

Chemical composition and bio-pesticidal values of essential oil isolated from the seed of *Heracleum persicum* Desf. ex Fischer (Apiaceae)

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Abstract

The present investigation was aimed to analyse the chemical composition of essential oil isolated from *Heracleum persicum* Desf. ex Fischer and assess its lethal and sub-lethal effects against *Tribolium castaneum* (Herbst). Essential oil from hydro-distilled seeds of *H. persicum* was analyzed by gas chromatography-mass spectrometry (GC-MS), and hexyl butyrate (50.58%), octyl acetate (9.80%) and hexyl hexanoate (8.75%) were found as principal constituents. Repellent activity, contact and fumigant toxicity and antifeedant effects of this oil were assessed against the adults of *T. castaneum*. The essential oil strongly repelled *T. castaneum* adults even at the lowest concentration (0.035 $\mu\text{L cm}^{-2}$). Complete repellency (100%) occurred when the highest concentration (0.212 $\mu\text{L cm}^{-2}$) was applied for 8 h. *T. castaneum* was very susceptible to *H. persicum* oil at both contact and fumigant bioassays. In the fumigant toxicity, essential oil killed the larvae, pupae and adults and significantly decreased larvae emerged from treated eggs. LC_{10} to LC_{40} values of fumigation adult's bioassay as sub-lethal concentrations were used to evaluate the antifeedant effects. *H. persicum* essential oil has significant antifeedant effects on *T. castaneum* adults and decrease of feeding happened when oil concentrations increased. The results of the present study indicate that essential oil of *H. persicum*, with wide bio-effects on *T. castaneum*, is a source of biologically active agents which may potentially prove to be efficient insecticides.

Additional key words: essential oils; *Heracleum persicum*; toxicity; repellent; antifeedant.

Introduction

Insect pests are a major constraint on crop production, especially in developing countries. The red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), is one of the most widespread and destructive stored-product insect pests throughout the world. Beetles and larvae feed on a very wide variety of dry vegetable substances, such as milled cereal products (Rees, 2004). Infestations not only cause significant

losses of grains, they also elevate their temperature and moisture conditions that lead to an accelerated growth of molds, including toxigenic species (Magan *et al.*, 2003).

Currently, the measures to control pest infestation in grain and dry food products rely on the use of liquid insecticides, such as organophosphates, pyrethroids and gaseous insecticides. This can lead to problems such as environmental toxicity, increasing application costs, pest resistance to pesticides, ozone layer deple-

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Abbreviations used: FDI (feeding deterrence index); GC (gas chromatography); LC_{50} (lethal concentration required to kill 50% of the population); MS (mass spectrometry); PR (percentage of repellency); RH (relative humidity).

tion and lethal effects on non-target organisms in addition to direct toxicity to the consumer (Zettler & Arthur, 2000; Khalfi *et al.*, 2008; Nyamador *et al.*, 2010; Wei *et al.*, 2014). The search for natural and environmentally friendly insecticidal substances is ongoing because of the negative effects of conventional chemical insecticides.

Essential oils are extracted by distillation and expression, and are popular as ingredients of perfumes, cosmetics and household cleaning products, as well as being used for flavouring food and drink. Recently, as an alternative pest control technology, essential oils have attracted particular attention because of their specificity to pests, biodegradable nature and potential for commercial application (Liu *et al.*, 2006). Essential oils generally have a broad spectrum of bioactivity because of the presence of several active ingredients that work through several modes of action. The toxicity of individual oils or compounds often exerts differential effects depending on both the mode of action and the target pest (Isman, 2006). Investigations in several countries confirm that some plant essential oils not only possess contact and fumigant toxicity against stored product insect pests, but repel insects as well as exhibit feeding inhibition or harmful effects on the reproductive system of insects (Isman, 2006; Rajendran & Srianjini, 2008).

Apiaceae (Umbelliferae) is one of the best known families of flowering plants, which comprise 300–450 genera and 3000–3700 species. They are aromatic plants and have a distinctive flavor with diverse volatile compounds present in the fruits and leaves (Pimenov & Leonov, 1993). *Heracleum persicum* Desf. Ex Fisher (syn. *H. pubescens* Rech. and *H. glabrescens* Boiss. & Hohen.) known as Persian Hogweed or “Golpar”, is an annual native plant to the Alborz region, the northern part of Iran with a wide distribution across the country. Its fruits are widely used as a spice and flavoring agent in foods and in the preparation of pickles (Amin, 1991) as well as carminative, antiseptic, digestive, analgesic, antioxidant and anticonvulsant herbal drug in Iranian traditional medicine (Souri *et al.*, 2004; Sayyah *et al.*, 2005; Radjabian *et al.*, 2013).

Due to the widespread use of *H. persicum* as a medicinal plant and flavoring agent, and as part of the search for bio-rational alternatives to synthetic insecticides, this study aims to evaluate the effects of *H. persicum* essential oil as a contact and fumigant insecticide, repellent, and antifeedant against *T. castaneum*. In addition, the chemical composition of this essential

oil was analyzed by gas chromatography-mass spectrometry (GC-MS).

Material and methods

Plant material and essential oil analysis

The seeds of *H. persicum* were collected from Meshkin shahr city, Ardabil province, Iran. The seeds were air-dried in the shade at room temperature (26–28°C) for 10 days and the essential oil was isolated by hydro-distillation method using a Clevenger apparatus. Conditions of extraction were: 50 g of air-dried sample, 1:10 in water (w/v), 3 h distillation. Anhydrous sodium sulfate was used to remove water after extraction and extracted essential oil was stored at 4°C.

GC-MS analysis was carried out on a HP 7890A GC (Hewlett-Packard, Palo Alto, CA, USA) equipped with a split injector and 5975C mass selective detector system. Chromatographic separation was carried out in a HP-5 capillary column (30 m × 0.25 mm, 0.25 µm in film thickness). The MS was operated in the EI mode (70 eV). The GC-MS interface, ion source, and quadruple temperatures were set at 280°C, 230°C, and 150°C, respectively. The injector temperature was set at 250°C, the column temperature program started at 50°C for 3 min, increased by 10°C min⁻¹ to 110°C and by 10°C min⁻¹ to 180°C, and was maintained for 2 min. Helium (99.999%) was used as the carrier gas with flow rate of 1 mL min⁻¹. Identification of spectra was carried out by studying their fragmentation and by comparison with standard spectra present in the library of the instrument. Area normalization was used for determination of composition percentage.

Insect rearing

Tribolium castaneum was reared in plastic rectangular containers (20 cm length × 14 cm width × 9 cm height) containing a wheat flour and wheat bran mixture (2:8 w/w). The mouth of the containers was covered with a fine mesh cloth for ventilation and to prevent the beetles from escaping. Cultures were maintained in an incubator at 27 ± 2°C and 60 ± 5% relative humidity (RH) in the dark. Parent adults were obtained from laboratory stock cultures maintained at the Department of Plant Protection, University of

Tehran, Iran. All experimental procedures were carried out under the same environmental conditions as the cultures.

Repellent activity

A choice bioassay system was used to study the repellency of *H. persicum* essential oil. One half of filter-paper disks (6 cm in diameter) was treated with 200 μL of acetonic solution of the essential oil and dried for 5 min. Half of the bottom of a Petri dish was covered with the treated filter paper (Whatman No. 1) with concentration of 0.035, 0.07, 0.106, 0.141 and 0.212 $\mu\text{L cm}^{-2}$, while the other half was covered with a filter paper disk impregnated with acetone. Ten unsexed adults were put into each Petri dish and the lid was sealed with parafilm[®]. Four replicates were run for each tested concentration, so that 40 adults were assayed per concentration. The number of insects on the two half paper disks was recorded after 2 and 4 h from the beginning of the test. Percentage of repellency (PR) was calculated as follows: $\text{PR} = [(C - T) / (C + T)] \times 100$, where C = numbers of insects on the untreated area, and T = numbers of insects on the treated area (Nerio *et al.*, 2009). Positive values express repellent and negative attraction values.

Contact toxicity

The contact toxicity of *H. persicum* essential oil against adults of *T. castaneum* was evaluated on filter paper discs of 6 cm in diameter which were treated with the substances diluted in acetone as a solvent. Range-finding studies were done to determine the fair testing concentrations. Concentrations of 1, 1.82, 3.32, 6.04, 10.99 and 20 μL of essential oil diluted in 0.5 mL of acetone were applied to the filter paper discs and the filter papers were placed in Petri's dishes of 6 cm diameter. These concentrations are equivalent to 0.03, 0.06, 0.11, 0.21, 0.38 and 0.71 $\mu\text{L cm}^{-2}$. The acetone was allowed to evaporate for 5 min before introduction of 10 unsexed insect adults and these were kept in darkness in the incubator at $27 \pm 2^\circ\text{C}$ and $60 \pm 5\%$ RH. The lids of Petri dishes were pierced (1 cm diameter) and then covered by a fine mesh cloth to avoid fumigant toxicity. In the control groups only acetone was applied to the filter papers. Each treatment was replicated three times and insect mortality was recorded after 24 h.

Fumigant toxicity

To determine the fumigant toxicity, filter paper (Whatman No. 1, cut into 2-cm diameter pieces) was impregnated with essential oil at doses calculated to give equivalent fumigant concentrations of 17.86, 24.64, 34, 46.92, 64.71 and 89.28 $\mu\text{L L}^{-1}$ air. The impregnated filter paper was then attached to the undersurface of the screw cap of a glass vial (280 mL). The caps were screwed tightly on a vial containing 10 insects. Each concentration and the control were replicated three times. Larvae (10-12 d-old), pupae (8-9 d-old) and unsexed adults (1-7 d-old) were tested as above. When no leg or antennal movements were observed, insects were considered dead. Mortality was determined after 24 h from commencement of exposure for larvae and adults, while adults emerging from pupae were noted after 10 days.

For bioassay of eggs, 50 newly emerged beetles (male and female) were placed in 280 mL glass jars containing wheat meal and after 24 h, the insects were removed. Then treated filter papers were placed in each glass jars containing meal and 24 h old eggs. The jars were checked after 14 days to compare the number of emerged larvae between treated and control groups.

Antifeedant activity

The antifeedant effect of sub-lethal doses on 1 to 7-day old adults was assessed as described for fumigant toxicity with concentrations ranging from LC_{10} to LC_{40} for 24 h exposure time. Surviving adults were removed and used immediately for the antifeedant assay. Ten grams of wheat flour and 50 insects previously treated with sub-lethal doses of oil was placed in each Petri dish (ϕ 6 cm). The lids of Petri dishes were pierced (ϕ 1 cm) and then covered by a fine mesh cloth for ventilation. Control groups were treated in the same way without oil. Each experiment was replicated three times. Reduction in the flour weight in each Petri dish was calculated after 72 h as follows: Feeding Deterrence Index or FDI (%) = $[(\text{Control} - \text{Treatment}) / \text{Control}] \times 100$, where 'Control' is the weight of meal consumed by the insects without essential oils and 'Treatment' the weight of meal consumed by the insects treated with essential oils.

The experiments were arranged in a completely randomized design and the data were analyzed with ANOVA. The means were separated using the Tukey's test at the

Table 1. Result of GC-MS analysis of essential oil from *Heracleum persicum* seed

No.	Compound	Retention time (min)	(%)	Quality	Molecular weight	Formula
1	n-hexanol	4.496	0.56	83	102.1748	C ₆ H ₁₄ O
2	isopropyl 2-methylbutyrate	4.864	0.49	80	144.2114	C ₈ H ₁₆ O ₂
3	isopropyl isopentanoate	5.025	0.36	90	144.2114	C ₈ H ₁₆ O ₂
4	butyl isobutyrate	6.520	0.21	90	144.2114	C ₈ H ₁₆ O ₂
5	butyric acid butyl ester	7.690	1.33	90	144.2114	C ₈ H ₁₆ O ₂
6	n-octanal	7.879	0.22	74	128.2120	C ₈ H ₁₆ O
7	n-hexyl acetate	8.206	0.40	90	144.2114	C ₈ H ₁₆ O ₂
8	o-cymene	8.532	1.10	97	134.2182	C ₁₀ H ₁₄
9	1,8-cineole	8.704	0.22	99	154.2493	C ₁₀ H ₁₈ O
10	butyl 2-methylbutanoate	9.025	0.39	90	158.2380	C ₉ H ₁₈ O ₂
11	butyl 3-methylbutanoate	9.162	0.25	90	158.2380	C ₉ H ₁₈ O ₂
12	γ-terpinene	9.530	0.26	96	136.2340	C ₁₀ H ₁₆
13	1-octanol	9.915	0.36	91	130.2279	C ₈ H ₁₈ O
14	linalool	10.752	0.74	91	154.2493	C ₁₀ H ₁₈ O
15	hexyl propionate	10.901	0.30	90	158.2380	C ₉ H ₁₈ O ₂
16	hexyl isobutyrate	12.171	3.85	91	172.2646	C ₁₀ H ₂₀ O ₂
17	hexyl butyrate	13.518	50.58	91	172.2646	C ₁₀ H ₂₀ O ₂
18	vinylcyclohexane	13.678	3.28	74	110.1968	C ₈ H ₁₄
19	octyl acetate	14.023	9.80	91	172.2646	C ₁₀ H ₂₀ O ₂
20	hexyl 2-methylbutanoate	14.717	5.89	83	186.2912	C ₁₁ H ₂₂ O ₂
21	hexyl isovalerate	14.836	0.95	90	186.2912	C ₁₁ H ₂₂ O ₂
22	hexyl valerate	16.159	0.16	47	186.2912	C ₁₁ H ₂₂ O ₂
23	octyl propionate	16.527	0.20	90	186.2912	C ₁₁ H ₂₂ O ₂
24	(-)-α-thujone	17.334	0.37	64	152.2334	C ₁₀ H ₁₆ O
25	octyl 2-methylpropanoate	17.643	2.41	91	200.3178	C ₁₂ H ₂₄ O ₂
26	(-)-β-thujone	18.474	0.89	64	152.2334	C ₁₀ H ₁₆ O
27	hexyl hexanoate	18.759	8.75	91	200.3178	C ₁₂ H ₂₄ O ₂
28	isopropylcyclopentane	18.996	0.44	38	112.2126	C ₈ H ₁₆
29	2-octyne	19.667	0.48	72	110.1968	C ₈ H ₁₄
30	octyl 2-methylbutanoate	19.946	4.08	90	214.3443	C ₁₃ H ₂₆ O ₂
31	2-(aminomethyl)-butyric acid	23.531	0.34	59	131.174	C ₆ H ₁₃ O ₂
32	heptyloctanoate	27.846	0.20	38	242.3975	C ₁₅ H ₃₀ O ₂
	Total		99.86			

5% level. The LC₅₀ values with confidence limits were calculated by probit analysis using the SPSS version 16.0 software package.

Results

The essential oil isolated by hydro-distillation from the seed of *H. persicum* was found to be pale yellow and the yield was 1.6% (w/w). The results of the chemical analysis are presented in Table 1. Thirty two compounds were positively identified in the oil. In this study, hexyl butyrate (50.58%), octyl acetate (9.80%), hexyl hexanoate (8.75%), hexyl 2-methylbutanoate (5.89%), octyl 2-methylbutanoate (4.08%), hexyl isobutyrate (3.85%) and vinylcyclohexane (3.28%)

were found to be the major constituents, accounting for 85.96% of the total oil (Table 1).

According to Fig. 1, the essential oil of *H. persicum* strongly repelled *T. castaneum* adults even at the lowest concentration, the repellency increased with increasing concentrations of essential oil. Complete repellency (100%) occurred when the highest concentration (0.212 μL cm⁻²) was applied for 8 h.

Fig. 2 shows that the adults of *T. castaneum* were very susceptible to the contact toxicity of essential oil of *H. persicum* and even the lowest concentration (0.03 μL cm⁻²) was significantly different from the control according to Tukey's test. The mortality was dose-dependent and increased with increasing concentrations (F = 284.57, p = 0.0001). Results of the probit analysis showed that the 24 h LC₅₀ with 95%

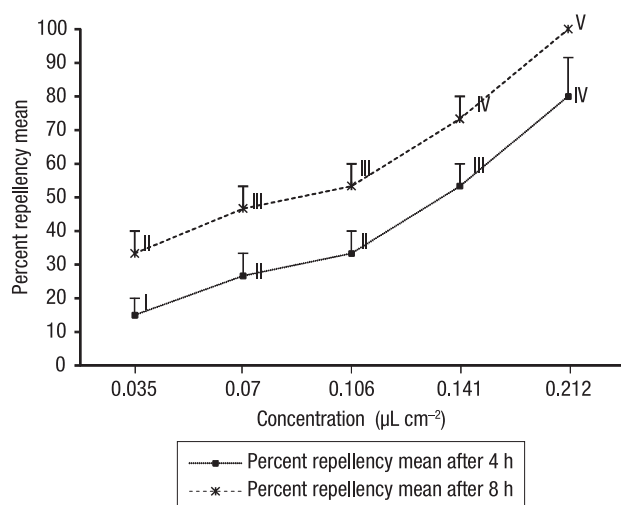


Figure 1. Repellency of essential oil of *Heracleum persicum* against the adults of *Tribolium castaneum* recorded 4 and 8 h after treatment. Repellency % = $(C - T) \times 100 / (C + T)$; C = number of insects on the control; T = number of insects on the treated. Ten adults were used per replicate.

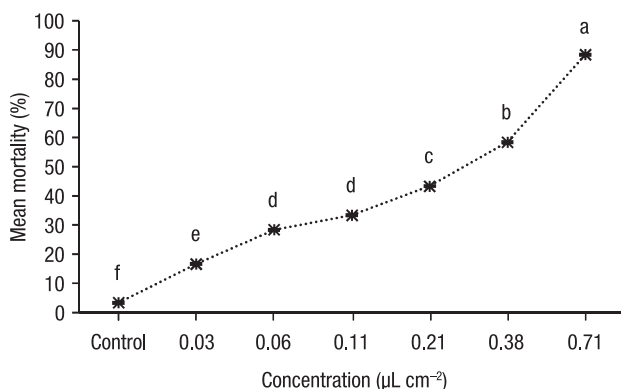


Figure 2. Contact toxicity of essential oil of *Heracleum persicum* against the adults of *Tribolium castaneum* after 24 h of exposition. Means with the same letter on standard error bars are not significantly different ($p = 0.05$, Tukey's test). Twenty adults were used per replicate.

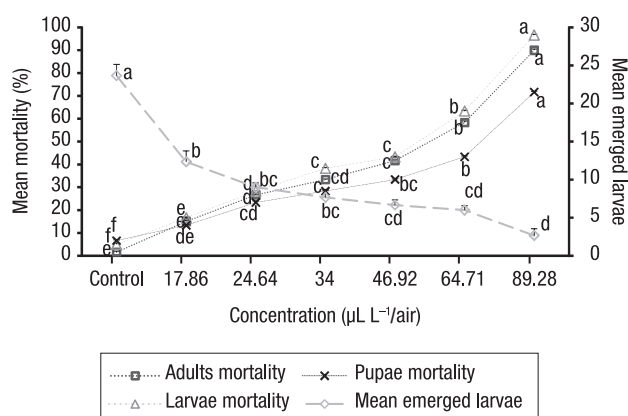


Figure 3. Fumigant toxicity of the essential oil of *Heracleum persicum* against the eggs, larvae, pupae and adults of *Tribolium castaneum* after 24 h. The mean emerged larvae from treated eggs after 14 days indicate the susceptibility of eggs. For each parameter studied means with the same letter on standard error bars are not significantly different ($p = 0.05$, Tukey's test). Twenty adults were used per replicate.

confidence limits was 0.194 (0.118-0.363) μL cm⁻² (Table 2).

The essential oil of *H. persicum* demonstrated fumigant toxicity to all stages of *T. castaneum*. This essential oil killed the larvae, pupae and adults and significantly decreased larvae emerging from treated eggs. The activity depended on essential oil concentrations ($F = 47.25, p = 0.0001$ for eggs, $F = 263.93, p = 0.0001$ for larvae, $F = 89.82, p = 0.0001$ for pupae, $F = 1591.57, p = 0.0001$ for adults) (Fig. 3). In the fumigant toxicity test, according to Table 2, the LC₅₀ values with their 95% confidence limits were 41.779 (29.991-60.831), 62.871 (53.333-79.427) and 46.005 (35.558-63.715) for larvae, pupae and adults, respectively. It is obvious that larvae were more susceptible than adults and pupae. On the other hand, pupae were more tolerant than larvae and adults (Table 2).

Probit analysis for fumigant toxicity on adults showed that sub-lethal concentrations from LC₁₀ to LC₄₀

Table 2. Results of probit analysis for contact and fumigant toxicity of the essential oil of *Heracleum persicum* against *Tribolium castaneum*

Test ¹	Insect stage	24-h LC ₅₀ with 95% confidence limits ²	Intercept ± SE	Slope ± SE	Chi-square (df = 4)
Contact toxicity	Adult	0.194 (0.118-0.363)	5.86 ± 0.15	1.35 ± 0.16	8.23
Fumigant toxicity	Larvae	41.779 (29.991-60.831)	0.09 ± 0.53	3.03 ± 0.33	14.33
	Pupae	62.871 (53.333-79.427)	1.22 ± 0.51	2.10 ± 0.31	5.065
	Adult	46.005 (35.558-63.715)	0.34 ± 0.52	2.80 ± 0.32	9.18

¹ N=420 for each test. ² μL cm⁻² for contact toxicity and μL L⁻¹ air for fumigant toxicity.

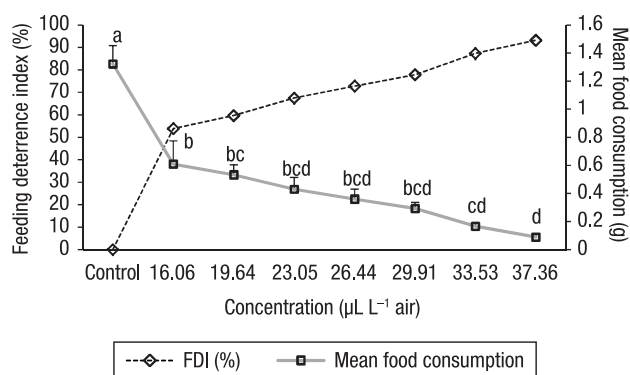


Figure 4. Feeding deterrence effect of sub-lethal concentrations of the essential oil of *Heracleum persicum* on adults of *Tribolium castaneum*. FDI was calculated as explained in M&M. Means with the same letter on standard error bars are not significantly different ($p = 0.05$, Tukey's test). Fifty adults were used per replicate.

were 16.06 to 37.36 $\mu\text{L L}^{-1}$ air respectively and these concentrations were used to assess feeding deterrent activity. Fig. 4 shows that the essential oil of *H. persicum* had significant antifeedant effects on adults of *T. castaneum* in a dose-dependant manner. The concentrations of 53.89, 59.71, 67.5, 72.79, 77.85, 87.38 and 93.2% were used in FDI bioassay. This effect was also dose-dependent ($F = 18.45$, $p = 0.0001$) and concentration of 37.36 $\mu\text{L L}^{-1}$ air (the LC_{40}) caused the highest antifeedant effect in which food consumption and FDI values were 0.09 g and 93.2% respectively (Fig. 4).

Discussion

There are many studies related to sensitivity of *Tribolium castaneum* to plant essential oils that support the results of the present study. For example, Zapata & Smagghe (2010) reported the repellent activity and the contact and fumigant toxicity of four essential oils extracted from the leaves and bark of *Laurelia sempervirens* Tul. (Monimiaceae) and *Drimys winteri* J.R. Forster and G. Forster (Winteraceae) against *T. castaneum*. After 4 h of exposure, >90% repellency was achieved with *L. sempervirens* oils at low concentrations of 0.032 $\mu\text{L cm}^{-2}$, while for *D. winteri* oils concentrations 3-10 times higher were needed to achieve it. LC_{50} values by topical application of *L. sempervirens* oils were from 39 to 44 $\mu\text{g mg}^{-1}$ insect; for *D. winteri* oils these were from 75 to 85 $\mu\text{g mg}^{-1}$ insect. By fumigation, LC_{50} values for *L. sempervirens* oils were

1.6-1.7 $\mu\text{L L}^{-1}$ air, while these were 9.0-10.5 $\mu\text{L L}^{-1}$ air for *D. winteri* oils. In the study of Abbasipour *et al.* (2011), the insecticidal activity of the essential oil from *Elettaria cardamomum* L. (Zingiberaceae) on the adults of *Ephestia kuehniella* Zeller (Pyralidae), *T. castaneum* and *Callosobruchus maculatus* (Fabricius) was investigated. Results indicated that essential oil of *E. cardamomum* was toxic to all insects and *E. kuehniella* adults were more sensitive than the Coleoptera and *T. castaneum* was more tolerant compared to *C. maculatus*. Also, the highest mortality of these insects was seen after 12 hours. Caballero-Gallardo *et al.* (2011) tested the repellent activity of essential oils from *Tagetes lucida* Cav., *Lepechinia betonicifolia* (Lam.), *Lippia alba* (Mill.), *Cananga odorata* (Lam.), *Rosmarinus officinalis* L. and several of their constituents against *T. castaneum*. All essential oils were repellents, followed a dose-response relationship, and had bioactivity similar to or better than that of commercial compound IR3535. Essential oils from *C. odorata* and *L. alba* were the most active. Compounds from essential oils, such as benzyl benzoate, β -myrcene, and carvone, showed good repellent properties. All mentioned studies emphasized the results of the present study for susceptibility of *T. castaneum* to plant essential oils from lethal to sub-lethal bio-effects.

Lethal and sub-lethal insecticidal effects of *H. persicum* essential oil have been reported in many studies. For example, the fumigant toxicity of this essential oil on *C. maculatus* was reported by Manzoomi *et al.* (2010). LC_{50} value in this study was 337.38 $\mu\text{L L}^{-1}$ that was much more than our LC_{50} values. In the study of Izakmehri *et al.* (2013), the lethal and sublethal effects of essential oils from *H. persicum* were evaluated on *C. maculatus* adults. The LC_{50} value of *H. persicum* was 219.4 $\mu\text{L L}^{-1}$ air after 12 h and 136.4 $\mu\text{L L}^{-1}$ air after 24 h of exposure, respectively. The results showed that low lethal concentration (LC_{20}) of essential oils negatively affected the longevity, fecundity, and fertility of female adults. In another study, Sedaghat *et al.* (2011) found that *H. persicum* essential oil exerted significant larvicidal potential with LC_{50} value of 26.30 ppm against four instar larvae of *Anopheles stephensi* Liston after 24 h and a positive correlation was observed between the essential oil concentrations and the mortality percentage. In the study of Faraji *et al.* (2012), the effect of *H. persicum* essential oil was tested on egg-laying of *Phthorimaea operculella* (Zeller) with fresh potato leaves and without them; the essential oil concentration in both

series of experiments affected to the female egg-laying rate, the number of eggs laid was significantly higher in the control than in the treated plants. The egg-laying inhibition observed in both series of experiments was 56.8% and 48% respectively. Furthermore, Amizadeh *et al.* (2013) studied the acaricidal activity of this oil against eggs and adults of *Tetranychus urticae* Koch (Acari: Tetranychidae). Their findings are in accordance with our results for lethal and sub-lethal effects of *H. persicum* essential oil.

The mechanisms of essential oil toxicity to insects have not been fully identified. Treating the insects with essential oils or pure compounds may cause symptoms due to neurotoxic activity. These symptoms include hyperactivity, seizures, and tremors followed by knocking down. Such symptoms are very similar to those produced by pyrethroid insecticides (Isman, 2006). Enan (2001) suggested that toxicity of the essential oil constituents is related to the octopaminergic nervous system of insects. Octopamine is a neurotransmitter, neurohormone, and circulating neurohormone-neuromodulator. Disruption of octopamine results in total breakdown of the nervous system in insects. The lack of octopamine receptors in vertebrates may provide the mammalian selectivity of essential oils as insecticides. Several reports illustrated that essential oils cause insect mortality by inhibiting acetylcholinesterase enzyme (AChE) activity (Kostyukovsky *et al.*, 2002; Houghton *et al.*, 2006; Abd El-Galeil *et al.*, 2009). However, some activity on the hormone and pheromone system and on the cytochrome P450 monooxygenase enzyme has also been detected (Tsao & Coats, 1995; De-Oliveira *et al.*, 1997). In our previous study (Ebadollahi *et al.*, 2013), the essential oil isolated from *Agastache foeniculum* (Pursh) Kuntze (Lamiaceae) not only showed high toxicity on *T. castaneum* larvae, but activity of esterase and glutathione S transferase enzymes were also decreased besides reducing total carbohydrate, lipid and protein contents. These studies show that the insecticidal activity of essential oils is due to several mechanisms that affect multiple targets.

Sefidkon *et al.* (2004) assessed the chemical composition of essential oils of the stems (before and at full flowering stage), unripe and ripe seeds of *H. persicum* by a combination of GC and GC/MS. Twenty-four components were characterized from the stem oil before flowering with (E)-anethole (47.0%), terpinolene (20.0%), γ -terpinene (11.6%) and limonene (11.5%) as the main constituents. At full flowering stage, 33

compounds were identified in the stem oil with (E)-anethole (60.2%), terpinolene (11.3%) and γ -terpinene (7.1%) as the major components. Among the 30 compounds identified in the seed oil of *H. persicum*, the major constituents were hexyl butyrate (22.5% and 35.5%), octyl acetate (19% and 27%) and hexyl isobutyrate (9.1% and 3.2%) in unripe and ripe seeds, respectively. In the study of Radjabian *et al.* (2013), the essential oils of flat-oval shaped fruits of 17 wild populations of *H. persicum* collected from different locations in Iran were obtained by hydro-distillation and analyzed by GC-MS. The oil yields varied greatly among populations and ranged from 1.6% to 4.9% based on dried plant material. Thirty-six compounds, which accounted for 89.7-99.0% of the total oil, were identified in the essential oils. Octyl acetate (7.5-40.8%), hexyl butyrate (13.3-43.0%), hexyl isobutyrate (2.9-7.2%) and hexyl 2-methyl butyrate (4.8-11.9%) were the major components. Aliphatic esters were the most abundant class of compounds identified in the essential oils and of them, both octyl acetate and hexyl butyrate were the characteristic constituents of fruit essential oils. In the present study, hexyl butyrate, octyl acetate and hexyl hexanoate were found as main constituents of essential oil from *H. persicum* seed and it confirms the results of previous mentioned studies. Generally, the chemical composition of essential oil varies with species, season, climate, soil type, age of the leaves, soil fertility regimen, the method used for drying the plant material and the method of oil extraction (Batish *et al.*, 2008).

The bioactivity of the essential oils depends on the type and nature of the constituents and their concentration. The toxicity, repellent, antifeedant and other biological activities of plant essential oils are mainly related to their constituents (Koul & Walia, 2009) and it may be concluded that the biological effects of *H. persicum* essential oil are related to its main components, such as hexyl butyrate, octyl acetate and hexyl hexanoate. The strong repellency, fumigant toxicity and the safety of essential oil suggest that *H. persicum* is a promising candidate to be used in insect pests' management. Furthermore, this oil is used as flavoring and medicinal agents, and is considered to reduce the harmful effect of conventional insecticides on humans and the environment. For the practical application of the essential oil as insecticide, further studies on formulation development are necessary to improve efficacy and stability, and cost reduction.

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