

Orijinal araştırma (Original article)

Contact toxicity and persistence of essential oils from *Foeniculum vulgare*, *Teucrium polium* and *Satureja hortensis* against *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) adults

Foeniculum vulgare, *Teucrium polium* and *Satureja hortensis* uçucu yağlarının *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) erginlerine kontakt ve residüyel etkisi

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Summary

Contact toxicity and persistence of essential oils extracted from *Foeniculum vulgare* Miller (Apiaceae), *Teucrium polium* L. (Lamiaceae) and *Satureja hortensis* L. (Lamiaceae) were evaluated against the adults of cowpea seed beetle, *Callosobruchus maculatus* (Fabricius) at 28±2 °C, 60±5% r.h. in dark conditions. Contact toxicity tests were conducted on one-day old adults of *C. maculatus* with 6 concentrations of essential oils at 24-h exposure time. The persistence of insecticidal activity of essential oils was examined for a period of 30 h at the LC₉₉ level. The results indicated that mortality of adults was dose related. Males were more susceptible to essential oils than females. The essential oil of *F. vulgare* was the most toxic to male and female adults of *C. maculatus* with LC₅₀ values of 390.38 and 513.46 µl.m⁻², respectively. Moreover, *S. hortensis* oil was more toxic to male and female adults of *C. maculatus* with LC₅₀ values of 535.69 and 640.99 µl.m⁻² than *T. polium* oil with LC₅₀ values of 1263.09 and 1469.72 µl.m⁻², respectively. The results of persistence tests revealed that essential oil of *S. hortensis* was the most persistent, while that of *T. polium* was the least. High persistence of *S. hortensis* oil could be attributed to its high proportion of oxygenated compounds compared to other oils. The present study demonstrated that these essential oils can be used as appropriate alternative to control of cowpea seed beetle.

Key words: Essential oils, persistence, contact toxicity, cowpea seed beetle

Özet

Foeniculum vulgare Miller (Apiaceae), *Teucrium polium* L. (Lamiaceae) ve *Satureja hortensis* L. (Lamiaceae)'den ekstrakte edilen uçucu yağların kontakt ve residüyel etkisi 28 ± 2 °C, % 60 ± 5 bağıl nemde, karanlık ortamda börülce tohum böceği, *Callosobruchus maculatus* (Fabricius) erginlerine karşı değerlendirilmiştir. Kontakt toksisite testleri *C. maculatus*'un bir günlük erginleri kullanılarak uçucu yağların 6 konsantrasyonuna 24 saat maruz bırakılarak yapılmıştır. Uçucu yağların insektisidal aktivitesinin kalıcılığı ise, LC₉₉ düzeyinde 30 saatlik süreyle incelenmiştir. Sonuçlar, yetişkinlerin ölüm oranının doz ile ilişkili olduğunu göstermiştir. Erkek bireyler, dişilere göre uçucu yağlara daha duyarlı bulunmuştur. *C. maculatus* dişi ve erkek erginlerine karşı en toksik olan ekstraktlar sırasıyla 390,38 ve 513,46 µl.m⁻² LC₅₀ değerleri ile *F. vulgare* uçucu yağı olmuştur. Ayrıca, *S. hortensis*'den elde edilen yağ, *C. maculatus* erkek ve dişi erginlerine karşı sırasıyla, 535,69 ve 640,99 µl.m⁻² LC₅₀ değerleri ile, 1263,09 ve 1469,72 µl.m⁻² LC₅₀ değerleri olan *T. polium*'dan elde edilen yağa göre daha toksik olarak bulunmuştur. Kalıcılık testi sonucunda, *S. hortensis* esansiyel yağının en kalıcı, *T. polium*'dan elde edilen yağın ise en az kalıcı yağ olduğu belirlenmiştir. *S. hortensis* yağının diğer yağlarla kıyaslandığında görülen yüksek kalıcılığının ihtiva ettiği yüksek orandaki oksijenli bileşiklerden olabileceği düşünülmektedir. Bu çalışmanın sonucunda, bu uçucu yağların börülce tohum böceğini kontrol etmek için birer alternatif olarak kullanılabilmesi belirlenmiştir.

Anahtar sözcükler: Uçucu yağlar, residüyel, kontakt toksisite, fasülye tohum böceği

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Introduction

Cowpea, *Vigna unguiculata* (L.) Walp. is one of the most adaptable and nutritious grain legumes (Ehlers & Halla, 1997), being consumed by humans in developing countries as a source of dietary proteins as well as vitamins and minerals (Singh et al., 2003). The cowpea seed beetle, *Callosobruchus maculatus* (Fabricius) is a major storage pest of pulses (Moravvej & Abbar, 2008; Aboua et al., 2010). This insect causes quantitative and qualitative losses due to perforation damage, lowered market value and poor viability of seeds (Ofuya & Osadahun, 2005). According to Rees (2004), loss of seed material is considerable; each adult *Callosobruchus* emerging from a cowpea would have consumed about 25% of the seed.

To prevent the loss of cowpea during storage and in fields, producers usually have relied on chemical insecticides but their widespread and intensive use has led to serious problems, including pollution of the environment, insecticide resistance, pest resurgence, pesticide residues, poisoning of workers and lethal effects on non-target organisms (Rahman & Talukder, 2006; Jovanovic et al., 2007; Lu & Wu, 2010). Therefore, research has been directed to develop user-friendly methods with low adverse effects on both environment and consumers. Recently, there has been a growing interest in research on the use of essential oils of aromatic plants for protection of stored products because of their complicated action mechanism to which insect pests find it difficult to develop resistance (Isman, 2008; Nerio et al., 2009). Moreover, local availability, rapid degradation and low mammalian toxicity are a few advantages of the essential oils for the environment as cost-effective control agents (Isman & Machial, 2006; Liu et al., 2007; Isman, 2008). The efficiency of some vegetable oils from the Apiaceae and Lamiaceae families against stored-product pests has been evaluated and well documented (Kim & Ahn, 2001; Sampson et al., 2005; Taghizadeh-Saroukolai et al., 2010). Many aromatic plant species are indigenous to Iran (Naghibi et al., 2005; Ebadollahi, 2011). However, the insecticidal activities of their essential oils have rarely been studied. The present study aimed to evaluate insecticidal activity and persistence of crude essential oils extracted from the three medicinal plants *Foeniculum vulgare* Miller (Apiaceae), *Teucrium polium* L. (Lamiaceae) and *Satureja hortensis* L. (Lamiaceae) grown in Iran against cowpea seed beetle.

Material and Methods

Insect culture

The insects used in this experiment were obtained from a culture of a non-flight form of *Callosobruchus maculatus* maintained in a glass jar containing seeds of cowpea in an incubator at $28 \pm 2^\circ\text{C}$, $70 \pm 5\%$ r.h. and under dark conditions. Fifty pairs of 1-2 day-old adults were introduced to a jar containing 100 g cowpea seeds for 24 h. After removing adults, the seeds containing eggs were maintained until the emergence of F_1 adults. One-day old adults were used for all bioassays.

Essential oils

The aerial parts of *T. polium* and *S. hortensis* and seeds of *F. vulgare* were collected from the Educational Farm of the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran during September 2010. Plant materials were dried at room temperature in the shade and powdered using a blender. A sample of 50 g of each plant was subjected to steam distillation for 3 h using a Clevenger-type apparatus. Water content of oils was removed using anhydrous sodium sulfate. Extracted oils were maintained in sterile tubes in a refrigerator at 4°C until the commencement of bioassays.

Gas chromatography- mass spectrometry

GC-MS analysis was carried out with a Thermoquest-Finnigan trace GC-MS instrument equipped with a DB-5 fused silica capillary column (60m × 0.25 mm inner diameter, film thickness 0.25 µm). The oven temperature was raised from 60 °C to 250 °C at a rate of 4 °C/min and held for 20 min. Transfer line temperature was 250 °C. Helium was used as the carrier gas at a flow rate of 1.1 cm³ min⁻¹. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds.

Procedure of contact toxicity test of oils

Filter paper discs (Whatman No. 1) (9 cm diameter) placed in the bottom of glass Petri dishes were impregnated with six concentrations of each essential oil diluted in 1 ml acetone. The experiments were performed on males and females separately. Oil concentrations ranged from 267-629 µl.m⁻² to 315-944 µl.m⁻² for *F. vulgare*, 944-1730 µl.m⁻² to 110-1887 µl.m⁻² for *T. polium*, and 315-991 µl.m⁻² to 315-1258 µl.m⁻² for *S. hortensis* against males and females, respectively. The range of concentrations was chosen on the basis of a number of preliminary trials. The acetone was allowed to evaporate for 10 min prior to introduction of insects. Ten one-day old males or females were transferred onto Petri dishes. Control dishes were treated with acetone only. Each bioassay was repeated six times. Mortalities were recorded after 24 h.

Procedure of persistence experiments

Persistence of insecticidal activity of oils was evaluated as described by Ngamo et al (2007) with slight modifications. The contact LC₉₉ values of essential oils for female and male adults were diluted in 1 ml acetone and pipetted onto filter paper discs (Whatman No. 1) (9 cm diameter) in Petri dishes. Six hours later, 10 male and 10 female one-day old adults were introduced separately into each Petri dish and then numbers of dead insects were recorded 24-h after commencement of the exposure. This procedure was also conducted at 6 h intervals (i.e. 12, 18, 24, 30-h). For each interval, separate series of Petri dishes were set up with five replications.

Data analysis

Concentration-mortality data was subjected to probit analysis (Finney, 1971) using a Maximum Likelihood Program (POLO-PC, LeOra Software, Berkeley, California) to determine the LC₅₀ and LC₉₀ (and other LCs if required) values, their 95% confidence limits, slope and intercept of probit mortality regressions, and the relevant statistical tests (such as "t" ratio, 'g' factor and heterogeneity). For comparison of the probit mortality lines of treatments, the program also provides the likelihood ratio tests of equality and parallelism (Russel et al., 1997). The resistance ratio and 95% confidence limits of this ratio were determined between data from different oil treatments, with comparisons made as described by Robertson & Preisler (1992). The estimates of parameters needed for computing confidence limits of the resistance ratio were provided by individual probit analysis in the POLO-PC output. The persistence data (i.e. percentage mortality of the insect every 6 h from the start) were transformed to arcsine square-root before performing three-way full factorial analysis of variance (ANOVA). The mean comparisons were made using the Duncan multiple range test. Means of untransformed data were reported. The relationship between mortality of *C. maculatus* adults and time after application of essential oils was examined using linear regression. Statistical analyses were carried out using SPSS (V. 16).

Results

Chemical composition of the essential oils

The chemical constituents of essential oils, the retention times and the percentages of the individual components are summarized in Tables 1, 2 and 3. The GC-MS analysis showed that the main constituents of *F. vulgare*, *T. polium* and *S. hortensis* oils were monoterpenoids. The main constituents of *F. vulgare* oil were *E*-anethole (60.61%), fenchone (12.14%) and limonene (8.92%) (Table 1) and those of *T. polium* oil were *E*-piperitenone oxide (21.72%), α -pinene (11.33%), carvone (11.29%), spathulenol (6.23%) and limonene (5.03%) (Table 2). The major components of *S. hortensis* oil were carvacrol (50.13%), thymol (27.77%), γ -terpinene (4.68%) and p -cymene (4.31%) (Table 3)

Table 1. Chemical constituents of essential oil from seed of *Foeniculum vulgare*

Compounds	RT*	Composition (%)
α -pinene	10.39	2.32
Camphene	10.81	0.17
Sabienne	11.37	0.45
β -pinene	11.55	2.09
Myrcene	11.67	0.78
α -phellandrene	12.20	0.2
p -cymene	12.3	2.12
Limonene	12.92	8.92
1,8-cineole	13.01	0.86
γ -terpinene	13.69	0.2
Fenchone	14.74	12.14
Camphor	16.33	0.43
Terpin-4-ol	17.20	0.07
p -allyl anisole	17.72	5.47
Fenchyl acetate	18.81	0.19
Carvone	19.06	1.13
<i>Z</i> -anethole	19.22	0.2
p -anisaldehyde	19.42	0.49
<i>E</i> -anethole	20.15	60.61

*: Retention time.

Table 2. Chemical constituents of essential oil from aerial parts of *Teucrium polium*

Compounds	RT*	Composition (%)
α -pinene	10.42	11.33
β -pinene	11.57	5.77
Myrcene	11.68	4.34
p -cymene	12.73	1.57
Limonene	12.9	5.03
<i>Z</i> -beta-ocimene	13.25	0.45
<i>E</i> -decaline	13.82	0.59
<i>Cis</i> -decaline	15.12	0.54
α -campholenal	15.66	0.84
<i>Cis</i> -verbenol	16.83	2.29
Myrtenal	17.79	1.18
Verbenone	18.17	1.67
Carvone	19.03	11.29
<i>E</i> -piperitenone oxide	19.36	1.58
<i>E</i> -anethole	20.17	1.38
Fenchyl acetate	20.26	1.68
Piperitenone	21.82	1.07
Eugenol	22.19	1.36
Piperitenone oxide	22.55	21.72
<i>Cis</i> -jasmone	23.26	0.52
Cinrolon	23.48	0.94
p -menthane-1,2,3-triol	23.53	0.45
β -caryophyllene	24.19	2.72
<i>Z</i> -beta-farnesene	24.64	1.61
Germacrene D	25.72	3.2
Bicyclogermacrene	26.10	0.92
Spathulenol	28.17	6.23
Caryophyllene oxide	28.33	3.4
β -eudesmol	29.91	4.28

*: Retention time.

Table 3. Chemical constituents of essential oil from aerial parts of *Satureja hortensis*

Compounds	RT*	Composition (%)
α -thujene	10.14	1.01
α -pinene	10.39	1.33
β -pinene	11.55	1.35
Myrcene	11.68	1.82
α -phellandrene	12.21	0.45
γ -terpinene	12.56	4.68
p -cymene	12.76	4.31
Limonene	12.89	0.48
β -phellandrene	12.95	0.3
Thymol	13.83	27.77
Terpin-4-ol	17.22	1.37
Carvacrol methyl ether	18.92	2.21
α -terpinene	20.16	0.33
Carvacrol	20.71	50.13
Carvacryl acetate	22.44	0.51
β -caryophyllene	24.19	1.15
β -bisabolene	26.12	0.8

*: Retention time.

Contact toxicity

The essential oils displayed variable toxicities against *C. maculatus* adults. Comparisons among the toxicity of three essential oils were obtained using probit analysis of data. The dose-mortality responses of *C. maculatus* adults to the oils were compared in terms of differences in slope and/or intercept of probit regressions and the LC₅₀s values (Table 4). The results for insecticidal efficacy of each essential oil showed that mortality of adults increased with oil concentration. The slopes of probit regressions were in the range of 3.68-11.52. The heterogeneity factors of all bioassays were less than 1 except those of *F. vulgare* and *T. polium* oils on males. A heterogeneity factor of more than 1 indicated that the result was not within the 95% confidence limits, requiring using the correction factor (g) for LC₅₀ estimates, whereas a heterogeneity factor less than 1 indicated that there was no sign of systematic deviations in the chi-square (χ^2) values. For all essential oils, the regression tests ("t" ratio) were greater than 1.96 and the potency estimation tests ("g" factor) were less than 0.5 at all probability levels (Table 4). The slopes of probit mortality regressions for the essential oils against male and female were significantly different, as revealed by rejection of the likelihood ratio test of parallelism ($\chi^2=88.48$, df=5, $P<0.001$). Further likelihood ratio tests on males revealed that the slope of probit mortality regression for *T. polium* oil was significantly greater than the corresponding slopes of *F. vulgare* ($\chi^2=13.08$, df=1, $P<0.001$) and *S. hortensis* oils ($\chi^2=47.32$, df=1, $P<0.001$). Furthermore, the slope of probit mortality regression for *F. vulgare* oil was significantly greater than that of *S. hortensis* oil ($\chi^2=13.36$, df=1, $P<0.001$). Similar comparisons on females showed that the slope of probit mortality regression for *T. polium* oil was significantly greater than those of *F. vulgare* ($\chi^2=8.36$, df=1, $P<0.05$) and *S. hortensis* oils ($\chi^2=47.32$, df=1, $P<0.001$). Furthermore, the slope of probit mortality regression for *F. vulgare* oil was significantly greater than that of *S. hortensis* oil ($\chi^2=10.96$, df=1, $P<0.001$) (Table 4).

Table 4. Probit analysis of contact toxicity of three essential oils to male and female adults of *Callosobruchus maculatus*

Essential oil	Adult sex	n ^a	Probit mortality-concentration		“t” ratio	Heterogeneity	g (0.95) factor	Lethal concentrations(µl.m ⁻²) (95% Confidence limit)	
			Slope±SE	Intercept±SE				LC ₅₀	LC ₉₀
<i>F. vulgare</i>	M	420	7.12±0.68	-18.45±1.77	10.48	1.39	0.097	390.38 (357.48-423.96)	590.74 (525.44-722.68)
	F	420	5.86±0.54	-15.87±1.48	10.77	0.65	0.033	513.46 (482.68-544.91)	849.87 (776.08-961.08)
<i>T. polium</i>	M	420	11.52±1.04	-35.74±3.22	11.09	1.41	0.088	1263.09 (1195.94-1332.59)	1631.67 (1515.82-1843.56)
	F	420	9.05±0.98	-28.66±3.08	9.27	0.12	0.044	1469.72 (1417.08-1525.97)	2063.44 (1903.40-2248.70)
<i>S. hortensis</i>	M	420	4.18±0.46	-11.41±1.25	9.13	0.47	0.046	535.69 (493.77-579.47)	1085.11 (944.26-1331.05)
	F	420	3.68±0.38	-10.34±1.08	9.56	0.73	0.042	640.99 (585.23-702.55)	1428.41 (1216.86-1800.12)

^a: Total number of 1-day-old adult insects tested (including control).

On the basis of likelihood ratio tests, there was no significant difference between the slopes of male and female probit mortality regressions for *F. vulgare* ($\chi^2=2.13$, df=1, $P=0.144$), *T. polium* ($\chi^2=3.05$, df=1, $P=0.081$) and *S. hortensis* ($\chi^2=0.69$, df=1, $P=0.405$) (Table 4).

The intercepts of probit mortality regressions for the three essential oils were significantly different, as revealed by rejection of the likelihood ratio test of equality ($\chi^2=585.29$, df=10, $P<0.001$). Further likelihood ratio tests of equality indicated that the intercepts between all possible paired combinations differed significantly from each other ($P<0.001$) (Table 4).

The above differences in slopes and/or intercepts of the probit mortality regressions among experimental treatments were reflected in the LC₉₀ or LC₅₀ estimates. The toxicity data with the LC₅₀ values and their respective 95% confidence limits indicated that *F. vulgare* oil had the highest contact toxicity to both sexes, while *T. polium* oil had the lowest contact toxicity (Table 5). Comparisons between susceptibility of male and female to each oil indicated that males were more susceptible than females to all three essential oils (Table 6).

Table 5. LC₅₀ ratios and their confidence limits calculated ^a for comparing contact toxicity of three essential oils to the adults of *Callosobruchus maculatus*

Variable	LC ₅₀ s ratio	95% Confidence limit
Adult sex	Tp- LC ₅₀ / Fv- LC ₅₀	
Male	3.24	3.05-3.43*
Female	2.86	2.67-3.07*
	Tp- LC ₅₀ / Sh- LC ₅₀	
Male	2.36	2.16-2.57*
Female	2.29	2.08-2.53*
	Sh- LC ₅₀ / Fv- LC ₅₀	
Male	1.37	1.25-1.51*
Female	1.25	1.12-1.39*

^a: Lower and upper 95% Confidence limits calculated as described by Robertson & Preisler (1992)

Tp: *Teucrium polium*, Fv: *Foeniculum vulgare*, Sh: *Satureja hortensis*

*: Significant difference at $P < 0.05$.

Table 6. LC₅₀ ratios and their confidence limits calculated^a for comparing susceptibility of males and females of *Callosobruchus maculatus* to contact toxicity of three essential oils

Variable	LC ₅₀ s ratios	95% Confidence limits
Essential oil	LC ₅₀ (female) / LC ₅₀ (male)	
<i>F. vulgare</i>	1.31	1.22-1.42*
<i>T. polium</i>	1.16	1.11-1.22*
<i>S. hortensis</i>	1.20	1.06-1.35*

^a: Lower and upper 95% Confidence limits calculated as described by Robertson & Preisler (1992).

*: Significant difference at $P < 0.05$.

Toxicity persistence of oils

The results of ANOVA indicated that both essential oil ($F_{2, 149} = 417.92$, $P < 0.001$) and time of introduction ($F_{4, 149} = 375.99$, $P < 0.001$) had a significant effect, but adult sex ($F_{1, 149} = 0.149$, $P = 0.700$) had no significant effect on mortality rates. All associated interaction effects, including oil vs. adult sex ($F_{2, 149} = 0.087$, $P = 0.917$), adult sex vs. time ($F_{4, 149} = 0.88$, $P = 0.479$) and oil vs. adult sex vs. time ($F_{8, 149} = 0.62$, $P = 0.764$) were not significant, except for oil vs. time ($F_{8, 149} = 22.17$, $P < 0.001$). During the 30-h treatment periods, the toxicity of tested oils did not remain constant. On the contrary, the activity of oils decreased with increasing time (Figure 1). There was significantly negative and linear association between the rates of adult mortality and exposure time for all oils (Table 7). The results revealed that the activity of *S. hortensis* oil was the most persistent on both adult sexes, while that of *T. polium* oil was the least persistent. The *T. polium* oil had very short duration toxicity; after 12 h its toxicity decreased to 34% and 38% and after 24 h, decreased to 2% and 0% on male and female, respectively. The *F. vulgare* oil was moderately persistent. Its toxicity decreased to 76% and 78% after 6 h, and to 12% and 14% after 24 h on male and female, respectively. *Satureja hortensis* oil was the most persistently toxic; mortality was 62% and 64% after 18 h and 46% and 44% after 30 h on male and female, respectively (Figure 1).

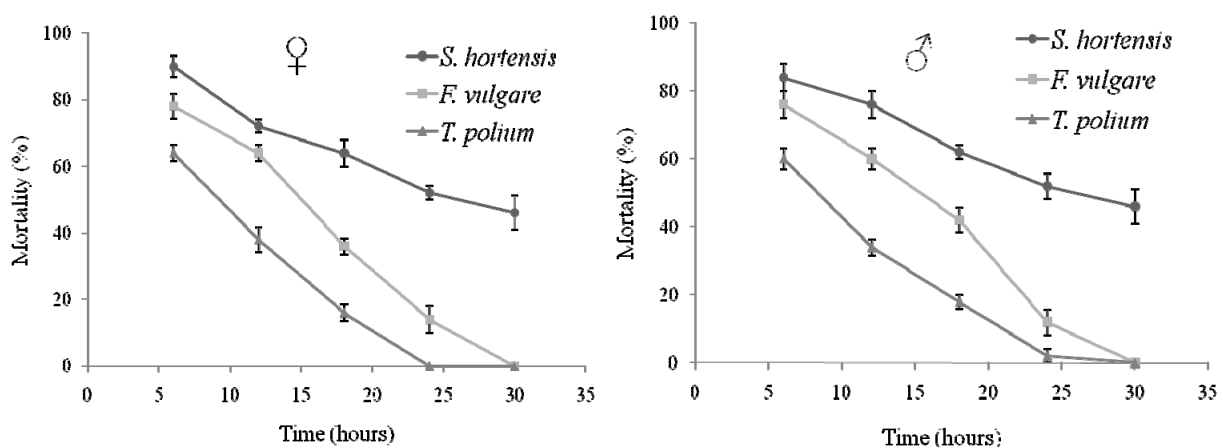


Figure 1. Mean mortality (\pm SE) of male (♂) and female (♀) adults of *Callosobruchus maculatus* exposed for 24 h to contact LC₉₉ of three essential oils at different times after oil application (untransformed data are shown).

Table 7. Linear regressions of adult mortality (%) of *Callosobruchus maculatus* on introduction time (h) of adults after application of contact LC₉₉ of three essential oils

Essential oil source	Adult sex	n ^a	Slope ± S.E. ^b	R-squared (adjusted)	F _(1,24)	P Value
<i>F. vulgare</i>	Female	250	-3.33±0.18	0.93	328.57	<0.001
	Male	250	-3.43±0.16	0.95	436.51	<0.001
<i>T. polium</i>	Female	250	-2.53±0.18	0.89	196.52	<0.001
	Male	250	-2.78±0.21	0.88	176.25	<0.001
<i>S. hortensis</i>	Female	250	-1.67±0.19	0.75	71.87	<0.001
	Male	250	-1.87±0.18	0.82	107.33	<0.001

^a: Total number of 1-day-old adult insects tested.

Discussion

Over 120 plants and plant products have been demonstrated to have insecticidal or deterrent activity against stored product pests (Dales, 1996). Many producers in parts of Asia and Africa have used some of these botanicals to protect their legumes from attack by bruchids, with varying degrees of success (Singh, 1990; Dharmasena et al., 1998). The essential oils extracted from many species of Apiaceae and Lamiaceae have shown strong insecticidal effects on stored-product pests (Kim & Ahn, 2001; Chaubey, 2006, 2007; Taghizadeh-Saroukolai et al., 2010). It has been reported that essential oils extracted from *Thymus persicus* Ronniger ex Rech. (Taghizadeh-Saroukolai et al., 2010), *Ziziphora clinopodioides* Boiss. (Lolestani & Shayesteh, 2009) and *Hyptis spicigera* L. (Ngamo et al., 2007) from Lamiaceae and *Trachyspermum ammi* L., *Anethum graveolens* L. and *Cuminum cyminum* L. (Chaubey, 2006, 2007) from Apiaceae, were relatively effective against several stored product insects.

In this study, the essential oils extracted from *F. vulgare*, *T. polium* and *S. hortensis* demonstrated insecticidal activity against male and female adults of *C. maculatus*. The insecticidal activity of *F. vulgare* and *S. hortensis* oils has been reported against *Lipaphis pseudobrassicae* Davis (Sampson et al., 2005), *Ephestia kuehniella* (Zell.) and *Plodia interpunctella* (Hübner) (Mollaei et al., 2011). Also, *T. polium* oil showed larvicidal effect on *Musca domestica* (Bigham et al., 2010).

For all oils, there was a positive relationship between oil concentration and adult mortality (Table 4). A similar relationship between the rate of mortality and concentration has been demonstrated in bioassays of many insects with various oils (Kim et al., 2003; Moravvej & Abbar, 2008; Moravvej et al., 2010; Ndomo et al., 2010). Based on the LC₅₀ values, the males were much more susceptible to all oils than the females (Tables 4 and 6). Our results are in accordance with those of Papachristos & Stamopoulos (2002) who reported a greater susceptibility of *Acanthoscelides obtectus* Say males than females to the essential oils extracted from *Mentha viridis* L. and *M. microphylla* C. Koch. In addition, Mollah and Islam (2007, 2008) reported a higher susceptibility of adult males of *C. maculatus* to *Thevetia peruviana* (Pers) Schum. and *Murraya paniculata* (L.) extracts, as compared to females. It is suggested that the differences in susceptibility of males and females are the result of the size difference and body weight, rather than an innate difference in mode of action (Weaver et al., 1991, 1994; Papachristos & Stamopoulos, 2002). Furthermore, it was demonstrated that reduction of body weight was an important factor in increasing susceptibility to insecticides in many insects (Selander et al., 1972). The *F. vulgare* oil exhibited the highest toxicity to adult beetles, by having the lowest LC₅₀ and LC₉₀ values at 24-h exposure (Tables 4 and 5). Based on LC₅₀ values, the contact toxicities of oils in descending order were from *F. vulgare*, *S. hortensis* and *T. polium* (Table 5). Similar results on varying toxicities among different plant species had been reported (Papachristos & Stamopoulos, 2002; Kim et al., 2003; Moravvej et al., 2010).

It is suggested that the variability of biological activities among essential oils extracted from different plant species could be due to differences in their chemical composition (Casida, 1990; Tapondjou et al., 2005; Ilboudo et al., 2010). The chemical composition of essential oils is generally characterized by the presence of mono- and sesquiterpene compounds. Even if essential oils extracted from different plant species share several compounds, the proportions of these compounds can vary and affect their biological activity. The essential oil of *F. vulgare* showed very high insecticidal activity, the most important component of which is *E*-anethole (Table 1). The oil of *T. polium* showed less activity than the other two oils. The main chemical constituents of this oil included *E*-piperitenone oxide, α -pinene and carvone (Table 2). The oil of *S. hortensis* exhibited relative strong insecticidal activity. The oil had a high content of carvacrol and thymol (Table 3). Taken together, these results suggest that *E*-anethole could be partly responsible for the high insecticidal activity of *F. vulgare* oil. The high toxic effect of this compound had been reported against *Sitophilus oryzae* L, *Callosobruchus chinensis* (L), *Lasioderma serricorne* F., *Tribolium castaneum* Herbst and *Spodoptera litura* Fab. (Ho et al., 1997; Hummelbrunner & Isman, 2001; Kim & Ahn, 2001). Hummelbrunner and Isman (2001) also reported that *E*-anethole acts synergistically with other monoterpenoid substances, including thymol, citronellal, α -terpineol and γ -terpinene against *Spodoptera litura*. It therefore seems that the insecticidal activity of each essential oil could be result of interaction between all its chemical constituents, rather than the activity of individual main components. It has been suggested that the components of oils found in low percentages may act as synergists that increase the effectiveness of the major constituents through a variety of mechanisms (Hummelbrunner & Isman, 2001; Ngamo et al., 2007; Ilboudo et al., 2010).

The steepness of slope in probit mortality regression in descending order was *T. polium*, *F. vulgare* and *S. hortensis* (Table 4). A steep slope value indicates that there is a large increase in the mortality of insects with a relatively small increase in the concentration of toxicants (Robertson & Preisler, 1992; Tiwari & Singh, 2004). That was true for the contact toxicity of *T. polium* oil within the experimental conditions of the present study. The higher LC₅₀ value of *T. polium* compared to other species was due to the higher concentrations of them needed for calculation of probit regression.

The results of persistence testing of oils showed that their toxicity decreased with time. Our findings are in accordance with those of Ngamo et al (2007) and Miresmailli & Isman (2006). The biological activity of all essential oils tested in the present study was lost in a relatively short time and did not exceed 30 h, except for that of *S. hortensis* oil (Figure 1). Ngamo et al. (2007) showed that the persistence of the biological activity of essential oils extracted from *Hyptis spicigera* and *Lippia rugosa* L. towards four stored-product pests, including *C. maculatus*, did not exceed 24 h. The loss of biological activity of essential oils was probably due to their high volatility and the quick degradation of active compounds. During use of oils, many factors negatively affect their activity. It was reported that oxidation of mono- and sesquiterpene compounds could occur, leading to the loss of their biological activity (Ngamo et al., 2007; Ilboudo et al., 2010).

The results of the current study showed that the rate of reduction in insecticidal activity was not the same for all three essential oils tested. The oil of *S. hortensis* was the most persistent and *T. polium* oil was the least persistent against male and female adults. It was suggested that persistence of the insecticidal activity of essential oils depends on its chemical composition (Obeng-Ofori et al., 1997). The essential oils with a high content of hydrogenated compounds are the most susceptible to oxidation and lose their activity quicker than those containing mainly oxygenated compounds (Huang & Ho, 1998; Regnault-Roger et al., 2002). Therefore, the higher persistency of *S. hortensis* oil was probably due to its high content of oxygenated monoterpenes compared to the other oils (Table 8).

Table 8. Relative amounts (%) of oxygenated and hydrocarbon monoterpene compounds of *Foeniculum vulgare*, *Teucrium polium* and *Satureja hortensis* essential oils

Essential oil Source	HMT(%)	OMT(%)	HMT	OMT
<i>F. vulgare</i>	17.25	75.37	α -pinene, camphene, sabienne, β -pinene, myrcene, α -phellandrene, p -cymene, limonene, γ -terpinene	1,8-cineole, fenchone, camphor, carvone, Z-anethole, E-anethole
<i>T. polium</i>	28.49	41	α -pinene, β -pinene, myrcene, p -cymene, limonene, Z-beta-ocimene	<i>cis</i> -verbenol, verbenone, carvone, E-piperitenone oxide, E-anethole, piperitenone, piperitenone oxide, cinerolon
<i>S. hortensis</i>	16.06	79.27	α -thujene, α -pinene, β -pinene, myrcene, α -phellandrene, γ -terpinene, p -cymene, limonene, β -phellandrene, α -terpinene	thymol, terpin-4-ol, carvacrol

OMT: Oxygenated monoterpenes
HMT: Hydrocarbon monoterpenes.

In conclusion, the results of present study demonstrated that the insecticidal activity of the three essential oils, especially that of *F. vulgare* and *S. hortensis*, was considerable, and that persistence of the insecticidal activity of the latter was the highest. Therefore, these oils can be considered as potential alternatives for the post-harvest control of *C. maculatus*. However, further research is needed in order to determine their mode of action and find practical methods of application that maximize their persistence.

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