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Chemical composition and insecticidal activity of essential oil from *Ziziphora clinopodioides* Lam. used against the Mediterranean flour moth, *Ephestia kuehniella* Zeller

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Abstract: The Mediterranean flour moth, *Ephestia kuehniella* Zeller, is a major pest of stored grain products, particularly flour. There have been major concerns over the application of conventional insecticides in stored products, which have strongly demonstrated the need for applying such alternative safe compounds as essential oils. The aim of the present study is to investigate the chemical composition and fumigant toxicity of essential oil from *Ziziphora clinopodioides* Lam. against the eggs, larvae, and adults of *E. kuehniella*. All toxicity tests were carried out under laboratory conditions set at 26±1°C and 70% relative humidity (RH). The results of gas chromatography--mass spectrometry (GC/MS) analysis indicated that the essential oil extracted from the leaves of *Z. clinopodioides*, is rich in pulegone (49.4%), piperitenone (10.7%), menthone (8.9%), and 1,8-cineol (6.9%). Based on the bioassay results, the LC_{50} value of the tested oil was estimated to be 54.61 μl · l^{-1} air for larvae and 1.39 μl · l^{-1} air for adults. Also, it is shown that increasing the oil concentration resulted in a significant increase in oviposition deterrency as well as a considerable reduction in the egg hatching percentage. These results suggest that *Z. clinopodioides* oil could be a potential candidate as a fumigant for managing *E. kuehniella* in stored products.

Key words: *Ephestia kuehniella*, *Ziziphora clinopodioides*, chemical composition, fumigant toxicity, oviposition deterrency, egg hatching

Introduction

The pyralid Mediterranean flour moth, *Ephestia kuehniella* Zeller, is distributed world-wide. This moth is a serious pest of stored grain products as well as flour and other milled products (Khebbeb *et al*. 2008). Many storage systems rely on the use of synthetic insecticides and fumigants like methyl bromide (MeBr) and phosphine (PH3) to control stored-product pests. These synthetic insecticides and fumigants are the most economical and convenient tools for managing these pests (Mueller 1990). Despite their satisfying results, the use of chemical fumigants is being restricted in many countries because of the fumigants' dangerous side effects such as contamination of stored products, the ecological consequences (Butler and Rodriguez 1996; MBTOC 1998), and an increased probability of insect resistance (Meaklim 1998; Haque *et al*. 2000). These problems have highlighted the need for developing new safe kinds of selective insect-control alternatives with fumigant action.

Plant materials, especially essential oils, have received special attention as alternative compounds to the currently used insecricides. The ability of the oils to control stored-product pests due to the low mammalian toxicity and sound environmental profile of the oils, is drawing a lot of attention to them (Isman 2000). In recent years, several experiments have been conducted to assess the efficacy of essential oils against stored-product insect species (Kim *et al*. 2013; Ziaee *et al*. 2014; Kim and Lee 2014; Nwachukwu and Asawalam 2014; Fatiha *et al*. 2014; Aref *et al*. 2015). For example, Karaborku *et al*. (2011) indicated that Marjoram (*Origanum majorana* L.) and Lemon (*Citrus limon* L.) oils were very effective against *E. kuehniella*. Also, Ghasemi *et al*. (2013) showed the fumigant toxicity of essential oils from *Callistemon viminalis* Gaertn. and *Ferula gummosa* L. on larvae of *E. kuehniella*.

Lamiaceae is a large plant family with many important species. In this family, *Ziziphora clinopodioides* Lam. with the Persian name "kakuti-e-kuhi", is a plant widely used in Iranian traditional medicine because of its antibacterial (Salehi *et al*. 2005; Sonboli *et al*. 2006; Murat and Pinar 2009), antifungal (Behravan *et al*. 2007), antioxidant (Meral *et al*. 2002; Salehi *et al*. 2005), and anti-inflammatory (Ghafari *et al*. 2006) properties. Also, the essential oil from *Z. clinopodioides* has been proved to possess insecticidal activity agains adults and eggs of one of the most destructive pests of stored products, *Callosobruchus maculatus* Fab. (Lolestani and Shayesteh 2009). Nevertheless, there has not yet been any report on the insecticidal activity of *Z. clinopodioides* oil against *E. kuehniella*.

We evaluated the chemical composition of the essential oil from *Z. clinopodioides* and the efficiency of the oil

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as a possible fumigant in the management of the Mediterranean flour moth.

Materials and Methods

Culturing insects

Eggs of *E. kuehniella* were provided by the Insectarium of Scientific and Industrial Research Organization of Iran. Three hundred of one-day-old eggs were reared in one plastic container (25 cm length, 15 cm width, and 10 cm height) half filled with a mixture of equal amounts of wheat flour and bran. Powdered yeast (5 g) was also added to each jar. The rearing conditions were 26±1°C and 75% relative humidity (RH) in darkness.

Plant materials

Aerial parts of *Z. clinopodioides* were collected from their natural habitats in Iran. The plant material was dried naturally at room temperature (23–25°C) for 5 days until crisp. The dried material was stored at 24°C until required.

Extraction and chemical analysis of the essential oil

Essential oil was extracted from the plant sample using a modified Clevenger-type apparatus (Negahban *et al.* 2007). The conditions of extraction were: 40 g of air-dried sample, 500 ml distilled water, and 3 h distillation. After extraction, anhydrous sodium sulfate was used to eliminate water. The extracted oil was placed in a sealed glass tubes and stored at 4°C for chemical analysis and bioassay tests.

Gas chromatographic (GC) analysis was performed using a Thermoquest-finnigan on a DB-1 fused-silica capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness). The temperature was programmed to rise to a 60°C and then to 250°C at 5°C · min^{-1}. Injector and detector temperatures were 250 and 280°C, respectively. The detector was a flame ionisation detector (FID) and nitrogen was used as a carrier gas at a flow rate of 1.1 ml · min^{-1}. Gas chromatography-mass spectrometry (GC/MS) was carried out using a Thermoquest-finnigan with the same characteristics as the one used in GC. The mass detector was Thermo-finnigan quadruple. Helium was used as a carrier gas at 1.1 ml · min^{-1}. The ionisation energy was 70 eV with a mass range of 35–465 amu.

The chemical constituents were identified by comparing their relative retention indices and mass spectra, with those published in the literature (Asawalam *et al*. 2006), and supplemented with NIST 1.7 and Wiley 7 GC/MS libraries. The relative proportion of the essential oil constituent was computed in each case from the GC/MS peak areas.

Fumigant toxicity bioassays

To determine the fumigant toxicity of *Z. clinopodioides* oil, 10 of two-day-old 5th instar *E. kuehniella* larvae were placed in one glass container of 280 ml volume. After preliminary dose-setting experiments, the final concentrations of the oil causing 5–95% mortality were obtained based on logarithmic distance (Robertson *et al*. 2007). The calculated concentrations of the oil were infused on the filter paper (Whatman No. 1, cut into 2 cm diameter pieces) and then were attached to the caps of the glass vials. Oils were applied as pure oils, and were applied using a micro-applicator. The caps of the vials were sealed tightly with Parafilm. The control larvae received no oil. The same experiment was designed to assess the fumigant toxicity of the tested oil against adult (0–24 h old) *E. kuehniella* insects.

In both assays, all treatments and controls were kept at 26±1°C and 70% RH in darkness. Each concentration and control was replicated three times. Mortality was determined after 24 h from the start of exposure. When no leg or antennal movements were observed, insects were considered dead. The percentage of insect mortality was calculated using the Abbott correction formula for natural mortality in the untreated controls (Abbott 1925).

Oviposition deterrence and egg hatching

In this experiment, two pairs (2 males and 2 females) of adult moths were selected for each concentration and for the control. Glass vials (250 ml), containing 5 g of mung bean grains, were treated with 1 ml concentrations of 500; 1,000; 2,000; 4,000, and 8,000 ppm *Z. clinopodioides* oil, as uniformly as possible. In the control, the food was treated only with a solvent (acetone). After evaporation of the acetone (~20 min), newly emerged *E. kuehniella* adults were transferred to the vials. Three replications were used for each concentration. The number of laid eggs per female was recorded after 96 h. Oviposition deterrence was calculated with the following formula (Pascual-Villalobos and Robledo 1998):

$$\%\text{Oviposition deterrence} = \left[1 - \frac{NE_t}{NE_c}\right] \times 100,$$

where NE_t – the number of eggs in a treatment, and NE_c – the number of eggs in the control.

Also, twenty eggs were selected from each replication and the effect of the essential oil on the egg hatching was recorded until all of the eggs in the control were hatched.

Statistical analysis

The LC values and 95% confidence limits were calculated from probit regressions using the POLO-PC computer program (LeOra Software). Data from oviposition deterrence and egg hatching assays were analysed using the SPSS programe version 16.0 for analysis of variance (ANOVA). Data obtained in percentages was subjected to the Arcsine $\sqrt{\frac{x}{100}}$ before ANOVA. The means were grouped using Tukey's test ($\alpha = 0.05$).

Results

Chemical constituents of *Ziziphora clinopodioides* oil

The yield of the essential oil from *Z. clinopodioides* was 2.3–2.5% (v/w based on dry weight). A total of 34 components from the essential oil of *Z. clinopodioides* were

identified, accounting for 94.48% of the total oil (Table 1). The major constituent in the oil was pulegone (49.4%), followed by piperitenone (10.7%), menthone (8.9%) and 1,8-cineol (6.9%), iso-menthone (3.9%), iso-pulegone (2.9%), and limonene (2.8%).

Toxicity tests

Data from the fumigant toxicity of *Z. clinopodioides* oil on *E. kuehniella* larvae and adults are presented in table 2. As is shown, LC_{50} of tested oil was determined to be 54.61 µl · · l^{-1} air for larvae and 1.39 µl · l^{-1} air for adults. According to LC_{50} values and estimated relative median potency (RMP), the adults were 42.17 times more sensitive than the larvae to *Z. clinopodioides* oil (Table 2).

Oviposition deterrence and egg hatching

The different concentrations of the *Z. clinopodioides* oil's oviposition deterrence activity on the females of *E. kuehniella* are shown in table 3. The results indicated that the number of eggs laid by the females decreased as concentrations of the oil were increased from 500 to 8,000 ppm. On the other hand, the oil's oviposition deterrency significantly increased with increased concentrations of the oil ($F = 295.26$; $df = 5$; $p = 0.000$). As is shown, treating female moths with concentrations of 2,000; 4,000, and 8,000 ppm of the oil caused a 51.11, 72.02, and 89.89% decline in the oviposition rate, respectively. Also, increasing the essential oil concentration resulted in a significant decrease in the egg hatching percentage ($F = 21.29$; $df = 5$; $p = 0.000$) (Table 3).

Table 1. Chemical constituents of the essential oil from *Ziziphora clinopodioides*

Compound	Retention index	%Composition
α-thujene	928	0.08
α-pinene	937	1.2
3-methylcyclohexanone	950	0.1
camphene	954	0.1
1-octen-3-ol	973	0.1
sabinene	976	1.0
β-pinene	983	1.5
β-myrcene	988	0.4
3-octanol	990	0.3
p-mentha-1(7)-dine	1008	0.1
p-cymene	1027	0.2
limonene	**1033**	**2.8**
1,8-cineol	**1037**	**6.9**
3-ethyl-2-hydroxy-2-cyclopenten-1-one	1053	0.1
trans-sabinene hydrate	1071	0.1
linalool	1100	0.1
3-octanol acetate	1119	0.1
cis-p-mentha-2,8-dien-1-ol	1142	0.1
menthone	**1162**	**8.9**
iso-menthone	**1172**	**3.9**
iso-pulegone	**1181**	**2.9**
2-(2-methylallyl) 2-cyclohexene-1-one	1217	0.2
coahuiolensol methyl ether	1234	0.4
pulegone	**1258**	**49.4**
piperitone	1265	1.9
iso-piperitenone	1279	0.2
lavandulyl acetate	1300	0.2
piperitenone	**1353**	**10.7**
piperitenone oxide	1372	0.1
α-copaene	1390	0.1
β-boubonene	1401	0.1
trans-geranyl acetone	1451	0.1
trans-β-lonone	1492	0.1
spathulenol	1596	0.1
Total identified		**94.48**

Bold values indicate the most abundant constituents of the oil

Table 2. Fumigant toxicity of *Ziziphora clinopodioides* oil against *Ephestia kuehniella*

Stage	n	LC_{50} values ($\mu l \cdot l^{-1}$ air)[a]	Slope±SE	χ^2 (df)	RMP (95% CL)[b]
Larvae	240	54.61 (35.07–83.47)	1.03±0.14	1.02 (5)	42.17 (5.99–85.71)
Adults	210	1.39 (1.15–1.66)	2.70±0.37	–	–

[a]LC values are expressed with their 95% confidence limits (CL)
[b]Relative Median Potency = LC_{50} of larvae divided by LC_{50} of adults

Table 3. Effect of *Ziziphora clinopodioides* oil on oviposition rate, percentage oviposition deterrence, and egg hatching of *Ephestia kuehniella,* after 96 h fumigation (the mean±SE)

Concentration [ppm]	Oviposition [number of eggs laid per female]	Oviposition deterrence [%]	Egg hatching [%]
0	330.33±17.53 f	0 f	95.12±2.88 c
500	296.00±7.57 e	10.30±2.29 e	90.05±5.02 c
1,000	236.00±9.84 d	28.48±2.98 d	81.66±4.40 bc
2,000	161.33±8.21 c	51.11±2.48 c	60.42±5.77 ab
4,000	92.33±4.66 b	72.02±1.41 b	35.12±2.88 a
8,000	33.33±5.36 a	89.89±1.62 a	25.21±2.88 a

Means followed by the different letters in each column indicate significant differences at $p < 0.05$, Tukey's test

Discussion

In the present study, we evaluated the insecticidal activity of the essential oil from *Z. clinopodioides* on the larvae and adults of the important *E. kuehniella*, stored-product pests. Our results demonstrated the fumigant toxicity of *Z. clinopodioides* oil to the studied stages of *E. kuehniella*, but the insecticidal activity varied with the developmental stages. Also, the tested oil showed outstanding oviposition deterrence and ovicidal activity agaist *E. kuehniella*.

Many essential oils extracted from different plant spices have already been screened for toxicity as potential insecticide agains *E. kuehniella*. The essential oil of *Satureja hortensis* L. showed fumigant toxicity against larvae of *E. kuehniella* (Maedeh *et al*. 2011). Essential oil derived from *Pistacia lentiscus* L. is known to have toxic effects on *E. kuehniella* (Bachrouch *et al*. 2010). The essential oil from oregano, *Origanum onites* L., and savory, *Satureja thymbra* L. has also been reported to have fumigant toxicity toward adults of *E. kuehniella* (Ayvaz *et al*. 2010). However, no report is available concerning the insecticidal property of *Z. clinopodioides* oil as a fumigant.

Essential oils are generally broad spectrum due to the presence of several active ingredients that may operate through various modes of action (Chiasson *et al*. 2004). The insecticidal activity of plant essential oils is reported to be mainly due to their content of monoterpenoids (Obeng-Ofori and Reichmuth 1999; Huang *et al*. 2002; Lee *et al*. 2002, 2003) with an indication of a neurotoxic mode of action (Isman *et al*. 2007), which is mainly due to inhibition of acetylcholinesterase activity (Abdelgaleil *et al*. 2009; Yeom *et al*. 2012). Pulegone, piperitenone, menthone, and 1,8-cineol are shown to be the main constituents of *Z. clinopodioides* oil which is in agreement with those reported by Ghafari *et al*. (2006), Sonboli *et al*. (2006), and Amiri (2009). Pulegone is about 50% of the total *Z. clinopodioides* oil and is the most powerful of the three insecticides naturally occurring in many mint species (Franzios *et al*. 1997). These volatile compounds, are more useful as insect fumigants against stored-product pests Numerous reports note the insecticidal activity of plant essential oils containing these chemical components against different species of pests (Ghasemi 2010, 2013; Hamzavi 2011).

In the present study, we observed that *E. kuehniella* adults (0–24 h old) were less tolerant than larvae (two-day-old fifth instar) to the oil. Similar results have been reported by Khodadoust and Moharramipour (2010), who showed that *E. kuehniella* adults were more susceptible than larvae instars to (*Cuminum cyminum* L.) and (*Carum copticum* C.B. Clarke) essential oils. In another experiment, Emamjomeh *et al*. (2014) indicated that essential oil of *Zataria multiflora* Boiss. was more toxic to *E. kuehniella* adults than to the larvae.

Conclusion

The essential oil investigated in this study is used as a pharmaceutical and in flavouring and is therefore considered less harmful to humans and non-target organisms than most conventional pesticides. It was shown that the essential oil from *Z. clinopodioides* was toxic to the eggs, larvae, and adults of *E. kuehniella* when applied as a fumigant in laboratory conditions. However, further semi-field and field evaluations are needed to confirm that this essential oil can be used as an effective safe insecticide in storage systems.

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