

Estragole-rich essential oil of summer savory (*Satureja hortensis* L.) as an eco-friendly alternative to the synthetic insecticides in management of two stored-products insect pests

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Abstract: The lesser grain borer [*Rhyzopertha dominica* (Fabricius, 1792)] and the red flour beetle [*Tribolium castaneum* (Herbst, 1797)] are among the cosmopolitan damaging pests on several stored-products. The overuse of chemical pesticides in the control of such pests caused several side-effects including environmental contaminations, human health problems, and insect pests' resistance. In this circumstance, researchers have focused on safe and effective alternatives to chemical pesticides. In the present study, the insecticidal efficiency of essential oil extracted from the summer savory (*Satureja hortensis* L.) was assessed on the *R. dominica* and *T. castaneum* adults. The chemical profile of essential oil was evaluated through a gas chromatography-mass spectrometer, in which estragole, β -ocimene and d-limonene were the main components. The essential oil had considerable fumigant toxicity on insect pests. The mortality of insects was dependent on the essential oil concentration and exposure time. Probit analysis indicated that *R. dominica* with low LC₅₀ values (Lethal Concentration to kill 50 % of tested insects) was more susceptible than *T. castaneum*. Accordingly, *S. hortensis* essential oil with a high level of phenylpropanoid and terpenic compounds can be recommended as an efficient and natural alternative to the detrimental chemicals in the management of *R. dominica* and *T. castaneum*.

Key words: essential oil; estragole; *Satureja hortensis*; fumigation; coleopteran pests

Na estragolu bogato eterično olje vrtnega šetrja (*Satureja hortensis* L.) kot okolju prijazna alternativa sintetičnim insekticidom pri zatiranju dveh vrst skladiščnih škodljivih žuželk

Izvleček: Žitni kutar [*Rhyzopertha dominica* (Fabricius, 1792)] in rižev moka [*Tribolium castaneum* (Herbst, 1797)] sta kozmopolitski vrsti škodljivcev, ki povzročata škodo na mnogih uskladiščenih pridelkih. Prekomerna raba insekticidov pri zatiranju takšnih škodljivcev ima številne stranske učinke, vključno z onesnaževanjem okolja, zdravstvenimi problemi ljudi in odpornostjo škodljivih žuželk. V tej raziskavi so se raziskovalci osredotočili na varno in učinkovito alternativo sintetičnim insekticidom. Insekticidna učinkovitost eteričnega olja iz vrtnega šetrja (*Satureja hortensis* L.) je bila preizkušena na odraslih osebkih obeh vrst zgoraj omenjenih škodljivcev. Kemična sestava eteričnega olja je bila ovrednotena s plinskim kromatografom in masnim spektrometrom, ugotovljeno pa je bilo, da so estragol, β -ocimen in d-limonen glavne sestavine. Zaplinjevanje z eteričnim oljem je imelo znaten toksični učinek na škodljivi žuželki. Smrtnost žuželk je bila odvisna od koncentracije eteričnega olja in časa izpostavitve. Analiza Probit je pokazala, da je vrsta *R. dominica* z manjšimi LC₅₀ vrednostmi bolj občutljiva kot vrsta *T. castaneum*. Glede na to bi lahko eterično olje iz vrtnega šetrja z veliko vsebnostjo fenilpropanoidov in terpenov priporočili kot učinkovito in naravno alternativo škodljivim kemikalijam pri zatiranju omenjenih škodljivcev.

Ključne besede: eterično olje; estragol; *Satureja hortensis*; zaplinjevanje; škodljivi hrošči

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1 INTRODUCTION

Secondary metabolites announce the evolution of chemical defenses in plants which are often formed as by-products throughout the production of primary metabolites. Secondary metabolites have several essential roles especially in the protection against herbivores and in the attraction of pollinators' (Dinan, 1995; Bohinc et al., 2012). Plant-derived essential oils as well-known secondary metabolites can be produced in several aerial parts including leaves, flowers, seeds, stems and the roots of aromatic plants. Essential oils are generally composed of isoprene units as terpenes and phenylpropane (Bakali et al., 2008). Although terpenes such as monoterpenes (2 units of isoprene, C_{10}), sesquiterpenes (3 units of isoprene, C_{15}), and diterpenes (4 units of isoprene, C_{20}) have a high quantity, the monoterpenoids (oxygenated monoterpenes) are often the most components of the many essential oils (Breitmaier, 2006; Abdel-Tawab, 2016). Along with the application of essential oils in the perfumery and pharmaceutical industries, their lethal and sub-lethal effects especially fumigant toxicity of essential oils have been approved toward different class and orders of main insect and acari herbivores (Regnault-Roger et al., 2012; Rojht et al., 2012; Ebadollahi & Jalali-Sendi, 2015).

Summer savory [*Satureja hortensis* L. (Lamiaceae)], as an aromatic spice and food preservative, widely distributed and/or cultivated in many countries. It used in Iranian traditional medicine to treat intestinal and stomach disorders such as indigestion and diarrhea, muscle pain, thrombosis, and cardiovascular diseases (Hajhashemi et al., 2000; Yazdanparast et al., 2008). Moreover, along with antibacterial, antifungal, antioxidant, and cytotoxic activities of *S. hortensis*, its potential on the insect pest management have also been documented (Mahboubi & Kazempour, 2011; Miladi et al., 2013; Gombac & Trdan, 2014; Farzaneh et al., 2015; Ghorbanpour et al., 2016).

R. dominica (lesser grain borer) and *T. castaneum* (red flour beetle) are among the cosmopolitan serious pests of stored-products such as cereal and legume grains, dried fruits, spices, flours, leather, and even packaging materials made from wood and paper. Further, the quality of infested products strongly reduces due to the residues of insect bodies and their unpleasant smell (Vilaverde et al., 2007; Edde, 2012).

As part of a program aimed at studying the insecticidal activity and chemical composition of plant essential oils, we have assessed the fumigant toxicity and chemical profile of *S. hortensis* essential oil against *R. dominica* and *T. castaneum*. Hope the range of introduced active bioagents derived from aromatic plants has extended by the results of the present study.

2 MATERIALS AND METHODS

2.1 ESSENTIAL OIL EXTRACTION AND ANALYSIS

Fresh 10 cm aerial parts from the shoots of *S. hortensis* were sampled for essential oil extraction. The specimens were collected during April and May 2019 from Parsabad region (Latitude: 39°38' N, Longitude: 47°52' E, and height: 52 m), Ardebil province, Iran. The samples were dried at room temperature within a week and then ground using an electric grinder. Fifty grams ground plant material was poured into a Clevenger apparatus equipped with a 1000 ml balloon. The essential oil was extracted within 3 h and the obtained oil was stored in a refrigerator at 4 °C.

Chemical profile of the *S. hortensis* essential oil was assessed using a gas chromatographic system (Agilent model 7890B) equipped with the mass spectrometer detector (Agilent model 5977A) according to Ebadollahi et al. (2017): chromatographic separation was performed on the HP-5MS (5 % phenyl-methyl-polysiloxane) capillary column (30 m length, 0.25 mm internal diameter, and 0.25 µm film thickness) with 70 eV ionization energy. The injected volume was 1.0 µl with 280 °C temperature. The temperature program of the column was set from 50 to 350 °C. Helium (99.999 %) was used as a carrier gas at 1 ml minute⁻¹. The component was identified by comparison of their mass spectra with those from Wiley's MS library (7th edition) and NIST (National Institute of Standards Technology) in the library.

2.2 TESTED INSECTS

The adult insects of *R. dominica* were obtained from the colonies at the Department of plant protection, University of Mohaghegh Ardebili, Ardabil, Iran. The adult insects of *T. castaneum* were collected from contaminated wheat grains in the warehouses of Parsabad city (Latitude: 39°38' N, Longitude: 47°52' E, and height: 52 m), Ardabil province, Iran. Adult insects were separately released on wheat grains in the breeding container. Adult insects were removed 48 h later and grains with insects' eggs were kept in an incubator at 25 ± 2°C and 65 ± 5 % relative humidity in dark (Arnaud et al., 2005). Synchronized adult insects with 1 - 7 old-days were selected.

2.3 BIOASSAY

The fumigation bioassay was done according to the study of Ebadollahi (2018): twenty adults of both insects

were separately located in 340 ml fumigant chambers. The tested concentrations of essential oil, based on the preliminary experiments, were from 11.76 to 47.06 $\mu\text{l l}^{-1}$ and from 21.00 to 55.15 $\mu\text{l l}^{-1}$ for *R. dominica* and *T. castaneum*, respectively. The essential oil concentrations were poured on the 2 × 3 cm piece of filter papers which were sealed to the inside of the container lids and the lids were closed using parafilm. Experiments were conducted for control groups without adding essential oil concentration. Each treatment was repeated 4 times and the insects' mortality was documented after 24, 48 and 72 h intervals.

2.4 STATISTICAL ANALYSIS

Variance analysis was used to assess the significant effects of essential oils' concentrations and the exposure times. To compare the effects of independent factors concentration and exposure time on the insects' mortality,

the ω^2 comparison was used. Calculation of lethal concentrations (LC), lethal times (LT) and linear regression analysis along with heterogeneity of the data by a Chi-squared test were done using SPSS software version 24 (IBM, Chicago, USA).

3 RESULTS

3.1 CHEMICAL COMPOSITION OF ESSENTIAL OIL

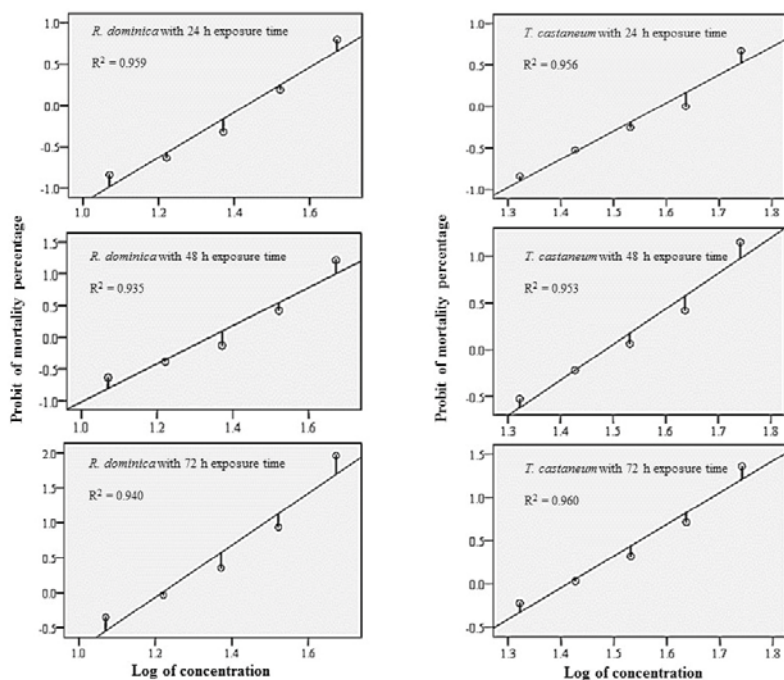
Chemical analysis of *S. hortensis* essential oil identified 17 components at 99.21 %, in which 83.02 % are phenylpropanoid constituents. Five different groups of terpenes were also recognized in the essential oil, in which the monoterpene hydrocarbons (15.38 %) had the highest amount followed by sesquiterpenoids (0.43 %), monoterpenoids (0.26 %), a sesquiterpene hydrocarbon (0.08 %), and a diterpene (0.04 %). Estragole (82.10 %) as

Table 1: Chemical composition of the essential oil isolated from Iranian *Satureja hortensis*

Compound	Retention Time (minute)	Formula and Classification	Percentage
α -Pinene	5.30	$\text{C}_{10}\text{H}_{16}^{\text{MH}}$	0.91
Camphene	5.57	$\text{C}_{10}\text{H}_{16}^{\text{MH}}$	0.04
Sabinene	6.03	$\text{C}_{10}\text{H}_{16}^{\text{MH}}$	0.06
β -Pinene	6.09	$\text{C}_{10}\text{H}_{16}^{\text{MH}}$	0.09
β -Myrcene	6.33	$\text{C}_{10}\text{H}_{16}^{\text{MH}}$	0.12
d-Limonene	7.08	$\text{C}_{10}\text{H}_{16}^{\text{MH}}$	2.25
β -Ocimene	7.46	$\text{C}_{10}\text{H}_{16}^{\text{MH}}$	11.86
α -Terpinene	8.27	$\text{C}_{10}\text{H}_{16}^{\text{MH}}$	0.05
Rosefuran	8.43	$\text{C}_{10}\text{H}_{14}\text{O}^{\text{M}}$	0.08
Estragole	11.51	$\text{C}_{10}\text{H}_{12}\text{O Ph}$	82.10
E,E-2,6-Dimethyl-3,5,7-octatriene-2-ol	11.54	$\text{C}_{10}\text{H}_{16}\text{O}^{\text{M}}$	0.07
Bornyl acetate	14.23	$\text{C}_{12}\text{H}_{20}\text{O}_2^{\text{M}}$	0.11
Methyl Eugenol	18.71	$\text{C}_{11}\text{H}_{14}\text{O}_2 \text{Ph}$	0.92
Germacrene-D	21.05	$\text{C}_{15}\text{H}_{24}^{\text{SH}}$	0.08
Spathulenol	23.76	$\text{C}_{15}\text{H}_{24}\text{O}^{\text{S}}$	0.31
Caryophyllene oxide	23.89	$\text{C}_{15}\text{H}_{24}\text{O}^{\text{S}}$	0.12
Eicosane	32.78	$\text{C}_{20}\text{H}_{42}^{\text{DH}}$	0.04
MH: Monoterpene Hydrocarbon			15.38
M: Monoterpenoid			0.26
SH: Sesquiterpene Hydrocarbon			0.08
S: Sesquiterpenoid			0.43
DH: Diterpene Hydrocarbon			0.04
Ph: Phenylpropanoid			83.02
Total			99.21

Table 2: Results of the variance analysis of *S. hortensis* essential oil fumigation on the adults of *R. dominica* and *T. castaneum* after 24, 48 and 72-h exposure times

Insect	Source of Variation	df	F	p-value	ω^2
<i>R. dominica</i>	Concentration	4	467.987 *	<0001	22.516
	Time	2	155.009 *	<0001	3.713
	Time × Concentration	8	1.594	0.154	0.057
<i>T. castaneum</i>	Concentration	4	324.572 *	<0001	17.793
	Time	2	142.271 *	<0001	3.884
	Time × Concentration	8	1.106	0.377	0.012

* Significant at $\alpha = 1\%$ **Figure 1:** Concentration – mortality lines for fumigant toxicity of *S. hortensis* essential oil against the adults of *R. dominica* and *T. castaneum* after 24, 48 and 72-h exposure times

a phenylpropanoid constituent had the highest amount and monoterpene hydrocarbons β -ocimene (11.86 %), and dl-limonene (2.25 %) were in the next points (Table 1).

3.2 FUMIGANT TOXICITY

Results of the fumigant toxicity indicated that essential oil of Iranian *S. hortensis* had considerable toxicity on the *R. dominica* and *T. castaneum* adults. The results of variance analysis were summarized in Table 2. Concentrations of essential oil and exposure times had statistically significant effects on the insects' mortality but

their interaction wasn't significant. Furthermore, based on the ω^2 values, among these factors, the effect of essential oil concentration was more effective.

The calculated R^2 values for concentrations-mortality correlation were 0.959, 0.935 and 0.940 for *R. dominica* and 0.956, 0.953 and 0.960 for *T. castaneum* after 24, 48 and 72-h exposure times, respectively. So, there is a direct correlation between the concentrations of essential oil and mortality of both insects (Figure 1).

Probit analysis indicated the calculated LC_{50} values (lethal concentration to kill 50 % of tested insects) of essential oil were significantly decreased from 24 h to 72 h for both insects (Table 3). For example, the 24 h- LC_{50} value of essential oil with 95 % confidence limits was 27.212

Table 3: Results of Probit analysis for fumigant toxicity of *S. hortensis* against the adults of *R. dominica* and *T. castaneum*

Insect	Time (h)	LC ₅₀ (95 % confidence limits) (µl l ⁻¹)	χ ² (df = 3)	Slope ± SE	Significance *
<i>R. dominica</i>	24	27.212 (24.657 - 30.361)	3.893	2.740 ± 0.294	0.273
	48	22.193 (20.140 - 24.385)	7.062	2.897 ± 0.298	0.070
	72	16.466 (12.128 - 20.013)	5.830	3.321 ± 0.329	0.120
<i>T. castaneum</i>	24	38.908 (35.951 - 42.688)	3.425	3.386 ± 0.412	0.331
	48	30.757 (28.377 - 33.070)	3.810	3.691 ± 0.419	0.283
	72	25.747 (23.020 - 28.021)	2.745	3.506 ± 0.429	0.433

Insect	Concentration (µl l ⁻¹)	LT ₅₀ (95 % confidence limits) (h)	χ ² (df = 1)	Slope ± SE	Significance *
<i>R. dominica</i>	47.06	10.301 (2.944 - 16.210)	1.765	2.060 ± 0.515	0.184
<i>T. castaneum</i>	55.15	12.682 (5.479 - 18.103)	2.023	2.282 ± 0.503	0.155

* Since the significance level is greater than 0.05, no heterogeneity factor is used in the calculation of confidence limits. The number of insects for calculation of LC₅₀ values is 400 for each time. The number of insects for calculation of LT₅₀ values is 240 for each concentration.

(24.657 - 30.361) µl l⁻¹ which was decreased to 16.466 (12.128 - 20.013) µl l⁻¹ after 72 h. Further, according to Table 3, adults of *R. dominica* with low LC₅₀ values were significantly susceptible than *T. castaneum* adults to the *S. hortensis* essential oil at all exposure times.

The lethal times to kill 50 % of tested insects (LT₅₀ values) are also shown in Table 3. At a high tested concentration of *S. hortensis* essential oil (47.06 µl l⁻¹), the LT₅₀ value was 10.301 (2.944 - 16.210) h against *R. dominica* adults. This value for *T. castaneum* adults with a concentration of 55.15 µl l⁻¹ was calculated as 12.682 (5.479 - 18.103) h.

4 DISCUSSION

The composition of *S. hortensis* essential oil have been investigated in the previous studies; carvacrol (11.0 %), *p*-cymene (19.6 %), sabinene (4.4 %), *γ*-terpinene (16.0 %), and thymol (28.2 %) were found as major compounds by Mahboubi and Kazempour (2011). Thymol, *p*-cymene, *γ*-terpinene, and carvacrol were not detected in the present study but a trace of sabinene (0.06 %) was determined. In contrast, estragole and *β*-ocimene as major components of present work were not detected in the study of Mahboubi and Kazempour (2011). Farzaneh et al. (2015) showed carvacrol (48.0 %), *p*-cymene (11.7 %), myrcene (2.5 %), *α*-pinene (2.5 %), *γ*-terpinene (24.2 %) were the main components. From these constituents, myrcene (0.12 %) and *α*-pinene (0.91 %) with different amounts were recognized in the essential oil of present study. In the other study, Miladi et al. (2013) also revealed that monoterpenoids (59.11 %) were the main chemical class of *S. hortensis* essential oil

which is parallel with our results but they announced other components such as carvacrol, *β*-caryophyllene, *p*-cymene, and *γ*-terpinene. In contrary, Mohammadhosseini and Beiranvand (2013) showed that the monoterpene hydrocarbons such as myrcene, *α*-pinene, *β*-pinene, *α*-terpinene, and *α*-thujene had the highest amount in the *S. hortensis* essential oil. These differences in the chemical profile of *S. hortensis* essential oil in the present and above-mentioned studies can be due to the variations in some of the influential factors, such as geographical and growing conditions, drying and extraction methods, ontogenetic stages, and season (Sefidkon et al., 2006; Pfeferkorn et al., 2008; Rezvanpanah et al., 2011; Ghorbanpour et al., 2016).

Insecticidal properties of *S. hortensis* essential oil were acknowledged in some recent studies; appropriate fumigant toxicity of this oil was proved against Mediterranean flour moth [*Ephesia kuehniella* (Zeller, 1879)], Indianmeal moth [*Plodia interpunctella* (Hubner, 1813)], and *T. castaneum* (Mollaei et al., 2011). The calculated 48 h-LC₅₀ value for *T. castaneum* in this work (192.350 µl l⁻¹) is much higher than the corresponding LC₅₀ in the present study (30.757 µl l⁻¹). In the study of Tozlu et al. (2011), the *S. hortensis* essential oil with a high amount of carvacrol, *β*-caryophyllene, *p*-cymene, *δ*-terpinene, and *α*-terpinene was very toxic against the broad bean weevil [*Bruchus dentipes* (Baudi, 1886)]. They concluded that the *S. hortensis* essential oil toxicity is directly related to its components. Along with the fumigant toxicity of *S. hortensis* essential oil, the contact toxicity, repellency, and disruption in the enzymes' activity were also described (Mollaei et al., 2011; Heydarzade & Moravvej, 2012; Magierowicz et al., 2019). The results of these studies indicated that *S. hortensis* essential oil has considerable

insecticidal activities against stored-product insect pests which are in accordance with our findings.

Estragole or methyl chavicol, as two major compounds identified in the present study, is a GRAS (Generally Recognized As Safe) nominated material and approved for food procedure (De Vincenzi et al., 2000). Its name originates from “estragon” which is a French word of tarragon (*Artemisia dracunculus* L.) (Misztal et al., 2010). Along with cytotoxic and antimicrobial properties of estragole (Bagamboula et al., 2004; Andrade et al., 2015), toxicity of this compound has also been approved against some of damaging stored-product insect pests including *T. castaneum*, the rice weevil [*Sitophilus oryza* (Linnaeus, 1763)], the maize weevil [*Sitophilus zeamais* (Motschulsky, 1855)], the booklice [*Liposcelis bostrychophila*, Badonnel, 1931], the cigarette beetle [*Lasioderma serricorne* (Fabricius, 1792)], and the adzuki bean beetle [*Callosobruchus chinensis* (Linnaeus, 1758)] (Kim & Ahn, 2011; Wang et al., 2011; Kim & Lee, 2014; Guo et al., 2015). Furthermore, the insecticidal properties of other main components identified in the present study including d-limonene and β -ocimene were also documented (Tripathi et al., 2003; Guo et al., 2015; Kang et al., 2018). Accordingly, the fumigant toxicity of *S. hortensis* essential oil may be attributed to such constituents. However, the existence of synergistic effects between other compounds is also possible.

5 CONCLUSION

Synthetic pesticide residues can be found in different parts of our surrounding environment from water and soil to everybody's foods and even human breast milk samples (Damgaard et al., 2006; Nicolopoulou-Stamati et al., 2016; Trdan, 2016). Regarding the pests' management, due to the overusing of synthetic chemicals, the other side-effects such as resurgence and outbreak of new pests, several pest-resistant reports on the different classes of synthetic pesticides, and detrimental effects on valuable non-target organisms including parasitoids and predators have also been documented (Köhler et al., 2013; Cruz et al., 2017; Sudo et al., 2018). Therefore, urgent efficacious tools for the reduction of synthetic chemical utilization and for announcing eco-friendly agents with fewer public health risks are required. Because of the low toxicity to the mammals and pose a minimum risk, the plant essential oils considered safe (Viciolle et al., 2012). The prospective pesticidal activity of several plants essential oils have been stated in recent years (Isman & Grieneisen, 2014), and the range of these eco-friendly bio-agents was extended in the present study through the introduction of Iranian phenyl-

propanoid-rich summer savory as a toxic agent against two damaging coleopteran insect pests *R. dominica* and *T. castaneum*. However, based on the short residual life-time (Isman, 2006), it is recommended that such essential oils be tested in the better applicable form such as “controlled release technique” through micro- and nano-encapsulation. Furthermore, the pesticidal ability of this plant essential oil on the other pests and its adverse effects on beneficial biocontrol agents should be more investigated.

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