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Fumigant and repellent properties of sesquiterpene–rich essential oil from Teucrium polium subsp. capitatum (L.)

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ABSTRACT

Objective: To test fumigant and repellent properties of sesquiterpene–rich essential oil from *Teucrium polium* subsp. *capitatum* (L.). **Methods:** The fumigant toxicity test was performed at (27±1) ℃, (65±5)% relative humidity, and under darkness condition and 24 h exposure time. The chemical composition of the isolated oils was examined by gas chromatography–mass spectrometry. **Results:** The major compounds were α-cadinol (46.2%), caryophyllene oxide (25.9%), α muurolol epi (8.1%), cadalene (3.7%) and longiverbenone (2.9%). In all cases, considerable differences in mortality of insect to essential oil vapor were observed in different concentrations and exposure times. *Callosobruchus maculatus* (*C. maculates*) (LC_{50}=148.9 μL/L air) was more susceptible to the tested plant product than *Teucrium castaneum* (*T. castaneum*) (LC_{50}=360.2 μL/L air) based on LC_{50} values. In the present investigation, the concentration of 3 μL/mL acetone showed 60% and 52% repellency against *T. casteneum* and *C. maculatus* adults, respectively. **Conclusions:** The results suggests that sesquiterpene–rich essential oils from the tested plant could be used as a potential control agent for stored–product insects.

1. Introduction

The invasion of food products by insects contribute greatly to the loss of quality and quantity. The cowpea weevil, *Callosobruchus maculatus* (*C. maculatus*) F. (Coleoptera: Bruchidae), and the rust red flour beetle, *Tribolium castaneum* (*T. castaneum*) (Herbst) (Coleoptera: Tenebrionidae), are among the most widespread and destructive stored product pests throughout the world. They cause significant losses of stored products, particularly in tropical and warm temperate regions[1].

Chemicals and fumigants play a vital role in controlling this problem but they have been known to cause serious toxicological and environmental problems. Persistent development of resistance is a serious threat to the future use of these materials, and consequently, there is an urgent need to develop affordable, safe and sound pest control agents and techniques[2]. Researchers are now focused on finding alternative insecticides which will be effective, environmentally friendly, biodegradable, safer and cheaper than existing ones. Plant oils, as an important natural resource of insecticides, are generally considered broad–spectrum and safe for the environment because the array of compounds they contain quickly biodegrade in the soil[2–4]. Their lipophilic nature facilitates their interference with basic metabolic, biochemical, physiological and behavioral functions of insects[4].

The genus *Teucrium* belongs to the family Lamiaceae (Labiatae), and consists of about 300 species, most of which are found in the dry and stony places of the hills and deserts of Mediterranean countries, South Western Asia, Europe and North Africa[5]. *Teucrium polium* (*T. polium*) subsp.

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capitatum L. is a perennial, pubescent, aromatic plant, 20-50 cm high, white or grey hairs on stems, with green-grayish leaves and white flowers. *T. polium* (locally called kalpooreh) is one of the wild-growing flowering species and is abundantly found in Iran. Various biological activities have been reported for *T. polium*, such as antiphytoviral[6], antibacterial[7], antifungal[8], repellent[9,10] and insecticidal properties[11,12].

Phytochemical investigations have shown that *Teucrium* genus contains various compounds such as terpenoids, flavonoids and iridoids[13]. The results of Bezic *et al*[6] showing that the major compounds in *T. polium* essential oil were the sesquiterpene hydrocarbons β-caryophyllene and germacrene D. However, there have been some variations in the constituents of this oil from different countries, and a chemo-geographical variation has been observed in essential oil composition of this species[14].

In the present study, the chemical components of essential oils from *T. polium* subsp. *capitatum* (Lamiaceae) aerial parts was determined, and the repellent and insecticidal activity of it was tested against the adult stages of the stored-products pests, *T. castaneum* Herbst (Coleoptera: Tenebrionidae) and *C. maculatus* F. (Coleoptera: Bruchidae).

2. Materials and methods

2.1. Insect material

C. maculatus and *T. castaneum* were reared in plastic containers (20 cm length, 14 cm width, and 8 cm height, covered by a fine mesh cloth for ventilation) containing bean grain and wheat flour mixed with yeast (10:1, w/w), respectively. The culture was maintained in the dark, in a growth chamber set at (27±1) ℃ and (65±5)% relative humidity. All experiments were carried out under the same environmental conditions.

2.2. Plants and essential oils

Aerial parts (leaves and flowers) of *T. polium* were collected in Kashmar (35° 14′ N, 58° 28′ E; 1 100 m a.s.l.) located in Khorasan Razavi province, Iran, from May to July 2010. Essential oil extraction was done following the method described by khani *et al*[15]. The extracted oil was dehydrated with anhydrous sodium sulphate (10 min) and immediately stored in airtight glassware in refrigerator at 4 ℃).

2.3. Gas chromatography (GC)-mass spectrometry

The essential oils was analyzed on a gas chromatograph mass spectrometer (GC-mass) (Shimadzu-17A-QP5050, Japan) following the method described by khani *et al*[15]. The GC column was DB-5 (30 m×0.25 mm *id.*, 0.25 μm film thickness). The column oven temperature was set at 60 ℃ for 3 min, and then increased to 260 ℃ at a rate of 5 ℃/min. Injector and detector temperatures were 230 ℃ and 245 ℃, respectively. The GC mass analysis was carried out with the same characteristics as used in GC. The ionization energy was 70 ev with a scan time of 1s and mass range of 40-500 amu.

2.4. Fumigant toxicity

Fumigant toxicity of the oil on adults of each species (1-7 days old of undefined sex) determined following the method described by khani *et al*[15]. Lethal effect assessed in glass vials (60 mL) at concentrations of 166.66, 250, 333.33, 416.67 and 450 μL/L air for *T. castaneum* and concentrations of 5, 50, 83.33, 166.66, 250 and 416.67 μL/L air for *C. maculatus*. Each concentration was replicated four or eight times with ten individuals per each replicate. Mortality was determined 24 h after exposure. When no signs of leg or antennal movement were observed, insects were considered dead. The treatment bottles were monitored for 48 h after recording the data, and no affected insect recovered.

To evaluation 50% lethal doses (LC_{50}) after an exposure time of 24 h, the concentrations of the essential oils causing 10% to 90% mortality were used. Data obtained from each dose response bioassay were subjected to probit analysis. LC_{50} values were determined by log-probit regression using SPSS 16.0 for Windows[16].

2.5. Repellency bioassay

Repellency was assessed as described by Fields *et al*[17]. The repellency tests were consisted of two clear plastic chambers (65 mL volume) joined to either side of a central main chamber with the same size by a small tubing (2 cm long and 3 mm in diameter). Concentrations of the test solutions were 0.2, 0.5, 1 and 3 μL of essential oil in 1 mL acetone. One milliliter of each solution was applied on 40 seeds of wheat for *T. castaneum* and 20 seeds of bean for *C. maculatus*. In the control, the food was treated with 1 mL acetone only. The treated and control seeds were kept for 20 min to evaporate the solvent completely, and placed in the center of treated and control chambers respectively. Three replications were used for each concentration. Fifty nonsexed adults of each species (1-7 days old) were introduced into the center of each main chamber. Central chamber was covered by plastic screen but the treated and control chambers were covered by lids and the whole set up was left in darkness. After 24 h, the number of beetles at each chamber was counted and the percentage repellency (R) values were computed using the formula of Liu *et al*[18]:

$$R(\%) = \frac{(C.E)}{T} \times 100$$

where C is the number of insects in control, E is the number of insects in oil treated chamber and T is the number of total released insects.

2.6. Statistical analysis

Statistical analysis of the mortality and repellency data was performed by one-way analysis of variance (ANOVA) with a *post-hoc* Tukey test using SPSS version 16.00. Normality of data was tested using, Kolmogorov-Smirnov test, a non-parametric test. The results were expressed as mean±SE, and considered significantly different at $P< 0.05$.

3. Results

3.1. Chemical composition of essential oils

The chemical composition of the essential oils of *T. polium* subsp. *capitatum* aerial parts (leaves and flowering heads) is presented in Table 1. The major compounds were α-cadinol (46.2%), caryophyllene oxide (25.9%), α muurolol epi (8.1%), cadalene (3.7%), longiverbenone (2.9%), carotol (2.7%) and diethyl phthalate (2.5%) in the essential oils of *T. polium* subsp. *capitatum* aerial parts.

Table 1
Chemical composition of essential oils of *T. polium* aerial parts (leaves and flowering heads).

Compounds[a,b]	Concentration (%)	Kovats index
Sabinene	0.11	973
Sesquisabinene hydrate	0.70	1 541
Diethyl phthalate	2.50	1 565
Caryophyllene oxide	25.9	1 580
Globulol	1.10	1 582
Epiglobulol	1.40	1 585
Viridiflorol	1.40	1 591
Carotol	2.70	1 598
δ-Cadinol	2.00	1 635
epi-α Muurolol	8.10	1 643
α-Cadinol	46.20	1 652
Cadalene	3.70	1 672
Longiverbenone	2.90	1 693
Other compounds	1.30	-

[a] Compounds are listed in the order of their elution.

[b] Identification based on authentic standards, Wiley libraries spectra, and literature.

3.2. Fumigant toxicity

The mortality increased significantly with rising concentrations (Figure 1) ($F_{4,27}=24.16$; P=0 for *T. castaneum* and $F_{5,35}=30.13$; P=0 for *C. maculatus*). Results indicated that the oil of tested plant was significantly more toxic against *C. maculatus* than *T. castaneum*, as inferred by the confidence intervals of LC_{50} (Table 2). Based on LC_{50} (Table 2) and fumigant toxicity experiments (Figure 1), the oil of *M. communis* displayed strong insecticidal activity against *C. maculatus* (LC_{50}=148.9 μL/L air), but revealed fewer activity against *T. castaneum* (LC_{50}=360.2 μL/L air) (Figure 2, Table 2).

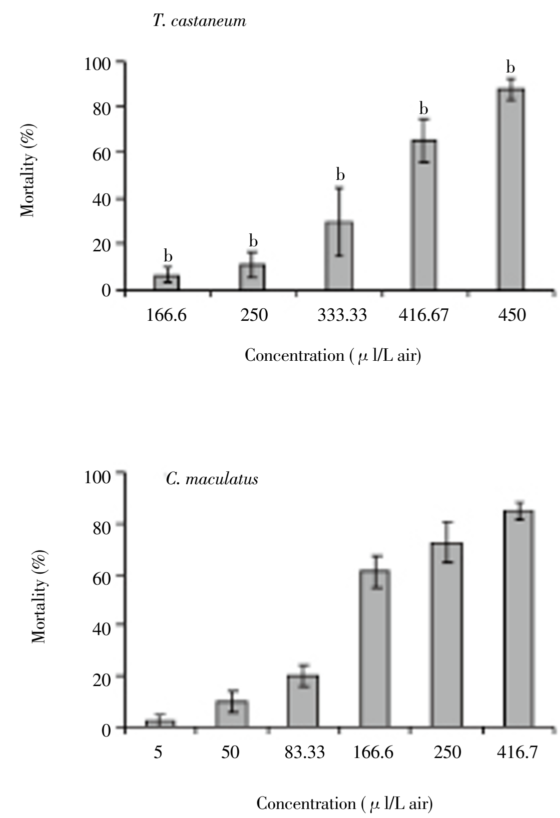

Figure 1. Lethal effect of essential oil from *T. polium* aerial parts on *T. castaneum* and *C. maculatus* after 24 h.

Table 2
Efficiency of essential oil extracted from *T. polium* against *T. castaneum* and *C. maculatus* adults.

Insects	95% *CL*	χ^2(*df*)	Probability	LC_{50}	Slope±SE
T. castaneum	(291.3-497.0)	3.96 (2)	0.14	360.25	8.40±1.03
C. maculatus	(127.2-170.9)	2.49 (3)	0.48	148.96	2.60±0.29

Ten individuals per replicate, 4 or 8 replicates per concentration, 4 and 5 concentrations per assay for *T. castaneum* and *C. maculatus*, respectively; LC: lethal concentration (μL/L air), *CL*: confidence limits.

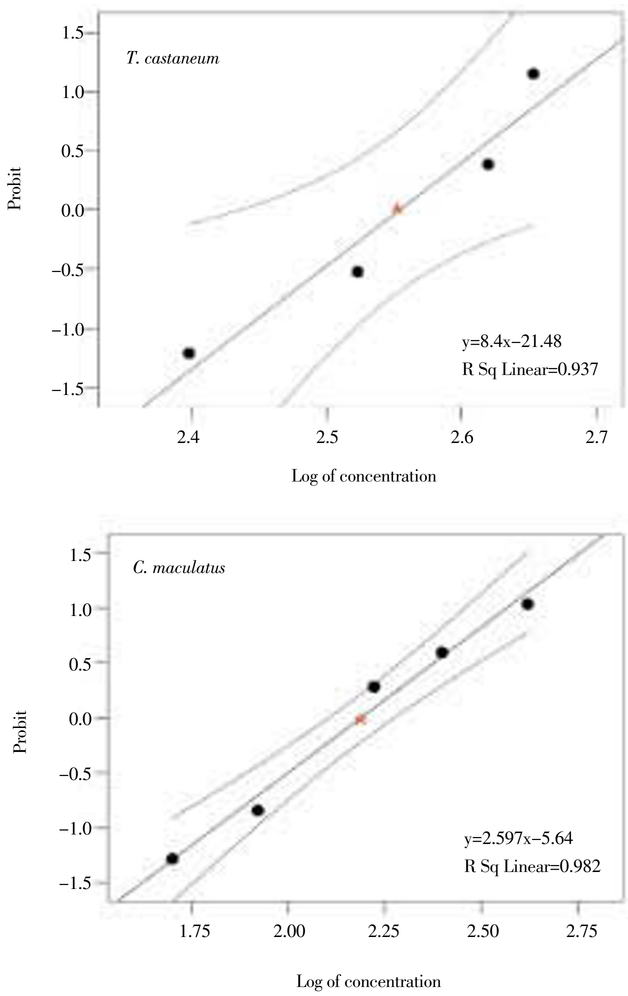

Figure 2. Mortality probit line and 95% confidence limits for *T. castaneum* and *C. maculatus* after 24 h exposure to essential oil extracted from *T. polium* aerial parts. LC_{50} point is shown by the star sign.

3.3. Repellent activity

T. polium essential oil moderately repelled *T. castaneum* and *C. maculatus* in the tested concentrations and the repellency for these two insects was not statistically different in all concentrations (t=−0.8, df= 22, P=0.43) (Table 3). The test showed that the repellency was concentration-dependent and the repellency increased significantly with increasing concentration of essential oil in all cases. In the present investigation, the concentration of 3 μL/mL acetone showed 60% repellency against *T. casteneum* beetle while it was 24.7% repellency at lower concentration 0.2 μL/mL acetone ($F_{3,11}$=52.38; P=0). Also the repellency for *C. maculatus* was obvious when compared to the lowest concentration and the highest one (20% and 52% respectively) ($F_{3,11}$= 5.03; P=0.3).

Table 3

Percent repellency (mean±SE) of the essential oil from *T. polium* on *T. castaneum* and *C. maculatus* adults.

Insect species	Concentration of essential oil (μL/mL acetone)			
	0.2	0.5	1	3
T. castaneum	24.7±2.4^{c}	34.7±2.4^{b}	40.0±0.0^{b}	60.0±2.3^{a}
C. maculatus	20.0±4.2^{b}	32.7±5.8^{ab}	36.0±6.1^{ab}	52.0±7.0^{a}

Means followed by the different letter within a row are significantly different using Tukey's test at P < 0.05.

4. Discussion

Essential oils of *T. polium* ssp. *capitatum* have been the subject of many studies. According to the plant origin studied, the amounts of main constituents differed notably[19]. Important differences were found with regard to the major constituents of the essential oils of five populations of *T. capitatum* grown in Portugal. τ-cadinol (24.1%) and α-cadinol (9.8%) were the major compounds in the oil from one population, whereas δ-cadinene (9.8%) and E-caryophyllene (4.6%) or α-muurolol (6.0%) was the major constituents in the other population[20]. Conversely α-pinene (7.7%), sabinene (11.2%) and β-pinene (10.3%) were the main compounds in the other sample[20]. α-pinene (28.8%), β-pinene (7.2%) and p-cymene (7.0%) were the main components of the essential oil of *T. polium* ssp. *capitatum* from Corsica, France[21]. Menichini *et al*[22] reported carvacrol (10.1%), (E)-β-caryophyllene (9.8%), α-cadinol (4.5%) and verbenone (3.7%) were the most abundant components in *T. polium* ssp. *capitatum* from Greece. In the oil of *T. polium* ssp. *capitatum* from Crete, Italy, the most abundant compounds were (E)-β-caryophyllene (10.1%) and the monoterpene, carvacrol (9.6%)[23]. The dominant constituents of the aerial parts of *T. polium* ssp. *capitatum* collected during the flowering period from Serbia were sesquiterpenes, germacrene D (31.8%), trans-caryophyllene (8.8%) and bicyclogermacrene (6.2%), and monoterpenes, linalool (14.0%) and β- pinene (10.7%)[24]. While the essential oil from Bulgaria was characterized by a high percentage of monoterpenes, β-pinene (26.8%), α-pinene (9.3%) and limonene (6.4%), and the sesquiterpene, germacrene D (17.7%)[24].

It is noticeable that the chemical composition of Iranian *T. polium* subsp. *capitatum* studied here was to some extent in accordance with some populations of *T. capitatum* grown in Portugal[20]. Furthermore, it is the first time that globulol and Longiverbenone (vulgarone B) have been characterized in *T. polium* oil. These discrepancies might account for geographical variation and explain the probability of the existence of several chemical races within the species.

Results indicated that the oil of tested plant was more toxic but not significantly against *C. maculatus* than *T. castaneum*, as inferred by the confidence intervals of LC_{50}. A difference in the response of the insect species to the essential oils has previously been reported for stored-product insects[15,25,26].

It has been reported that the oil and extract of the tested plant had insecticidal activity against other insects. For example, An LC_{50} of 80 ppm was estimated with the larvae of *Musca domestica* in bioassays when the essential oil of *T. polium* was admixed with artificial diet. Furthermore a reduction of carbohydrase activity of α-amylase, α-glucosidase and β-glucosidase were detected in larval midgut extract[12]. The contact LD_{50} of *T. polium* essential oil against house fly larvae was 0.028 μL/larvae[27]. The LC_{50} of essential oil of *T. polium* for adults of *C. maculatus* and *Tribolium confusum* were 9.6 μL/L and 35.5 μL/L, respectively[27].

Terpenes have been well documented as active fumigants, repellents, and insecticides toward stored-product insects[4]. The insecticidal activity of the essential oils investigated in our study may be attributed to their having major terpenoids components. There are numerous reports on the toxicity of the essential oils from various plants species with major components similar to *T. polium* in our study, such as α-cadinol[28], caryophyllene oxide[29], Longiverbenone (vulgarone B)[30,31], viridiflorol[32-34] which are classified as active toxic compounds.

Furthermore, insecticidal activity of major constitutes of the tested oil were before reported. α-cadinol possessed the strong antitermitic activity with 70% mortality of *C. formosanus* Shiraki at 10 mg/g dosage for 14 days[35]. In addition, α-cadinol possessed the strong antimite activities. *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, the dominant species of house dust mites, were all killed at a low concentration (6.3 mg/cm^2) dosage of α-cadinol after 24 h of exposure[36]. Caryophyllene oxide showed high insecticidal activity (LC_{50}=0.18 mg/L) against *T. castaneum* (Coleoptera: Tenebrionidae) adults[37]. LC_{50} value of caryophyllene oxide in *Cinnamomum osmophloeum* leaf essential oil against mosquito species, *Aedes albopictus* larvae was 65.6 μg/mL[29]. Caryophyllene oxide inhibited the mitochondrial electron transport chain in mammalian cells[38].

The essential oil assayed was not only active insecticide against *T. castaneum* and *C. maculatus*, but it was also effective repellent. Based on our results, adults of these two beetles were found to be repelled by essential oil tested even at very low concentration. Our results are in accordance with those of Heydarzade *et al*[9] who reported the mean repellency percentage of *T. polium* at the highest concentration (3 μL/mL acetone) was 84.9% and 76.1% on males and females of *C. maculatus*, respectively. Other study has shown that 35% effective repellent doses of *T. polium* essential oil against adults of *C. maculatus* and *Tribolium confusum* were 6.45 and 2.91 μL/mL acetone, respectively[10]. The strong repellent activity of the tested oil could be due to caryophyllene oxide, one of the main constituent of this oil (>25%). In previous research, this compound has shown to be a powerful repellent towards *T. castaneum* with complete repellency at 0.03 mg/cm^2[37].

The essential oil from the plant could become a viable alternative to conventional chemical control strategies. However, further studies need to be conducted in order to evaluate the safety of the oil before practical use in stored-product insect control.

Conflict of interest statement

We declare that we have no conflict of interest.

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