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Short Communication

Influence of winter savory (*Satureja montana*) aqueous extract on mortality of lesser grain borer (*Rhyzopertha dominica*)

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ABSTRACT: Losses of grain quality and quantity as a result of insect activity during storage impose the need for proper pest control. One of the major pests of stored wheat is the lesser grain borer (LGB), *Rhyzopertha dominica*. This pest is usually controlled with insecticides. However, there is recently a growing interest in the use of plant-based products in pest management. Plants are a rich source of active compounds and for centuries have been used for the control of harmful insects. This study was carried out in order to evaluate the effects of *Satureja montana* aqueous extract on LGB adults in contact and contact-digestive tests. The mortality of LGB adults during contact exposure to *S. montana* extract was low, regardless of the concentration (16.7–33.3% after 24 h and 30.0–33.3% after 48 h). After 24 h of contact-digestive exposure, the highest mortality was caused by 2% *S. montana* extract (23.3%), while after 48 h the level of mortality increased in all treatments with *S. montana* extract (0.5, 1, and 2%) and was 28.4, 28.4, and 41.7%, respectively. After 72 h of contact-digestive exposure, it ranged from 57.5 to 63.5%, while in the control it remained the same (2.7%). After 7 days of such exposure, it was 91.6–98.4%, depending on the concentration. Based on the obtained results, we conclude that *S. montana* aqueous extract caused mortality of LGB adults, the level of this mortality depending on concentration of the applied extract and duration of the experiment.

Keywords: insecticidal effect, *Rhyzopertha dominica*, *Satureja montana*

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In 1984 Rise defined alleopathy as the effect of one plant on growth of another plant through release of chemical compounds into the environment (Inderjit & Duke 2003). Allelopathic compounds are generally considered to be secondary plant metabolites (Soliman & Zatout 2014) and have positive or negative effects on target organisms (Cheng & Cheng 2015). Allelochemicals can potentially be used as growth regulators, herbicides, insecticides, and antimicrobial plant protection products (Cheng & Cheng 2015). Plants are a very important source of a variety of chemicals that can be successful pest control agents. The mechanisms of plant activity against herbivore insects can be direct or indirect. Direct defence includes mechanical protection on the surface of plants (e.g., hairs, trichomes, thorns, spines, and thicker leaves). Alternatively, plants can act indirectly by producing toxic chemicals such as terpenoids, alkaloids, anthocyanins, phenols, and quinines that either kill or retard the development of herbivores (War *et al.* 2012). *Satureja montana* L., commonly known as winter savory or mountain savory, belongs to the family Lamiaceae. Species of the genus *Staureja* are well known for their aromatic and medicinal character. Phytochemical researchers have discovered volatile oils, tannins, phenolic compounds, sterols, acids, gum, mucilage, and pyrocatechol as the main compounds of *Satureja* species (Ćavar

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Table 1. Mortality of LGB adults treated with *S. montana* extract (0.5, 1, and 2%) compared to the control during contact exposure.

Treatments	Mortality (%)	
	24 h	48 h
S. montana (0.5%)	16.7 ± 0.75 c	33.3 ± 0.51 a
S. montana (1%)	20.0 ± 2.01 b	31.5 ± 2.02 a
S. montana (2%)	33.3 ± 0.35 a	33.3 ± 0.51 a
Control	3.3 ± 0.30 d	3.4 ± 0.15 b
F value	304**	1284**

Values with the same letters are on the same level of significance; $p > 0.05$ ns; $p < 0.05$ *; $p < 0.01$**.

et al. 2014). Among biological properties, *Satureja* species have been reported to possess antibacterial, antifungal, cytotoxic, insecticidal, herbicidal, antidiabetic, antiviral, and insect-repellant activities (Tape 2015).

The quantitative and qualitative losses of grains and grain products during prolonged storage may amount to 20–30% in the tropical zone and 5–10% in the temperate zone as a result of insect attack (Rajendran & Sriranjini 2008). One of the most important insect pests of stored grains worldwide is the lesser grain borer (LGB), *Rhyzopertha dominica* Fabricius, which causes large economic losses (Guzzo *et al.* 2006; Arthur *et al.* 2012). The damage to wheat is visible as reduction in quality and quantity (Ileke & Oni 2011). That imposes the need for prevention of postharvest losses (Rajashekar *et al.* 2012) and efficient pest control (Guzzo *et al.* 2006). The use of synthetic insecticides and fumigants is the most commonly used strategy against this pest (Gurusubramanian *et al.* 2008). However, these compounds can have negative consequences like residues in cereal products, the occurrence of insect resistance, ecological and health risks, increased costs of storage, etc. Recently, there has been a growing interest in the use of plant products for elimination of harmful insects (Rajendran & Sriranjini 2008; Ashamo et *al.* 2013). Many plant secondary metabolites have already been formulated as botanical pesticides.

The aim of this study was to evaluate effectiveness of *S. montana* aqueous extract as a potential insecticide and/or grain protectant against LGB adults in order to determine the potential of this plant species as a bioinsecticide and wheat grain protectant.

Plant material and preparation of the aqueous extract. *Satureja montana* plants were collected in Montenegro, around the city of Podgorica (42° 32' 23.21'' N, 19° 20' 02.17'' E) at an altitude of 123 m a.s.l. in June of 2012. The voucher specimen of collected plants is deposited in the herbarium of the Department of Biology and Ecology, Faculty of Science, University of Novi Sad under the identification number 2-1544. The plant material was dried at room temperature for two weeks. The air-dried plant material was ground into powder. The powdery material (10 g) was added to 100 mL of boiling distilled water (10%, w/v) and left for 24 h. After 24 h, the extract was filtered through Whatman No. 4 filter paper and kept at 4°C in a refrigerator until application. The employed working concentrations (0.5, 1, and 2%) were obtained by diluting the 10% *S. montana* aqueous extract.

Insects. The original population of *R. dominica* (Coleoptera: Bostrichidae) was reared in laboratory conditions, on wheat kernels in a thermostat chamber at 27 ± 1°C and 60 ± 5% RH, at the Faculty of Agriculture, University of Novi Sad, Serbia.

Bioassay. The insecticidal effect of *S. montana* applied in different concentrations was assessed in contact and contact-digestive tests.

Contact test: For assessment of contact insecticidal activity, we used the method described by Kouniki *et al.* (2007) as modified according to Betancure *et al.* (2010). Ten adults of lesser grain borer (seven to 10 days old) were inserted in 6-mL glass tubes previously "rinsed" with 1 mL of plant extracts. Glass tubes "rinsed" with distilled water were used as the control. The tubes were sealed with para-film and placed in a horizontal position so the insects could move along the tube wall. The tubes were incubated in a thermostat at 28°C in the dark. Mortality was assessed after 24 and 48 h by counting the number of dead and paralysed adults.

Contact-digestive test: The contact-digestive insecticidal effect of tested extracts was evaluated in a "no-choice" test according to Obeng-Ofer & Reichmuth (1997) using wheat grains. On 100 g of wheat grains, 3 mL of tested *S. montana* extracts was applied, homogenised in a rotary shaker for 1 hour, and air-dried for 2 h at room temperature. Distilled water was used in the control. Twenty adults were placed on 10 g of treated grains in Petri dishes. The Petri dishes were incubated in a thermostat chamber in the dark at 29±1°C. Insects were starved 24 h prior to testing. Mortality was recorded after 24 h, 48 h, 72 h, and 7 days of exposure by counting the number of dead and paralysed adults. The experiment was set in four replications.

Statistical analysis. The results were analysed using Duncan's multiple range test for a confidence interval of 95% in SPSS 17 statistical software.

The present study was carried out for evaluation of *S. montana* aqueous extract against LGB in order to find some safer alternatives for the control of insect pests of stored grains.

Table 2. Mortality of LGB adults treated with *S. montana* extract (0.5, 1, and 2%) compared to the control during contact-digestive exposure.

Treatments	Mortality (%)			
	24 h	**48 h**	**72 h**	**7 days**
S. montana (0.5%)	6.6 ± 0.51 c	28.4 ± 0.35 c	57.5 ± 2.0 b	91.6 ± 0.51 b
S. montana (1%)	15.0 ± 0.58 b	38.4 ± 0.51 b	62.5 ± 0.50 a	93.3 ± 0.31 b
S. montana (2%)	23.3 ± 0.26 a	41.7 ± 9.51 a	63.5 ± 1.0 a	98.4 ± 0.51 a
Control	2.7 ± 0.63 d	2.7 ± 0.63 d	2.7 ± 0.60 c	3.5 ± 0.60 c
F value	269**	12078**	6502**	63050**

Values with the same letters are on the same level of significance; p > 0.05 ns; p < 0.05 *; p < 0.01**.

In our previous research, we found that the main constituent of phenolic components in *S. montana* aqueous extract was caffeic acid (78.17 µg g^{-1}), the second most prevalent one was gallic acid (15.36 µg g^{-1}), while quercetin, p-coumaric acid, chlorogenic acid, and ferulic acid were present in lower concentrations (2.36, 1.59, 1.36, and 0.50 µg g^{-1}, respectively) (Šućur *et al.* 2015).

In the present work, we evaluate the insecticidal activity of *S. montana* aqueous extract against LGB. During contact exposure, the tested extract of *S. montana* caused low mortality regardless of the concentration. The obtained mortality ranged only from 16.7 to 33.3% after 24 h and from 30.0 to 33.3% after 48 h (Table 1), although it was significantly higher compared to the control (3.3–3.4%). During contact-digestive exposure (Table 2), the highest mortality after 24 h was caused by 2% *S. montana* extract (23.3%), which was significantly higher compared to other treatments, including the control (2.7–15.0%). After 48 h, mortality increased in all treatments with *S. montana* extract (0.5, 1, and 2%) and was 28.4, 28.4, and 41.7%, respectively. After 72 h, it ranged from 57.5 to 63.5%, while in the control it remained the same (2.7%). After 7 days, the obtained mortality ranged from 91.6 to 98.4%, depending on the concentration.

Phenols are a large group of secondary metabolites produced by plants. Among them, flavonoids, quercetin, chlorogenic acid, caffeic acid, and rutin are the ones most frequently found among diverse crop species and are the basis of the plants' resistance (Martens *et al.* 2010; Hichri *et al.* 2011). They affect herbivore larval growth and development mainly by causing inhibition (Treutter 2006; Page *et al***.** 2012).

The dominant phenolic compound in extract of *S. montana* was caffeic acid, which was shown to possess insecticidal activity (Harrison *et al.* 2003; Pavela *et al.* 2009). Caffeic acid is one of the many phenolics considered to be an important part of the defence mechanism of plants against microbial infection, insects, and other predators (Dehghani & Ahmadi 2013). According to Pavela *et al.* (2009), the presence of caffeic acid in *Impatiens parviflora* could be responsible for insecticidal activity of this plant against the green peach aphid. Stamp *et al.* (1994) proved negative effects of caffeic acid on early stadiums of *Manduca sexta*.

As for LGB, Ahmad *et al.* (2016) suggested that *Piper nigrum* extract may be a potential grain protectant against *Rhyzopertha dominica*.

Our results showed that *S. montana* aqueous extract had a toxic effect in the contact-digestive test, with mortality rates between 57 and 63% against lesser grain borer adults. After 7 days, all the extract concentrations used caused high mortality (91–98%). In conclusion, it can be stated that *S. montana* aqueous extract caused mortality of LGB adults, the level of mortality depending on concentration of the applied extract and duration of the experiment. This supports the view that natural substances present in plant extracts are good candidates to be developed as biopesticides. Further research on action of the tested extract against other insect pests should be conducted in the future.

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REZIME

Uticaj vodenog ekstrakta *Satureja montana* na stopu smrtnosti jedinki žitnog kukuljičara (*Rhyzopertha dominica*)

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Gubici u kvalitetu i prinosu tokom skladištenja žita ukazuju na potrebu zaštite žita odgovarajućom kontrolom štetočina u skladištima. Žitni kukuljičar, *Rhyzopertha dominica*, je glavna štetočina uskladištenih žitarica. Konvencionalni način da se kontrolišu štetočine je upotreba pesticida. Međutim, postoji konstantna potraga za prirodnim jedinjenjima za njihovu kontrolu. Biljke su bogat izvor biološki aktivnih jedinjenja. U ovom radu vršeno je ispitivanje uticaja vodenog ekstrakta *Satureja montana* na jedinke žitnog kukuljičara primenom kontaktnog i kontaktno-digestivnog testa. Smrtnost jedinki žitnog kukuljičara nakon izlaganja vodenom ekstraktu *S. montana* bila je niska (16,7–33,3% nakon 24 h i 30,0–33,3% nakon 48 h). U kontaktno-digestivnom testu, nakon 24 h, najveća smrtnost postignuta je u tretmanu sa koncentracijom od 2%, dok je smrtnost jedinki povećana u svim tretmanima (koncentracije 0,5, 1 i 2%) nakon 48 h i iznosila je 28,4, 28,4 i 41,7%, redom. Nakon 72 h, kretala se u intervalu od 57,5% do 63,5% u tretmanima, dok je smrtnost u kontroli ostala ista. Nakon 7 dana iznosila je od 91,6 do 98,4% u zavisnosti od primenjene koncentracije. Na osnovu dobijenih rezultata zaključujemo da je vodeni ekstrakt *S. montana* prouzrokovao smrtnost jedinki žitnog kukuljičara, koja je zavisila od primenjene koncentracije ekstrakta i vremena izloženosti insekata uticaju ekstrakta.

Ključne reči: Insekticidni efekat, *Rhyzopertha dominica*, *Satureja montana*