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Original article (Orijinal araştırma)

Repellency of three plant essential oils against red flour beetle *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae)

Un biti, *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae)'a karşı üç bitki esansiyel yağının kaçırıcı etkileri

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Summary

Tribolium castaneum (Herbst, 1797) is an insect pest found in stored products. To control this pest, it is necessary to develop safe alternatives to replace hazardous fumigants. This research aimed to determine the repellency of essential oils from three different plants, *Hypericum hemsleyanum*, *Mentha haplocalyx* and *Stemona japonica*, against *T. castaneum* adults under laboratory conditions. The repellency of essential oils was determined by area preference method at a concentration of 31.5 μg/cm^2. Filter paper disks were placed in petri dishes, one half was treated with essential oil and other half served as control. Twenty *T. castaneum* adults were placed in the center of each paper disk. Insects were counted in treated and control areas at 12, 24, 48, and 72 h after insect release. *Hypericum hemsleyanum* and *M. haplocalyx* showed the strong repellency at all assessment times, with values of 94, 71, 69 and 70%, and 91, 65, 73 and 83% at 12, 24, 48 and 72 h, respectively, followed by *S. japonica*. This research showed that these oils are strong repellents and can potentially be used to repel *T. castaneum* in stored products.

Keywords: Ethanol extracted, *Hypericum hemsleyanum*, *Mentha haplocalyx*, red flour beetle, repellence, *Stemona japonica*

Özet

Tribolium castaneum (Herbst, 1797) depolanmış ürünlerde bulunan zararlı bir böcek türüdür. Bu zararlıyı kontrol etmek için, tehlikeli fumigantlar yerine güvenli alternatiflerin geliştirilmesi gerekmektedir. Bu araştırma laboratuvar koşullarında *T. castaneum* erginlerine karşı üç farklı bitki, *Hypericum hemsleyanum, Mentha haplocalyx* ve *Stemona japonica* esansiyel yağların kaçırıcı etkilerini belirlemek amacıyla yapılmıştır. Uçucu yağların kaçırıcı özelliği, alan tercihi yöntemi ile 31.5 μg/cm^2'lik bir konsantrasyonda belirlenmiştir. Filtre kağıdı diskleri petri kabına yerleştirilmiş ve bir yarısı uçucu yağ ile muamele edilmiş, diğer yarısı ise kontrol olarak kullanılmıştır. Yirmi adet *T. castaneum* ergini her bir kağıt diskin ortasına yerleştirilmiştir. Böceklerin sayımı, kontrol ve uygulama alanında böcek salınmasından 12, 24, 48 ve 72 saat sonra yapılmıştır. *Hypericum hemsleyanum* ve *M. haplocalyx*, tüm değerlendirme zamanlarında en güçlü kaçırıcı etkiyi göstermiş; 12, 24, 48 ve 72 saat sonra sırasıyla %94, 71, 69, 70 ve %91, 65, 73, 83 değerleri elde edilmiş olup; ve *S. japonica* bunları takip etmiştir. Bu araştırma, bu yağların güçlü kaçırıcılar olduğunu ve depolanmış ürünlerde *T. castaneum*'u uzaklaştırmak için potansiyel olarak kullanılabilir olduğunu göstermiştir.

Anahtar sözcükler: Ethanol ekstraktı, *Hypericum hemsleyanum*, *Mentha haplocalyx,* un biti, kaçırıcı etki, *Stemona japonica*

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Introduction

In stored products worldwide, insect pest infestation may cause up to 40% damage (Matthews, 1993). *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) is known as the most common insect pest in stored food for human and animal consumption. It affects a wide range of products, including beans, cacao, dried, flour, fruits, grain, nuts, peas and spices. The presence of both adults and larvae in stored food directly affects the quality and quantity of products (Campbell & Runnion, 2003). Insects may cause damage to seed embryos, resulting in decreased germination (Baier & Webster, 1992; Moino et al., 1998). Therefore, control of stored-product pests is necessary to provide a stable and safe food supply at affordable prices (Nadeem et al., 2012; Ukeh et al., 2012; Jahromi et al., 2014). Control of pests in stored products relies on gaseous fumigants, including hazardous chemicals, such as methyl bromide or phosphine. There is a global concern about the negative effects of these chemicals, including direct toxicity to users, increasing cost of application, environmental pollution, resistance to pesticides and pest resurgence, and toxicity to non-target organisms (Lee et al., 2004; Isman, 2006).

Considering the damage caused by *T. castaneum*, there is a need to develop and commercialize safe alternatives to replace hazardous insecticides. To protect the environment and avoid bad ecological effects, researchers have focused on the new ways of carrying out insect pest management in grain stores; moreover, they have diverted their attention toward the use of organic products as pesticides, such as plant extracts (Rajendran & Sriranjini, 2008). Plant essential oils are complex mixtures of a large number of chemical constituents in variable proportions (Van Zyl et al., 2006), they have bioactivities against bacteria fungi, insects, nematodes and viruses (Negahban et al., 2007; Kotan et al., 2008; Park et al., 2008; Razzaghi-Abyaneh et al., 2008). Plant essential oils are environmentally friendly and biodegradable; furthermore, they do not persist in soil and water and are easily extractable (Isman, 2000; 2006).

In the current study, the repellency of essential oils from three different plants, *Hypericum hemsleyanum* H. Lév. & Vaniot, *Mentha haplocalyx* Briq., *Stemona japonica* (Blume) Miquel against *T. castaneum* adults was determined under laboratory conditions. The results will provide data useful for the development of new repellants for stored-product pests.

Materials and Methods

Insects

Tribolium castaneum was reared on wheat flour in the Hubei Insect Resource Utilization and Sustainable Pest Management Key Laboratory of Huazhong Agricultural University, China. *Tribolium castaneum* adults were maintained in glass jars (250 mL) containing wheat flour and 5% yeast; jars were covered with black cloth to provide air and darkness at the top. The insects were reared in the laboratory at 25°±2°C and 50±5% RH with a 14 L:10 D photoperiod.

Ethanol-extracted botanical oils

Plant materials were bought from a franchised outlet of Beijing Tongrentang Group, China. The plant materials, foliage of *M. haplocalyx*, and roots of *H. hemsleyanum* and *S. japonica,* for essential oil extraction were dried in the sunlight then stored at 25°C. Samples were extracted according to the methods of Su et al. (2009) and Yao et al. (2011). Before extraction, plant parts were dried in an oven at 45°C for up to 3 days. Plant parts were crushed into a powder then passed through a 0.425 mm aperture sieve. Samples (1 g) were then suspended in 95% ethanol (5 mL) and incubated in dark at 20±5°C for 7 days, mixing with a vortex mixer twice per day. The solvent was removed with a Buchner funnel, the filtrate was stored at room temperature and solid residue re-extracted in 2.5 mL of 95% ethanol using the same procedure. Filtrates from the first and second extraction were combined and concentrated to dryness with rotary evaporator. The extracts were then weighed and stored in brown collection bottles and stored at 4°C. Crude oils were dissolved in dimethyl sulfoxide (0.05 g oil + 0.3 mL dimethyl sulfoxide + 1% Tween-20), then distilled water added to a final volume of 50 mL, to produce the working solution of essential oil.

Area preference test

Area preference tests were performed using the area preference method of Tapondjou et al. (2005) with modifications. Working solution (0.1 mL) of essential oil was uniformly applied to half a filter paper disk to a final concentration of 31.5 µg/cm^2. The same volume of the solute without essential oil was applied to the other half to serve as a control. Paper disks were placed in 90-mm petri dishes and the solvent allowed drying. One hour after the application, 20 adults of *T. castaneum* were placed in the center of each paper disk. The dishes were covered with black plastic to provide darkness and placed in the same environmental conditions as for rearing. Insects were counted in treated and control areas at 12, 24, 48 and 72 h after insect release. With 20 insects per dish and 8 replicate dishes of each essential oil, a total of 480 insects were used.

Gas chromatography and mass spectrometry

The essential oil components were separated and identified by gas chromatography mass spectrometry (GC-MS) on a Varian 450-GC/320-MS (Varian Medical Systems, Inc. Palo Alto, CA, USA) according to Karina *et al*. (2014). The constituents were identified from the gas chromatography using MANLIB, REPLIB, PMWTox3N and Wiley (NIST, 2011).

Data analysis

The following equation was used to calculate the percent repellency: PR (%) = [(C-T) / (C+T)] × 100 (Liu et al., 2013). Analysis of variance (ANOVA) and Tukey's post hoc tests were used to compare the mean percentage of repellencies (PRs) between essential oils. Paired t-tests were used to compare the mean number of insects in the treated and untreated areas of the filter paper disk. Statistical analysis was performed using SPSS version 20 (IBM Corp. Armonk, NY, USA), with a significance level of $p < 0.05$. The percentage data were arcsine square root transformed, and all count data were square root (x+1) or log10(x+1) transformed before analysis. The untransformed means are presented in the results.

Results

Repellency of essential oils

The results of the *t*-test show that *M. haplocalyx* oil maintained strong repellency during the entire assessment period. Its repellency was significantly higher than the control at all assessment times. It was the least repellant at 12 h (F=30.92, df=7, *P*<0.01) and 24 h (F=24.32, df=7, *P*<0.01) after insect release, but its repellency to best at 48 h (F=29, df=7, *P*<0.01) and 72 h (F=33, df=7, *P*<0.01) (Figure 1). The repellency of *H. hemsleyanum* oil was significantly higher than the control during the entire assessment period. *Hypericum hemsleyanum* oil had the strongest repellency of *T. castaneum* adults at 12 h (F=51.23, df=7, *P*<0.01) and 24 h (F=31.45, df=7, *P*<0.01), but its repellency decreased to second at 48 h (F=23.30, df=7, *P*<0.01) and 72 h (F=26.19, df=7, *P*<0.01) (Figure 1). Essential oil from *S. japonica* was significantly different from the control; its repellency was second at 12 h (F=51.23, df=7, *P*<0.01) and 24 h (F=25.18, df=7, *P*<0.01). However, at 48 and 72 h, its repellency had declined, and it was no longer significantly different from the control (Figure 1).

Duration of repellency

The essential oils of *M. haplocalyx*, *H. hemsleyanum* and *S. japonica* tested against *T. castaneum* exhibited high repellency at 12 h (F=0.53, df=4, *P* > 0.01), 24 h (F=50.02, df=4, *P*<0.01), 48 h (F=272.24, df=4, *P*<0.01) and 72 h (F=619.81, df=2, *P*<0.01).

Mentha haplocalyx acted as a strong repellent against *T. castaneum* adults at 12, 24, 48 and 72 h after insect release, with mean percentages of 91, 65, 73, and 83, respectively (Figure 2). However, *H. hemsleyanum* had second repellency; it was the strongest repellent at 12 and 24 h, with values of 94 and 71%, respectively, and second repellent at 48 and 72 h with values of 69 and 70%, respectively (Figure 2). Finally, *S. japonica* exhibited 94 and 66% repellency at 12 and 24 h after insect release, but it had lost all repellency by 48 and 72 h (Figure 2).

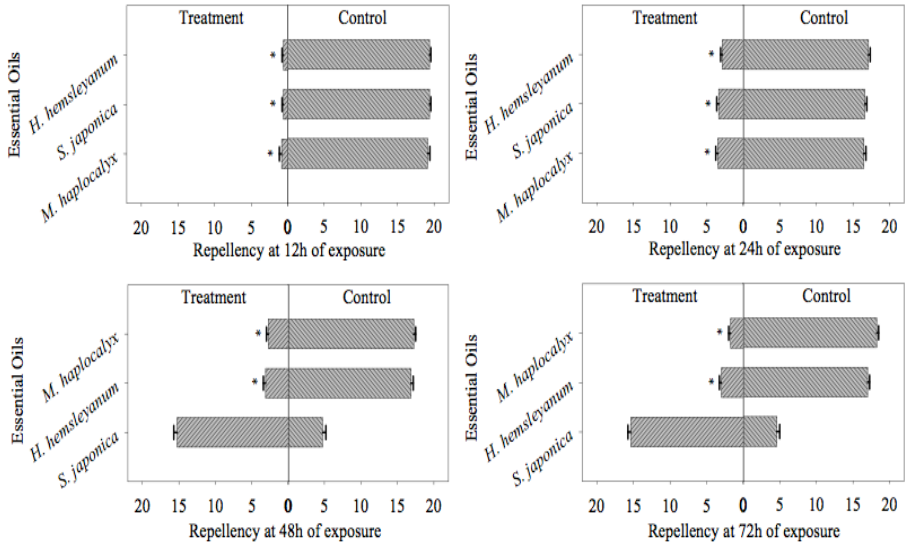

Figure 1. Mean number and SE of repellency at 12, 24 48 and 72 h of *Tribolium castaneum* adults' release. Values are means of 8 replicates (20 insects/replicate). The mean numbers of adults in the treated and control were analyzed by paired t-test at significance level of $P < 0.05$.

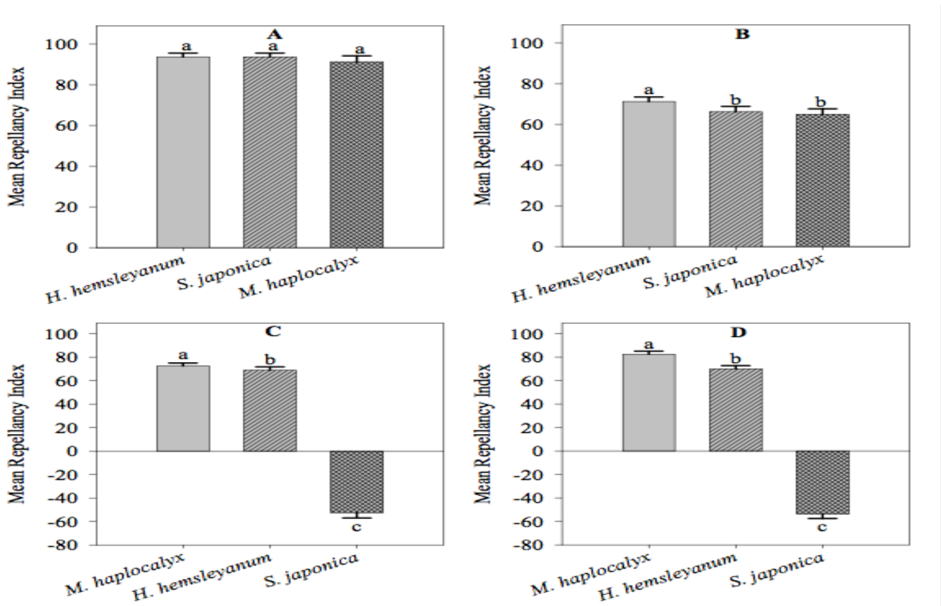

Figure 2. Percentage (mean ± SE) of repellency of *Tribolium castaneum*: A: 12 h after insect release, B: 24 h after insect release, C: 48 h after insect release, D: 72 h after insect release. Values are means of 8 replicates. The mean numbers of adults were analyzed by one-way ANOVA, using a Tukey HSD post-hoc test at significance level of $P < 0.05$.

Gas chromatography and mass spectrometry

After recording strong repellency for the essential oils tested, the oils were analyzed by GC-MS. The results revealed complex mixtures of chemical constituents with nine major components identified in each oil. The primary chemicals identified from *H. hemsleyanum*, *M. haplocalyx* and *S. japonica* oils are presented in Table 1.

Table 1. Chemical components of essential oils based on GC-MS assay

Components	Retention Time (minutes)	Percent of Total (%)
Hypericum hemsleyanum oil		
Phenol, 3-methyl	7.26	2.4
Palmitic acid	12.64	2.6
Hexadecanoic acid, ethyl ester	12.73	1.0
2H-1-benzopyran	13.45	0.7
9,12-Octadecadienoic acid (Z,Z)	13.49	5.1
Linoleic acid ethyl ester	13.55	1.9
Osthole	13.61	35.6
Lomatin acetate	14.47	0.3
1,2-dihydrocyclobuta[b]anthracen-1-one	15.05	6.7
Mentha haplocalyx oil		
L-(-)-menthol	7.85	8.3
Cyclohexanol, 5-methyl-2-(1-methylethyl)	7.89	2.0
2-Hexadecen-1-ol, 3,7,11,15-tetramethyl	11.94	1.2
Hexadecanoic acid	12.64	4.4
Hexadecanoic acid, ethyl ester	12.74	1.2
Phytol	13.33	1.3
9,12-Octadecadienoic acid (Z,Z)	13.49	1.6
9,12,15-Octadecatrienoic acid, (Z,Z,Z)	13.52	4.5
24(Z)-Methyl-25-homocholesterol	23.88	1.4
Stemona japonica oil		
4-Vinylphenol	8.40	0.3
2-Furancarboxaldehyde, 5-(hydroxymethyl)	8.53	0.7
dl-Stenine	14.17	0.9
9,10-Anthracenedicarbonitrile	16.26	0.4
1-Tert-butyl-5-methoxy-2,2-dimethylindan	17.59	7.6
Benzo[a]naphthacene	21.13	1.6
Methyl 4,5,7-trimethoxy-2-naphthoate	21.55	5.7
Stemonine	22.33	31.2
Syn-7-benzhydrylbicyclo[2.2.1]heptan-2-one	23.28	3.3

Discussion

In this study the repellency of the three essential oils, *M. haplocalyx* oil was the most repellent for the targeted insect species, and its repellency was maintained throughout the assessment period. Previous studies showed repellency effects from *Mentha* sp. against many insect and non-insect pests. El-Seedi et al. (2012) investigated the oils of *Mentha* sp. which showed strong repellency (93.2% using a 15 μg/cm^2 concentration in a lab test and 59.4% using 6.5 μg/cm^2 on test cloths in the field) against ticks, *Ixodes ricinus* (L., 1758). A 14-d experiment was conducted in Ebeling choice boxes to determine the toxicity and repellency of *Mentha* oil to American cockroaches (*Periplaneta americana* (L., 1758)) and German cockroaches (*Blattella germanica* (L., 1767)); it showed 100% repellency to both species during each day of the experiment (Appel et al. 2001). Ren et al. (2007) reported that *M. haplocalyx* oil showed bioactivity, such as repellency, insecticidal properties, and growth and reproduction regulation, against numerous insect pests. *Mentha haplocalyx* contains the active components of hexadecanoic acid ethyl ester, menthol and phytol. Hexadecanoic acid ethyl ester (palmitic acid ester) and linoleic acid ethyl ester possess antioxidant, pesticide, and nematicide properties (Jananie et al., 2011). Menthol blocks voltage-sensitive sodium channels, reducing neural activity that may stimulate muscles (Haeseler et al., 2002). Phytol chemical is a natural bioactive compound in plants that acts in a defense systems against diseases (Krishnaiah et al., 2009). At 12 and 24 h *M. haplocalyx* oil had a slightly lower repellency index compared to the other two oils; however, its repellency persisted for a longer time and showed maximum repellency on the repellency index at 48 and 72 h. So it is concluded that *M. haplocalyx* oil is a strong and persistent repellent against the target insect. The components of *M. haplocalyx* essential oil, which are active against T. castaneum, need to be studied further.

Hypericum hemsleyanum oil was the second most repellent to *T. castaneum* adults. Previous studies have also shown its repellency and toxicity against some insect pests. Moore & Debboun (2007) reported that most of the *Heracleum* species suppress the growth of some flies and mosquitoes, because they contain furanocoumarins. However, aqueous and aqueous-alcohol extracts from the *Hypericum* sp. were not repellent to *Sitophilus oryzae* (L., 1763), but rather they were attractants for this stored-product pest (Ciepielewska et al., 2005). Major chemical components of *H. hemsleyanum* were identified with GC-MS analysis in the current study included hexadecanoic acid ethyl ester, linoleic acid ethyl ester, methyl phenol and osthole. Jegadeeswari et al. (2012) demonstrated that hexadecanoic acid has antioxidant activity. Hexadecanoic acid ethyl ester (palmitic acid ester) and linoleic acid ethyl ester possess antioxidant, pesticide and nematicidal characteristics (Jananie et al., 2011). In our GC-MS result, osthole and methyl phenol were the major chemical components of *H. hemsleyanum* essential oil, probably these chemicals are important for repellency against *T. castaneum*, but there are no previous reports on bioactivity of osthole and methyl phenol.

Stemona japonica showed repellency up to 24 h. However, by 48 h its repellency was lost. So it had no long-lasting repellency against the target pest. Extracts of roots and leaves of *Stemona* sp. have been shown to have repellent, antifeedant, and insect toxicity activities against larvae of *Spodoptera littoralis* (Boisduval, 1833) (Brem et al., 2002). In the GC-MS result, the dominate chemical components of *S. japonica* were 1-tert-butyl-5-methoxy-2,2-dimethylindan, stemonine, and methyl 4,5,7-trimethoxy-2-naphthoate. Brem et al. (2002) demonstrated that tuberostemonine has outstanding repellent activity against larvae of *S. littoralis.* There is no published research on the bioactivity of 1-tert-butyl-5-methoxy-2,2-dimethylindan and methyl 4,5,7-trimethoxy-2-naphthoate. This is the first report of repellent activity of *S. japonica* oil against *T. castaneum*.

Conclusion

The results of our research on the repellency of essential oils proved that these oils are strong repellents that can be effectively used to repel adults of *T. castaneum*. *Mentha haplocalyx* and *H. hemsleyanum* showed repellency throughout the duration of the experiment. However, the repellency of *S. japonica* was lost with 48 h. These results could be helpful for the management of pests in stored products. Further research is required to determine the individual bioactivity of the chemical components of these essential oils against store-product pests, including the fumigant and contact toxicity of these essential oils and their chemical components against larval stage of *T. castaneum*.

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