INSECTICIDAL ACTIVITY OF ESSENTIAL OIL OF *AGASTACHE FOENICULUM* AGAINST *EPHESTIA KUEHNIELLA* AND *PLODIA INTERPUNCTELLA* (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT: In the present study, the essential oil isolated from aerial parts of *A. foeniculum* by Clevenger apparatus was analyzed by gas chromatography mass spectrometry. Methyl chavicol was main component of oil. Insecticidal activity of this essential oil was evaluated by fumigation method against *E. kuehniella* and *P. interpunctella*. Mortality was recorded daily for four days after treatment. *P. interpunctella* (LC_{50} = 16.535 µlL^{-1}) was more susceptible than *E. kuehniella* (LD_{50} = 23.075 µlL^{-1}) at 24 h after treatment. In general, mortality, increased as the doses of essential oil and exposure period increased. These results showed that the *A. foeniculum* oil could be used in grain storage in order to decrease detrimental effects and risk of utilization synthetic insecticides.

KEY WORDS: *Agastache foeniculum*, essential oil, fumigant toxicity, *Ephestia kuehniella, Plodia interpunctella.*

Current stored-product pest management has relied on the use of chemicals. However, chemical control methods using fumigants are restricted because of development of pest resistance, health hazards and risk of environmental contamination (Zettler & Cuperus, 1990; Tunc & Sahinkaya, 1998; Isman, 2000). Therefore, in the current scenario, there is an urgent need to develop safer, environmentally friendlier and efficient alternative that have potential to replace synthetic pesticides and are convenient to use. Essential oils are potential alternatives to current stored-grain fumigants because of their low toxicity to warm-blooded mammals and their high volatility (Shaaya et al., 1997; Li & Zou, 2001). Plant oils have insecticidal (Shaaya et al., 1997), antifungal (Kordali et al., 2008), nematicidal (Oka et al., 2000), virucidal (Schuhmacher et al., 2003), anti-bacterial (Kotan et al., 2008) effects.

Indian meal moth, *Plodia interpunctella* Hubner (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 et al., 2004). Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), is an important pest in stored products worldwide (Khebbeb et al., 2008).

The aim of this study was to analyze the chemical composition of essential oil extracted from aerial parts of blue giant hyssop, *Agastache foeniculum* (Pursh) Kuntze and to evaluate the insecticidal effect of this oil against *P. interpunctella* and *E. kuehniella*. It is hoped that this information will be useful in selection of plant-derived insecticides for control of insect pests.

MATERIALS AND METHODS

Aerial parts from 1.5 cm of top of *A. foeniculum* were collected at the flowering stage from plants grown in the experimental farm of the Department of Horticultural, University of Urmia, Urmia, Iran. The specimen plants were air dried in the shade at room temperature (26-28 ºC) for 14 days. The essential oil was isolated from dried plant samples by hydrodistillation method using a Clevenger apparatus for 4 h. The oil was dried over anhydrous Na_2so_4.

The constituents of *A. foeniculum* essential oil were analyzed by gas chromatography mass spectrometry (GCMS) (Thermo-UFM). The GC-MS conditions were as follows: capillary column ph-5 (10 m × 0.1 mm, film thickness 0.4 μm); helium as the carrier gas (0.5 $mlMin^{-1}$); oven temperature program, initially 60 °C rising to 285 °C; injector and detector temperatures of 280 °C. The identification of individual compounds was based on comparison of their relative retention index with those of authentic samples on capillary column.

The colony of *P. interpunctella* was reared on a diet of 80% ground rice, 10% glycerin, 5% yeast and 5% honey and *E. kuehniella* were reared on a diet of a 10:2:1 mixture of oat bran: wheat germ: yeast 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 ºC, 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.

To determine the fumigant toxicity of the *A. foeniculum* oil, filter papers (Whatman No. 1, cut into 3-cm diameter pieces) were impregnated with oil at doses calculated to give equivalent fumigant concentrations from 3 to 30 μlL^{-1} and 5 to 40 μlL^{-1} for *P. interpunctella* and *E. kuehniella*, respectively. Then the impregnated filter paper was attached to the under-surface of the screw cap of a glass jar (1 liter). No material was applied in control jars. The caps were screwed tightly on the jar containing fifteen moths of each species of insect separately. Each test was replicated three times. Mortality was recorded daily for four days after treatment. The insect will be considered as dead when they did not move, fly or response to gentle touch.

Percentage insect mortality was calculated using the Abbott correction formula for natural mortality in untreated controls (Abbott, 1925). To equalize variances, mortality percentages of insects were transformed using the square root of the arcsin. The experiments were arranged by completely randomize design. The data were analyzed with analysis of variance (ANOVA) by SAS. The means were separated by using the Tukey test, $\alpha = 0.05$. Probit analysis was used to estimate LC_{50} (Lethal concentration of 50% of population of insects) values with their fiducially limits by SPSS software.

RESULTS AND DISCUSION

Results of the GC-MS analysis for *A. foeniculum* essential oil are presented in Table 1. The four major constituents, in order of decreasing amounts, were methyl chavicol (94.003%) 1,8-cineole (3.334%), 1-octen-3-ol (0.461%) and germacrene D (0.430%) (Table 1).

The essential oil vapours of this plant species tested was toxic to both *P. interpunctella* and *E. kuehniella* pest species. According to the results of ANOVA, the effect of doses and exposure time interactions of the essential oil obtained from *A. foeniculum* on moths were significant at $P< 0.01$ (Table 2). There were significant differences in the mortality of *P. interpunctella* and *E. kuehniella* exposed to different concentrations of *A. foeniculum* oil for 24, 48, 72 and 96 hours (Fig 1).

The 24-h LC_{50} values against the moths were 16.535 and 23.075 μlL^{-1} for *P. interpunctella* and *E. kuehniella* respectively (Table 3). In general, mortality, increased as the doses of essential oil and exposure period increased. On the other hand, the LC_{50} decreased with the duration of exposure to the essential oil concentrations (Table 3 & Fig 1).

The essentials oil caused the highest mortality in 30 and 40 μlL^{-1} doses and at 96 h of exposure on *P. interpunctella* and *E. kuehniella* respectively. From the probit analyses, the calculated regression line equation of the fourth day data was $Y = 1.860X + 3.464$ for *P. interpunctella* and $Y = 2.328X + 2.191$ for *E. kuehniella*. In all of the times, *E. kuehniella* was noted to be relatively more tolerant than *P. interpunctella* (Table 3).

Many plant species produce various chemical compounds that could be repellent or deterrent or even toxic for plant feeding insects. Some of these compounds are also toxic to the plant itself, and therefore they are stored in special organs such as flowers and seeds. Phytophagous insects use plant volatiles to recognize their host plants. Therefore, the use of essential oils as a non-host volatile emission to repel insect pests is a viable alternative for control (Mauchline et al., 2005). Therefore, large quantities of plant material have to be processed in order to obtain essential oils in quantities sufficient for laboratorial and commercial scale tests.

Some constituents may exhibit a much higher activity than the whole essential oil. Mazza & Kiehn (1992) investigated characterization of essential oil of *A. foeniculum* and find out that methylchavicol was the major constituent this oil, which was confirmed in this study. Toxicity of methylchavicol was not detected against any insect pest of stored products, but 1,8-cineole (another major constituent of *A. foeniculum* oil) reported as a toxic agent against some of insect pests (Kordali et al., 2006; Stamopoulos et al., 2007).

The most promising botanical groups are Meliaceae, Rutaceae, Asteraceae, Annonaceae, Lamiaceae, Aristolochiaceae and Malvaceae (Regnault-Roger, 1997). *A. foeniculum* is perennial member in plants family Lamiaceae. The susceptibility of *P. interpunctella* and *E. kuehniella* against plant essential oils has been investigated to a limited extent (Tunc et al., 2000; Shojaaddini et al., 2008; Isikber et al., 2009) but toxicity of essential oil of *A. foeniculum* has not studied against these insects and others.

Conclusively, *A. foeniculum* essential oil showed potent fumigant toxicity against *P. interpunctella* and *E. kuehniella*. The essential oil of *A. foeniculum* could be used as an environmentally friend fumigant to control adults of moth pest on stored product.

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Table 1. Chemical components of the essential oil of *A. foeniculum*.

Component	Retention index	Percentage (%)
1-octen-3-ol	977	0.461
3-octanone	985	0.407
1,8-cineole	1058	3.334
octen-3-yl-acetete	1108	0.386
methyl chavicol	1200	94.003
α-copaene	1375	0.029
β-boarbonene	1386	0.084
E-caryophyllene	1418	0.058
germacrene D	1485	0.430
bicyclogermacrene	1500	0.020
spathulenol	1570	0.039
α-maurolol	1643	0.014
β-eudesmol	1650	0.015
dihydro eudesmol	1664	0.013
Total		99.293

Table 2. The results of ANOVA belonging to dose and time.

Source	*P. interpunctella*			*E. kuehniella*		
	df	Mean square	*F*	df	Mean square	*F*
Time (h)	3	642.380	61.040*	3	860.486	192.982*
Dose (μLl^{-1})	4	2980.138	283.179*	4	2607.301	584.741*
Time × Dose	12	59.644	5.669*	12	47.310	10.610*
Error	40	10.524		40	4.450	
Total	59			59		

*indicate significant difference at $P < 0.05$.

Table 3. LC_{50} calculated for mortality within 4 days of exposure of *p. interpunctella* and *E. kuehniella* on fumigation of essential oil of *A. foeniculum*.

Insects	Time [h]	LC50 (μlL^{-1})	95% Confidence Limits	χ^2 (df= 3)	Intercept [a]	Slope [b]
p. interpunctella	24	16.535	(12.545 – 24.079)	2.059 *	3.236	1.447
	48	12.343	(9.520 – 16.358)	4.685 *	3.286	1.570
	72	9.529	(2.596 – 22.727)	7.641 *	3.363	1.671
	96	6.690	(0.000 – 16.776)	12.448**	3.464	1.860
E. kuehniella	24	23.075	(17.188 – 35.658)	1.834 *	3.179	1.335
	48	16.358	(12.443 – 27.556)	4.772 *	3.161	1.514
	72	12.359	(3.804 – 23.148)	6.126 *	3.125	1.935
	96	8.60.56	(0.168 – 16.341)	9.273 **	3.190	1.935

*No heterogeneity factor is used in the calculation of confidence limits.
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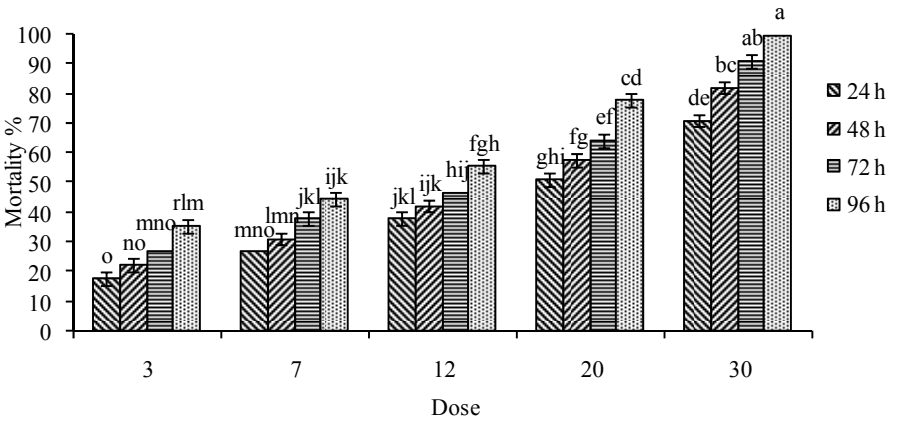
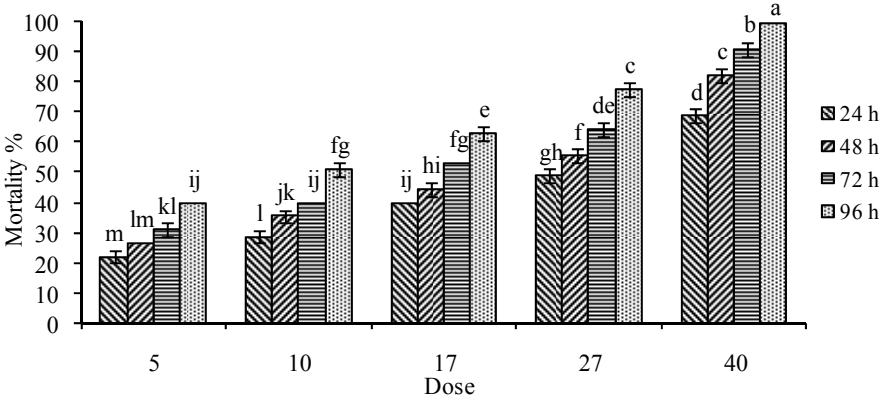


P. interpunctella



E. kuehniella

Figure1. Percent mortality ± SE of *P. interpunctella* and *E. kuehniella* exposed to different concentrations of *A. foeniculum* oil for 24, 48, 72 and 96 hours. Different letters in the top of columns are significantly different at 5% level.