

Toxicity of essential oil from *Artemisia haussknechtii* (Boiss), to larvae and adults of *Tribolium confusum* (Jacquelin du Val)

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Abstract. Fumigant toxicity of the essential oil from *Artemisia haussknechtii* (Boiss) belonging to the family Asteraceae, was assessed against the larvae (10 and 25 days old) and adults (1–7 days old) of the confused flour beetle *Tribolium confusum* (Jacquelin du Val) (Coleoptera: Tenebrionidae). Dry ground plant was subjected to hydrodistillation using a Clevenger-type apparatus. The resulting oil contained camphor (29.24%), 1, 8-cineole (27.62%), and yomogi alcohol (5.23%) as the major compounds. The essential oil was toxic on three insect stages with LC₅₀ values ranging between 27.37 and 115.93 µl/l air, depending on the insect stage, concentration, and exposure time. From the three stages of *T. confusum*, the 25-day-old larvae were more tolerant, and adults were more susceptible than other stages. These results suggested that the essential oil of *A. haussknechtii* have potential as a control agent against larvae and adults of *T. confusum*.

Key words: *Artemisia haussknechtii*, essential oil, fumigation, *Tribolium confusum*.

Introduction

In stored grains, insect damage may account for 10-40% of loss worldwide (Matthews 1993). Fumigation plays a very important role in insect pest elimination in stored products (Zettler & Arthur 2000). Plant essential oils and their components have been shown to possess potential for development as new fumigants. The essential oils may have advantages over conventional fumigants in terms of local availability, rapid degradation, and low mammalian toxicity (Isman 2008).

Confused flour beetle, *Tribolium confusum* (Jacquelin du Val) (Coleoptera: Tenebrionidae), is one of the most serious pests of stored products. Laboratory evaluations were done on the insecticidal activity of essential oils and their major components, often various monoterpenoids, against *T. confusum* (Ojimelukwe & Adler 1999, Erler 2005, Isikber et al. 2006, Stamopoulos et al. 2007, Kotan et al. 2008).

The genus *Artemisia* (Asteraceae family), with the common Persian name 'dermane' and the common English name 'wormwood' includes 34 species that are found in wild form all over Iran. Two of these species are endemic (Mozaffarian 1996).

The *Artemisia* species are popular plants, which are used for the treatment of diseases such as hepatitis, cancer, inflammation and infections by fungi, bacteria, and viruses (Kim et al. 2002). Furthermore, *Artemisia* species are widely used as medicinal plants in folk medicine (Tahraoui et al. 2007). Local people in the western part of Iran use *Artemisia haussknechtii* (Boiss) in the treatment of dyspepsia and other gastrointestinal disorders (Khanahmadi et al. 2009).

Fumigant toxicity of *A. haussknechtii* oil evaluated on *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.), and *Callosobruchus maculatus* (Fab.) (Hashemi & Safavi 2012a). However, there are no reports on the insecticidal activity of essential oil of *A. haussknechtii* on larvae and adults of *T. confusum*.

Materials and methods

Insect culture

All stages of *T. confusum* were obtained from laboratory stock cul-

tures maintained at the Department of the Plant Protection of the University of Urmia, Iran. Larvae and adults were reared on wheat flour (*Triticum aestivum* L.) mixed with yeast (10:1, w/w). The cultures were maintained in the dark in a growth chamber set at 27±2°C and 60±5% RH. The larvae of the *T. confusum* used in the experiments were 10 and 25 days old and adults were 1–7 days post-eclosion. All experiments were carried out under the same environmental conditions.

Plant material

The aerial parts (stem and leaves) *A. haussknechtii* were collected from the Karand region (Kermanshah province) [lat.: 34°16', long.: 46°15'; 2042 m a.s.l.]. The plant material was collected during July 2010. Plant taxonomists in the Department of Biology at Urmia University, confirmed the taxonomic identification of the plant species. The voucher specimens have been deposited at the herbarium of the Department of Plant Protection in Urmia University. The plant material was dried naturally on laboratory benches at room temperature (23–24°C), for 5 days until crisp (Negahban et al. 2007).

Extraction and analysis of essential oil

Essential oil was extracted from the plant samples using a Clevenger-type collector; the plant material was subjected to hydro-distillation. Conditions of extraction were 50 g of air-dried sample; 1:10 plant material/water volume ratio, 2 h distillation. Anhydrous sodium sulphate was used to remove the water after extraction. Extracted oil was stored in a refrigerator at 4°C.

The constituents of essential oil from *A. haussknechtii* were analyzed by gas chromatography mass spectrometry (GC-MS) (Thermo-UFM). The GC condition was 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); and injector and detector temperature of 250°C. The identification of individual compounds was based on a comparison of their relative retention times with those of authentic samples on a capillary column. The compound identification was also done by matching their mass spectra of peaks with those obtained from authentic samples and published data (Davies 1990).

Fumigant toxicity

In order to test the toxicity of essential oil, concentrations of 35, 47, 62, 83 and 110 µl/l air of the oil were dissolved in 100 µl acetone, air-dried for 2 min and applied on a filter-paper (Whatman No.1) strip measuring 4 × 5 cm that was attached to the lower side of the jar's lid. Twenty adults and larvae (10 and 25 days old) were placed in small plastic tubes (3.5 cm diameter and 5 cm height) whose open ends were covered with cloth mesh. The tubes were hung at the

Table 1. Result of the probit analysis to estimate LC₅₀ and LC₉₅ values of *Artemisia haussknechtii* essential oil on adult, and 10-and 25-day-old larvae of *T. confusum*.

Insect stage	Time [h]	LC ₅₀ ^a	LC ₉₅ ^a	χ ² [df = 3]	p	Intercept	Slope
Adult	24	61.19 (44.52–83.24)	300.55 (159.01–4126.88)	1.90 ^b	0.59	0.75	2.38
	48	39.38 (27.32–47.20)	104.22 (80.47–200.80)	2.15 ^b	0.54	-1.20	3.89
	72	27.37 (9.18–35.02)	59.13 (48.70–117.41)	0.43 ^b	0.93	-2.06	4.91
Larva (10 d)	24	101.25 (78.18–218.03)	440.13 (209.31–9122.33)	3.29 ^b	0.34	-0.16	2.57
	48	66.42 (57.71–77.59)	149.57 (115.33–254.44)	2.42 ^b	0.48	-3.50	4.66
	72	31.27 (16.93–38.54)	72.66 (58.94–130.64)	0.44 ^b	0.93	-1.67	4.46
Larva (25 d)	24	115.93 (94.50–194.13)	285.56 (177.97–1244.00)	0.77 ^b	0.85	-3.67	4.20
	48	102.18 (79.29–211.03)	424.14 (207.41–6844.45)	1.04 ^b	0.78	-0.34	2.66
	72	45.21 (25.24–57.63)	210.13 (125.24–1654.93)	1.62 ^b	0.65	0.92	2.46

^aNinety-five percent lower and upper fiducial limits are shown in parenthesis.

^bSince Chi square goodness-of-fit is not significant ($P > 0.15$), no heterogeneity factor is used.

Table 2. Result of probit analysis to calculate LT₅₀ and LT₉₅ values. Lethal time values were calculated at the highest concentration (110 µl/l air).

Insect stage	LT ₅₀ h	LT ₉₅ h	χ ² [df = 1]	p	Intercept	Slope
Adult	13.37	39.18	0.64 ^a	0.42	1.04	3.52
Larva (10 day)	20.30	55.03	0.71 ^a	0.40	0.04	3.79
Larva (25 day)	31.39	180.58	2.39 ^b	0.12	1.76	2.16

^aSince Chi square goodness-of-fit is not significant ($P > 0.15$), no heterogeneity factor is used.

^bSince Chi square goodness-of-fit is significant ($P < 0.15$), a heterogeneity factor is used.

geometrical center of 1 L glass jars. The jars were then sealed with air-tight lids. Thus, there was no direct contact between the oil and the insects (Hashemi & Safavi 2012b). In the control jars, only acetone was applied on the filter papers. Mortality was determined after 24, 48, and 72 h from commencement of the exposure. Each experiment was replicated four times for each concentration. When no leg or antennal movements were observed, an insect was considered dead.

Statistical analysis

The mortality data were corrected using Abbott's formula (Abbot 1925) for the mortalities in the controls, and then subjected to probit analyses using SPSS (vers. 16.0) software to estimate LC₅₀, LC₉₅, LT₅₀ and LT₉₅ values. The percentage mortality values for different exposure times were subjected to analysis of variance (one-way ANOVA) using the SPSS (version 16.0) software statistical program. Data were transformed using arcsine \sqrt{x} to meet normality, before ANOVA. Comparison of means were done through Tukey's (HSD) test at 0.05.

Results

Yield and chemical constituent

The essential oil obtained was pale yellow with a yield of 0.9%. The main compounds of *A. haussknechtii* were camphor (29.24%), 1, 8-cineole (27.62%), yomogi alcohol (5.23%), and

camphene (4.80).

Fumigant toxicity

Toxicity data for essential oil from *A. haussknechtii* indicate a remarkable difference in susceptibility between the life stages of *T. confusum* (Tables 1, 2, and Fig. 1). Larvae (25 days old) were the most resistant stage to the essential oil with LC₅₀ value of 115.93 µl/l air, whereas the adult insects were more susceptible with LC₅₀ value of 61.19 µl/l air, at a 24 h exposure time. Furthermore, with the increase of exposure time to 72 h, mortality increased and LC₅₀ values decreased to 31.27 µl/l air for 10-day-old larvae; 45.21 µl/l air for 25-day-old larvae; and 27.37 µl/l air for adults (Table 1). The 95% fiducial limits of the LC₅₀ and LC₉₅ values for three stages are shown in Table 1.

Lethal time values and their corresponding information were calculated at the highest concentration (110 µl/l air). From Table 2, it can be seen that adult insects (LT₅₀ = 13.37 h) were more susceptible and 25-day-old larvae (LT₅₀ = 31.39 h) were more tolerant than other stages to the essential oil.

Discussion

The yield of essential oil, from our study, was relatively

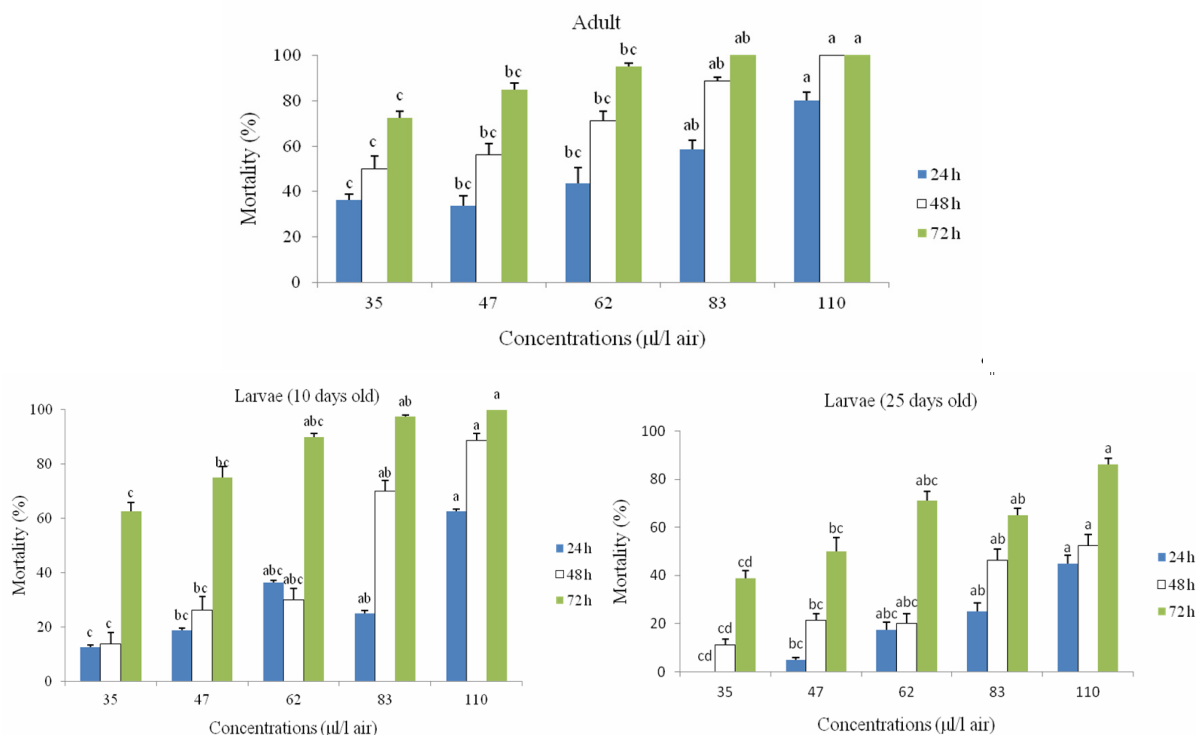


Figure 1. Mean mortality (%) of adults and 10 and 25 days old larvae of *Tribolium confusum* exposed to different concentrations of the *Artemisia haussknechtii* oil. (Different letters over columns indicate significant differences according to Tukey test at $\alpha=0.05$. Columns with the same letter are not significantly different. Vertical bars indicate standard error (\pm)).

lower than other studies on *A. haussknechtii* in Iran (Sereshti & Samadi 2007, Jalali heravi & Sereshti 2007). The oil had compositions similar to those of other *A. haussknechtii* essential oils analyzed in Iran. Jalali Heravi & Sereshti (2007) and Sereshti & Samadi (2007) reported the main components of *A. haussknechtii* to be camphor (32.70), 1, 8-cineole (31.40), and cis-davanone (7.46). The oil Khanahmadi et al (2009) studied contained camphor (12.40), α - terpineol (9.93), and davana ether (6.24) as the main components. In the *A. haussknechtii* oil studied by Rustaiyan et al (2009), the major constituents were 1, 8-cineole (16.5%), camphor (14.5%), and artemisia ketone (10.5%).

When comparing results of published data, it can be clearly seen that ingredients of the essential oil of *A. haussknechtii* are similar. The differences in the percentages depend distinctly on the region in which they are grown. This study has demonstrated that the essential oil yield and the amount of each component varied among the different studies done in Iran. It is known that many factors affect the essential oil yield and composition, such as season, herbivore, temperature, and reproductive stage (Putievsky et al 1986, Figueiredo et al 1997).

Based on the results from fumigant bioassays, the essential oil tested showed high toxicity when it was applied against insects. The insecticidal activity depended on the oil concentration, insect stage, and exposure time. When insects were fumigated for 48 h, a concentration of 35 $\mu\text{l/l}$ oil was necessary to cause 50% mortality for the adults, while a concentration of up to 62 $\mu\text{l/l}$ for 10-day-old larvae, and for 25-day-old larvae a concentration 110 $\mu\text{l/l}$ was the amount used to cause equal mortality (Fig. 1). Moreover, slopes of probit lines estimated that any increase in essential oil concentra-

tion, inflicted the highest mortality on adults when compared to other tested stages at 72 h exposure time. Furthermore, the intercept of the probit line for this stage at a 24 h exposure time was higher than for 10 and 25-day-old larvae, showing the higher response threshold (Table 1). Fast insecticidal activity by the vapor of oil also became evident for the adults when determining the medium lethal time values (LT_{50}). Considering the R^2 values, a linear model was fitted for lethal time analysis. Comparing the slopes of regression lines for three stages showed that 25-day-old larvae mortality was slowly influenced by time, and conversely, 10-day-old larvae mortality was highly affected by time spent when compared with other stages. Intercept of time analysis lines showed that the mortality of 10-day-old larvae started faster than the other tested stages (Table 2).

The experiment has shown that adults were more susceptible than the other stages (Tables 1, 2, and Fig. 1). These results, and those reported earlier, indicate that the insecticidal activity of the essential oil varies depending on the stage of the insect (Papachristos & Stamopoulos 2002; Isikber et al 2006). In addition, it is not surprising that susceptibility to the essential oil varied with age. In fact, as the larvae develop, they become less susceptible (Tables 1, 2, and Fig. 1). Similar trends have been obtained for several stored product insects. Stamopoulos et al (2007) tested the vapor form of five monoterpenoids against different stages of *T. confusum*. The LC_{50} values to the selected monoterpenoids (terpinen-4-ol, 1, 8-cineole, linalool, R-(+)-limonene, and geraniol) for 10-day-old larvae were 1.1, 3.5, 8.6, 2.6, and 607 $\mu\text{l/l}$ air, respectively, and ranged from 14.4, 12.1, 31.5, 12.3 and 1358 $\mu\text{l/l}$ air for 25-day-old larvae, respectively. Papachristos & Stamopoulos (2002) assessed fumigant toxicity of three essential

oils against the larvae of *A. obtectus*. The LC₅₀ values, from *Lavandula hybrid* Rev. (Lamiaceae), *Rosmarinus officinalis* (L.) (Lamiaceae), and *Eucalyptus globulus* (Labill.) (Myrtaceae) against third/fourth instar larvae were 8.0, 10.6, and 18.7 µl/l air and third/fourth instar larvae were significantly more tolerant than first instar larvae 0.6, 1.1, and 3.4 µl/l air, respectively. Huang et al (2000) tested methyl allyl disulfide and diallyl trisulfide; the major constituents of the essential oil of *Allium sativum* (L.) (Amaryllidaceae) against larvae of the *Tribolium castaneum*. They reported that younger larvae were more susceptible than older larvae, to the fumigant toxicity of these compounds. Wang et al (2006) studied fumigant toxicity of essential oil from *Artemisia vulgaris* against *T. castaneum* adults and larvae. Their study showed that adults were much more susceptible than the larvae. However, they reported that the age of the larvae was not significant in the fumigant activity of *A. vulgaris* oil. Our experiment showed, that toxicity of the essential oil of *A. haussknechtii* varied with the age of larvae (10 and 25 days old) (Fig. 1).

The monoterpene camphor (29.24%) is the main component in *A. haussknechtii* essential oil. This monoterpenoid might have broad insecticidal activity against stored-product insects, and act as the fumigant in *Artemisia* species essential oil. Camphor from several *Artemisia* species is reported to be toxic against stored-product beetles. Kordali et al (2006) reported toxicity of the oils of the three *Artemisia* species; *A. absinthium*, *A. santonicum*, and *A. spicigera* against *Sitophilus granarius*. In that study, camphor as the predominant component was more toxic on insect. In another study by Negahban et al (2007), fumigant toxicity of *Artemisia sieberi* was evaluated on *C. maculatus*, *S. oryzae*, and *T. castaneum*. The toxic effects of *A. sieberi* could be attributed to the major constituents such as camphor (54.7%), and the bioefficacy of essential oil extracted from the leaves of *Artemisia herba-alba* toward the *Acanthoscelides obtectus* (Tani et al 2008).

In conclusion, even if large-scale trials are necessary to determine an application method for the fumigant of this oil against stored product insect pests, in an integrated approach that could represent a possible alternative to chemical insecticides against stored product pests.

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