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Insecticidal activity of essential oil of *Artemisia herba-alba* Asso against three stored product beetles

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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 *A. herba-alba* Asso were subjected to hydrodistillation using a Clevenger-type apparatus and the chemical composition of the volatile oils was studied by GC-MS. β-thujone (35.66) was the main component of the essential oil. Insecticidal activity of the oil was evaluated against *Tribolium castaneum* (Herbst), *Callosobruchus maculatus* (F.) and *Rhyzopertha dominica* (F.). After 24 h exposure time, *R. dominica* (LC_{50}= 76.48 µl/l air) was more susceptible and *T. castaneum* (LC_{50}= 564.40 µl/l air) was more tolerant than other species. In general, mortality increased as the doses of essential oil and exposure time increased. These results suggested that *A. herba-alba* oil might have potential as a control agent against *T. castaneum, C. maculatus*, and especially *R. dominica*.

Key words: *Artemisia herba-alba*, essential oil, fumigant toxicity, stored product beetles.

Introduction

Stored products of agricultural and animal origin were attacked by more than 600 species of beetle pests causing quantitative and qualitative losses (Rajendran 2002). Fumigation plays a major role in insect pest elimination in stored products. Chemical control of stored products' pests with current chemical pesticides may cause special problems on stored products (Collins et al. 2002). These problems have highlighted the need for the development of new types of selective insect-control alternatives with fumigant action (Negahban et al. 2006). It is believed that essential oils have the advantage over conventional fumigants in terms of low mammalian toxicity, rapid degradation and local availability (Rajendran & Sriranjini 2002). They do not leave residues toxic to the environment and have medicinal properties for humans (Isman 2006).

Asteraceae family, especially *Artemisia* species essential oils have insecticidal or repellent properties (Kordali et al. 2006, Negahban et al. 2006, Wang et al. 2006, Negahban et al. 2007, Derwich et al. 2009). Several *Artemisia* species were medicinally used and hence are of commercial values (Evans 2001). *Artemisia herba-alba* Asso inhibited the asexual reproduction of *Aspergillus niger* Tiegh, *Penicillium italicum* Wehmer and *Zygorrhychus* sp. (Tantaoui-Elaraki et al. 1993). *Artemisia herba-alba* and two other species of *Artemisia* genus have insecticidal effects against *Acanthoscelides obtectus* (Say) (Derwich et al. 2009). This plant characterizes steppes and Middle East deserts (Iran, Egypt and Sinai desert), North Africa (Morocco, Algeria), Spain and the North-West of Himalaya (Paolini et al. 2010). In some studies on chemical compositions of *Artemisia* species, *A. herba-alba* had higher essential oils yield than other experimental species (Derwich et al. 2009).

In this study, we investigated the chemical composition of the essential oil from Iranian *A. herba-alba*. In addition, we evaluated its efficacy as a fumigant toxic agent in the management of the red flour beetle *Tribolium castaneum* (Herbst), cowpea weevil *Callosobruchus maculatus* (F.), and lesser grain borer *Rhyzopertha dominica* (F.) that are three of the most important beetle pests of stored products in Iran.

Material and Method

Insect culture

All culturing insects were obtained from laboratory cultures from stored product pest chamber room, Department of Plant Protection, Urmia University. *Tribolium castaneum*, *C. maculatus*, and *R. dominica* were reared on flour mixed with yeast (5% W/W), Cowpea and Wheat at 12-13% m.c., respectively. All culturing media were purchased from a local market and kept in a freezer at -(5)±2 °C for 24 h and then were used as culturing medium. Experimental procedures were carried out at 28±2 °C in a dark room, and 65±5% r.h. was provided only for culturing media.

Plant material

The visible parts (stem and leaves) of *A. herba-alba* have been collected during July 2010 in the region of Nazlo, 12 km in the west of Urmia (latitude: 37° 32', longitude: 45° 05'; altitude: 1313 m), Iran. The plant material was dried naturally on laboratory benches at room temperature (23-24°C) for 5 days until crisp (Negahban et al. 2006). Plant samples were steam distilled for 2 h using a Clevenger-type collector.

Extraction and analysis of essential oil

The constituents of *A. herba-alba* essential oil were analyzed by gas chromatography mass spectrometry (GC-MS) (Thermo-UFM). The GS conditions were as follows: capillary column 1-ph (30 m x 0.25 mm, film thickness 0.25 µm); helium as a carrier gas (0.5 ml/min); oven temperature program, initially 40°C rising to 250°C (80°C/min, 3 min); injector and detector temperature of 250°C. The identification of individual compounds was based on comparison of their relative retention times with those of authentic samples on a capillary column, and by matching their mass spectra of peaks with those obtained from authentic samples and published data (Davies 1990).

Bioassay

Each concentration was applied to filter paper strips (Whatman No. 1, cut into 4 × 5 cm paper strip). Treated filter papers were placed at the bottom of 1000 ml glass jars. Twenty adult of insects (1-7 days old) were placed in small plastic tubes (3.5 cm diameter and 5 cm height) with open ends covered with cloth mesh. Tubes were hung at the geometrical centre of the glass jars and then sealed with air-tight lids. Thus, there was no direct contact between the oil and the insects (Ebadollahi 2011). In the control jars, oil was not applied on the filter papers. Mortality was determined after 24, 48, and 72 h after expo-

sure commencement. Each experiment was replicated three times for each concentration. Insects were considered dead when no leg or antennal movements were observed.

Data analysis

Mortality percentages were calculated by the Abbott correction formula for natural mortality in the untreated control (Abbott, 1925). The trials were arranged using randomized complete block design and the data were subjected to the analysis of variance (ANOVA) using the SPSS 16.0 software. Differences between means were tested by Tukey's test ($p < 0.01$). LC_{50}, LC_{95}, LT_{50} and LT_{95} values were estimated by probit analysis by SPSS 16.

Results

Chemical constituents of essential oil

Results of the GC-MS analysis for *A. herba-alba* essential oil are presented in Table 1. Major constituents of essential oil in order of decreasing amount, were β-thujone (35.66%), Camphor (34.94%), 1, 8-Cineole (7.42%) and α-thujone (4.12%).

Table 1. Major chemical composition (%) of essential oil of *Artemisia herba-alba.*

Component	Retention index	Percentage (%)
β-Thujone	1037	35.66
Camphor	1064	34.94
1,8-Cineole	953	7.42
α-Thujone	1012	4.12
Total		82.16

Bioassay

The insecticidal activity varied with different concentrations of the oil, insect species and exposure time. Probit analysis showed that at exposure time of 24 h, *R. dominica* was more susceptible than *C. maculatus*, and *T. castaneum*. Furthermore, with the increase of exposure time to 72 h, mortality increased and LC_{50} values decreased in all three species. Lethal time values were calculated at the highest concentrations (360 µl/l air for *Tribolium castaneum*, 130 µl/l air for *Callosobruchus maculatus*, and 110 µl/l air for *Rhyzopertha dominica*) (Table 2, Fig. 1).

According to the results of ANOVA, the mortality percentages significantly increased depending on the increase in the essential oil concentration. Adults of *R. dominica* were significantly influenced in different manners exposing to essential oil.

Discussion

The yield of essential oil of *A. herba-alba* collected in the region of Gigou was 0.59% (Derwich et al. 2009). The oil yield in Haouari & Ferchichi (2009) research from areal parts of 18 individual from *A. herba-alba* varied between 0.68 and 1.93% v/w. Oil yields of 16 individual plants of *A. herba-alba* gathered from four different locations of southern Spain ranged between 0.41 and 2.30% (Salido et al. 2004). Sixteen *A. herba-alba* samples harvested in full bloom from eight East Moroccan locations yielded of the essential oils were 1.3–3.3 % (Paolini et al. 2010). The essential oil yield of *A. herba-alba* from Nazlo, West Azerbaijan, was 1.1% that is a moderate oil yield.

Several studies have been reported on the chemical composition of the essential oils of *A. herba-alba* belonging to different regions in the world (Haouari & Ferchichi 2009, Salido et al. 2004, Paolini et al. 2010). Major constituent of *A. herba-alba* in Derwich et al. (2009) research had been α-thujone, and this composition had been similar between three *Artemisia* species. The main components of *A. herba-alba* in different regions were davanone, 1,8-cineole, chrysanthenone and cis-chrysanthenol (Salido et al. 2004), cineole, thujones, chrysanthenone, camphor, borneol, chrysanthenyl acetate, sabinyl acetate, davana ethers and davanone (Haouari & Ferchichi 2009), camphor, chrysanthenone, and α- and β-thujone (Paolini et al. 2010).

Numerous authors (Negahban et al. 2006, Kordali et al. 2006, Negahban et al. 2007, Derwich et al. 2009) performed

Table 2. Result of the *Artemisia herba-alba* oil probit analysis to calculate LC_{50}, LC_{95}, LT_{50}, and LT_{95} values.

Insect species	Time	LC_{50}	LC_{95}	$\chi 2$ [df = 3]	*P*	Intercept	Slope
T. castaneum	24	564.40	2451.00	2.04	0.56[a]	-2.08	2.57
	48	183.71	857.16	0.60	0.89[a]	-0.56	2.45
	72	99.75	233.47	7.19	0.06[b]	-3.90	4.45
C. maculatus	24	99.35	303.62	0.41	0.93[a]	-1.77	3.39
	48	81.79	260.70	0.50	0.91[a]	-1.25	3.26
	72	56.61	104.66	3.57	0.31[a]	-5.80	6.16
R. dominica	24	76.59	213.04	0.32	0.95[a]	-1.97	3.70
	48	58.68	109.84	7.45	0.05[b]	-5.68	6.04
	72	48.15	72.51	0.84	0.83[a]	-10.56	9.24

Insect species	LT_{50} [h]	LT_{95} [h]	$\chi 2$ [df = 1]	*P*	Intercept	Slope
T. castaneum	32.05[c]	66.37	4.26	0.03[b]	-2.83	5.20
C. maculatus	19.17	77.89	10.29	0.00[b]	1.53	2.70
R. dominica	19.90	32.61	0.10	0.75[a]	-4.96	7.66

[a]Since goodness-of-fit Chi square is not significant ($P > 0.15$), no heterogeneity factor is used.
[b]Since goodness-of-fit Chi square is significant ($P < 0.15$), a heterogeneity factor is used.
[c]Lethal time values were calculated at the highest concentrations (360 µl/l air for *Tribolium castaneum*, 130 µl/l air for *Callosobruchus maculatus*, and 110 µl/l air for *Rhyzopertha dominica*).

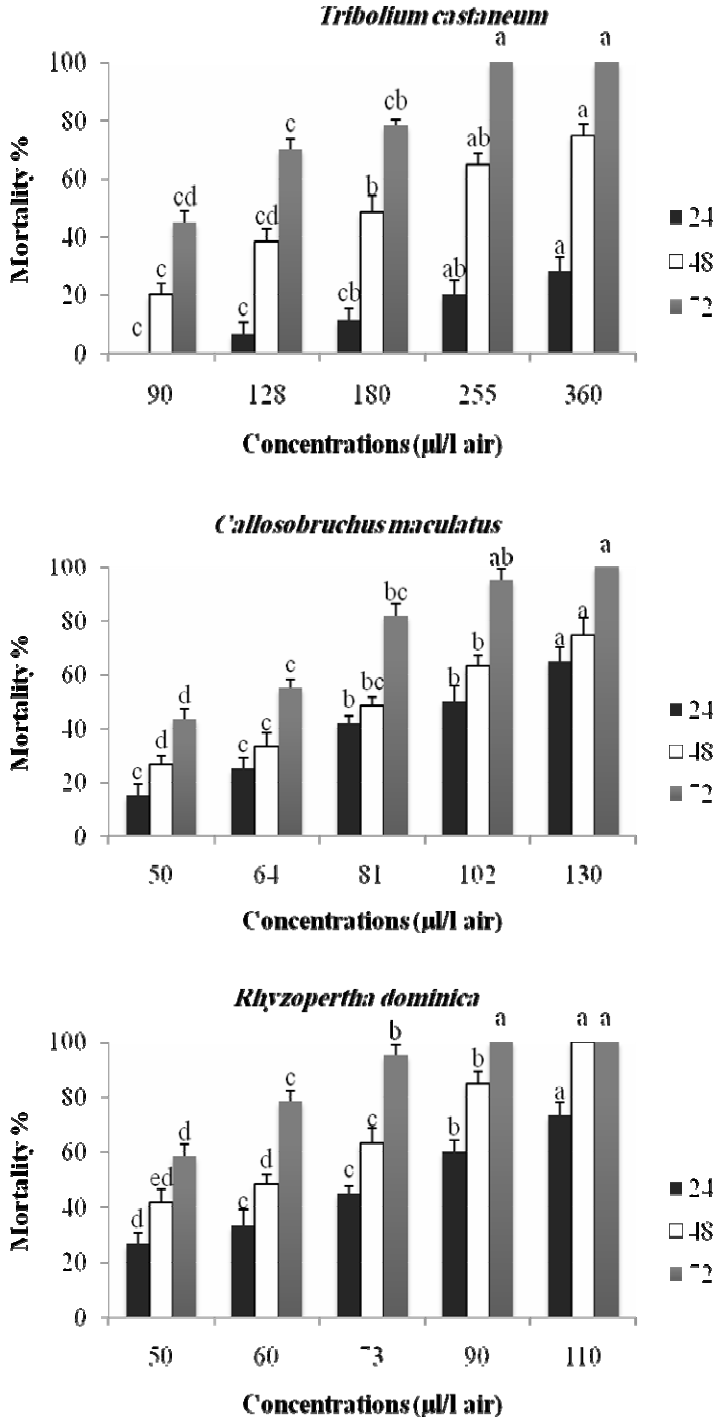

Figure 1. Mean mortality (%) of *Tribolium castaneum, Callosobruchus maculatus*, and *Rhyzopertha dominica* exposed to different concentrations of *Artemisia herba-alba* essential oil (Different letters over columns indicate significant differences according to Tukey test at α= 0.01. Columns with the same letter are not significantly different. Vertical bars indicate standard error (±); very small values are not represented).

several studies on the fumigant toxicity of the essential oils of different *Artemisia* species against stored product insects. Fumigant toxicity of *A. herba-alba* was investigated against *Acanthoscelides obtectus*, one of the stored product pest (Derwich et al. 2009), but its toxicity was not reported against present beetles. Derwich et al. (2009) showed *A. herba-alba* essential oils had higher fumigant toxicity (LD_{50}= 15.1 mg/g) between other *Artemisia* species against *Acanthoscelides obtectus*. Fumigant toxicity of three *Artemisia* species (*A. absinthium, A. santonicum*, and *A. spicigera*) was investigated against *Sitophilus granarius* (L.). In this study at 24-hour exposure time, concentration of 9 µl/l air caused 73.3,

66.7, and 80.0% mortality, respectively (Kordali et al. 2006). Evaluated LC_{50} and LC_{95} in present study are more than estimated values in earlier studies, due to lack of Acetone and other solvents. In general, the essential oil of *A. herba-alba* possesses a potential for use in the management of *T. castaneum*, *C. maculates*, and especially *R. dominica*.

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