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Chemical composition and insecticidal activity of essential oil of *Artemisia herba alba* (Asteraceae) against *Ephestia kuehniella* (Lepidoptera: Pyralidae)

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Abstract

Plant secondary metabolites play an important role in plant-insect interactions and therefore such compounds may have insecticidal or biological activity against insects. In the present study, aerial parts of *Artemisia herba alba* Asso were subjected to hydro distillation using a Clevenger-type apparatus and the chemical composition of the volatile oils was studied by GC-MS. Forty two compounds were detected. The main components of the essential oil were D-camphor (34.34%), Eucalyptol (13.49%), α-thujone (8.37%), Camphene (8.26%), Chrysanthenone (6.40%), Isoborneol (5.94%), β thujone (4.24%). Insecticidal activity of the oil was evaluated for *Ephestia kuehniella*. The essential oil was tested with various amounts (1, 3 and 5 µl/ml of acetone) on the adults of *E. kuehniella*; this oil present an insecticidal activity and induce in the females of insect a very significant reduction of the laying compared with that in the control. In addition, eggs hatching rate laid by treated females is reduced. It will be interesting to test theses essential oils *in situ* to assess their efficiency in the conservation of stored food.

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Introduction

The Genus *Artemisia* L. comprises important medicinal plants, which are currently the subject of phytochemical attention due to their biological and chemical diversity (Abad *et al.*, 2012). The white wormwood, *Artemisia herba-alba* Asso (Asteraceae) is a greenish-silver perennial dwarf shrub growing in arid and semi-arid climates. It occurs through the Mediterranean region in North Africa, Spain, deserts of Sinai Peninsula, the Middle East, Northwestern Himalayas, and in India. *A. herba alba* Asso (desert wormwood, *Armoise blanche* (Fr.), *Chih* (Arab.) is a dwarf shrub with a rapid growth in dry and warm climates and in muddy areas (Belhattab *et al.*, 2014). The various species are morphologically different from each other depending on geographical, environmental, and climatic conditions. The plant is green to light green, with strong, sturdy roots. The flowering time and harvesting is around May/June and continues until October in some areas (Vernin *et al.*, 1995).

Over the last decades, studies on *A. herbaalba* were focused on its essential oils. Their composition through the world revealed a high level of polymorphism and led to the definition of several chemotypes. Because of their importance in the fragrance industry, numerous studies on *A. herba alba* essential oils have been published. *A. herba-alba* has been traditionally used in the treatment of diabetes, bronchitis, diarrhea, neuralgias and hypertension (Zouari *et al.*, 2010). The plant is reported to possess hypoglycemic (Awad *et al.*, 2012), anticancer (Tilaoui *et al.*, 2011), antiangiogenic (Jaouadi *et al.*, 2014), insectisidal (Nia*et al.*, 2015), hypotensive and diuretic (Zeggwagh *et al.*, 2014), anti-inflammatory (Salgueiro, 2015), antimicrobial activity (Fedhila *et al.,* 2015), and many other biological activities.

The pyralid Mediterranean flour moth, *Ephestia kuehniella* Zeller, is distributed world-wide. This moth is a serious pest of stored grain products as well as flour and other milled products (Khebbeb *et al.*, 2008). In recent years, several experiments have been conducted to assess the efficacy of essential oils against stored-product insect species (Aref *et al.*, 2015).

According to Keita *et al.* (2000) the essential oils are part of the ways most explored in the regulation of the ravagers. Their applications in the protection and conservation of stocks were the object of many works. Their toxicity is expressed various manners: ovicide activity, larvicide, anti-nutritional and inhaling. Thus, the aims of the present study are to investigate the chemical composition and to determine the insecticidal activity of the essential oil of *A. herba-alba* against *Ephestia kuehniella* Zeller.

Material and methods

Plant material

The plant material consists of the aerial part of the plant *A. herba alba* Asso; harvested in Souk Ahrass (Algeria) in October.

Extraction of essential oils

The essential oil was obtained by steam distillation using a Linkens- Nickerson type apparatus. The distillation was carried out by boiling 100 g of dry matter in 1 liter of water in a 2 liter flask surmounted by a column of 60 cm length connected to a Condenser (Bruneton, 1999). After an extraction of 2 hours, the oil is recovered in small opaque bottle and stored at 4°C.

Chemical characterization of essential oil by GC/MS

The essential oil of *A. herba alba* Asso was analyzed by chromatography phase gas (Trace GC Ultra) coupled to a mass spectrometer (Polaris Q ion trap MS). The database used for the identification of chemical compounds and measurements of peak areas obtained is that of NIST/EPA/NIH MS LIBRARY (NIST 05).

Breeding insects

Studied specie is *Ephestia kuehniella* belonging to Pyralidae family, it is holometabole. Mass breeding is done in the laboratory in drying oven under optimal development conditions, at 27°C and 70% humidity in darkness.

Treatment application

Essential oil was tested by topical application on abdominal ventral of female pupae of *E. kuehniella* with the doses of 1, 3 or 5µl/ml of acetone, immediately after their nymphal exuviation which represents the beginning of ovarian development in Lepidoptera. Control will not have treatment. Five repetitions are necessary for statistical treatment. Treatment was applied to test its bio pesticidal effect on female fertility and the percentage of laid eggs.

Repellency test

This test consists on the study of the repulsive effect of the essential oil of *A. herba alba* on the adults of *E. kuehniella*. For that purpose, we have followed this protocol: We Cutted two equal parts of *Canson* paper with a length equivalent to the length of the box. We pulverized part of the paper with an amount of selected essential oil (1, 3 or 5µl/ml of acetone) and kept the other part of paper without treatment. After evaporation of solvent, the two parts of the *Canson* paper are gathered by anadhesive ribbon. Ten adults of the same age (as soon as exuviated) were deposited in the middle of the box. We counted, after half an hour, the insects found on each side of the paper. The percentage repellency (PR) is calculated as follows: PR=[(NC-NT)/(NC+NT)]*100

Where,

NC: The number of insects on the untreated part of the paper

NT: The number of insects on the treated part of the paper with different doses of the essential oil (1, 3 or 5µl/ml of acetone)

The average repulsion percentage for each dose is calculated. Thus, the oil will be allocated to one of several repulsive classes as ranked by Mc Donald *et al.* (1970).

Statistical analysis

The values of the different tested parameters of the control and the treated groups are expressed as the average ± standard deviation. The *Student t* test enabled us comparing the pairwise averages of control and treated groups. The Minitab software was used for statistical analysis.

Results

Yield and chemical composition of the essential oil

Hydro distillation of the leaves of *A. herba-alba* Asso yielded 1.19% with yellow liquid oil with a strong penetrating pleasant herbaceous odor characteristic of the plant.

The chemical composition of the oil was investigated using GC/MS technique. The percentages and the components identified are listed in Table 1, in the order of their elution.

Our results indicated that the essential oil is containing 42 identified compounds, representing 99.91% of the oil. Components were identified as following: D-Camphor (34.34%), Eucalyptol (13.49%), α-thujone (8.37%), Camphene (8.26%), Chrysanthenone (6.40%), Isoborneol (5.94%), β-thujone (4.24%) were the most abundant components.

Table 1. Principal compounds of the essential oil of *Artemisia herba alba* Asso from Souk Ahras – Algeria (Total percentage = 99.91%).

Pic	RT (min)	Compound	%
1	3.056	Santolinatriene	1.69
2	3.294	Tricyclene	0.44
3	3.459	α-pinene	2.02
4	3.665	Camphene	8.26
5	4.051	Sabinene	0.61
6	4.111	Β-pinene	0.29
7	4.312	Hemimellitene	0.56
8	4.394	2,5dimethyl-3-methylene-1,5-Hexadine,3,3,6-Trimethyl-1,4-heptadien-6-ol	0.30
9	4.513	Yomorgi alcohol	0.15
10	4.646	α-Phellandrene	0.19
11	4.855	Psi-cumene	0.44
12	4.992	O-Cymene	1.20
13	5.184	Eucalyptol	13.49
14	5.293	Trans-2,7-dimethyl-4,6-octadien-2ol	0.20
15	5.896	Γ-terpinene	0.42
16	6.808	Chrysanthenone	6.40
17	6.899	Β-thujone	4.24

Pic	RT (min)	Compound	%
18	7.262	α-thujone	8.37
19	7.994	D-comphor	34.34
20	8.032	L-trans pinocarveol	0.87
21	8.206	Cis-Verbenol	0.80
22	8.308	Menthone	0.16
23	8.432	Pinocarvone	1.42
24	8.563	Isogeraniol	0.18
25	8.829	Isoborneol	5.94
26	9.037	Isothujol	0.10
27	9.253	4-terpineol	1.39
28	9.425	(IR)-(-)-Myrtenal	0.18
29	9.636	α-terpineol	0.30
30	9.845	L-Verbenone	0.74
31	10.282	Cis-Piperitol	0.43
32	11.040	Pulegone	0.27
33	11.483	Piperitone	0.29
34	11.952	Γ-mentha-1,8-dien-3-one(+)	0.37
35	12.312	[+]-trans-Chrysanthenyl Acetate	0.25
36	12.931	Cyclohexane-1-methanol-4-[1-methylethenylacetate]	0.28
37	13.157	Bornyl-acetate	0.76
38	13.418	1.6-Dimethylhepta-1.3.5-triene	0.60
39	15.483	Eugenol	0.17
40	16.921	Cis-jasmone	0.33
41	20.798	Germacrene D	0.28
42	25.078	Spathulenol	0.19

Insecticidal effect of essential oil

Essential oil effect, administered by topical application, on the fertility of the females

Once, after coupling, female started immediately to lay eggs during the oviposition period. A female witness lays. On average 253.30 eggs, whereas the administration of the essential oil reduced in a significant way this number which reaches 81.00±6.56 (p=0.004), 34.67±2.52 (p= 0.002) and 22.67±2.31 (p= 0.002) respectively with the three administered doses (Table2).

Table 2. Insecticidal effect of essential oil of *Artemisia herba alba* , administred by topical application, on fertility (number of laid eggs) of females *Ephestia kuehniella* (m± s, n= 5 repetitions).

Parameter	Control	1 µl/ml	3 µl/ml	5 µl/ml
Fertility of females	253.30 ± 7.50	81.00 ± 6.56**	34.67 ± 2.52**	22.67 ± 2.31**

**: highly significant difference (p<0.001).

Essential oil effect, administered by topical application, on the hatching eggs rate

Table 3 shows that the treatment reduces significantly of hatching eggs rate laid by females treated by the three doses compared to Witnesses.

Repulsive effect of essential oil, of Artemisia herba alba

Repulsivity rates with regard to adults and larvae are mentioned on Table 4.

Table 3. Insecticidal effect of essential oil of, *Artemisia herba alba* administred by topical application, on the hatching rate (%) of the laid eggs by females *Ephestia kuehniella* (m±s, n=5)**.**

Parameter	Control	1 µl/ml	3µl/ml	5µl/ml
Hatching eggs (%)	66.89 ± 2.33	19.54 ± 0.93***	13.56 ± 2.39***	10.00 ± 2.23***

***: difference very highly significant (p<0,0001).

Table 4. Average repellency of *Artemisia herbaalba* essential oil on the adults and the larvae of *Ephestia kuehniella*.

Parameter	Adults	Larvae
Repellencyrate (%)	72 .00 ± 22.80	62.00 ± 10.95

The behavior of insects under test was visible after half an hour of exposure to treatment. According to Mc Donald classification, essential of A. *herbaalba* tested in our study, is classified in (class 4) as repulsive oil.

Discussion

Yield and chemical composition of the essential oil

According to literature, our yield (1.19%) is situated within the ranges reported (0.1- 4.9%) by Belhattab *et al.* (2014). According to Benjilali (1986) and Boutedjiret *et al.* (1994) there exists several essential oil chemotypes of *Artemisia herba alba* containing: camphene, cineol 1.8 (Eucalyptol), chrysanthenone α and β-thujone, camphor and sometimes the acetate of chrysanthenyl and the davanone. Essential oil of *Artemisia* is characterized by an important chemical polymorphism (Benabdellah *et al.*, 2006).

Chemical variability of *A. herba-alba* Asso seems to depend on the genetic characteristics of the plant(Skoula *et al.,* 1999), geographical locations, consequently different climatic conditions under which it has grown (Mighri *et al.,* 2010), part of the plant, phonological stage and the method used to obtain the essential oil (Hussain *et al.*, 2008 ; Singh and Guleria, 2013). In fact, these factors influence the plant's biosynthetic pathways and, consequently, the relative proportion of the main characteristic compounds (Gardeli *et al.,* 2008). On the other hand, these major components determine the biological properties of the essential oil (Jemâa, 2014). Akrout (2004) reported that the biological activities of an essential oil are not only due to the majority compounds but to the whole of made up containing in this oil. Therefore it is necessary to conduct a study detailed on the biological activities of these oils to show their importance and the possibility of their exploitation in certain fields: pharmaceutical, cosmetic, insecticidal, food.

Essential oil contains compounds known for their insecticidal properties such like Comphene and the Thujone (major component) and α-pinene. Accordingto Duke (1998) the α-thujone presents several biological activities: it is abortive, antibacterial, emmenagogue, insecticide and larvicide. Bouchikhi *et al.* (2010) reported that the α-pinene and camphene are two major components with insecticidal properties.

Insecticidal effect of essential oil

Essential oil extracted of *Artemisia herba alba* of Souk Ahras (Northeastern Algeria) by hydro distillation, allowed to get an insecticidal effect on female of *Ephestia kuehniella* as it affects the fertility of females corresponding to a decrease of laid eggs number by a treated female. During topical application of essential oil at different doses (1, 3 and 5µl/ml of acetone) on pupae of 0 day, results on the affectation of this insect reproduction. Indeed, Delimi *et al.* (2013) reported that the essential oil extracted from the white wormwood *A. herba alba* is considered a reproduction disruptive insecticide. They showed that the toxic effect varies according to the dose given and by extending the preoviposition duration and the length of pupal development and reducing the laying period. Consulted literature showed that essential oil of cloves affects fertility of *C. maculatus* at dose 5µl/ml, there were no laid eggs (Kellouche and Soltani, 2004). According to Kellouche (2005) chickpeas grains and leaves powder of plants rich in essential oils (Fig tree, Olive tree, Lemon tree, Eucalyptus) reduce *Callosobruchus maculatus* females' fertility. While essential oil extracted of cloves completely inhibits laying. However, essential oils of *Romarinus offinalis* and *Thymus vulgaris* disturb reproduction of *Acous celides obtectus* and *Teneola bisselliella* by inhibiting completely their fertility (Bouchikhi *et al.*, 2008).

In addition, eggs hatching rate laid by treated females is reduced compared to witness after the application of number of doses of essential oil. According to the applied dose, daily monitoring of the experimentation, allowed us to note that even eggs hatched, new larvae cannot live more than 24 hours, these observations are compatible with other researchers since Hill and Schoonhoven (1981) showed that essential oil extracted from palm leaves and cotton grains have an important effect on eggs hatching of bean weevil.

Repulsive effect of some vegetable oil has been noted by many studies, thus our test on the effect of essential oil of *Artemisia herba alba* confirms that it has a repulsive effect on pupae and adults Lepidoptera *Ephestia kuehniella* due to its exposition, during half an hour, to several doses of tested biopesticides. Boumendjel *et al.* (2017) have shown that the essential oil of *Origanum vulgaris* has a repellent effect against adults and larvae of *Ephestia kuehniella*. Toxic and repulsive effect of essential oil depends on chemical composition and sensitivity of insects (Casida, 1990). Essential oils of *Eucalyptus globulus, Myrtus communis, Pogostemon cablin* and *Cypressus sempervirens* have been classified as moderately repellent (Class III) (Kellouche *et al.,* 2010).

Conclusion

In the present research, we performed a phytochemical study of *Artemisia herba alba*, determined the chemical composition of essential oils and assessed its insecticidal activity. Themajor components were; D-camphor, Eucalyptol**,** α-thujone**,** Camphene, Chrysanthenone, Isoborneo**,** β thujone**.** Essential oil contains compounds known for their insecticidal properties like the comphene and the thujone (major component) and the α-pinene. The study indicates that the essential oil of *A. herba alba* has a potential to be developed to anatural insecticide for the control of grain storage insects. In fact, it affects reproduction of insects by the reduction of the laying and eggs hatching rate laid, so the fecundity was significantly reduced. In addition, essential oil has a repulsive effect on pupae and adults of *Ephestia kuehniella* (Lepidoptera).

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