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## Essential oil composition and insecticidal activity of *Satureja boissieri* against *Sitophilus granarius* (Coleoptera:Curculionidae) and *Spodoptera littoralis* (Lepidoptera: Noctuidae)

*Satureja boissieri*'nin *Sitophilus granarius* (Coleoptera:Curculionidae) ve *Spodoptera littoralis* (Lepidoptera: Noctuidae)'e insektisidal aktivitesi ve uçucu yağ kompozisyonu

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### ABSTRACT

In the scope of this study, we have investigated fumigant and contact toxicity effects of *Satureja boissieri* Hausskn. ex Boiss. essential oil against *Sitophilus granarius* L. (Coleoptera: Curculionidae) and *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). The fumigant and contact toxicity experiments were carried out with the adults of *Sitophilus granarius* whereas the contact toxicity experiment was carried out with the 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> larvae of *S. littoralis*. Also, various fractions of *S. boissieri* essential oil were investigated for fumigant activity against *S. granarius*. In fumigant toxicity studies, the highest activity was obtained at 36 hours with a dose of 0.1 µl/ml with a 100% mortality rate. Fractions of the essential oil had significant fumigant activity on *S. granarius* (100% fumigant activity at 0.1 µl/ml). Contact toxicity of 100 µl/ml essential oil on the 3<sup>rd</sup> and 4<sup>th</sup> larval stage of *S. littoralis*, was determined to be 100%, however, on the 5<sup>th</sup> larval stage it was 90.17%. The activity at 25 µl/ml dose was 71.12% in the 3<sup>rd</sup> larval stage whereas the activity remained very low (6.39- 22.20%) in other larval stages. These results indicated that essential oil of *S. boissieri* have significant potential which merits to be studied further in possible applications as fumigant and contact insecticide in the control of *S. granarius* and *S. littoralis*.

### INTRODUCTION

There are 574 species and 741 taxa belonging to the Lamiaceae family in Turkey (Davis 1982, Erik and Tarikahya 2004).

Many important genus including *Satureja* sp., *Origanum* sp., *Thymus* sp. that have extensive uses such as tea, spice

are members of Lamiaceae family (Kurkcuoglu et al. 2001). In Turkey, genus *Satureja* has 15 species, five of which are endemic. Previous reports indicate that essential oils obtained from plants belonging to the genus *Satureja* have various activities (i.e., antioxidant, antimicrobial, antibacterial, and antidiabetes) (Azaz et al. 2002, Ayvaz et al. 2010, Momtaz and Abdollahi 2010). In addition, the insecticidal activities of essential oils from *Satureja* species on insects have been reported by some other researchers (Maede et al. 2011, Taban et al. 2017, Tozlu et al. 2011). *Satureja boissieri* (Lamiales: Lamiaceae) is distributed in Adiyaman, Bingöl, and Malatya in Turkey and is not an endemic species (Davis 1982). The main components of essential oils obtained from *S. boissieri* were previously reported to be thymol, carvacrol,  $\gamma$ -terpinene and *p*-cymene (Kurkcuoglu et al. 2001). However, the quantities and respective ratios of the main components vary according to the regions and geographical locations (Kurkcuoglu et al. 2001, Sajjadi and Baluchi 2002, Sefidkon and Jamzad 2006). *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is a polyphagous pest, which spreads over a wide area in Turkey (Aydin and Gurkan 2006). This pest can cause a high-level loss in vegetables and fruits as well as in industrial and field crops with high economic value (Ünlü and Kornoşor 2003, Yıldırım and Başpınar 2008). Significant quality and quantitative losses are observed due to biotic and abiotic factors in agricultural products. Therefore, there is an increasing need for new effective substances and new strategies to be used in pest management. Microorganisms, rodents and harmful insects cause large losses in stored products due to the lack of adequate protection measures in grain storages. *Sitophilus granarius* L. (Curculionidae: Coleoptera) is one of the primary pests causing damage in stored cereal crops (Karakoç et al. 2006). Shaaya et al. (1997) reported that in countries where modern storage techniques is not present, the losses from insects in grains and legumes can reach up to 40%.

In the scope of this study, insecticidal activity and chemical composition of essential oil obtained from *S. boissieri* was determined. Previously, there is no report on the insecticidal activity of *S. boissieri* against *S. littoralis* and *S. granarius* in the literature. Therefore, the insecticidal activity studies carried out against these pest species are reported for the first time.

## MATERIALS AND METHODS

### *Insect rearing*

*Sitophilus granarius* adults used in the experiments were

cultured in the Biology Department of the Faculty of Science of Çankırı Karatekin University. Five-liter glass jars were used for growing adults. In order to obtain single age populations of the insects, females and male insects were placed into jars that contain wheat and stored in a dark climate room at  $27\pm 2$  °C for 48 hours. At the end of 48 hours, adult individuals were removed from the jars and only eggs laid by the insects were left. After 45 days, a new generation of adult individuals emerged. In the experiments, 3-4 weeks old adults were used. *Spodoptera littoralis* were continuously reared on lettuce plants at Karatekin University in Çankırı, Turkey during the study as described in Karakoç and Gökçe (2012). In the rearing of *S. littoralis*, 150-200 larvae were transferred to plastic containers and daily fresh lettuce leaves were given. The larvae were fed with lettuce leaves for ten days. Adults were fed with 15% honey solution to lay eggs on wax paper sheets placed in plastic containers. Honey solution was absorbed in cotton plug and placed in the container. All experiments on *S. littoralis* were conducted at 16:8 (light/dark) light regime condition at 25 °C.

### *Plant material*

Plant material (*Satureja boissieri*) was collected at the flowering stage from its natural habitat in Ortaköy Village (38° 51' 31.77" N, 40° 21' 50.32" E) in July 2012. The plants collected were dried in a room without direct sunlight and air circulation. Plant samples were prepared for identification and to be stored in a herbarium. Plant species was identified at Biology Department of Bingöl University and the voucher specimen was registered in the Herbarium of the department with a voucher number: L. Behçet 176.

### *Isolation of essential oil*

The dried aerial parts of *S. boissieri* were cut into small pieces. Two kilograms of the plant is weighed and used in distillation. Distillation was done in the Clevenger type apparatus for approximately three hours (100 g x 20). A total of 28 ml (~27.3 g) of essential oil was obtained. After the distillation the resulting oil was dried on the anhydrous sodium sulfate. Water was removed from the oil than its removed from sodium sulfate with filtration, which was performed with blue band filter paper. The resulting essential oil was stored until the time of analysis in dark glass containers at +4 °C.

### *Essential oil separation*

Approximately 110 g of silica gel were activated in the oven at 100 °C for 24 hours to separate the essential oils. 7680 mg (8 ml) of crude essential oil material was separated by

column chromatography using analytical grade solvents. Chromatography with 100% n-hexane solvent was initiated and followed by increasing polarity, diethyl ether: n-hexane (1:99, v/v), diethyl ether: n-hexane (2:98, v/v), diethyl ether: n-hexane (3:97, v/v), diethyl ether: n-hexane (4:96, v/v), diethyl ether: n-hexane (5:95, v/v), diethyl ether: The separation process was terminated using hexane (6:94, v/v), diethyl ether: n-hexane (10:90, v/v) and finally 100% diethyl ether solvent system. A total of 1770 fractions were obtained. Combination of similar fractions was made in some series based on the results of GC-MS analysis. Fractions that are sufficient for biological activity tests were investigated for fumigant activity against *S. granarius*. The fractions between 450-550 contained thymol (52.04%) + carvacrol (31.35%) and the fractions between 580-720 contained thymol (100%) were used in this part of the experiment.

#### *Gas chromatography-mass spectrometry analysis of the fractions*

Constituents of *S. boissieri* essential oil fractions was analyzed with the Agilent Technologies 7890A GC System coupled to a 5975C inert MSD with Triple-Axis Detector (Agilent Technologies; Capillary column HP-5-MS (30 m x 0.25 mm I.D. x 0.25 µm). GC temperature program was as follow: From 60 °C to 150 °C at a rate of 3 °C/min and holding for 10 min, then from 150 °C to 200 °C at a rate of 5 °C/min and holding for 3 min, and from 200 °C to 250 °C at a rate of 15 °C/min. Inlet temperature was 250 °C. Spectra were obtained for the range 50–550 m/z, following electron impact ionization at 70 eV. The carrier gas was helium at a flow rate of 1 ml/min and injections were in split mode (50:1). The mass-spectrometer interface temperature was set at 250 °C. The temperature of the ion source was 230 °C, electron energy 70 eV and quadruple temperature 150 °C. Injection volume was 1 µl.

#### *Fumigant toxicity*

In this study, the fumigant effect of essential oil extracted from *S. boissieri* on *S. granarius* was investigated in three different doses (0.025, 0.05, 0.1 µl/ml). Glass tubes with 10 ml volume and compressible cap were used in these experiments. Glass tubes were filled with 1/3 wheat and 10 adult individuals were placed in each. 10 mm in diameter discs were cut from Whatman filter paper and fixed with a pin to the caps of the glass tubes used in the experiment. Essential oil dilutions in acetone at different concentrations were applied with a micropipette to the filter paper to achieve final essential oil solution 0.05; 0.1 and 0.2 µl/ml air in the tubes. The applied filter papers were then placed in the fume hood for 5 minutes to allow the acetone to evaporate. At the end of this period,

the filter papers were fixed to the caps of the glass tubes using a pin and the caps were put on the glass tubes and closed, and tubes were incubated in the dark in a climatic cabinet for 36 hours at 25 °C. Dead insects were counted and recorded after 12, 24 and 36 hours. The study was established according to the randomized block design. The entire trial was repeated three times and each replicate consisted of 3 replicates.

#### *Contact toxicity*

*Satureja boissieri* essential oil was diluted with acetone to obtain 25, 50 and 100 µl/ml stock solution. Stock concentrations were applied to *S. granarius* (1 µl/insect) and *S. littoralis* (1 µl/insect for 3<sup>rd</sup> larval stage, 2 µl/insect for 4<sup>th</sup> larval stage and 3µl /insect for 5<sup>th</sup> larval stage) with a micro applicator. The acetone was applied (1 µl/insect) as blank control. Essential oil concentrations were applied with micro applicator on the dorsal surface of the thorax of the insects Karakoç and Gökçe (2012). After the treatment, insects were transferred to 9 cm petri dishes containing 10 g of wheat. The insects were incubated at 25±2 °C in a climatic chamber. The mortality was recorded after 24 h. Each treatment was replicated three times and the whole experiment was repeated three different times. Twenty larvae was used for each replication.

#### *Statistical analysis*

Results were firstly converted into % death values, and then they were subjected to arcsine transformation. Variance analysis was carried out with transformed data at alpha: 0.05 level, and additionally, the differences among treatments were analyzed by means of Tukey multiple comparison test (α:0.05). All statistical analyses were conducted with MINITAB® Release 16 package program.

## **RESULTS AND DISCUSSION**

#### *Fumigant toxicity against S. granarius*

Three different doses of essential oil of *S. boissieri* were tested for fumigant toxicity against *S. granarius* (Table 1). According to the results obtained, mortality rates increased with time and dose increase. The highest effect was determined with 100% mortality rate at 0.1 µl/ml dose after 36 hours. Mortality rates of fumigants were reduced as fumigant exposure time decreased, and this decrease was 76.72% at 24 hours and 36.64% at 12 hours, respectively (F=1766.64; df:3,8; P<0.05). When the application concentration was reduced, essential oil of *S. boissieri* did not show considerable fumigant toxicity.

**Table 1.** Fumigant toxicity of essential oils from *Satureja boissieri* on *Sitophilus granarius*

Dose (µl/ml)	% Mortality±SE*		
	12 hour	24 hour	36 hour
0 (Control)	0.00±0.00b <sup>1</sup> A <sup>2</sup>	0.00±0.00cA	0.00±0.00dA
0.025	0.00±0.00bB	0.00±0.00cB	7.71±0.12cA
0.05	0.00±0.00bB	9.83±0.32bA	16.58±0.20bA
0.1	36.64±0.12aC	76.72±0.16aB	100.00±0.00aA

<sup>1</sup>Means in a column followed by a different lowercase letter are significantly different (Anova P < 0.05, Tukey test). <sup>2</sup>Means in a line followed by a different uppercase letter are significantly different (Anova P < 0.05, Tukey test). \*Standard Error

#### Contact toxicity bioassay

Experiments were carried out at different doses to determine the contact toxicity of essential oil of *S. boissieri* against *S. granarius* after 24 hours. The data at the end of 24 hours were evaluated, mortality rates increased with increase in the dose. The mortality rate at 100 µl/ml concentration was determined as 97.82% and was statistically different from the control and other doses (F=199.39; df:3,8; P<0.05). Contact toxicity of essential oil obtained from *S. boissieri* plant was also tested on different larval stages of *S. littoralis*. The essential oil of *S. boissieri* had high contact toxicity for the all larval stages at the dose of 100 µl/ml. Mortality rate was observed as 100%, 100% and 90.17% for the 3<sup>rd</sup> (0.1 µl essential oil dose per larvae), 4<sup>th</sup> (0.2 µl essential oil dose per larvae) and 5<sup>th</sup> (0.3 µl essential oil dose per larvae) larval stages, respectively (F=96.01; df:2,6; P<0.05). After, the dose was reduced in the experiments (50 µl/ml), the highest effect was observed in the 3<sup>rd</sup> larval stage with 95.68% and the mortality rates in the 4<sup>th</sup> and 5<sup>th</sup> larval stages were 86.79% and 53.34%, respectively (F=125.39; df:2,6; P<0.05). Finally, when the dose was lowered to 25 µl/ml, the mortality rate was 71.12% for the 3<sup>rd</sup> larval stage and activity decreased in the other larval stages. (F=227.10; df:2,6; P<0.05) (Table 2).

#### Chemical composition and insecticidal activity of fractions

Chemical composition of the fractions which were tested for fumigant activity against *S. granarius* was also analyzed with GC/MS. The essential oil fractions showed considerable fumigant activity. The combined fractions between 450-550 caused 70.33% death at 5% (v:v) application concentration and the mortality rate in *S. granarius* reached 100% at 10% (v:v) concentration. When the combined fractions between 580-720 were tested in fumigant contact toxicity against *S. granarius*, 53.35% mortality was determined in 5% (v:v) concentration and 100% mortality was achieved in 10% (v:v) application concentration (Table 3).

The main constituents of the essential oil obtained from the *S. boissieri* plant collected from the Bingöl region were determined as *p*-cymene,  $\gamma$ -terpinene, thymol, and carvacrol. The main constituent in *S. boissieri* used in this study was *p*-cymene with 23.15%, while in other studies this rate was 35.5%, 17.1%, and 6.3%, respectively (Azaz et al. 2002, Sajjadi and Baluchi 2002, Sefidkon et al. 2006). Azaz et al. (2002) reported that carvacrol was the major component of this plant with 44.8%. Similarly, in the study conducted by Sajjadi and Baluchi (2002), the main component of *S. boissieri* was reported to be carvacrol 70.1%. In another study *S. boissieri*

**Table 2.** Contact toxicities of essential oil from *Satureja boissieri* on *Spodoptera littoralis*

Dose (µl/ml)	% Mortality±SE*		
	3 <sup>rd</sup> instar larvae	4 <sup>th</sup> instar larvae	5 <sup>th</sup> instar larvae
0 (Control)	0.00±0.00d <sup>1</sup> A <sup>2</sup>	0.00±0.00dA	0.00±0.00dA
25	71.12±0.05cA	6.39±0.48cC	22.20±0.05cB
50	95.68±0.20bA	86.79±0.24bB	53.34±0.11bC
100	100.00±0.00aA	100.00±0.00aA	90.17±0.32aB

<sup>1</sup>Means in a column followed by a different lowercase letter are significantly different (Anova P < 0.05, Tukey test). <sup>2</sup>Means in a line followed by a different uppercase letter are significantly different (Anova P < 0.05, Tukey test). \*Standard Error

**Table 3.** Fumigant toxicity of essential oil fractions obtained from *Satureja boissieri* on *Sitophilus granarius*

Fractions	% Mortality±SE*	
	%5	%10
450-550	70.33±1.22a	100.00±0.00a
580-720	53.35±0.34a	100.00±0.00a
Control	0.00±0.00b	0.00±0.00b

\*Means in a column followed by a different lowercase letter are significantly different (Anova  $P < 0.05$ , Tukey test). \*Standard Error

essential oil main components were determined as thymol (29.6%) was identified as the major component in the highest proportion (Sefidkon et al. 2006). The results obtained in this study have concluded that there may be a chemotypic variation in *S. boissieri* when compared with previous studies.

*S. boissieri* showed the highest fumigant activity against *S. granarius*. This activity is thought to originate from the main components of the essential oil obtained from the plant. Although there is no study on this insect species with *S. boissieri* essential oil, studies with other *Satureja* species have shown that essential oils obtained from this genus exhibit fumigant activity (Ayvaz et al. 2010, Maede et al. 2011, Yıldırım et al. 2011). Furthermore, the fumigant activity of these major components found in *S. boissieri* was also determined by researchers (Isman 2000, Lee et al. 2001, Rozman et al. 2007). Contact toxicity studies were also carried out on *S. granarius* at the same time and showed high insecticidal activity. Tapondjou et al. (2005) who studied *p*-cymene from the main components of *S. boissieri* demonstrated contact toxicity of this compound on *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). It is suggested that contact toxicity can arise from this compound.

Considerable results were obtained in contact effect studies on *S. littoralis*. The dose of 100 µl/ml used in the experiments showed very high insecticidal activity for all larval stages. Pavela (2010) was determined that *p*-cymene,  $\gamma$ -terpinene, thymol and carvacrol, the major components of the plant, exhibit insecticidal activity on this species. When the dose was reduced by 1/4, high activity was still observed for the 3<sup>rd</sup> larval stage, while activity for the 4<sup>th</sup> and 5<sup>th</sup> larval stages remained very low. This may be interpreted as a decrease in activity of the essential oil as the larval period progresses depending on the physiology of the insect. Alkan et al. (2017) found that the activity changed due to the larval period in the study conducted with *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). Similarly, Karakoç and Gökçe (2012) were reported that as larval stages progressed activity

was decreased. Thus, these results are consistent with our study.

As a result, the essential oils obtained from *S. boissieri* plants have the potential to be used as insecticide in both insect species used in the experiments. But the main components of the plant need to be tested on these insect species. However, conducting synergistic activity studies with the main active ingredients will lead to the presence of more effective insecticides.

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#### ÖZET

Bu çalışmada *Satureja boissieri* Hausskn. ex Boiss. uçucu yağının *Sitophilus granarius* L. (Coleoptera: Curculionidae) ve *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae)'e karşı fumigant ve kontakt etkileri araştırılmıştır. *Sitophilus granarius* erginleri ile fumigant ve kontakt toksisitesi denemeleri; *S. littoralis*'in 3., 4. ve 5. dönem larvaları ile sadece kontakt toksisitesi denemeleri yürütülmüştür. Ayrıca, *S. boissieri* uçucu yağının fraksiyonları, *S. granarius*'a karşı fumigant aktivitesi açısından araştırılmıştır. *Sitophilus granarius* ile yapılan fumigant etkisi çalışmalarında en yüksek etki 0.1 µl/ml uygulama dozunda %100 ölüm oranıyla 36 saatte elde edilmiştir. Fraksiyonların *S. granarius* üzerinde önemli oranda fumigant aktiviteye sahip olduğu ve fraksiyonların zararlıya %10 uygulama dozunda önemli bir aktivite gösterdiği tespit edilmiştir. *Spodoptera littoralis*'in 3. ve 4. dönem larvalarına 100 ul/ml uçucu yağ uygulandığında, kontakt aktivite %100 olarak tespit edilirken, ölüm oranı 5. dönem larvada %90.17 olarak belirlenmiştir. Tüm larva dönemlerine 25 ul/ml uçucu yağ dozu uygulandığında, 3. larva döneminde kontakt aktivite

%71.12 olarak belirlenirken, diğer larval dönemlerde aktivite çok düşük kalmıştır. Bu sonuçlar, *S. boissieri* uçucu yağının, *S. granarius* ve *S. littoralis*'in mücadelesinde fumigant ve kontakt insektisitler olarak önemli bir potansiyele sahip olabileceğini göstermiştir.

Anahtar kelimeler: uçucu yağ, fumigant aktivite, kontakt aktivite, GC-MS, Lamiaceae, fraksiyon, kolon kromatografisi

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