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Toxicity of Essential Oils Isolated from Achillea millefolium L., Artemisia dracunculus L. and Heracleum persicum Desf. Against Adults of Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae) in Islamic Republic of Iran

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Abstract. The environmental problems caused by overuse of synthetic insecticide have been the matter of concern in recent years. Essential oils from aromatic plants are recognized as proper alternatives to conventional insecticides. Therefore, this study was conducted to determine the Fumigant toxicity of essential oils from *Achillea millefolium*, *Artemisia dracunculus* and *Heracleum persicum* against adults of *Plodia interpunctella* under laboratory conditions and mortality was determined after 12, 24, 36 and 48 h from beginning of exposure. The essential oils were extracted from seeds of *H. persicum* and aerial parts from 1.5 cm of top of *A. millefolium*, *A. dracunculus* by hydrodistillation method using a Clevenger apparatus. All essential oil were highly effective against *P. interpunctella* and the mortality values reached 100% when the adults were exposed to 50, 65 and 80 µl/ l concentrations of *A. dracunculus*, *A. millefolium* and *H. persicum* essential oil, respectively. The LC_{50} (lethal concentration to kill 50% of the population) values of essential oils from *A. dracunculus*, *A. millefolium* and *H. persicum* were 22.24, 34.80 and 36.96 µl/ l after 24 h fumigation, respectively. On the other hand, *A. dracunculus* oil was more effective than the other essential oils against *P. interpunctella* adults. The LC_{50} values decreased with increasing of exposure times. In all cases, considerable differences in the mortality of insect to essential oils were observed with different concentrations and exposure times. These results suggest that the essential oils of *A. millefolium*, *A. dracunculus* and *H. persicum* have merit further studies as potential fumigants for the management of *P. interpunctella* or probably other stored-product insects.

Key words: *Achillea millefolium*, *Artemisia dracunculus*, *Heracleum persicum*, essential oil, Fumigant toxicity, *Plodia interpunctella.*

Introduction

Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), is distributed world-wide and is a serious stored-product pest of grain and seeds as well as flour and other milled products (NANSEN & PHILLIPS, 2004). It prefers to feed on broken grains and more especially on milled cereal, dried fruits and almonds, pistachios and walnuts and groundnuts. It is found in warehouses (PEREZ-MENDOZA & AGUILERA-PENA, 2004). In recent years, it was considered as the most important pest of stored pistachios in Iran which cause severe qualitative and quantitative losses in this fruit crop (SHOJAADDINI *et al.,* 2005). Because of its high incidence, synthetic insecticides have been used to control it. Synthetic pesticides have been considered the most effective and accessible means to control insect pests of stored products (HUANG & SUBRAMANYAM, 2005). The

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indiscriminate use of synthetic pesticides has given rise to many serious problems, including genetic resistance by pest species, toxic residues, increasing cost of application, pollution of storage environment, and hazard from handling (BARNARD *et al*., 1997; ISMAN, 2000; HANSEN & JENSEN, 2002; KOUL *et al*., 2008).In view of these facts, researchers for the last two decades or so have diverted their attention towards age-old practices of using alternative eco-friendly insect pest control methods which are readily biodegraded, less toxic to mammal, easy to use and specific in their action. Therefore, the use of essential oils extracted from aromatic plants to control insect pests has been investigated and is well documented (ISMAN, 2006; KOUL *et al*., 2008; RAJENDRAN & SRIRANJINI, 2008).

Heracleum persicum Desf. Belongs to Apiaceae family, known as "golpar", is native to Iran. Fruits of the plant are used as a constituent of the daily diet of general population in Iran. *Artemisia dracunculus* L. (Asteraceae), known as "tarkhun" in Iran, is a small shrubby perennial herb. It is cultivated for the use of its aromatic leaves in seasoning, salad, and soup. *Achillea millefolium* L. (Asteraceae), known as "yarrow", is a perennial herb that has been widely used in folk medicine in many countries (BENEDEK *et al*., 2008). Consequently, the objective of this research was to evaluate the insecticidal activities of essential oils from *A. dracunculus*, *A. millefolium* and *H. persicum* against *P. interpunctella* under laboratory conditions. On the other hands, the goal of this study was to seek much safer and cheaper agents for controlling insect pests.

Material and methods

Insect cultures and experimental conditions

The colony of *P. interpunctella* was reared on a diet of 80% ground rice, 10% glycerin, 5% yeast and 5% honey in plastic containers (30 cm length × 20 cm width × 8 cm height). Mouth of the containers was covered with fine mesh cloth for ventilation as well as to prevent escape of the insects. The cultures were maintained in the laboratory at 27 ± 1 °C, 60 ± 5% Relative Humidity (RH) and 16:8 h light: dark. Adult insects, 1-2 days old, were used for fumigant toxicity tests. Parent adults were obtained from laboratory stock cultures maintained at the Entomology Department, University of Urmia, Iran. All experimental procedures were carried out under the same environmental conditions as the cultures.

Plant materials and extraction of essential oils

The ripe seeds of *H. persicum* and aerial parts from 1.5 cm of top of *A. dracunculus* and *A. millefolium* were collected at the flowering stage from Ardabil city, Ardabil province, Iran. The specimen plants were air dried in the shade at room temperature (26-28 °C) for 14 days. The essential oil was isolated from dried plant samples by hydrodistillation method using a Clevenger apparatus. Conditions of extraction were: 50 g of air-dried sample, 1:10 plant material/water volume ratio, 4 hrs distillation. Anhydrous sodium sulfate was used to remove water after extraction. Extracted oils were stored in a refrigerator at 4 °C.

Insecticidal activity

The insecticidal effects of the essential oils were evaluated by fumigation method. The fumigant bioassays were conducted as described by NEGAHBAN *et al*. (2007), with slight modifications. In this study, tested insects were confined in the plastic tubes. Thus, there was no direct contact between the oil and the insects. Different concentrations were prepared to evaluate mortality of insects after an initial dose-setting experiment. *P. interpunctella* were exposed to *A. dracunculus* oil at 10, 14.8, 22.3, 33.4 and 50 μl/ l, to *A. millefolium* oil at 20, 27, 36.2, 48.5 and 65 μl/ l and to *H. persicum* oil at 25, 33.5, 44.7, 59.8 and 80 μl/ l. Each concentration was applied to filter paper stripe (4 × 5 cm, Whatman N° 1) and the impregnated filter papers were then attached to the screw caps of glass jars with volumes of 1 L. Ten insect adults were placed in small plastic tubes (3.5 cm diameter and 5 cm height) with open ends covered with clothes mesh and each tube were placed at the bottom of glass jar. Caps of glass jars were screwed tightly. The jars were kept in the incubator and mortality was determined after 12, 24, 36 and 48 hrs from beginning of exposure. Each experiment was replicated for four times at each concentration. The control insects were maintained under the same conditions without any essential oil. When no leg or antennal movements were observed, insects were considered dead. The experiments were arranged in a completely randomize design and the data were analyzed with ANOVA. The means were separated using the Duncan's test at the 5% level. The LC_{50} values with their fiducial limits were calculated by probit analysis using the SPSS version 16.0 software package.

Results and Discussion

The essential oils from *A. dracunculus*, *A. millefolium* and *H. persicum* showed strong fumigant activity against *P. interpunctella* adults at different concentrations and exposure times. Figure 1 displays the mortality percentages of five different concentrations from essential oils in four times on *P. interpunctella*. This figure also shows that there are significant differences between insect mortality induced by essential oils with different concentrations and exposure times. These differences obtained by Duncan's test at $p \leq 0.05$. The mortality values reached 100% when the adults were exposed to 50, 65 and 80 µl/ l concentrations of *A. dracunculus*, *A. millefolium* and *H. persicum*, respectively (Fig. 1).

Essential oils showed variable toxicity to adults of *P. interpunctella*. The concentration for the essential oil to cause 50% mortality (LC_{50}) for *A. dracunculus* essential oil was 31.50 µl/ l after 12 h from commencement of fumigation (Table 1), whereas with *A. millefolium* and *H. persicum* essential oils 12 h LC_{50} values were 43.07 and 46.25 µl/ l respectively. The susceptibility of insect increased with exposure time and essential oil concentrations and LC_{50} values decreased within 48 h. On the other hand, increase susceptibility of insect associated with increase of the different concentrations of all oils and time of exposure. For example, LC_{50} value for *A. millefolium* essential oil decreased from 34.80 µl/ l at 24 h exposure time to 22.07 µl/ l after 48 h (Table 1). Based on LC_{50} values, essential oil of *A. dracunculus* was more potent than *A. millefolium* in the all times and it is obvious that *A. millefolium* oil had stronger toxicity than *H. persicum* oil on *P. interpunctella* (Table 1).

The failure to discover a significant new class of insecticides has led many researchers back to biodiscovery studies in the search for new and economically viable alternatives. It has been recognized that some plant-derived insect-control agents could be developed into products suitable for integrated pest management because they are selective to pests, have no or little harmful effect against non-target organisms or the environment (ISMAN, 2000). The most promising botanical groups are Meliaceae, Rutaceae, Asteraceae, Annonaceae, Lamiaceae, Aristolochiaceae and Malvaceae (REGNAULT-ROGER, 1997). *A. dracunculus* and *A. millefolium* are belonging to Asteraceae family and they are candidate for toxic agents on insect pests. However, the insecticidal activity of some essential oils from Apiaceae has been evaluated against a number of stored product insects (SAHAF *et al.*, 2007; CHAUBEY, 2008; LOPEZ *et al.*, 2008). In the present study the insecticidal activity of *H. persicum*, as one of the Apiaceae family, has been evaluated.

The effect of many essential oils as insecticides in protecting *P. interpunctella* infestation has been studied, and this insect has shown susceptibility to the some plant derived chemicals. AYVAZ *et al.* (2010) stated that the essential oils from oregano, *Origanum onites* L., savory, *Satureja thymbra* L., and myrtle, *Myrtus communis* L. were highly effective against *P. interpunctella*. In accordance with this study, our earlier study indicated that the essential oil of *Agastache foeniculum* Kuntze had fumigant toxicity against *P. interpunctella* (EBADOLLAHI *et al.*, 2010) and LC_{50} value decreased from 16.535 µl/ l at 24 h to 6.690 µl/ l at 96 h exposure time.

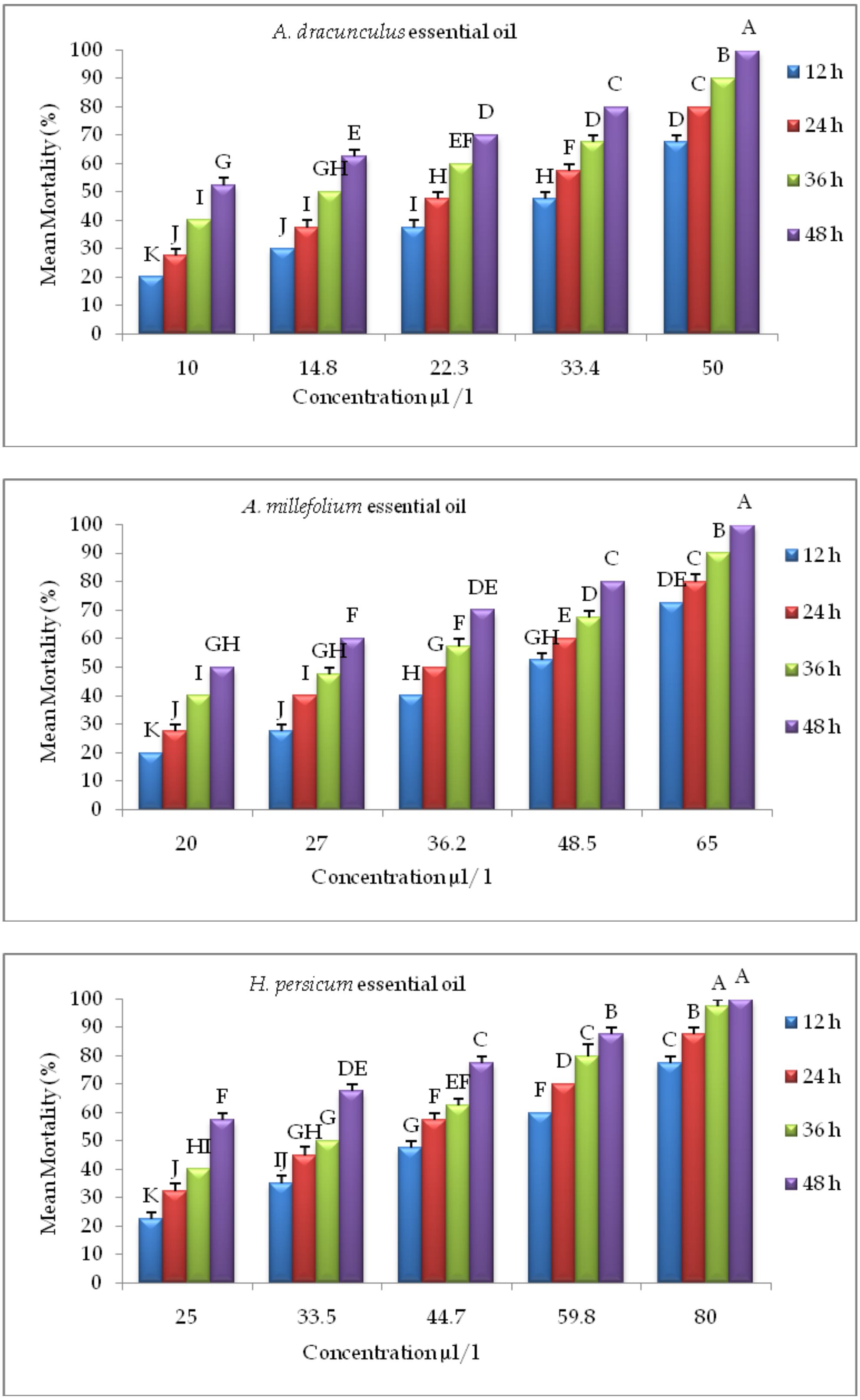

Fig. 1. Mean mortality (%) of *Plodia interpunctella* exposed to different concentrations of the essential oils from *Achillea millefolium*, *Artemisia dracunculus* and *Heracleum persicum*. Different letters on top of columns are significant differences according to Duncan's test at $p \leq 0.05$. Columns with the same letter are not significantly different. Vertical bars indicate standard error of the mean (±); very small values are not represented.

Table 1. Results of probit analysis from toxicity of the essential oils from *Achillea millefolium*, *Artemisia dracunculus* and *Heracleum persicum* against *Plodia interpunctella* at four different times.

Essential oil	Time (h)	LC_{50} (µl/l air)[a]	Slope	Intercept	Chi-square (χ^2)[b]
A. dracunculus	12	31.50 (24.75 – 46.04)	1.75	2.39	0.69
	24	22.24 (17.54 – 28.22)	1.91	2.43	1.32
	36	14.97 (10.44 – 18.80)	1.90	2.78	2.43
	48	10.68 (0.19 – 16.97)	2.22	2.71	5.57
A. millefolium	12	43.07 (37.0 – 52.62)	2.78	0.46	0.61
	24	34.80 (29.07 – 41.17)	2.62	0.97	0.82
	36	27.79 (21.74 – 32.69)	2.63	1.21	2.79
	48	22.07 (3.92 – 30.13)	3.20	0.69	5.33
H. persicum	12	46.25 (39.72 – 54.32)	2.89	0.19	0.24
	24	36.96 (30.64 – 42.60)	2.99	0.32	0.72
	36	32.42 (27.05 – 36.81)	3.62	-0.43	3.40
	48	23.72 (16.43 – 28.58)	3.30	0.47	3.05

[a]95% lower and upper fiducial limits are shown in parenthesis [b]df = 3

Similar to our results, MAHMOUDVAND *et al*. (2011) studied the fumigant toxicity of essential oils extracted from *Rosmarinus officinalis* L., *Mentha pulegium* L., *Zataria multiflora*, and *Citrus sinensis* (L.) Osbeck var. *hamlin* on adults of *P. interpunctella* and discovered that *Z. multiflora* and *R. officinalis* had fumigant toxicity on *P. interpunctella* adults. RAFIEI-KARAHROODI *et al*. (2011) investigated insecticidal effect of six native medicinal plants essential oil on *P. interpunctella*. They results demonstrated strong toxicity of essential oils extracted from *Melissa officinalis* L., *Mentha piperata* L., *Petroselinum sativum* Hoffmann, *Lavandula angustifolia* Mill., *Ziziphora clinopodioides* Lam., and *A. dracunculus*, on first instar larvae and eggs of *P. interpunctella*. These findings are parallel with the results of present study for susceptibility of *P. interpunctella* to plant essential oils.

Previous studies have showed that, in general, the toxicity of plant essential oils against stored product pests is related to their major components (TAPONDJOU *et al*., 2002; SINGH *et al*., 2003). 1,8-cineol, camphor and borneol in the *A. millefolium* essential oil (HAZIRI *et al*., 2010), (Z)-anethole, methyl-eugenol, (Z)-β-ocimene and limonene in the *A. dracunculus* essential oil (AYOUGHI *et al*., 2011) and (E)-anethole, γ-terpinene and limonene in the *H. persicum* essential oil (FIRUZIA *et al*., 2010), were major components. LEE *et al*. (2001) showed 1,8-cineole (LC_{50} = 23.5 µl/l air) was the most toxic fumigant on *S. oryzae*, followed by limonene (LC_{50} = 61.5 µl/l air) and α-terpinene (LC_{50} = 71.2 µl/l air). CHANG & AHN (2002) studied fumigant activity of (E)-anethole *Blattella germanica*. (E)-Anethole caused 100% mortality at 0.398 mg cm-2 4 h after treatment. HUANG *et al*. (2002) indicated contact toxicity of eugenol, isoeugenol and methyleugenol on *Sitophilus zeamais* Motsch. and *Tribolium castaneum* (Herbst). PAPACHRISTOS *et al*. (2004) investigated relationship between the chemical composition of the essential oils from *Lavandula hybrida* Rev, *Rosmarinus officinalis* L and *Eucalyptus globulus* Labill and their insecticidal activity against *Acanthoscelides obtectus* Say. They found among the main constituents, only linalyl and terpinyl acetate were not active against

A. obtectus, while all the others (including teprinen-4-ol, camphor, 1,8-cineol, verbenone, p-cymene, S(-)limonene, R(+)limonene, γ-terpinene, α-terpineol) exhibited insecticidal activity against both male and female adults. OGENDO *et al.* (2008) introduced (Z)-β-ocimene as a toxic agent against adults of *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst), *Oryzaephilus surinamensis* (L.), *Rhyzopertha dominica* (F.) and *Callosobruchus chinensis* (L.). Moreover, Repellency of Bornyl acetate, Borneol, Linalool, p-Cymene and Camphene against *Myzus persicae* (Sulzer) (Aphididae) was proved (MASATOSHI, 1998). Therefore, the insecticidal activity of *A. dracunculus*, *A. millefolium* and *H. persicum* essential oils could be related to these constituents.

The essential oils investigated in this study are used as pharmaceuticals and in flavoring and are therefore considered less harmful to humans than most conventional insecticides and they can use as safe fumigants for controlling *P. interpunctella*. However, the possibility of employing this natural insecticides in the management of Indian meal moth is plausible, but is worthy of further investigation. Future research should focus on residues on target commodity and the influence of any residues on product acceptability.

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