Lethal and Sublethal Toxicities of Annona sylvatica (Magnoliales: Annonaceae) Extracts to Zabrotes subfasciatus (Coleoptera: Chrysomelidae: Bruchinae)

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Lethal and sublethal toxicities of *Annona sylvatica* (Magnoliales: Annonaceae) extracts to *Zabrotes subfasciatus* (Coleoptera: Chrysomelidae: Bruchinae)

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**Abstract**

Plant secondary metabolites comprise a diverse range of compounds (allelochemicals) that affect insect–plant interactions; many function in plant defense against herbivory. Thus, allelochemicals constitute an important source of insecticidal molecules that potentially can be used in different forms in integrated pest management programs. The objective of this study was to evaluate the bioactivity of ethanolic extracts and partially purified fractions of these extracts obtained from the leaves, branches, and seeds of *Annona sylvatica* A. St.-Hil. (Magnoliales: Annonaceae), a native Brazilian species, against *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae: Bruchinae). In the screening assay, the ethanolic extract of *A. sylvatica* seeds was the most promising treatment, causing lethal (LC₅₀ = 753.47 and 701.06 mg kg⁻¹ for males and females, respectively) and sublethal effects, mainly oviposition deterrence (EC₅₀ = 438.70 mg kg⁻¹). On the other hand, ethanolic extracts prepared from branches and leaves caused only sublethal effects including mainly oviposition deterrence (EC₅₀ = 1,168.90 and 1,010.70 mg kg⁻¹, respectively) and reduction in number of offspring. Based on these results, the extracts were submitted to liquid–liquid partitioning, and their fractions were tested against *Z. subfasciatus* to verify their bioactivity. Overall, the results of the fraction bioassays showed evidence of synergistic interactions among compounds of different chemical classes and polarities. Chemical analyses of active fractions revealed the presence of triglycerides, alkaloids, and acetogenins in the seed fractions; alkaloids, lignans, and long-chain fatty acid ethyl esters in the branch fractions; and glycosides, flavonoids, terpenoids, and long-chain fatty acid ethyl esters in the leaf fractions. Thus, *A. sylvatica* is an interesting and potentially important source of structurally diverse grain-protective compounds.

**Key Words:** Mexican bean weevil; bioactivity; allelochemicals; acetogenins; alkaloids; lignans

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The development of sustainable practices that can be incorporated into food production systems currently constitutes one of the greatest challenges for the scientific community (Prasifka & Gray 2012). In Brazil, this is even more evident when taking into consideration that current pest management programs of major agricultural commodities are based on the systematic and routine application of synthetic
pesticides instead of an integrated pest management approach, which takes into account the economic, environmental, and social aspects of pest control interventions (Norris et al. 2003).

Recently, with the decreased availability of synthetic molecules with insecticidal action from the market of compounds with broad-spectrum action, interest in botanical insecticides as an alternative method for insect pest control has been revived (Dayan et al. 2009). The advantages of botanical insecticides compared with conventional insecticides are related to their lower mammalian toxicity and decreased health risk to applicators and their rapid degradability, which reduces residues in the environment and in the treated products (Isman 2006). Additionally, they generally contain various biologically active compounds capable of synergistic interactions that may reduce the selection of resistant pest populations in the field (Akhtar & Isman 2013). Owing to the ease of obtaining botanical derivatives (as plant powders, crude extracts, or oils), these botanical derivatives have been used for various purposes in the management of insect pests of stored grains in many countries, especially Latin America, Africa, and Asia (Isman 2008). Another important aspect of research on botanical derivatives with insecticidal potential is the discovery of model prototypes that can be used in the synthesis of new insecticides, especially those with different modes/mechanisms of action and less impact on the environment and human health (Regnault-Roger et al. 2012).

Several phytochemicals with insect-associated biological properties are known; these chemicals can act as feeding and oviposition deterrents, repellents, attractants, growth inhibitors, and insecticides (Al Lawati et al. 2002). Brazilian scientists are in a prime position to identify such active phytochemicals, as plant genetic diversity is higher in Brazil than in all other countries, with more than 55,000 vascular plant species catalogued in Brazil (Simões & Schenkel 2002).

Among botanical families from tropical regions, plants of the family Annonaceae are one of the most promising sources of bioactive molecules because of the wide variety of biologically active metabolites produced by this family (Leboeuf et al. 1980; Bermejo et al. 2005). The acogenins, a chemically diverse class of compounds found in some genera of Annonaceae (Alai et al. 1998), are promising molecules because of the wide variety of biologically active secondary plant species catalogued in Brazil (Simões & Schenkel 2002).

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The tree species Annona sylvatica A. St.-Hil. (Magnoliales: Annonaceae), previously assigned to the genus Rollinia, is native to southern-central Brazil (LoBao et al. 2005). It produces edible fruits with considerable health benefits due to the presence of phenolic compounds, antioxidants, carotenoids, and vitamin C (Pereira et al. 2013), making it capable of providing health benefits due to the presence of phenolic compounds, antioxidants, carotenoids, and vitamin C (Pereira et al. 2013), making it a potential food source. The tree species Annona sylvatica A. St.-Hil. (Magnoliales: Annonaceae), previously assigned to the genus Rollinia, is native to southern-central Brazil (LoBao et al. 2005). It produces edible fruits with considerable health benefits due to the presence of phenolic compounds, antioxidants, carotenoids, and vitamin C (Pereira et al. 2013), making it a potential food source.

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derived from a population maintained under laboratory conditions for approx. 15 generations. Adult survival was assessed on the 5th day after infestation. Insects with legs completely extended that showed no reaction to contact with a thin brush for 1 min of observation were considered dead.

**ASSESSMENT OF SUBLETHAL EFFECTS**

The same experimental arrangement used in the previous test was used to evaluate the sublethal effects of the crude extracts. For this assessment, the adults were removed after 5 d of infestation, and the eggs on each bean seed surface were counted with the aid of a stereomicroscope. Then, the sampling units were maintained under the climatic conditions mentioned previously. At 60 d after the initial infestation, the adults that emerged were separated by gender and counted. At this time, the percentage of damaged seeds (with holes) in each sample was determined through visual assessment of each seed. Similar to the previous bioassay, 10 replicates per treatment were used.

**CONCENTRATION–RESPONSE CURVES OF THE PROMISING EXTRACTS**

The extracts that showed the most promising results were tested for LC₉₀ and LC₅₀ estimations, which correspond to the concentrations required to kill 50 and 90%, respectively, of the weevil population. Here, preliminary tests were performed using these extracts to determine the basic concentrations that caused 95% adult mortality and a mortality rate similar to the control. Based on this data, a range of concentrations (100 to 2,500 mg kg⁻¹ [mg of extract per kg of seeds]) was set to determine the LC₅₀ values for both males and females, and this was accomplished by applying the formula proposed by Finney (1971). Concentration–response curves were constructed to estimate the EC₅₀ (the effective concentration required to reduce the number of eggs laid per sample by 50% [oviposition deterrence]). The same procedures described previously were used.

**LIQUID–LIQUID PARTITIONING OF THE SELECTED CRUDE EXTRACTS**

Based on the results from the bioassays described previously, the most promising extracts were selected and subjected to liquid–liquid partitioning. Specifically, the selected extracts were solubilized separately in methanol:water (hydro–methanol fraction; 1:3 [v/v] for the leaf and branch extracts and 8:2 [v/v] for the seed extracts) and partitioned in a separatory funnel, using hexane for the seed extract and organic solvents of increasing polarity (hexane, dichloromethane, and ethyl acetate, and hydro–methanol, respectively; and branch fractions yielded 16.59, 3.88, 5.98, and 48.38% for the hexane, dichloromethane, ethyl acetate, and hydro–methanol, respectively).

**ASSESSMENT OF THE BIOACTIVITY OF THE OBTAINED FRACTIONS**

Each of the obtained fractions (phases) was tested to evaluate its lethal and sublethal effects on *Z. subfasciatus*. In this assessment, the sample units (10 g of beans) were treated with these fractions using the respective LC₉₀ estimated for the crude extract, and the same experimental procedures described previously for the screening assay were adopted. For cases where LC₉₀ could not be estimated (> tested range for extracts of leaves and branches), the fractions were tested at the same concentration that was used in the bioassays with the crude extracts (1,500 mg kg⁻¹). In these tests, the same variables and experimental procedures adopted in the previous tests were used, and for each treatment, 10 replicates containing 5 pairs of *Z. subfasciatus*, aged between 0 and 24 h, were used.

**CHEMICAL ANALYSIS OF BIOACTIVE FRACTIONS**

To identify the class of compounds present in the bioactive fractions, hydrogen nuclear magnetic resonance (¹H NMR) was performed with a BRUKER DRX 400 instrument operating at 400 MHz for ¹H nucleus (9.4 Tesla) and the deuterated solvents CDCl₃ and CD,O,OD.

The chemical profiles of the bioactive fractions were analyzed using thin-layer chromatography (TLC). The TLC analyses were carried out by testing the proportions of solvents of different polarities with the aim of identifying the best mobile phase for the separation of the components present in the samples including hexane:dichloromethane 1:1 (v/v), hexane:acetone 8:2, 7:3, and 1:1 (v/v), dichloromethane:acetone 7:3 and 1:1 (v/v), dichloromethane (100%), and acetone (100%). For the detection of the spots of the constituents present in each sample, the following tools were used: ultraviolet light (254 and 365 nm), vanillin sulfuric solution (general developer), ethanolic solution of 1% aluminum chloride (for detection of flavonoids [Jácome et al. 2010]), Dragendorff reagent (specific to alkaloids [Sherma 2000]), and Kedde reagent (indicating the presence of the g-lactone-a,b-unsaturated sub-unit present in acetogenins [Caloprisco et al. 2002]).

**DATA ANALYSES**

Generalized linear models (GLMs) belonging to the exponential family of distributions (Nelder & Wedderburn 1972) were used to assess the biological variables of *Z. subfasciatus* exposed to the extracts and fractions of *A. sylvatica*. The quality of the fit was verified using a half-normal probability graph with a simulation envelope (Hinde & Demétrio 1998). When significant differences were observed between treatments, multiple comparisons (Tukey test, P < 0.05) were performed using the glht function of the multcomp package with adjusted P values. These analyses were performed using the statistical software R, version 2.15.1 (R Development Core Team 2012).

To estimate the lethal concentrations (LC₅₀ and LC₉₀), we used a binomial model with a complementary log-log link function (gompit model), using the Probit Procedure of SAS software, version 9.2 (SAS Institute 2013). To estimate the average effective concentration (EC₅₀), i.e., the concentration required to reduce the number of eggs per sample by 50%, we employed a non-linear logistic model using the Nlin Procedure of SAS software version 9.2 (SAS institute 2013). Finally, the mean lethal time (LT₅₀) was estimated using the method proposed by Throne et al. (1995) for the Probit analysis of correlated data.

**Results**

**BIOLOGICAL ACTIVITY OF ETHANOLIC CRUDE EXTRACTS**

The ethanolic extract from *A. sylvatica* seeds was the only treatment that caused significant mortality of *Z. subfasciatus* adults (Table 1). There was no significant difference in mortality between the sexes (LC₉₀ for females: 701.06 mg kg⁻¹ [CI 95%: 664.85–727.67], χ² = 2.21, df = 5; LC₅₀ for males: 753.47 mg kg⁻¹ [CI 95%: 636.53–804.31], χ² = 10.36, df
and hydro–methanol fractions of the crude seed extract caused significantly lower and high polarity.

The hexane fraction of the ethanolic seed extract, present especially the hexane fraction, in both cases) caused a significant reduction in the number of eggs per sample and the number of \( F_1 \) progeny, and in the damage caused to the treated bean samples (Table 4). Moreover, the hydro–methanol and hexane fractions from the partitioning of the ethanol extract of \( A. \) sylvatica seeds significantly affected all parameters (Table 4). However, there was no difference in effects between these 2 fractions, except for the percentage of damaged seeds, where the hydro–methanol fraction reduced the damage caused to the treated bean samples to a much greater extent than the hexane fraction of the ethanol seed extract.

### CHEMICAL ANALYSIS OF BIOACTIVE FRACTIONS

The analysis of the hydro–methanol fraction of the seeds using TLC (hexane:acetone 7:3 [v/v]) reacted positively with Kedde reagent (spots with a reddish color), as brown spots appeared after the use of sulfuric vanillin solution, indicating the presence of acetogens. The analysis using Dragendorff reagent revealed orange spots at the base of the analytical plate, indicating the presence of polar alkaloids in this fraction, and absorption occurred at 365 nm after visualization using UV light (confirming the presence of compounds with a high conjugation of \( n \) bonds, observed in the alkaloids). This fraction was analyzed using \(^1H\) NMR, which allowed the identification of the major classes of compounds present in this fraction as acetogens. Thus, \( d_1 \), \( 7,28, d_2 \), 5,05, and \( d_3 \), 1,38 refer to the lactone \( \alpha,\beta \)-unsaturated unit, signals at the region of \( d_4 \), 4,42 to \( d_3 \), 3,08 are related to the oxymethylene hydrogens of their substituents, and signals at \( d_4 \), 2,49 and \( d_5 \), 2,37 refer to diastereotopic hydrogens adjacent to the lactone ring (Cortes et al. 1993; Colman-Saizarbitoria et al. 1995).

The hexane fraction of the ethanolic seed extract, present as an oil at room temperature, was similarly analyzed using TLC (hexane:dichloromethane 1:1 [v/v]) and developed using a vanillin/sulfuric acid solution; the appearance of blue spots indicated the presence of \( \pi \) bonds in the material, as observed in the alkaloids. This fraction was analyzed using TLC (hexane:acetone 7:3 [v/v]), and absorption occurred at 365 nm after visualization using UV light (confirming the presence of compounds with a high conjugation of \( n \) bonds, observed in the alkaloids). This fraction was analyzed using \(^1H\) NMR, which allowed the identification of the major classes of compounds present in this fraction as acetogens. Thus, \( d_1 \), 7,28, \( d_2 \), 5,05, and \( d_3 \), 1,38 refer to the lactone \( \alpha,\beta \)-unsaturated unit, signals at the region of \( d_4 \), 4,42 to \( d_3 \), 3,08 are related to the oxymethylene hydrogens of their substituents, and signals at \( d_4 \), 2,49 and \( d_5 \), 2,37 refer to diastereotopic hydrogens adjacent to the lactone ring (Cortes et al. 1993; Colman-Saizarbitoria et al. 1995).
ence of triglycerides. These compounds are the major constituents of vegetable seed oils, which are also classified as triacylglycerols, where in each functional ester group contains a saturated or unsaturated hydrocarbon chain (Fernandes et al. 2002). The presence of these compounds in each functional ester group contains a saturated or unsaturated hydrocarbon chain (Fernandes et al. 2002). The presence of these compounds in this fraction was confirmed by the analysis of signals in the H NMR spectrum, showing the characteristic signals observed in the H NMR spectrum, showing the characteristic signs of lignans and alkaloids as observed in the literature (Tantisewi et al. 1989; Biavatti et al. 2001; Pinheiro et al. 2009).

The dichloromethane fraction of the branches was eluted in dichloromethane:acetone 7:3 (v/v), and after the staining of the chromatographic plate with Dragendorff reagent, orange spots that interacted strongly with the silica (retention factor: intermediate to high) could be visualized, indicating the presence of alkaloids as constituents of this fraction. This evidence was confirmed by the analysis of the signals observed in the 1H NMR spectrum, which presented a signal for this class of compound as observed by Colzato et al. (2008).

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Due to the non-polar nature of the hexane fractions from the leaves and branches, their chromatographic profiles were analyzed using TLC and 1H NMR. The TLC analysis allowed the comparison of the chemical profiles of both fractions, which were characterized by the presence of spots with the same retention factor for both samples (indicating the similarity of compounds present in both fractions) after elution with hexane:acetone 8:2 (v/v) and staining with vanillin/sulfuric acid solution. The analysis of the 1H NMR spectrum showed signals of chemical shifts that were characteristic of furofuranlignans, as described by Biavatti et al. (2001). Additionally, signals similar to those described for the hexane fraction of seeds were observed, which indicated the presence of triacylglycerols (Colzato et al. 2008).

Table 3. Mortality (mean ± SE) of Zabrotes subfasciatus adults exposed to samples of bean seeds treated with fractions prepared by liquid–liquid partitioning from ethanolic extracts of different Annona sylvatica parts after they had been steeped for 5 d in ethanol.

| Fractions                | Males         | Females        | Total          |
|--------------------------|---------------|----------------|----------------|
| A. sylvatica leaves      |               |                |                |
| Hexane                   | 4.00 ± 2.67   | 6.00 ± 4.27    | 5.00 ± 3.07    |
| Dichloromethane          | 6.00 ± 3.05   | 6.00 ± 4.20    | 6.00 ± 3.09    |
| Ethylacetate             | 4.00 ± 2.67   | 8.00 ± 4.42    | 6.00 ± 2.21    |
| Hydro–methanol           | 2.00 ± 2.00   | 8.00 ± 3.27    | 4.00 ± 1.63    |
| Control (acetone)        | 8.00 ± 4.42   | 8.00 ± 4.42    | 8.00 ± 3.59    |
| Control (methanol)       | 10.00 ± 4.47  | 8.00 ± 3.27    | 9.00 ± 2.77    |
| F value                  | 0.81**        | 0.07**         | 0.33**         |
| P value                  | 0.5479        | 0.9968         | 0.8911         |
| A. sylvatica branches    |               |                |                |
| Hexane                   | 24.00 ± 5.81  a| 30.00 ± 5.37 a | 27.00 ± 4.95   |
| Dichloromethane          | 22.00 ± 10.08 a| 14.00 ± 6.70 a | 18.00 ± 7.27   |
| Ethylacetate             | 12.00 ± 3.26  a| 12.00 ± 4.42 a | 12.00 ± 3.26   |
| Hydro–methanol           | 2.00 ± 2.00   a| 28.00 ± 8.54 a | 15.00 ± 4.53   |
| Control (acetone)        | 8.00 ± 4.42   a| 8.00 ± 4.42    a| 8.00 ± 3.59    |
| Control (methanol)       | 10.00 ± 4.47  a| 8.00 ± 3.27    a| 9.00 ± 2.77    |
| F value                  | 2.62          | 2.78           | 2.21**         |
| P value                  | 0.0342        | 0.0263         | 0.0668         |
| A. sylvatica seeds       |               |                |                |
| Hexane                   | 30.00 ± 9.45  a| 6.00 ± 2.89 ab | 15.00 ± 5.96 a |
| Hydro–methanol           | 44.00 ± 12.22 a| 18.00 ± 3.59 a | 31.00 ± 6.57 a |
| Control (acetone:methanol 1:1 [v/v]) | 2.00 ± 2.00 b | 4.00 ± 2.67 b | 3.00 ± 1.53 b |
| F value                  | 16.32         | 4.40           | 17.11          |
| P value                  | < 0.0001      | 0.0221         | < 0.0001       |

*Means followed by different letters within columns (each plant part) indicate significant differences between treatments (GLM with a quasi-binomial distribution followed by Tukey’s post hoc test, P < 0.05).

**Non-significant (P > 0.05).
Table 4. Sublethal effects (mean ± se) on Zabrotes subfasciatus of fractions prepared by liquid–liquid partitioning from ethanolic extracts of different parts of Annona sylvatica at 1,500 mg kg⁻¹.

| Fraction | No. eggs/sample | F₁ progeny* | Viability (%) (egg–adult)$^a$ | Sex ratio$^a$ | Grains damaged (%)$^a$ |
|----------|----------------|-------------|--------------------------------|--------------|----------------------|
|          | Males          | Females     | Total                          |              |                      |
| A. sylvatica leaves |
| Hexane   | 1.20 ± 0.72 c  | 0.60 ± 0.43 d | 0.60 ± 0.34 c                  | 1.20 ± 0.73 c | 100.00 ± 0.00$^c$  | 0.50 ± 0.00$^c$ | 3.20 ± 1.80 d |
| Dichloromethane | 79.60 ± 8.96 a  | 30.40 ± 3.78 b   | 34.10 ± 3.77 a                  | 64.50 ± 6.92 a | 82.15 ± 2.21       | 0.53 ± 0.03 | 66.75 ± 4.64 b |
| Ethyl acetate | 27.90 ± 7.32 b | 9.40 ± 2.53 c    | 11.30 ± 3.36 b                  | 24.70 ± 5.77 b | 72.08 ± 4.82       | 0.42 ± 0.08 | 32.17 ± 7.73 c |
| Hydro–methanol | 100.40 ± 6.70 a | 39.50 ± 2.10 ab  | 40.20 ± 2.39 a                  | 79.70 ± 4.12 a | 77.27 ± 1.69       | 0.50 ± 0.01 | 92.32 ± 1.60 a |
| Control (acetone) | 103.50 ± 7.84 a | 48.80 ± 3.96 a   | 42.30 ± 2.84 a                  | 87.10 ± 6.26 a | 84.40 ± 1.32       | 0.49 ± 0.01 | 93.16 ± 1.93 a |
| Control (methanol) | 82.30 ± 4.90 a | 36.90 ± 2.10 ab  | 36.00 ± 2.84 a                  | 72.90 ± 4.59 a | 88.35 ± 2.07       | 0.48 ± 0.01 | 89.02 ± 2.79 a |

| A. sylvatica branches |
| Hexane   | 15.80 ± 4.44 d | 5.00 ± 1.48 c   | 7.40 ± 2.31 a                   | 12.40 ± 3.47 d | 81.91 ± 3.99       | 0.61 ± 0.09$^c$ | 22.13 ± 4.82 d |
| Dichloromethane | 37.90 ± 7.03 c  | 13.80 ± 2.75 b  | 16.70 ± 2.60 b                  | 30.50 ± 5.17 c | 84.26 ± 3.65       | 0.55 ± 0.03 | 49.28 ± 6.75 c |
| Ethyl acetate | 83.90 ± 5.87 ab | 37.90 ± 3.26 a  | 36.30 ± 3.13 a                  | 74.20 ± 5.81 ab | 88.01 ± 1.50       | 0.49 ± 0.02 | 86.23 ± 2.76 ab |
| Hydro–methanol | 61.80 ± 4.57 b  | 20.20 ± 2.46 b  | 20.20 ± 2.46 b                  | 40.50 ± 3.87 b | 92.72 ± 1.78       | 0.45 ± 0.02 | 68.99 ± 5.67 b |
| Control (acetone) | 103.50 ± 7.84 a | 44.80 ± 3.96 a  | 42.30 ± 2.84 a                  | 87.10 ± 6.26 a | 84.40 ± 1.32       | 0.49 ± 0.01 | 93.16 ± 1.93 a |
| Control (methanol) | 82.30 ± 4.90 ab | 36.90 ± 2.10 a  | 36.00 ± 2.84 a                  | 72.90 ± 4.59 ab | 88.35 ± 2.07       | 0.48 ± 0.01 | 89.02 ± 2.79 a |

| A. sylvatica seeds |
| Hexane   | 21.50 ± 4.11 b | 3.50 ± 0.49 b   | 5.00 ± 0.70 b                   | 8.50 ± 1.00 b  | 37.89 ± 3.73 b     | 0.53 ± 0.04$^c$ | 62.07 ± 9.78 b |
| Hydro–methanol | 11.60 ± 4.57 b | 3.10 ± 0.64 b   | 1.60 ± 0.74 b                   | 2.90 ± 1.33 b  | 15.93 ± 4.67 b     | 0.36 ± 0.13$^c$ | 7.55 ± 3.02 c |
| Control (acetone:methanol 1:1 [v/v]) | 72.10 ± 4.86 a | 23.10 ± 2.18 a  | 22.20 ± 2.12 a                  | 45.30 ± 3.87 a | 62.52 ± 2.84 a     | 0.49 ± 0.02 | 95.00 ± 1.63 a |

*Means followed by different letters within columns indicate significant differences between treatments (GLM with a quasi-Poisson distribution followed by Tukey’s post hoc test, P < 0.05).

*Sex ratio can be inferred from the female fraction data, which are presented here.

*Not included in the analysis due to a small sample unit.

Many oil products are used as adjuvants in the application of pesticides. They may act as a vehicle that helps the active ingredient come into contact with the target (kidney bean) and achieve better coverage of its surface, thereby assisting the principal compound in exerting its insecticidal action. Therefore, it is possible that triglycerides only help the active ingredients to express their insecticidal effect. However, if triglycerides promote the mortality of exposed insects, this may be due to asphyxia caused by the blocking of weevil spiracles (Hewlett 1947).

By contrast, if the insecticidal effect is due to the presence of minor chemical compounds or compounds derived from the breakdown of triglycerides, then in the latter case, fatty acids probably are responsible for this bioactivity. A study conducted by Ramsewak et al. (2001) supports this hypothesis, as these authors identified 5 compounds in the hexane extract of the seeds of Dirca palustris L. (Malvales: Thymeleaceae). Three triglycerides (1,3-dilinoleoyl-2-olein; 1,3-dioleoyl-2-linolen; 1,2,3-trilinolein) showed no bioactivity, but the other 2 compounds (oleic acid and linoleic acid) isolated from the methanol fraction of the hexane extract of the seeds promoted larval mortality of the 4th instar of Aedes aegypti (L.) (Diptera: Culicidae), and reduced the growth of Helicoverpa zea Boddie (Lepidoptera: Noctuidae), Lymantria dispar L. (Lepidoptera: Lymantriidae), Orgyia leucostigma (Smith) (Lepidoptera: Lymantriidae), and Malacosoma disstria Hübner (Lepidoptera: Lasiocampidae) larvae, likely due to the phagodeterrent action of these compounds. These results demonstrate that fatty acids may exert bioactivity on the insects, which was also reported by Fatope et al. (2000).
Similarly, Annonaceae species have the same fatty acids in their seeds. Egydio & Santos (2011) observed little variation in the fatty acids of various species of *Annona* (*A. crassiflora*, *A. coriacea*, *A. montana*, *A. cherimola*, *A. squamosa* “Pink Mammoth” and *A. cherimola* “A. squamosa ‘Gefner’”), and they found palmitic, stearic, oleic, and linoleic acids to be the major components in all species. Chemical characterization of seed fatty acids from *A. sylvatica* showed the presence of palmitic (20.84%), stearic (4.26%), oleic (54.41%), and linoleic acids (20.49%) (Andrade et al. 2012). Annonaceae species, including *A. sylvatica*, produce certain fatty acids with insecticidal effects, as reported by Peñaflor et al. (2006). Moreover, in the current study, the hexane extract of the seeds of *A. sylvatica* drastically decreased the oviposition of *Z. subfuscatus* females. Thus, some fatty acids present in *Annona* seeds may deter oviposition. Indeed, Peñaflor et al. (2006) demonstrated that fatty acids that have between 5 and 9 carbon atoms in their chains are capable of affecting the behavior of insects; more specifically, these fatty acids can repel workers of *Atta sexdens rubropilosa* Forel (*Hymenoptera: Formicidae*).

During the evaluation of the experiments investigating the effect of exposure to the hydro–methanol fraction of the *A. sylvatica* seed extract, a decrease in muscle coordination of the beetles was observed, and this symptom is characteristic of the effect promoted by acetogenins (González-Coloma et al. 2002). The acetogenins (C-35/C-37) are derived from long-chain fatty acid (C-32/C-34) units that combine with 2-propenal, and they have a wide range of biological effects, including insecticidal activity (Alali et al. 1999). Mikolajczak et al. (1990) reported that the insecticidal action of the hexane extract of *A. sylvatica* fruit on *O. nubilalis* and *A. vittata* was due to the presence of sylvaticin, an acetogenin. Acetogenins act by interrupting energy production in the mitochondria through the inhibition of complex I (NADH: ubiquinone oxireductase) of the electron transport system and NADH oxidase at the plasma membrane (González-Coloma et al. 2002).

The presence of several active principles that act at different sites by exerting effects on the physiology and behavior of insects potentially reduces the number of resistant pest populations (Rattan 2010), which is an important advantage of botanical insecticides. Alali et al. (1999) showed that 6 acetogenins exhibited insecticidal effects that were equivalent to or greater than the effects of 5 commercial products (synthetic amidinohydrazone [hydramethylnon], carbamate [propoxur, bendiocarb], organophosphate [chlorpyrifos], and pyrethroid [cypermethrin]) was due to the presence of sylvaticin, an acetogenin. Acetogenins act by exerting effects on the physiology and behavior of insects potentially through the inhibition of complex I (NADH: ubiquinone oxireductase) of the electron transport system and NADH oxidase at the plasma membrane.

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