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Chemical composition and insecticidal activity of essential oil of *Zataria multiflora* Boiss. (Lamiaceae) against *Ephestia kuehniella* (Lepidoptera: Pyralidae)

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ABSTRACT

This work aims to investigate chemical composition and toxic potential of Zataria multiflora essential oil on Ephestia kuehniella (Lepidoptera,pyralidae).The essential oil of dried leaves and flowers was extracted using Clevenger-type apparatus. The essential oil was analyzed by GC/MS.The major compounds were Thymul (26.32%)and Carvacrol (25.51%). Toxicity of this essential oil against Ephestia kuehniella was evaluated by fumigation method. The appropriate concentrations of essential oil were tested against adults and larvae at 27±1˚C and 65±5% RH under dark conditions. Probit analysis showed that the LC_{50} values for adults were 0.98 µl/l air and for larvae was 20.67 µl/l air. Results showed that Zataria multiflora has a potential toxicity against Ephestia kuehniella and could be useful for integrated pest management of Ephestia kuehniella.

Key words: clevenger, fumigation method, GC/MS.

INTRODUCTION

The Mediterranean flour moth, *Ephestia kuehniella* is one of the major pests in industrial flour mills in temperate climates [3]. Larvae reduce product quality by their presence and by the production of frass and webbing , and they also cause direct damage by feeding [10]. Fumigation is an essential tool for control of insect pests in stored products. Fumigants are mostly used against stored products insect pests,not only because of their broad activity spectrum, but also because of their penetrating power resulting in minimal or no residues on the treated products. Although effective fumigants(e.g.methyl bromide and phosphine) are available ,there is global concern about their negative effects,such as ozone depletion ,environmental pollution ,toxicity to non-target organisms,pest resistance and pesticide residues [10, 7, 9, 6]. More recent showed that essential oils and their constituent may have potential as alternative compounds to currently used fumigants [8]. In this study fumigant effect of essential oil of *Z. multiflora* on larvae and adult of *E. kuehniella* was evaluated.

MATERIALS AND METHODS

Insects
The Mediterranean flour moth eggs, *E. kuehniella* was taken from Iranian Research Organization for Science and Technology and was reared on flour.

The culture were maintained in the dark conditions in growth chamber set as 27±1˚C and 65±5% RH.

Plant material
Aerial part and leaves of *Z. multiflora* was harvested at the flowering stage from Eghlid and was air-dried at room temperature (20-25˚C), for one week.

Essential oil extraction and chemical analysis
The essential oil was extracted by hydrodistillation of dried plant material(100gr of air-dried sample, 1:12 plant material /water volume ratio).

The essential oil was analyzed using an Agilent-Technologies 6890 N Network GC system equipped with a flame ionization detector and Hp-5MS capillary column(30m×0.25 mm, film thickness 0.25 µm; Agilent –Technologies, Little Falls, CA, USA).The injector and detector temperatures were set at 250˚C. Column temperatures was programmed from 60 to230˚C at a rate of 6˚C with the lower and upper temperatures being held for 3 min .

The ionization energy was 70evwith a scan time of 1s and mass range of 40-300amu.Unknown essential oil was identified by their GC retention time, expressed by koat́s index, which was calculated by the van den Dool and kratz equation using a hydrocarbon homologous series and by comparison of test compounds mass spectra with GC retention time of known compounds or those present in published spectra.

Fumigation bioassay
To assess fumigant toxicity of *Z. multiflora* essential oil,2 cm diameter filter papers (Whatman No.1) were impregnated with the different oil doses(0.4 , 0.6 , 0.92 , 1.36 , 2 µl/l air)for adult (0-24h old)and (13.5 , 17.88 , 23.51 , 31.08 , 40.54 µl/l air)for larvae(7-14 days old) then treated filter papers were placed on the underside of the screw cap of a glass vial (volume 250ml for adult and volume 37ml for larvae) and the whole system was sealed by parafilm. Each concentration and control had 10 insects and was replicated four times. Control insects were maintained under the same conditions with out any essential oil. Mortality was recorded after 24 hours. Percentage insect mortality was calculated using Abbot́s [1]. The LC_{50} value was calculated by probit analysis [6].

RESULTS

Results of the chemical analysis are shown in the table1. The total of 19 components were present in the essential oil and the major components were Thymol(26.32%), Carvacrol(25. 51%), PARA-cymene(8. 86%)and γ-terpinen (7.93%)(Table1).

Fumigant toxicity
The fumigant toxicity of essential oil against *E. kuehniella* larvae(7-14 days old) and adult (0-24h old) were showed in Table 2. Probit analysis showed that LC_{50} values for larvae and adults were 20.67 and 0.98 µl/l air respectively. *E. kuehniella* adults (0-24h old) were less tolerant than larvae(7-14 days old).

Table1.Chemical constituents of the essential oil extracted from *Zataria multiflora*

Compound	Retention Index	%Composition
Thymol	1306	26.32
Carvacrol	1319	25.51
PARA-cymene	1036	8.86
γ -Terpinene	1070	7.93
α -Pinene	944	4.63
β-Caryphyllene	1454	4.14
α-Terpinene	1027	2.70
Carvacrol acetate	1384	2.47
Thymol acetate	1366	2.16
Allo-Aromadendrene	1474	2.13
Linalool	1105	2.08
Carvacrol methyl ether	1254	1.87
Viridiflorence	1528	1.62
Myrcene	993	1.52
Thymol methyl ether	1243	1.47
Terpinene-4-ol	1195	1.43
β -Pinene	989	1.28
Spathulenol	1618	1.02
α -Thunjen	935	0.87

The curve in Figure1, 2 show mortality was increased with increase in the concentration of the essential oil .

Table2. Fumigant toxicity of *Zataria multiflora* essential oil against *E. kuehniella* larvae and adults

Insect stage	N	Intercept±SE	Slope±SE	Chi square($\%^2$)	df	P-value	LC_{50}^*(µl/l air)	Lower	Upper
larvae	240	-5±0.82	3.80±0.59	0.43	3	0.933	20.67	18.08	23.17
adult	240	0.01±0.09	2.26±0.39	2.62	3	0.454	0.98	0.81	1.21

**95% lower and upper confidence intervals are shown in parenthesis*

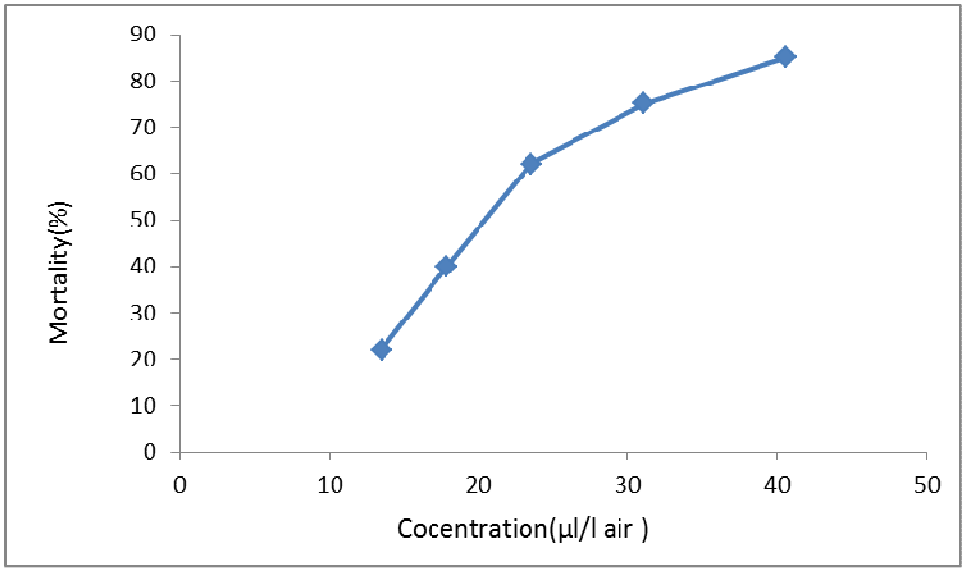

Figure 1. Fumigant activity of *Zataria multiflora* essential oil against *E. kuehniella* larvae

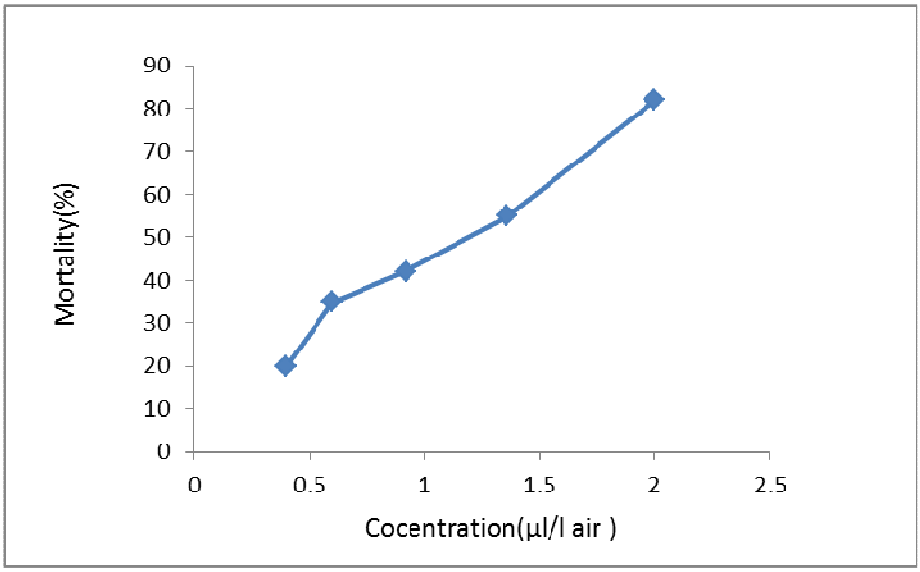

Figure 2. Fumigant activity of *Zataria multiflora* essential oil against *E. kuehniella* adults

DISCUSSION

Our study has shown the chemical composition of *Zataria multiflora* essential oil. The main components of *Zataria multiflora* oil were Thymol(26.32%), Carvacrol (25. 51%), PARA-cymene(8. 86%)and γ-terpinen (7.93%). Rastegar et al. [15] obtained similar result by evaluating essential oil compounds from *Z. multiflora* and major compounds were Thymol(30.72%), Carvacrol(29.95%), PARA-cymene (11.38%)and γ-terpinen (8.86%). Plant extracts contain compounds that show ovicidal, repellent, antifeedant, sterilization and toxic effects on insects [8, 4].

It is clear from our result that *Z. multiflora* essential oil was toxic to *E. kuehniella* larvae and adults . On the basis of the LC_{50} values and the same experimental conditions for adults, as compared with the result reported by Median-Ben Jemaa et al . [14], *Z. multiflora* demonstrate a higher toxicity in compare with *Laurus nobilis* .Our study has shown that *E. kuehniella* adults (0-24h old) were less tolerant than larvae(7-14 days old). Similar results by Khodadoust and Moharramipour, [11], showed that *E. kuehniella* adults were less tolerant than larvae instars towards (*Cuminum cyminum*) and (*Carum copticum*) essential oils. Previous studies have shown the toxicity of essential oils from various aromatic plants against the Mediterranean flour moth. Findings of this study showed that essential oil of *Z. multiflora* have potent fumigant toxicity against larvae and adults of *E. kuehniella* . On the basis of the LC50 values and the same experimental conditions for adults, as compared with the result reported by Akrami et al. [3], *Z. multiflora* demonstrate a lower toxicity in compare with *Thymus kostchyanus* and *Mentha longiflodia*.

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