

**BIOACTIVITY OF EXTRACTS FROM SOLANACEAE AGAINST
*Zabrotes subfasciatus*****Bioactividad de extractos de Solanaceae contra
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The botanical family Solanaceae has many species producing compounds with insecticidal properties, e.g. nicotine and capsaicin, which are used for pest management in agriculture. This fact provides perspectives to identify insecticidal compounds in Brazilian native species of Solanaceae. In this study, we performed a screening with 25 ethanolic extracts from 17 Solanaceae species in order to evaluate their bioactivity against the Mexican bean weevil, *Zabrotes subfasciatus* (Coleoptera: Chrysomelidae: Bruchinae). The bioactivity of Solanaceae ethanolic extracts (2,500 mg kg⁻¹) was tested with residual contact bioassays. Adults of *Z. subfasciatus* were exposed to treated bean grains, and adult mortality, oviposition, F1 progeny and damages on grains were quantified. Most of the ethanolic extracts from Solanaceae reduced the number of eggs per sample, the egg-adult viability, the F1 progeny and the damages on bean grains promoted by *Z. subfasciatus*, but none of them interfered on its sex ratio. Ethanolic extract from leaves of *Solanum lycocarpum* A. St.-Hil promoted the most promissory effects on *Z. subfasciatus*. This ethanolic extracts can be a suitable alternative to control *Z. subfasciatus* in stored beans, mainly for small farmers and organic farmers.

Keywords: botanical insecticides, Mexican bean weevil, *Phaseolus vulgaris*.**RESUMEN**

La familia botánica Solanaceae tiene muchas especies que producen compuestos con propiedades insecticidas, e.g. nicotina y capsaicina, que se utilizan para el control de plagas en la agricultura. Este hecho proporciona perspectivas promisorias para identificar compuestos insecticidas en especies nativas brasileñas de Solanaceae. En el presente estudio se llevó a cabo un cribado con 25 extractos etanólicos de 17 especies de Solanaceae con el propósito de evaluar su bioactividad sobre el gorgojo pinto del frijol, *Zabrotes subfasciatus* (Coleoptera: Chrysomelidae: Bruchinae). La bioactividad de los extractos etanólicos se evaluó por experimentos de contacto residual. Los adultos de *Z. subfasciatus* se expusieron a los frijoles tratados con los extractos y se midió la mortalidad de los adultos, la ovoposición, la progenie F1 y el daño en los granos. La mayoría de los extractos etanólicos redujeron el número de huevos, la viabilidad de los huevos, la progenie y el daño en los granos, pero ninguno de ellos interfirió en la proporción sexual de los insectos. El extracto etanólico de las hojas de *Solanum lycocarpum* A. St.-Hil promovió los efectos más prometedoros sobre *Z. subfasciatus*. Este extracto puede ayudar a controlar a *Z. subfasciatus* en frijoles almacenados, principalmente a los pequeños agricultores y a los agricultores orgánicos.

Palabras clave: insecticidas botánicos, gorgojo mexicano del frijol, *Phaseolus vulgaris*.

INTRODUCTION

The Bruchinae beetles are major pests worldwide; globally widespread species such as *Zabrotes subfasciatus* (Boheman, 1833) promote high levels of damages on stored bean grains (*Phaseolus vulgaris* L.), mainly at warehouses of developing countries in Sub-Saharan Africa and Latin America, where common beans are an important source of proteins (Tuda, 2007; Daglish, 2018; Tripathi, 2018). The larvae of *Z. subfasciatus* consumes grains internally and promotes high levels of damages on dried beans at warehouses resulting on severe economic losses to farmers (Gonçalves *et al.*, 2017; Tigest *et al.*, 2018). In Southern Ethiopia, for example, it is estimated that 44.6 % of the losses of common bean production occur during storage due to inappropriate infrastructure and the presence of *Z. subfasciatus*. And most farmers (48 %) apply botanical insecticides to control populations of the Mexican Bean Weevil (Mesele *et al.*, 2019).

Unfortunately, chemical control of insect pests of stored products is practically restricted to phosphine, organophosphates and pyrethroid insecticides and many of these products are not easily accessible and available for farmers in developing countries (Boyer *et al.*, 2012). Moreover, there are many reports of resistant insect-pest populations to synthetic insecticides worldwide (Pimentel *et al.*, 2010; Zettler and Arthur, 2010; Boyer *et al.*, 2012). Therefore, it is important to develop botanical insecticides with new modes of action 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 *Z. subfasciatus* (Ribeiro-Costa *et al.*, 2007; Gonçalves *et al.*, 2015; Gonçalves *et al.*, 2017; Lüthi *et al.*, 2018). The use of botanical insecticides can be an efficient and accessible tool to control infestations of *Z. subfasciatus* at warehouses, mainly for small farmers (Mulungu *et al.*, 2007; Pessoa *et al.*, 2016; Silva *et al.*, 2016; Tamiru *et al.*, 2016; Bayih *et al.*, 2018; Bernardes *et al.*, 2018; Zekarias and Haile, 2018).

Worldwide, many botanical insecticides such as neem oil, pogram oil, rotenone and essential oils have been sprayed on crops, and two major classes of insecticides, pyrethroids and neonicotinoids are based on plant insecticidal chemical compounds (Pavela, 2016; Sparks *et al.*, 2017). Regarding botanical insecticides based on Solanaceae species, there are products such as Hot Pepper Wax (Vitova Insectaries, USA) formulated with *Capsicum annum* L., and Nico Dust (Nico Orgo Manures, India) based on *Nicotiana tabacum* L. (Pavela, 2016). Solanaceae is a promissory botanical family to discover new insecticidal molecules. It is widely distributed in both the temperate and tropical zones with about 2,300 species presenting secondary metabolites (flavonoids, alkaloids, whitanolides, capsinoids etc.) (Shonle and Bergelson, 2000; Alves *et al.*, 2003; Martins and Barkman, 2005; Veleiro *et al.*, 2005; Luo *et al.*, 2011; Pinto *et al.*, 2011;

Ohyama *et al.*, 2013). These secondary metabolites can promote different effects on insect pests, such as mortality, feeding deterrence and repellence (Chowanski *et al.*, 2016). Recent studies demonstrated the efficacy of botanicals from *Datura stramonium* L. (Solanaceae) and *Capsicum frutescens* L. (Solanaceae) to control *Z. subfasciatus* (Bayih *et al.*, 2018; Zekarias and Haile, 2018). Thereby, in the present study, it was performed toxicological bioassays with ethanolic extracts of Solanaceae species in order to evaluate their bioactivity against *Z. subfasciatus*. To perform a suitable selection of Solanaceae species it was adopted the following criteria: i) scientific data regarding their chemical diversity and bioactivity, ii) focus on including species from different genera (8 in total), and iii) plants that may be easily available for farmers.

MATERIALS AND METHODS

Collecting plant material and preparing ethanolic extracts

In the present research, 25 ethanolic extracts from 17 Solanaceae species were studied (Table 1). The selection of Solanaceae species used were based on i) scientific data regarding their chemical diversity and bioactivity, ii) focus on including species from different genera (eight in total), and iii) plants that may be easily available for farmers. The process adopted to obtain ethanolic extracts was cold maceration in ethanol solvent (analytical grade, 99.5 %). All plant parts were dried in an air flow drying oven (40 °C for 48 to 72 h) and separately drilled in a knife mill to produce a powder. These plant powders (200 g) were separately immersed in 1 L of ethanol (1:5 w/v), stirred for ten minutes, stored at 25 °C for 72 hours to finally be filtered with qualitative filter paper (80 g m⁻², porosity of 3 µm). Such process was repeated three times with each plant powder. Afterwards, the remaining solution (ethanol with extracted compounds from plant powders) was placed in a rotary evaporator at 50 °C and -600 mmHg in order to eliminate the ethanol solvent and acquire the plant extract (with paste or oil consistence).

Toxicological bioassays with ethanolic extracts from Solanaceae

The colony of *Z. subfasciatus* was initiated with individuals acquired from warehouses of Piracicaba municipality, São Paulo, Brazil. They were maintained in the laboratory under controlled conditions (25±2 °C, 60±10 % R.H. and a photoperiod of 14 L: 10 D hours) in glass containers (2.6 L) with *P. vulgaris* grains cv. 'Bolinha' as substrate for adults to lay their eggs.

It was employed five couples of *Z. subfasciatus* adults (aging 24 hours) per sample unit (100 insects per treatment) to perform residual contact toxicological bioassays. The treatments consisted of samples of bean grains treated with

Table 1. Identification of Solanaceae species, localization, date of collecting and extraction yield.

Species	Collecting local	Collecting date	Voucher number	Herbarium number	Ethanollic extraction yield (%)
<i>Acnistus arborescens</i> (L.) Schltldl.	Campus CENA/USP, Piracicaba, SP (22°42'30.2"S 47°38'38.2"W)	16/11/15	D. S. Gissi 46	ESA 126582	Leaf: 9.84 Stem: 2.82
<i>Brugmansia suaveolens</i> (Willd.) Sweet.	Campus ESALQ/USP, Piracicaba, SP (22°42'27.4"S 47°37'46.0"W)	31/10/15	G. Padoan 01	ESA 139306	Flower: 17.31
<i>Brunfelsia uniflora</i> (Pohl) D. Don	Campus ESALQ/USP, Piracicaba, SP 22°42'34.0"S 47°37'53.4"W	04/08/15	F. Rocha 02	ESA 127276	Leaf: 11.38
<i>Cestrum intermedium</i> Sendtn.	Campus ESALQ/USP, Piracicaba, SP (22°42'27.4"S 47°37'46.0"W)	22/08/15	D. S. Gissi 275	BOTU 34709	Leaf: 6.25
<i>Cestrum nocturnum</i> L.	Louveira, SP (23°04'48.0"S 46°57'00"W)	10/08/15	F. Rocha 03	ESA 127279	Leaf: 12.96
<i>Lycianthes repens</i> (Spreng.) Bitter	Campus ESALQ/USP, Piracicaba, SP (22°42'54.4"S 47°37'50.5"W)	16/11/15	D. S. Gissi 47	ESA 126584	Leaf: 16.96
<i>Lycianthes rantonnetii</i> (Carrière) Bitter	Campus ESALQ/USP, Piracicaba, SP (22°42'27.4"S 47°37'46.0"W)	17/08/15	A. Fabri 03	ESA 139304	Leaf: 8.16
<i>Nicandra physalodes</i> (L.) Gaertn	Campus ESALQ/USP, Piracicaba, SP (22°42'28.9"S 47°37'48.9"W)	02/09/15	D. S. Gissi 36	ESA 126576	Leaf: 9.32
<i>Nicotiana longiflora</i> Cav.	Campus ESALQ/USP, Piracicaba, SP (22°42'27.4"S 47°37'46.0"W)	05/01/15	D. S. Gissi 274	ESA 139299	Leaf: 14.19
<i>Solanum americanum</i> Mill.	Campus ESALQ/USP, Piracicaba, SP (22°42'27.4"S 47°37'46.0"W)	16/11/15	A. Fabri 04	ESA 139305	Leaf: 11.07 Stem: 5.12
<i>Solanum cernuum</i> Vell.	Campus ESALQ/USP, Piracicaba, SP (22°42'28.9"S 47°37'50.4"W)	17/11/15	D. S. Gissi 38	ESA 126547	Leaf: 5.61
<i>Solanum granulosoaleprosum</i> Dunal	Campus ESALQ/USP, Piracicaba, SP (22°41'59.4"S 47°37'59.8"W)	05/01/16	D. S. Gissi 271	ESA 139296	Leaf: 12.01 Stem: 5.74 Fruit: 18.14
<i>Solanum lycocarpum</i> A. St.-Hil	Sítio Retiro, Lavras, MG (21°12'08.3"S 45°09'52.2"W)	31/12/15	A. Fabri 02	ESA 139303	Leaf: 8.64
<i>Solanum paniculatum</i> L.	Campus ESALQ/USP, Piracicaba, SP (22°42'28.9"S 47°37'48.9"W)	10/09/15	D. S. Gissi 35	ESA 126577	Leaf: 8.49
<i>Solanum scuticum</i> M.Nee.	Campus ESALQ/USP, Piracicaba, SP (22°41'59.5"S 47°37'59.8"W)	05/01/16	D. S. Gissi 270	ESA 139295	Leaf: 8.7 Stem: 4.09 Fruit: 14.9
<i>Solanum seaforthianum</i> Andr.	Campus ESALQ/USP, Piracicaba, SP 22°42'36.1"S 47°37'58.5"W	16/11/15	F. Rocha 01	ESA 127275	Leaf: 9.86 Fruit: 9.96
<i>Solanum viarum</i> Dunal	Campus ESALQ/USP, Piracicaba, SP (22°42'28.9"S 47°37'48.9"W)	16/11/15	D. S. Gissi 37	ESA 126575	Leaf: 17.57 Stem: 1.86

Solanaceae ethanolic extracts [(Petri dishes dimensions of 6.5 cm diameter × 2 cm high) with 10 g of bean cv. "Bolinha" per sample] in a completely randomized design, with 10 repetitions per treatment.

For each treatment, 100 g of beans were placed inside a plastic bag (2 L) and sprayed with an ethanolic extract using a microatomizer pistol coupled to a pneumatic pump adjusted to provide a spray pressure of 0.5 kgf cm⁻² and a volume of 30 L t⁻¹ [3 mL of solution (solvent + Solanaceae extract) per each 100 g of beans]. The solvents methanol and acetone were used to solubilize ethanolic extracts at a ratio of 1:1 (v/v). After applying ethanolic extracts (2,500 mg kg⁻¹), treated beans were softly shaken for one minute

to homogeneously distribute Solanaceae extracts on grains surface. Afterwards, they were kept in an airflow chamber during two hours for solvent evaporation before insects (five couples of *Z. subfasciatus*) were inserted in treated bean samples (10 g of beans). The concentration (2,500 mg kg⁻¹) used to apply ethanolic extracts in toxicological bioassays was defined based on preliminary tests. The preliminary tests were performed adopting the same procedures described above but applying different concentrations (500-3,000 mg kg⁻¹) of the fraction on bean grains.

For each experiment a negative control with acetone:methanol (1:1, v/v) was included. In addition, the botanical insecticide Azamax® 1.2 EC {azadiractin A/B

[12 g.L⁻¹ (1.2 % m/m)]} was included in bioassays to compare its activity with the ethanolic extracts. Azamax[®] 1.2 EC, a botanical insecticide registered in Brazil to control many insect pests, presents limonoids (azadiractin A and 3-tigloylazadirachtol) that can cause phagodeterrence and hormonal disbalance on insects. Azamax[®] 1.2 EC was applied adopting the same concentration (2,500 mg kg⁻¹) used for spraying ethanolic extracts.

The mortality of adults and number of eggs per sample were assessed five days after infestation (adults were withdrawn from sample units), and insects were considered dead when they did not react to a brush touch after one minute. Moreover, the F₁ progeny and damages on grains were assessed after 56 days of infestation. Bean grains were considered damaged when they presented galleries due to the feeding of insect larvae. The percentage of egg-adult viability was calculated based on the formulae: (n° of F₁ individuals/n° of eggs) * 100, for each repetition.

Data analysis

The data were analyzed with the software "R", version 3.3.1. It was used generalized linear models (GLM) with quasibinomial or quasipoisson family distribution together with a half-normal probability plot with simulation envelope to verify generalized linear models' fit (Nelder and Wedderburn, 1972; Demétrio and Hinde, 1997; Hinde and Demétrio, 1998). In the case of significant differences among treatments, a multiple comparison test (Tukey's test, $p < 0.05$) was performed to identify such differences.

RESULTS

The Solanaceae ethanolic extracts affected *Z. subfasciatus* mainly by reducing the number of eggs per sample, the F₁ progeny and the damages on bean grains (Table 2). The adults of *Z. subfasciatus* do not feed on bean grains; thereby reducing the number of eggs and inhibiting its F₁ progeny are key aspects to avoid infestations at warehouses and damages on grains. Species from different genus promoted statistically significant effects on *Z. subfasciatus*, but at different intensities. None of the ethanolic plant extracts interfered on sex ratio, but many of them reduced egg-adult viability (Table 2).

The extract of *Solanum lycocarpum* A. St.-Hil leaves almost reduced the number of eggs per sample to zero and completely protected bean grains against damages resulting from larvae feeding (Table 2). In addition, the extract from flowers of *Brugmansia suaveolens* (Willd.) Sweet provided a good protection of beans grains against *Z. subfasciatus*. It promoted an expressive decrease in the number of eggs per sample (11.0), the F₁ progeny (6.6), egg-adult viability (57.6 %) and damaged grains (15.6 %) (Table 2). Only the extract of *Solanum paniculatum* L. (leaves) promoted significant mortality

on *Z. subfasciatus* adults comparing to the negative control, but it did not expressively reduce oviposition, F₁ progeny and damages on grains comparing to other extracts (Table 2). However, some extracts (stems and leaves of *Solanum viarum* Dunal, and leaves of *Solanum seafortianum* Andr., *Solanum scuticum* M.Nee, *Solanum americanum* Mill. and *Lysianthes repens* (Spreng.) Bitter) intensively reduced both the number of eggs per sample and the F₁ progeny. They also promoted a moderate toxicity to *Z. subfasciatus* eggs expressed by the reduction in egg-adult viability and, consequently, by the reduction of damages on grains (Table 2).

Some ethanolic extracts such as *Acnistus arborescens* (L.) Schltdl. (leaves), *Nicandra physalodes* (L.) Gaertn. (leaves), *Solanum granulosoleprosum* Dunal (stems), *Solanum cernuum* Vell. (leaves) and *Solanum americanum* (leaves) promoted a good reduction of damages caused by larvae on bean grains, but less intensively than *S. lycocarpum* and *B. suaveolens* (Table 2). Nonetheless, the highest mortality was promoted by the positive control with azadiractin and it also presented grain-protective properties (Table 2).

DISCUSSION

Many toxicological studies with insecticidal plants to control *Z. subfasciatus* focus on terpenes such as limonoids (azadiractin) and essential oils that act as fumigants (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). However, an advantage of non-volatile compounds is that they can protect bean grains for a longer period and avoid new infestations of *Z. subfasciatus*. In general, Solanaceae ethanolic extracts affected *Z. subfasciatus* mainly by reducing the number of eggs per sample, the F₁ progeny and the damages on bean grains (Table 2). Considering that only the larvae of *Z. subfasciatus* feed on bean grains, reducing its oviposition and F₁ progeny is a strategically aspect to reduce damages on bean grains.

The ethanolic extracts of *N. physalodes* (leaves), *S. scuticum* (leaves) and *A. arborescens* (leaves) reduced oviposition to 13.9, 15.8 and 16.6 eggs.sample⁻¹, respectively (Table 2). Females of *Z. subfasciatus* may have laid less eggs due to oviposition deterrence or due to active compounds disrupting egg formation. The insecticidal whitanolide nicandrenone produced by *N. physalodes* and cytotoxic whitanolides produced by *A. arborescens* may be responsible for the reduced oviposition (Bates and Eckert, 1972; Cordero *et al.*, 2009; Roumy *et al.*, 2010). Whitanolides promote feeding deterrence on insects and can antagonize ecdysteroid hormones (Dinan *et al.*, 1996; Mareggiani *et al.*, 2000). Earle *et al.* (1970) verified that some ecdysone analogues are able to induce sterility on females of *Anthonomus grandis* Boheman (Coleoptera: Curculionidae). Solanaceae ethanolic extracts not only reduced the number of eggs laid on beans' surface

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

Treatment	Mortality (%) ¹	N° eggs/ sample ²	F ₁ progeny ²	Viability (%) (egg-adult) ¹	Damaged grains (%) ¹
<i>Solanum paniculatum</i> (leaves)	11.0 \pm 5.86 c	35.8 \pm 4.71 d	30.1 \pm 3.84 d	84.5 \pm 1.49 b	57.2 \pm 5.96 e
<i>Cestrum nocturnum</i> (leaves)	7.0 \pm 2.13 bc	26.2 \pm 3.74 c	22.7 \pm 3.34 c	88.5 \pm 3.51 ab	42.3 \pm 4.96 cd
<i>Brunfelsia uniflora</i> (leaves)	6.0 \pm 2.21 bc	29.8 \pm 3.92 cd	23.7 \pm 3.48 c	78.9 \pm 3.86 c	45.7 \pm 6.55 cd
<i>Lycianthes rantonnetii</i> (leaves)	4.0 \pm 3.06 ab	31.3 \pm 5.00 cd	24.6 \pm 4.20 cd	77.6 \pm 2.43 c	47.0 \pm 6.21 d
<i>Cestrum intermedium</i> (leaves)	3.0 \pm 2.13 ab	25.7 \pm 6.43 c	23.0 \pm 5.95 c	79.2 \pm 9.61 a	36.3 \pm 8.87 c
<i>Nicandra physalodes</i> (leaves)	1.0 \pm 1.00 a	13.9 \pm 3.05 b	12.6 \pm 2.95 b	86.1 \pm 4.45 a	25.1 \pm 6.70 b
Control (acetone:methanol (1:1))	4.0 \pm 1.63 ab	82.0 \pm 4.16 e	70.0 \pm 3.91 e	85.3 \pm 1.71 b	88.6 \pm 2.49 f
Azamax [®] (2,500 mg kg ⁻¹)	28.0 \pm 6.29 d	0.8 \pm 0.42 a	0.5 \pm 0.22 a	30.0 \pm 13.33 **	1.6 \pm 0.66 a
F	5.087	27.930	25.934	2.469	19.606
p value	<0.0001	<0.0001	<0.0001	0.03317	<0.0001
<i>Solanum granulosoleprosum</i> (stems)	5.0 \pm 2.24 b	24.1 \pm 5.32 b	15.5 \pm 3.52 b	63.5 \pm 4.78 a	25.0 \pm 6.2 a
<i>Solanum granulosoleprosum</i> (fruits)	4.0 \pm 1.63 b	50.4 \pm 6.68 c	40.9 \pm 5.47 c	80.6 \pm 3.01 c	60.9 \pm 5.5 c
<i>Nicotiana longiflora</i> (leaves+stems)	4.0 \pm 2.21 b	28.0 \pm 8.11 b	19.6 \pm 3.58 b	79.1 \pm 13.75 ab	39.7 \pm 6.1 b
<i>Solanum granulosoleprosum</i> (leaves)	1.0 \pm 1.00 a	25.9 \pm 4.89 b	18.0 \pm 3.67 b	62.6 \pm 7.14 b	34.6 \pm 6.4 b
<i>Solanum lycocarpum</i> (leaves)	1.0 \pm 1.00 a	0.1 \pm 0.10 a	0.0 \pm 0.0*	0.0 \pm 0.0*	0.0 \pm 0.0*
Control (acetone:methanol)	0.0 \pm 0.00*	66.0 \pm 7.01 d	53.8 \pm 5.79 d	81.7 \pm 1.05 c	81.7 \pm 4.3 d
Azamax [®] (2,500 mg kg ⁻¹)	36.0 \pm 10.46 c	0.7 \pm 0.70 a	0.5 \pm 0.50 a	7.1 \pm 7.14**	0.0 \pm 0.0*
F	10.915	26.565	25.455	4.485	13.837
p value	0.0001	0.0001	0.0001	0.003984	0.0001
<i>Solanum scuticum</i> (leaves)	10.0 \pm 2.98 c	15.8 \pm 5.58 a	11.6 \pm 4.02 b	46.1 \pm 13.0 b	19.8 \pm 6.97 ab
<i>Solanum scuticum</i> (fruits)	7.0 \pm 2.13 bc	30.8 \pm 7.12 b	24.6 \pm 5.88 cd	70.8 \pm 8.5 bd	37.3 \pm 8.18 cd
<i>Solanum cernuum</i> (leaves)	5.0 \pm 2.69 ab	16.2 \pm 4.02 a	12.5 \pm 3.05 b	69.3 \pm 8.6 bc	23.7 \pm 6.31 ab
<i>Solanum scuticum</i> (stems)	4.0 \pm 2.21 ab	24.6 \pm 5.88 b	19.4 \pm 4.68 c	64.6 \pm 11.2 bd	28.3 \pm 7.29 bc
<i>Lysianthes repens</i> (leaves)	4.0 \pm 2.21 ab	30.0 \pm 5.44 b	6.7 \pm 1.45 a	21.3 \pm 4.7 a	16.6 \pm 3.74 a
<i>Solanum americanum</i> (leaves)	2.0 \pm 2.00 a	14.8 \pm 4.93 a	12.1 \pm 4.19 b	55.7 \pm 12.4 cd	19.4 \pm 6.51 ab
<i>Solanum americanum</i> (stems)	0.0 \pm 0.00*	32.1 \pm 4.70 b	26.9 \pm 4.44 d	82.2 \pm 2.9 d	44.5 \pm 4.64 d
Control (acetone:methanol)	2.0 \pm 1.33 a	61.2 \pm 4.58 c	49.8 \pm 4.13 e	81.1 \pm 2.9 cd	75.8 \pm 4.43 e
Azamax [®] (2,500 mg kg ⁻¹)	27.0 \pm 5.97 d	0.0 \pm 0.0*	0.0 \pm 0.0*	0.0 \pm 0.0*	0.0 \pm 0.0*
F	5.733	5.957	8.728	26.529	8.390
p value	0.0001	0.0001	0.0001	0.0001	0.0001
<i>Brugmansia suaveolens</i> (flowers)	8.0 \pm 2.00 b	11.0 \pm 3.22 b	6.6 \pm 1.80 d	57.6 \pm 11.23 a	15.6 \pm 3.82 b
<i>Acnistus arborescens</i> (stems)	4.0 \pm 2.67 a	26.1 \pm 5.29 d	20.9 \pm 4.04 b	76.3 \pm 9.29 c	37.8 \pm 6.58 d
<i>Acnistus arborescens</i> (leaves)	3.0 \pm 1.53 a	16.6 \pm 3.23 c	11.2 \pm 2.38 c	66.0 \pm 5.87 b	23.2 \pm 3.92 c

(Continued)

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

Treatment	Mortality (%) ¹	N° eggs/ sample ²	F ₁ progeny ²	Viability (%) (egg-adult) ¹	Damaged grains (%) ¹
Control (acetone:methanol (1:1))	7.0 \pm 2.13 b	67.1 \pm 8.48 e	55.4 \pm 6.94 a	83.0 \pm 1.88 d	83.9 \pm 6.05 e
Azamax® (2,500 mg kg ⁻¹)	21.0 \pm 6.23 c	2.4 \pm 1.11 a	0.3 \pm 0.21 e	5.3 \pm 3.69**	0.8 \pm 0.57 a
F	4.686	28.237	42.652	5.742	37.265
p value	0.003	<0.0001	<0.0001	0.00266	<0.0001
<i>Solanum viarum</i> (stems)	12.0 \pm 3.27 b	5.6 \pm 1.74 b	2.9 \pm 0.98 b	46.8 \pm 11.72 b	6.1 \pm 1.8 b
<i>Solanum viarum</i> (leaves)	11.0 \pm 4.07 ab	10.4 \pm 3.47 c	6.6 \pm 2.25 c	56.8 \pm 18.86 c	12.3 \pm 3.9 c
<i>Solanum seaforthianum</i> (leaves)	10.0 \pm 4.47 ab	13.1 \pm 3.74 c	1.0 \pm 0.39 a	4.6 \pm 1.67 a	2.2 \pm 0.8 a
<i>Solanum seaforthianum</i> (fruits)	7.0 \pm 3.35 a	56.2 \pm 6.56 d	38.1 \pm 4.17 d	68.3 \pm 2.25 d	56.4 \pm 5.5 d
Control (acetone:methanol)	12.0 \pm 2.91 b	76.3 \pm 5.70 e	57.9 \pm 5.13 e	75.4 \pm 1.98 e	83.5 \pm 2.6 e
Azamax® (2,500 mg kg ⁻¹)	42.0 \pm 4.42 c	2.1 \pm 0.89 a	1.2 \pm 0.59 a	21.6 \pm 10.02**	2.4 \pm 1.1 a
F	7.716	47.224	76.596	44.158	81.068
p value	0.0001	0.0001	0.0001	0.0001	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 included in the analysis (null variance);

** Not analyzed due to small sample unit;

^{ns} Not significant ($p > 0,05$).

but also the egg-adult viability of *Z. subfasciatus* (Table 2). The highest reduction of egg-adult viability (4.6 %) was performed by the ethanolic extract of *S. seaforthianum* (leaves) (Table 2). Differently from the above-mentioned species, *S. seaforthianum* produces glycoalkaloids (solasodine, solasonine and solamargine) and the reduction of egg-adult viability could be related to the contact of eggs with the treated surface of bean grains (Alsherbiny *et al.*, 2018).

The bioactivity of Solanaceae extracts was species-specific and varied according to the plant-structure (leaf, stem, flower and fruit). Bean samples treated with the ethanolic extract from leaves of *S. seaforthianum* had 2.2 % of damaged grains whereas samples treated with its fruits extract had 56.4 %, for example (Table 2). The ethanolic extract from *A. arborescens* leaves (16.6 eggs.sample⁻¹) reduced more the oviposition of *Z. subfasciatus* than its stem extract (26.1 eggs.sample⁻¹) (Table 2). The ethanolic extract from stems (25 % of damaged grains) of *S. granulosoleprosum* presented better grain-protectant properties than its fruit ethanolic extract (60.9 % of damaged grains) (Table 2). This factor is also reported for Annonaceae species promoting toxicity on *Z. subfasciatus*. The ethanolic extract from leaves of *Annona sylvatica* A. St.-Hil. (Annonaceae), applied at 1,500 mg kg⁻¹, did not promote mortality of *Z. subfasciatus* adults but promoted sublethal effects (11.9 eggs sample⁻¹; 21.11 % of damaged grains) whereas the ethanolic extract from

A. sylvatica seeds promoted 100 % mortality and completely inhibited damages on grains (Gonçalves *et al.*, 2015). This species-specific bioactivity is probably due to the specific distribution of secondary metabolites among plant structures and tissues (Berenbaum, 1995). In addition, plants can inductively or constitutively produce secondary metabolites and their production suffers interference from biotic and abiotic factors (Mithöfer and Boland, 2012).

Based on the percentage of damaged grains, in the present study the most bioactive species tested against *Z. subfasciatus* was *S. lycocarpum* (leaves) (Table 2). The ethanolic extract from leaves of *S. lycocarpum* presented grain-protectant characteristics and completely inhibited damages on grains, despite the fact that it did not kill adults (Table 2). The powder from leaves of *Datura stramonium* (Solanaceae) (tested at 2,000 mg kg⁻¹) promoted 71.28 % mortality of *Z. subfasciatus* adults after 5 days, but it did not inhibit F₁ progeny (11.33 insects.sample⁻¹) or avoid damages on bean grains (Weevil Perforation Index: 21.06) (Bayih *et al.*, 2018). Nonetheless, in the present study, the ethanolic extract from *S. lycocarpum* (tested at 2,500 mg kg⁻¹) did not promote significant mortality, but it inhibited F₁ progeny and damages on bean grains (Table 2). It demonstrates that killing adults of *Z. subfasciatus* is not essential to avoid damages on grains and bioinsecticides with sublethal effects can be efficient.

S. lycocarpum naturally occurs in Brazilian savannas and degraded pastures (Bueno *et al.*, 2002) and it produces the steroidal alkaloids solasonine and solamargine, two defensive allelochemicals (Miranda *et al.*, 2012; Al Sinani and Eltayeb, 2017). These were able to block the larval development of *Earias insulana* (Boisd.) (Lepidoptera: Noctuidae) when incorporated in artificial diet at 0.2 % (Weissenberg *et al.*, 1986); and they reduced the larval growth of *Tribolium castaneum* (H.) (Coleoptera: Tenebrionidae) at 1 $\mu\text{mol g}^{-1}$ diet (Weissenberg *et al.*, 1998). Solasonine and solamargine present membrane-disrupting properties and can affect phosphatidylcholine/cholesterol liposomes (Roddick *et al.*, 1990). The bioactivity promoted by the ethanolic extract from *S. lycocarpum* may be due to the presence of such alkaloids. Alkaloids share equal amino acid precursors with insect neurotransmitters enabling them to interact with neurotransmitters' molecular binding sites (Wink and Schimmer, 2018). Other species, such as *S. viarum*, presents the steroidal alkaloid solasodine (Mola *et al.*, 1997; Shakirov *et al.*, 2012); and *B. suaveolens* (flowers) produces the alkaloids hyoscyamine, atropine and scopolamine (Andreola *et al.*, 2008). Nonetheless, in the present study it was verified nor high levels of mortality neither symptoms of neurotoxicity on *Z. subfasciatus* (Table 2). This absence of adult mortality may be due to low concentrations of alkaloids in ethanolic extracts of *S. lycocarpum*, *B. suaveolens* and *S. viarum*.

The present study provides good perspectives for exploring insecticidal compounds in Solanaceae species to produce a botanical insecticide for the management of *Z. subfasciatus*. Nonetheless, it is necessary to perform bioguided chemical fractionations with Solanaceae ethanolic extracts in order to identify which chemical compounds are responsible for the observed bioactivity against *Z. subfasciatus*. Moreover, it is also important to perform toxicological studies to verify the presence of toxic compounds to humans and non-target organisms. Many Solanaceae species present alkaloids that are toxic to humans (Smith *et al.*, 2008; Chowanski *et al.*, 2016). These studies are essential to assure safety for both farmers and consumers before recommending the application of a botanical insecticide on stored products. Ethanolic extracts are a simple, cheap and efficient way to extract and apply insecticidal chemical compounds from plants. Therefore, the ethanolic extract from *S. lycocarpum* might be an accessible solution to the management of *Z. subfasciatus* to small-scale agriculture.

CONCLUSIONS

Most of the 25 ethanolic extracts from 17 different Solanaceae species promoted significant interference on *Z. subfasciatus* oviposition, F_1 progeny or egg-adult viability resulting in less damaged grains (Tables 1 and 2). The most promissory species was *S. lycocarpum* that completely

inhibited the F_1 progeny and damages on bean grains when applied at 2,500 mg kg^{-1} . It demonstrates that Solanaceae species can be a source of botanical insecticides to control *Z. subfasciatus*.

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