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

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Abstract

Aretemisia herba alba Asso (*A.herba alba*) (Asteraceae) is widely used in herbal medicine it has a real mine of natural molecules like davanone which is a very interesting product on the international market. The present research proposes a method for controlling the pre-imaginary stages of *Culex pipiens* (L4 and pupae) based on essential oil *of Artemisia herba alba*. The aerial part of this plant was extracted by hydrodistillation which gave a yield of 1.5. Then it was analyzed by gas chromatography coupled to the mass spectrometry (CPG / SM) for the determination of its chemical composition. The results of the analysis showed that the oil of *A.herba alba* is a davanone chemotype which consists mainly of davanone (48.8%).

Three concentrations (1µl/ml, 5µl/ml and 10µl/ml) are prepared and directly tested on larvae (L4) and pupae. The results show that the essential oils have an important larvicidal and pupicidal activity. This efficiency is expressed by the calculated toxicological parameters which are successively LC50 and LC90, for larvae 3.278 µl/ml and 7.573 µl/ml, and for pupae 1.213 µl/ml and 2.288 µl/ml.

Introduction

The control of immature mosquitoes considered as an advantageous means for the prevention of the transmission of vector diseases, 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 the presence of residues in various environments and in food (Air parif 2016).

Today, in order 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 included in the industry of new bioinsecticides (Acheuk et al. 2017). *Artemisia herba alba* is a silvery perennial dwarf shrub that grows in arid areas and semi-arid climates. With rapid growth in dry and hot climates and in muddy areas (Tilaoui et al. 2015). In Algeria it represents an important fodder resource (Belhattab et al. 2014). The essential oil of this herb has antioxidant, disinfectant, antibacterial, antileshmanial, anthelmintic, nematicide and antispasmodic properties (Abu-Darwish et al. 2015)

In Algeria, the studies on the insecticidal activity of plant extracts against the mosquito larva are very limited (Benhissen et al. 2018) but in recent years has started to develop, through a multitude of recent works (Habbachi et al. 2013; Belhattab et al. 2014; Merabti et al. 2015, 2016; Acheuk et al. 2017; Matoug et al. 2017; Benhissen et al. 2018)

This study is therefore oriented towards biological control by the use of active natural substances, non-polluting and used in a less harmful and more reasoned fight, by developing an extract that is the least expensive and the most effective possible. However, our choice fell on the essential oil of *Artemisia herba*

alba and this in order to evaluate its toxic activities on the larvae of the fourth stage and the pupae of *Culex pipiens*.

Materials And Methods

Insect

Culex pipiens are completely metamorphic insects; they pass successively through very different stages: egg, larva, nymph 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 in 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) *Culex pipiens* larvae are found in the most diverse roosts in urban and peri-urban environments, especially those rich in organic matter (Jiafeng et al. 2011).

Mosquito Rearing

In the laboratory, the captured larvae are 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, humidity of 75 ± 10% and a scotophase 12 hours. A mixture of biscuit and dry yeast ensures the nutrition of the larvae (Rehimi and Soltani 2002).

Only the larvae having 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) While the adults feed on raspberry and cotton swabs soaked in sugar water, However the blood meal, essential for the laying was provided by the introduction of a Petri dish containing about 5 ml of blood of horse mixed with heparin (anticoagulant) (Couzin 2006).

Plant material

The plant material used in this study consists of the aerial part of *Artemisia herba alba* its determination is made by comparing to the one of New York garden herbarium; voucher number (02708325), then the identification has been confirmed by Mr. Brague A., Principal Forest Inspector at the National Institute of Forest Research of the province of Djelfa, harvested in May from the Medjbara (34° 30' N, 3° 28' E) region in Djelfa (Fig. 1). After recovery of the plant, the aerial part was well cleaned. The drying was carried out naturally, protected from light and humidity, at room temperature (around 24 °C), for 15 days, in order to preserve the integrity of the molecules as much as possible.

Extraction

The essential oil was obtained after 4 main stages, hydrodistillation, liquid-liquid extraction, elimination of water and elimination of solvent.

Hydrodistillation: A quantity of 50 g of the dried plant previously cut is introduced into a balloon of 1000 ml, then a quantity of 500 ml of distilled water is transferred and the whole is stirred. The balloon is then

placed in 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 is put in a separatory funnel, then the solvent is added and the funnel is closed, vigorous stirring is practiced for a time necessary to establish a concentration equilibrium between the two phases and degassed, after it is fixed on a support with the removal of the cover. At the end, each phase is collected in an appropriate container (Abe et al. 2010).

Removal of water: To remove all traces of water, the organic phase is dried by adding a few grams of anhydrous magnesium sulfate MgSo4, then filtered using filter paper (Feknous et al. 2014).

Removal of the solvent: The liquid obtained in the previous step is poured into an appropriate flask, then fixed to a rotary evaporator to carry out a simple distillation under reduced pressure with a temperature of 37 ° C (Mecquenem et al. 2018) The oil obtained is stored in sterile glass bottles hermetically sealed, protected from light and at a temperature of 4 °C.

Extraction efficiency of essential oil

The extraction yield is calculated by the following formula (Falleh et al. 2008):

R (%) = (Mext / Méch.) * 100

R = 3/200 = 1.5%

R is the yield in%.

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

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 compounds part of the sample (2-5 µl) was transferred to a GC vial, diluted in hexane (1-2 ml), then sealed with a high performance septum (Delazar et al. 2004).

The identification of the constituents was carried out by coupling of a Chromatograph ingas 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) is 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 240 °C. The ionization of components of the sample was performed in EI mode

(70 eV). MS scan range was going 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, version of May 19, 2011.

Treatment

The sensitivity tests were carried out in accordance with the protocol recommended by the World Health Organization, adopted to test the sensitivity of the larvae towards insecticides used in control campaigns (World Health Organization 2005). This test is carried out on 2 larval stages, the larvae of the 4th stage and the pupae of Culex pipiens. Preliminary tests with different doses are carried out, in order to select a range of concentrations before starting the toxicity test.

Three dilutions of 10% = 1µl/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, then adding a milliliter of each solution thus diluted in the goblets previously prepared. The same number of individuals was 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. The exploitation of these results was subjected by probit analysis to calcul the lethal concentrations and lethal times (LC50% LC90%, LT50% and LT 90%). This analysis was performed using the IBM SPSS Satistics program23 on Windows.

Results

The effect of A. herba alba on the mortality of C. pipiens

The two stages of *C. pipiens* are sensitive to *A. herba alba*. This sensitivity is reflected by higher or lower mortality rates depending on the concentrations used, and especially according to the time of exposure to the extract (Fig. 2).

In the fourth stage of larvae the mortality rate ranges between 8.87% and 28.87% for the lowest concentration (1 µl/ml) while it reaches 100% when the larvae are exposed to the highest concentration (10 µl/ml) after 48h.

In the pupae the mortality rate ranges between 6.67% and 40% for the lowest concentration (1 µl/ml) while it reaches 100% when the pupae are exposed to the medium concentration (5 µl/ml) after 72h.

Toxicological parameters of A. herba alba

The results also show that there is 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 stage of larvae after 24h, the concentration of *A. herba alba* must be equal to 5.081µl/ml, on the contrary, 9.128 µl/ml *of A. herba alba* insures the mortality of 90% (Table 1A).

After 48h, the calculations show that the LC50% is 4.241µl/ml, while the LC90% is 9.166µl/ml. After 72h of treatment, the LC50% is 3.278 µl/ml and the LC90% is 7.573 µl/ml.

On the lethal times, the concentration 1 µl/ml of *A. herba alba* can eliminate 50% of the population of *C. pipiens* in the 4.37 day and 90% during 7.70 days of treatment (Table 1B). When 5µl/ml of *A. herba alba* extract is applied, LT50% is 0.75 days, while the LT90% is 9.77 days.

Table 1
Toxicological parameters of *A. herba alba* essential oil in larvae treated with *C. pipiens* (A: exposed time; B: used concentration)

A			
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
B			
Concentration (µl/ml)	**1**	**5**	**10**
Regression line	Y = -1.71 + 0.02x	Y = -0.11 + 5.92E-3x	*
LT50% (hours)	104.910	18.025	*
LT90% (hours)	184.869	234.389	*
* Mortality equal to 100% for this dose for the 3 repetitions			

To ensure a 50% mortality of the pupae after 24h, 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* insures the mortality of 90% (Table 2A).

After 48h, the calculations show that the LC50% is 2.579µl/ml, while the LC90% is of 6.075 µl/ml. After 72h of treatment, the LC50% is 1,213 µl/ml and the LC90% is 2,288 µl/ml.

On the lethal times, the concentration 1 µl of *A. herba alba* can eliminate 50% of the population of *C. pipiens* in the 3.3 days and 90% during 5.52 days of treatment (Table 2B). When 5 µl/ml of *A. herba alba* extract is applied, LT50% is 0.82 days, while the LT90% is 0.99 days.

Table 2
Toxicological parameters of *A. herba alba* essential oil in pupae treated with *C. pipiens* (A: exposed time; B: used concentration)

A			
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
B			
Concentration (µl/ml)	**1**	**5**	**10**
Regression line	Y = -2.02 + 0.03x	Y = -0.33 + 0.02x	*
LT50% (hours)	79.077	19.693	*
LT90% (hours)	132.479	53.257	*
* Mortality equal to 100% for this dose for the 3 repetitions			

Average Yield Of Aeo And Its Chemical Characterization

The yield of *Atremisia herba alba* essential oil obtained in this study was 1.5% Further, twenty-nine main molecules were extracted within forty minutes, we note that the large proportions were monopolized for the Davanone molecule by 48.84%, which is approximately half, followed by chrysanthenone with 15.97%, then by camphor with 14.84%, then the remaining proportions from 0.04–5.69% (Table 3, Fig. 3)

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

Ret. Time	Compound Name	%
35.621	(-)-Spathulenol	0.82
36.086	**Davanone**	**48.84**

Discussion

More than 2,000 plant species with insecticidal activity have already 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 and polyphenols, terpenoids, alkaloids) that can be isolated using various extraction methods (Dubey 2010).

The experiences of 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 realized by Kaura et al. (2019) reveals the larvicidal and pupicidal effect of the essential oil of *Eucalyptus globulus* which acts quickly on the larvae and pupae of *Aedes aegypti* and *Aedes albopictus* with LC 50 of 93.3 and 144.5ppm and LC90 was found to be 707.9 and 741.3 ppm respectively.

The results of the present study reveal a considerable and variable sensitivity 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 made by Aksorn and Mayura (2018) on the larvae and nymphs of Aedes aegypti, which showed that the mortality is correlated with the doses used is all the more increased as the exposure of larvae and nymphs to insecticides is extended over time.

This activity can be expressed by the diversification of the bioactive molecules which compose this essential oil being able to carry out a singular action of one of the major components, of which it is dominated by Davanone (48.8%), or a synergistic effect between several compounds towards the larvae and the nymphs of mosquitos which are exposed to it.

The oil yield recorded in the present study was relatively higher to those extracted from the same species collected in the region of Spain with 0,8% (Salido et al. 2001) and Tunisia 0,7% (Haouari and Ferchichi 2009). While it equal to those extracted in Tunisia per Zouari et al. (2010) and by Boutemak et al. (2009) in Algeria. Also it is lower at the one extracted 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 country 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)(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), also 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).

In the other hand, with the exception to 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 Sharifianet al. (2012) reported the [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 *Artemisia 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 (32–41%), camphor (16–25%), cineol (0.1– 10%)

However, Boutekedjiret et al. (1992), bring out that there is a variation of the volatile component of Artemisia 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 combined with the influences of geographical locations and climatic conditions as well as the difference of the developmental stages of the plant and method used to obtain the essential oil (Belhattab et al. 2014; Lakehal and A 2016).

Indeed, several previous studies have revealed the different bioactivities of the components of Artemisia 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), another study of Tani et al. (2008) on bean leaf beetle *Acanthoscelides obtectus*(Say) and of Hifnawy et al. (2001) on Cotton Worm *Spodoptera littoralis* (Boisduval) revealed also the toxic effect against insects. Moreover an acaricidal activity was been 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 Pavela (2016) the most important neurotoxic mode of action symptoms are hyperactivity followed by hyperarousal leading to rapid reversal and immobilization as well as the insects' mouthparts become paralyzed and stop feeding and starve.

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 B. Isman (2015) note that insecticidal activity is the result of 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 an 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 *Artemisia herba alba* on the proper functioning and survival of the organism after a cutaneous exposure

Conclusion

This study indicates that essential oil of *Artemisia herba alba* having toxic properties on larvae and pupae of *Culex pipiens*. These results are encouraging and open up interesting and promising horizons for its application in the production of bioinsecticides, these are readily available and the cost constraint can be overcome by the low value of the LC50. However, another deep chemical study would be necessary in order to precise and to isolate the molecule responsible for this toxic effect, in addition a histological study is desirable in order to know the mode of action of this oil on the tissues of larvae and pupae.

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Figures

Figure 1

Chromatographic profile of A. herba alba essential oil analyzed by CG/SM

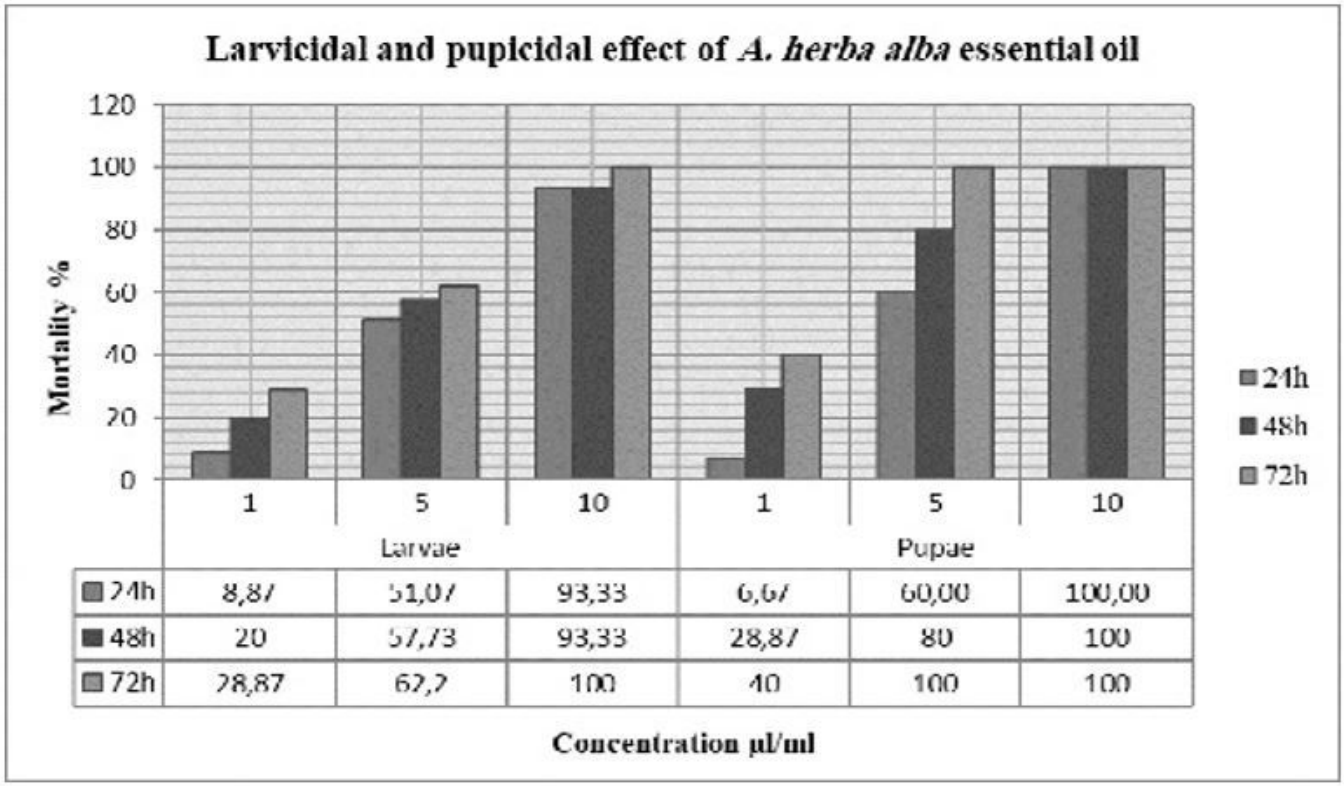

Figure 2

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

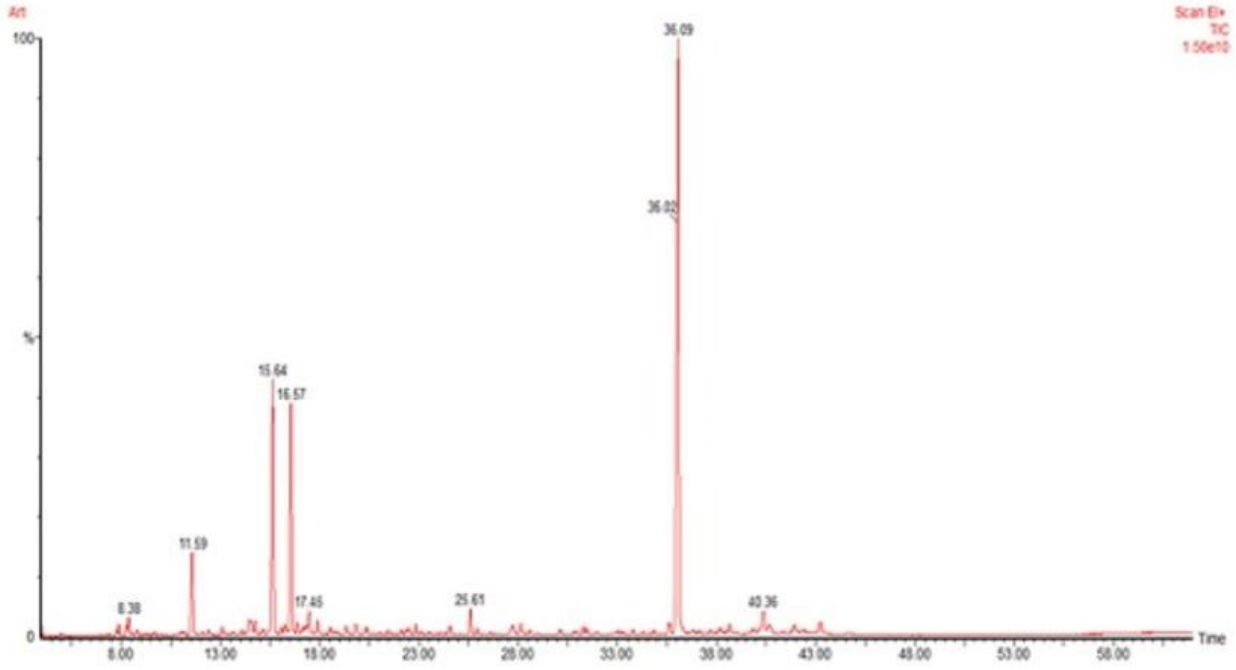

Figure 3

Chromatographic profile of A. herba alba essential oil analyzed by CG/SM