CHEMICAl COMPOSITION AND FUMIGANT TOXICITY OF ESSENTIAL OIL FROM *THYMUS CARMANICUS* AGAINST TWO STORED PRODUCT BEETLES

Azadeh Jarrahi*, Saeid Moharramipour and Sohrab Imani***

* Department of Entomology, Faculty of Agriculture, Science and Research Branch, Islamic Azad University, Tehran, IRAN. E- mail: jarrahiazadeh@yahoo.com
** Department of Entomology, Faculty of Agriculture, Tarbiat Modares University, Tehran, IRAN. E- mail: moharami@modares.ac.ir

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ABSTRACT: Chemical composition of the essential oil from *Thymus carmanicus* Jalas, and its fumigant activity was investigated against two stored product insects, *Callosobruchus maculatus* (F.) and *Sitophilus granarius* (L.). Essential oil was isolated via hydrodistillation from dry leaves of *T. carmanicus* using a modified Clevenger-type apparatus and the chemical composition of the oil was assessed via GC and GC-MS. Twenty one compounds (100% of the total composition) were identified. Carvacrol (41.14%), *p*-cymene (12.09%), Thymol (6.35%) and γ-terpinene (6.21%) were found to be the major compounds of the essential oil. The fumigant toxicity of the essential oil was tested against 1-3 day-old adults of *C. maculatus* and *S. granarius* at 27±1°C and 65±5% r.h. in darkness. The mortality of adults was tested at different concentrations (28.12, 40.62, 53.12 and 65.62 µl/l air) and different exposure times. The highest concentration (65.62 µl/l air), caused 84% and 56% mortalities with a 5 h exposure On *C. maculatus* and *S. granarius*, respsctively. Based on LC_{50} values, *C. maculatus* (9.28 µl/l air) was significantly more susceptible than *S. granarius* (12.71 µl/l air). These results showed that the essential oil of *T. carmanicus* can play an important role in stored-product protection.

KEY WORDS: Fumigant toxicity, Essential oil, *Thymus carmanicus*, *Callosobruchus maculatus*, *Sitophilus granarius*.

Stored products are attacked by many species of pests (Rajendran, 2002). Hence, there is a need to develop new types of selective insecticides with fumigant action. Nowadays, management of stored product pests, using substances of natural origion, is the subject of many studies (Isman, 2006). Most of the essential oils have been tested against stored product pests, in order to find new control substances with lower mammalian toxicity, low persistence in the environment and potential for commercial application (Liu et al., 2005). *C. maculates* causes extensive loss, especially to cowpea in storages (Hu et al., 2008). The granary weevil, *S. granarius* is an important pest of stored cereals (Rees, 1996). Larva of *S. granarius* consume approximately half of a wheat kernel (Hurlock, 1965).

Labiatae family is best known for their essential oils common to many members of the family. (Naghibi et al., 2005). The genus *Thymus* L. (Labiatae) is represented in Iranian flora by 14 species, four of which including *T. carmanicus* are endemic in Iran. (Rechinger, 1982; Nickavar et al., 2005). In Iranian folk medicine leaves of this plant is used in treatment of Rheumatism, skin disorders and as an antibacterial agent (Zargari, 1990). Recently, several studies have assessed the ability of the *Thymus* essential oils as fumigants against a number of pests. The insecticidal activity of *Thymus persicus* (Ronniger ex Rech. f.) has been reported against *Tribolium castaneum* (Herbst) and *Sitophilus oryzae* (L.) (Taghizadeh et al., 2010). *Thymus numidicus* (Poiret) has contact toxicity against

Rhizopertha dominica (F.) (Saidj et al., 2008). The insecticidal and fumigant activities of *Thymus vulgaris* L. have been reported against *S. oryzae* (Lee et al., 2001), *Plodia interpunctella* (Hübner) (Passino et al., 2004), *T. castaneum* (Clemente et al., 2003), *Lasioderma serricorne* (F.) (Hori, 2003), *Spodoptera litura* (F.) (Hummelbrunner & Isman, 2001). Moreover *Thymus mandschuricus* Ronniger has insecticidal activity against *S. oryzae* (kim et al., 2003). However, There are no reports in the insecticidal bioactivity of *T. carmanicus*. Therefore the present study was carried out to determine the fumigant toxicity of the essential oil of *T. carmanicus* against *C. maculatus* and *S. granarius*.

MATERIALS AND METHODS

Insect cultures

Callosobruchus maculatus was reared in 1-liter jars containing cowpea seeds. *S. granarius* was reared in a 0.5-liter jars containing whole kernels of wheat, which were covered by a fine mesh cloth for ventilation. The cultures were maintained in the dark in a growth chamber set at 27±1°C and 65±5% r.h. Adult insects, 1-3 day-old, were used for the fumigant toxicity test. All experimental procedures were carried out under the same environmental conditions as the cultures.

Plant materials

Aerial parts of *T. carmanicus* were collected at full flowering stage in may 2009 from the farm of Shahid Fozveh Research station in Isfahan Center for Research of Agricultural Science and Natural Resources. The plant material was dried naturally on laboratory benches at room temprature (23-24°C) for 5 days. The dried material was stored at -24°C until needed and then hydrodistilled to extract its essential oil.

Fumigant toxicity bioassay

To determine the fumigant toxicity of the *T. carmanicus* oil and the median effective time to cause mortality in 50% of test insects (LT_{50} values), filter papers (Whatman No.1, cut into 6 cm diameter pieces) were impregnated with an appropriate concentration (28.12 to 65.62 µl/l air) of the oil without using any solvent. The impregnated filter paper was then attached to the undersurface of the screw cap of a 320 ml glass vial. The caps were screwed tightly onto the vials, each of which contained ten adults of each species of insect separately. The combination of each concentration and exposure time (1-10 h) was replicated five times. When no leg or antennal movements were observed, insects were considered dead. The data was computed using SPSS 16.0 software package. The estimates were compared using overlap of the 95% fiducial limits. Non-overlap at the 95% fiducial limits is equivalent to a test for significant differences.

Another experiment was designed to assess 50% and 95% lethal doses. Ten adult insects of *C. maculatus* were put into 90 ml glass bottles, Whereas *S. granarius* adults were held in 60 ml glass bottles. *C. maculatus* adults were exposed to the essential oil at the doses of 3.33, 4.44, 6.66, 10, 15.55 and 22.22 µl/l air and *S. granarius* at 5, 6.66, 10, 13.33, 18.33, 25 and 33.33 µl/l air. Control insects were kept under the same conditions without any essential oil. Each dose was replicated five times. The insects were exposed for 24 h to the essential oil vapor and after 24 h the dead insects were counted. Probit analysis (Finney, 1971) was used to estimate LC_{50} and LC_{95} values with their fiducial limits by SPSS. 16.0.

Samples for which the 95% fiducial limits did not overlap were considered to be significantly different.

Extraction and analysis of essential oil

Dried leaves and flowers were subjected to hydrodistillation using a modified Clevenger- type apparatus in order to obtain essential oil. Condition of extraction was: 50 g of leaves and flowers; 600 ml distilled water and 3 h distillation. Anhydrous sodium sulfate was used to remove water after extraction. *T. carmanicus* oil yield was 1.8±0.09 % v/w, as calculated on a dry weight basis . Extracted oil was stored in a refrigerator at 4°C. GC analysis was carried out on a HP-6890 gas chromatograph equipped with a HP-5 MS (non-polar) capillary column (30m×0.32mm; 0.25μm film tickness). The oven temperature was held at 60°C for 3 min then programmed at 6°C/min to 220°C. Other operating conditions were as follows: carrier gas He, at a flow rate of 1 ml/min; injector temperature 250°C, Mass system, the operating conditions was as the same as described above. Mass spectra were taken at 60 eV. Quantitative data were abtained by comparison of their mass spectra and retention indices with those published in the literature (Adams, 1995) and presented in the MS computer library.

RESULTS

Fumigant toxicity

In all cases, considerable differences in mortality of insects to essential oil vapor were observed with different concentrations and times. From the graph in Fig.1 it can be seen that, *T. carmanicus* oil was relatively more toxic to *C. maculatus* than to *S. granarius*. The lowest concentration (28.12 μl/l) of the oil yielded 84% mortality of *C. maculatus* after a 7.5 h exposure but mortalities of *S. granarius* at the lowest concentration were 60% after 7.5 h. At 40.62 μl/l air *T. carmanicus* oil against *C. maculatus* caused about 50% mortality with a 5 h exposure and 80% mortality after 6 h. At this concentration 80% mortality was achieved after 8 h for *S. granarius*. The oil at 53.12 μl/l air caused 85% morality for *C. maculatus* and *S. granarius* with 5.5 and 7.3 h exposure, respectively. At the highest concentration (65.62 μl/l air) kills of *C. maculatus* reached 84% with a 5 h exposure. By contrast only about 56% mortality was achieved for *S. granarius* at the same time exposure. Probit analysis showed that *C. maculatus* was more susceptible (LC_{50}=9.28 μl/l air) to *T. carmanicus* oil than *S. granarius* (LC_{50}=12.71 μl/l air). The corresponding LC_{90} were 18.79 and 24.92 μl/l air, respectively (Table 1).

Chemical constituents of essential oil

The chemical constituents of the essential oil of *T. carmanicus*, the retention indices and the percentage of the individual components are summarized in Table 2. Twenty-one components were identified. Carvacrol (41.14%) and *p*-cymene (12.09%) were the major constituents of the oil.

DISCUSSION

The potential for pest control or crop protection using *T. carmanicus* essential oil has not been investigated previously. Shaaya et al. (1997) reported that essential oil extracted from Labiatae family has shown fumigant toxicity against stored-product insects. Carvacrol, is the major component in *T. carmanicus* essential oil. Carvacrol has fumigant toxicity against agricultural and stored-product insects (Ahn et al., 1998 ; Isman, 2000). Carvacrol is very effective in inhibiting *Acanthoscelides obtectus* (say) reproduction (Regnault-Roger & Hamraoui, 1995). This compound is also effective against *Oryzaephilus surinamensis* (L.) (Shaaya et al., 1990). *p*- cymene (12.09%) is the second main component of *T. carmanicus* oil that has insecticidal activity. *p*- cymene had fumigant toxicity on *A. obtectus* (Regnault-Roger & Hamraoui, 1995). Therefore, the toxicity of this oil could be attributed to these chemicals. As major compounds of *T. carmanicus* are montoterpenoids, they are typically volatile and rather lipophilic compounds that can penetrate into insects rapidly (Lee et al., 2002). In our study, *T. carmanicus* was characterized by a rapid knockdown effect, hyperactivity, convulsion and paralysis and death. These effects show that this essential oil could resemble traditional fumigants. Results of this study and other studies indicate that some plant essential oils might be useful for insect control in enclosed spaces because of their fumigant action (Iamsn , 2000). In traditional medicine, leaves and flowering parts of *Thymus* species are used as tonic and herbal tea, antiseptic and carminative as well as treating colds (Amin, 2005), so it had fewer risk to human healthy and can be less harmful than insecticides. There is a need to conduct further studies on the essential oil against other stored-product pests, like *R. dominica* and against all life stages of the insects, particularly in the presence of the commodity load to establish its efficacy as a fumigant. There is a global interest by agro-chemical companies in developing plant-based pesticides. However, further studies are necessary to develop formulations to improve their efficacy and stability.

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Table 1. LC_{50} values of *Thymus carmanicus* essential oil to *Callosobruchus maculatus* and *Sitophilus granaries*.

Insect species	LC_{50} (µl/l air)	LC_{90} (µl/l air)	Slope ± SE	Degree of freedom	Chi-square (χ^2)	*P*-Value
C. maculatus	9.28 (8.40 – 10.28)*	18.79 (16.21 – 22.91)	4.18 ± 0.38	4	6.01	0.198
S. granarius	12.71 (11.63 – 13.87)	24.92 (22.0 – 29.34)	4.38 ± 0.37	5	4.25	0.513

*95% lower and upper fiducial limits are shown in parenthesis.

Table 2. Chemical constituents of the essential oil from *Thymus carmanicus*.

Compounds	Retention Index	% Composition
α-phellandrene	1092	1.10
α-pinene	1101	1.26
Camphene	1118	1.84
3-octanone	1144	1.77
Myrcene	1149	0.90
α-terpinene	1180	1.48
p-cymene	1187	12.09
1,8-cineole	1196	2.48
γ-terpinene	1221	6.21
trans-sabinene hydrate	1232	1.53
Borneol L	1339	9.65
Terpinene-4-ol	1346	1.09
Carvacrol methyl ether	1402	1.83
Cyclohexasiloxane, dodecamethyl	1414	0.98
Thymol	1445	6.35
Carvacrol	1460	41.14
β-pinene	1509	0.85
β-caryophyllene	1599	0.82
β-bisabolene	1668	0.95
Naphtalene	1788	0.87
α-cadinol	1811	4.83

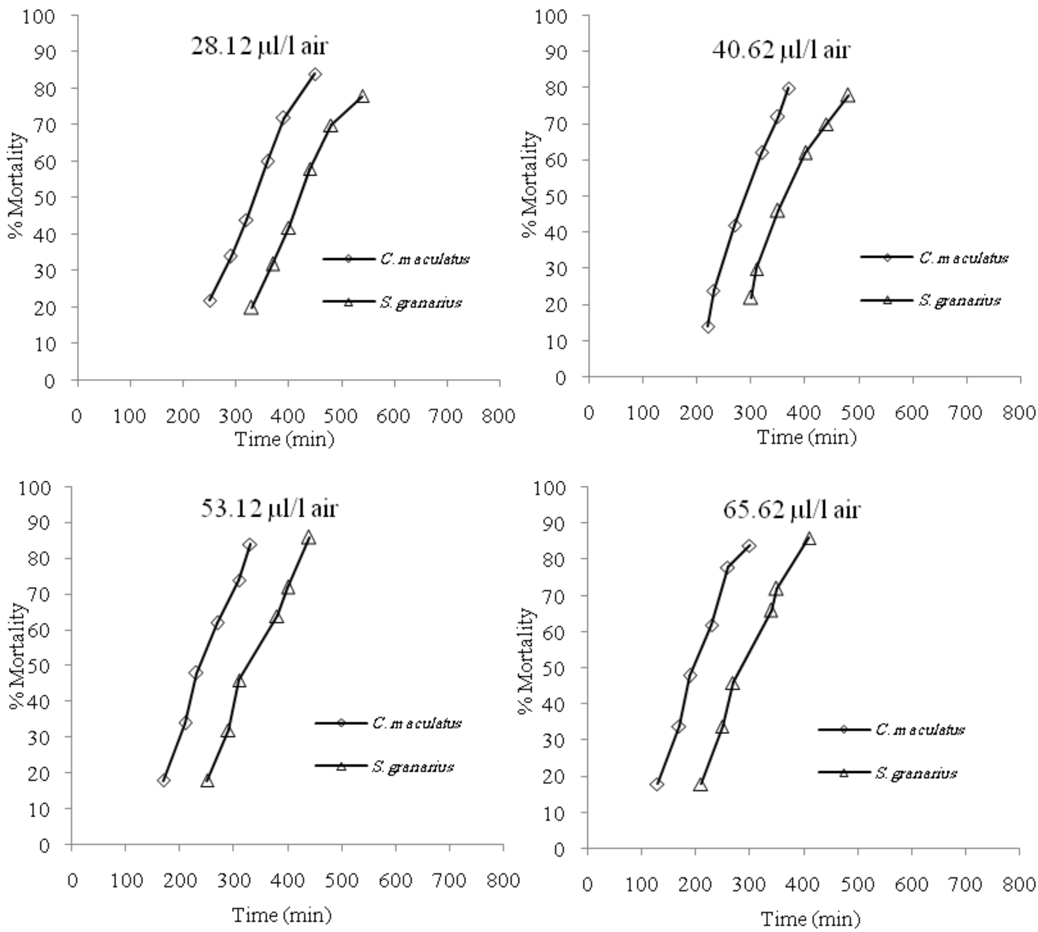

Figure 1. Mortality (%) of *Callosobruchus maculatus* and *Sitophilus granarius* exposed to essential oil from *Thymus carmanicus* for various periods.