



## Chemical profile and insecticidal activity of an Iranian endemic savory *Satureja isophylla* Rech.

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### ARTICLE INFO

Article :

Reçu le : 12/01/2020

Accepté le : 03/07/2020

**Keywords:** Chemical composition, essential oil, fumigant toxicity, *Satureja isophylla*.

### ABSTRACT

Regarding the ongoing searches for eco-friendly alternatives to the harmful synthetic pesticides, the fumigant toxicity of essential oil extracted from aerial parts of *Satureja isophylla* Rech. was assessed against two cosmopolitan stored-product insect pests *Rhyzopertha dominica* (F.) and *Tribolium castaneum* Herbst. The chemical composition of extracted essential oil was analyzed through Gas Chromatography – Mass Spectrometry. *S. isophylla* essential oil had statistically significant toxicity on both insect pests. Probit analysis revealed that *R. dominica* is more susceptible than *T. castaneum* to the essential oil. The LC<sub>50</sub> (Lethal Concentration to kill 50% insects) values were significantly declined and/or the toxicity augmented with increasing exposure times. Monoterpene hydrocarbons (50.57%) and monoterpenoids (43.55%) had high amounts in the essential oil, in which thymol, cymene,  $\gamma$ -terpinene,  $\beta$ -myrcene, and  $\alpha$ -terpinene were the main components. Consequently, the essential oil isolated from aerial parts of *S. isophylla* may be considered as one of the natural insecticides for *R. dominica* and *T. castaneum* management.

### 1. INTRODUCTION

The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), and the red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), are among the most economical insect pests all over the world. Although the direct damage of these pests is evident due to the intensive feeding on various stored-products such as cereals, spices, leather and flours, their refuses, corpses, unpleasant smell and even mechanically associated microbes severely diminish the quality of storage products (Edde, 2012; Li et al., 2013; Bosly and Kawanna, 2014). Although the use of synthetic chemicals is a typical method of such insect pest management, their misuse caused various side-effects such as the emergence of resistant pest populations, the spread of secondary pests, the harmful effects on the environment and the threat to human and non-target organisms (Jeyasankar and Jesudasan, 2005; Damalas and Eleftherohorinos, 2011). Therefore, the search for and introduction of reliable and eco-friendly alternatives to hazardous chemical pesticides are necessary.

Plant-derived essential oils as well-known secondary metabolites are generally composed of complex components that mainly fall into two groups: the highest amount of isoprene units and the lowest amount of phenylpropanes (Bakkali et al., 2008). In recent years, the possibility of essential oils' application in the management of insect pests has been described with different plant genera and families (Isman and Grieneisen, 2014; Ebadollahi and Jalali-Sendi, 2015; Pavela and Benelli, 2016).

Among several aromatic plants from the Lamiaceae family, the *Satureja* L., known as savory, with up to 200 species in Asia, America, and the Mediterranean area, is one of the most distributed genera (Rustaiyan et al., 2004). Along with diverse therapeutic importance of *Satureja* genus principally in folk medicine (Jafari et al., 2016), its promising biological effects including antioxidant, antibacterial, antifungal, and cytotoxic activities has also been recognized

(Zarrin et al., 2010; Yousefzadi et al., 2012; Hassanein et al., 2014; Falsafi et al., 2015). Further, previous studies revealed that the essential oils isolated from some *Satureja* species have considerable insecticidal efficiency. For example, the insecticidal properties of *S. thymbra* L. essential oil against Mediterranean flour moth (*Ephesia kuehniella* (Zell.)), Indian meal moth (*Plodia interpunctella* (Hübner)), and the bean weevil (*Acanthoscelides obtectus* Say), *S. spicigera* Boiss essential oil against granary weevil (*Sitophilus granarius* (L.)), *S. khuzistanica* essential oil against Colorado potato beetle (*Leptinotarsa decemlineata* (Say)), and *S. hortensis* L. essential oil against the grey knot-horn (*Acrobasis advenella* (Zinck.)) were approved (Ayvaz et al., 2010; Yildirim et al., 2011; Taghizadeh-Saroukolai et al., 2014; Magierowicz et al., 2019).

With aiming to find the healthy and efficient agents in the pest management strategies, the present study was established to investigate the chemical profile and the toxicity of essential oil of an Iranian endemic savory *S. isophylla* against *T. castaneum* and *R. dominica*.

## 2. MATERIALS AND METHODS

### 2.1. Insects

*Tribolium castaneum* adults were gathered from infested stored grains of the wheat in the Moghan region (Ardabil province, Iran). The initial colony of *Rhyzopertha dominica* acquired from the department of plant protection, University of Mohaghegh Ardebili (Ardabil province, Iran). In the breeding containers, fifty pairs of adult insects were released onto wheat grains and removed after 48 h. Grains contaminated with insect eggs were separately held in an incubator at  $25 \pm 2^\circ\text{C}$  and  $65 \pm 5\%$  relative humidity in the dark (Arnaud et al., 2005).

### 2.2. Plant materials and the extraction of essential oil

Aerial parts of savory were gathered from April to June from the Heiran regions, Ardebil province, Iran, and designated *S. isophylla* based on the keys provided by Jamzad (2009). The fresh leaves and flowers were dried at room temperature in a week and ground utilizing an electric grinder. Fifty grams of the specimen with 500 ml distilled water were poured into a 1000 ml balloon of the Clevenger apparatus. The essential oil extraction was fulfilled during three h. The achieved essential oil was dried through anhydrous sodium sulfate and deposited in a refrigerator at  $4^\circ\text{C}$ .

### 2.3. Fumigant toxicity

Based on the preliminary experiments, the concentration ranges from 10.29 to 35.29  $\mu\text{l/l}$  and from 14.71 to 55.00  $\mu\text{l/l}$  of *S. isophylla* essential oil were acknowledged for *R. dominica* and *T. castaneum*, respectively. The glass containers (340 ml) were used as a fumigation chamber and twenty 1 to 7 day-old adult insects were separately transferred to them. The concentrations of essential oil were poured on the  $2 \times 2$  cm filter paper pieces (Whatman Grade 1). The treated filter papers adhered to the internal of the lid of containers, which were immediately closed. All experiments were conducted without essential oil concentrations for control groups. Each experiment was repeated 4 times and the mortality was recorded after 24, 48 and 72 h exposure times.

### 2.4. Essential oil analysis

Chemical analysis of the essential oil of *S. isophylla* was investigated through a Gas Chromatography (Hewlett–Packard, Palo Alto, CA) equipped with a mass spectrometer (model 5975C) according to the study of Ebadollahi (2017).

### 2.5. Statistical analysis

Analysis of variance (ANOVA) was used to evaluate the significance of essential oil concentration and the time factors on the insects' mortality with SPSS Version 24 software. The Tukey's test was performed on the fumigant toxicity data to distinguish significant differences at  $\alpha = 0.05$  among concentrations and exposure times. Lethal concentrations (LC), the data heterogeneity and linear regression analysis were also accomplished using SPSS software.

## 3. RESULT AND DISCUSSION

Analysis of variance (ANOVA) displayed that the concentrations of *S. isophylla* essential oil ( $F = 289.359$ ,  $p < 0.0001$  for *R. dominica* and  $F = 120.221$ ,  $p < 0.0001$  for *T. castaneum*) and the exposure times ( $F = 429.673$ ,  $p < 0.0001$  for *R. dominica* and  $F = 85.185$ ,  $p < 0.0001$  for *T. castaneum*) had statistically significant effects on the mortality of both insect pests. As shown in Table 1, the essential oil concentration of 35.29  $\mu\text{l/l}$  made the highest mortality (98.75%)

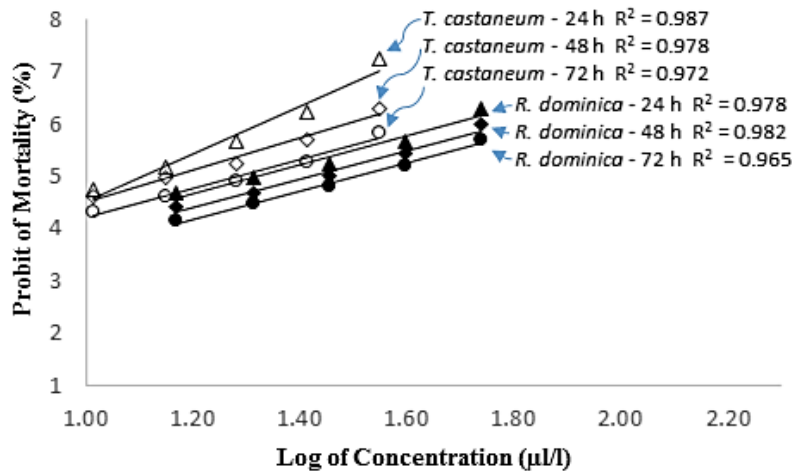
after 72 h for *R. dominica*. At the same time, the oil concentration of 55.00 µl/l created 90.00% mortality on the *T. castaneum*.

**Table 1.** Fumigant toxicity of *Satureja isophylla* essential oil against *R. dominica* and *T. castaneum* adults after 24, 48 and 72 h exposure times.

Insect	Time (h)	Concentration (µl/l)				
		10.29	14.12	19.12	25.88	35.29
<i>R. dominica</i>	24	25.00 ± 0.67 <sup>j</sup>	35.00 ± 0.67 <sup>i</sup>	46.25 ± 0.92 <sup>g</sup>	61.25 ± 0.92 <sup>e</sup>	80.02 ± 1.33 <sup>c</sup>
	48	35.00 ± 0.67 <sup>i</sup>	48.72 ± 0.92 <sup>g</sup>	60.00 ± 1.33 <sup>e</sup>	76.25 ± 0.92 <sup>cd</sup>	90.00 ± 1.33 <sup>b</sup>
	72	40.00 ± 0.67 <sup>h</sup>	56.25 ± 0.92 <sup>f</sup>	75.00 ± 0.67 <sup>d</sup>	88.75 ± 0.25 <sup>b</sup>	98.75 ± 0.25 <sup>a</sup>
<i>T. castaneum</i>		Concentration (µl/l)				
		14.71	20.59	28.53	39.71	55.00
	24	20.00 ± 0.67 <sup>j</sup>	30.00 ± 0.67 <sup>i</sup>	42.50 ± 0.33 <sup>g</sup>	58.75 ± 0.25 <sup>e</sup>	76.25 ± 0.92 <sup>c</sup>
	48	27.50 ± 0.33 <sup>i</sup>	37.50 ± 0.33 <sup>h</sup>	50.00 ± 0.67 <sup>f</sup>	66.25 ± 0.25 <sup>d</sup>	83.75 ± 0.92 <sup>b</sup>
	72	37.50 ± 1.67 <sup>h</sup>	48.75 ± 0.92 <sup>f</sup>	60.00 ± 0.67 <sup>e</sup>	75.00 ± 0.67 <sup>c</sup>	90.00 ± 0.67 <sup>a</sup>

Data that do not have the same letters are statistically significant difference at  $P < 0.05$  based on the Tukey's test. Each data represents mean ± SD of four replicates with eighty adult insects.

The least mortalities were achieved with the lowest tested concentrations for both insects. According to high  $R^2$  values, there is a positive correlation between essential oil concentrations and both insect pests' mortalities in all exposure times (Figure 1).



**Figure 1.** Concentration-mortality lines of the fumigant toxicity of *S. isophylla* essential oil against *R. dominica* and *T. castaneum*.

Probit analysis indicated revealed that the essential oil of *S. isophylla* has promising fumigant toxicity against the adults of *R. dominica* and *T. castaneum*. The  $LC_{50}$  values were 19.289 (17.485 – 21.307) µl/l for *R. dominica* and 31.833 (28.831 – 35.473) µl/l for *T. castaneum* after 24-h exposure time. So, the adults of *R. dominica* were more susceptible than *T. castaneum* to the fumigation of *S. isophylla* essential oil.  $LC_{50}$  values were also significantly reduced from 24 h to 72 h for both insects. For example, the  $LC_{50}$  value was decreased to 12.487 (11.267 - 13.552) µl/l after 72 h for *R. dominica* (Table 2).

**Table 2.** LC<sub>50</sub> and their related values of the fumigant toxicity of *S. isophylla* essential oil against *R. dominica* and *T. castaneum*.

insect	Time (h)	LC <sub>50</sub> with 95% confidence limits (µl/l)	χ <sup>2</sup> (df = 3)	Slope ± SE	Significant
<i>R. dominica</i>	24	19.289 (17.485 – 21.307)	1.728	2.760 ± 0.324	0.631*
	48	14.501 (12.900 – 15.946)	1.529	2.984 ± 0.338	0.676*
	72	12.487 (11.267 – 13.552)	2.575	4.055 ± 0.404	0.462*
<i>T. castaneum</i>	24	31.833 (28.831 – 35.473)	0.813	2.700 ± 0.305	0.846*
	48	27.367 (24.318 – 30.661)	0.328	2.354 ± 0.297	0.955*
	72	20.845 (18.196 – 23.218)	2.308	2.639 ± 0.312	0.511*

\* 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 LC<sub>50</sub> values is 400 for each time.

The chromatogram of chemical profile and the chemical composition of the *Satureja isophylla* essential oil are shown in Figure 2 and Table 3. Both terpenes and phenylpropene groups were identified, in which monoterpene hydrocarbons (50.57 %) and monoterpenoids (43.55%) had high amounts and the terpenes accounted for 97.87 of the total essential oil components. The terpenes thymol (41.46%), cymene (25.85%), γ-terpinene (16.92%), β-myrcene (2.12%), α-terpinene (1.57%) and α-phellandrene (1.46%) were the main components.

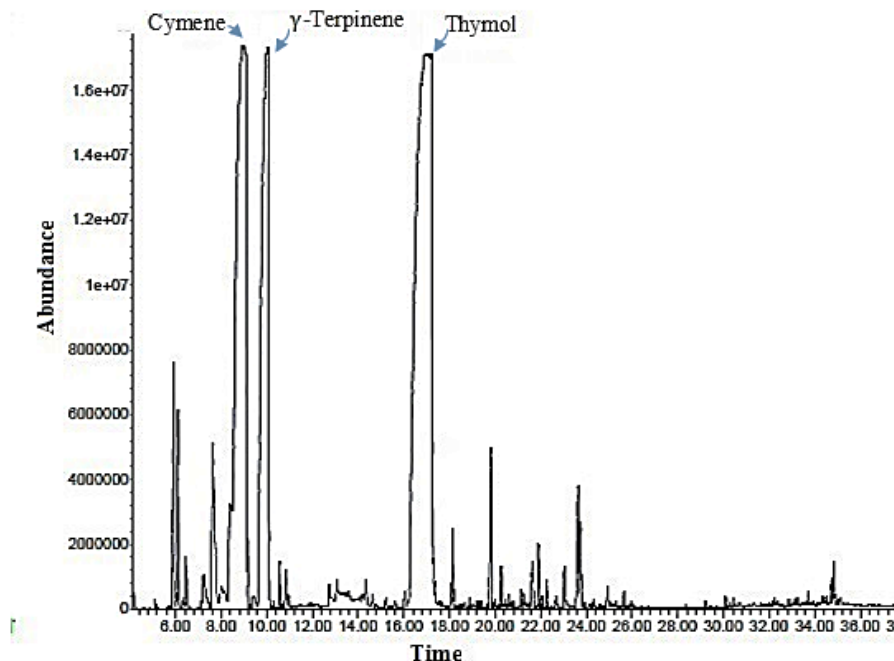
Some recent studies have shown that it is possible to manage *R. dominica* and *T. castaneum* using plant-derived essential oils (Kim et al., 2010; Bahrami et al., 2016; Ebadollahi et al., 2016; Ebadollahi, 2018; Saad et al., 2019). Furthermore, the toxicity of essential oils isolated from different species of *Satureja* genus had been assessed on the several insect pests (Zarrin et al., 2010; Yildirim et al., 2011; Taghizadeh-Saroukolai et al., 2014; Magierowicz et al., 2019). However, the toxicity of *S. isophylla* essential oil, as one the aromatic pant endemic to Iran, was evaluated for the first time in the present study.

There are few studies on the evaluation of the chemical components of *S. isophylla* essential oil. In the study of Sefidkon and Jamzad [30], α-eudesmol (11.3%), β-eudesmol (9.6%), camphor (7.1%), β-caryophyllene (6.1%), and γ-eudesmol (5.8%) were recognized as the main components in the *S. isophylla* essential oil. Of these compounds, the only caryophyllene in much lower quantity (0.98%) was existent in the present study. Thymol as a major component in the essential oil of present work had not any trace in the study of Sefidkon and Jamzad (2006), and γ-terpinene and cymene as the two of other main components, had the only 0.5% and 1.4%, respectively, in this study. Ghorbanpour et al. (2016) displayed that α-eudesmol (47.8%), β-eudesmol (9.2%), γ-terpinene (3.8%), and cymene (1.3%) had the high amount and the thymol had only 0.1% of the total *S. isophylla* essential oil. There are many differences in the recognized components of *S. isophylla* essential oil in the studies mentioned above and the present research. These differences may be due to the plant-related factors such as genetic origin and ontogenetic stage, and the external factors such as harvesting time, geographical location, climatic conditions, seasonal variation and distillation methods (Chorianopoulos et al., 2006; Sefidkon et al., 2007; Pfefferkorn et al., 2008).

The insecticidal effects of main components of *S. isophylla* including thymol, cymene, β-myrcene and α-terpinene had been documented (Kordali et al., 2008; Kordali et al., 2012; Andrade-Ochoa et al., 2018). Although the synergistic effects of other compounds should also be considered, it can be said that the promising toxicity of *S. isophylla* essential oil may be due to the presence of such components.

**Table 3.** Chemical composition of the essential oil isolated from *Satureja isophylla*.

Compound	Formula	RT (minute)	Percentage
$\alpha$ -Phellandrene	C <sub>10</sub> H <sub>16</sub>	5.903	1.46
$\alpha$ -Pinene	C <sub>10</sub> H <sub>16</sub>	6.087	0.99
Camphene	C <sub>10</sub> H <sub>16</sub>	6.467	0.31
$\beta$ -Pinene	C <sub>10</sub> H <sub>16</sub>	7.262	0.52
<i>p</i> -Mentha-1,5-diene	C <sub>10</sub> H <sub>16</sub>	7.087	0.46
$\beta$ -Myrcene	C <sub>10</sub> H <sub>16</sub>	7.666	2.12
$\alpha$ -Terpinene	C <sub>10</sub> H <sub>16</sub>	8.402	1.57
Cymene	C <sub>10</sub> H <sub>14</sub>	8.853	25.85
$\beta$ -Ocimene	C <sub>10</sub> H <sub>16</sub>	9.447	0.17
$\gamma$ -Terpinene	C <sub>10</sub> H <sub>16</sub>	9.898	16.92
$\alpha$ -Terpinolene	C <sub>10</sub> H <sub>16</sub>	10.556	0.20
Linalool	C <sub>10</sub> H <sub>18</sub> O	10.841	0.19
Sabinene hydrate	C <sub>10</sub> H <sub>18</sub> O	11.500	0.18
Borneol	C <sub>10</sub> H <sub>18</sub> O	12.800	0.28
Terpinene-4-ol	C <sub>10</sub> H <sub>18</sub> O	13.215	0.34
<i>p</i> -Cymen-8-ol	C <sub>10</sub> H <sub>14</sub> O	14.343	0.26
$\alpha$ -Terpineol	C <sub>10</sub> H <sub>18</sub> O	14.628	0.14
2-Methyl-3-phenylpropanal	C <sub>10</sub> H <sub>12</sub> O	16.041	0.16
Thymol	C <sub>10</sub> H <sub>14</sub> O	16.830	41.46
Carvacrol	C <sub>10</sub> H <sub>14</sub> O	17.382	0.21
Carvol	C <sub>10</sub> H <sub>14</sub> O	17.554	0.06
Thymyl acetate	C <sub>12</sub> H <sub>16</sub> O <sub>2</sub>	18.124	0.43
Isoledene	C <sub>15</sub> H <sub>24</sub>	18.575	0.06
$\beta$ -Bourbonene	C <sub>15</sub> H <sub>24</sub>	18.901	0.05
Caryophyllene	C <sub>15</sub> H <sub>24</sub>	19.792	0.98
Germacrene D	C <sub>15</sub> H <sub>24</sub>	19.982	0.14
Aromadendrene	C <sub>15</sub> H <sub>24</sub>	20.255	0.25
$\alpha$ -Humulene	C <sub>15</sub> H <sub>24</sub>	20.617	0.08
$\alpha$ -Amorphene	C <sub>15</sub> H <sub>24</sub>	21.139	0.15
Ledene	C <sub>15</sub> H <sub>24</sub>	21.632	0.39
$\beta$ -Bisabolene	C <sub>15</sub> H <sub>24</sub>	21.887	0.28
$\delta$ -Cadinene	C <sub>15</sub> H <sub>24</sub>	22.255	0.13
$\alpha$ -Bisabolene	C <sub>15</sub> H <sub>24</sub>	22.664	0.08
Spathulenol	C <sub>15</sub> H <sub>24</sub> O	23.620	0.90
Isolongifolene, 7,8-dehydro-8a-hydroxy-	C <sub>15</sub> H <sub>24</sub> O	24.320	0.05
Isospathulenol	C <sub>15</sub> H <sub>24</sub> O	24.926	0.13
$\alpha$ -Bisabolene epoxide	C <sub>15</sub> H <sub>24</sub> O	25.626	0.08
Monoterpene Hydrocarbons			50.57
Oxygenated Monoterpenes (Monoterpenoids)			43.55
Sesquiterpene Hydrocarbons			2.59
Oxygenated Sesquiterpenes (Sesquiterpenoids)			1.16
Phenylpropenes			0.16
Total			98.03



**Figure 2.** Chromatogram of the chemical profile of *Satureja isophylla* essential oil from Iran.

#### 4. CONCLUSION

The use of detrimental synthetic chemicals is still the main method in pest management strategies. The destructive side-effects of these chemicals on the environment and human health have led researchers to look for novel ways to find eco-friendly and, at the same time, active agents. Since the plant essential oils have been produced as secondary metabolites in the interaction of plants with herbivores, they can have diverse insecticidal effects. These natural agents have complex components and have multiple modes of action against insect pests. Therefore, the chances of insect pests resistant are low. The toxicity of essential oil isolated from an endemic Iranian savory *S. isophylla*, contain a high percentage of terpenes thymol, cymene,  $\beta$ -myrcene, and  $\alpha$ -terpinene, was approved on the two cosmopolitan damaging insect pests of storage grains *R. dominica* and *T. castaneum*. Accordingly, *S. isophylla* essential oil can be suggested as a new bio-agent to organize more investigations in stored-product pest management strategies.

#### ACKNOWLEDGMENT

All supports by the University of Mohaghegh Ardabili are hereby gratefully appreciated.

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