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# Toxicity of the Hexane Fraction of Fruits and Seeds of *Ricinus communis* to Caterpillars of the *Spodoptera* Complex

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**Abstract:** The objective of this work was to evaluate the effect of the hexane fraction of the crude extract of the fruits and seeds of *Ricinus communis* (HFFSRC) on eggs of *Spodoptera cosmioides* and *Spodoptera frugiperda* and on caterpillars of *Spodoptera eridania*, *S. frugiperda*, and *S. cosmioides*, under laboratory conditions through topical application and ingestion, as well as to identify the compounds in the hexane fraction through high-performance chromatography (HPLC-ESI-Q-TOF-MS/MS). To do so, three bioassays were conducted: (1) the effect of HFFSRC at 2% (20,000 mg·mL$^{-1}$) on eggs of *S. cosmioides* and *S. frugiperda*, (2) the lethal effect of HFFSRC at 2% applied topically, and (3) the lethal effect of HFFSRC at 2% applied to soybean-leaf discs on first-, second-, third-, and fourth-instar caterpillars of the three insect species. It was found that 2% HFFSRC had an ovicidal effect on *S. frugiperda* and *S. cosmioides*, completely reducing larvae hatching and the insecticidal effect for the four instars of the three insect species when applied topically and on food. Five compounds were identified in the HFFSRC: three flavonoids, one ricinoleic acid, and one cinnamic acid. HFFSRC at 2% had an acute ovicidal and insecticidal effect on caterpillars of the studied species, configuring itself as a potential insecticide.

**Keywords:** botanical insecticide; *Spodoptera frugiperda*; *Spodoptera cosmioides*; *Spodoptera eridania*; IPM

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## 1. Introduction

Lepidoptera stands out among the complex insect pests affecting crops. Among the Lepidoptera species, the *Spodoptera* complex, the black armyworm *Spodoptera cosmioides* (Walker, 1858), the southern armyworm *Spodoptera eridania* (Cramer, 1782), and the fall armyworm *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) are considered key pests of crops of economic importance due to the potential for defoliation and destruction of reproductive structures [1,2] These insects are polyphagous and difficult to control, with synthetic insecticides being the most used method. However, it is known that the excessive use of synthetic insecticides can cause several negative effects, such as the selection of resistant populations, the emergence of secondary pests, the resurgence of pests, and biological imbalances [3–5]. In addition, they can cause negative effects on several non-target organisms, such as parasitoids [6,7], pollinators [8,9], and farmers.

Humankind's use of plant derivatives is an ancient practice due to their therapeutic, insecticidal, repellent, and antimicrobial properties. Nevertheless, since the 1990s, research on plant compounds has intensified [3,10–13] as a strategic alternative of viable technological innovation for the development of more sustainable agriculture. The perspectives are promising in the context of integrated pest management (IPM), extrapolating the frontiers

of use by small producers, since botanical insecticides associated with other control strategies can contribute to the reduction of doses and applications of synthetic insecticides [14]. Furthermore, the botanical insecticides studied are usually environmentally safer compared to synthetic insecticides, as reported for bees [15,16] and parasitoids [17].

The properties of botanical derivatives result from substances originating from the secondary metabolism of plants, developed throughout the evolutionary process as a defense strategy against attack by pathogens and herbivores, especially insects [11,12,18]. Such substances can be obtained from different parts of the plant, such as stems, flowers, leaves, bark, roots, and fruits [19], and may have direct effects, such as insecticidal activity on adults [20,21] and younger instars [22–25], feeding inhibition [21], and ovicidal effects [26]. In addition, they may have side effects such as reduced oviposition [25,27], reduced fertility, and the occurrence of developmental anomalies [27], among others.

A plant that has gained prominence in research into the control of insects and diseases and due to its applications in industry is the castor-bean plant, *Ricinus communis* L. (Malpighiales: Euphorbiaceae). It is a cosmopolitan species in tropical regions [28], and in Brazil, the main producing regions are the Southeast, Northeast, and South [29]. It has a high oil content, with wide applicability in industry. It is characterized by producing ricinoleic acid and other compounds with medicinal properties and microbial and insecticidal effects, depending on the parts of the plant and the extractor solvents used [28]. The main toxic compounds present in castor-bean leaf and fruit extracts are ricin and ricinolein, both of which have an insecticidal effect [30] that can act on insects, causing several effects such as reduced growth and changes in ecdysis, including effects on their feeding behavior [31]. Some studies have already shown the insecticidal effect of castor-bean extracts, such as on the malaria-vector mosquito, *Anopheles gambiae* (Giles, 1902) (Diptera: Culicidae) [32]; the corn weevil, *Sitophilus zeamais* (Mots., 1855) (Coleoptera: Curculionidae) [33]; the coffee berry *Hypothenemus hampei* (Ferrari, 1867) (Coleoptera: Scolytineae) [34]; the red flour beetle *Tribolium castaneum* (Herbst, 1897) (Coleoptera: Tenebrionidae) and the cigarette beetle *Lasioderma serricorne* (Fabricius, 1792) (Coleoptera: Anobiidae) [35], and the fruit flies *Ceratitis capitata* (Wiedemann, 1824) and *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae) [36]. The insecticidal potential of the hexane fraction of the fruits and seeds of *R. communis* (HFFSRC) has already been verified for second-instar caterpillars of the soybean looper *Chrysodeixis includens* (Walker, 1857) (Lepidoptera: Noctuidae), with an average lethal concentration ($LC_{50}$) of 20,000 mg·$L^{-1}$ [37].

In this context, considering the importance of the *Spodoptera* complex as an insect pest, the demand for more sustainable control alternatives, and the potential of HFFSRC as a botanical insecticide, it is necessary to investigate the possible effects of HFFSRC on caterpillars of this complex. Thus, the present work aimed to evaluate the effect of the HFFSRC on eggs of *S. cosmioides* and *S. frugiperda* and caterpillars of different instars of *S. cosmioides*, *S. frugiperda*, and *S. eridania* under laboratory conditions in two application modalities (topical and ingestion), in addition to identifying the compounds present in the hexane fraction.

## 2. Materials and Methods

Solvents of HPLC grade (methanol, acetonitrile, and acetic acid) were acquired from J.T. Baker (Phillipsburg, NJ, USA); hexane and ethanol were purchased from Dinamica (Diadema, SP, Brazil).

The bioassays were conducted in insect-rearing rooms, with a controlled environment, at a temperature of 26 ± 2 °C, a relative humidity (RH) of 65 ± 10%, and a 12 h photophase. The hexane fraction of the crude ethanolic extract of *R. communis* fruits and seeds (HFFSRC) at 2% was evaluated on insects of the *Spodoptera* complex in three different bioassays: (1) the topical effect of HFFSRC on eggs of *S. cosmioides* and *S. frugiperda*; (2) the lethal effect of HFFSRC applied topically on first-, second-, third-, and fourth-instar larvae of *S. cosmioides*, *S. eridania*, and *S. frugiperda*, and (3) the lethal effect of HFFSRC applied on food offered to first-, second-, third-, and fourth-instar larvae of *S. cosmioides*, *S. eridania*, and *S. frugiperda*.

### 2.1. Obtaining Insects and Soybean Plants

Eggs of *S. cosmioides*, *S. eridania*, and *S. frugiperda* were provided by the company Corteva Agriscience™, based in Toledo—PR, Brazil. For the first bioassay, part of the eggs was placed in a refrigerator for a maximum of 24 h at a temperature of approximately 4 °C. At the same time, to conduct the other bioassays, the eggs were kept in disposable plastic cups, with an artificial diet developed by Greene at al. [38] and modified by Hoffmann [39] containing beans, brewer's yeast, casein, soy protein, wheat germ, vitamins, antibiotics, sorbic acid, ascorbic acid, and nipagim and supplied until the first-, second-, third-, and fourth-instar stages were reached, and were kept in a rearing room.

To obtain the soybean plant *Glycine max* L. Merrill (Fabaceae), which was used in the experiment in the first half of September 2021, three conventional soybean seeds of the cultivar BRS 525 were sown in pots with a 254 mm diameter × 250 mm height, containing soil from an organic-cultivation area and kept in a greenhouse on a rural property on Carlos Gomes road in Dois Vizinhos, PR, Brazil, at latitude and longitude coordinates of 25°41′37″ S 53°02′04″ W. Until the bioassays were conducted, the plants did not receive any phytosanitary treatment during development. Soybean leaves for feeding the caterpillars were removed 40 to 55 days after plant emergence.

### 2.2. Obtaining the Hexane Fraction of Fruits and Seeds of R. communis (HFFSRC)

The fruits (capsule and seeds) of *R. communis* were collected in an area of native vegetation located on a rural property in the municipality of Dois Vizinhos, PR, Brazil, at latitude and longitude coordinates of 25°42′14″ S 53°05′34″ W and an altitude of 521 m in March 2021 in the afternoon between 5:00 p.m. and 6:30 p.m. The collected material was sorted and the castor-bean fruits were individualized. The castor-bean fruits were then packed in kraft paper (60 × 80 cm), remaining in a forced-circulation drying oven for 48 h at 60 °C. After completely drying the material, the fruits and seeds were crushed in a Willey knife mill (ALPAX) until a fine powder with a granulometry of 0.5 mm was obtained.

To prepare the crude ethanolic extract of *R. communis*, 100 g of the powder were diluted in 1 L of 80% ethanol in Erlenmeyer flasks with a capacity of 1 L. The mixture was transferred to 1 L Becker flasks and placed in a bath of water thermostated at 60 °C for 30 min. Then, the obtained solution/extract was filtered with an 8 µ filtering membrane in a Kitasato flask coupled to a vacuum pump (TECNAL-TE058) at a constant pressure of 1.2 kgf/cm$^2$, obtaining the crude ethanolic extract.

The crude ethanolic extract was fractionated using the liquid–liquid extraction technique [40]. With a separation funnel with a volumetric capacity of 1 L, 250 mL of crude ethanolic extract of *R. communis* and 250 mL of hexane extract were placed. The mixture was manually stirred for about one minute to obtain greater homogenization, and after 15 min of rest, the two phases separated. The crude extract was deposited at the bottom of the funnel and the hexane fraction at the top, and the part of interest was collected through the tap of the separation funnel.

The HFFSRC was then subjected to a rotary evaporator at 42 to 45 °C to remove the solvent completely. Then, it was stored in a refrigerator (4 °C) without light until the bioassays were conducted. When the hexane fraction was used, it was diluted in 90% ethanol to obtain a 2.0% concentration (20,000 mg·mL$^{-1}$). The choice of hexane-fraction concentration for the study was based on a previous study by [37], wherein it was determined that the average lethal concentration ($LC_{50}$) of *R. communis* for *C. includens* caterpillars was 20,000 mg·L$^{-1}$ (2.0%).

### 2.3. Evaluation of the Effect of HFFSRC on S. cosmioides, S. eridania, and S. frugiperda

In all bioassays, the evaluated treatments were (T1) distilled and sterilized water, (T2) commercial product Bazuka 216 SL based on methomyl (216 g/L ai.; evaluated according to the package-insert recommendation (0.33 mL/100 mL/water)), (T3) HFFSRC (2%), and (T4) 90% ethanol. The experimental design used for all bioassays was completely randomized.

2.3.1. Bioassay 1: Effect of 2% HFFSRC on Eggs of *S. cosmioides* and *S. frugiperda*

Postures laid out on butter paper were separated into cards (approximately 2.0 × 2.0 cm), each containing 30 eggs, which were subsequently immersed in the treatments for five seconds, as adapted by Matharu and Mehta [41]. For each treatment, 10 cards (replications) were prepared, with 30 eggs of each insect species per card. After immersion, the cards containing eggs of *S. cosmioides* and *S. frugiperda* were individualized in glass tubes (25 mm diameter × 100 mm height) and placed in a climate-controlled room under the described conditions. The evaluation was conducted daily for five days, quantifying the number of hatched caterpillars.

2.3.2. Bioassay 2: Lethal Effect of 2% HFFSRC Applied Topically on First-, Second-, Third-, and Fourth-Instar Caterpillars of *S. cosmioides*, *S. eridania*, and *S. frugiperda*

For the evaluation of the lethal effect of HFFSRC at 2% applied topically on caterpillars of *S. cosmioides*, *S. eridania*, and *S. frugiperda*, four bioassays were conducted separately, with first-, second-, third-, and fourth-instar caterpillars. Acrylic plates with 12 cell-culture wells were used. In each well, soybean-leaf discs of approximately 5.4 ± 0.1 $cm^2$ were placed according to the method described by Escoubas et al. [42] and lined with filter-paper discs moistened with distilled water.

With a brush with fine bristles, a caterpillar from the evaluated instar was inserted into each well. Then, with a micropipette, 5 µL of each treatment was applied to the back of each caterpillar. The plates were identified and kept in a breeding room under the described conditions. Five acrylic plates (replications) were used for each treatment, with 12 caterpillars placed in each one, totaling 60 caterpillars per treatment.

The evaluation was conducted daily every 24 h until the 10th day after applying the treatments, quantifying the number of dead insects. Caterpillars that did not respond to three touches of a soft-bristle brush were considered dead.

2.3.3. Bioassay 3: Lethal Effect of HFFSRC at 2%, Applied to Soybean-Leaf Discs to Be Ingested by First-, Second-, Third-, and Fourth-Instar Caterpillars of *S. cosmioides*, *S. eridania*, and *S. frugiperda*

To evaluate the lethal effect of HFFSRC at 2% applied to food and offered to *S. cosmioides*, *S. eridania*, and *S. frugiperda* caterpillars, four bioassays were carried out separately, with first-, second-, third-, and fourth-instar caterpillars. Soybean-leaf disks were obtained as described in bioassay 2, immersed in the treatments, and arranged in Petri dishes (150 × 25 mm). The disks were placed in a laminar-flow chamber for 20 min until the water evaporated and then placed in 12-well acrylic plates, and a caterpillar of the corresponding instar was placed in each well. Conditioning, experimental design, and evaluation were similar to those described in bioassay 2.

*2.4. Analysis of the HFFSRC by High-Performance Liquid Chromatography—HPLC-ESI-Q-TOF-MS/MS*

A 20 mg HFFSRC sample was resuspended in 2 mL of water/acetonitrile (1:1), and 10 µL were injected and analyzed in a high-performance liquid chromatograph (Shimadzu, Japan) coupled to the Phenomenex Luna C18 (250 × 4.6 mm–5 µm) with a flow rate of 1 mL $min^{-1}$. The solvent-gradient mixture for negative analysis was solvent A ($H_2O$ with 0.5% acetic acid, *v*:*v*), and solvent B, acetonitrile:methanol (70:30), was as follows: 15% B 0–5 min, 20% B 5–18 min, 40% B 18–40 min, and 100% B 40–50 min and held at 100% B for up to 50 min at 30 °C, with the final nine minutes devoted to column reconstitution for the next analysis.

MS/MS experiments were performed on a Q-TOF MAXIS 3G high-resolution mass spectrometer (Bruker Daltonics Corporation, Breemen, Germany) equipped with an electrospray-ionization source. The ionization source was operated in negative-ionization mode and set to 4500 V with an end-plate compensation potential of −500 V. Drying-gas parameters were set to 8 L $min^{-1}$ at 250 °C and gas pressure of mist at 2 bar. Data were collected from $m/z$

50 to 1800 with a 5 Hz acquisition rate, with the 5 most intense ions selected for automatic fragmentation (Auto MS/MS).

### *2.5. Statistical Analysis*

The data obtained in the bioassays were submitted to exploratory analyses to evaluate the assumptions of normality in the residues (Lilliefors test) and the homogeneity of the variance of the treatments (Bartlett test). The data did not show normal distribution, so the Kruskal–Wallis non-parametric test was performed at a 5% significance level. Mortality tests were compared with each other using Mann–Whitney. All statistical analyses were performed using the Rbio software [43].

## 3. Results

### *3.1. Evaluation of the Effect of HFFSRC on S. cosmioides, S. eridania, and S. frugiperda*

#### 3.1.1. Bioassay 1: Effect of 2% HFFSRC on Eggs of *S. cosmioides* and *S. frugiperda*

It was found that the 2% HFFSRC applied on eggs of the *S. frugiperda* and *S. cosmioides* moths completely prevented the hatching of the larvae, as was also verified in the positive control (synthetic insecticide). In the negative controls (water and ethanol), the larvae hatching was 100% and 99%, respectively. No difference was observed in response to treatments when comparing the species *S. frugiperda* and *S. cosmioides* (Table 1).

**Table 1.** Percentage of hatching (±SE) of caterpillars of *Spodoptera frugiperda* and *Spodoptera cosmioides* from eggs immersed in the hexane fraction of the fruits and seeds of *Ricinus communis* at 2% and controls.

| % Hatching of Caterpillars at 5 Days | | | |
|---|---|---|---|
| **Treatment** | ***S. frugiperda*** | ***S. cosmioides*** | ***p*=** |
| Water | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 0.0001 |
| HFFSRC at 2% | 0.00 ± 0.00 bA | 0.00 ± 0.00 bA | - |
| BAZUKA 216 SL | 0.00 ± 0.00 bA | 0.00 ± 0.00 bA | - |
| Ethanol 90% | 99.66 ± 0.31 aA | 99.00 ± 0.67 aA | 0.0003 |
| *p*= | 0.0002 | 0.0002 | |

Means followed by the same lowercase letter in the column do not differ per the Kruskal–Wallis test ($p \leq 0.05$). Means followed by the same capital letter in the row do not differ per the Mann–Whitney test ($p \leq 0.05$).

#### 3.1.2. Bioassay 2: Lethal Effect of 2% HFFSRC Applied Topically on First-, Second-, Third-, and Fourth-Instar Caterpillars of *S. cosmioides*, *S. eridania* and *S. frugiperda*

The HFFSRC at 2% caused significant mortality for the three species of caterpillars evaluated in all their instars, with the first- and second-instar caterpillars being the most susceptible with a mortality of 100%, and differing from the negative controls and not differing from the positive control (BAZUKA 216SL). It was observed that the mortality caused by HFFSRC at 2% in fourth-instar caterpillars of *S. frugiperda* and *S. cosmioides* was lower, differing significantly from the other instars. In the case of *S. eridania*, the mortality of third- and fourth-instar caterpillars was lower, not differing from each other but differing from first- and second-instar caterpillars in the 2% HFFSRC treatment (Table 2).

#### 3.1.3. Bioassay 3: Lethal Effect of HFFSRC at 2% Applied to Soybean-Leaf Discs and Arranged to Feed Caterpillars of First, Second, Third, and Fourth Instars of *S. cosmioides*, *S. eridania*, and *S. frugiperda*

An insecticidal effect of 2% HFFSRC was found on all instars of the three evaluated insect species, with mortality differing from the respective negative controls (water and ethanol) and not differing from the positive control (BAZUKA 216 SL) (Table 3).

**Table 2.** Mortality (%) (±SE) at 10 days of first-, second-, third-, and fourth-instar caterpillars of *Spodoptera frugiperda*, *Spodoptera eridania*, and *Spodoptera cosmioides* after topical application of the hexane fraction of the fruits and seeds of *Ricinus communis* at 2% and controls.

| ***Spodoptera frugiperda*** | | | | | |
|---|---|---|---|---|---|
| **Treatment** | **% Mortality Accumulated in 10 Days** | | | | |
| | **1st Instar** | **2nd Instar** | **3rd Instar** | **4th Instar** | ***p*=** |
| Water | 9.99 ± 0.20 bA | 14.99 ± 0.58 bA | 5.00 ± 0.37 cB | 3.33 ± 0.24 cB | 0.0070 |
| HFFSRC at 2% | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 78.33 ± 0.24 bA | 68.33 ± 0.49 bB | 0.0024 |
| BAZUKA 216 SL | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 96.66 ± 0.24 aA | 0.0013 |
| Ethanol 90% | 4.99 ± 0.24 bB | 6.66 ± 0.37 bB | 15.00 ± 0.37 cA | 11.66 ± 0.40 cA | 0.0241 |
| *p*= | 0.0002 | 0.0004 | 0.0010 | 0.0032 | |
| ***Spodoptera eridania*** | | | | | |
| **Treatment** | **% Mortality Accumulated in 10 Days** | | | | |
| | **1st Instar** | **2nd Instar** | **3rd Instar** | **4th Instar** | ***p*=** |
| Water | 9.99 ± 0.49 bA | 9.99 ± 0.49 bA | 11.66 ± 0.51 cA | 13.33 ± 0.24 cA | 0.0115 |
| HFFSRC at 2% | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 68.33 ± 0.49 bB | 49.99 ± 0.55 bB | 0.0235 |
| BAZUKA 216 SL | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 96.66 ± 0.40 aA | 0.0025 |
| Ethanol 90% | 10.00 ± 0.58 bB | 8.33 ± 0.45 bB | 18.33 ± 0.37 cA | 16.66 ± 0.31 cA | 0.0157 |
| *p*= | 0.0005 | 0.0020 | 0.0051 | 0.0019 | |
| ***Spodoptera cosmioides*** | | | | | |
| **Treatment** | **% Mortality Accumulated in 10 Days** | | | | |
| | **1st Instar** | **2nd Instar** | **3rd Instar** | **4th Instar** | ***p*=** |
| Water | 3.33 ± 0.40 bC | 10.00± 0.58 bA | 6.66 ± 0.37 cB | 8.33 ± 0.45 cA | 0.0281 |
| HFFSRC at 2% | 100.00 ± 0.00 aA | 100.00 ± 0.20 aA | 78.33 ± 0.24 bA | 48.33 ± 0.49 bB | 0.0051 |
| BAZUKA 216 SL | 100.00 ± 0.00 aA | 100.00 ± 0.00aA | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 0.0002 |
| Ethanol 90% | 5.00 ± 0.40 bA | 5.00 ± 0.40 bA | 6.66 ± 0.37 cA | 8.33 ± 0.45 cA | 0.0017 |
| *p*= | 0.0002 | 0.0002 | 0.0004 | 0.0002 | |

Means followed by the same lowercase letter in the column do not differ per the Kruskal–Wallis test ($p \leq 0.05$). Means followed by the same capital letter in the row do not differ per the Mann–Whitney test ($p \leq 0.05$).

**Table 3.** Mortality (%) (±SE) at 10 days of first-, second-, third-, and fourth-instar caterpillars of *Spodoptera frugiperda*, *Spodoptera eridania,* and *Spodoptera cosmioides* after immersion of soybean-leaf discs in the hexane fraction of the fruits and seeds of *Ricinus communis* at 2% and controls.

| ***Spodoptera frugiperda*** | | | | | |
|---|---|---|---|---|---|
| **Treatment** | **% Mortality Accumulated in 10 Days** | | | | |
| | **1st Instar** | **2nd Instar** | **3rd Instar** | **4th Instar** | ***p*=** |
| Water | 6.66 ± 0.37 bA | 0.00 ± 0.00 cB | 0.00 ± 0.00 cB | 8.33 ± 0.31 bA | 0.0031 |
| HFFSRC at 2% | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 0.0001 |
| BAZUKA 216 SL | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 0.0001 |
| Ethanol 90% | 6.66 ± 0.37 bB | 6.66 ± 0.37 bB | 6.66 ± 0.37 bB | 13.33 ± 0.51 bA | 0.0028 |
| *p*= | 0.0008 | 0.0005 | 0.0008 | 0.0009 | |
| ***Spodoptera eridania*** | | | | | |
| **Treatment** | **% Mortality Accumulated in 10 Days** | | | | |
| | **1st Instar** | **2nd Instar** | **3rd Instar** | **4th Instar** | ***p*=** |
| Water | 0.00 ± 0.00 cB | 0.00 ± 0.00 bB | 0.00 ± 0.00 bB | 5.00 ± 0.40 bA | 0.0038 |
| HFFSRC at 2% | 100.00 ± 0.00aA | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 0.0001 |
| BAZUKA 216 SL | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 0.0001 |
| Ethanol 90% | 10.00 ± 0.58 bA | 5.00 ± 0.40 bB | 5.00 ± 0.40 bB | 13.33 ± 0.51 bA | 0.0025 |
| *p*= | 0.0003 | 0.0002 | 0.0001 | 0.0098 | |
| ***Spodoptera cosmioides*** | | | | | |
| **Treatment** | **% Mortality Accumulated in 10 Days** | | | | |
| | **1st Instar** | **2nd Instar** | **3rd Instar** | **4th Instar** | ***p*=** |
| Water | 5.00 ± 0.40 cA | 0.00 ± 0.00 cB | 0.00 ± 0.00 cB | 5.00 ± 0.40 bA | 0.0275 |
| HFFSRC at 2% | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 93.33 ± 0.20 aA | 0.0018 |
| BAZUKA 216 SL | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 0.0001 |
| Ethanol 90% | 16.66 ± 0.31 bA | 15.00 ± 0.37 bA | 6.66 ± 0.37 bB | 15.00 ± 0.37 bA | 0.0221 |
| *p*= | 0.0006 | 0.0005 | 0.0001 | 0.0063 | |

Means followed by the same lowercase letter in the column do not differ per the Kruskal–Wallis test ($p \leq 0.05$). Means followed by the same capital letter in the row do not differ per the Mann–Whitney test ($p \leq 0.05$).

### *3.2. Analysis of HFFSRC by High-Performance Liquid Chromatography—HPLC-ESI-Q-TOF-MS/MS*

The presence of five compounds was verified in the hexane fraction of the crude extract of the fruits and seeds of *R. communis*: three flavones, one fatty acid, and one cinnamic acid (Table 4).

**Table 4.** Chemical characterization of the hexane fraction of the fruits and seeds of *Ricinus communis* by UHPLC-ESI-QTOF-MS/MS obtained in negative mode.

| | | | | *m/z* | | | |
|---|---|---|---|---|---|---|---|
| **No.** | **Compound** | **Retention Time (min)** | **Molecular Formula** | **Measured Mass (Da)** | **Theoretical Mass (Da)** | **Ion Fragments** | **Class** |
| 1 | Naringenina | 42.6 | C15H12O5 | 271.0552 | 271.0610 | 150.9997 (100)<br>119.0456 (36) | Flavone |
| 2 | Prunin 3-p-coumarate | 44.3 | C30H28O12 | 579.1356 | 579.1580 | 271.0550 (100)<br>307.0772 (35)<br>145.0258 (46) | Flavone |
| 3 | Ricinoleic acid | 48.3 | C18H34O3 | 297.2375 | 297.2435 | 183.1350 (72)<br>279.2271 (8) | Fatty acid |
| 4 | Melilotoside | 49.2 | C15H18O8 | 325.1765 | 325.1840 | 183.0071 (46)<br>119.0485 (19) | Cinnamic acid |
| 5 | 5,6,2′-Trimethoxyfl avone | 50.2 | C18H16O5 | 311.1626 | 311.1685 | 183.0070 (36)<br>119.0484 (16) | Flavone |

## 4. Discussion

The insecticidal potential of HFFSRC at 2% for *S. eridania*, *S. cosmioides*, and *S. frugiperda* was verified. There was a toxic effect on *S. frugiperda* and *S. cosmioides* eggs, as well as an acute insecticidal effect on first-, second-, third-, and fourth-instar caterpillars of the three insect species studied, regardless of the form of exposure (topical or ingestion).

Regarding the ovicidal effect, the HFFSRC at 2% made the eggs of *S. frugiperda* and *S. cosmioides* unviable, with a total reduction in larval hatching, demonstrating the ability of HFFSRC to penetrate the egg and act on the embryo. This ability of HFFSRC to penetrate the egg had already been verified by [44] when evaluating the selectivity of HFFSRC to the egg parasitoid *Trichogramma pretiosum* (Riley, 1879) (Hymenoptera: Trichogrammatidae) inside eggs of *C. includens*. According to the author, HFFSRC at 2% applied on eggs of *C. includens* parasitized by *T. pretiosum* penetrated the egg, killing the parasitoid embryo. In a similar study, with aqueous, hexane, methanolic, and ethyl-acetate extracts from four different plants, the methanolic and hexane extracts of *Acorus calamus* (Araceae) at 7.5% inhibited 100% of the hatching of caterpillars of *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) [45].

The ovicidal effect of *R. communis* extracts was also observed on Diptera. In an evaluation of aqueous, methanolic, and ethyl-acetate extracts of bark and leaves of *R. communis* on eggs of *Phlebotomus duboscqui* (Neveu-Lemaire, 1906) (Diptera: Phlebotominae), the methanolic extract (500 ppm) reduced 95% of larvae hatching, indicating the corruption of the chorion reaching the embryo [46]. The authors also highlighted that the extracts damaged the egg surface, reducing the roughness and even causing the loss of the outer layer of the chorion.

It is important to point out the ovicidal potential of different extracts obtained with different solvents. In the case of lepidopterans, the ovicidal effect may also be related to the fact that the chorion of these eggs has a rough texture composed of lipoproteins. This layer may favor the fixation or retention of chemical substances, keeping them on the surface for a longer time, increasing the possibility of the substance penetrating the chorion and killing the embryo [47], as [26] suggested in a study conducted with the pear-tree aqueous extract *Aspidosperma pyrifolium* (Apocynaceae) applied on *P. xylostella* eggs. The HFFSRC (0.5, 1, and 2% concentrations) did not affect the emergence, the sex ratio, the egg–adult period, or the longevity of the emerged egg parasitoid *Telenomus podisi* (Ashmead,

1893) (Hymenoptera: Scelionidae) when HFFSRC was applied on eggs of *Euschistus heros* (Fabricius, 1794) (Hemiptera: Pentatomidae) containing the parasitoid [48], showing that the substances present in the HFFSRC did not penetrate eggs of *E. heros*.

The effects of 2% HFFSRC on the studied caterpillars further demonstrate the insecticidal potential of this compound. All instars of *S. cosmioides*, *S. eridania*, and *S. frugiperda* were susceptible when treatments were applied topically. It is essential to point out that for the three species of caterpillars, the mortality of individuals in the fourth instar was lower compared to the mortality observed in the other instars. In the case of *S. eridania*, lower mortality was also observed in the third-instar caterpillars compared to the first- and second-instar caterpillars. However, when HFFSRC at 2% was applied to the food and offered to the insects, all species in different instars were susceptible, with lower mortality, similar to the positive control.

The plant *R. communis* has been studied in relation to its insecticidal effect. It has several chemical properties with different modes of action that can act on insects, causing a lethal effect and several sublethal effects, such as reduced growth, changes in ecdysis, and effects on their eating behavior. All parts of this plant can be used to extract substances with an insecticidal effect, but the fruits and seeds stand out due to their high concentrations of ricin and ricinin [31]. In the present work, the fruits and seeds of *R. communis* were used, as in most studies with this plant available in the literature. Nonetheless, studies considering obtaining compounds with apolar solvents, such as hexane, are scarce.

Among the five chemical compounds found in HFFSRC, there are three flavonoids, one fatty acid, and one cinnamic acid, all with insecticidal potential, which may explain the results obtained in this study. Flavonoids play a fundamental role in several biological processes in plants, such as the germination of the pollen tube, seed development and growth, fruit growth and ripening, hormone transport, chemical attractants for pollinators, allelopathic functions, chemical messengers between mycorrhizae and bacteria, and prevention of damage caused by fungi, viruses, bacteria, and herbivores [49–51].

Flavonoids can act on insects in various ways, such as deterrent and repellent action, causing histological and histochemical damage to their digestive system [52,53], as well as inactivating enzymatic activity [54]. They can also interfere with the molting process, inhibiting the formation of the hormone ecdysone [55]. There are also reports of some flavonoids, such as naringenin (identified in this study as a component of HFFSRC), with ovicidal and lethal effects on insect pests and sublethal effects such as reduced oviposition, fecundity, reduced larval weight, and the emergence of adults [56,57], and with satisfactory results for the control of nymphs and adults of *Eriosoma lanigerum* (Hausmann, 1802) (Hemiptera: Aphididae). Another aspect to consider is that the action of flavonoids in the metabolism of insects may also be related to lipid degradation since they constitute cellular structures [58], as observed in studies with the addition of rutin in the diet of *Anticarsia gemmatalis* (Hübner, 1818) (Lepidoptera: Erebidae) [59] and *S. frugiperda* [60], wherein reduced larval and pupal weight of insects was observed. Due to the range of flavonoid molecules available in nature (281) with insecticidal potential, as well as the diverse mode of action, flavonoids are considered benign substitutes for chemical insecticides. However, there is still a large gap regarding insecticidal activities [61].

The other two compounds identified in the HFFSRC were ricinoleic acid (fatty acid) and cinnamic acid. When ingested by the insect, ricinoleic acid inhibits protein synthesis, irreversibly affecting ribosomes and causing cell death. Furthermore, these toxins can cause gastrointestinal hemorrhagic inflammations and renal tubular necrosis [30,62], which, together with flavonoids, configures a greater insecticidal effect, as observed in this study. In addition, cinnamic acid acts as an antioxidant, a structural component of lignin, and a precursor of flavonoids [63]. It has low toxicity but a broad spectrum of biological activity as a fungicide and antiviral. However, there is little information about toxicity, pharmacokinetic properties, and mode of action [64]. Few studies have reported the effect of cinnamic-acid derivatives on insects, such as insecticidal activity for larvae of *Aedes*

*aegypti* (L., 1762) (Diptera: Culicidae) [65,66] and insecticidal activity, larvicidal activity, and inhibition of larval growth of *T. castaneum* [67].

In studies conducted with the hexane fraction of different plants, satisfactory mortality results were obtained for caterpillars. The hexane extract of *Annona mucous* Jacq (Annonaceae) at concentrations of 2.0%, 4.0%, and 8.0% caused mortality greater than 93.33% in first- and third-instar caterpillars of *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae) [39]. The hexane fraction of *Andira paniculata* Benth (Fabaceae) at a concentration of 0.5% caused mortality of 85% of third-instar caterpillars of *S. frugiperda* [68], and the hexane fractions of *Syzygium aromaticum* L. (Myrtaceae) and *Annona atemoya* (a hybrid of *Annona cherimola* Mill and *Annona squamosa* L.) (Annonaceae) caused, respectively, 90% and 77% mortality in second-instar caterpillars of *S. frugiperda* [69].

This study verified that the present compounds' ingestion caused significant mortality in fourth-instar caterpillars, which was not observed in the topical-application bioassay. As discussed above, this effect may be associated with the mode of action of the compounds present in HFFSRC and different modes of action at the enzymatic and cellular levels. Other works that demonstrated the insecticidal potential of *R. communis* by ingestion were also published.

Toxicity by ingesting the aqueous extract of *R. communis* was also observed in other lepidopteran species. Said extract, at concentrations of 5% and 10%, when sprayed on cabbage leaves and offered to the insects, caused, respectively, 33.9% and 100% mortality in caterpillars of third-instar *P. xylostella* ([70,71]) and when applied as 1% oil of *R. communis* on pumpkin leaves *Cucurbita moschata* (Cucurbitales: Cucurbitaceae) it caused significant mortality of *Diaphania nitidalis* (Cramer, 1781) (Lepidoptera: Pyralidae) [71].

The demand for plant-based products (extracts and essential oils) to control insect pests reappears, after the era of synthetic chemicals, as a strategic alternative for viable technological innovation for developing more sustainable agriculture, so the demand for such products is increasingly imminent. Although research has increased exponentially in recent years, there are still information gaps related to phytotoxicity, the isolation of active principles, the selection of extractor solvents, conservation techniques and application of these products, and the specific mode of action on insect pests, non-target organisms, and compatibility with other control strategies. According to Turchen et al. [13], in an analysis of 2500 articles published between 1945 and 2020, the main gaps observed are the concentration of studies with few botanical families and the little attention paid to the effects on non-target organisms and sublethal effects, which is key information in the context of the IPM.

In general, the results of the present study show that HFFSRC at 2% had a toxic effect on caterpillars of *S. cosmioides*, *S. eridania*, and *S. frugiperda*, as well as on eggs of *S. cosmioides* and *S. frugiperda*. Thus, considering the insecticide potential of HFFSRC for pest control, further laboratory studies are suggested to identify the mode of action of HFFSRC. In this study, a concentration of 2% was evaluated. However, dose–response analyses may be interesting to understand the sublethal effects and possible enzymatic effects on the nervous system and/or histological damage in the insect's gut.

In this vein, another important factor to consider for improving the effects of substances on insects and, in this case, the effects of HFFSRC, is the evaluation at the semi-field (greenhouse) and field level since the results found in the present work may be different when evaluated in the agroecosystem. Experimentation in the laboratory is subject to the assertiveness of the applications, as well as the standardization of variables. Still, in the field, several abiotic factors can prevent the replication of results.

Under field conditions, information about the effect of the fraction mentioned above on natural enemies, emphasizing the egg parasitoids *T. podisi* and *T. pretiosum*, is also important. In this sense, studies on strategies for using biological agents and HFFSRC in the field are also fundamental for managing these insect pests. Added to this is the need to evaluate HFFSRC on bees, insects of high importance that have been declining due to using non-selective insecticides. The analysis of the effects of this compound on vertebrates

is also of paramount importance in order to assess biosafety and prospect its effects on humans.

Moreover, it is interesting to use methods that evaluate the substance/environment interaction to obtain results regarding environmental degradation. Linked to this, assessing food deterrence through assessments of consumption of the leaf area of these insects in contact with HFFSRC is also relevant since information on the subject is scarce.

## 5. Conclusions

HFFSRC at 2% is toxic for eggs of *S. cosmioides* and *S. frugiperda* and caterpillars of first, second, third, and fourth instars of these insects and *S. eridania* when applied topically and when offered via contaminated food.

The compounds identified in HFFSRC are flavonoids, fatty acid, and cinnamic acid, which have an insecticidal effect on the studied species.

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