Effects of *Brugmansia suaveolens* fractions on *Zabrotes subfasciatus* (Coleoptera: Chrysomelidae: Bruchinae)

Gabriel Luiz Padoan Gonçalvesa; Eduardo José Crevelinb; Simone Possedente de Lira; José Djair Vendramim

**ABSTRACT**

The high costs of developing new insecticide molecules combined with stringent regulatory laws for pesticide registration have encouraged more research with insecticidal compounds of plant origin. Moreover, insecticidal plant compounds may be less harmful to humans and to non-target organisms, and present new modes of action to control insect pests. Thereby, bio-guided chromatographic fractionations of the ethanolic extract from flowers of *Brugmansia suaveolens* (Willd.) (Solanaceae) were performed in order to identify insecticidal chemical compounds able to protect stored beans against Bruchinae beetles. The chromatographic fractionations using silica column chromatography was based on results from toxicological bioassays (residual contact) using *Zabrotes subfasciatus* (Boheman) as a model insect. During the bio-guided chromatographic fractionations, the bioactive fractions expressed their effects mainly by inhibiting the F1 progeny of *Z. subfasciatus*. Consequently, damages on bean grains were completely inhibited. Moreover, some fractions also killed adults of *Z. subfasciatus*, which demonstrated signs of hyperexcitation. The fraction BSHidAcF1-1 (150 mg Kg⁻¹) killed 56% of adults of *Z. subfasciatus*, promoted egg deterrence, and drastically reduced egg-adult viability.

**Keywords**: Mexican bean weevil; *Phaseolus vulgaris*; secondary metabolites; botanical insecticides.

**INTRODUCTION**

The problems related to quantitative and qualitative damages on stored grains promoted by insects date back to antiquity. Consequently, the elaboration of controlling methods for these pests has emerged more than 3,000 years ago with the use of ash and soil dust (dehydration and mechanical damage), plant materials (resins, powders etc.) and sulfur dioxide for fumigation and repellency of different stored grain pests (Levinson and Levinson, 1998; Hagstrum and Phillips, 2017). Bean beetles species (Coleoptera: Chrysomelidae: Bruchinae), mainly from the genera *Acanthoscelides*, *Callosobruchus*, *Caryedon* and *Zabrotes*, are important pests of stored dried legumes [beans, peanuts and groundnuts (*Vigna, Phaseolus, Glycine* etc.)] worldwide (Tuda, 2007; Tripathi, 2018). Beans (*Vigna* and *Phaseolus*) present a huge economic and nutritional importance in the world and Bruchinae beetles, such as the Mexican bean weevil (*Zabrotes subfasciatus* (Boheman)) (Coleoptera: Chrysomelidae: Bruchinae), promote high quantitative losses (up to 99.3%) on stored beans and their quality in both tropical areas and the Mediterranean region (Barbosa et al., 2000; Tuda, 2007; Ribeiro-Costa and Almeida, 2012; Daglish et al., 2018). Therefore, it is important to develop control methods that not only kill adults of *Z. subfasciatus* but also prevent its larvae to
penetrate bean grains. Studies concerning the use of plant-based insecticides and resistant plant varieties have been performed in order to minimize the damages promoted by such pest’s larvae (Ribeiro-Costa et al., 2007; Luethi et al., 2013; Gonçalves et al., 2015; Gonçalves et al., 2017). However, currently, synthetic insecticides (pyrethroids and phosphine) are the major adopted tool to control insect-pests of stored products, and there are resistant insect-pest populations for some insecticides (Zettler and Arthur, 2000; Pimentel et al., 2010; Boyer et al., 2012; Sparks and Nauen, 2019). Nonetheless, to replace environmentally aggressive molecules of synthetic insecticides by plant-based insecticides, it is necessary to perform bioprospection studies (toxicological bioassays along with chromatographic separation techniques) in order to isolate and identify new insecticidal compounds from plants. Earth have suffered a high rate of deforestation in the last few centuries resulting in an immensurable loss of genetic patrimony that could have been explored to discover and develop new synthetic and botanical insecticides (Hansen et al., 2013). In this context, Brazil, as the owner of an enormous plant genetic diversity, with more than 56,000 catalogued plant species (Giulietti et al., 2005), can assume a leading role in prospecting insecticidal molecules from plants. The Solanaceae botanical family includes several species of economic relevance and it is widely present in both the temperate and tropical zones, with around 2,300 species distributed in 92 genera (Martins and Barkman, 2005). In Brazil, there are 450 species (150 endemic ones) of Solanaceae distributed in 31 genera (Giulietti et al., 2005). Such family presents a great diversity of alkaloids with direct application in the control of agricultural pests, both in the form of botanical insecticides, e.g. nicotine, as well as its synthetic derivatives, e.g. neonicotinoids (Elbert et al., 2008; El-Wakeil, 2013). Thereby, the Solanaceae family is a promising source of secondary metabolites (whitanolides, capsinoioids, alkaloids and flavonoids) with insecticidal properties suitable for both the formulation of botanical insecticides and synthetic insecticides with novel mechanisms of action (Silva et al., 2003; Veleiro et al., 2005; Luo et al., 2011; Chowański et al., 2016). The plant angel's trumpet, Brugmansia suaveolens (Humb. & Bonpl. ex. Willd.) Bercht. & C. Presl (Syn. Datura suaveolens) (Solanaceae), is a native shrub from South America and it is largely used in landscape projects for ornamental purposes due to its beautiful white or pink blossoms. Regarding to its chemical composition, it presents a diverse range of secondary metabolites including tropane alkaloids, pyrrolizidine alkaloids and kaempferol glycosides isolated from its leaves (Geller et al., 2014). The alkaloids hyoscyamine, atropine and scopoline occur in B. suaveolens flowers (Andreola et al., 2008), and the later can promote insecticidal effects (Roesler et al., 2007). Therefore, in the present study, it was evaluated the lethal and sublethal effects of fractions from B. suaveolens on the Mexican bean weevil with residual contact bioassays.

**MATERIAL AND METHODS**

**Insecticidal Bioassays**

In all bioassays, it was used individuals of Z. subfasciatus from laboratory colonies established with specimens collected in warehouses of Piracicaba municipality, SP, Brazil. The laboratory colonies were maintained in glass containers (2.6 L) containing Phaseolus vulgaris grains cv. Bolinha and kept in acclimatized room (25±2°C, 60±10% RH and a photoperiod of 14 L: 10 D hours). Both residual contact bioassays and oviposition deterrence bioassay were conducted under controlled conditions (25±2°C, 60±10% RH and a photoperiod of 14 L: 10 D hours) with a completely randomized experimental design.

The effects of B. suaveolens on Z. subfasciatus were verified by evaluating different variables. It was accounted the number of dead insects (insects were considered dead if they did not respond to a brush touch after 1 minute) and the number of eggs deposited on bean grains surface after five days of exposition to treated bean samples (adults were withdrawn from
The number of insects in the first generation (Z. subfasciatus) (aging 0-24 hours after emerging from beans) were introduced at the central chamber. The number of eggs on grains was assessed after five days of introducing adults.

**Statistical analysis**

The data from bioassays with ethanolic extracts and *B. suaveolens* fractions was analyzed using the software "R", version 3.3.1. Generalized linear models (GLM) with quasibinomial or quasipoisson family distribution were applied, and a half-normal probability plot with simulation envelope of the hnp package was applied to verify the model’s fit quality (Nelder and Wedderburn, 1972; Demétrio and Hinde, 1997; Hinde and Demétrio, 1998). In the instance of significant differences between treatments (ethanolic extracts and fractions), multiple comparisons tests (Tukey's test, p<0.05) were executed using the glht function of the multcomp package.

The egg deterrence bioassay was analyzed using a Deterrence Index (DI). The DI was calculated using the formula $DI = \frac{2G}{P}$, where $G$ is the % of eggs in treated unit samples, and $P$ is the % of eggs in control. Based on DI values for each repetition and their Standard Deviation (S.D.) a Classification Interval (CI) was calculated using the following formula $CI = 1 \pm t \left( \frac{S.D}{\sqrt{n}} \right)$, where $t$ is the Student's t distribution value (n-1; $\alpha$: 0.05), S.D. the standard deviation, and $n$ the number of repetitions. Treatments are considered neutral when the DI and CI values overlap, stimulant when DI values are superior to CI values, and deterrent when DI values are lower than CI values.

**Bioguided fractionations of *B. suaveolens***

The ethanolic extract from flowers of *B. suaveolens* was submitted to a liquid-liquid partitioning to produce fractions presenting different chemical affinities, one with more polar compounds [methanol:water (1:3, v.v⁻¹)] and other with less polar compounds (hexane). The ethanolic extract was solubilized in methanol:water (1:3, v.v⁻¹), adding 100 mL for each gram of extract. To perform the liquid-liquid partitioning it was added 100 mL of

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**Oviposition deterrence bioassay**

The oviposition deterrence effect of the fraction BSHidF1Ac-1 (applied at 25 mg Kg⁻¹) was evaluated in a choice bioassay with completely randomized design with 10 repetitions. The bean’s grains were sprayed using the same method described above. It was used an acrylic choice-arena (square-shape) containing five interconnected circular chambers(6.5 cm diameter × 2 cm high, one central and four in the corners). Inside each corner chamber it was placed 5 g of bean grains, two opposite chambers with treated bean grains (BSHidF1Ac-1) and two with the control (ethyl acetate). Ten couples of Z. subfasciatus were included in bioassays as a positive control.

The bean’s grains were sprayed with fractions from *B. suaveolens*. Fractions were solubilized using organic solvents and applied on samples of 100 g of beans (10 replicates with 10 g) per treatment placed inside plastic bags (2 L). A microatomizer pistol attached to a pneumatic pump adjusted to provide a spray pressure of 0.5 kgf cm⁻² with a volume of 30 L t⁻¹ [3 mL of solution (solvent + fraction) per each 100 g of beans] was used to spray fractions on bean grains. After this, bean grains were softly shaken inside their plastic bags to promote a more homogeneous adherence and distribution of fractions on their surface. Afterwards, treated beans were placed in an airflow chamber during two hours for solvent evaporation. For each bioassay, a negative control (solvent used for suspension of *B. suaveolens* fractions) was included. Moreover, the botanical insecticide Azamax® 1.2EC [azadiractin A and 3-tigloylazadirachtol [12 g.L⁻¹ (1.2% m/m)]] that causes phagodeterrence and hormonal disbalance on insects, was included in bioassays as a positive control.

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hexane for each gram of ethanolic extract in the separation funnel for three times. The hexane fraction (BSHex) and the remaining hydroalcoholic phase (BSHid) were both concentrated in a rotary evaporator (50°C and -600 mmHg). The hexane (52.67% yield) and hydroalcoholic (39.67% yield) fractions from the flowers of *B. suaveolens* were tested against *Z. subfasciatus* adopting the bioassay procedures described above. They were applied at a concentration of 2,500 mg kg\(^{-1}\), the same one used in the bioassay with crude extracts.

The previous hydroalcoholic fraction from *B. suaveolens* flowers was separated using a Sep-Pak Silica Classic Cartridge (Strata-Phenomenex, 10 g). A mass of 0.5 g of the hydroalcoholic fraction was solubilized in acetone and inserted in the silica cartridge, and it was applied 150 mL of acetone and 150 mL of methanol. The acetone fraction (BSHidAc) yielded 43.12% and the methanol fraction (BSHidMet), 51.8%. They were applied on bean grains surface using acetone:methanol (1:1, v:v).

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The scheme of chromographic separations performed with the ethanolic extract from flowers of *B. suaveolens* is presented in Fig. 1.
**Brugmansia suaveolens** fractions on *Zabrotes subfasciatus*  

Table 1. Bioactivity (mean ± SE) of fractions from *Brugmansia suaveolens* (tested at 2,500 mg kg⁻¹) against *Zabrotes subfasciatus*, by residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mortality (%) ¹</th>
<th>N° eggs/sample ²</th>
<th>F₁ progeny ²</th>
<th>Viability (%) (egg-adult) ¹</th>
<th>Sex ratio ¹</th>
<th>Damaged grains (%) ¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroalcohol (BSHid)</td>
<td>6.0±2.67</td>
<td>33.5±6.21</td>
<td>20.6±3.98</td>
<td>56.8±7.91</td>
<td>0.43±0.08</td>
<td>30.8±5.38</td>
</tr>
<tr>
<td>Hexane (BSHex)</td>
<td>2.0±1.33</td>
<td>68.7±8.21</td>
<td>48.4±6.37</td>
<td>70.6±2.98</td>
<td>0.51±0.03</td>
<td>61.5±6.71</td>
</tr>
<tr>
<td>Control (acetone: methanol (1:1))</td>
<td>3.0±1.53</td>
<td>87.5±6.21</td>
<td>63.0±5.64</td>
<td>71.3±2.38</td>
<td>0.51±0.02</td>
<td>78.6±3.36</td>
</tr>
<tr>
<td>Azamax® (2,500 mg kg⁻¹)</td>
<td>5.0±2.24</td>
<td>28.4±5.45</td>
<td>4.0±0.77</td>
<td>5.4±2.34</td>
<td>0.10±0.07</td>
<td>7.2±1.42</td>
</tr>
</tbody>
</table>

F value: 0.896  <0.0001  52.049  0.268  43.266  <0.0001

¹Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey’s post hoc test, p<0.05);
²Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey’s post hoc test, p<0.05);

* Not significant (p>0.05).

Based on the similarities of their chemical profiles of silica thin layer chromatography, they were grouped in 7 fractions: BSHidAcF1 (15.12% yield), BSHidAcF2 (9.71%), BSHidAcF3 (4.32%), BSHidAcF4 (9.21%), BSHidAcF5 (1.78%), BSHidAcF6 (15.48%) and BSHidAcF7 (39.26%). The organic solvent ethyl acetate was used to apply these fractions on bean grains.

Based on toxicological bioassays results, the fraction BSAcF1 (0.41 g) was selected to the next chromatographic separation in a Sep-Pak Silica Classic Cartridge (Strata-Phenomenex, 10 g). A range of organic solvents was sequentially used to separate chemical compounds in fraction BSHidAcF1. The solvents used were: 100 mL of 8dcm:2 hex (8 dichloromethane:2hexane), 100 mL of dichloromethane, 100 mL 98dcm:2EtAc (96 dichloromethane:4 ethyl acetate), 100 mL 96dcm:4EtAc, 100 mL 94dcm:6EtAc, 100 mL 92dcm:8EtAc, 100 mL of ethyl acetate, 100 mL of acetone, and finally 100 mL of methanol. Based on the similarities and differences of their chemical profiles using thin layer chromatography, they were grouped in five fractions, BSHidAcF1-1 (30.43% yield), BSHidAcF1-2 (17.87%), BSHidAcF1-3 (30.91%), BSHidAcF1-4 (14.52%) and BSHidAcF1-5. The organic solvent ethyl acetate was used to apply these fractions on bean grains.

**RESULTS**

**Residual contact bioassays**

Preliminary tests with the ethanolic extract from *B. suaveolens* flowers revealed its effect on reducing the number of eggs laid by *Z. subfasciatus* females and inhibiting its F₁ progeny. Thereby, it was selected for a set of chromatographic fractionations based on results from toxicological bioassays using *Z. subfasciatus* as model insect.

The first fractionation, a liquid-liquid partition, divided *B. suaveolens* ethanolic extract in two fractions, one in hexane (BSHex) and a remaining hydroalcoholic (75% H₂O + 25% methanol) phase (BSHid). Neither hexane nor hydroalcoholic fractions from *B. suaveolens* flowers promoted significant mortality of *Z. subfasciatus* adults (Table 1). Nevertheless, both of them interfered on *Z. subfasciatus* development, mainly the hydroalcoholic one. It reduced females’ oviposition on beans surface, F₁ progeny, egg-adult development and damages on grains (Table 1). The BSHid fraction was separated in two fractions, one using acetone (BSHidAc) and other methanol (BSHidMet). When tested at 2,500 mg kg⁻¹, the BSHidAc fraction reduced the number of eggs on beans and it completely blocked egg development, thereby inhibiting the F₁ progeny and damages on grains (Table 2). The fraction BSHidMet also reduced the number of eggs and F₁ progeny but less intensely than BSHidAc (Table 2).

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Table 2: Bioactivity (mean ± SE) of fractions from Brugmansia suaveolens (tested at 2,500 mg kg⁻¹) against Zabrotes subfasciatus, by residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mortality (%)¹</th>
<th>N° eggs/sample²</th>
<th>F₁ progeny²</th>
<th>Viability (%) (egg-adult)¹</th>
<th>Sex ratio¹</th>
<th>Damaged grains (%)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone (BSHidAc)</td>
<td>13.0±4.73 b</td>
<td>14.7±2.57 b</td>
<td>0.0±4.0*</td>
<td>0.0±4.0*</td>
<td>0.0±4.0*</td>
<td>0.0±4.0*</td>
</tr>
<tr>
<td>Methanol (BSHidMet)</td>
<td>11.0±3.79 b</td>
<td>36.7±5.16 c</td>
<td>23.0±3.05 b</td>
<td>63.3±3.57 b</td>
<td>0.47±0.02 a</td>
<td>49.4±5.35 b</td>
</tr>
<tr>
<td>Control</td>
<td>3.0±1.53 a</td>
<td>69.6±6.86 d</td>
<td>56.6±5.35 c</td>
<td>71.3±2.38 c</td>
<td>0.54±0.02 b</td>
<td>79.7±5.75 c</td>
</tr>
<tr>
<td>Azamax® (2,500 mg kg⁻¹)</td>
<td>17.0±4.23 c</td>
<td>4.8±1.20 a</td>
<td>0.6±0.27 a</td>
<td>9.8±4.59 a</td>
<td>0.20±0.13**</td>
<td>2.2±0.82 a</td>
</tr>
<tr>
<td>F</td>
<td>3.0727</td>
<td>50.547</td>
<td>106.990</td>
<td>27.513</td>
<td>8.361</td>
<td>65.517</td>
</tr>
<tr>
<td>p value</td>
<td>0.0419</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0097</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

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It was observed that the eggs deposited on grains treated with the BSHidAc fraction did not acquire the specific white coloration of healthy eggs. Thus, the BSHidAc fraction was fractionated in seven fractions using a silica cartridge with organic solvents of different polarities, and they were applied at 250 mg Kg⁻¹. The compound(s) that previously inhibited egg development was/were exclusively separated to the fraction BSHidAcF1. This fraction reduced the number of eggs and inhibited their development resulting in no damage to bean grains (Table 3). The fraction BSHidAcF3 also promoted some reduction of Z. subfasciatus eggs while others did not (Table 3). Therefore, the BSHidAcF1 was submitted to another column chromatography resulting in five fractions. The bioactivity was concentrated only in the less polar fraction BSHidAcF1-1.

Table 3: Bioactivity (mean ± SE) of fractions from Brugmansia suaveolens (tested at 250 mg kg⁻¹) against Zabrotes subfasciatus, by residual contact bioassay. Temp.: 25±2°C; R. H.: 60±10%; Photoperiod: 14L:10D.

<table>
<thead>
<tr>
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<th>Viability (%) (egg-adult)¹</th>
<th>Sex ratio¹</th>
<th>Damaged grains (%)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSHidAcF1</td>
<td>2.0±1.33</td>
<td>18.3±2.58 b</td>
<td>0.0±0.0*</td>
<td>0.0±0.0*</td>
<td>0.0±0.0*</td>
<td>0.0±0.0*</td>
</tr>
<tr>
<td>BSHidAcF2</td>
<td>2.0±1.33</td>
<td>33.3±2.92 cd</td>
<td>27.0±2.25 c</td>
<td>81.3±1.28 bc</td>
<td>0.50±0.01</td>
<td>41.0±2.31 b</td>
</tr>
<tr>
<td>BSHidAcF3</td>
<td>1.0±1.00</td>
<td>28.3±1.15 c</td>
<td>22.6±3.45 b</td>
<td>78.8±2.35 bc</td>
<td>0.50±0.04</td>
<td>43.8±5.71 b</td>
</tr>
<tr>
<td>BSHidAcF4</td>
<td>1.0±1.00</td>
<td>41.7±6.32 cd</td>
<td>28.8±4.23 cd</td>
<td>72.5±5.85 c</td>
<td>0.51±0.01</td>
<td>54.5±7.48 c</td>
</tr>
<tr>
<td>BSHidAcF5</td>
<td>1.0±1.00</td>
<td>29.7±1.94 cd</td>
<td>25.0±1.53 bc</td>
<td>85.1±2.92 b</td>
<td>0.51±0.02</td>
<td>55.0±3.76 c</td>
</tr>
<tr>
<td>BSHidAcF6</td>
<td>1.0±1.00</td>
<td>34.9±3.06 de</td>
<td>27.0±2.02 c</td>
<td>78.1±1.60 cd</td>
<td>0.52±0.02</td>
<td>57.3±5.39 c</td>
</tr>
<tr>
<td>BSHidAcF7</td>
<td>5.0±2.69</td>
<td>35.1±2.39 de</td>
<td>28.0±2.64 cd</td>
<td>79.5±3.60 cd</td>
<td>0.48±0.01</td>
<td>54.5±5.05 c</td>
</tr>
<tr>
<td>Control</td>
<td>1.0±1.00</td>
<td>44.1±5.42 f</td>
<td>32.4±2.58 d</td>
<td>77.4±5.48 de</td>
<td>0.50±0.13</td>
<td>61.8±3.74 c</td>
</tr>
<tr>
<td>Azamax® (250 mg kg⁻¹)</td>
<td>1.0±1.00</td>
<td>6.3±1.38 a</td>
<td>1.3±0.60 a</td>
<td>17.8±6.02 a</td>
<td>0.28±0.13**</td>
<td>2.9±1.41 a</td>
</tr>
</tbody>
</table>

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This fraction, applied at 150 mg Kg⁻¹, killed 56% of adults of Z. subfasciatus and completely inhibited female oviposition resulting in no damage on bean grains (Table 4). With this fraction, the adults of Z. subfasciatus presented signs of muscle hyperexcitation followed by difficult to move and paralysis. The surviving insects reacted with spasms and tremors when touched by a brush and presented great difficulty of moving coordinately.

Egg deterrence bioassay

The fraction BSHidAcF1-1 applied at 25 mg Kg⁻¹ promoted an oviposition deterrent effect in a free-choice bioassay. Treated beans presented an average of 58.1 ± 3.34 eggs whereas untreated ones presented 108.4 ± 3.46 eggs resulting in a Deterrence Index of 0.7 ± 0.09.
DISCUSSION

The fractions from flowers of *B. suaveolens* killed adults of *Z. subfasciatus*, reduced the number of eggs on bean grains, inhibited the F₁ progeny and the damages on them. As *Z. subfasciatus* is an oviparous species, the onset of its embryonic development is linked to the deposition of the egg on bean grains; thereby inhibiting its F₁ progeny is a key-factor to avoid its larvae to penetrate grains. The females of *Z. subfasciatus* lay their eggs individually in a transparent gelatinous droplet previously deposited on the surface of bean grains, and viable eggs acquire a white coloration. It was observed that eggs on treated beans stayed with a transparent aspect whereas eggs on untreated grains had white color. Therefore, the contact of the egg with the treated surface of the grain was enough to prevent its F₁ progeny to penetrate in bean grains. This is a desirable aspect because it is hard to control *Z. subfasciatus* larvae after they penetrate grains, remaining in this case theoption of using extreme temperatures, modified atmosphere (saturated with CO₂) and radiation, which are more expensive and inaccessible methods for most farmers (Boyer *et al.*, 2012; Zaugg *et al.*, 2013). Nonetheless, it may be possible to associate the use of fractions from *B. suaveolens* with resistant bean varieties presenting arcelyn, which can be a cheap and accessible control method (Eduardo *et al.*, 2016). During the chromatographic fractionation process, the efficacy of *B. suaveolens* bioactivity increases. It went from 6% mortality and 30.8% of damages on bean grains (BSHid applied at 2,500 mg Kg⁻¹) to 56% mortality and 0% of damage (BSHidAcF₁-1 applied at 150 mg Kg⁻¹). The concentrations used in bioassays resemble to the concentrations recommended for the insecticides K-Obiol 2P (500-1,000mg Kg⁻¹), Kaolin (4 g Kg⁻¹), and neem oil (2 g Kg⁻¹) to control *Z. subfasciatus*, which demonstrates its technical viability (Barbosa *et al.*, 2002; Costa *et al.*, 2014; Agrofit, 2017). The fraction BSHidAcF₁-1 applied at 150 mg Kg⁻¹ killed 56% of adults of *Z. subfasciatus*, which demonstrated signs of hyperexcitation (Table 4). The plant *B. suaveolens* presents tropane alkaloids, pyrrolizidine alkaloids and kaempferol glycosides (Geller *et al.*, 2014). It produces atropine and scopolamine in its flowers (Geller *et al.*, 2011 and 2014), and it was verified that scopolamine promotes insecticidal effect against *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) (Roesler *et al.*, 2007). Alkaloids are known for their neurotoxicity; they can modulate ion channels, interfere on enzymes activity and act as agonists or antagonists of insect neuroreceptors (Wink and Schimmer, 2010). Therefore, the observed signs of hyperexcitation in *Z. subfasciatus* adults can be due to the action of tropane alkaloids present in the flowers of *B. suaveolens*. Most studies to control *Z. subfasciatus* are focused...
on the use of plant essential oils that act after the insects have infested the warehouse (Weaver et al., 1994; Silva et al., 2008; Zewde et al., 2010; França et al., 2012; Brito et al., 2015; Jairoce et al., 2016; Bernardes et al., 2018). On the other hand, the present study provides a grain protectant that can avoid the initial infestation and damages of bean grains. In Brazil, the only registered product able to avoid infestations of *Z. subfasciatus* in stored beans is the synthetic insecticide K-Obiol 2P (a.i. 0.02% deltamethrin); it acts as a modulator of voltage gated sodium channels in the neuron axon of insects promoting hyperexcitation on them (Agrofit, 2017; Sparks and Nauen, 2019). Thus, a botanical insecticide based on *B. suaveolens* could be a viable and accessible solution to control *Z. subfasciatus* for organic farmers.

Tropine alkaloids, scopolamine and atropine, can promote loss of body coordination, muscular paralysis, hallucinations and respiratory distress in humans (Smith et al., 1991); and there are some reports of human intoxication due to the exposure to plants containing tropine alkaloids such as hyoscine, atropine and scopolamine (Adamse et al., 2014). It demonstrates the importance of performing bioguided chromatographic fractionations and chemical analysis in order to verify which compounds are actually bioactive in complex chemical mixtures of plant extracts and fractions. It is essential to verify possible toxic compounds to humans, and establish safe standards and develop purification processes to formulate botanical insecticides because the toxicity of a chemical compound depends on its concentration applied, time and form of exposition, and the target-organism. For example, the average fatal dose (humans) for atropine is 50 mg, but its therapeutic dose is 1-3 mg for acute organophosphorus pesticide poisoning (Smith et al., 1991; Eddleston et al., 2008; Moudgilet al., 2018). In addition, atropine at low doses (0.01%) can be used to treat myopia (Gonget al., 2018). This demonstrates how complex is the interaction of bioactive compounds with live organisms and the importance of formulating botanical insecticides with minimum risks to humans despite the general public perception regarding the safety of botanical insecticides.

The results obtained in the present study are an important step to identify an insecticide compound from *B. suaveolens* flowers that can be applied to control Bruchinae beetles in stored beans. More chromatographic fractionations and chemical analysis are necessary in order to isolate and identify the chemical compound(s) from flowers of *B. suaveolens* that is responsible for the observed bioactivity on *Z. subfasciatus*. Not only research with insecticidal plant compounds has been growing in recent years but also legislative and bureaucratic regulations to facilitate their registration and use for pest management (Isman and Grieneisen, 2014; Handford et al., 2015; Villaverde et al., 2016). This legislative trend can open many opportunities for developing commercial botanical insecticides and synthetic insecticides based on plant chemical compounds.

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