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Potential use of *Annona* by products to control *Drosophila suzukii* and toxicity to its parasitoid *Trichopria anastrephae*

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ABSTRACT

Extracts of *Annona* (Annonaceae) plants have been shown to satisfactorily control several insect pest species, especially on Neotropical regions. Here, we tested the potential use of formulated ethanolic seed-extracts of three *Annona* species (i.e., *A. mucosa* Jacq., *A. muricata* L. and *A. sylvatica* A. St.-Hil) to control the invasive pest *Drosophila suzukii* Matsumura, 1931 (Diptera: Drosophilidae). Using a discriminatory concentration (i.e., 2000 mg L^{-1}) for all the extracts, our results revealed that only the formulation based on *A. mucosa* seed-extract caused mortality above 85%, which was equivalent to the mortality caused by the synthetic insecticide spinetoram, our positive control. However, *D. suzukii* laid significantly fewer eggs in strawberry fruits treated with any of the formulated *Annona* extracts when compared to fruits that were not subjected to insecticide applications. The concentration-mortality curves for *A. mucosa* formulated extracts on *D. suzukii* were determined, and the application of this extract at LC_{90} (i.e., 1,995.04 mg L^{-1}) caused low mortality (33%) of the parasitoid *Trichopria anastrephae* Lima (Hymenoptera: Diapriidae) though ingestion exposure; however, it was toxic (70% mortality) to this natural enemy of *D. suzukii* by means of contact exposition. Behavior of parasitoids that survived exposure to the LC_{90} of *A. mucosa* formulated extract or of spinetoram (i.e., 105.34 mg L^{-1}) was not significantly affected. Thus, the *A. mucosa* formulated extract exhibited potential to be used in the management programs of *D. suzukii*, especially in organically based production systems where there are fewer control tools available.

1. Introduction

Among the many plant families occurring in the Neotropical region, the Annonaceae is considered one of the best sources of compounds with insecticidal properties, especially due to the high diversity of secondary compounds (allelochemicals) that are synthesized and accumulated in different plant parts (Ribeiro et al., 2016). Phytochemical studies have identified a wide array of bioactive compounds in species of Annonaceae plants, particularly acetogenins (ACG), which have diverse chemical structures and potent insecticidal/acaricidal properties (Blessing et al., 2010). The acetogenins comprise a series of natural products (C-35/C-37) derived from long-chain fatty acids (C-32/C-34) combined with a unit of 2-propanol (Alali et al., 1999). These molecules have been found in high concentration in the seeds of some plant genera [*Annona*, *Anomianthus*, *Asimina*, *Desepalum*, *Goniothalamus*, *Rollinia* (now *Annona*), *Polyalthia*, *Porcelia*, *Uvaria*, and *Xylopia*] of the Annonaceae family (Johnson et al., 2000).

Acetogenins are potent mitochondrial poisons, inhibiting cellular energy production (Isman and Seffrin, 2014). More specifically, acetogenins block the respiratory chain at complex I (NADH:ubiquinone oxidoreductase) of the mitochondrial electron transport system and the enzyme NADH:oxidase in the cell membrane of target arthropods, directly affecting electron transport in the mitochondria and causing apoptosis as result of ATP deprivation (Alali et al., 1999). Moreover, in a recent study, acetogenins at sub-lethal doses caused damage in the insect midgut epithelium and digestive cells, decreasing the expression of genes associated with transport and absorption of nutrients, metabolites and nonelectrolytes and increasing the expression of genes linked with autophagy induction (Costa et al., 2016). In addition to lethal toxicity (Ansante et al., 2015; Gonçalves et al., 2015; Ribeiro et al., 2013, 2014a,b,c,d, 2015, 2016; Souza et al., 2017), acetogenin-based extracts or isolated compounds also affect development, feeding

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and oviposition behavior in insects (Ansante et al., 2015; Ribeiro et al., 2015; Gonçalves et al., 2017; Souza et al., 2017). However, to date, we are unaware of any published studies that describe the bioactivity of annonaceous derivatives or isolated acetogenins in the realm of *Drosophila suzukii* Matsumura, 1931 (Diptera: Drosophilidae), an invasive pest that has caused severe losses in small berry or soft fruit fields in all producing countries (Cini et al., 2012; Asplen et al., 2015).

Native to Asia, *D. suzukii* dispersed rapidly throughout North America, Europe and South America (Cini et al., 2012; Santos, 2014; Schlesener et al., 2014; Asplen et al., 2015; Andreazza et al., 2016b). This species is recognized as a serious phytosanitary threat in many countries (Hamby et al., 2016) because of the many factors related to its biology, such as its wide host range (Lee et al., 2015; Poyet et al., 2015), dispersion capability (Haye et al., 2016) and its short generation time (Tochen et al., 2014; Asplen et al., 2015). As an emergency control strategy, the application of synthetic organophosphorous, pyrethroid and spinosyn insecticides are the most used approach to manage *D. suzukii* worldwide (Bruck et al., 2011; Haviland and Beers, 2012; Van Timmeren and Isaacs, 2013; Haye et al., 2016).

Although effective, the continuous use of synthetic insecticides results in environmental contamination, toxicological risks, selection for insecticide-resistant populations and adverse effects on beneficial fauna (Roubos et al., 2014). Additionally, many berry fields in large production countries, such as Brazil, are small properties cultivated under organic or low residue policies in which the use of synthetic substances are banned or restricted (Zanardi et al., 2015). Thus, the development of new management alternatives that meet the standards of organic production or the low residue policies of the international trade market has become sorely needed. In this context, botanical insecticides might have an important role in integrated pest management (IPM) programs for *D. suzukii*, particularly as alternatives to the synthetic insecticides (Isman and Grieneisen, 2014).

Thus, the aim of the present investigation was to assess the toxicity of formulated ethanolic extracts obtained from the seeds of *Annona* species (e.g., *A. mucosa* Jacq., *A. muricata* L. and *A. sylvatica* A. St.-Hil) on *D. suzukii* adults. A spinosyn-based (i.e., spinetoram) and an acetogenin-based (i.e., annonin) commercial insecticide were used as positive controls. Furthermore, we evaluated residues of these products on the oviposition behavior of *D. suzukii* and whether the most promising extracts were selective against the *D. suzukii* parasitoid, *Trichopria anastrephae* Lima (Hymenoptera: Diapriidae). This parasitoid was recently found parasitizing *D. suzukii* pupae in Brazil (Wollmann et al., 2016; Andreazza et al., 2017b).

2. Materials and methods

2.1. Formulated extracts: sources and preparation

Information on the origin of *Annona* species used in this study (Table 1) is detailed in Ribeiro et al. (2014c, 2016). Voucher specimens of these species, previously identified by Prof. Dr. Renato Mello-Silva [Department of Botany, Biosciences Institute/University of São Paulo (IB/USP)], are deposited in the herbarium of the Department of Biological Sciences at "Luiz de Queiroz" College of Agriculture/University of São Paulo, Piracicaba Municipality, São Paulo State, Brazil.

To prepare the extracts, seeds collected from ripe fruits were oven-dried at 38 °C for 48 h. Seeds were then milled to a powder in a knife mill. Powders were stored in sealed glass containers and stored at approximately −10 °C until use. Organic extracts were obtained by cold maceration using ethanol as a solvent (5:1, v:w), with the seed powder held in ethanol for three days, followed by filtering through filter paper. The filtrate was immersed in solvent again, being this process repeated three times. Remaining solvent was removed *in vacuo* at 50 °C. After complete evaporation of the solvent in an airflow chamber, the extraction yield for each species was determined. For preparation of the formulations that would produce aqueous emulsions when diluted with water, the extracts were solubilized in acetone:methanol (1:1, v:v) (100 g L^{-1}), with the subsequent addition of Tween® 80 emulsifier at a concentration of 10 g L^{-1}. All the dilutions were done to a final volume of 100 mL of each desired concentrations (Table 1).

2.2. Test insects

The *D. suzukii* (20th generation) and *T. anastrephae* (10th generation) individuals used in all bioassays were obtained from laboratory stock colonies maintained at 25 ± 2 °C, 70 ± 5% relative humidity and a 12 h photophase. The flies were reared and fed on artificial diet following the methodology described by Andreazza et al. (2016a). The *T. anastrephae* parasitoids were reared on *D. suzukii* pupae and fed honey/water (80%, w:v). Before each bioassay, four-day-old adults of both species were deprived of food for 8 h, but with water provided.

2.3. Bioassays

All bioassays were performed under laboratory conditions at 25 ± 2 °C, 70 ± 10% relative humidity and a 12 h photophase, using a completely randomized design. The formulated synthetic insecticide Delegate 250WG™, which contained the active ingredient spinetoram, was used at the field rate (75 mg of a.i. L^{-1}) as a positive control (Table 1). In addition to the laboratory extracts of the three selected *Annona* species (described in 2.1 and Table 1), a commercial bioinsecticide containing acetogenins was tested [Anosom® (AgriLife SOM

Table 1
Insecticides evaluated for the management of *Drosophila suzukii*.

Treatments	Description	Discriminatory concentration tested[a]	Origin/manufacturer
Anosom 1 EC	*Annona squamosa* L. and *Annona reticulata* L. extracts based bioinsecticide [acetogenin annonin (10.000 mg L^{-1})]	2000	Agrilife Biosolutions, Ltda. (Hyderabad, Andhra Pradesh, India)
ESE Annona mucosa	Aqueous emulsion of ethanolic seed-extract of *Annona mucosa* Jacq. (pre-commercial)	2000	Laboratory extraction and formulation
ESE Annona muricata	Aqueous emulsion of ethanolic seed-extract of *Annona muricata* L. (pre-commercial)	2000	Laboratory extraction and formulation
ESE Annona sylvatica	Aqueous emulsion of ethanolic seed-extract of *Annona sylvatica* A. St.-Hil. (pre-commercial)	2000	Laboratory extraction and formulation
Delegate 250WG™	Spinetoram (250 g Kg^{-1})	300[b]	Dow AgroSciences Industrial Ltda., São Paulo, SP, Brazil

*The concentration of 4000 mg L^{-1} of each extract was used for the oviposition deterrence test; ESE = Formulated ethanolic seed extract.
[a] Concentration: mg of extract or commercial product per L of water.
[b] 75 mg of a.i. per L of water.

Phytopharma Ltda., Hyderabad, Andhra Pradesh State, India]. This formulation is prepared from seed-extracts of *Annona squamosa* L. and *Annona reticulata* L. and has the acetogenin annonin (10,000 mg L^{-1}) as the primary active ingredient.

2.3.1. Annona toxicities and lethal concentrations of the active treatments on D. suzukii

To test the toxicity of the selected extracts, initial tests were performed using discriminatory concentrations on *D. suzukii* adults (Table 1). For these initial tests and further lethal concentrations bioassays, the product's toxicities on *D. suzukii* adults were assessed using both ingestion and contact exposure procedures.

For the ingestion bioassays, insects were separated into groups (sample units) of 20 adults per cage. Each cage was made of a clear plastic cup (1 L) flipped upside down on a plastic Petri dish (25 cm in diameter) with its top (cup bottom) sealed with a voile mesh for ventilation. After appropriate dilutions in water, the products were offered to the insects via capillarity in a saturated cotton rolls, held inside a 10 mL glass vial for 24 h. After this period, 10 mL glass vial containing the treated cotton roll was replaced by food and distilled water until the end of the evaluation period.

For the ingestion bioassays, insects were separated into groups (sample units) of 20 adults per cage. The cages consisted of transparent plastic containers (1 L) flipped upside down on a plastic Petri dish (25 cm in diameter). The cagés upper side (i.e, container bottom) was sealed with a *voile* mesh (i.e., organza) allowing ventilation inside the cages. During the exposure time (i.e., 24 h), the flies could feed in cotton rolls (product traits) saturated with product-containing solutions placed inside a 10 mL glass vial that were held inside the cages. After the exposure period, the 10 mL glass vial containing the treated cotton roll was replaced by food and distilled water until the end of the evaluation period.

In these initial tests, it was used 4 replicates of 20 flies per treatment (products) (n = 80) in the ingestion bioassay and, 10 replicates of 10 insects per treatment (products) (n = 100) in the contact bioassay. Mortality was evaluated at 120 h after initial exposure to the treatment. A fly was considered dead when no movement occurred even after the touch of a fine brush.

Based on the results from this initial test, the most promising treatment (formulated ethanolic seed-extract from *A. mucosa*) and the positive control (spinetoram) were further evaluated to estimate the concentration required to kill 50% and 90% of exposed flies [Lethal concentration (LC), LC_{50} and LC_{90}, respectively]. Therefore, seven concentrations (range: 125–4000 mg L^{-1} for the *A. mucosa* formulated extract and 10–100 mg L^{-1} for spinetoram) were tested for each product and mode of exposure based on the Finney (1971) procedure. The exposure and evaluation procedures and criteria were identical to those of the initial tests. In the ingestion bioassays, four replicates of 20 flies (n = 80) for each concentration of each product (n = 560) were used, whereas in the contact bioassays, 10 replicates of 10 flies (n = 100) for each concentration of each product (n = 700) were used.

2.3.2. Effects of formulated Annona seed-extracts on D. suzukii oviposition

Ripe and undamaged 'Aromas' strawberries without previous insecticide exposure were dipped in the treatment solutions (Table 1) for 5 s and then dried on filter paper for three hours. Later, each fruit (sample unit) was placed inside a cage containing two four-day-old pairs of *D. suzukii* flies for oviposition. The cages were clear plastic cups (200 mL) flipped upside down on a Petri dish (8 cm in diameter). After 24 h, the adults were removed, and the eggs in the fruit (internal or external epidermis) were counted with the aid of a stereoscopic microscope (40x). Fifty replicates were examined per treatment.

2.3.3. Toxicities and sub-lethal effects of the active treatments on T. anastrephae

To evaluate the selectivity of the active treatments on the *D. suzukii* parasitoid *T. anastrephae*, the values for the LC_{90} of the formulated *A. mucosa* seed-extract and spinetoram were used in each exposure procedure, which were determined previously (120 h of exposure time). Adult wasps were submitted to both exposure procedures described in 2.3.1. Wasp mortality was evaluated at 120 h following the beginning of exposure using the same criteria to evaluate death as in *D. suzukii*.

To evaluate the sub-lethal effects of the treatments on the wasps, ten pupae of *D. suzukii* (24-h-old pupae) were offered, per day, for seven days (beginning at 120 h), to each surviving *T. anastrephae* female from the ingestion bioassay. The pupae, obtained from the laboratory colony (as described in 2.2), were exposed to the wasps on a wet hydrophilic cotton layer on an acrylic Petri dish. Pupae were removed daily, and placed in plastic cups (100 mL) sealed on top with *voile* until the fly or wasp emergence. During the evaluation period, the wasps were fed 80% honey/water (w/v). Percentage parasitism was determined by dividing the total number of wasp offspring per total number of pupae offered, multiplied by 100.

2.4. Data analyses

Generalized linear models of the exponential family of distributions (Nelder and Wedderburn, 1972) were used for the analyses of studied variables. The verification of quality adjustment was performed through the half-regular graph odds with simulation envelope (Hinde and Demétrio, 1998). When significant differences were detected among treatments, multiple comparisons (Tukey's test, $P < 0.05$) were performed using the glht function in the Multicomp package, with adjustment of *p*-values. All analyses were performed using the "R" statistical software version 2.15.1 (R Development Core Team, 2012).

A binomial model with a complementary log–log link function (gompit model) was used to estimate the lethal concentrations (LC_{50} and LC_{90}) using the *Probit Procedure* in the SAS statistical software package version 9.2 (SAS Institute, 2011).

3. Results

At 120 h after exposure, the formulation based on ethanolic extract of *A. mucosa* seeds (2000 mg L^{-1}) showed toxicity similar ($F_{5,12} = 75.32$, $P < 0.0001$) to that with spinetoram (75 mg L^{-1}), with approximately 90% fly mortality via ingestion (Fig. 1). These values were significantly higher than those of the commercial acetogenin-based bioinsecticide and the formulated seed-extracts from *A. sylvatica*

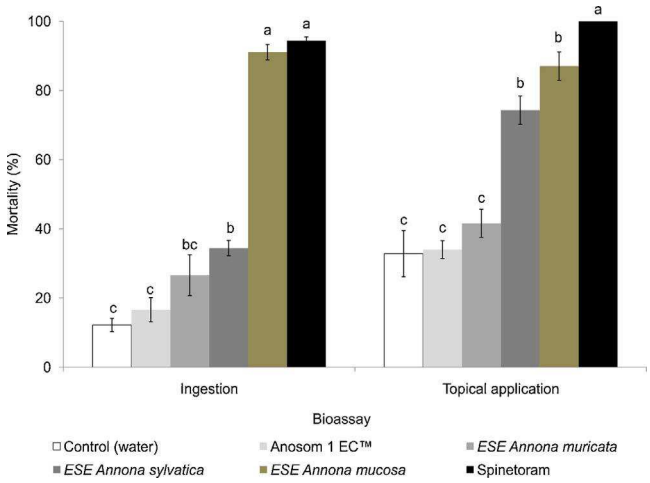

Fig. 1. Mortality (%) (± SE) of *Drosophila suzukii* at 120 h after exposure to treatments in laboratory bioassays. Means followed by different letters on the columns (within each exposition bioassay) indicate significant differences between treatments (GLM with quasi-binomial distribution followed by *post hoc* Tukey test, $P < 0.05$); ESE = Formulated ethanolic seed extract.

Table 2
Insecticidal activity of *Annona mucosa* (Annonaceae) formulated seed extract and spinosyn-based insecticide on *D. suzukii* adults in ingestion bioassay.

Exposure time (hours)	Treatments	*n*	*Slope* ± SE	LC_{50} (CI 95%)[a]	LC_{90} (CI 95%)[b]	χ^{2}[c]	d.f.[d]
12	ESE *Annona mucosa*	80	2.79 ± 0.60	1,666.13 (1,010.11–2,400.08)	4,785.12 (3,068.11–11,984.10)	10.15	4
	Spinetoram	80	3.99 ± 1.25	67.51 (25.55–86.00)	141.25 (103.96–487.11)	15.27	5
24	ESE *Annona mucosa*	80	3.32 ± 1.04	984.88 (726.13–1,816.10)	3,394.10 (1,134.16–7,136.13)	7.86	4
	Spinetoram	80	2.43 ± 0.29	39.89 (32.40–46.79)	134.02 (109.48–179.81)	8.39	5
48	ESE *Annona mucosa*	80	1.60 ± 0.34	584.11 (220.93–1,305.11)	2,691.00 (1,538.07–5,195.70)	9.17	4
	Spinetoram	80	1.85 ± 0.48	29.09 (5.09–50.81)	133.19 (75.55–308.11)	12.26	4
72	ESE *Annona mucosa*	80	1.83 ± 0.49	569.75 (411.56–1,195.79)	2,837.05 (1,239.08–5,674.10)	12.15	4
	Spinetoram	80	1.39 ± 0.21	22.00 (15.88–29.42)	132.51 (109.64–439.83)	1.54	4
96	ESE *Annona mucosa*	80	2.13 ± 0.71	571.46 (410.56–1231.18)	2,214.19 (1,611.14–4,684.90)	10.80	4
	Spinetoram	80	1.50 ± 0.21	18.03 (13.00–23.59)	127.75 (83.33–258.01)	5.55	4
120	ESE *Annona mucosa*	80	2.13 ± 0.54	500.43 (367.06–914.89)	1,995.04 (1,067.03–4,452.3)	8.38	4
	Spinetoram	80	1.51 ± 0.37	15.08 (1.61–34.29)	105.34 (42.79–355.14)	9.47	4

[a] LC_{50} and LC_{90}: Concentrations (mg L^{-1}) required to kill 50 or 90% of the adults of *D. suzukii*, respectively, CI: confidence interval at 95%.
[b] LC_{50} and LC_{90}: Concentrations (mg L^{-1}) required to kill 50 or 90% of the adults of *D. suzukii*, respectively, CI: confidence interval at 95% "a" and "b" have the same sentence. I suggest to include the same letter ("a") in the table and remove the second sentence.
[c] χ^{2}: Pearson's chi-square value.
[d] df: degrees of freedom; ESE = Formulated ethanolic seed extract.

and *A. muricata* (< 35%; Fig. 1). By contrast, using the contact bioassay on *D. suzukii*, the formulated extracts from both *A. mucosa* and *A. sylvatica* caused higher mortality (> 74%) ($F_{5,12} = 54.66$, $P < 0.0001$) than the commercial acetogenin-based bioinsecticide and the formulated extract from *A. muricata* (< 42%; Fig. 1). However, both of formulated extracts were less toxic than the spinetoram insecticide (100% mortality; Fig. 1).

Based on the probit analysis from the ingestion bioassay, the toxicity of a formulated *A. mucosa* seed-extract was dependent on the evaluation time, with the LC_{50} differing significantly between 12 and 120 h (Table 2). For the contact bioassays, the LC_{50} at 120 h significantly differed from both the LC_{50} at 12 and 24 h (Table 3). The pattern was similar for the insecticide spinetoram in the ingestion bioassay (Table 2). However, in the contact bioassay the LC_{50} values for spinetoram decreased significantly only among the first 48 h (Table 3).

The dried residues of all the products (*Annona* formulated extracts, acetogenin-based bioinsecticide and spinetoram) deterred oviposition of *D. suzukii* on the strawberry fruits (from 12.86 to 17.06 eggs per fruit) compared with the control (23.14 eggs per fruit; $F_{5,29} = 27.55$, $P < 0.0001$; Fig. 2). An approximate 45% reduction in oviposition was recorded in fruits previously treated with formulation based on *A. mucosa* extract, whereas the reduction was only approximate 26% when

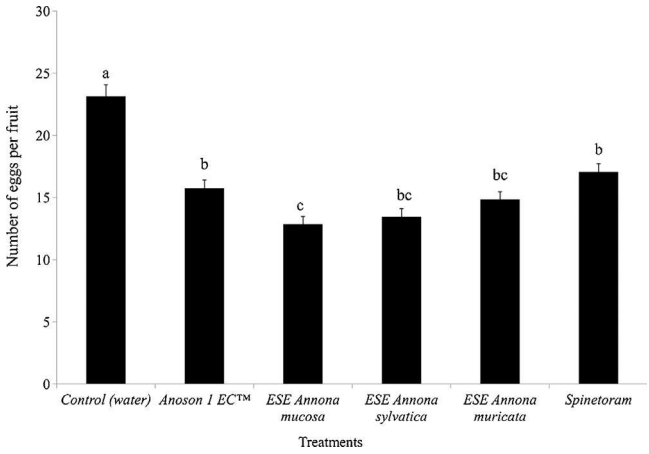

Fig. 2. Effect of a formulated ethanolic seed-extract from *Annona* species and the spinosyn-based synthetic insecticide on the oviposition of *Drosophila suzukii* females in strawberry fruits. Bars (± SE) with the same letter are not significantly different (GLM with a quasi-binomial distribution followed by Tukey *post hoc* test: $P < 0.05$). ESE = Formulated ethanolic seed extract.

Table 3
Insecticidal activity of *A. mucosa* (Annonaceae) formulated seed extract and spinosyn-based insecticide on *D. suzukii* adults in contact bioassay.

Exposure time (hours)	Treatments	*n*	*Slope* ± SE	LC_{50} (CI 95%)[a]	LC_{90} (CI 95%)[b]	χ^{2}[c]	d.f.[d]
12	ESE *Annona mucosa*	761	1.51 ± 0.16	2,623.02 (2,160.02–3,169.01)	–	4.02	5
	Spinetoram	1181	2.42 ± 0.33	69.19 (58.07–83.05)	233.16 (166.24–436.74)	14.52	8
24	ESE *Annona mucosa*	761	1.56 ± 0.16	1,826.01 (1,485.00–2,192.08)	–	6.17	5
	Spinetoram	1181	2.96 ± 0.25	41.10 (36.52–45.32)	111.33 (98.51–130.42)	10.61	8
48	ESE *Annona mucosa*	761	1.52 ± 0.21	1,176.03 (796.80–1,521.03)	8,129.00 (6,008.03–13,144.10)	8.06	5
	Spinetoram	1181	3.81 ± 0.32	29.41 (25.79–32.61)	63.80 (58.54–70.65)	10.12	8
72	ESE *Annona mucosa*	761	1.86 ± 0.50	1,336.11 (891.61–2,199.25)	6,483.19 (3,961.73–10,935.24)	12.51	5
	Spinetoram	1181	4.03 ± 0.42	26.28 (22.05–29.81)	54.57 (49.84–60.67)	10.75	8
96	ESE *Annona mucosa*	761	1.81 ± 0.61	1,083.27 (926.09–1,977.13)	5,512.36 (3,365.17–6,648.25)	11.93	5
	Spinetoram	971	3.94 ± 0.52	25.03 (20.04–28.91)	52.92 (47.40–61.13)	7.52	6
120	ESE *Annona mucosa*	761	1.15 ± 0.27	440.71 (270.47–1,045.13)	5,013.12 (2,763.84–6,398.25)	12.29	5
	Spinetoram	971	4.06 ± 1.14	25.96 (17.27–34.74)	53.62 (42.29–94.73)	14.59	6

[a] LC_{50} and LC_{90}: Concentrations (mg L^{-1}) required to kill 50 or 90% of the adults of *D. suzukii*, respectively, CI: confidence interval at 95%.
[b] LC_{50} and LC_{90}: Concentrations (mg L^{-1}) required to kill 50 or 90% of the adults of *D. suzukii*, respectively, CI: confidence interval at 95%. Idem Table 2.
[c] χ^{2}: Pearson's chi-square value.
[d] df: degrees of freedom; ESE = Formulated ethanolic seed extract.

Table 4
Mortality and parasitism (± SE) of *Trichopria anastrephae* at 120 h after exposure to selected treatments in a laboratory ingestion and contact bioassays.

Treatments	Concentration (mg L^{-1})[a]	Mortality (%)[b]		Parasitism (%)[b]
		Ingestion bioassay	Contact bioassay	
ESE *Annona mucosa*	1995.04	31.5 ± 1.50b	70.9 ± 5.94a	55.5 ± 2.21a
Spinetoram	105.34	60.0 ± 4.26a	64.9 ± 6.96a	51.9 ± 5.51a
Control (Water)	–	6.5 ± 2.18c	23.1 ± 4.70b	55.5 ± 0.43a

[a] Concentration estimated based on LC_{90} values in ingestion bioassay on *Drosophila suzukii*.
[b] Means within a column followed by the same letter do not differ significantly (GLM with a quasi-binomial distribution followed by Tukey *post hoc* test: $P < 0.05$). ESE = Formulated ethanolic seed extract.

previously treated with the spinetoram insecticide ($F_{5,29} = 27.55$, $P < 0.0001$; Fig. 2).

The contact exposure at the LC_{90} of both the formulated *A. mucosa* seed-extract and spinetoram resulted in similar mortality of the parasitoid *T. anastrephae*, with the mortality of both higher than that of the negative control (water) ($F_{2,12} = 17.02$, $P < 0.0003$; Table 4). However, in the ingestion bioassay, the mortality caused by the formulated *A. mucosa* seed-extract was significantly lower than that of the spinetoram insecticide ($F_{2,12} = 65.59$, $P < 0.0001$), although still higher than that of the control (Table 4). Finally, the parasitism rate of *D. suzukii* pupae by *T. anastrephae* was not significantly affected by either treatment ($F_{2,11} = 0.65$, $P < 0.5403$) compared with the control (Table 4).

4. Discussion

In the present study, the toxicities of selected Annonaceae seed extracts on *D. suzukii* and its parasitoid *T. anastrephae* were assessed. The bioactivity of the formulated *A. mucosa* extract was equivalent to that of a commercial insecticide containing spinetoram, and for the first time, the action of Annonaceae derivatives against *D. suzukii* was demonstrated. Several studies demonstrate the toxicity of *A. mucosa* extracts to other pest species, including *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) (Ribeiro et al., 2013), *Panonychus citri* (McGregor) (Prostigmata: Tetranychidae) (Ribeiro et al., 2014b), *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) and *Myzus persicae* (Sulzer) (Aphidomorpha: Aphididae) (Ribeiro et al., 2014c), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (Ansante et al., 2015) and *Helicoverpa armigera* (Souza et al., 2017). Subsequently, bio-monitored fractionations indicated that the acute and chronic toxicity of this botanical derivative is due to the synergistic interaction of structurally diverse acetogenins, with the acetogenin rolliniastatin-1 being the main active ingredient (Ansante et al., 2015; Souza et al., 2017).

Although some synthetic insecticides, including organophosphates, pyrethroids and spinosyns, are highly toxic to *D. suzukii* (Bruck et al., 2011; Haviland and Beers, 2012; Van Timmeren and Isaacs, 2013; Andreazza et al., 2017a), the use of such products can leave chemical residues on fruits. The concern about these residues increases particularly during the pre-harvest and fruit-ripening periods, which are the periods most preferred for infestation by *D. suzukii* (Bruck et al., 2011; Van Timmeren and Isaacs, 2013; Abraham et al., 2015; Lee et al., 2015; Bernardi et al., 2017). Thus, given the ease of production and rapid degradation and low residual effect (Ribeiro et al., 2016), formulations based on *A. mucosa* seed-extracts can be an additional tool for use in the management of *D. suzukii*.

The acetogenins are the likely primary active ingredient responsible for arthropod pest mortality in the tested ethanolic extracts (Ribeiro et al., 2013). These molecules are inhibitors of the complex I (NADH:ubiquinone oxidoreductase) in the mitochondrial electron transport system, with inhibition inducing cellular death (Tormo et al., 1999). Because most insecticides used in the control of *D. suzukii* act on acetylcholine receptors or on sodium channels (Casida and Durkin, 2013), the search for products with a different mode of action (e.g., acetogenins) might assist in the management of resistant populations (Audsley and Down, 2015). Currently, populations of *D. suzukii* resistant to insecticides have not been reported, however, with high polyphagy (Lee et al., 2015; Poyet et al., 2015), a short generation time (Asplen et al., 2015), and rapid dispersion (Haye et al., 2016) associated with the continued use of insecticides, the selection pressure is high for the appearance of resistant populations (Wilson, 2001; Haye et al., 2016).

In addition to the similar lethal toxicity of the formulated *A. mucosa* seed-extract and a spinosyn-based commercial insecticide on *D. suzukii* adults, all tested formulations deterred oviposition by *D. suzukii* females. The level of oviposition deterrence observed in this study (i.e., 45%), if alone, would probably not fully contribute to this species management at field, especially at zero-tolerance regions (Van Timmeren and Isaacs, 2013). Nonetheless, its combining effect with the adult mortality could be an important part of an IPM program, where the deterrence of oviposition is beneficial because of the negative effect on the population dynamics in future generations (Kogan, 1998). Furthermore, products that decrease oviposition in *D. suzukii* reduce the incidence of epidermis rupture by oviposition, which consequently reduces the infestation of pathogens that increase the processes of fruit decay (Mitsui et al., 2006; Walsh et al., 2011; Calabria et al., 2012).

The formulation of *A. mucosa* seed extract through dietary exposure caused low mortality to the pupal parasitoid *T. anastrephae*, which highlights the potential use of this extract in toxic baits. Although the mortality caused by the contact exposure could be considered relatively high, the mortality of the parasitoid was lower than that of *D. suzukii*, and after ingestion exposure, no sublethal effects on the rate of parasitism by surviving wasps were detected. Thus, the formulated ethanolic seed-extract of *A. mucosa* is a promising tool that is compatible with future biological control programs of *D. suzukii* (Biondi et al., 2012), particularly because *T. anastrephae* were recently found parasitizing pupae of this pest in strawberry and blackberry fields in Brazil (Wollmann et al., 2016; Andreazza et al., 2017b). Furthermore, the compatibility of *A. mucosa* derivatives with isolates of the entomopathogenic fungi *Beauveria bassiana* (Bals.-Criv.) Vuill and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Ribeiro et al., 2014a), which are also considered promising control agents of *D. suzukii* (Cuthbertson et al., 2014; Gargani et al., 2014), is a positive aspect to include their use in *D. suzukii* management programs.

In this study, the formulation with the ethanolic seed-extract of *A. mucosa* had promising bioactivity against *D. suzukii*, with toxic effects equivalent to a spinosyn-based commercial insecticide, in addition to a less toxic effect on its pupal parasitoid *T. anastrephae*. Thus, this formulated extract can be a useful and a promising component for the management of *D. suzukii*, particularly in organic or low residue production systems. This study highlights the importance of future studies to investigate the cropping of *A. mucosa* and efficient derivative extraction and processing.

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