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Chemical composition and insecticidal activity of essential oil of *Zataria multiflora* Boiss. (Lamiaceae) against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)

Fahimeh Rastegar[1,3]*, Saeid Moharramipour[2], Mahmood Shojai[1], Habib Abbasipour[3]
[1]Department of Entomology, Science and Research Branch, Islamic Azad University, Tehran, Iran; [2]Department of Entomology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran; [3]Department of Plant Protection, Faculty of Agricultural Sciences, Shahed University, Tehran, Iran
* *Corresponding Author e-mail: Rastegar_Fa@Yahoo.com*

Abstract: In recent years essential oils of medicinal plants have received much attention as pest control chemical agents. The discovery of active compounds that are less persistent will be beneficial for both the environment and agricultural product consumers. *Zataria multiflora* is a plant belonging to the Iran, Pakistan and Afghanistan. The essential oil of dried leaves and flowers were obtained by Clevenger-type apparatus. The composition of the essential oil was analyzed by GC- MS. Sixteen compounds representing 95.91% of total oil were identified. The major components in the oil were Thymol (30.72%) and Carvacrol (29.95%). Fumigant toxicity of essential oil from *Z. multiflora* was tested against adults, different ages of larvae and eggs. The experiments were carried out at 25 ± 1ºC and 65 ± 5% RH under dark condition. The essential oil showed strong adulticidal, larvicidal and ovicidal activity. Probit analysis showed that the LC_{50} values for adults were 8.81µl/l air, for 1, 7 and 14 days larvae were 8.47, 10.37, 13.36µl/l air and for 1, 3 and 6 days eggs were 4.55, 3.63, 3.01µl/l air, respectively. On the basis of these LC_{50} values, eggs were much more susceptible than adults and larvae. The essential oil *Z. multiflora* may be suitable as fumigants because of its high volatility and safety.

Key words: *Zataria multiflora*, essential oil, insecticidal activity, *Callosobruchus maculatus*

Introduction

During the last years natural products with insecticidal activity and low mammalian toxicity have been put forward as protective agents for stored staple crops (Shaaya et al., 1997; Elhag, 2000; Boeke et al., 2004). Plant essential oils in general have been recognized as an important natural resource of insecticides (Gbolade et al., 2000; Adebayo et al., 1999). They have the potential of being acute ovicidal, fumigant, insect growth regulator and insecticidal against various insect species (Tsao et al., 1995) and concurrently developed as ecologically sensitive pesticides (Isman, 2000). Prakash and Rao (1997) described 866 plant species that produce chemicals useful against insects and listed their 256 biologically active chemical components. Among many essential oils, these from plants within the Lamiaceae family have received considerable attention in the search for biologically active natural products against agricultural as well as stored products pests (e.g., Mansour et al., 1986; Dube et al., 1989; Shaaya et al., 1991; Tunc and Sahinkaya, 1998; Isman, 2000; Lee et al., 2001). *Zataria multiflora* Boiss. is a plant belonging to the labiata family that geogeraphically grows only in Iran, Pakistan and Afghanistan. This plant with the vernacular name of Avishan Shirazi (in Iran) has several traditional uses such as antiseptic, anesthetic and antispasmodic (Zargari, 1990). Stored products of agricultural and animal origin are attacked by more than 600 species of beetle pests, 70 species of moths and about 355 species of mites causing quantitative and qualitative losses (Rajendran, 2002). Among stored-product pests species of the genus *Callosobruchus* (Coleoptera: Bruchidae) seriously damage beans or legume seeds, especially in warm parts of the old world from which it originates (Borowiec, 1987; Udayagiri and Wadhi, 1989; Singal and Pajni, 1990). One of the most important insect pests of the cowpea [*Vigna unguiculuta* (L.) Walp] is the bruchid beetle *Callosobruchus maculatus* (F.) that attacks the seeds during storage, severely affecting the quality and storability of the produce (Hall et al., 1997). Previous studies have assessed fumigant activity of essential oils on adults and larvae and recently authors have described the contact and fumigant toxicity of essential oils or their major components against eggs of stored-product insect (Shaaya et al., 1993; Ho et al., 1997; Huang et al., 1997, 2000; Tune et al., 2000). In this study fumigant effect of essential oil of *Z. multiflora* on the eggs, larvae and adults of *C. maculatus* was evaluated.

Material and methods

Insect cultures
Some samples of adults *C. maculatus* was taken from Nuclear Research Center of Medicine and Agriculture. *C. maculatus* was reared on mung bean seeds. The culture were maintained in the dark condition in growth chamber set as 27 ± 2°C and 65 ± 5% RH.

Plant materials
Aerial parts of *Z. multiflora* at the flowering stage were collected around Noor Abad in the Shiraz region and shade dried on laboratory benches at room temperature (23-27°C) for 5 days before extraction.

Extraction of essential oil
The essential oil of *Z. multiflora* was extracted by hydrodistillation using a modified Clevenger-type apparatus. Conditions of extraction were: 50g of air-dried sample, 1:12 plant material/water volume ratio and 4h distillation. Oil yield (1.6% w/w) was calculated on the basis of dry weight. The obtained oil was dried over anhydrous sodium sulphate and stored in the refrigerator at + 4°C until used.

Analysis of the essential oil
Gas chromatographic analysis was performed with a Shimadzu GC-9A with helium as a carrier gas with a linear velocity of 30cm/s on DB-5 Column (30m ×0.25 mm i.d, 0.25µm film thickness). The oven was programmed to rise 60°C (3min) isotherm, and then to 210$^{°C}$ at a rate of 3°C/min. Injector and detector temperatures were 300 and 270°C, respectively. The GC mass analysis was carried out on a Varian 3400 equipped with a DB-5 column with the same characteristics as the one used in GC. The transfer line temperature was 260°C. The ionization energy was 70ev with a scan time of 1s and mass range of 40-300amu. Unknown essential oil was identified by comparing its GC retention time to that of known compounds and by comparison of its mass spectra, either with known compounds or published spectra.

Fumigant activity of C. maculatus
Adults: In this experiment the fumigant toxicity of *Z. multiflora* was tested on 1-7 days old adults, different concentrations (0.714, 1.785, 2.5, 3.571, 10.714, 17.857, 25, 35.714 and 53.571µl/l air) of essential oil were applied on a filter papers (2cm diameter), then treated filter papers were placed under the surface of the screw cap of a glass vial (Volume 280ml).

Each concentration and control was replicated five times. Control insects were without using essential oil. Number of dead and live insects in each vial was counted 24h after initial exposure to the essential oil. The dead insects were monitored for at least 48h after recording the data and no affected insects recovered. Mortality of control was evaluated by using Abbott's formula (Abbott, 1925). The LC_{50} and LC_{95} values were calculated by probit analysis (Finney, 1971).

Larvae and eggs**:** Another experiment was designed to assess 50% lethal dose for larvae and eggs. Fifty pair of newly emerged beetles (i.e., a male and female, sexed as adults: Bandara and Saxena, 1995) was placed on 150g on infected mung bean and after 24h, the insects were removed. Only one egg was left on each mung bean seed and others were deleted. Ten eggs (1, 3 and 6 days old) and larvae (1, 7 and 14 days old) were placed into the vials (70ml volume) and the same procedure for adult experiment, was followed. Different concentrations (0.714 to 28.571µl/l air) of *Z. multiflora* oil were tested for 24h on the basis of Wang et al. (2006) method after 24h, the eggs and larvae were transferred to clean vials with culture media and kept in the incubators.

Results

Chemical constituents of *Z. multiflora* results of the component analyses by GC-MS are summarized in Table 1. Sixteen constituents were identified, accounting for the 95.91% of the oil. The major components were Thymol (30.72%), Carvacrol (29.95%), PARA-cymene (11.38%) and γ-terpinen (8.86%).

Table 1. Chemical composition of the essential oil from *Zataria multiflora*.

Compound	Retention Index	%Composition
Thymol	1290	30.72
Carvacrol	1299	29.95
α-phellendrene	1005	0.74
α-pinene	939	3.61
β-pinene	979	0.36
Myrcene	991	1.12
α-terpinolen	1089	2.23
PARA-Cymene	1025	11.38
γ-terpinene	1060	8.86
Thymol acetate	1352	2.02
β-Caryphyllene	1425	3.08
t(-)Arom adendrene	1441	0.77
α-humulene	1455	0.23
Myrtenal	1196	0.23
Linalyl propionate	1184	0.15
α-copaene	1377	0.46
Other Compound		4.54

Fumigant toxicity

Adults: *Z. multiflora* oil had strong fumigant activity against *C. maculatus* adults. The mortality increased with rising concentration from 0.714 to 35.714µl/l air and with exposure 24h. Table 2 shows the LC_{50} and LC_{95} values for essential oil against adults. The LC_{50} value calculated was 8.81µl/l air.

Table 2. Fumigant toxicity of *Zataria multiflora* essential oil against *C. maculatus* eggs, larvae and adults.

Insect stages	Old (day)	LC_{50}* (µl/l air)	LC_{95}* (µl/l air)	Slope±SE	df	Chi square ($\%^2$)	P-value
Eggs	1	4.55 (3.08-8.71)	32.38 (13.69-41.35)	1.93 ± 0.38	4	8.60	0.071
	3	3.63 (3.05-4.38)	22.56 (14.97-43.42)	2.07 ± 0.25	4	4.11	0.390
	6	3.01 (2.51-3.60)	19.35 (13.11-35.66)	2.03 ± 0.24	4	4.02	0.402
Larvae	1	8.42 (7.24-9.71)	38.11 (28.03-61.77)	2.50 ± 0.30	4	4.91	0.296
	7	10.37 (8.86-12.30)	56.20 (37.76-109.23)	2.24 ± 0.30	4	4.0	0.404
	14	13.36 (11.45-15.88)	80.06 (53.80-150.26)	2.11 ± 0.25	5	4.34	0.800
Adults	1-7	8.81 (4.83-16.46)	220.59(74.79-2725)	1.18 ± 0.11	7	10.58	0.158

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

Larvae and eggs: The fumigant toxicity of essential oil against *C. maculatus* eggs (1, 3 and 6 days old) and larvae (1, 7 and 14 days old) were showed in Table 2. Data probit analysis showed that LC_{50} values for 1, 3 and 6 days eggs were 4.55, 6.63 and 3.01µl/l air, respectively. These results indicated that 6-day eggs were more susceptible than 1 and 3 days old eggs. At the highest tested concentration 7.142µl/l air, on 1, 3 and 6 days old eggs had 76, 82 and 86% mortality, respectively (Fig .1).

Based on this tested essential oil, the 14-days old were the most tolerant (Table 2). The curve in Figure 2 shows mortality was increased with increase in the concentration of essential oil.

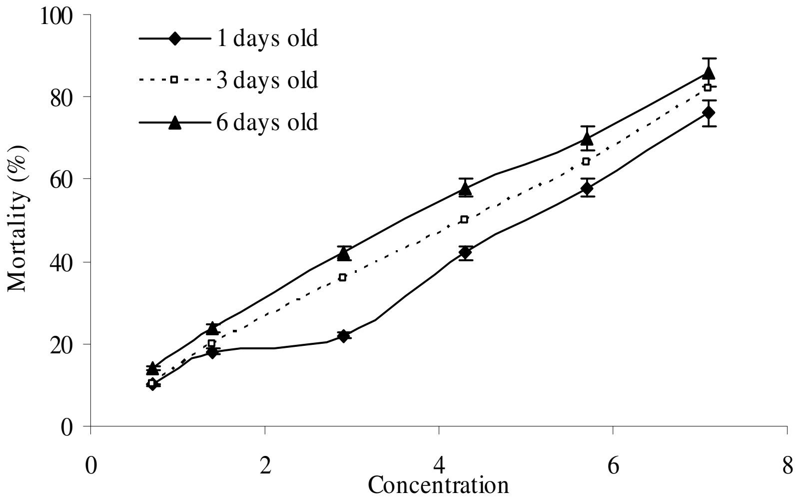

Figure 1. Fumigant activity of *Zataria multiflora* essential oil to *Callosobruchus maculatus* eggs.

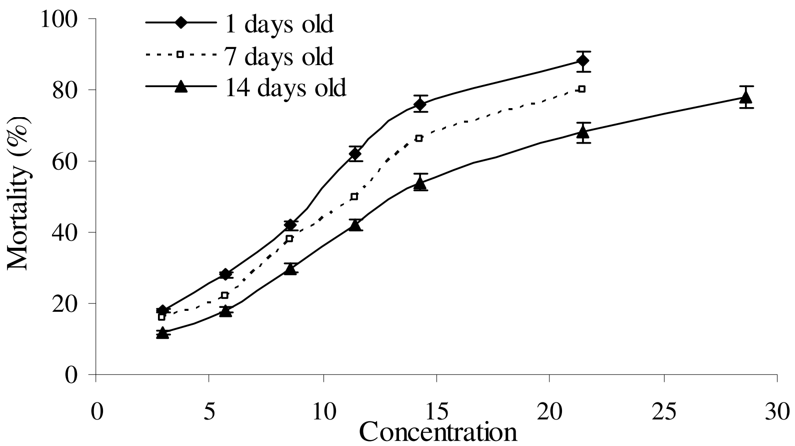

Figure 2. Fumigant activity of *Zataria multiflora* essential oil *Callosobruchus maculatus* larvae.

Discussion

Zataria multiflora is a thyme-like plant and a member of Labiata family that grows wild in central and southern Iran (Amin, 1991). The major components of the oil from *Z. multiflora* were Thymol (30.72%), Carvacrol (29.95%), PARA-cymene (11.38%) and γ-terpinen (8.86%). Sharififar et al. (2007) obtained similar results by evaluating essential oil compounds from *Z. multiflora* and the major compounds were Thymol (37.59%), Carvacrol (33.65%), P-cymene (7.72%) and γ-terpinen (3.88%). Many plant extracts are known to have ovicidal, repellent, antifeedant and insecticidal activities against various insect species (Arnason et al., 1989; Hedin et al., 1997). Additionally, some plant extracts or their constituents can be highly effective against insecticide-resistant insect pests (Schmutterer, 1992; Ahn et al., 1997). For example, Arabi et al. (2008) showed that essential oil of

Perovskia abrotanoides (Lamiaceae) had strong fumigant activity against *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) adults. Furthermore 1, 8-cineole, borneol, thymol were highly more effective against *S. oryzae*, while for *Rhyzopertha dominica*, camphor and linalool were more effective (Rozman et al., 2007). Findings of this study showed that essential oil of *Z. multiflora* have potent fumigant toxicity against eggs, larvae and adults of *C. maculatus*. On the basis of the LC_{50} values and the same experimental conditions for adults, as compared with the result reported by Negahban *et al.* (2006), *Z. multiflora* demonstrate a lower toxicity in compare with *Artemisia sieberi* Besser. Also toxicity of studied plant on the adults of *C. maculatus* was less than *Cymbopogon schoenanthus* L. (Ketoh et al., 2005), *Carum copticum* and *Vitex pseudo-negundo* (Sahaf and Moharramipour, 2004). In addition, in compare with Sahaf (2008) study, our results showed the effect of *Z. multiflora* essential oil on 1 day old eggs and 1 day old larvae was weaker.

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