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Research Article

Annona mucosa Jacq. (Annonaceae): A Promising Phytoinsecticide for the Control of *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae)

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Abstract

Background: *Annona mucosa* Jacq. is a promising species for pest control due to its effectiveness against some insects. The insecticidal effects of crude extracts of *A. mucosa* seeds on the soybean looper, *Chrysodeixis includens* (Walker) an insect pest of several crops was evaluated. **Materials and Methods:** The extract was prepared and diluted in water with solubilizer at the concentrations of 0.5, 1.0, 2.0, 4.0 and 8.0% and applied topically and orally to first, third and fifth instar larvae. **Results:** The extract of *A. mucosa* exhibited insecticidal properties against all instars of *C. includens*. Mortality of first instars was high after 24 h for both application methods. No differences were observed in mortality of third instar larvae exposed by ingestion of leaves treated with the extract at different concentrations. Mortality reached 80% of third instar larvae 24 h after exposure by contact with the highest extract concentration. Among fifth-instar caterpillars fed on leaves treated with the extract, mortality was significant only after 72 h at the concentrations between 2.0 and 8.0%. Topical application of *A. mucosa* extract resulted in mortality of 93.3% at the highest concentration after 24 h of treatment. **Conclusion:** The results show that *A. mucosa* is a promising species for the development of new toxic molecules for the control of *C. includens*.

Key words: Annonaceae, bioinsecticide, alternative control, defoliator

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Annona mucosa Jacq. is a native species of the Amazon and Atlantic forests. It belongs to the Annonaceae family, commonly known as biriba, wild sweetsop and cachiman[1]. Several studies have shown that chemical compounds known as "Annonaceous acetogenins" present in the Annonaceae family exhibit important biological activities, such as cytotoxicity, antitumor, antimicrobial, insecticidal, among other properties[2,3]. The antimicrobial and antifungal activities of *A. mucosa* have been demonstrated by Caetano and Dadoun[4] as well as the inhibition of ATP synthesis of the mitochondrial complex I and antitumor[5-9], leishmanicidal[10] and insecticidal effects[2,11-13].

Plants with insecticidal properties have drawn considerable attention as alternatives to chemical insecticides as they are less toxic to the environment and humans[14]. In addition, the excessive and continuous use of neurotoxic products promotes the selection of pest resistance to active compounds[15,16].

Boethel *et al.*[17] examined insecticide resistance in populations of Plusiinae (Noctuidae), in Southwestern United States since the 1960s. Resistance in these populations could be the result of high insecticide use in crops such as chrysanthemum and tomatoes where the number of applications of synthetic products can reach as much as 100 annually.

Among the Plusiinae, *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae) commonly known as soybean looper, is a pest that occurs from the northern United States to Southern South America[18]. This species has been reported infesting nearly 70 plant species including soybean, cotton, bean, sunflower, tobacco, passion fruit, tomato and several leafy greens[19,20,21].

In Brazil, the main states producinsoybeans, *C. includens* was considered a secondary pest controlled naturally by parasitoids and entomopathogenic fungi[22]. However, since 2013, outbreaks of this species have been observed in the states of Mato Grosso do Sul, Goiás, São Paulo, Paraná and Mato Grosso[23].

Chemicals are often used for the control of this caterpillar but they are not always effective as the caterpillars prefer to stay on the abaxial leaf surface at the middle third of plants and often do not get in direct contact with the insecticide[24]. As a result the dosages of chemicals are drastically increased and thus reducing the populations of natural enemies and promoting the selection of resistant strains of the pest. Thus further studies on new molecules and new modes of action that are environmentally safer and less toxic to natural enemies are needed in integrated programs for the control of *C. includens*[25].

Given that the insecticidal properties of *A. mucosa* have been reported on species such as *Aedes aegypti* L. (Diptera: Culicidae)[11], *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae)[12], *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae)[13], its insecticidal effect on *C. includens* caterpillars was evaluated under laboratory conditions.

MATERIALS AND METHODS

Extract preparation: Seeds of *A. mucosa* were collected in Tangará da Serra, Mato Grosso State, Brazil (14°18′44″S and 57°45′18″W) for identification. After confirmation of the species, voucher specimen were deposited in the Herbarium of the University of Mato Grosso, Tangará da Serra campus (UNEMAT) and assigned the record number 92. From the collected fruits, seeds were removed and dehydrated in an air circulating oven at 40°C for 72 h. After drying, seeds were ground in a knife mill. The resulting powder was taken to the Laboratory of Biochemistry of carbohydrates, Federal University of Paraná for lipid extraction for three days with chloroform-methanol (2:1) with a soxhlet extractor under a heating pad at 60°C until complete exhaustion. The extracted material was rotoevaporated at 40°C for removal of solvents, resulting in the crude extract. From this extract, dilutions were prepared for the concentrations used in the bioassays.

Rearing of *Chrysodeixis includens*: Caterpillars of *C. includens* used in bioassays were obtained from a colony established at the Laboratory of Entomology of UNEMAT. All stages were reared in a climatized room at 25±3°C and Relative Humidity (RH) of 70±10%.

Adults were maintained in PVC cages (100×200 mm) internally lined with sulphite paper for oviposition. The top was closed with voil fabric with a rubber band and the bottom with a petri dish lined with filter paper and two containers one with cotton embedded in 10% solution of honey for feeding and another with water, both replaced three times a week.

The eggs obtained were used in bioassays and for the maintenance of the colony. For the latter, the substrate with eggs (sulphite paper and voil fabric) was cut in stripes and placed on the lid of 145 mL plastic containers with an artificial diet[26]. In these containers, caterpillars hatched and remained

for approximately 10 days and then were transferred to 50 mL cups with acetate lids with three larvae per container and fed the same diet until they reached the pupal stage. Pupae were removed from the cups and placed in PVC cages. After adult emergence, moths were maintained in the above described cages until death.

Bioassays-contact and ingestion toxicity: The experiments were conducted at the Laboratory of Entomology of UNEMAT/CUTS in climate chambers BOD at 25±1°C, RH 70±10% and 12 h photoperiod. The treatments for all bioassays used the extract of *A. mucosa* seeds at the concentrations of 0.5, 1.0, 2.0, 4.0 and 8.0%. The crude extract was solubilized in water with polysorbate 80 (Tween 80®) at 5% and mixed for 5 min with a magnetic agitator. Randomized bioassays were conducted with five concentrations and two control groups (water and water+tween 80). Each treatment was carried out with 15 replicates of one caterpillar.

Twenty four hour old eggs were removed from the colony and placed in the above described conditions. Caterpillars were maintained in 145 mL plastic containers lined with filter paper and were fed on soybean leaves (var. conventional Tucunaré) grown in a greenhouse. Soybean leaves were washed under tap water and placed inside the container with the caterpillar. The petiole was wrapped in moistened cotton to prevent desiccation.

Some first instar caterpillars were treated 24 h post egg emergence. The remaining caterpillars were reared to third and fifth instars which were determined by the presence of head capsules in the container.

Two methods of application of the extract were evaluated: Ingestion of treated leaves and by direct contact. For the ingestion method the extract was applied to the adaxial surface of leaves. The solubilized extract was placed in a petri dish and the adaxial face of the soybean leaf was immersed in the solution for 3 sec and allowed to dry for approximately 30 min with the treated surface facing up. Leaves were then offered to caterpillars for 24 h. After this period, the first assessment was carried out and a new untreated leaf was provided.

For contact activity, the extract was applied on the dorsal surface of each caterpillar with an Hamilton microsyringe (1 µL for first-instar caterpillars and 2 µL for third and fifth-instar caterpillars). The application of the extract to first instars was carried out under stereomicroscope. After application, caterpillars were maintained under controlled conditions and mortality was recorded daily.

Statistical analysis: Data were tested for normality with the Shapiro-Wilk test. Since, assumptions of normality were not met, the results were analyzed with the Kruskal-Wallis test with significance set at 5% and comparisons between methods of application were analyzed with the Mann-Whitney test with significance set at 5%. The lethal concentrations for 50 and 90% of treated caterpillars (LC_{50} and LC_{90}) were calculated by Probit analysis[27]. The analyses were conducted with the software Statistica 7.0.

RESULTS

The extract of *A. mucosa* seeds exhibited insecticidal effect on *C. includens* caterpillars on all instars evaluated. Daily assessments were carried out during five consecutive days. However, since the results were similar at 48 and 96 h intervals, we presented the results as 24, 72 and 120 h after application (HAA).

Effect of the extracts on first instar larvae: Although, higher concentrations provided higher mortality, no significant difference was recorded among the treatments on first instar larvae, either by contact or ingestion treatments. Concentrations of 4 and 8% killed significantly more larvae than the controls at all evaluation periods after treatment both by ingestion and contact methods (Table 1). At 0.5% none of

Table 1: Percentage of mortality (±SD) of first-instar caterpillars of *Chrysodeixis includens* exposed to different concentrations of *Annona mucosa* extract by ingestion and by contact after 24, 72 and 120 h

	Ingestion*			Contact		
Treatment	24 h	72 h	120 h	24 h	72 h	120 h
Water	0.0±0.0^{b}	6.6±2.5^{b}	6.6±2.5^{b}	00.0±0.0^{c}	0.0±0.0^{c}	0.0±0.0^{c}
Water+tween	0.0±0.0^{b}	6.6±2.5^{b}	6.6±2.5^{b}	20.0±4.1^{bc}	20.0±4.1^{bc}	20.0±4.1^{bc}
0.5 (%)	13.3±3.5^{ab}	60.0±5.0^{ab}	60.0±5.0^{ab}	40.0±5.0^{abc}	40.0±5.0^{abc}	53.3±5.1^{abc}
1.0 (%)	13.3±3.5^{ab}	80.0±4.1^{a}	86.6±3.5^{a}	60.0±5.0^{aabc}	66.6±4.8^{ab}	66.6±4.8^{ab}
2.0 (%)	33.3±4.8^{ab}	93.3±2.5^{a}	93.3±2.5^{a}	80.0±4.1^{ab}	80.0±4.1^{ab}	86.6±3.5^{a}
4.0 (%)	73.3±4.5^{a}	100.0±0.0^{a}	100.0±0.0^{a}	93.3±2.5^{a}	93.3±2.5^{a}	93.3±2.5^{a}
8.0 (%)	26.6±4.5^{ab}	100.0±0.0^{a}	100.0±0.0^{a}	86.6±3.5^{a}	93.3±2.5^{a}	93.3±2.5^{a}
H	32.8	66.2	68.7	45.4	49.2	50.4

*Means followed by the same letter in columns are not significantly different (p>0.05) according to the Kruskal-Wallis test at 5%

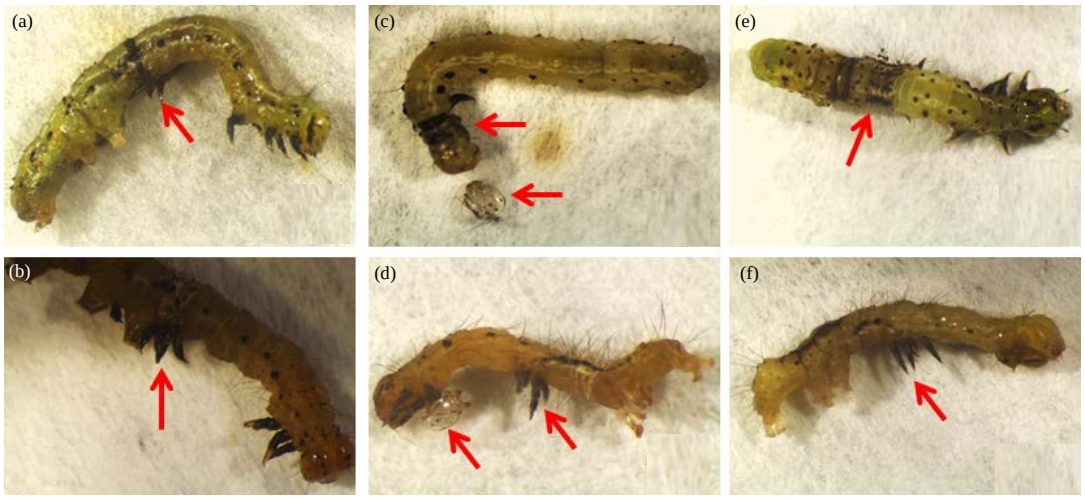

Fig. 1(a-f): Third-instar *Chrysodeixis includens* caterpillars after ingestion of leaves treated with *A. mucosa* extract dead in the exuvium 72 HAA, (a-b) Concentration of 2.0%, (c-d) Concentration of 4.0% and (e-f) Concentration of 8.0%. Arrows indicate the old cuticle

Table 2: Percentage of mortality (±SD) of third-instar caterpillars of *Chrysodeixis includens* exposed to different concentrations of *Annona mucosa* extract by ingestion and by contact after 24, 72 and 120 h

	Ingestion*			Contact		
Treatment	24 h^{ns}	72 h^{ns}	120 h^{ns}	24 h	72 h	120 h
Water	0.0±0.0	6.6±2.5	13.3±3.5	0.0±0.0^{b1}	6.6±2.5^{b}	6.6±2.5^{c}
Water+tween®	0.0±0.0	13.3±3.5	20.0±4.1	0.0±0.0^{b}	20.0±4.1^{b}	20.0±4.1^{bc}
0.5 (%)	13.3±3.1	26.6±4.5	26.6±4.5	46.6±5.1^{ab}	60.0±5.0^{ab}	86.6±3.5^{a}
1.0 (%)	26.6±4.5	53.3±5.1	53.3±5.1	6.6±2.5^{b}	66.6±4.8^{ab}	73.3±4.5^{ab}
2.0 (%)	13.3±3.5	46.6±5.1	53.3±5.1	40.0±5.0^{ab}	60.0±5.0^{ab}	80.0±4.1^{ab}
4.0 (%)	0.0±0.0	46.6±5.1	46.6±5.1	60.0±5.0^{ab}	86.6±3.5^{a}	93.3±2.5^{a}
8.0 (%)	13.3±3.5	46.6±5.1	60.0±5.0	80.0±4.1^{a}	93.3±2.5^{a}	93.3±2.5^{a}
H	10.5	13.7	13.0	40.4	37.4	50.7

*Means followed by the same letter in columns are not significantly different (p>0.05) according to the Kruskal-Wallis test at 5%

the treatments were significantly different from the controls at any time after application or treatment method. At concentrations of 1 and 2% significantly higher mortalities than the controls were recorded at 72 and 120 HAA, when larvae ingested the extract. By contact, no differences were recorded between treated larvae with 1% and the controls and at 2% only after 120 HAA contact treated larvae showed higher mortality than the controls (Table 1).

Effect of the extracts on third instar larvae: Although, mortality in relation to the controls was higher on larvae that ingested soybean leaves treated with the extract, none of the treatments showed statistical difference between treated and control treatments of third instar larvae (Table 2). The extract when ingested affected the ecdysis of the larvae hampering the shedding of the old cuticle (Fig. 1).

Contact toxicity was significantly higher than the controls at the concentrations of 4 and 8% after 72 and 120 HAA, while the concentration of 8% showed significantly higher mortality already after 24 HAA. Concentrations between 0.5 and 2% did not differ from the water and tween control, except at 0.5% 120 HAA (Table 2).

Unlike mortality observed by the ingestion of the extract, contact toxicity did not affect ecdysis.

Effect of the extracts on fifth instar larvae: The ingestion of soybean leaves containing the extract of *A. mucosa* had little effect on the larvae after 24 h. At 72 HAA all concentrations had larval mortality higher than the controls, although only the 2% concentration had mortality significantly higher than the controls (Table 3). The two higher concentrations killed more than 75% of fifth instar larvae, well above the 13.3% of the water and tween control but without statistical difference between them. After 120 HAA concentrations between 1 and 8% caused mortalities of *C. includens* above 70%, all significantly higher than the water control (Table 3). Similarly,

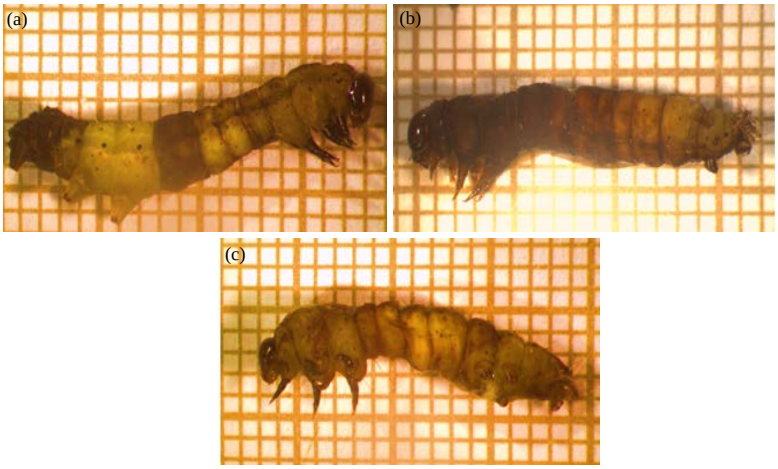

Fig. 2(a-c): Dead caterpillars in pre-pupal stage fed leaves treated with *A. mucosa* extract at the concentration of 0.5%, 120 h after ingestion

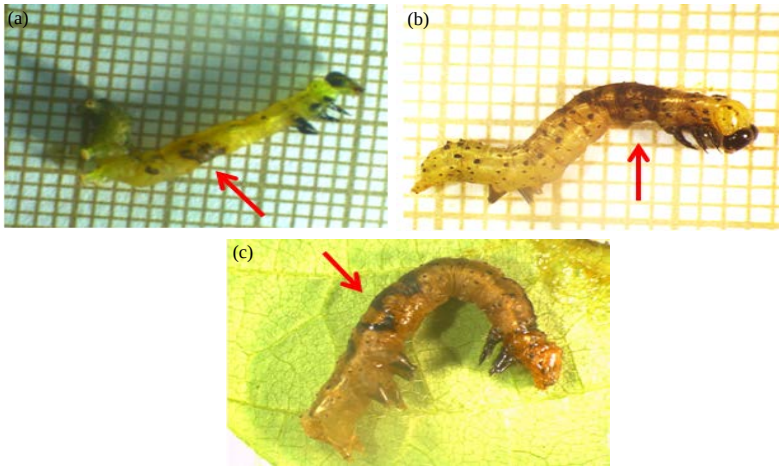

Fig. 3(a-c): Fifth-instar caterpillars of *Chrysodeixis includens* dead after topical application of *Annona mucosa* extract exhibiting wound on the epidermis. Arrows indicate wound in the cuticle of dead larvae

to what was observed with third instars, larvae were affected after ingestion of *A. mucosa* extract and were unable to pupate due to disturbance in the process of ecdysis (Fig. 2).

Mortality by contact reached 93.3% with the highest concentration of the extract and after 72 HAA all concentrations caused mortalities above 60% in comparison to 6.6% recorded in the control treatments (Table 3). After 120 HAA all extract concentrations showed mortalities above 70% all significantly higher than the controls.

When fifth instar larvae were treated by contact, death was caused by the inability of the specimens to pupate and wound were seen in the cuticle of dead larvae (Fig. 3).

The LC_{50} and LC_{90} were calculated for 24, 72 and 120 h (Table 4). After 72 and 120 h of application the LC_{50} was lower (Table 4). The LC_{90} values remained high only for third-instar caterpillars. For caterpillars after 24 h following topical application, LCs were lower and gradually decreased with time. Third-instar larvae were more resistant to the *A. mucosa* extract compared to first and fifth-instar ones and therefore LCs were higher, especially for exposure by ingestion (Table 4).

DISCUSSION

Our findings indicate that *A. mucosa* has strong insecticidal properties against *C. includens*, in agreement with results reported by Ribeiro *et al.*[13] using ethanolic extract of *A. mucosa* on larvae and other species of the genus *Annona.* Few studies have been conducted with extracts

Table 3: Percentage of mortality (±SD) of fifth-instar caterpillars of *Chrysodeixis includens* exposed to different concentrations of *Annona mucosa* extract by ingestion or by contact 24, 72 and 120 h after application

	Ingestion			Contact		
Treatments	24 h^{ns}	72 h^{ns}	120 h^{ns}	24 h	72 h	120 h
Water	0.0±0.0	6.6±2.5^{c}*	6.6±2.5^{c}	0.0±0.0^{b}	6.6±2.5^{b}	6.6±2.5^{b}
Water+tween®	0.0±0.0	13.3±3.5^{bc}	13.3±3.5^{bc}	0.0±0.0^{b}	6.6±2.5^{b}	6.6±2.5^{b}
0.5%	13.3±3.5	40.0±5.0^{abc}	66.6±4.8^{abc}	33.3±4.8^{ab}	80.0±4.1^{a}	86.6±3.5^{a}
1.0%	6.6±2.5	66.6±4.8^{abc}	73.3±4.5^{ab}	13.3±3.5^{b}	66.6±4.8^{ab}	73.3±4.5^{a}
2.0%	40.0±5.0	86.6±3.5^{a}	93.3±2.5^{a}	33.3±4.8^{ab}	73.3±4.5^{a}	73.3±4.5^{a}
4.0%	20.0±4.1	73.3±4.5^{ab}	93.3±2.5^{a}	53.3±5.1^{ab}	80.0±4.1^{a}	86.6±3.5^{a}
8.0%	13.3±3.5	73.3±4.5^{ab}	93.3±2.5^{a}	93.3±2.5^{a}	100.0±0.0^{a}	100.0±0.0^{a}
H	14.8	35.8	54.2	44.9	51.0	56.9

$^{n.s}$ Not significant. *Means followed by the same letter, lowercase in columns not significant different ($p<0.05$) by the Kruskal-Wallis test at 5%

Table 4: Lethal concentration (LC_{50} and LC_{90}) (%) of *Annona mucosa* extract for exposure by ingestion or by contact of *Chrysodeixis includens* caterpillars (Lepidoptera: Noctuidae)

		Ingestion			Contact		
Treatments	LC (%)	24 h	72 h	120 h	24 h	72 h	120 h
1st	LC_{50} (IC95)	7.70*(7.3-8.0)**	1.65 (0.5-2.8)	1.61 (0.4-2.7)	2.05 (1.7 -2.3)	1.68 (1.1-2.1)	1.35 (0.8-1.9)
	LC_{90} (IC95)	15.70 (14.9-16.4)	3.80 (2.4-5.2)	3.74 (2.3-5.1)	6.38 (5.8-6.9)	5.65 (4.8-6.4)	5.52 (4.6-6.3)
3rd	LC_{50} (IC95)	109.38 (107.3-111.4)	6.41 (6.3-6.5)	4.59 (4.5-4.6)	4.05 (3.9-4.1)	1.55 (1.1-1.9)	0.40 (-0.1-1.0)
	LC_{90} (IC95)	215.30 (211.3-219.3)	17.05 (16.7-17.3)	13.23 (13.1-13.3)	8.36 (8.1- 8.5)	6.12 (5.4-6.7)	5.47 (4.6-6.2)
5th	LC_{50} (IC95)	25.65 (24.5-26.7)	2.06 (1.9-2.1)	0.85 (0.2-1.4)	3.83 (3.7-3.9)	1.37 (0.7-2.0)	1.14 (0.3-1.9)
	LC_{90} (IC95)	51.20 (48.9-53.4)	8.18 (7.9-8.4)	5.33 (4.4- 6.9)	7.48 (7.2-7.7)	5.28 (4.3- 6.2)	4.91 (3.8- 5.9)

*Values calculated by the Probit analysis and **Confidence intervals of 95% (CI)

of this species and the present study is the first one to assess the insecticidal properties of *A. mucosa* on *C. includens* caterpillars.

Initial mortality 24 h after the treatments was higher when the extract was topically applied to the larvae. The same pattern was observed for either first, third or fifth instars. Ingestion of treated leaves resulted in lower initial mortality but at the end of the experimental period percent mortality was comparable to the values recorded for the contact action.

All instars evaluated were susceptible to the extract, although third instar larvae were less affected by *A. mucosa* by ingestion. Mortalities between 80 and 100% were recorded in most treatments at concentrations between 2 and 8% of the crude extract except for third instar larvae.

Third and fifth instar larvae showed symptoms of growth inhibition caused by the ingestion of the extract; larvae were unable to shed the old cuticle and died before completing ecdysis. Topical application on fifth instar larvae caused the appearance of edemas on the cuticle hampering pupation.

The effects of *A. mucosa* have been previously evaluated on the maize weevil *S. zeamais* by Ribeiro *et al.*[12]. The authors evaluated the effects of different parts of the plant (leaves, branches and seeds) using different solvents (hexane, dichloromethane and ethanol) and concluded that the extract of *A. mucosa* seeds at the concentration of 300 mg kg^{-1} in hexane resulted in a mortality of 98.0% and in dichloromethane, 85.5%. At the concentration of 1500 mg kg^{-1}, mortality was 100% for both solvents.

The effect of *A. mucosa* seeds was also evaluated on *A. aegypti* L. by Costa *et al.*[11]. These authors reported that methanolic extract at a concentration of 0.1 mg mL^{-1} resulted in 100% mortality 24 h after application. In first-instar larvae of *C. includens*, mortality was also high (over 70%) 24 h after application for both methods, confirming the insecticidal properties of *A. mucosa*.

Ribeiro *et al.*[13] also examined the effects of ethanolic extract of *A. mucosa* added to an artificial diet fed to *T. ni* at a concentration of 1000 mg kg^{-1} and reported a mortality of 97.5 %. When the extract was topically applied, the mortality was 96.6%, while exposure by contact with a surface impregnated with the extract resulted in mortality of 16.6%, showing a stronger response compared to other *Annona* species examined. Ribeiro *et al.*[13] evaluated the same extract for the control of *M. persicae* and reported that the extract reduced the population of aphids from 74.8 insects per plant to 10.5 under laboratory conditions and from 97.1 aphids per plant to 1.8 under greenhouse conditions, demonstrating the insecticidal properties of *A. mucosa*.

Despite the few studies of *A. mucosa* on lepidopterans, other species of the genus *Annona* have shown insecticidal properties against caterpillars. Seffrin *et al.*[28] evaluated the methanolic extracts of *A. atemoya* (hybrid between

A. cherimola x *A. squamosa*) and *A. squamosa* by exposing third-instar *T. ni* caterpillars through ingestion and by topical application and concluded that *A. squamosa* was a stronger feeding deterrent and more effective in inhibiting larval growth. In the present study, exposure by ingestion of *A. mucosa* extracts did not have satisfactory results on third-instar caterpillars. However, larvae died before completing ecdysis, which could be associated to the inhibition of larval growth as observed by Seffrin *et al.*[28] in *T. ni.*

The ethanolic extract of *A. muricata* leaves produced 100% mortality in *Plutella xylostella* larvae fed collard green leaves treated with the extract at the concentration[29] of 5 mg mL^{-1}. The dead insects exhibited dark coloration and small size a sign of larval growth inhibition. Also, several larvae died during ecdysis as they were stranded in the exuvia, probably due to the effects of the chemical components of the extract on the hormonal system of the pest. Similar results were observed in the present study for third instar larvae, with dead individuals exhibiting dark coloration, while others died during ecdysis and some fifth-instar caterpillars were not able to enter the pupal stage, which may be directly associated with growth hormones.

Acetogenins present on extracts of *Annona* species have shown strong interference with insect hormones. Blessing *et al.*[30] evaluated the effects of *A. montana* acetogenins (annonacin, cis-annonacin-10-one, densicomacin-1, gigantetronenin, murihexocin-B and tucupentol) on *Spodoptera frugiperda* and observed that all acetogenins added to an artificial diet resulted in 100% mortality of the pest in the larval or pupal stages. Blessing *et al.*[30] found no correlation between toxicity and the capacity of the acetogenins, annonacin, cis-annonacin-10-one and gigantetronenin to inhibit NADH oxidation indicating that this is not the only cause of larval mortality. This explains the morphological alterations observed in caterpillars and pupae of *S. frugiperda*, which were typical of compounds that interfere with the hormonal activity of lepidopterans and that were also observed in study with *C. includens*.

The acetogenins of *A. cherimola* have also been tested on *S. frugiperda* caterpillars by Colom *et al.*[31]. Among acetogenins, squamocin exposure resulted in 100% of mortality at the larval stage while the remaining acetogenins tested (itrabin, cherimolin-1 and -2, neoannonim, almuñequin, motrilin, tucumanin and asimicin) significantly prolonged the larval phase, mortality in the pupal phase and malformations in the abdomen and wings of adults, preventing the development of the next generation.

Findings of this study confirm the insecticidal activity of *A. mucosa* as reported by other authors. This property may be associated to acetogenins present in *A. mucosa* seeds[5,8,32-34].

Acetogenins are strong inhibitors of ATP production of the mitochondrial complex I (NADH ubiquinone oxidoreductase) in insects, which reduces the production of energy and leads to programmed cell death (apoptosis)[35-37]. They also exhibit characteristics of compounds that interfere in the hormonal activity of lepidopterans[30] and may explain the mortality rates of *C. includens* in the study.

The Lethal Concentrations (LC) 50 and 90 for caterpillars exposed by ingestion were high, especially for third-instar caterpillars. However, at the end of the treatments, LCs of larvae exposed by ingestion and by contact were similar as reported by Lima *et al.*[38]. These authors evaluated the effects of essential oil of *Piper hispidinervum* (Piperaceae) on *S. frugiperda* and observed that high LCs in the first assessments may be associated with the knock-down effect, in which larvae initially first try to feed and then stop for a few hours. Only after a certain length of time they resume eating, thus ingesting the extract.

Based on the knowledge about molecules and chemical structures of active compounds it will be possible to produce new products from synthetic molecules in large scale for commercial purposes, resulting in an effective control of the pest with less impact to the environment.

Semi-field and field studies are also needed to confirm the results obtained in the laboratory. Further studies may evaluate *A. mucosa* extract at the concentration of 4.0% as it produced good results for all instars examined 72 HAA for both methods of application.

Further studies are needed to assess the insecticidal effects of *A. mucosa* extracts on other lepidopteran pests as well as natural enemies, so that they can be used in integrated pest management programs.

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