methodes-et-outils/utilisation-de-l-evaporateur-rotatif

Merabti, B., Ismahane, L., Adamou, A. -E., & Ouakid, Mohammed. L. (2015). Effet toxique de l'extrait aqueux des fruits de *Citrullus colocynthis* (l.) Schrad sur les larves des culicidae. *Revue des BioRessources, 5*, 120-130. DOI:10.12816/0046302

Merabti, B., Lebbouz, I., Ouakid, M. L. (2016). Larvicidal activity and influence of Azadirachtin (Neem Tree Extract) on the Longevity and fecundity of mosquito species. *Acta Zoologica Bulgarica, 69*(3), 429−435.

Michaelakis, A., Mihou, A. P., Couladouros, E. A., Zounos, A. K., & Koliopoulos, G. (2005). Oviposition Responses of *Culex pipiens* to a Synthetic Racemic *Culex quinquefasciatus* Oviposition Aggregation Pheromone. *Journal of Agricultural and Food Chemistry, 53*(13), 5225-5229. DOI:10.1021/jf0504871

Mills, C., Cleary, B. J., Gilmer, J. F., & Walsh, J. J. (2004). Inhibition of acetylcholinesterase by Tea Tree oil. *The Journal of Pharmacy and Pharmacology, 56*(3), 375-379. DOI:10.1211/0022357022773

Paolini, J., Ouariachi, E., Bouyanzer, A., Hammouti, B., Desjobert, J. M., Costa, J., & Muselli, A. (2010). Chemical variability of *Artemisia herba-alba* Asso essential oils from East Morocco. *Chemical Papers, 64*(5), 550556. DOI:10.2478/s11696-010-0051-5

Pavela, R. (2016). History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects–a review. *Plant Protection Science, 52*(4), 229-241. DOI:10.17221/31/2016-PPS

Pranati, D., Jyoti, R., Preeti, D., Panda, S., Pallabi, P., Rath, C., Pradhan, C., & Sahoo, S. (2018). Larvicidal and Pupicidal activity of *Clerodendrum philippinum* Schauer Leaf Extracts against *Anopheles stephensi* and *Aedes aegypti. Pharmacognosy Journal, 10*, 1137-1142. DOI:10.5530/pj.2018.6.194

Priestley, C. M., Williamson, E. M., Wafford, K. A., & Sattelle, D. B. (2003). Thymol, a constituent of thyme essential oil, is a positive allosteric modulator of human GABA(A) receptors and a homo-oligomeric GABA receptor from *Drosophila melanogaster. British Journal of Pharmacology, 140*(8), 1363-1372. DOI:10.1038/sj.bjp.0705542

Rageau, J., & Delaveau, P. (1979). Toxic effects of plant extracts on mosquito larvae. *Societe de pathologie exotique et de ses filiales, 72*(2), 168-171.

Rattan, R. S. (2010). Mechanism of action of insecticidal secondary metabolites of plant origin. *Crop Protection, 29*(9), 913-920. DOI:10.1016/j.cropro.2010.05.008

Rehimi, N., & Soltani, N. (2002). Laboratory evaluation of andalin, an insect growth regulator interfering with cuticle deposition, against mosquito larvae. *Sciences & Technologie 18,* 106-110. http://revue.umc.edu.dz/index.php/a/article/view/1820

Resseguier, P. (2011). Contribution à l'étude du repas sanguin de *Culex pipiens pipens* [Other, Université Paul – Sabatier.]. https://oatao.univ-toulouse.fr/5079/

Salido, S., Altarejos, J., Nogueras, M., & Sánchez, A. (2001). Chemical Composition of the Essential Oil of *Artemisia herba-alba* Asso ssp. Valentina (Lam.) Marcl. *Journal of Essential Oil Research, 13*(4), 221-224. DOI:10.1080/10412905.2001.9699675

Salido, S., Luis, R., Valenzuela, B., & Joaqun Altarejos, A. (2002). Composition and infraspecific variability of Artemisia herba-alba from southern Spain. *Biochemical Systematics and Ecology, 32*, 265–277.

Sharifian, I., Hashemi, S.M., Aghaei, M., & Alizadeh, M. (2012). Insecticidal activity of essential oil of *Artemisia herba-alba* Asso against three stored product beetles. *Biharean Biologist, 6*(2), 90-93.

Soliman, M. M. M. (2007). Phytochemical and toxicological studies of *Artemisia L.* (Compositae) essential oil against some insect pests. *Archives of Phytopathology and Plant Protection, 40*(2), 128-138. DOI:10.1080/03235400500355808

Tani, Z. B., Hassani, F., & Khelil, M. A. (2008). Bioefficacy of essential oils extracted from the leaves of *Rosmarinus officinalis* and *Artemisia herba-alba* towards the bruche bean *Acanthoscelides obtectus* (Coleoptera: Bruchidae). *Journal of Pure and Applied Microbiology, 2*, 165-170.

Tilaoui, M., Ait Mouse, H., Jaafari, A., & Zyad, A. (2015). Comparative phytochemical analysis of essential oils from different biological parts of *Artemisia herba-alba* and

their cytotoxic effect on cancer cells. *PloS One, 10*(7), e0131799. DOI:10.1371/journal.pone.0131799

Touil, S., & Benrebiha, F. Z. (2014). Composition chimique et activite antimicrobienne des huiles essentielles d'*Artemisia herba-alba* asso et *Artemisia campestris* l de la region aride de Djelfa. *Agrobiologia, 4*(1), 40-45. https://www.asjp.cerist.dz/en/article/119039

World Health Organization. (2005). *Guidelines for laboratory and field testing of mosquito larvicides* (WHO/CDS/WHOPES/GCDPP/2005.13). World Health Organization. https://apps.who.int/iris/handle/10665/69101

Zouari, S., Zouari, N., Fakhfakh, N., Bougatef, A., Ayadi, M,. & Neffati, M. (2010). Chemical composition and biological activities of a new essential oil chemotype of Tunisian *Artemisia herba-alba* Asso. *Journal of Medicinal Plants Research, 4*(10), 871−880.