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Research Article

Insecticidal activities of some essential oils against larval ectoparasitoid, *Habrobracon hebetor* (Hymenoptera: Braconidae)

Jabraeil Razmjou*, Vahid Mahdavi, Hooshang Rafiee-Dastjerdi, Arash Farhoomand and Samira Molapour

Department of Plant Protection, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran.

Abstract: *Habrobracon hebetor* Say is an idiobiont and gregarious larval ectoparasitoid of many moths. In this study, lethal and sublethal effects of *Eucalyptus camaldulensis, Carum carvi* and *Heracleum persicum* essential oils on the demographic parameters of *H. hebetor* were assessed at 26 ± 2 °C, 60 ± 5% RH, and a photoperiod of 16:8 (L: D) h. Essential oils were obtained from these plants by hydro-distillation method using a Clevenger apparatus. The chemical constituents of essential oils were detected by Gas Chromatography-Mass spectrometry (GC-MS). 250ml Glass vials were used for the fumigant toxicity experiments. In order to assess the sublethal effects, adult wasps were exposed to an LC_{25} of each essential oil and then the demographic parameters of live parasitoid wasps were studied. Fumigant toxicity with adults indicated that the lethal concentration (LC_{50}) values of the above essential oils against *H. hebetor* females were 1.116, 0.34 and 3.416µl/l air, respectively. Chemical analysis by GC-MS displayed o-Cymene (15.11%), Carvone (55.8%) and Hexyl butyrate (41.78%) were main constituents of the essential oils of *E. camaldulensis*, *C. carvi* and *H. persicum*, respectively. The results showed that the intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R_0) and gross reproductive rate (GRR) were significantly affected by the essential oils. The highest and the lowest r values were 0.226 and 0.130 day^{-1} in control and *C. carvi*-treated insects, respectively. According to these results, essential oils have suitable potential for the integrated management of stored product pests.

Keywords: Essential oils, fumigant toxicity, GC-MS, parasitoid wasp

Introduction

Plants may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals (Wink, 1993). Essential oils are volatile natural complex secondary metabolites characterized by a strong odor and have a generally lower density than that of water (Bakkali *et al.,* 2008). In stored-product insect pest control, essential oils may have numerous types of effects (Papachristos and Stamopoulos, 2002): they may have a fumigant activity (Lee *et al.,* 2004; Mahdavi *et al.,* 2017), they may act as repellents (Ndungu *et al.,* 1995), they may act as anti-feedants (Huang *et al.,* 1997) or may affect some biological parameters such as growth rate, life span and reproduction (Pascual-Villalobos, 1996). Since these oils are often biodegradable to non-toxic products, and are potentially suitable for use in integrated pest management, knowledge of their composition could lead to the development of new classes of safer insect-control agents (Kim and Ahn, 2001).

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* **Corresponding author**, e-mail: razmjou@uma.ac.ir
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It has been suggested that essential oils could be used in conjunction with biological control on the integrated pest management program (González *et al.,* 2013). On the other hand, integrating the application of biocontrol agents and pesticides for pest management requires knowledge about the impact and selectivity of the pesticides, on natural enemies (Croft, 1990). *Habrobracon hebetor* Say (Hymenoptera: Braconidae) is one of the most important parasitoids of the larval stage of many important agricultural pests such as the Noctuid and Pyralid moths (Baker *et al.,* 1995; Dweck *et al.,* 2008). In 1961, in Varamin, Iran, the *H. hebetor* was first collected by Farahbakhsh (Navaei *et al.,* 2002). In Iran, mass rearing of *H. hebetor* is done on Mediterranean flour moth, *Anagasta kuehniella* (Zeller) (Attaran, 1996). Although a high volume of informative literature has been published regarding the effects of essential oils on pest species (Moawed and Ebadah, 2007; Negahban *et al.,* 2007; Ayvaz *et al.,* 2010) little information is available on its effects on parasitoids (González *et al.,* 2013).

Since populations of different insect species respond differently to equal levels of stress (Stark and Banks, 2004), lethal concentrations of pesticides cannot be used to compare the effects of toxicants when the time interval for analysis is more than a few days. In fact, population effects will largely depend on life history traits. It is difficult to measure both lethal and sublethal effects in a meaningful way that scientists and IPM practitioners can understand. Demographic toxicology is usually considered to be an important tool for accurate assessment of global effects of pesticides (Stark and Banks, 2004). The intrinsic rate of increase (*r*) has been recommended to assess overall effects of pesticides, because it is based on both survivorship and fecundity parameters (Stark and Wennergren, 1995). Limited information is available on the lethal and sublethal effects of essential oils on *H. hebetor* (Hashemi *et al.,* 2014).

The purpose of this study was to assess the lethal and sublethal effects of *Eucalyptus camaldulensis, Carum carvi* and *Heracleum persicum* essential oils on the ectoparasitoid *H. hebetor* for determining possible compatibility of these essential oils with *H. hebetor* in IPM programs.

Materials and Methods

Insect rearing

The *H. hebetor* colony was obtained from the Department of Plant Protection, University of Mohaghegh Ardabili, Iran. The parasitoid wasps were reared on fifth-instar larvae of *A. kuehniella* at 26 ± 2 °C, 60 ± 5% relative humidity (RH) and under a photoperiod of 16:8 (L: D) h. Honey, smeared on a strip of paper, was provided to the adults as a food source.

Plant materials and extraction of essential oils

C. carvi was purchased from local market of Ardabil city. *E. camaldulensis* and *H. persicum* were collected from Sari and Meshkin-Shahr cities, respectively. Essential oil was extracted from the plant samples using a Clevenger-type apparatus where the plant material is subjected to hydro-distillation. Conditions of extraction were 50g of samples, 1: 10 plant material/water volume ratio, and a three-hour distillation. The oil was dehydrated with anhydrous sodium sulphate (10min). Extracted oil was stored in a refrigerator at 4 °C.

Analysis of essential oils

The oil composition was analyzed by gas chromatography-mass spectrometry (GC-/MS; Agilent, 7890 B series, USA). Identification of the spectra was carried out by studying their patterns of fragmentation and also their coincidence with the standard spectra present in the library of the instrument.

Fumigant toxicity

Bioassay trials were carried out following Negahban *et al.,* (2007) technique. The fumigant toxicity of *C. carvi*, *E. camaldulensis* and *H. persicum* essential oils on *H. hebetor* female adult stage were tested in glass vials (250mL) in which 20 young females (< 24h old) were released. Filter paper disks (Whatman

No. 1) were cut into 2-cm diameter pieces and fixed under the glass vial screw caps. Filter papers were impregnated with a series of concentrations of each essential oil. Four replicates were run for each concentration and for the control groups. Numbers of dead and live insects were also counted after 24 h from the start of exposure. The control did not show any mortality. The result of each trial was tested for curve fit using PROC GENMOD procedures (Robertson *et al.,* 2007; SAS Institute, 2002), and the data were analyzed using PROC PROBIT (SAS Institute, 2002) in order to determine lethal concentrations (LC_{25}, LC_{50}, and LC_{90} values) with associated 95% fiducial limits.

Sublethal effects

To determine the life table parameters of *H. hebetor* after exposure to sublethal concentrations of essential oils, mated young females (< 24h old) were exposed to an LC_{25} concentration of essential oils (0.92µl/l air for *E. camaldulensis,* 0.2µl/l air for *C. carvi* and 2.788µl/l air for *H. persicum*). Directly after 24 h exposure period, 25 randomly selected live females were transferred individually to plastic Petri dishes (60 mm in diameter) and then paired with a young male (< 24h old) to ensure sufficient mating during their life span. The wasps were provided with honey as food. The survival and fecundity of each female were recorded daily. Seven fifth instar host larvae were supplied daily for oviposition. Parasitized larvae were kept at the rearing conditions described above. The total number of eggs, hatched number of eggs and the sex of the emerged wasps at adulthood were recorded. Daily schedules of mortality and fecundity were integrated into a life table format (Carey, 1993) and used to calculate net reproductive rate (R_0), mean generation time (T), intrinsic rate of increase (r), and finite rate of increase (λ) values. Statistical analysis of the life table parameters was performed using the SAS software (SAS Institute, 2002). Total egg, egg hatch rate, offspring sex ratio [female/(male + female)] and longevity data were analyzed by ANOVA with mean separation at a 5% level of significance by Tukey test.

Results

Chemical constituents of essential oil

The chemical constituents of the essential oil of *E. camaldulensis*, the retention indices and the percentage of the individual components are summarized in Table 1. Thirty-one components were identified representing 92.73% of the total detected constituents. o-Cymene (15.11%), Spathulenol (14.73%), Cryptone (9.23%) and Terpinene-4-ol (7.08%) were the major constituents of the essential oil. Also, Chemical analysis of the essential oil of *C. carvi* revealed 15 components (98.61%) in which Carvone (55.8%) and Limonene (29.17%) were the major constituents (Table 2). 22 compounds were identified in the essential oil of *H. persicum* representing 96.99% of the total essential oil, with Hexyl butyrate (41.78%) and Octyl acetate (27.83%) as the major constituents (Table 3).

Table 1 Chemical composition of essential oil of *Eucalyptus camaldulensis*.

Compound	RI[1]	Content (%)
α-Pinene	939	1.48
Sabinene	975	4.49
β-Pinene	979	1.91
β-Myrcene	991	0.41
α-Phellandrene	1003	3.18
α-Terpinene	1017	0.69
o-Cymene	1026	15.11
α-Terpinolene	1089	0.38
Linalool	1097	3.19
β-Thujone	1114	0.31
Terpineol	1134	2.11
Terpinene-4-ol	1177	7.08
Cryptone	1186	9.23
Myrtenol	1196	1.14
Verbenone	1205	0.11
Trans-Carveol	1217	0.24
Cuminaldehyde	1242	4.16
Phellandral	1251	2.17
Carvacrol	1299	2.18
Bicyclo elemene	1324	0.69
α-Copaene	1377	0.49
Cis-Caryophyllene	1409	1.58
α-Gurjunene	1410	0.48
Trans-Caryophyllene	1419	3.91
Aromadendrene	1439	3.11
Germacrene D	1485	0.38
Bicyclogermacrene	1500	1.89
Δ-Cadinene	1523	0.68
Spathulenol	1578	14.73
Isospathulenol	1621	3.21
γ-Eudesmol	1632	2.01

[1]. Retention index.

Table 2 Chemical composition of essential oil of *Carum carvi.*

Compound	RI[1]	Content (%)
α-Pinene	939	1.07
β-Pinene	979	0.02
β-Myrcene	991	2.01
Limonene	1029	29.17
γ-Terpinene	1060	0.32
α-Terpinolene	1089	0.3
Linalool	1097	0.88
Trans limonene oxide	1159	2.81
Trans carveol	1217	0.41
Cuminaldehyde	1242	0.09
Carvone	1243	55.8
Carvacrol	1299	0.34
β-Caryophyllene	1441	0.51
Germacrene D	1485	1.87
Spathulenol	1578	3.01

[1] Retention index.

Table 3 Chemical composition of essential oil of *Heracleum persicum.*

Compound	RI[1]	Content (%)
Propyl butyrate	939	0.9
Ethyl(2E)-pentenoate	941	0.6
Butyl isobutyrate	975	0.9
Butyl butyrate	986	0.7
Isobutyl isovalerate	988	0.4
Hexyl acetate	992	1.1
Octanal	998	0.8
Buthyl-2-methylbutyrate	1024	1.2
ρ-Cymene	1025	2.7
Isoamyl butyrate	1027	0.7
γ-Terpinene	1060	3.2
Isoamyl,2-methyl-butyrate	1087	0.4
Linalool	1097	0.5
Hexyl isobutyrate	1132	4.3
Hexyl butyrate	1179	41.78
Hexyl-2-methylbutyrate	1182	2.3
Decanal	1186	0.9
Octyl acetate	1199	27.83
Octyl butyrate	1230	1.1
(E)-Anethol	1261	0.5
Hexyl hexanoate	1368	2.08
Octyl-2-methylbutyrate	1373	2.1

[1] Retention index.

Fumigant toxicity

The LC_{25}, LC_{50} and LC_{90}, 95% fiducial limits, slope and Chi-square (χ^2) of different tested essential oils against female parasitoid wasp are shown in Table 4. Probit analysis showed that LC_{50} values of these essential oils against *H. hebetor* were 1.116, 0.34 and 3.416 µl/l air for *E. camaldulensis, C. carvi* and *H. persicum*, respectively. The toxicity of essential oils on *H. hebetor* female adults was different, as inferred by the confidence intervals of LC_{50}.

Sublethal effects

The number of eggs laid by *H. hebetor* was significantly affected by essential oil treatments in comparison with control ($F = 99.36$; $df = 3,99$; $p < 0.0001$) (Table 5). Egg hatch rate was reduced by essential oil treatments. Mean female longevity was significantly affected by essential oil treatments compared with control ($F = 68.74$; $df = 3,99$; $p < 0.0001$) (Table 5). No difference in the sex ratio was found in the offspring of females of *H. hebetor* that were exposed to the essential oils ($F = 1.77$; $df = 3,99$; $p = 0.158$). The effects of exposure to LC_{25} concentration of essential oils on stable population parameters of *H. hebetor* are shown in Table 6. Computed net reproductive rate (R_0) and gross reproductive rate (*GRR*) illustrated that essential oil treatments reduced female population (Table 6). The intrinsic rate of increase (r) was significantly different between control and essential oil treatments ($F = 124.69$; $df = 3,99$; $p < 0.0001$). Our results showed that *Carum carvi* essential oil and control had the lowest (0.130 day^{-1}) and maximum (0.226 day^{-1}) r values, respectively. The finite rate of increase (λ) ($F = 132.69$; $df = 3,99$; $p < 0.0001$) was affected by essential oils. The highest and lowest value of finite rate of increase was observed in control (1.25 day^{-1}) and *Carum carvi* essential oil (1.14 day^{-1}), respectively. The LC_{25} of essential oils influenced the mean generation time (T) ($F = 72.89$; $df = 3,99$; $p < 0.0001$) compared to the control (Table 6). The doubling time (DT) was significantly affected by essential oil treatments compared with control ($F = 61.90$; $df = 3,99$; $p < 0.0001$).

Table 4 Toxicity of essential oils to adult stage of *Habrabracon hebetor*.

Essential oils	n	Slope ± SE	χ^2	Lethal concentrations (µl/l air)		
				LC_{25} (95% FL)	LC_{50} (95% FL)	LC_{90} (95% FL)
Eucalyptus camaldulensis	480	7.65 ± 0.93	2.62^{ns}	0.920 (0.84-0.96)	1.116 (1.072-1.168)	1.644 (1.508-1.876)
Carum carvi	480	3.17 ± 0.39	2.85^{ns}	0.200 (0.172-0.236)	0.340 (0.308-0.376)	0.880 (0.720-1.200)
Heracleum persicum	480	7.64 ± 0.90	3.2^{ns}	2.788 (2.584-2.94)	3.416 (3.272-3.572)	5.028 (4.624-5.720)

Lethal concentrations and 95% fiducial limits (FL) were estimated using logistic regression (SAS Institute 2002).

Table 5 The mean of biological parameters of adults *Habrobracon hebetor* exposed to LC_{25} of essential oils.

Insecticide	Egg laid	Hatched eggs (%)	longevity (day)	Sex ratio ♀ /(♀ + ♂)
Control	326.76 ± 26.53 a	89.40 ± 0.62 a	29.92 ± 2.101 a	0.486 ± 0.021 a
Heracleum persicum	87.68 ± 5.38 b	83.64 ± 1.38 ab	15.00 ± 0.678 b	0.451 ± 0.034 a
Eucalyptus camaldulensis	53.64 ± 4.92 bc	76.13 ± 4.79 bc	10.52 ± 0.466 c	0.397 ± 0.043 a
Carum carvi	26.88 ± 2.28 c	70.33 ± 4.61 c	8.24 ± 0.487 c	0.366 ± 0.056 a

Table 6 The mean of population parameters of *Habrobracon hebetor* treated with LC_{25} of essential oils.

Treatment	*GRR* (offspring)	R_0 (offspring)	r (day^{-1})	λ (day^{-1})	T (day)	DT (day)
Control	179.324 ± 0.362a	158.346 ± 0.521a	0.226 ± 0.0001a	1.25 ± 0.0001a	22.43 ± 0.0096a	3.07 ± 0.001c
Heracleum persicum	45.429 ± 2.001b	42.392 ± 2.62b	0.174 ± 0.0027b	1.19 ± 0.0032b	21.58 ± 0.173b	3.99 ± 0.063b
Eucalyptus camaldulensis	28.258 ± 1.57c	24.590 ± 2.26c	0.16 ± 0.0045b	1.17 ± 0.0053b	20.03 ± 0.211 c	4.33 ± 0.119b
Carum carvi	13.346 ± 0.739d	11.751 ± 0.954d	0.130 ± 0.0048c	1.14 ± 0.0055c	18.91 ± 0.248 d	5.31 ± 0.192a

Means in a column followed by different letters are significantly different. ANOVA with Tukey post hoc test ($P < 0.05$).

Discussion

Essential oils are phytochemicals with large insecticidal activities (Lee *et al.,* 2008; Chu *et al.,* 2011). Due to their high volatility under ambient temperatures, they have fumigant activity that might be important for controlling stored-product insect pests (Tripathi *et al.,* 2002; Ayvaz *et al.,* 2010). This study was aimed at assessing lethal and sublethal effects of *C. carvi*, *E. camaldulensis* and *H. persicum* essential oils on *H. hebetor* female adult stage. Experiments were conducted to determine whether the insecticidal activity of *C. carvi*, *E. camaldulensis* and *H. persicum* essential oils against *H. hebetor* female adults was attributable to fumigant action. The LC_{50} values that were determined in this study indicated that *C. carvi* essential oil has very high toxicity to adults of *H. hebetor* (LC_{50} = 0.34µl/l air) (Table 4). Our results showed that essential oils had toxic effects on female adult stage of *H. hebetor*. There are few studies for toxicity of the essential oils against *H. hebetor*. For example, the toxicity of *Ferula assafoetida* essential oil was demonstrated on biological characteristics of *H. hebetor* (Hym.: Braconidae) in vitro by Hashemi *et al.,* (2014). González *et al.,* (2013) examined the lethal and sublethal effects of four essential oils on the egg parasitoid *Trissolcus basalis* and reported that the plant essential oils had a negative effect on the population growth parameters of this parasitoid wasp. The reasons for the insecticidal effects of plant essential oils can be attributed to their chemical compounds. There are reports on insecticidal activity of o-Cymene (Jemâa *et al.,* 2012), Carvone (Ibrahim *et al.,* 2001) and Hexyl butyrate (Comisi *et al.,* 2009).

Several approaches have been utilized to study the effects of pesticides on natural enemies, such as lethal effect, and exposure of

natural enemies to sublethal doses (Desneux *et al.,* 2006). Evaluation of only acute toxicity (lethal effect) only provides a partial assessment of potential effects of pesticides in exposed organisms (Walthall and Stark, 1996) and therefore studying sublethal effects of phyto-insecticides is of primary importance (Desneux *et al.,* 2007) for IPM. Biological parameters such as fecundity, fertility and longevity were significantly and adversely affected by *C. carvi* essential oil compared to the control treatment. But none of the essential oils had effects on the sex ratio of offspring (Table 5). Demographic toxicology has been considered as a better measure of response to toxicants than individual life history traits (Forbes and Calow, 1999). The use of intrinsic rate of increase (r) has been recommended for evaluating the total effects of phyto-insecticides, because it is based on both survivorship and fecundity (Stark and Wennergren, 1995). In our study, the life table parameters showed significant differences in population growth performance between the treated and untreated females of *H. hebetor*. The net reproductive rate (R_0), the intrinsic rate of increase (r), and the finite rate of increase (λ) of the treated females were inferior to the control and these were achieved within a shorter mean generation time (T). This in turn resulted in a longer doubling time (DT). Several researchers have reported that life-table parameters of natural enemies were affected by sublethal concentrations of pesticides (essential oils and insecticides) (Mahdavi *et al.,* 2011; Wang *et al.,* 2012; Poorjavad *et al.,* 2014).

The high activity of these compounds could make them a potential substitute for methyl bromide in various uses in stored-product control programs and can be used in coordination with biological agents as a component of the integrated pest management. The observed fumigant activity demonstrates that essential oils are a source of biologically active vapors which may potentially prove to be efficient insecticides. The results showed that *C. carvi* essential oil had more adverse acute and sublethal effects on the ectoparasitoid *H. hebetor*. Our results were based on extended laboratory studies. Based on these laboratory results it seems that *H. persicum* essential oil is potentially more compatible with a chosen IPM approach. After laboratory studies, more attention should be devoted on storage environment experiments to obtain more applicable results under storage conditions.

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فعالیت حشره‌کشی تعدادی از اسانس‌های گیاهی علیه زنبور پارازیتوئید خارجی لارو *Habrobracon hebetor* (Hymenoptera: Braconidae)

جبرائیل رزمجو*، وحید مهدوی، هوشنگ رفیعی‌دستجردی، آرش فرهومند و سمیرا مولاپور

گروه گیاه‌پزشکی، دانشکده کشاورزی و منابع طبیعی، دانشگاه محقق اردبیلی، اردبیل، ایران.

* پست الکترونیکی نویسنده مسئول مکاتبه: razmjou@uma.ac.ir

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چکیده: *Habrobracon hebetor* Say، زنبور پارازیتوئید خارجی ایدیوبایونت و اجتماعی تعدادی زیادی از شب‌پره‌ها می‌باشد. در این مطالعه، اثرات کشندگی و زیرکشندگی اسانس‌های اکالیپتوس *Eucalyptus camaldulensis*، زیره‌ی سیاه *Carum carvi* و گلپر *Heracleum persicum* روی پارامترهای دموگرافیک *H. hebetor* در شرایط ۲ ± ۲۶ درجه‌ی سلسیوس، رطوبت نسبی ۵ ± ۶۰ درصد و دوره‌ی نوری ۱۶ ساعت روشنایی و ۸ ساعت تاریکی مورد ارزیابی قرار گرفت. اسانس‌های گیاهی به روش تقطیر با بخار آب با استفاده از دستگاه کلونجر استخراج شدند. ترکیبات شیمیایی اسانس‌های گیاهی با استفاده از دستگاه کروماتوگرافی گازی متصل به طیف‌سنج جرمی (GC-MS) شناسایی شدند. برای انجام آزمایش‌های تدخینی از ظروف شیشه‌ای ۲۵۰ میلی‌لیتری استفاده گردید. به‌منظور ارزیابی اثرات زیرکشندگی، حشرات کامل زنبور در معرض مقادیر LC_{25} هر یک از اسانس‌ها قرار داده شدند و سپس پارامترهای دموگرافیک زنبورهای پارازیتوئید زنده مورد مطالعه قرار گرفتند. سمّیت تدخینی نشان داد که مقادیر غلظت کشنده‌ی LC_{50} اسانس‌های مذکور علیه حشرات کامل ماده‌ی زنبور *H. hebetor* به‌ترتیب ۱/۱۱۶، ۰/۳۴ و ۳/۴۱۶ میکرولیتر بر لیتر هوا بودند. تجزیه‌ی شیمیایی اسانس‌ها با استفاده از GC-MS نشان داد که به‌ترتیب o-Cymene به میزان ۱۵/۱۱ درصد، Carvone به میزان ۵۵/۸ درصد و Hexyl butyrate به میزان ۴۱/۷۸ درصد، ترکیبات اصلی اسانس‌های اکالیپتوس، زیره ی سیاه و گلپر بودند. نتایج نشان داد که نرخ ذاتی افزایش جمعیت (r)، نرخ متناهی افزایش جمعیت (λ)، نرخ خالص تولیدمثلی (R_0) و نرخ ناخالص تولیدمثلی (GRR) زنبور پارازیتوئید، به‌طور معنی‌داری تحت تأثیر اسانس‌ها بودند. بالاترین و پایین‌ترین مقدار r به‌ترتیب در تیمارهای شاهد (۰/۲۲۶ بر روز) و حشرات تیمار شده با اسانس‌های زیره‌ی سیاه (۰/۱۳۰ بر روز) بودند. مطابق نتایج، اسانس‌های گیاهی پتانسیل مناسبی برای مدیریت تلفیقی آفات انباری دارند.

واژگان کلیدی: اسانس‌های گیاهی، سمّیت تدخینی، GC-MS، زنبور پارازیتوئید