Research Article

Chemical composition and fumigant toxicity of essential oil from *Thymus daenensis* against two stored product pests

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Abstract: Plant essential oils and their constituents are recognized as proper alternatives to fumigants. Thymus daenensis Celak is one of these plants that have medicinal properties and is endemic to Iran. The essential oil was isolated by hydrodistillation from dry leaves using a modified clevenger-type apparatus and the chemical composition of the oil was assessed via GC and GC-MS. Fourteen compounds (100% of the total composition) were identified. Thymol (57.4%), carvacrol (9.8%), β -caryophyllene (6.9%), γ -terpinene (6.7%) and pcymene (6.3%) were found to be the major compounds of the essential oil. The fumigant toxicity of the essential oil was tested against 1-3 days old adults of Callosobruchus maculatus (F.) and Sitophilus granarius (L.) at 27 ± 1 °C and $65 \pm 5\%$ 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. At the highest concentration (65.62 µl/l air), T. daenensis oil caused 90 and 60% mortality with a 3 h exposure on C. maculatus and S. granarius, respectively. Based on LC50 values, C. maculatus (4.22 µl/l air) was significantly more susceptible than S. granarius (6.55 μ l/l air). These results show the efficacy of T. daenensis oil for stored-products protection.

Keywords: Fumigant toxicity, Essential oil, *Thymus daenensis, Callosobruchus maculatus, Sitophilus granarius*

Introduction

Synthetic insecticides have been considered the most effective means to control stored-product pests (Huang and Subramanyam, 2005) but, these insecticides may involve serious health hazards for mammals (Lamiri *et al.*, 2001). In addition, their undesirable side effects are ozone depletion and environmental pollution (WMO, 1995), toxicity to non-target organisms and pest resistance (Mohan and Fields, 2002). These problems have highlighted the need for

the development of selective insect-control alternatives.

Plant essential oils and their constituents have been well demonstrated against storedproduct pests. Especially their main compounds, monoterpenoids, offer promising alternatives to classical fumigants (Papachristos and Stamopoulos, 2003).

Biological activity of essential oils can vary for many reasons. Bioactivity depends on chemical composition and interactions among structural components in the essential oil. Even minor compounds can have a critical function owing to synergism between chemical constituents (Hummelbrunner and Isman, 2001; Lahlou, 2004; Sampson *et al.*, 2005; Angioni *et al.*, 2006; Bakkali *et al.*, 2008).



Handling Editor: Yaghoub Fathipour

^{*} Corresponding author, e-mail: moharami@modares.ac.ir Received: 23 April 2014, Accepted: 9 January 2016 Published online: 11 April 2016

Callosobruchus maculatus is a cosmopolitan pest of stored grain legumes (Fabaceae) particularly of the genus *Vigna* (Cope and Fox, 2003). Indeed, *C. maculatus* infestation on stored legumes may reach upto 50% within 3-4 months (Pascual-Villalobos and Ballesta-Acosta, 2003). Granary weevil *S. granarius*, is an important stored grain pest (kucerova, *et. al.*, 2003). *Sitophilus granarius* reduces amount of available grain to sell and quality of wheat flour too. (Toews *et al.*, 2006).

The plant *Thymus* is one of the most famous genera of Lamiaceae. Thymus species are commonly used as herbal tea, condiment, spice and medicinal plants. T. daenensis is an endemic species grown in Iran (Gasemi Pirbalouti et al., 2011). Studies concerning the composition of T. daenensis essential oil are limited. In the previous investigation, Sajjadi and Khatamsaz (2003) reported that thymol (73%), carvacrol (6.7%) and *p*-cymene (4.6%) are the main constituents of T. daenensis Celak. subsp. Loncifolius (Celak.) Jalas. oil. Nickavar et al. (2005) reported that the major compounds of the essential oil from aerial parts of T. daenensis subsp. daenensis were thymol (74.7%), *p*-cymene (6.5%), β -caryophyllene (3.8%) and methyl carvacrol (3.6%).

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.) Jalas. 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 Plodia interpunctella (Hübner) (Passino et al., 2004), T. castaneum (Clemente et al., 2003), Lasioderma serricorne (F.) (Hori, 2003), Spodoptera litura (F.) (Hummelbrunner and Isman, 2001) and S. oryzae (Lee et al., 2003). Moreover, Thymus mandschuricus (Ronniger) has insecticidal activity against S. oryzae (Kim et al., 2003).

Consequently, the objective of this research was to evaluate the insecticidal properties of the

essential oil of *T. daenensis* against adults of *C. maculatus* and *S. granarius* under laboratory conditions.

Materials and Methods

Insect cultures

Callosobruchus maculatus was reared in 1-liter jars containing cowpea seeds. *S. granarius* was reared in 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 $65 \pm 5\%$ R.H. Adult insects, 1-3 days old, were used for the fumigant toxicity tests. All experimental procedures were carried out under the same environmental conditions as the cultures.

Plant materials

Aerial parts of *T. daenensis* were collected at full flowering stage in May 2009 from the farm of Shahid Fozveh in Isfahan Research Station Center. The plant material was dried on laboratory benches at room temperature (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

An experiment was designed to assess 50% and 90% lethal doses. Ten adult insects of C. maculatus and S. granarius were put into 320 ml glass bottles. C. maculatus adults were exposed to the essential oil at doses of 2.18, 2.81, 3.43, 4.06, 5 and 6.25 μ l/l air and S. granarius at 5.31, 5.93, 6.56, 7.18 and 7.81 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 LC50 and LC90 values with their fiducial limits by SPSS. 20.0. Samples for which the 95% fiducial limits did not overlap were considered to be significantly different.

To determine the fumigant toxicity of T. daenensis 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 which contained ten adults of each species of insect in separate vials. The combination of each concentration and exposure time (1-10 h) was replicated five times independently. When no leg or antennal movements were observed, insects were considered dead. The probit analysis (Finney, 1971) was computed using SPSS 20.0 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.

Extraction and analysis of essential oil

Dried leaves and flowers were subjected to hydrodistillation using a modified Clevengertype 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. Extracted oil was stored in a refrigerator at 4°C. GC analysis carried out on a HP-6890 was gas chromatograph equipped with a HP-5 MS (nonpolar) capillary column (30m×0.32mm; 0.25µm film thickness). 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 were the same as described above. Mass spectra were taken at 60 eV. Quantitative data were obtained 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 were observed in mortality of insects due to essential oil vapor with different concentrations and times. From the graph in (Fig. 1), it can be seen that, T. daenensis oil was relatively more toxic to C. maculatus than to S. granarius. The lowest concentration (28.12 µl/l) of the oil yielded 90% mortality of C. maculatus after a 4.5 h exposure but mortalities of S. granarius at the lowest concentration were 75% after 4.5 h. At 40.62 µl/l air T. daenensis oil caused about 55% mortality against C. maculatus with a 2 h exposure and 80% mortality after 3 h. At this concentration, 80% mortality was achieved after 4 h for S. granarius. The oil at 53.12 µl/l air caused 85% morality of C. maculatus and S. granarius with 3 and 4.5 h exposure, respectively. At the highest concentration (65.62 μ l/l air), kills of C. maculatus reached 90% with a 3 h exposure. By contrast, only about 60% mortality was achieved for S. granarius at the same exposure time. On the basis of the LT₅₀s, C. maculatus was killed more quickly than S. granarius. For C. maculatus, LT₅₀ values ranged from 0.94 h at the highest dose (65.62 μ l/l air) to 1.93 hat the lowest dose (28.12 μ l/l air). Whereas for S. granarius, LT₅₀ values ranged from 4.30 h at the highest dose to 4.93 h at the lowest dose (Table 2).

Probit analysis showed that *C. maculatus* was more susceptible ($LC_{50} = 4.22 \mu l/l air$) to *T. daenensis* oil than *S. granarius* ($LC_{50} = 6.55 \mu l/l air$). The corresponding LC_{90} were 8.21 and 8.73 $\mu l/l air$, respectively (Table 1).

Yield and chemical constituents of the essential oil

Thymus daenensis oil yield was 2.3 ± 0.13 % v/w, as calculated on a dry weight basis. The chemical constituents of *T. daenensis* essential oil, the retention indices and the percentage of the individual components are summarized in (Table 3). Fourteen components were identified of which Thymol (57.35%) and carvacrol (9.75%) were the major constituents of the oil.



Figure 1 Percentage mortality of *Callosobruchus maculatus* and *Sitophilus granarius* exposed to essential oil from *Thymus daenensis* for various periods.

Table 1 LC₅₀ and LC₉₀ values of *Thymus daenensis* essential oil to *Callosobruchus maculatus* and *Sitophilus granarius*.

Insect species	LC_{50} (µl/l air) ¹	LC ₉₀ (µl/l air)	Slope \pm SE	df	Chi-square (χ^2)	P-Value
C. maculatus	4.22 (3.90 - 4.63)	8.21 (6.95 - 10.72)	4.44 ± 0.56	4	2.68	0.612
S. granarius	6.55 (6.30 - 6.81)	8.73 (8.11 - 9.90)	10.27 ± 1.50	3	1.07	0.783

¹. 95% lower and upper fiducial limits are shown in parenthesis.

Table 2 LT₅₀s of *Thymus daenensis* essential oil against *Callosobruchus macolatus* and *Sitophilus granarius*.

Insect species	Concentration (µl/l air)	$LT_{50}(h)^{1}$	Slope ± SE	Chi-square ($\chi 2$) df = 4
C. maculatus	28.12	1.93 (1.71 - 2.14)	3.55 ± 0.41	0.71
	40.62	1.61 (1.42 - 1.82)	3.02 ± 0.35	1.05
	53.12	1.28 (1.09 - 1.46)	2.79 ± 0.32	0.37
	65.62	0.94 (0.78 - 1.10)	2.52 ± 0.28	0.49
S. granarius	28.12	3.07 (2.84 - 3.30)	4.93 ± 0.58	1.4
0	40.62	2.82 (2.60 - 3.04)	4.66 ± 0.56	0.77
	53.12	2.54 (2.32 - 2.76)	4.25 ± 0.55	0.41
	65.62	2.36 (2.15 - 2.56)	4.30 ± 0.52	0.53

¹95% lower and upper fiducial limits are shown in parenthesis.

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Compounds	Retention Index	%Composition ¹	
Myrcene	1150	0.9	
α-terpinene	1180	1.2	
<i>p</i> -cymene	1188	6.3	
1,8-Cineole	1196	0.6	
γ-terpinene	1222	6.7	
Linalool L	1258	1.5	
1-borneol	1339	2.1	
Terpinene-4-ol	1347	0.7	
Thymol	1452	57.4	
Carvacrol	1460	9.8	
β -caryophyllene	1600	6.9	
β -bisabolene	1668	1.6	
α-bisabolene	1698	2.4	
Caryophyllene oxide	1765	2.1	

Table 3 Chemical constituents of the essential oil from

 Thymus daenensis.

¹ Major constituents are shown in bold.

Discussion

In regard to the previous studies on the essential oil composition of *T. daenensis*, there are some qualitative and quantitative differences between the present work and those of the earlier studies. The major component of *T. daenensis* essential oil in our research was same as previous works but the amount of thymol in this study was lower than that found in the other works (Sajjadi and Khatamsaz, 2003; Nickavar *et al.*, 2005). The differences between this analysis and other works can be related to the time and place of the plant harvested that might influence the chemical composition of the plant essential oil.

There have been numerous research studies on plant products as fumigants against insect pests of stored products (Rajendran and Sriranjini, 2008). Previous studies have shown that the toxicity of plant essential oils against stored product pests is related to the main components of EOs (Isman *et al.*, 2001; Lee *et al.*, 2003). The main constituents of many plant essential oils are monoterpenoids. They are typically volatile and rather lipophilic compounds, which can rapidly penetrate into insects (Lee *et al.*, 2002). 1,8–cineole, carvacrol, eugenol, linalool and thymol are known compounds to show effects against various insect species (Koul *et al.*, 2008).

Thymol, a monoterpenoid is the major component in T. daenensis essential oil. There are numerous reports on insecticidal activity of thymol. Erler (2005), reported the fumigant activity of thymol against adults and eggs of Tribolium confusum (Jacquelin Du Val.), and larvae and eggs of Ephestia kuehniella (Zeller.) Carvacrol and thymol, the major components of oregano and savory, were highly effective against P. interpunctella and E. kuehniella (Ayvaz et al., 2010). Moreover, the essential oil of Origanum glandulosum (Lamiaceae) showed toxicity against R. dominica. The main components of this oil were thymol, carvacrol, *p*-cymene and γ -terpinene (khalfi et al., 2008). Rozman et al. (2006) reported that S. granarius showed very high susceptibility to thymol and carvacrol. The major constituent of Ocimum gratissimum essential oil (Lamiaceae) was thymol that had insecticidal effect on Sitophilus zeamais (Motsch.) (Jirovetz et al., 2005). Thymol showed fumigant toxicity against larvae and adults of T. castaneum (Mondal and Khaleguzzaman, 2010). Rozman et al. (2007) reported that thymol was highly effective against S. oryzae. Therefore, the toxic effects of T. daenensis oil could especially be attributed to thymol and carvacrol.

The results of this study suggest that essential oil of *T. daenensis* or its major constituents could be used as alternatives to develop less hazardous treatment systems to protect stored product from pests.

In our study, essential oil of *T. daenensis* caused rapid knockdown, hyperactivity, convulsion, paralysis and death of insects. These effects show that, this essential oil could resemble traditional fumigants. The high activity of this essential oil renders it as a potential substitute for chemical insecticides in stored-product control programs and could be used as a component of the integrated pest management if the cost-effective commercial problems can be solved. However, there is a need for more elaborate studies on the essential oil against other stored-product pests, 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 plantbased pesticides. However, further studies are necessary to develop formulations to improve their efficacy and stability.

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ترکیب شیمیایی و سمیت تنفسی اسانس آویشن دنایی Thymus daenensis روی دو گونه آفت انباری

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 دریافت: ۳ اردیبهشت ۱۳۹۳؛ پذیرش: ۱۹ دی ۱۳۹۴

چکیده: اسانسهای گیاهی و اجزای تشکیلدهنده آنها بهعنوان ترکیبات جایگزین برای آفتکشهای تدخینی محسوب میشوند. آویشن دنایی . Thymus daenensis Celak یکی از گیاهان دارویی و بومی ایران میباشد. اسانس برگ و گل گیاه آویشن دنایی با استفاده از دستگاه کلونجر و به روش تقطیر با آب استخراج شد و ترکیبات آن با استفاده از دستگاه GC-MS مورد شناسایی قرار گرفت. ۱۴ ترکیب در اسانس شناسایی شد. تیمول (٪/۹/۵)، کارواکرول (٪/۹/۹)، بتا-کاریوفیلن (٪/۹/۹)، آلفا-ترپینن (٪/۶/۹) و پی-سایمن (٪/۶/۳) بهعنوان ترکیبات اصلی در اسانس بودند. سمیت تنفسی اسانس روی حشرات بالغ پی-سایمن (٪/۶/۳) بهعنوان ترکیبات اصلی در اسانس بودند. سمیت تنفسی اسانس روی حشرات بالغ یک تا ۳ روزه سوسک چهار نقطهای حبوبات (.F) درجه سلسیوس، رطوبت نسبی ۵ ± ۶۵ درصد و در (.C) کانجام گرفت. مرگومیر حشرات بالغ در غلظتها (۲۸/۱۲، ۶۰/۶۲، ۲۱/۳۲ و ۲۵/۶۹ میکرولیتر بر لیتر هوا) و زمانهای مختلف مطالعه شد. درصد مرگومیر سوسک چهار نقطهای حبوبات و شپشه گندم در بالاترین غلظت (۶/۶۹/۹ میکرولیتر بر لیتر هوا) بهترتیب ۹۰ و ۶۰ درصد در طول ۳ ساعت بهدست آمد. براساس مقادیر رکیهای سوسک چهار نقطهای حبوبات (۴/۲۲ ایلار) نیمای معوبات و شپشه گندم در بالاترین غلظت (۲/۶۹) بهطور معنیداری حساستر است. نتایج حاکی از کارایی اسانس آویشن دنایی در حفاظت از محصولات انباری میباشد.

واژگان کلیدی: سـمیت تنفـسی، اسـانس، Callosobruchus maculatus ،Thymus daenensis، Sitophilus ، Callosobruchus maculatus ،