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Susceptibility of *Tribolium castaneum* (Coleoptera: Tenebrionidae) to the Fumigation of Two Essential *Satureja* Oils: Optimization and Modeling

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Abstract: Due to the numerous side effects of synthetic pesticides, including environmental pollution, threats to human health, harmful effects on non-target organisms and pest resistance, the use of alternative healthy, available and efficient agents in pest management strategies is necessary. In this paper, the susceptibility of the cosmopolitan, polyphagous, stored-product pest *Tribolium castaneum* (red flour beetle) to the fumigation of the essential oils of two important medicinal and food additive plants, *Satureja hortensis* and *S. intermedia*, was investigated. The insecticidal properties of the essential oils were modeled and optimized using response surface methodology. It was found that a maximum significant mortality of 94.72% and 92.97% could be achieved within 72 h with the applications of 55.15 µL/L of *S. hortensis* (with the linear model) and 58.82 µL/L of *S. intermedia* (with the quadratic model), respectively. There were insecticidal terpenes and phenylpropanoids in both essential oils, including thymol (50.8%), carvacrol (11.2%) and *p*-cymene (13.4%), in the *S. intermedia* and estragole (68.0%) and methyl eugenol (5.6%) in the *S. hortensis*. It was suggested that the essential oils of *S. hortensis* and *S. intermedia* could be offered as promising pesticidal agents against *T. castaneum* for further studies in the management of such pests instead of detrimental synthetic pesticides.

Keywords: biorational pesticides; chemical profile; fumigant toxicity; modeling; optimization; *S. hortensis*; *S. intermedia*

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1. Introduction

The globally distributed insect pest, the red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), attacks several stored grain products such as beans, cereals, chocolate, flour, meal, nuts, seeds and spices [1]. The adults of *T. castaneum* are long-lived; they can live for up to three years [2]. In addition to qualitative nutritional damage, contaminated products also have reduced quality due to the creation of an unpleasant odor by the secretion of benzoquinone compounds from abdominal glands and remains of various molting stages and cadavers [3]. Furthermore, *T. castaneum* can transmit phytopathogenic microbial agents such as *Aspergillus*, *Pseudomonas* and *Staphylococcus* to infested storage products [4,5]. Bosly and Kawanna [6] demonstrated that *T. castaneum* can actually carry and distribute some *Aspergillus* species, particularly *A. flavus*, the main producer of carcinogenic aflatoxins in agricultural products [7], in stored flour.

Although the use of synthetic pesticides is the principal approach in pest management, the widespread use of these compounds has resulted in numerous side effects, such as environmental contaminations, the accumulation of hazardous residues in food and high toxicity to non-target organisms [8–10]. In addition, the resistance of pests to frequently used chemical pesticides is increasing. For example, the resistance of *T. castaneum* to

phosphine, as one of the commonly used fumigants against stored grain insect pests, was reported [11–13]. Therefore, the introduction of low-risk, available and effective pesticides is essential to alternate with synthetic chemicals in the management of such pests.

The effectiveness of plant-derived essential oils in insect pest control has been demonstrated in numerous studies in recent years [14,15]. Along with the prospective insecticidal effects of essential oils on insect pests from various species, genera, families and orders, their biodegradable nature, safety compared to synthetic chemicals and multiple modes of action in targeted pests indicate that they can be suitable alternatives to detrimental chemical pesticides [16,17]. In fact, the volatile oils obtained from different species of the *Satureja* genus were enriched with terpenes such as 1,8-cineole, γ-terpinene, thymol and β-caryophyllene, which were grouped into four primary classifications: monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes [18–20]. Promising insecticidal effects of *Satureja* essential oils against insect pests of storage products were also reported. These included, for example, insecticidal activities of *S. bachtiarica* Bung and *S. khuzestanica* Jamzad essential oils against *T. castaneum* [21], *S. hortensis* L. essential oil against the bean weevil (*Bruchus dentipes* (Baudi)) [19] and the cowpea weevil (*Callosobruchus maculatus* (Fabricius)) [22] and *S. spicigera* Boiss essential oil against the granary weevil (*Sitophilus granarius* (L.)) [23] and the maize weevil (*Sitophilus zeamais* Motschulsky) [24].

Response surface methodology (RSM) is a set of mathematical and statistical tools for the optimization of independent factors and modeling of dependent factors [25]. The optimization of agricultural and chemical processes requires the simultaneous optimization of several objective functions [26]. Therefore, RSM has been used in numerous agricultural and pharmacological fields [27–30]. For example, RSM was used to find mathematical models and optimized conditions for the toxicity of *Eucalyptus globulus* Labill and *Teucrium polium* L. essential oils against *T. castaneum* [31,32].

Given the importance of using natural and low-risk compounds in pest management, the main purpose of the present study is to investigate the possibility of using *S. intermedia* C. A. Mey and *S. hortensis* L. essential oils in the control of *T. castaneum*. The insecticidal properties of essential oils are modeled and optimized using response surface methodology. Due to changes in essential oil composition under different environmental and geographical conditions [18], the chemical profiles of the *S. intermedia* and *S. hortensis* essential oils were also assessed.

2. Materials and Methods

2.1. Plant Materials and Extraction of the Essential Oils

S. intermedia was gathered from its wild populations in the Heiran regions in Ardebil Province, Iran (38°230′ N, 48°350′ E and an elevation of 910 m). *S. hortensis* was collected from Parsabad, Ardebil Province, Iran (39°38′ N, 47°52′ E and an elevation of 52 m). The *Satureja* species were identified based on the work of Jamzad [33]. The fresh leaves and flowers of *S. intermedia* and *S. hortensis* were used for essential oil extraction. All specimens were allowed to dry in the shade over a 10-day period. For each plant sample, 100 g of plant material was added to a 2-L flask of the Clevenger apparatus and subjected to 3 h hydrodistillation. The extracted essential oils were then individually poured into glass vials, covered with aluminum foil, dried over anhydrous Na_2SO_4 and kept in the refrigerator at 4 °C until use.

2.2. Chemical Profiles of the Essential Oils

The *Satureja* essential oils were analyzed by gas chromatography–mass spectrometry using an Agilent 7890B GC with an Agilent 5977A mass selective detector (MSD), operated in the EI mode (electron energy = 70 eV, scan range = 50–550 amu and scan rate = 3.99 scans/s). The GC column was an HP-5ms fused silica capillary column (30 m in length, 0.25 mm internal diameter, (5% phenyl)-polymethylsiloxane stationary phase and 0.25 µm film thickness). Helium was the carrier gas, with a 52.8 kPa column head pressure and 1.0 mL/min flow

rate. The inlet temperature was 200 °C, and the interface temperature was 280 °C. The GC oven temperature was programed to hold for 1 min at 50 °C and then increase at a rate of 6 °C/min to a final temperature of 290 °C, with a total run time of 50 min. A 10% *w*/*v* solution of the sample in methanol was prepared, and 1 µL was injected using a 100:1 split ratio. Identification of the oil components was based on their retention indices, determined by reference to a homologous series of *n*-alkanes and by comparison of their mass spectral fragmentation patterns with those reported in the databases [34–36].

2.3. Insect Rearing

Adult *T. castaneum* specimens were collected from contaminated wheat grains in warehouses in Parsabad, Ardabil Province, Iran (39°38′ N, 47°52′ E and an elevation of 52 m). The adults were reared on wheat grains in cylindrical glass containers (720 mL) covered by a fine mesh cloth for ventilation. Contaminated grains were kept in the incubator at 27 ± 2 °C and 60 ± 5% RH in the dark. Newly emerged adult insects 1–10 days old were selected for the bioassays.

2.4. Fumigant Toxicity

A series of concentrations from 20.59 to 58.82 µL/L and from 21.00 to 55.15 µL/L was selected to assess the fumigant toxicity of *S. intermedia* and *S. hortensis* essential oils, respectively. Filter papers (Whatman No. 1; 2 × 2 cm) separately treated with the concentrations of the essential oils were glued to the inner surface of the screw caps of fumigant glass containers (340 mL). Twenty unsexed adults (1–10 days old) were transferred to containers before screwing their caps. Then, the caps were sealed to be impermeable to air with parafilm. The bioassay was repeated four times, and in the control groups, all steps were performed except for adding essential oils. The fumigant containers were kept in an incubator with 27 ± 2 °C and 60 ± 5% RH, and insect mortality was recorded after 24, 48 and 72 h of exposure. The mortality in the control groups was corrected by the following formula: $Pt = [(Po - Pc)/(100 - Pc)] \times 100$, in which *P*t is the corrected mortality percentage, *P*o is the mortality of the insects treated by essential oil concentrations and *P*c is the mortality of the insects in the control groups [37].

2.5. Modeling and Optimization by RSM

Using RSM under the historical data design by the statistical software Design Expert 8.0.6 (Stat-Ease, Inc., Minneapolis, MN, USA), various concentrations of essential oils were analyzed in five levels, with the exposure times in three levels as the independent variables and with the mortality of *T. castaneum* as the dependent variable.

Multiple linear regression analysis for the interactions of the independent and dependent variables was used to achieve the statistically appropriate mathematical model in the following second-order polynomial equation [38]:

$$y = \beta o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_j X_j + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} X_i X_j + \sum_{i=1}^{k} \beta_{jj} X_j^2 \quad (1)$$

where y is the mortality of *T. castaneum* (dependent variable), X_i and X_j are the exposure times and essential oil concentrations, respectively (independent variables), k is a number of independent variables (2), β_o is the intercept of the model, β_i and β_j are the coefficients of the linear parameters and β_{ij} specifies the quadratic parameter coefficient. The statistical relationship between the independent and dependent variables was evaluated by the correlation coefficient of determination (R^2), adjusted R^2 and predicted R^2. Optimization of the insect pest mortality caused by the fumigation of *S. intermedia* and *S. hortensis* essential oils was performed to the maximum desirability using Design Expert software with RSM. The statistical significance of the independent variables on the response variables was examined at a 95% confidence level ($p < 0.05$), and only the variables with a significant effect on the response variable were used in the proposed regression equation.

3. Results

The results of the GC-MS analyses of the essential oils of *S. intermedia* and *S. hortensis* are presented in Table 1. It can be seen that the essential oil of *S. intermedia* was rich in the phenolic monoterpenes thymol (50.8%) and carvacrol (11.2%) along with monoterpenes *p*-cymene (13.4%) and 1,8-cineole (4.3%). The essential oil of *S. hortensis*, on the other hand, was dominated by phenylpropanoids, especially estragole (68.0%) as well as methyl eugenol (5.6%) and (*E*)-*p*-methoxycinnamaldehyde (4.4%) (Table 1 and Figure 1).

Table 1. Chemical profiles of the essential oils isolated from the aerial parts of *S. intermedia* and *S. hortensis*.

RIcalc	RIdb	Compound	Percent Composition	
			S. intermedia	*S. hortensis*
933	933	α-Pinene	1.3	1.3
978	982	1-Octen-3-ol	0.9	—
1005	999	3-Octanol	0.4	—
1025	1024	Limonene	—	0.9
1027	1025	p-Cymene	13.4	—
1031	1032	1,8-Cineole	4.3	—
1031	1032	(Z)-β-Ocimene	—	1.2
1048	1044	(E)-β-Ocimene	—	0.9
1054	1057	γ-Terpinene	1.0	—
1097	1091	Rosefuran	—	0.2
1101	1101	Linalool	0.2	0.2
1105	1102	6-Methylhepta-3,5-dien-2-one	—	0.2
1129	1127	allo-Ocimene	—	1.3
1137	1138	cis-p-Mentha-2,8-dien-1-ol	—	0.2
1143	1142	(E)-Myroxide	—	0.2
1149	1145	trans-Verbenol	—	0.1
1174	1171	p-Mentha-1,5-dien-8-ol	—	0.2
1174	1173	Borneol	0.2	0.1
1181	1180	Terpinen-4-ol	0.7	0.1
1203	1201	Estragole (=Methyl chavicol)	—	68.0
1211	1211	β-Cyclocitral	—	0.4
1223	1223	trans-Carveol	—	0.1
1237	1239	Thymyl methyl ether	1.8	—
1248	1244	Carvacryl methy ether	3.1	—
1256	1252	Chavicol	—	0.2
1259	1257	p-Anisaldehyde	—	0.5
1288	1289	Thymol	50.8	0.3
1296	1298	Carvacrol	11.2	0.8
1361	1356	Eugenol	—	0.1
1362	1365	Carvacryl acetate	0.1	—
1407	1405	Methyl eugenol	—	5.6
1424	1424	(E)-β-Caryophyllene	1.1	—
1478	1478	γ-Muurolene	0.2	—
1490	1490	(E)-β-Ionone	—	0.3
1497	1491	Viridiflorene	0.4	—
1500	1500	α-Muurolene	0.1	—
1508	1508	β-Bisabolene	0.9	—
1516	1514	γ-Cadinene	0.2	—
1524	1518	δ-Cadinene	0.4	—
1540	1541	(E)-α-Bisabolene	0.1	—
1544	1544	α-Calacorene	0.1	—
1552	1554	Thymohydroquinone	0.4	—
1575	1567	(E)-p-Methoxycinnamaldehyde	—	4.4
1576	1576	Spathulenol	1.0	0.5
1587	1583	allo-Spathulenol	—	3.5
1589	1587	Caryophyllene oxide	0.8	0.8
1611	1614	1,10-di-epi-Cubenol	—	0.4
1613	1613	Humulene epoxide II	0.1	—

Table 1. *Cont.*

RIcalc	RIdb	Compound	Percent Composition	
			S. intermedia	*S. hortensis*
1629	1631	1-epi-Cubenol	tr	—
1635	1638	cis-Cadin-4-en-7-ol	0.1	—
1636	1629	iso-Spathulenol	—	0.2
1639	1636	Caryophylla-4(12),8(13)-dien-5β-ol	0.1	—
1641	1640	τ-Cadinol	0.1	—
1646	1645	α-Muurolol (=δ-Cadinol)	tr	—
1655	1655	α-Cadinol	0.1	—
1658	1656	14-Hydroxy-9-epi-(Z)-caryophyllene	0.1	—
1667	1668	ar-Turmerone	0.5	0.2
1667	1668	β-Turmerone	tr	—
1672	1666	14-Hydroxy-9-epi-(E)-caryophyllene	0.2	—
1675	1677	Cadalene	tr	—
1681	1679	epi-α-Bisabolol	tr	—
1687	1683	Germacra-4(15),5,10(14)-trien-1α-ol	tr	—
1699	1701	Curlone	0.1	—
1841	1841	Phytone	0.1	0.4
1926	1925	Methyl palmitate	—	0.2
2109	2109	Phytol	—	1.2
		Monoterpene hydrocarbons	15.7	5.7
		Oxygenated monoterpenoids	72.8	2.8
		Sesquiterpene hydrocarbons	3.5	0.0
		Oxygenated sesquiterpenoids	3.1	5.6
		Benzenoid aromatics	0.0	78.7
		Others	1.4	2.3
		Total identified	96.4	95.0

RIcalc = Retention index, determined with respect to a homologous series of n-alkanes on an HP-5 ms column; RIdb = Retention index from the databases.

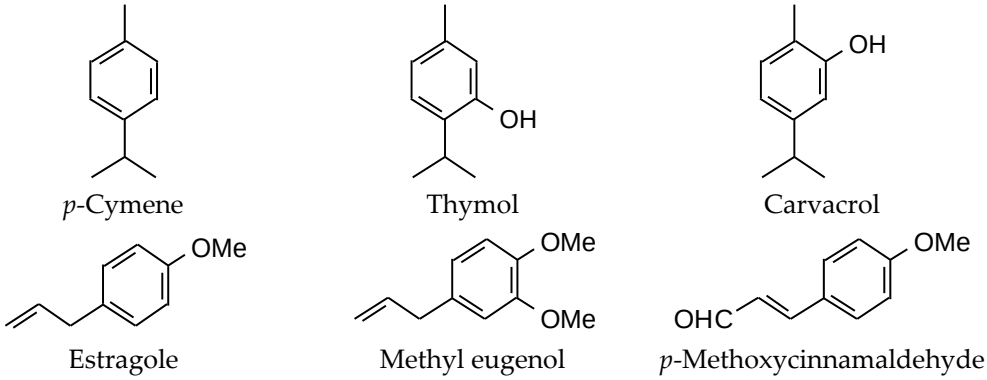

Figure 1. The structures of the major components in the essential oils of *Satureja intermedia* and *S. hortensis*.

Table 2 shows the results of the analysis of variance (ANOVA), which indicated that the mortality of *T. castaneum* adults was significantly affected by A (exposure time) and B (essential oil concentration) in the *S. hortensis* treatment and by A, B, and B^2 in the *S. intermedia* treatment ($p < 0.01$). The lack-of-fit test for both essential oils was non-significant, demonstrating the validation of treatments (Table 2).

In Table 3, the fitting effect of different levels of the essential oil concentration and exposure time on the mortality amount is presented. The model's fitting was evaluated on the basis of the coefficient of determination (R^2), adjusted R^2 and prediction R^2 as well as the coefficient of variation (CV). It can be seen that all the R^2 values were high (>0.94), meaning that the response surface methodology models were suitable. Furthermore, the coefficient of variation for almost all parameters was below 8.4%. This means that the results demonstrated good accuracy and precision with the reliability of experiments. As

is shown in Table 3, it was found that the linear effects of the dependent variables on the mortality amount were significant ($p < 0.05$) under the essential oil of *S. hortensis*. The effect on the mortality value under the essential oil of *S. intermedia* was significant according to the quadratic equation ($p < 0.05$). The mortality was strongly influenced by the essential oil concentrations, based on the higher coefficient for B in the equation of Table 3.

Table 2. Results of analysis of variance for prediction of the fumigant toxicity of *S. intermedia* and *S. hortensis* essential oils against *T. castaneum*.

Essential Oil	Source	Sum of Squares	df	Mean Square	F Value	*p*-Value
S. intermedia	Model	21,460.07	3	7153.36	287.26	<0.0001
	A	4305.62	1	4305.62	172.90	<0.0001
	B	16,798.40	1	16,798.40	674.58	<0.0001
	B^2	518.89	1	518.89	20.84	<0.0001
	Residual	1394.51	56	24.90		
	Lack of Fit	250.76	11	22.80	0.90	0.5504 NS
	Pure Error	1143.75	45	25.42		
	Model	25,853.86	2	12,926.93	760.04	<0.0001
S. hortensis	A	4622.50	1	4622.50	271.78	<0.0001
	B	21,231.36	1	21,231.36	1248.30	<0.0001
	Residual	969.47	57	17.01		
	Lack of Fit	231.97	12	19.33	1.18	0.3262 NS
	Pure Error	737.50	45	16.39		
	Cor Total	26,823.33	59			

A: exposure time (h); B: essential oil concentrations (µL/L); NS: non-significant.

Table 3. RSM modeling results for predicting the mortality percentage under different concentrations of essential oils and exposure times.

Essential Oil	Equation	R^2 Value	Adj R^2	Pred R^2	C.V. (%)
S. intermedia	$-34.33772 + 0.43229A + 2.77692B - 0.019410B^2$	0.9390	0.9357	0.9297	8.40
S. hortensis	$-23.11885 + 0.44792A + 1.55199B$	0.9639	0.9626	0.9600	7.59

A: exposure time (h); B: essential oil concentrations (µL/L).

Figure 2 presents the effect of different concentrations of essential oils (in five levels) and exposure times (in three intervals) on the mortality of *T. castaneum* adults. It can be seen that the susceptibility or the mortality of the insect pest was increased by increasing the concentrations of both the essential oils and the exposure times. According to the color points in Figure 2, the mortality of *T. castaneum* was increased from 15% to 95% and from 20% to 100% by increasing the concentrations of the *S. hortensis* and *S. intermedia* essential oils from 21.00 to 55.15 µL/L and from 20.59 to 58.82 µL/L, respectively, and the exposure times from 24 to 72 h.

Plots of the residuals versus the predicted mortality of *T. castaneum* adults caused by the fumigation of *S. intermedia* and *S. hortensis* essential oils are shown in Figure 3. It can be seen that the introduced models for predicting *T. castaneum* mortality were accurate and consistent with the variation hypothesis.

The optimized conditions for the significant maximum mortality of *T. castaneum* adults treated by the essential oils of *S. intermedia* and *S. hortensis* after different exposure times are shown in Table 4. After 72 h of exposure time, the maximum mortality of the pest (92.973%) may be achieved with 58.820 µL/L of *S. intermedia* essential oil with a desirability of 0.9121, while a concentration of 55.150 µL/L would be sufficient to attain the maximum mortality (94.72%) with *S. hortensis* essential oil with desirability of 0.997%. This indicated that the *T. castaneum* adults were more susceptible to the essential oil of *S. hortensis* than the *S. intermedia* (Table 4).

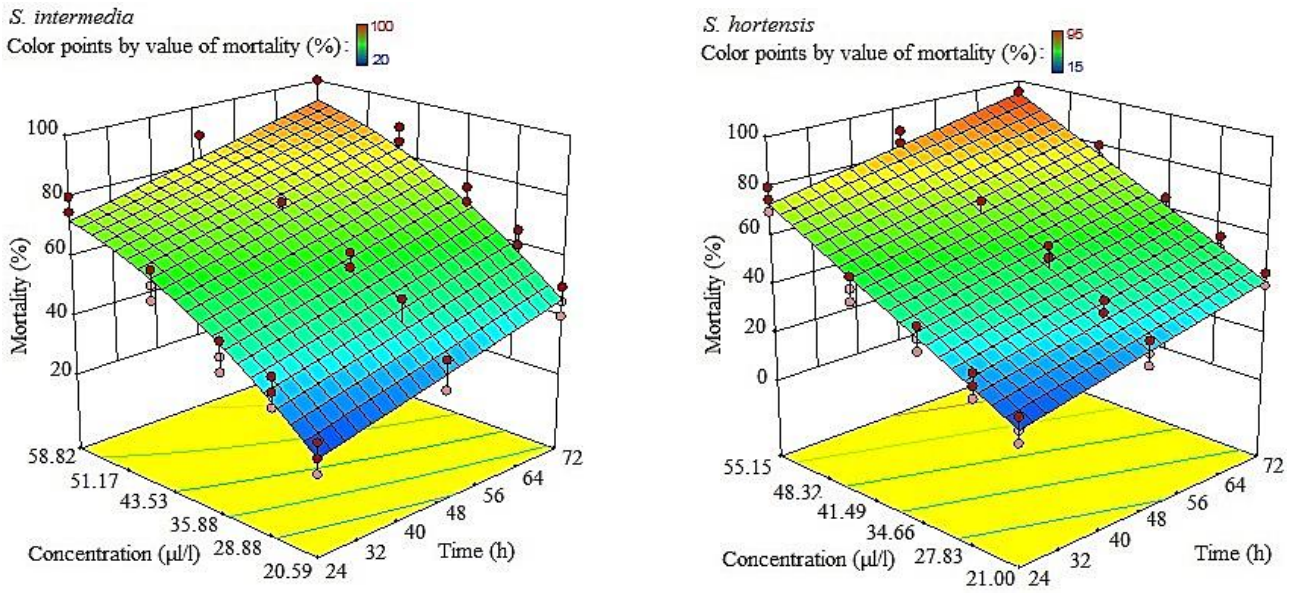

Figure 2. Three-dimensional diagrams of the mortality of *T. castaneum* caused by the fumigation of *S. intermedia* and *S. hortensis* essential oils.

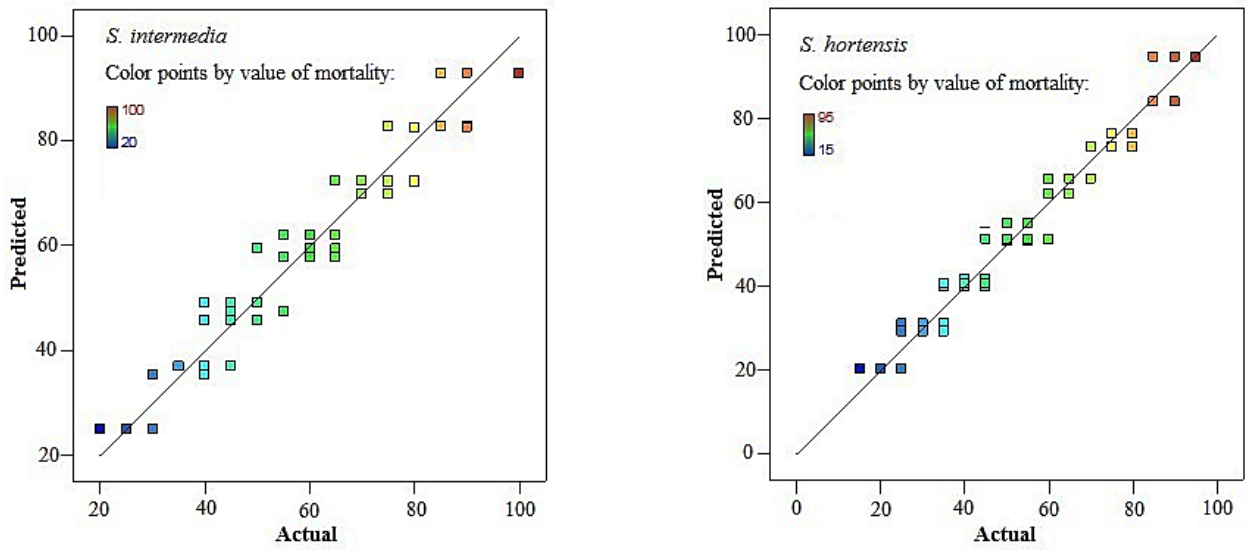

Figure 3. Plots of residual vs. predicted mortality of *T. castaneum* caused by the fumigation of *S. intermedia* and *S. hortensis* essential oils.

Table 4. Optimization of the mortality of *T. castaneum* caused by the fumigation of *S. intermedia* and *S. hortensis* essential oils.

Essential Oil	Mortality (%) *	Time (h)	Concentration (µL/L)	Desirability
S. intermedia	50.000	31.569	33.127	1.000
	92.973 *	72.000	58.820	0.912
S. hortensis	50.000	61.952	29.233	1.000
	94.723 *	72.000	55.150	0.997

* The maximum significant mortality percentage based on high desirability, calculated by Design Expert software.

4. Discussion

In the *S. intermedia* essential oil, 95.1% of the identified compounds were terpenes, in which oxygenated monoterpenoids such as thymol (50.8%) and carvacrol (11.2%) had high concentrations. In contrast, the terpenes had only 14.1% of the total *S. hortensis*

essential oil, and this essential oil was rich in benzenoid aromatics such as estragole (68.0%), methyl eugenol (5.6%) and (*E*)-*p*-methoxycinnamaldehyde (4.4%). Therefore, the essential oils studied in the present study were completely different in terms of chemical components. It was reported that γ-terpinene (42.3%), carvacrol (32.8%), *p*-cymene (8.1%), β-pinene (2.3%), β-caryophyllene (2.2%), α-thujene (1.9%) and α-pinene (1.5%) were the main components of the essential oil isolated from the aerial parts of *S. intermedia* at the flowering stage from Romania [39]. In the present study, only carvacrol (0.8%) and α-pinene (1.3%) in low concentrations were found in *S. hortensis* at the pre-flowering stage. In contrast, estragole and methyl eugenol, identified in high amounts in the present study, were not detected in the essential oil investigated by Chambre et al. [39]. The chemical composition of *S. intermedia*, as one of the *Satureja* species endemic to Iran, was assessed [40], and γ-terpinene (37.1%), thymol (30.2%), *p*-cymene (16.2%), limonene (3.9%), α-terpinene (3.3%) and β-myrcene (2.5%) were reported as the main components. In the present study, γ-terpinene, thymol and *p*-cymene were also identified, but there were no traces of limonene, α-terpinene or β-myrcene. Accordingly, differences in the chemical compositions of essential oils may be related to the different species and stages of plants as well as the temperature, humidity, height and other geographical conditions of the cultivation locales [18,41].

The insecticidal effects of the essential oils extracted from *S. hortensis* and *S. intermedia* against stored-product insect pests were also reported in some recent studies. The fumigant toxicity of *S. hortensis* essential oil against *C. maculatus*, *S. zeamais* and the Mediterranean flour moth (*Ephestia kuehniella* Zeller) was studied [22,24,42]. Additionally, the fumigant toxicity of *S. intermedia* essential oils against the lesser grain borer (*Rhyzopertha dominica* (Fabricius)) and the khapra beetle (*Trogoderma granarium* Everts) was observed in one of our previous works [43]. These results are consistent with the current findings about the insecticidal potential of essential oils of the *S. hortensis* and *S. intermedia* species. Overall, the insecticidal effects of plant essential oils are related to their active compounds such as terpenes and phenylpropanoids [44,45]. As is shown in Table 5, the strong insecticidal effects of the components were identified in high percentages in the *S. intermedia* and *S. hortensis* essential oils, including 1,8-cineole, carvacrol, estragole, methyl eugenol, *p*-cymene, thymol and α-pinene (in both essential oils). The insecticidal activity of *S. intermedia* and *S. hortensis* essential oils may be highly associated with these active compounds.

Table 5. Review of insecticidal effects for the main compounds identified in *S. intermedia* and *S. hortensis* essential oils.

Compound	Reported Insecticidal Effects
p-Cymene	Significant toxicity against the third and fourth instar larvae and pupae of mosquito *Culex quinquefasciatus* Say [46].
1,8-Cineole	High contact toxicity to the third instar larvae of the diamondback moth (*Plutella xylostella* (L.)), which was synergistically increased by terpene pulegone [47]. Significant fumigant toxicity against the adults of rice weevil (*Sitophilus oryzae* (L.)) and *T. castaneum* [48].
Thymol	High contact toxicity to the third instar larvae of the diamondback moth, which was synergistically increased by the terpene pulegone [47]. The 100% mortality of adults of *S. granarius* by the fumigation of 163.3 µL/L after 96 h [49].
Carvacrol	The 100% mortality of adults of *S. granarius* by the fumigation of 166.7 µL/L after 96 h [49]. Significant toxicity against the third and fourth instar larvae and pupae of *C. quinquefasciatus* [46]. Significant fumigant toxicity against the adults of *T. castaneum* after 24 h [44].
Estragole	Significant fumigant toxicity and repellent action against the adults of *S. zeamais* after 168 and 1 h, respectively [45]. Strong fumigant toxicity against *S. zeamais* [50].
Methyl eugenol	Toxic to the third instar larvae of cigarette beetle (*Lasioderma serricorne* (F.)) in the feeding test after 168 h [51].
α-Pinene (presence in both essential oils)	The 100% mortality of adults of *S. granarius* by the fumigation of 72.5 µL/L after 96 h [49]. Significant toxicity against the third and fourth instar larvae and pupae of *C. quinquefasciatus* [46].

Furthermore, according to the study of Kim et al. [44], estragole had more fumigant toxicity against *S. zeamais* (LC_{50} = 0.004 mg/cm^3) and *T. castaneum* (LC_{50} = 0.013 mg/cm^3) adults than the terpenes limonene, linalool, β-myrcene, α-pinene and α-humulene. Therefore, having a high percentage of estragole in *S. hortensis* essential oil can be the main reason for its higher toxicity than *S. intermedia*. However, the synergistic and antagonistic effects between all essential oil components should be considered. For example, the toxicity of 1,8-cineole and thymol against the third instar larvae of diamondback moths (*Plutella xylostella* (L.)) were synergistically enhanced in combination with another terpene, pulegone, while the pulegone alone had no significant toxicity to the pest [47].

According to our recent studies, the *T. castaneum* adults were susceptible to the fumigation of *S. hortensis* and *S. intermedia* essential oils [43,52]. However, the optimization and modeling of the fumigant toxicity of these essential oils, through RSM, are reported for the first time in the current study. The use of RSM in the optimization and modelling of the insecticidal efficiency of essential oils can be found in a few other investigations. For example, optimization of the fumigant toxicity of *Thymus vulgaris* L. essential oil indicated that a concentration of 25.86 µL/L and a 59.00-h exposure time were sufficient to achieve 50% mortality of *R. dominica* adults [53]. In another work on the essential oil of *Thymus kotschyanus* Boiss. & Hohen, the optimized condition for 50% mortality against *R. dominica* adults was 24.62 µL/L and a 57.98-h exposure time [54]. Based on the above-mentioned results, the essential oil of *T. kotschyanus* was more toxic than the *T. vulgaris* oil. Regarding the toxicity of *S. hortensis* and *S. intermedia* essential oils against *T. castaneum*, 29.23 and 33.13 µL/L of essential oils after 61.95 and 31.57 h, respectively, can result in 50% mortality of the pest. In one of our other studies, modeling and optimization of the fumigant toxicity of *Teucrium polium* L. essential oil against *T. castaneum* adults was evaluated through RSM, and it was found that 97.97% mortality of the insect pest could be attained by a 20 µL/L essential oil concentration after 72 h with the model $+0.71 - 0.047A - 8.84E - 3B + 3.89E - 4AB + 3.27E - 3A^2 + 8.38E - 5B^2$ (A and B are the time and essential oil concentration, respectively) [31]. In comparison with these results, and after 72 h of exposure time, 55.15 and 58.82 µL/L of *S. hortensis* and *S. intermedia* essential oils could result in 94.72% and 92.97% mortality of *T. castaneum*, respectively. The differences may be associated with different tested essential oils and subjected insect pests. In addition, the above-mentioned and present findings indicated that RSM is a useful method for the optimization and modeling of the insecticidal effects of essential oils.

The disadvantage of such essential oils (i.e., low persistence in environmental conditions) can be improved by micro-and nano-encapsulation formulations based on controlled release techniques [55,56].

5. Conclusions

The present study has shown that the essential oils isolated from the aerial parts of *S. intermedia* and *S. hortensis* had strong fumigant toxicity against the adults of the red flour beetle *T. castaneum*. GC-MS analyses indicated that there was a high percentage of insecticidal components such as estragole and methyl eugenol in the *S. hortensis* essential oil and thymol, carvacrol, *p*-cymene and 1,8-cineole in the *S. intermedia* essential oil. However, additional studies are required to determine which components have efficient insecticidal activity. Based on RSM, it was found that the best model for the prediction of mortality was linear and the quadratic equation for the essential oil of *S. hortensis and S. intermedia*, respectively. Up to 94.72% and 92.97% mortality of the insect pest, as the maximum significant mortality, can be attained with 55.15 and 58.82 µL/L of *S. hortensis* and *S. intermedia* essential oils, respectively, after 72 h. The essential oils of *S. hortensis* and *S. intermedia* can be proposed for further research in the management of *T. castaneum* and probably other stored-product insect pests.

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