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Lethal and sublethal toxicities of acetogenin-based bioinsecticides on *Ceratitis capitata* and the parasitoid *Diachasmimorpha longicaudata*

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Abstract In this study, was evaluated the action lethal and sublethal of three formulations of Annona species extracts (*A. mucosa*, *A. muricata* and *A. sylvatica*) on adults of *Ceratitis capitata* (Wiedemann, 1852) (Diptera: Tephritidae) and yours parasitoid *Diachasmimorpha longicaudata* (Ashmead, 1905) (Hymenoptera: Braconidae). In addition, the efficacy of the selected toxic bait formulations was evaluated by mixing them with different food attractants (Anamed™, 3% Biofruit and 7% sugarcane molasses). From the use of the discriminatory concentration (2000 mg L-1), only *A. mucosa* caused mortality of *C. capitata* of more than 85% in ingestion and topical application bioassays, equivalent a spinosyn-based insecticide (control positive). In toxic bait formulation with the tested food attractants, the extracts had a residual effect (mortality ≥80% *C. capitata*) up to 14 days after applying treatments (DAAT) in the absence of rain. Based on the LC90 (1984.20 mg L^{-1}) values estimated from the concentration-response curves for *C. capitata*, *A. mucosa* caused less than 40% adult mortality of the parasitoid *D. longicaudata* in the ingestion bioassay. However, both treatments showed no sublethal effects (parasitism reduction) over time. In addition, in bioassays with and without choice, there was a significant reduction in the number of punctures and galls caused by females and larvae of *C. capitata*, respectively, in grape berries in the presence of dry residues of all evaluated treatments. The *A. mucosa* seed extract was considered the most promising product for use in *C. capitata* management programs, particularly in organic-based systems, due to its selectivity to *D. longicaudata*.

Keywords Annonaceae · Botanical insecticides · Parasitoid larvae · Mediterranean fruit fly · Integrated pest management

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Introduction

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann, 1852) (Diptera: Tephritidae), is one of the main phytosanitary problems of fruit growing in various parts of the world (Morelli et al. 2012). Among their main features, the development potential of Mediterranean fruit flies of approximately 360 hosts from 63 distinct families worldwide (Urbaneja et al. 2009; McQuate and Liquido 2017), while Brazil is associated with 100 hosts (Zucchi and Moraes 2012). The damage is caused by females, who pierce the fruits when performing oviposition, and by the larvae, that consume

the pulp, induce early ripening and consequently lead to premature fruit drop (Nava and Botton 2010).

Due to the economic damage caused by this pest species to Brazilian fruit crops, synthetic insecticides (i.e. pyrethroids, spinosyn and phosphorus) have been widely used for their management, either through full area applications or even as a lethal agent in the formulation of toxic baits (Navarro-Llopis et al. 2013; Botton et al. 2016; Baronio et al. 2018, 2019a, b). Despite the effectiveness of these management strategies in population suppression, some of these insecticides are either not allowed for use in integrated fruit production (IFP) or have recently been withdrawn from the market (Botton et al. 2016). This was due to the possibility of residues remaining in the fruits and the need for a longer postharvest interval of orchards treated with such insecticides (Chueca et al. 2007). In addition, adverse effects of these broad-spectrum insecticides on natural enemies have often been noted (Urbaneja et al. 2009; Harbi et al. 2017; Bernardi et al. 2019). Given this, one of the greatest demands of integrated pest management (IPM) programs in tropical and subtropical fruit orchards is the development of new products and/or effective strategies for the management of *C. capitata*, especially more environmentally appropriate and sustainable tools compatible with the beneficial entomofauna present in these agroecosystems (Navarro-Llopis et al. 2011).

In this context, botanical insecticides have stood out, as they fit the IPM precepts and certifying norms of organic production (Navarro-Llopis et al. 2011; Zanardi et al. 2015; Pretty et al. 2018; Amoabeng et al. 2019). The botanical families Annonaceae, Asteraceae, Canellaceae, Lamiaceae, Malvaceae, Meliaceae, and Rutaceae stand out as the main sources of bioactive compounds for Mediterranean fruit fly mitigation (Ribeiro et al. 2013; Moghadamtousi et al. 2015; Isman 2017). In the Neotropical region, the Annonaceae family is considered to be one of the most promising sources of bioactive compounds to different pests of agricultural importance (Ribeiro et al. 2013), especially from the seeds of the acetogenin-rich *Anonna* species (Isman and Grieneisen 2014; Ribeiro et al. 2016). Acetogenins cause pronounced lethal effects on different pests and sublethal effects on arthropods, especially on the eating behavior and oviposition of the target insects (Ribeiro et al. 2015; Bernardi et al. 2017; Geisler et al. 2019).

Botanical insecticides can affect beneficial entomofauna (Ndakidemi et al. 2016; Monsreal-Ceballos et al. 2018) or even have toxicological effects on nontarget organisms. Thus, the selectivity of these derivatives over the biological control agents present in agroecosystems should be carefully evaluated before their recommendation and implementation in pest management programs (Desneux et al. 2007; Biondi et al. 2013).

The present study aimed to i) evaluate the lethal toxicity of ethanolic extracts rich in acetogenins (precommercial) to adults of *C. capitata* in topical and ingestion bioassays; ii) evaluate the effect of oviposition deterrence on grape berries; and iii) determine the adult selectivity of *Diachasmimorpha longicaudata* (Ashmead, 1905) (Hymenoptera: Braconidae), the largest fruit fly larval parasitoid worldwide (Nunes et al. 2013; Meirelles et al. 2016). For comparison purposes, a spinosyn-based formulation (spinetoram) was used as a positive control for lethal toxicity comparison.

Materials and methods

Botanical extracts: sources, method of preparation and formulation procedures

Information about *Annona* species used in this study is detailed in Table 1. *Voucher specimens*, previously identified by Prof. Dr. Renato Mello-Silva [Departamento de Botânica, Instituto de Biociências/Universidade de São Paulo (IB / USP)], were deposited in the herbarium of the Departamento de Ciências Biológicas da Escola de Agricultura "Luiz de Queiroz"/Universidade de São Paulo, in Piracicaba, SP, under the registered numbers 120,985 (*A. mucosa*), 121,205 (*A. sylvatica*), and 121,892 (*A muricata*).

For the preparation of crude ethanolic extracts, the seeds collected from ripe fruits were dried in an oven with forced air circulation at 38 °C for 48 h and ground in a knife mill until a fine powder was obtained. The powders were placed in glass containers, sealed and stored at approximately −10 °C until use.

Organic extracts were obtained by cold maceration using ethanol grade analysis as solvent (5:1, $v.v^{-1}$). For this, the seed powder of the respective species was added in above-mentioned solvent (separately), stirred for 10 min, and kept at rest for three days. After this period, the solution was filtered and the remaining cake subjected again to the same solvent and ratio, repeating this process three times. The remaining solvent in the sample was removed by rotary evaporator at 50 °C and

600 $mmHg^{-1}$. After complete evaporation of the solvent in an airflow chamber, the extraction yield for each species was determined. For the preparation of the formulations (aqueous emulsions), the ethanolic extracts of the seeds were solubilized in acetone: methanol (1: 1, $v.v^{-1}$) at 100 g L^{-1}, with the addition of the emulsifier Tween® 80 at a concentration of 10 g. L^{-1}.

Insects

The insects used from the bioassays were obtained from a population of *C. capitata* were from mango fruits (*Mangifera indica* L.) collected in the municipality of Pelotas, Rio Grande do Sul, Brazil (31°38′20″ S and 52°30′43″ W) and maintained in the laboratory (~ 20 generations) under controlled conditions (Temp.: 25 ± 1 °C, R.H.: 70 ± 5% and 12 h photophase). *C. capitata* was reared in an artificial diet proposed by Nunes et al. (2013). The individuals of *D. longicaudata* were obtained from field collections of *C. capitata* larvae in the municipality of Pelotas, RS, Brazil and maintained in the laboratory for approximately 30 generations under controlled conditions (Temp.: 25 ± 1 °C, R.H.: 70 ± 5% and 12 h photophase). In order to maintain the *D. longicaudata* population, we used the procedure proposed by Meirelles et al. (2013).

Bioassays

All bioassays were performed under controlled conditions (Temp.: 25 ± 1 °C, R.H.: 70 ± 5% and a 12 h photophase) in a completely randomized design. The treatments and discriminatory concentrations used are detailed in Table 1. A spinetoram-based formulation (Delegate ™ 250 WG, 250 g active ingredient (a.i). kg^{-1}) (Corteva Agriscience, São Paulo, SP, Brazil) was used as a positive control for lethal toxicity comparison (Table 1). As a negative control, the solvents used in the solubilization of the respective treatments were used.

Initial screening: lethal toxicity to adult C. capitata via ingestion and topical application

Ingestion bioassay The insecticides (Table 1) were mixed in different food attractants: a) Anamed™ [40% SPLAT ™ + 24.2% food containing fruit extracts and phytostimulants (Isca Tecnologias Ltd. Ijuí, RS, Brazil)]; b) 3% Biofruit at 3% [hydrolyzed protein (BioControle Métodos de Controle de Pragas Ltd. Indaiatuba, São Paulo, Brazil)] and c) 7% sugar cane molasses. Therefore, the insects were separated into groups (sampling units) of 20 couples (eight days of age) and placed in cages made of transparent plastic cups (1 l) facing downward in plastic Petri dishes (25 cm diameter) and sealed on top (bottom of cup) with a voile mesh for ventilation. After preparation of the toxic bait dilutions (insecticide + food attractant), the products were offered to the insects by capillary on hydrophilic cotton rolls in 10 mL glass bottles. After 24 h, the toxic baits were removed, and the adults were fed an artificial diet and distilled water (Nunes et al. 2013). Mortality was assessed daily for 5 days. Insects were considered dead when no movement occurred after touching with a thin brush. For each treatment, 4 replications were used, each composed of 20 adults ($n = 80$).

Topical bioassay Groups of 10 *C. capitata* couples (8 days old) were separated and placed in clear glass

Table 1 Treatments evaluated for the management of *Ceratitis capitata*

Treatments	Description	Discriminatory concentration tested[a]	Origin/manufacturer
EES Annona mucosa	Aqueous emulsion of ethanolic seed-extract of *Annona mucosa* Jacq. (pre-commercial)	2000	Laboratory extraction and formulation
EES Annona muricata	Aqueous emulsion of ethanolic seed-extract of *Annona muricata* L. (pre-commercial)	2000	Laboratory extraction and formulation
EES 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]	Corteva AgriScience™, São Paulo, SP, Brazil

[a] Concentration: mg^{-1} of extract or commercial product per L^{-1} of water

[b] 75 mg of a.i. per L^{-1} of water

EES = Formulated ethanolic seed extract

tubes (2.5 cm diameter × 8 cm long) sealed with cotton plugs. The insects were sedated in a freezer (~ −10 °C) for 30 s and placed on a glass petri dish (9 cm in diameter) lined with filter paper. Subsequently, the insects were sprayed using a Potter tower (Burkard Scientific, Uxbridge, Reino Unido), applying 1 mL of solution per sample unit at a working pressure of 7 lb. in^{-2}, resulting in an average residue deposition of 3 $mg.cm^{-2}$. Once this was done, the insects were placed in transparent plastic cages (1000 mL) sealed with a vent lid (a voile mesh sealed opening in the cup lid) as described above. Adults were fed an artificial diet and distilled water in the same way as mentioned above until the end of the evaluation period. Again, mortality was assessed daily for 5 days. For each treatment, 10 replications were used with 10 adults per repetition ($n = 100$). For both bioassays, mortality was corrected according to Abbott (1925).

Concentration-response curves

Based on the initial screening, the most promising treatments were selected and subjected to a new bioassay to estimate the concentration needed to kill 50% and 90% of the exposed flies (LC_{50} and LC_{90}, respectively). For this, seven concentrations were tested (range: 125–4000 mg. L^{-1} for the formulated *A. mucosa* seed extract and 10–100 mg. L^{-1} for spinetoram (positive control)). Exposure and application modes, as well as mortality criteria and exposure times, were the same as those used for initial screening (ingestion bioassay and topical application). In the intake bioassays, four repetitions were used, with each repetition composed of 20 adults ($n = 80$). In the topical bioassays, 10 replications were performed with 10 adults each ($n = 100$).

Evaluation of oviposition deterrence

To evaluate the effect of the treatments (Table 1) on oviposition behavior, *C. capitata* adults approximately 12 days old were submitted to bioassays with and without choice. As oviposition substrate, ripe grape berries cv. Italy (intact and free from insecticide contamination).

For the bioassay with no choice, grape berries were dipped in syrup (Table 1) diluted in water for 5 s. Subsequently, they were placed on a filter paper for 3 h to remove the excess slurry. After this time, grape berries were placed inside cages made of transparent plastic cups (1000 mL) inverted over a Petri dish (8 cm diameter) (one per cage). Each experimental unit was then infested with two 13-day-old *C. capitata* couples. The insects were fed artificial diet + distilled water (Nunes et al. 2013).

The bioassay with choice was conducted in plastic cages under the same conditions as the bioassay with no choice. However, two intact grape berries were internally placed in each cage (one treated berry (immersed in the slurry of the treatments as described above) and one untreated berry (one without contact with the treatments)). Subsequently, each cage was infested with two *C. capitata* couples.

In the both bioassays, after 24 h of exposure the adults were removed and the grape berries were individually placed in plastic cups (50 mL) on a vermiculite layer (1 cm), sealed on the top with parafilm.™. They were then placed in a climate-controlled room (Temp.:25 ± 1 °C, U.R. 70 ± 5% and 12 h photophase). After 5 days, the number of punctures per berry was counted, that is, oviposition marks caused by *C. capitata* female and presence of galleries by larval development. The number of punctures and galleries was counted using a stereoscopic microscope (40 times). In all bioassays, 50 berries / treatment ($n = 50$) were used.

Toxicity of C. capitata adult toxic bait formulation

To evaluate the residual effect of toxic baits in absence of rainfall on medfly kill, 2-year-old citrus *Citrus sinensis* L. (Rutaceae) seedlings (1.5 m height) were grown in pots inside a greenhouse (temp.: 25 ± 2 °C, R.H.: 70 ± 10% and a 12 h photophase). Toxic baits were applied on the leaves, which were collected after drying, on the day of the application (two hours after application) and at 7, 14, 21 and 30 days after application of the treatments (DAAT); transported to the laboratory and supplied to five males and five females of *C. capitata* adults 5 to 8 days old, food deprived for 12 h, in each cage. Plastic containers with cotton wool soaked in distilled water were offered inside 5 mL plastic containers during the evaluation period. The cage top was lined with a white "voile" type fabric to facilitate the viewing and counting of dead insects. The number of dead individuals in each treatment was evaluated at 120 h after the exposure to treatments (HAET). The mortality was evaluated by counting the number of insects that did not show any reaction to the touch of a fine-tipped brush. All the experiments were

conducted in a completely randomized design with 10 replicates with 10 fruit flies adults. The efficacy of each treatment was calculated using the formula of Abbott (1925).

Toxicities and sublethal effects on D. longicaudata (ingestion bioassay)

To evaluate the selectivity of treatments on the *C. capitata* parasitoid *D. longicaudata*, the same concentrations of the previous bioassays were used. Three-day-old insects from the maintenance rearing were starved for a period of 12 h. At the end of this time, 10 couples were placed inside a cage consisting of a plastic container (500 mL) inverted on an acrylic plate (12 cm in diameter), with the top cut out and covered with fine mesh net to allow for ventilation. Subsequently, one drop (10 mm) of each treatment (Table 1) was placed with a micropipette (100 μL) on a plastic plate (2.5 cm in diameter) made of plastic paraffin film and Parafilm™ paper (Bemis Company, Inc. USA). The treatments were available to the insects for feeding for a period of 24 h. At 120 h after exposure, the numbers of alive and dead insects were recorded. Insects that showed no reaction to being touched with a fine-tipped brush were considered dead.

To evaluate the lethal and sublethal effects of the treatments on the wasps, the wasps alive at 120 h in the ingestion bioassay were used. Daily, from the seventh day after emergence, third instar larvae of *C. capitata* (30 larvae per female) were offered for 7 consecutive days. During the evaluation period, the wasps were fed honey/water 80% (*w*/*v*). After 1 h of daily parasitism, the larvae were removed and stored in plastic containers (100 mL) containing a layer of fine vermiculite (1 cm) until adult emergence. After the emergence of the first insect (*C. capitata* or *D. longicaudata*), the puparia were evaluated daily. At the end of the bioassay, pupae that remained intact were dissected to assess the presence of nonemergent flies or parasitoids to determine the true parasitism rate. The reductions in the parasitism capacity (%) for each treatment were determined by comparison with the negative control and calculated using the following formula: RP = [(1 - T/C) * 100], where T is the mean parasitism or mean emergence in the treatment and C is the mean parasitism or emerged insects observed in the negative control (water + honey).

Statistical analysis

For the analysis of the studied variables, generalized linear models (GLM) belonging to the exponential family of distributions were used (Nelder and Wedderburn 1972). The quality of the fit was verified by means of the half-normal probability envelope (Hinde and Demétrio 1998). When significant differences between treatments were detected, multiple comparisons (Tukey post hoc test, $p < 0.05$) were performed using the Multicht package glht function, with adjustment of p values. For comparisons of the mean treatments in the oviposition deterrence bioassay (with choice), Student's t test was used. All analyzes were performed with "R" statistical software (R.D.C.T. 2012). A binomial model with a complementary log-log binding function (gompit model) was used to estimate lethal concentrations (LC50 and LC90) using the Probit package using SAS version 9.2 statistical software (SAS Institute 2011).

Results

Lethal toxicity and concentration-response curve for *C. capitata*

After 120 h of application via topical exposure, the aqueous emulsion of the ethanolic extract of *A. mucosa* seeds was equitoxic ($F = 55.922$, d.f. = 5, 24; $p < 0.0001$) with the synthetic spinosyn-based insecticide (spinetoram) used as a positive control (Fig. 1), causing adult *C. capitata* mortality levels of above 80%. In contrast, aqueous emulsions from the ethanolic seed extracts of *A. muricata* and *A. sylvatica* provided mortalities of less than 65% (Fig. 1), with activity levels below that of the positive control (spinetoram). For this mode of contamination, median lethal concentration (LC_{50}) values of 640.45 and 39.44 mg L^{-1} were estimated for the aqueous emulsion of the ethanol extract from *A. mucosa* seeds and for the spinetoram, respectively (Table 3).

By ingestion (120 h of exposure), an aqueous emulsion of an *A. mucosa* seed extract in combination with Anamed ™ food attractants and sugarcane molasses caused the mortality level of *C. capitata* adults of 70%, being significantly superior ($F = 47.50$, d.f. = 5, 24; $p < 0.0001$) to the other botanical derivatives tested but inferior to the positive control constituted by the spinetoram insecticide (Table 2). However, when mixed with the 3% Biofruit, all botanical derivatives provided

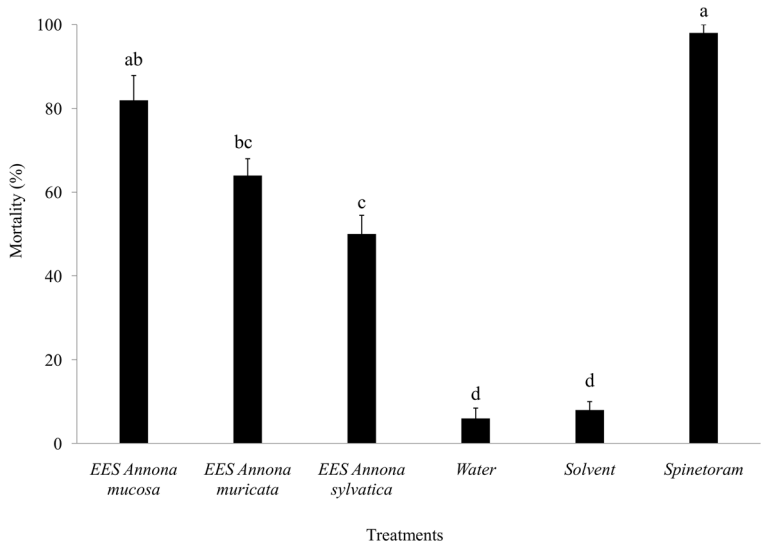

Fig. 1 Mortality (%) (± SE) of *Ceratitis capitata* at 120 h after exposure to treatments in laboratory bioassays by means of topical application, Means followed by different letters on the columns indicate significant differences between treatments (GLM with quasi-binomial distribution followed by post hoc Tukey test, $p < 0.05$); EES = Formulated ethanolic seed extract

mortality below 40%, indicating an antagonistic interaction with this food attractant (Table 2).

By ingestion, using the Anamed™ food attractant for emulsion solubilization, LC_{50} values of 927.33 and 40.23 mg L^{-1} were estimated for the aqueous emulsion of the ethanol extract from *A. mucosa* seeds and for spinetoram, respectively (Table 3).

Toxicity and residual effect of toxic bait formulations on *C. capitata* adults

For the toxic bait formulations, an aqueous emulsion of an *A. mucosa* seed extract in combination with Anamed ™, 3% Biofruit and 7% sugarcane molasses provided mortality levels ranging from 40 to 100% of the *C. capitata* adults at 0, 7, 14, 21 and 30 days after application (Fig. 2a, b, and c). In addition, they had a residual effect up to 14 DAAT (mortality ≥80%). For toxic baits formulated with the food attractants + spinetoram, the mortality levels ranged from 80 to 10% on the same assessment dates (Fig. 2a, b, and c). In addition, for the spinetoram-based baits, the residual effect was up to 7 DAAT with mortalities of less than 80% during this period.

Table 2 Corrected mortality percentage (± SE) of *Ceratitis capitata* after 120 h of exposure to Annonaceae-based and spininosam-based formulations (spinetoram) in admixture with different food and water attractants via ingestion

Tratamentos	Food attractives			Water (Control)
	Biofruit	Sugarcane	Anamed™	
EES *A. mucosa*	38.00 ± 5.83 b	70.00 ± 3.16 b	70.00 ± 4.47 b	82.00 ± 3.74 a
EES *A. muricata*	6.00 ± 2.45 c	8.00 ± 3.74 d	20.00 ± 3.16 c	26.00 ± 4.00 b
EES *A. sylvatica*	34.00 ± 4.00 b	34.00 ± 4.00 c	28.00 ± 3.74 c	24.00 ± 2.45 b
Spinetoram (controle positivo)	94.00 ± 4.00 a	90.00 ± 3.16 a	88.00 ± 4.90 a	90.00 ± 3.16 a
F	29.431	36.881	47.50	30.404
d.f.	3, 24	3, 24	3, 24	3, 24
p values	<0.0001	<0.0001	<0.0001	<0.0001

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: $p < 0.05$)

EES, Formulated ethanolic seed extract

Table 3 Estimate of the LC_{50} and LC_{90} (in mg L^{-1}) as well as the confidence interval of a formulated ethanolic extract from *Annona mucosa* seeds (Annonaceae) and a spinosyn-based synthetic insecticide (spinetoram) to *Ceratitis capitata* adults at 120 HAE in topical application and ingestion bioassays

Treatments	*n*	*Slope* ± SE	LC_{50} (CI 95%) [a]	LC_{90} (CI 95%)[b]	χ^{2c}	d.f.[d]
Topical application bioassay						
EES *A. mucosa*	640	2.80 ± 0.27	640.45 (470.47–945.13)	6215.20 (3763.84 – 8398.25)	6.70	5
Spinetoram	712	4.06 ± 1.14	39.44 (27.27–54.74)	81.54 (52.29–104.21)	9.72	5
Ingestion bioassay						
EES *A. mucosa*	640	2.80 ± 0.14	927.33 (670.47–1185.38)	1984.20 (1763.84 – 2298.30)	9.13	5
Spinetoram	712	3.06 ± 0.36	40.23 (27.14–48.58)	91.36 (62.15–120.36)	6.34	5

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

Lethal and sublethal effect on *D. longicaudata* parasitoid

The aqueous emulsions of the ethanolic extracts *A. mucosa* (27% mortality), *A. muricata* (36% mortality) and *A. sylvatica* (30% mortality) caused lower mortalities on adults of *D. longicaudata* when compared ($F = 3.14$; d.f. = 5, 594 $p < 0.001$) with that of the spinosyn insecticide (69% mortality) after 120 h of exposure (Table 4). However, only the aqueous emulsion of the *A. mucosa* seed extract did not differ statistically from the negative control (10% mortality) (Table 4), showing its selective character. In assessing the sublethal effect, it was found that all treatments tested did not cause parasitism reduction in *C. capitata* third instar larvae (PR <20%) ($F = 10.19$; d.f. = 4, 495; $p = 0.755$) (Table 4).

Oviposition deterrence

In the no-choice test, residues from all treatments significantly reduced ($F = 265.23$; d.f. = 5, 593; $p < 0.0001$) the number of punctures per berry caused by *C. capitata* females when compared to those in the negative control groups (water and solvents). (Fig. 3a). This trend was also observed in the choice bioassay (Fig. 3b). The reduction in the number of punctures had a significant impact ($F = 104.50$; d.f. = 5,93; $p < 0.0001$) on the number of galleries per grape berry (Table 5). The antifeedant level of the aqueous emulsion of the *A. mucosa* seed extract was comparable to that of the positive control constituted by the spinosyn-based insecticide, both in the number of punctures per berry (with and no-choice, Fig. 3) and in the average number of galleries (Table 5).

Discussion

This is the first study to report the lethal toxicity and oviposition inhibition of acetogenin-rich botanical derivatives for one of the world's leading fruit fly species (*C. capitata*). Furthermore, our results demonstrated the potential use of the aqueous emulsion of an *A. mucosa* seed extract in toxic bait formulations in a manner superior to a spinosyn-based formulation. In addition to its selective action for *D. longicaudata*, it is considered the main parasitoid of *C. capitata* larvae in Brazil (Nunes et al. 2013; Meirelles et al. 2016).

The efficacy of allelochemicals extracted from Annonaceae species of the genus *Annona* has already been demonstrated for different arthropod pests, such as *Panonychus citri* (McGregor, 1916) (Prostigmata: Tetranychidae) (Ribeiro et al. (2014a), *Trichoplusia ni* (Hübner, 1803) (Lepidoptera: Noctuidae) (Ribeiro et al. 2014b), *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) (Ansante et al. 2015), *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae) (Souza et al. 2019), *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) and *Zaprionus indianus* (Gupta, 1970) (Diptera: Drosophilidae) (Bernardi et al. 2017; Geisler et al. 2019). Thus, our study broadens the spectrum of action of these derivatives on pests of agricultural importance and the possibility of application in attracting-and-kill strategies.

The high lethal toxicity of the aqueous emulsion of the ethanolic extracts of *A. mucosa* seeds is related to the presence of structurally diversified acetogenins, with the major component being the bis-tetrahydrofuran acetogenin rollinistatin-1 (Ansante et al. 2015; Souza et al. 2017). Acetogenins comprise a series of natural

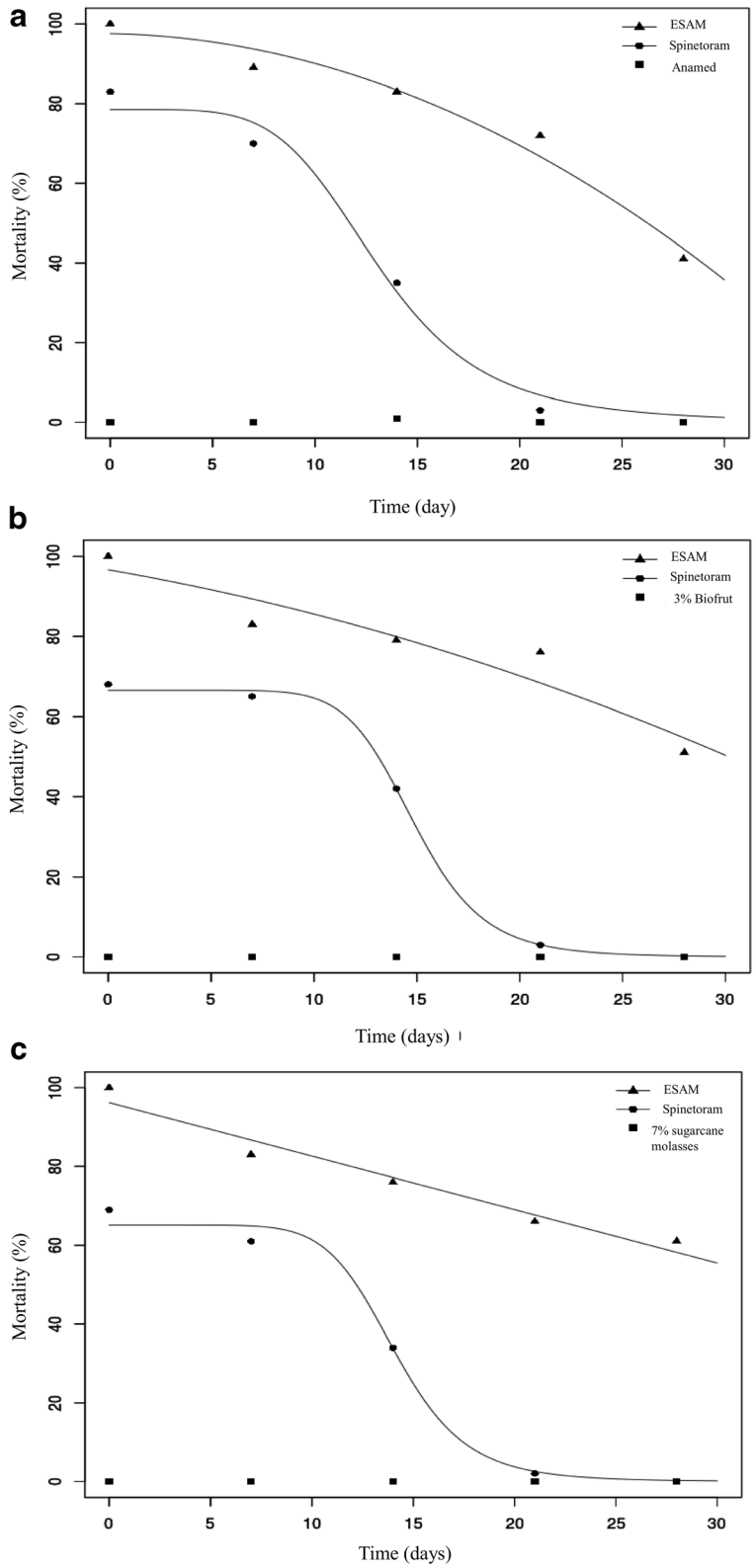

Fig. 2 Mortality (%) of *Ceratitis capitata* after 24 of exposure to toxic bait residues with different food attractives at 0 (after treatment), 7, 14, 21 and 30 days after application of treatments (DAAT), in absence of rain. **a** Anamed; **b** Biofruit and **c** Molasses of sugarcane

Table 4 Porcentage of mortality (± SE) and parasitism reduction (PR) of *Diachasmimorpha longicaudata* at 120 h after exposure to treatments in laboratory in ingestion bioassay

Treatments	Concentration (mg L^{-1})	Mortality (%)[a]	PR (%)[a]
EES *Annona mucosa*	2.000	27.0 ± 0.15 bc	10.85 ± 1.03 a
EES *Annona muricata*	2.000	36.0 ± 0.16 b	15.85 ± 0.66 a
EES *Annona sylvatica*	2.000	30.0 ± 0.70 b	9.27 ± 0.74 a
Spinetoram (positive control)	250	69.0 ± 1.00 a	10.13 ± 0.36 a
80% Honey-water + Tween 20% (negative control)	–	20.0 ± 0.55 c	13.85 ± 1.03 a
80% Honey-water (negative control)	–	10.0 ± 0.35 c	–
F		3.14	10.19
d.f		5, 594	4, 495
P values		< 0,0001	= 0,7550

[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: $p < 0.05$)

EES, Formulated ethanolic seed extract

(C-35/C-37) products derived from long chain (C-32/C-34) fatty acids combined with a 2-propanol moiety (Alali et al. 1999). Acetogenins are potent mitochondrial inhibitors that affect cellular energy production

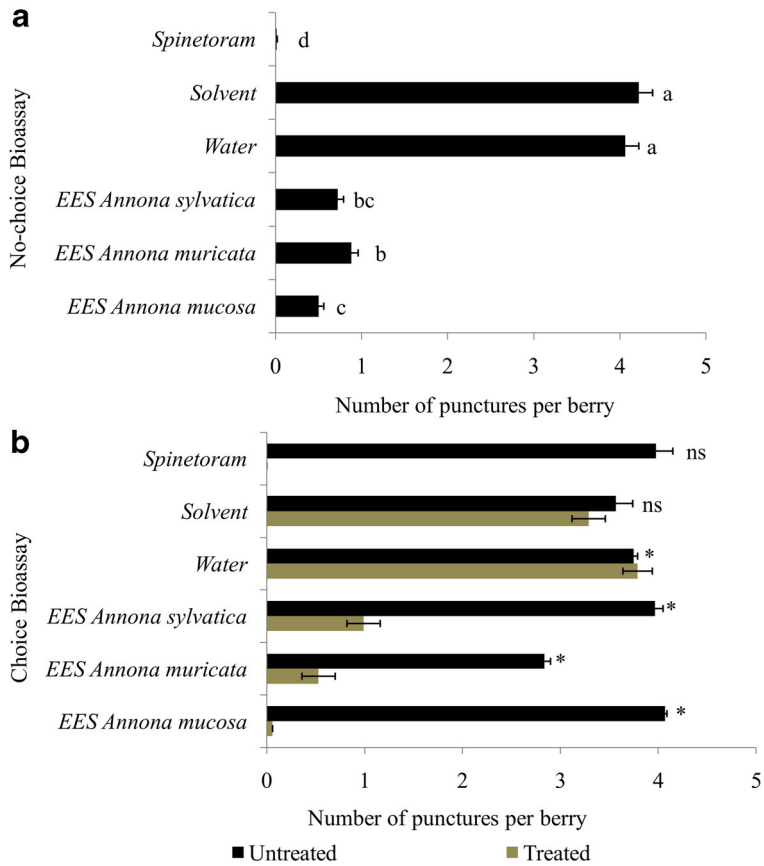

Fig. 3 Averages (± SE) of the number of punctures caused by *Ceratitis capitata* females in berries of grapes treated with different (bio) insecticides: **a** bioassay without choice (Averages followed by distinct letters in the columns indicate differences between treatments (GLM with quasi-Poisson distribution followed by Tukey's post hoc test, $p < 0.05$). **b** choice bioassay. (Asterisks indicate significant differences between treatments according to the t-test of Student ($p < 0.05$) and non-significant "ns" according to Student's t test ($p < 0.05$))

Table 5 Average number of galleries (± SE) caused by *Ceratitis capitata* larvae in grape berries treated with different treatments

Treatments	Number of galleries
EES *Annona mucosa*	0.05 ± 0.02 c
EES *Annona muricata*	0.59 ± 0.06 b
EES *Annona sylvatica*	0.72 ± 0.06 b
Water	1.56 ± 0.07 a
Solvents	1.45 ± 0.07 a
Spinetoram (controle positivo)	0.00 ± 0.00 c
F	104.52
df	5, 593
P values	<0.0001

Means followed by different letters on the columns indicate significant differences between treatments (GLM with quasi-Poisson distribution followed by post hoc Tukey test, $p < 0.05$);

EES, Formulated ethanolic seed extract

(Isman and Seffrin 2014). More specifically, acetogenins block the respiratory chain in complex I (NADH: ubiquinone oxidoreductase) of the mitochondrial electron transport system and the enzyme NADH oxidase in the target arthropod cell membrane, directly affecting electron transport in the mitochondria and causing apoptosis (programmed cell death) as a result of ATP deprivation (Alali et al. 1999; Tormo et al. 1999). Furthermore, it has been found that some alkaloids present in crude or semipurified extracts may cause a synergistic effect by increasing the activity of the compound (Ribeiro et al. 2013).

Although there are no reports of the evolution of insecticide resistance in *C. capitata* populations in Brazil (Raga and Galdino 2018), factors related to high pest polyphagy (Sá et al. 2019) associated with selection pressure exerted by the use of chemical insecticides (i.e. pyrethroids, spinosyns and phosphorus) in full-area spraying (up to 5 sprayings per crop) (Raga and Galdino 2018) may trigger this process, a fact already confirmed in Spain (Magaña et al. 2007; Couso-Ferrer et al. 2011; Vontas et al. 2011; Arouri et al. 2015). Therefore, studies aimed at detecting new molecules with different modes of action are extremely important for the management of resistance evolution (Vontas et al. 2011; Baronio et al. 2019b).

In the present study, all aqueous emulsions of the ethanolic seed extracts of the tested Annonaceae seeds (rich in acetogenins) caused oviposition deterrence for *C. capitata* females, both in bioassays with and without choice using grape berries as oviposition substrates. The negative impact caused by such treatments on oviposition behavior can be exploited in grape orchards to prevent females from causing damage (punctures) to fruits in the oviposition process and, consequently, decreasing the entry of pathogens that may accelerate fruit spoilage and product shelf life (Machota Júnior et al. 2013).

In addition to demonstrating high lethal toxicity to *C. capitata* adults and the deterrent effect of oviposition, the aqueous emulsion of the ethanolic extract of *A. mucosa* seeds mixed with Biofruit (3%), sugar cane molasses and Anamed ™ showed high toxicity and a toxic residual effect on *C. capitata* adults after application to citrus leaves in the absence of rain. The characterization of another sustainable alternative may contribute to the replacement of the active ingredient malathione, the most commonly used lethal agent in the formulation of toxic baits in the world (Manrakhan et al. 2013; Botton et al. 2016; Baronio et al. 2019b; Bernardi et al. 2019).

Although the residual period of the aqueous emulsion of *A. mucosa* extract was 14 days (approximately 80% mortality) in the absence of rain, some factors may accelerate the degradation or washing of the active lethal agent of the formulation (acetogenins) in the field. This may be due to the constant presence of solar radiation or the occurrence of rainfall, as verified with spinosyn-based bait formulations (Revis et al. 2004; Flores et al. 2011; Härter et al. 2015; Baronio et al. 2019b). Therefore, complementary experiments should be conducted under field conditions and/or simulated rainfall so that the residual effect is known for regions with high rainfall (Härter et al. 2015; Baronio et al. 2019b).

Another factor that may contribute positively to the management of *C. capitata* populations through the use of acetogenin-rich extracts was the low mortality rate on adults of *D. longicaudata*, which is the most prevalent exotic species found in the countryside in areas with *C. capitata* and temperate climate fruit crops (Montoya et al. 2013; Meirelles et al. 2016). In addition to providing low mortality, the acetogenins did not cause sublethal effects to adults, making it an obvious alternative for use in IPM programs.

Considering the high toxicity of the seed extracts to *C. capitata* adults in different exposure forms, the deterrence effects of oviposition, the possibility of use in toxic baits and the compatibility with *D. longicaudata* adults, the aqueous emulsion (precommercial formulation) of the ethanolic extract of *A. mucosa* seeds is a

promising alternative for the management of *C. capitata* in orchards, either in conventional or organic cultivation systems that meet the requirements of IPM and integrated production. In this context, seeds of this Annonaceae species (considered a waste in fruit processing industries) can enable the development of more environmentally friendly products, turning waste into solutions for more sustainable agriculture.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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