Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article doi: 10.12980/jclm.4.2016J6-19

Effects of *Artemisia herba-alba* essential oils on survival stored cereal pests: *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae) and *Trogoderma granarium* (Everst) (Coleoptera, Dermestidae)

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ARTICLE INFO

Article history:
Received 18 Jan 2016
Received in revised form 28 Jan, 2nd revised form 16 Feb, 3rd revised form 26 Feb 2016
Accepted 15 Apr 2016
Available online 9 May 2016

Keywords:
Artemisia herba-alba
Essential oil
Stored-grain insects
Toxicity

ABSTRACT

Objective: To assess the chemical components and toxicity of *Artemisia herba-alba* (*A. herba-alba*) essential oil against two major stored cereal pests, *Tribolium castaneum* (*T. castaneum*) and *Trogoderma granarium* (*T. granarium*).
Methods: Two bioassay actions were tasted: repellent and fumigant actions against adult and larvae, respectively, to assess the effect of *A. herba-alba* essential oil.
Results: Gas chromatography-mass spectrometer analyses of the essential oil contained β-thujone (12.50%), α-thujone (8.78%), sabinyl acetate (8.56%), terpinene-4-ol (8.51%), α-terpineol (3.35%), 1,8-cineol (5.45%), γ-terpene (4.82%), camphor (4.52%), dimethyl-ethylbenzene (3.93%) and α-terpinene (3.35%) as the major components. Fumigant toxicity tests showed that *A. herba-alba* oil was more toxic than *T. granarium* (LC_{50} = 2.09 mg/mL, LC_{90} = 4.12 mg/mL) and *T. castaneum* (LC_{50} = 6.39 mg/mL, LC_{90} = 10.10 mg/mL).
Conclusions: This study has highlighted a bioinsecticide activity of *A. herba-alba* against two insect pests of stored foodstuffs (*T. castaneum* and *T. granarium*). The *Artemisia* essential oil offers an interesting potential insecticide that could be studied more deeply to isolate and identify the active substances, to study their physiological impact on other insects

1. Introduction

Certain kinds of beetles (Buchidae and Cucurlionaidae) attack grains. They are a major thread to a wide range of seeds[1-4]. Without specific protection, stored cereals are destroyed by pests. Furthermore, feeding on seeds, insect pests can cause quantitative and qualitative losses leading to a low market value. Chemical control using conventional insecticides with serious drawbacks are considered as unfriendly to the environment. These problems lead to increasingly stringent environmental regulation of pesticides[5,6]. The essential oils are being assessed as potential candidates for pests and disease management. Their toxicities as well as arresting and repellent effects on pests were of special interest during the last decade. All these gentle properties of essential oils allow their use even in responsive areas. Peasant farmers traditionally use spices and other aromatic plant material against insect pests of stored products. *Artemisia herba-alba* (*A. herba-alba*) is one of the aromatic herbs widely distributed in Tunisia in old days. It was used as a folk medicine to delight diseases such as cough, stomach and intestinal disturbances, common cold, measles, diabetes, yellowed skin (jaundice), anxiety, irregular heartbeat and muscle weakness. It is also used for parasitic infections as an antihelmintic agent.

The target of this study was to investigate the composition of *A. herba-alba* essential oil and to evaluate their toxic, antifeedant activities and their insecticidal activity to control stored product insects *Tribolium castaneum* (*T. castaneum*) and *Trogoderma granarium* (*T. granarium*).

2. Materials and methods

2.1. Insect rearing

T. castaneum and *T. granarium* were the subject of this study. The insects were cultured in the Laboratory of Entomology of the Regional Research Center in Horticulture and Organic Agriculture

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The journal implements double-blind peer review practiced by specially invited international editorial board members.

of Chatt Meriem during the period from December 2011 to April 2012. They were cultured in a nutrient medium consisting of wheat semolina and corn meal. The rearing conditions were 26 °C, a photoperiod of 15:9 h (L:D) and 60% relative humidity.

2.2. Plant material

A. herba-alba leaves were collected in February 2012 in the region of Boughrara Medenine, Southeast Tunisia, 33°32'16" N, 10°40'34" E. The harvested material was air-dried (20–25 °C) and stored until it was used.

2.3. Chemical characterization of the essential oil

A total of 100 g of dry matters were subjected to hydrodistillation using a modified Clevenger-type apparatus to remove remaining water. Na_2SO_4 was added after extraction. The distilled oil was then stored at 4 °C.

The essential oil was analyzed by gas chromatography-mass spectrometer with electron impact ionization (70 eV) coupled with an HP-5890 series II gas chromatograph using a Hewlett-Packard 6890 gas chromatograph (Agilent Technologies, Palo Alto, California, USA) equipped with a flame ionization detector and an electronic pressure control injector. A polar HP Innowax column (30 m × 0.25 mm, 0.25 μm film thickness). An HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness) was used. The oven temperature was programmed to rise from 50 to 240 °C at a rate of 5 °C/min. Analyses were performed using the following temperature program. Injector and detector temperatures were held at 250 and 300 °C , respectively.

Essential oil volatile compounds based on the comparison of mass with other published mass spectra (Adams, 2001).

2.4. Fumigant toxicity

To test the toxicity of essential oils on insects, 10 specimens (of one or other of the insect) were put into a 44 mL plexiglas bottle. Doses of the essential oils were applied on a filter-paper attached to the screw cap. Each concentration and control was replicated four times. The mortality was recorded when no insect movements observed and was calculated using Abbott's formula correction[7].

The second experiment was designed to assess 50% and 90% lethal doses. A series of dilutions was prepared to evaluate the mortality of insects after an initial dose-setting experiment. *T. castaneum* and *T. granarium* were exposed to the essential oil vapors 1, 2, 4 and 8 μL for 24 h. The lethal concentration of the essential oil needed to kill 50% or 90% of the insects was determined. The mortality data were corrected for control mortality by using Abbott formula[7] and probit analysis[8] was used to estimate the LC_{50} and LC_{90} values.

2.5. Repellent activity

This activity has been tested only on *T. castaneum*. The repellent effect of the essential oil against adults of *T. castaneum* was evaluated using the method of the preferred area on filter papers as described by McDonald *et al.*[9]. Thus, the filter paper discs of 9 cm in diameter used for this purpose have been cut into two equal parts. Four doses were prepared (1, 2 , 4 and 8 μL/mL) and diluted with acetone. Then, 0.5 mL of each solution thus prepared was spread evenly over one half of the disc. After 15 min, the time required for completing evaporation of the solvent dilution, the two halves of the discs were glued together using adhesive tape. The filter paper disc was restored and placed in a box and kneaded a batch of 20 adult insects was placed in the center of each disk. Three repetitions were performed for each dose. After 2 h, the number of insects on the part of filter paper treated with essential oil (Nt) and the number of those present on the treated only with acetone (Nc) part were identified. The percentage of repulsion (RP) was calculated using the following formula:

$$PR = \frac{Nc - Nt}{Nc + Nt} \times 100$$

The average percentage of essential oil repellency was calculated and assigned as ranked by McDonald *et al.*[9] by a repulsive different classes varying from 0 to V [Class 0 (RP < 0.1%), class I (RP = 0.1%–20.0%), class II (RP = 20.1%–40.0%), class III (RP = 40.1%–60.0%), class IV (RP = 60.1%–80.0%) and class V (RP = 80.1%–100.0%)].

2.6. Statistical analysis

Analysis of variance was performed by ANOVA. The comparison of means was based on Duncan's test using SPSS 11 software for Windows. The LC_{50} values were calculated using the method of probit analysis program by Finey.

3. Results

3.1. The major components of the essential oil

Gas chromatography-mass spectrometer analysis of *A. herba-alba* essential oil led to the identification of 58 components (Table 1). The oil profile is characterized by β-thujone (12.50%), α-thujone (8.78%), sabinyl acetate (8.56%), terpinen-4-ol (8.51%), 1,8-cineole (5.45%), γ-terpinene (4.82%), camphor (4.52%), dimethyl-ethyl-benzene (3.93%) and α-terpinene (3.35%).

3.2. Fumigant toxicity

The essential oil was more toxic to *T. granarium* than to *T. castaneum*. No mortality was observed in the control. The lowest oil concentration (2 μL) achieved 5.0% of *T. castaneum* and 77.5% mortality of *T. granarium* after 24 h of exposure (Figures 1 and 2). Furthermore, at the concentration of 8 μL, 70.0% mortality was recorded for *T. castanium*, by cons 75.0% mortality was recorded at 4 μL after 24 h of exposure for *T. granarium* adult and only 35% for *T. granarium* larvae (Figure 3).

T. granarium was more susceptible to *A. herba-alba* essential oil than *T. castaneum*. The corresponding LC_{50} and LC_{90} were 2.09 and

4.12 mg/mL for *T. granarium*, respectively, and 6.39 and 10.10 mg/mL for *T. castaneum,* respectively. The corresponding LC_{50} and LC_{90} were 10.14 and 19.50 mg/mL for *T. granarium* larvae, respectively.

Table 1
Chemical compositions of the analyzed *A. herba-alba* essential oil.

Number	Retention time	Peak area (%)	Compounds
1	4.33	0.08	1,3-Cyclopentadiene
2	4.72	0.17	7-Methyl-1-octene-3-heptene
3	5.79	0.09	Terpinolene
4	6.11	0.11	Tricyclene
5	6.22	0.23	Alpha-thujene
6	6.37	0.64	Alpha-pinene
7	6.71	1.79	Camphene
8	7.25	1.03	Sabinene
9	7.32	0.22	1-Beta-pinene
10	7.63	0.46	Myrcene
11	7.94	0.19	Alpha-phellandrene
12	8.23	3.35	Alpha-terpinene
13	8.43	3.80	O-cymene
14	8.58	5.45	1,8-Cineol
15	9.19	4.82	Gamma-terpinene
16	9.40	0.41	Trans-sabinene hydrate
17	9.82	1.43	Terpinolene
18	10.32	12.50	Beta-thujone
19	10.36	0.19	1,3,8-Para-menthatriene
20	10.56	8.78	Alpha-thujone
21	10.64	1.49	Cis-p-2-menthen-1-ol
22	10.70	2.68	Chrysanthenone
23	10.90	0.23	Camphene
24	11.03	2.00	P-menth-2-en-1-ol
25	11.14	4.52	Camphor
26	11.36	0.19	Trans-chrysanthemal
27	11.48	0.62	3-Nopinenone
28	11.60	1.89	Borneol
29	11.90	8.51	Terpinene-4-ol
30	12.09	0.47	L-alpha-terpineol
31	12.30	1.11	Piperitol
32	13.07	0.55	Alpha-terinene
33	13.50	3.93	Dimethylethylbezene
34	13.98	0.78	L-bornylacetate
35	14.15	8.56	Sabinylacetate
36	14.27	0.67	Gamma-terpinene
37	14.32	0.05	Chrysanthenone
38	14.37	0.06	Phenol,2-ethyl-4,5-dimethyl
39	15.20	0.30	Camphene
40	15.72	0.52	Ethylcinnamate
41	16.16	0.37	Cis-jasmone
42	16.59	0.48	Beta-caryophyllene
43	17.37	0.94	Ethylcinnamate
44	17.72	3.47	Germacrene-d
45	18.19	2.09	Davanaether
46	17.98	2.10	Bicyclogermacrene
47	18.40	0.25	Delta-cadinene
48	19.03	0.28	Farnesol
49	19.45	2.12	Cis-davanone
50	19.50	0.52	(-)-Caryophulleneoxide
51	19.66	0.17	Viridiflorol
52	20.14	0.28	Beta-myrcene
53	20.38	0.29	Isospathulenol
54	20.65	0.40	T-muurolol
55	20.88	0.42	Ethanone
56	21.17	0.36	Gamma-trans-aeaqui-cyclocitral
57	25.09	0.34	Palmiticacid
58	27.10	0.26	Phytol

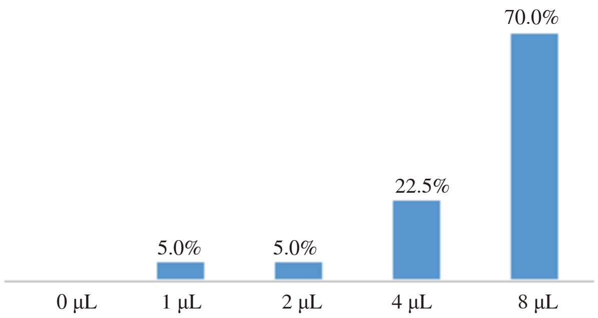

Figure 1. *T. castaneum* adult mortality.

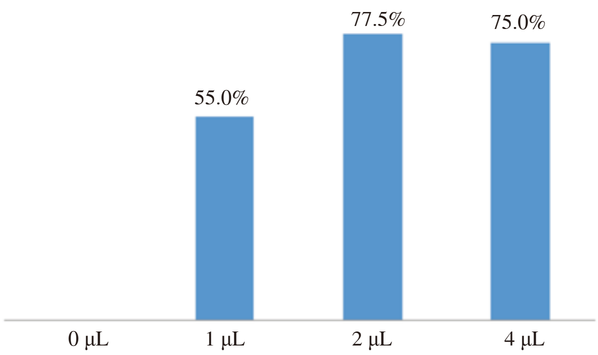

Figure 2. *T. granarium* adult mortality.

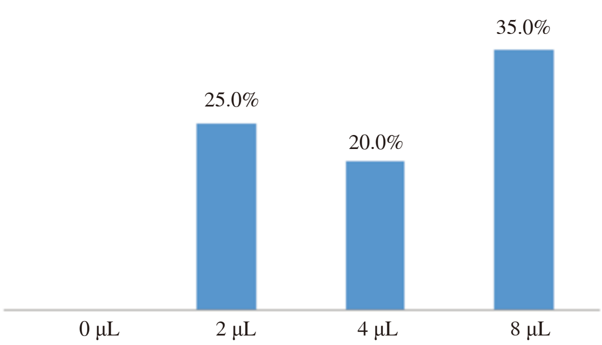

Figure 3. *T. granarium* larvae mortality.

3.3. Repellent activity

The repellent activity is a physiological phenomenon that occurs in insects as a defense mechanism against toxins secreted by plants. Studying this phenomenon allows us to identify potential repellents in *A. herba-alba* essential oil. These repellents can be used to fight against this insect-grains damage. In this study, this test was applied only on *T. castaneum* adult. The maximum repellency rate is 73.33% with a dose of 4 μL (Figure 4). According to McDonald *et al.*[9], this plant belongs to the repulsive class IV.

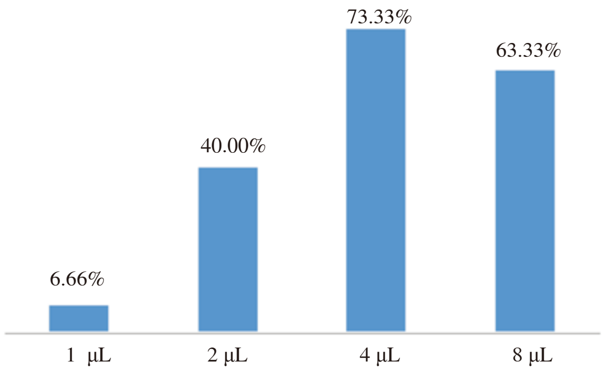

Figure 4. Repellency rate of *A. herba-alba* essential oil to *T. casteneum* adult.

4. Discussion

Several studies confirm our results showing that the selected aromatic plant essential oils have a repellent effect on the stored grain insects including *T. castaneum*[10-13].

The repellent activity of the essential oil of the genus *Artemisia* could be attributed to its major constituents. For example, camphor (24.81%) for *A. absinthium* has been reported as a toxic repellent against some stored product beetles[14]. Many authors have reported that the *Artemisia* genus could have insecticidal properties and/or repellent against many insects in stored cereals[15,16].

Thujone (β and α) is the major component of absinthe essential oil causing "central nervous system cholinergic receptor binding activity" in the brain, which improves the brain's cognitive functions claimed by scientists. They have been reported to be toxic to several insect species. The sabinyl acetate used in the past, mainly in veterinary medicine, also has been used to induce abortion in humans. Furthermore, this terpene is the main component of *Juniperus sabina* essential oil and it has an implantation inhibiting effect. The terpinen-4-ol representing the major component of *A. herba-alba* essential oil was reported to be toxic to several insect species. Kordali *et al.*[17] indicated that terpinen-4-ol was the most toxic component of *Artemesia* oil to *Sitophilus granarius* adults exposed for 12 h. Furthermore, this component showed promising fumigant toxicity on rice weevils *Sitophilus oryzae* (L.)[18] and relatively strong toxicity on both larvae and adults of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), as compared with the toxicities of other oxygenated monoterpenes. It was also reported that terpinen-4-ol which was present in the oil of *Salvia hydrangea* (DC. ex Benth.) showed toxic effects on *Tribolium confusum* (du Val) as well as *Sitophilus granarius*[19]. The 1,8-cineol listed in several essential oils of aromatic plants has been reported to be toxic to several insect species. Cavalcanti *et al.*[20] mentioned that monoterpenoids eugenol and 1,8-cineol from *Ocimum gratissimum* showed a good larvicidal activity against *Aedes aegypti*. Prates also reported that the monoterpene element demonstrated insecticidal activity by penetrating the insect cuticle (contact effect), by respiration (fumigant effect) and through the digestive system (ingestion effect)[21]. The camphor is a terpenic substance found in a number of essential oils extracted from aromatic plants (such as *Eucalyptus* sp., *Cinnamomum camphora* (L.) Sieb., *Rosmarinus officinalis* L., *Chrysanthemum coronarium* L., *Artemisia* sp., *Carum carvi* L., *Thymus* sp., *etc*.). It is one of the highly biologically active substances which possess insect, fungi and bacteria control management[22-25]. Essential oils are known to possess both antifeedant and larval growth inhibiting potential[26]. All of these data can explain the effectiveness of *A. herba-alba* oil on *T. granarium* and *T. castaneum* development. Furthermore, results from this study demonstrated that the selected aromatic plant essential oil has excellent larvicidal activity. However, there is little information about the mechanism of essential oils action. One of the hypotheses is that the components of the essential oils act on other vulnerable sites, such as nervous system. However, it would be difficult to link the insecticide and repellent activities of this oil only to some of their major constituents; it could be due to the synergistic effect of several elements of the oil. Thus, the use of natural products may be considered as an important alternative insecticide for the control of stored-product pests.

This study has highlighted a bioinsecticide activity of *A. herba alba* on two insect pests of stored foodstuffs (*T. castaneum* and *T. granarium*). This bioinsecticide action is manifested by a repulsive toxicity of the selected plant essential oil activity. In this study, adults of *T. granarium* seems to be more sensitive to essential oil than those of *T. castaneum*. The *T. granarium* larvae are much less affected by the essential oil than their adults. This result is confirmed by mortality rate and lethal doses tests.

These results show that the *Artemisia* genus offers an interesting potential insecticide that could be studied more deeply to isolate and identify the active substances to study their physiological impact on other insects.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

Authors would like to thank the High Institute of Environmental

Science and Technology of Borj-Cedria, Tunisia for technical help.

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