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Salvia officinalis essential oil in bean weevil control[1]

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ABSTRACT

Bean weevil [*Acanthoscelides obtectus* Say (Coleoptera: Bruchidae)] is considered the main storage pest of the bean crop. Its control is performed mainly by chemical treatment, which has potential to cause resistance in pests, as well as environmental contamination. This study aimed at evaluating the insecticidal and repellent effect of *Salvia officinalis* L. essential oil against bean weevil. The doses used for the insecticidal test were: 0 L t^{-1}, 0.5 L t^{-1}, 1.0 L t^{-1}, 1.5 L t^{-1}, 2.5 L t^{-1} and 5.0 L t^{-1} of bean grains. For the mortality test, the experimental design was completely randomized, in a 6 × 7 (dose × time) factorial scheme, with five replications. The number of dead insects was counted at 2, 6, 12, 24, 48, 72 and 96 h after the insect introduction. The repellency test was conducted in arenas, under a completely randomized design, using the same doses applied to evaluate the insecticidal effect. Counts were performed 24 h after the introduction of insects. The insecticidal effect of the *S. officinalis* essential oil on *A. obtectus* resulted in mortality rates higher than 95 %, after 6 h of insect introduction, for all doses tested. Repellency effect was also detected for all doses tested.

KEY-WORDS: *Acanthoscelides obtectus*; *Phaseolus vulgaris* L.; storage pests; bioactive plants.

RESUMO

Óleo essencial de *Salvia officinalis* no controle de caruncho do feijão

O caruncho do feijão [*Acanthoscelides obtectus* Say (Coleoptera: Bruchidae)] é considerado a principal praga de armazenagem da cultura do feijão, sendo o seu controle realizado, principalmente, por meio de tratamento químico, o qual possui potencial para causar resistência em pragas, bem como contaminação ambiental. Objetivou-se avaliar o efeito inseticida e repelente do óleo essencial de *Salvia officinalis* L. sobre o caruncho do feijão. As doses testadas para o ensaio inseticida foram: 0 L t^{-1}; 0,5 L t^{-1}; 1,0 L t^{-1}; 1,5 L t^{-1}; 2,5 L t^{-1}; e 5,0 L t^{-1} de grãos de feijão. Para o teste de mortalidade, o delineamento experimental utilizado foi o inteiramente casualizado, em esquema fatorial 6 x 7 (dose x tempo), com cinco repetições. A contagem do número de insetos mortos foi realizada às 2, 6, 12, 24, 48, 72 e 96 h após a introdução dos insetos. O teste de repelência foi conduzido em arenas, sob delineamento inteiramente casualizado, sendo utilizadas as mesmas doses citadas para avaliar o efeito inseticida. As contagens foram realizadas 24 h após a introdução dos insetos. O efeito inseticida do óleo essencial de *S. officinalis* sobre *A. obtectus* resultou em taxas de mortalidade superiores a 95 %, após 6 h da introdução dos insetos, para todas as doses testadas. Efeito de repelência também foi constatado para todas as doses testadas.

PALAVRAS-CHAVE: *Acanthoscelides obtectus*; *Phaseolus vulgaris* L.; pragas de armazenagem; plantas bioativas.

INTRODUCTION

Brazil is one of the world's major producers of beans (*Phaseolus vulgaris* L.). For this reason, the crop has an economic and social importance for many farmers, since it can be grown in different regions of the country (Smiderle et al. 2014).

Insect pests attack different developmental stages of beans in the field, as well as in storage. The bean weevil (*Acanthoscelides obtectus*) is a pest that occurs mainly in temperate regions and has a potential for cross infestation, causing direct losses in grain quantity and quality, and indirect losses as vectors of storage fungi (Gallo et al. 2002, Lorini et al. 2010).

Chemical methods are commonly used to control *A. obtectus* in stored grains. Phosphine is the main molecule used for controlling bean weevil. However, chemical control is associated with the increase in resistance of storage pests, and has a high potential to cause environmental pollution and poisoning, especially in the applicants (Soares et al. 2009). Furthermore, the adoption of alternative control methods is important, given the increasing

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demands for high quality food exempted from the application of chemical inputs, and the increase in organic areas and agroecological farming (Lima Júnior et al. 2012).

Among the alternatives to control this pest, the essential oils extracted from plants stand out with confirmed efficiency. *Hypothenemus hampei* Ferrari (Coleoptera, Scolytidae) has been controlled using *Schinus terebinthifolius* essential oil (Santos et al. 2013) and *Zabrotes subfaciatus* Boheman (Coleoptera: Bruchidae) using *Laurus nobilis* essential oils (Silva et al. 2013). Moreover, due to the fact that essential oils are composed of different substances, they can be important tools for insect resistance management, since these compounds may act on different physiological routes of the insect (Bakkali et al. 2008, Regnault-Roger et al. 2012).

Salvia officinalis L. has the potential for essential oil extraction, and it can be cultivated under Brazilian soil and weather conditions. The insecticide potential of *S. officinalis* has been already verified in *Spodoptera littoralis* Boisduval (Noctuidae: Lepidoptera) (Souguir et al. 2013). However, there are few studies on *S. officinalis* essential oil activity on insects. Furthermore, studies have demonstrated its acaricide potential on *Tetranychus urticae* (Acari: Tetranychidae) and fungicide activity against *Alternaria* spp. and *Phakopsora pachyrhizi* (Dellavalle et al. 2011, Borges et al. 2013, Laborda et al. 2013).

In this context, the present study aimed at evaluating the insecticide and repellent effects of *S. officinalis* essential oil on bean weevil (*A. obtectus*).

MATERIAL AND METHODS

The experiment was conducted at the Universidade Federal da Fronteira Sul, in Erechim, Rio Grande do Sul State, Brazil, in January 2014, in BOD chambers with 25 ºC ± 2 ºC and relative humidity (RH) of 70 % ± 10 %, with a 12-h photoperiod.

Plants were collected in the Alto Uruguai region and shade dried until constant weight. Then, the essential oil was extracted by hydrodistillation, using a Clevenger apparatus, with extraction time of 2 h.

The insects used were obtained by laboratory rearing conducted in glass pots covered with fabric and maintained in BOD chamber at 25 ºC ± 2 ºC, under relative humidity of 70 % ± 10 %. Creole bean grains were used as substrate, free from treatment with insecticides and disinfected in ultrafreezer at -50 ºC, for 48 h.

The mortality test was conducted under completely randomized design, in a 6 × 7 (dose × time) factorial scheme, with five replications. For the test, 20 g samples of bean grains were put in plastic pots (6.8 cm diameter and 7 cm height). Subsequently, pure essential oil was applied on the grains, using an automatic pipettor, at doses of 0 μL 20 g^{-1}, 10 μL 20 g^{-1}, 20 μL 20 g^{-1}, 30 μL 20 g^{-1}, 50 μL 20 g^{-1} and 100 μL 20 g^{-1} of grains, which correspond to values equivalent to 0.0 L t^{-1}, 0.5 L t^{-1}, 1.0 L t^{-1}, 1.5 L t^{-1}, 2.5 L t^{-1} and 5.0 L t^{-1} of grains, respectively. Uniform oil distribution to the grains was achieved by hand shaking, for 1 min. After applying the essential oil, 20 unsexed adult insects, aged between 20 and 40 days, were put in each pot. Dead weevils were counted and recorded at 2, 6, 12, 24, 48, 72 and 96 h after insect introduction. The data were adjusted as described by Abbott (1925), with results expressed as percentage of dead insects.

The repellency test was conducted in arenas under completely randomized design, with five replications. Each arena consisted of six plastic pots (6.8 cm diameter and 7 cm height) arranged symmetrically and diagonally to a central pot, and interconnected by plastic tubes (Figure 1). The doses were the same used to evaluate the insecticide effect and each replicate consisted of one arena (Mazzonetto & Vendramim 2003, Procópio et al. 2003, Tavares & Vendramim 2005).

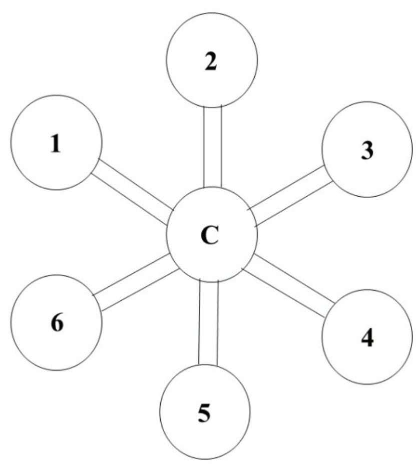

Figure 1. Arena scheme used for the repellency test.

In each pot (1, 2, 3, 4, 5 and 6), 20 g of bean grains pre-impregnated with each oil dose were randomly deposited. Oil application on the grain was conducted in the same way as described for the mortality test. Then, in the central pot (C), 50 adult insects not sexed, aged between 20 and 40 days, were introduced. The evaluation was performed 24 h after insect introduction. The number of insects present in each pot was counted and the results expressed as percentage of repellency. Furthermore, the repellency data were evaluated using the Preference Index (PI), as proposed by Procópio et al. (2003), described by the following equation:

$$PI = \frac{\text{\% of insects in the tested plant - \% of insects in the control}}{\text{\% of insects in the tested plant + \% of insects in the control}}$$

where: PI = -1.00 to -0.10: plant test repellent; PI = -0.10 to +0.10: plant test neutral; PI = +0.10 to +1.00: plant test attractive.

Mortality and repellency percentage data were submitted to analysis of variance by the F test, with the Statistica 10.0 software. Mortality data were analyzed employing the response surface methodology, with the Table Curve 3D 4.0 software. The percent repellency results were submitted to regression analysis, with the Sigma Plot 10.0 software.

RESULTS AND DISCUSSION

The results showed that the *S. officinalis* essential oil has insecticidal effect on *A. obtectus*. According to the response surface graph (Figure 2), insect mortality was high for all doses. Mortality varied with varying exposure time. Regardless of the dose used, *A. obtectus* mortality was not satisfactory before 2 h of exposure into impregnated beans. However, from 6 h, exposure mortality rates were higher than 95 % for all doses tested.

A. obtectus mortality rates higher than 95 % were also observed by Santos et al. (2007) 48 h after the application of *Schinus terebinthifolius* essential oil with dilutions higher than 10^{-2}, and by Savaris et al. (2012) after 24 h of *Cunila angustifolia* essential oil application at doses greater than 0.001 mL cm^{-2}. Campos et al. (2014) observed, after 2 h of insect infestation, a mortality rate higher than 95 %, when *Baccharis articulata* essential oil at a dose of 5.0 L t^{-1} of grains was applied.

The insecticidal action mechanism of essential oils can occur in different ways, causing mortality, deformations in different developmental stages and repellency (Knaak & Fiúza 2010). The toxic effect of essential oils involves many factors, such as the toxins entrance point, which can occur by inhalation routes, ingestion and contact (Regnault-Roger 1997). Therefore, the toxicity caused to insects probably occurred through contact action of the essential oil and inhalation, because adult *A. obtectus* does not feed. In addition, the insecticidal action can be directly related to the monoterpenes present in the essential oil, which act mainly on the insect acetylcholinesterase inhibition (Viegas Júnior 2003).

According to Pierozan et al. (2009), the oil from *S. officinalis* cultivated in southern Brazil has as major compounds α-thujone, camphor and 1,8-cineol. Thus, the insecticidal action exerted by the *S. officinalis* essential oil against *A. obtectus* is possibly associated with the presence of these compounds.

The α-thujone compound is classified as a neurotoxic pesticide (Ratra et al. 2001). The insecticidal activity of this compound was demonstrated by Rojht et al. (2009) on *Corythucha ciliata* Say (Heteroptera: Tingidae), Pavlidou et al.

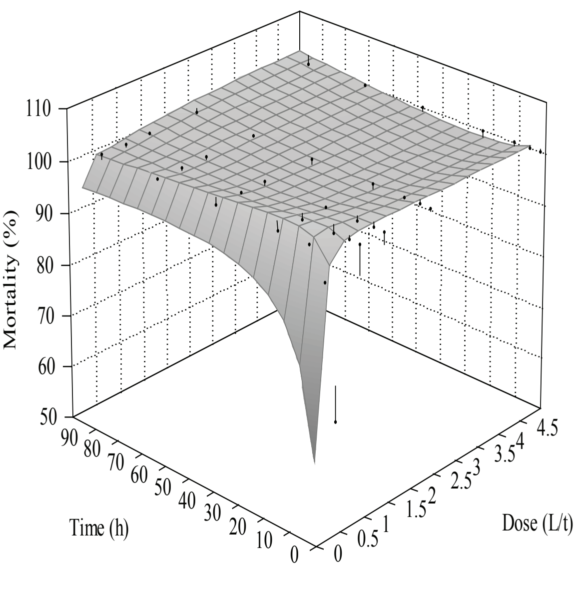

Figure 2. *Acanthoscelides obtectus* mortality after *Salvia officinalis* essential oil application. $Z = 100.21 - 9.19/X + 3.13 * LnY - 4.21/X^2 - 2.62 * (LnY)^2 + 10.42 * LnY/X - 0.90/X^3 + 0.45 * (LnY)^3 - 2.18 * (LnY)^2/X + 1.87 * LnY/X^2$, with $R^2 = 0.71$ and $p < 0.0001$, where: X = dose, Y = time, Z = mortality.

(2004) on *Drosophila melanogaster* Fallén (Diptera: Drosophilidae) and *Bactrocera oleae* Rossi (Diptera: Tephritidae), and Creed et al. (2015) on *Cydia pomonella* Linnaeus (Lepidoptera: Tortricidae).

The insecticidal effect of the *Rosmarinus officinalis* essential oil, which has 20.8 % of camphor, was observed on *A. obtectus* by Papachristos et al. (2004). In addition, studies by Lamiri et al. (2001) on the control of *Mayetiola destructor* Say (Diptera: Cecidomyiideae) and Chu et al. (2010) on *Sitophilus zeamais* Linnaeus (Coleoptera: Curculionidae), with the application of the *Artemisia herba-alba* (60.8 % camphor) and *Artemisia vestita* (11.3 % camphor) essential oils, respectively, showed the camphor insecticidal action.

The pesticide action of the 1,8-cineole compound was found in studies by Lima et al. (2011) on *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae) and Rossi & Palacios (2015) on *Musca domestica* Linnaeus (Diptera: Muscidae). Moreover, 1,8-cineole has proven insecticidal effect on *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae), *Rhyzopertha dominica* Fabricius (Coleoptera: Bostrychidae) and *Sitophilus oryzae* Linnaeus (Coleoptera: Curculionidae) (Aggarwal et al. 2001). Against *A. obtectus*, the study by Bittner et al. (2008) showed that the *Gomortega Keule* essential oil, whose composition has about 35 % of 1,8-cineole, caused a mortality rate of 100 %, after 96 h, when applying the dose of 8 µL L air^{-1} in fumigation.

Furthermore, the association between 1,8-cineole and camphor compounds promotes synergistic effect, thereby increasing the penetration of these components in the cuticle of insects (Tak & Isman 2015).

According to the Preference Index, all doses of *S. officinalis* essential oil tested showed repellency for *A. obtectus*. The higher the dose the higher the repellency rate (Figure 3).

The regression model shows an increase in the percentage of insect repellency with increasing doses of *S. officinalis* essential oil (Figure 4). All doses obtained repellency rates above 90 %, 24 h after insect inoculation.

Jumbo et al. (2014) also observed a repellent action against *A. obtectus* with the application of *Cinnamomum zeylanicum* essential oil at doses of 46.8 µL kg^{-1} and 122.4 µL kg^{-1} in beans. Likewise, Papachristos & Stamopoulos (2002) found that the essential oils of *Mentha microphylla*, *Mentha viridis*, *Lavandula hybrida* and *Rosmarinus officinalis* promoted repellency on *A. obtectus*. Campos et al. (2014) observed repellency of *Baccharis trimera* essential oil on *A. obtectus* at doses above 0.5 L t^{-1}.

The repellent activity caused by essential oils in insects is mainly due to the major compounds present in its composition (Knaak & Fiúza 2010), through contact action, interacting with the insect integument, as well as acting in digestive and neurological enzymes (Soares et al. 2012). Thus, the *S. officinalis* essential oil repellent action on *A. obtectus* can also be attributed to the presence of α-thujone, camphor and 1,8-cineole compounds.

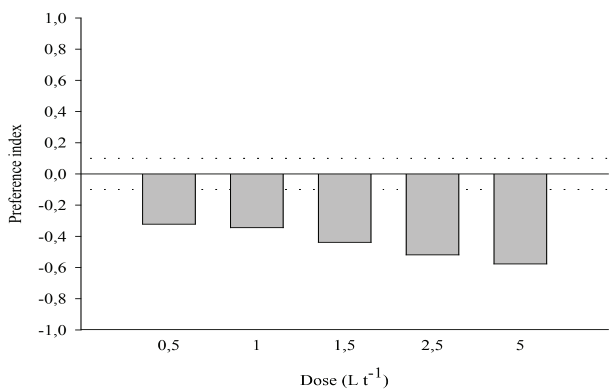

Figure 3. Repellency Index of *Salvia officinalis* essential oil on *Acanthoscelides obtectus*, according to the Preference Index (-1.00 to -0.10 = plant test repellent; -0.10 to +0.10 = plant test neutral; +0.10 to +1.00 = plant test attractive).

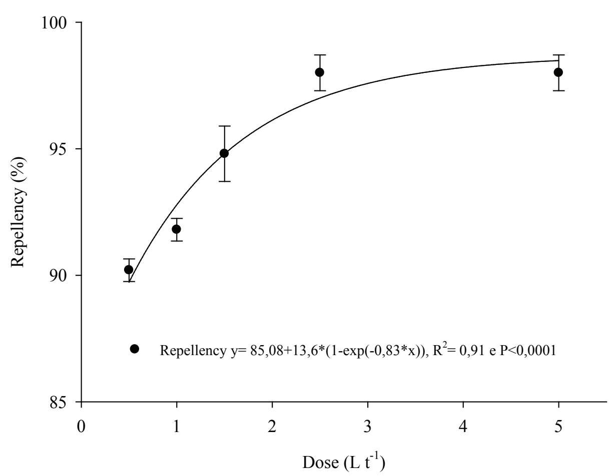

Figure 4. Percentage of repellency of *Acanthoscelides obtectus* after application of *Salvia officinalis* essential oil.

Furthermore, the synergistic effect between camphor and 1,8-cineole could increase the penetration of these constituents in the insect integument, as aforementioned.

According to Nerio et al. (2010), the camphor compound has a high repellent activity on insects. Zhang et al. (2014) found that, when camphor was applied alone, it had a repellent effect on *Solenopsis invicta* Buren (Hymenoptera: Formicidae).

Tampe et al. (2015) showed that the α-thujone compound presented a repellent effect when applied alone on *Aegorhinus nodipennis* Hope (Coleoptera: Curculionidae).

Aggarwal et al. (2001) concluded that 1,8-cineole showed repellency rates higher than 70 % on *C. maculatus* and *R. dominica*, at the dose of 4 μL mL^{-1}, 1 h after application. However, there are few studies about the effects of these compound on *A. obtectus*, thus underlining the importance of further research in this context.

CONCLUSIONS

1. The *Salvia officinalis* essential oil has insecticide and repellent effects on *Acanthoscelides obtectus*.
2. Doses of *S. officinalis* essential oil greater than 0.5 L t^{-1} of grains increase mortality rates above 95 %, 6 h after application.
3. *S. officinalis* essential oil applied with 0.5 L t^{-1} of grains induces a repellency rate above 90 % for *A. obtectus*.

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