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An Interesting Relationship between the Insecticidal Potential of *Simarouba* sp. in the Biology of Diamondback Moth

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Abstract: Alternative methods of insect management are an important field of study for agriculture. The current study aimed to determine the effect of aqueous extracts from *Simarouba* sp. (AE-S) on the biology of *Plutella xylostella* and to determine the toxicity of the extract to the nematode *Caenorhabditis elegans* (an important in vivo alternative assay system for toxicological study). Lyophilized AE-S was chemically investigated by Ultrahigh-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). We evaluated the effect of the botanical extract on the life cycle of *P. xylostella*, from larval stage to adult stage, at concentrations of 10%, 5%, 1%, 0.1%, 0.05%, and 0.01% and a control. Subsequently, we analyzed the toxicity of the extract in an in vivo model. AE-S showed high amount of phenolic and flavonoid compounds. Six compounds were identified based on UHPLC-MS/MS analysis, including flavanone, kaempferol, 4,5-dimethoxycanthin-6-one, 11-acetylamarolide, ailanthinone, and glaucarubinone. The median lethal time for *P. xylostella* was estimated to be 96 h in all concentrations of AE-S, and at 120 h, 100% of the individuals were dead. Larvae exposed to AE-S at concentrations of 0.01, 0.05, and 0.1% showed a reduction in leaf area consumption, underdevelopment, and reductions in movement and pupal biomass. The lowest concentrations of AE-S (0.1%, 0.05%, and 0.01%) did not cause mortality in nematodes. Thus, the aqueous extract of *Simarouba* sp. could be an effective control tool because it mainly acts in the larval stage, the stage at which the insect causes damage to brassicaceae.

Keywords: biopesticide; bioactive compounds; botanical insecticide; toxicity; selectivity; integrated pest management; *Caenorhabditis elegans*

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1. Introduction

For years, chemical products have been sought as attractive alternatives for pest control [1,2]. However, in addition to their benefits, many consequences should be considered when applying chemical products: environmental problems, human health concerns, pest resistance, mortality of beneficial insects [3–5], toxic residue accumulation, water and soil contamination, toxicity to landholders [6,7], augmentation of secondary pests, pest population explosions, and selection loss of insecticide efficacy [8,9].

Plutella xylostella L. (Lepidoptera: Plutellidae) is one of the most destructive pests worldwide, and its high migratory capacity, biotic potential, and short life cycle, as well as a lack of proper management practices [10], have made the insect resistant to more than 101 active ingredients of pesticides registered worldwide [11]. This requires frequent rotation of synthetic insecticides, generating an estimated cost to control this pest of USD 4–5 billion a year [12]. Consequently, there is greater environmental pollution and accumulation of residues in vegetables [13,14]. Therefore, the elevated cost of agricultural production and the risks to the environment demand novel alternatives to control these insects that are less harmful to the environment and humans [15,16]. In addition, higher awareness by farmers and consumers and policy implementation have resulted in demands for reduced pesticide use during food production and the use of practices that support agroecological intensification [17,18].

Therefore, eco-friendly attitudes and the production of pesticide-free organic products have promoted the search for alternative products that do not harm the environment [19] and need to be aligned with the global demands of enterprises that are willing to achieve Environmental Social Governance (ESG) indices, as well as the Sustainable Development Goals (SDG), among them, we cite SDG 1—no poverty, SDG 2—zero hunger, SDG 3—good health and well-being, and ODS 12—responsible consumption and production. Among these alternatives are natural botanical insecticide products, derived from the secondary metabolism of plants, that make food repellant, inhibit oviposition and growth, or have larvicidal effects in insect pests of agricultural importance [20–27]. The mode of action of these natural insecticides is based on several compounds; they hinder the evolution of resistance in herbivorous insects [28,29] and, with some exceptions, are less toxic to non-target organisms, such as natural predators, pollinators, and vertebrates [30]. They also cost less and are easy to obtain, apply, and manage [31]. Thus, botanical insecticides can be important alternatives for controlling insects in laboratory experiments [21–27], in agricultural production areas, as in the use of *Azadirachta* [32], or in the plastic tunnel condition [33]. Another advantage is that botanical insecticides do not remain in nature after application, which drastically reduces the chances of environmental contamination [34] and promotes greater environmental conservation [35].

The Simaroubaceae family is composed of 32 genera, constituting of approximately 250 species of shrubs and trees, and some *Simarouba* species have been reported to possess insecticidal and repellent activity. For example, *S. amara* Aubl., used as a repellent against larvae of the mosquito *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) [36], and *S. versicolor* showed clear activity against the leaf-cutting ant *Atta sexdens rubropilosa*, Forel, 1908 (Hymenoptera: Formicidae), and antifungal activity against the symbiotic fungi *Leucoagaricus gongylophorus* (Möller) Singer (Agaricales, Agaricaceae) and *Rhodnius milesi* (Hemiptera: Reduviidae) [37–39]. Phytochemical studies on major compounds from *S. versicolor* A. St-Hill, including quassinoids, triterpenoids, alkaloids, steroids, and coumarins, have mainly concentrated on stem, branches, fruits, and bark [39,40]. Quassinoid compounds are almost exclusive to Simaroubaceae, and are considered taxonomic markers of the group [41]. Although our knowledge of the chemical composition of extract generated from the leaves of this plant is still limited, Simote [38] reported the presence of flavonoids.

Plutella xylostella was selected for the present investigation based on its economic importance. We hypothesized that *Simarouba* sp. would present insecticide activity against *P. xylostella* with low toxicity to the environment and could be used as an alternative pest control agent.

To test the selective toxicity of plant extracts, many studies were performed using *Caenorhabditis elegans* (Maupas, 1900) as a model non-target organism [42–45]. Organophosphorus insecticides with eight active ingredients that were tested on *C. elegans* inhibited the enzyme cholinesterase through the accumulation of acetylcholine, a neurotransmitter [45–47]. This finding demonstrates that *C. elegans* is a good model for neurotoxicity testing [48]. *C. elegans* is a free-living nematode present in soil, plant litter [49,50], and rarely in aquatic environments [51]. It is responsible for maintaining soil quality and recycling

nutrients [52], is extremely sensitive to the presence of pesticides such as herbicides and insecticides [45–47,53,54], and is considered an important bioindicator of soil pollution [55] and water pollution [56]. Using mammals for toxicity testing is not feasible, as it takes years to evaluate the results and the cost is high due to the long-life cycle [57,58]. The advantage of using *C. elegans* is that, because it has a short life cycle, it is possible to observe the results and effects in the short term [59].

In this work, we aimed to determine the action of *Simarouba* sp. aqueous leaf extract on the biology of *P. xylostella* and the toxicity in *C. elegans*, and to determine its chemical composition, by LC-MS/MS, in order to scientifically support its biological action. The results of this study will be important to our understanding of the in vivo toxicity of *Simarouba* sp.and the possibility of using a botanical extract of this species to minimize damage to the environment. We evaluated the extract from leaves, as they are renewable materials and removing them does not compromise the plant's development.

2. Materials and Methods

2.1. Botanical Material

Fully expanded leaves of *Simarouba* sp. were collected in the morning during January 2020 at Pousada das Abelhas, in the municipality of Campo Grande, MS (21°13′28″ S, 54°11′28″ W, 437 m altitude), placed in plastic bags moistened with filter paper, taken to the laboratory for sorting and identification by an expert, and then deposited in the herbarium of the Faculty of Biological and Environmental Sciences of the UFGD under number 6481. The collection of botanical material was authorized by the National Management System. Genetic heritage and associated traditional knowledge (SISGEN) were filled under number AF5E2AA.

2.2. Preparation of Aqueous Extract

The *Simarouba* sp. leaves were cleaned in running water and dried in a forced air circulation greenhouse for 72 h at a maximum temperature of 40 °C (±1 °C), then the completely dried leaves were crushed in an industrial mill until they turned into a fine powder. The powder was protected from light and moisture during storage and stored at room temperature (25 ± 2 °C). To obtain the aqueous extract (AE-S) by maceration, 3 g of vegetable matter was added to 30 mL of distilled water. After homogenization, for 24 h the mixture was maintained in a refrigerated (10 °C) and filtered with filter paper before the conduction of the experiments.

The assays against *P. xylostella* were conducted with aqueous extract (AE-S) at a concentration of 10% in different concentrations (5, 1, 0.1, 0.05, and 0.01%).

To chemical analysis of the aqueous extract (AE-S) was conducted by process of lyophilization, also known as freeze-drying, which is typically used for water removal, and sample preservation, resulting in lyophilized AE-S.

2.3. Chemical Composition

The total phenolic content in lyophilized AE-S (1 mg/mL, dissolved in water) was determined by using Folin–Ciocalteu reagent [60]. A 100 µL sample of AE-S was mixed with 0.5 mL of Folin–Ciocaleu' s (1:10 *v*/*v*), and after 3 min, 1.5 mL of aqueous sodium bicarbonate (2%) was added. The absorbance was measured at 765 nm using a spectrophotometer, after 30 min. A calibration curve of gallic acid was prepared (2.5–125 µg/mL, in water). We then used these data to generate a linear regression model, and the line equation was obtained and used for the calculation of the experimental samples. The equation of the gallic acid curve was Y = −0.052 + 7.5x, with a determination coefficient of R^2 = 0.99727, and the results are expressed in milligrams of gallic acid equivalent (GAE) per gram of extract.

To measure the level of flavonoids, 500 µL of lyophilized AE-S (1 mg/mL, dissolved in water) was mixed with 1.50 mL of ethanol (95%), 0.10 mL of aluminium chloride (10%), 0.10 mL of sodium acetate (1 M), and 2.80 mL of distilled water. At room temperature, without the presence of light, it remained for 40 min, and absorbance was measured at

415 nm. The quantification was carried out using a standard curve of quercetin to obtain a line equation (Y = 0.3546 + 12.8030X; R^2 = 0.99972). The results were expressed as quercetin equivalent (QE) in mg per gram of extract [60]. Total flavanol in AE-S was estimated using a method reported previously [60]; absorbance was read at 440 nm, and expressed as quercetin equivalent (QE) in mg per gram of extract using the quercetin calibration curve.

The condensed tannin content was measured with vanillin−HCl reagent [61]. The lyophilized AE-S (1 mg/mL, dissolved in water) was mixed with 5 mL vanillin–HCl (8% conc. aq. HCl and 4% vanillin). Absorbance was read at 500 nm after 20 min. Quantification was performed using a calibration curve with catechin as the standard (Y = 0.00896 + 0.84392X; R^2 = 0.98978). The condensed tannin concentration was expressed as catechin equivalent (CAE) in mg per gram of extract. All the assays were carried out in triplicate.

2.4. HPLC-MS/MS Analysis

The lyophilized AE-S was solubilized in methanol–acetonitrile (1:1, *v*:*v*) at a concentration of 0.5 mg/mL centrifuged (1200× *g*, 5 min), and the supernatant was analyzed with a UHPLC system (Shimadzu Nexera X2) equipped with a CBM-20A system controller, two LC-30AD pumps, a CTO-30A column oven, and an SIL-30AC autosampler coupled to an HRMS system (QTOF Impact II, Bruker Daltonics Corporation, Harvard, Cambridge, MA, USA) equipped with an electrospray ionization source, quadrupole time-of-flight (QTOF) analyzer, and multichannel plate (MCP) detector (Impact II, Bruker Daltonics Corporation, Cambridge, MA, USA). The capillary voltage was operated in positive ionization mode and was set to 4500 V with an endplate offset potential of −500 V. The dry gas parameters were set to 8 L/min at 200 °C with a nebulization gas pressure of 4 bar. Data were collected from *m*/*z* 50–1300 with an acquisition rate of 5 spectra per second, and the ions of interest were selected by automatic MS/MS scan fragmentation. Chromatographic separation was performed using a C18 column (Column, LC, Shim-pack XR-ODS III 1.6 µm particle size, 2.0 mm i.d. × 75 mm length).

The gradient mixture of solvents A (H_2O) and B (acetonitrile with 0.1% formic acid; *v*:*v*) was as follows: 5% B 0–1 min, 30% B 1–3 min, 95% B 3–12 min, maintained at 95% B 12–16 min, and 5% B 16–17 min. The flow rate was 0.2 mL/min, the column temperature was 40 °C, and the injection volume was 3 µL. The data were processed by Bruker Compass DataAnalysis 4.3 software. The compounds were proposed based on a bibliographic review of the genus and species, as well as the error value of the mass [62].

2.5. Rearing of P. xylostella

Larvae and pupae of *P. xylostella* were collected from cabbage fields in the city of Dourados (22°13′16″ S and 54°48′20″ W), state of Mato Grosso do Sul, and reared at the Insect–Plant Interaction Laboratory of the Faculty of Biological and Environmental Sciences at the Federal University of Grande Dourados (UFGD), Mato Grosso do Sul, Brazil. Individuals were kept under constant temperature (25 ± 2 °C) and relative humidity (70 ± 5%) with a photophase of 12 h.

The pupae were deposited in plastic containers (9 cm × 19 cm × 19 cm) for the emergence of adults. The adults were fed honey diluted in 10% distilled water using cotton soaked in the solution. Cabbage and filter paper discs moistened with distilled water, both 9 cm in diameter, were added to the same container for egg deposition; the set was changed daily and replaced with new discs.

After eggs were laid, the discs were transferred to transparent plastic containers (30 cm × 15 cm × 12 cm) in which the larvae remained from hatched eggs until they reached the pupa stage. They were fed with organic cabbage leaves (*Brassica oleracea* var. *acephala*) previously sanitized with 5% sodium hypochlorite solution.

Cabbage leaves were arranged with the adaxial side of the first leaf facing the plastic container, and the free abaxial side was used to place the larvae. Then, the second leaf was positioned with the abaxial side facing down. Every day, the leaf with the adaxial side facing the plastic container was discarded and replaced by the second leaf with the abaxial

face facing down [63]. Newly formed pupae were removed from the plastic containers and transferred back to the adult cage.

2.6. Bioactivity of Aqueous Extract on P. xylostella

Organic cabbage leaves (*B. oleracea* var. *acephala*) were cut into discs measuring 4 cm^2, which were immersed for 15 s in AE-S at concentrations of 10, 5, 1, 0.1, 0.05, and 0.01%; the control consisted of distilled water. Subsequently, the discs were kept at 25 °C for 40 min to remove excess moisture and then immediately transferred to Petri dishes. In each Petri dish (12 cm × 2 cm), a cabbage disc was inserted under a wet filter paper disc with a neonate (0–24 h) larva of *P. xylostella*. Cabbage discs were replaced every 24 h by new discs immersed in the respective treatment, and filter paper discs were changed every 48 h. The control treatment larvae were fed with cabbage discs (4 cm^2) immersed in distilled water. *Plutella xylostella* larvae were monitored daily, and the number of dead individuals, characterized by immobility, was counted. The surviving larvae remained in Petri dishes until they reached the pupal stage. They were weighed 24 h after pupation (pupal biomass) and subsequently isolated in individual test tubes until they emerged as adults. Pupal duration was assessed according to the time (days) that individuals remained in the pupal stage. Pupal survival was calculated according to the percentage of adults that emerged.

After the adults emerged in the test tubes and were sexed, two *P. xylostella* (one male and one female) were transferred into a transparent cage with untreated cabbage and moistened filter paper discs as an oviposition substrate. Diluted honey solution was used as food source.

Discs with eggs were removed daily and replaced, and the number of deposited eggs (fecundity) was counted. After counting, the discs were transferred to Petri dishes to count the number of hatched larvae (egg survival). The moths remained in the cage until both died, and during this period, the number of days that males and females remained alive (longevity) and that females oviposited (oviposition period) were counted (Figure 1). The data evaluations of the pupal and adult phase were carried out in individuals that were exposed to different tested concentrations of AE-S and control (distilled water) and survived in the larval phase.

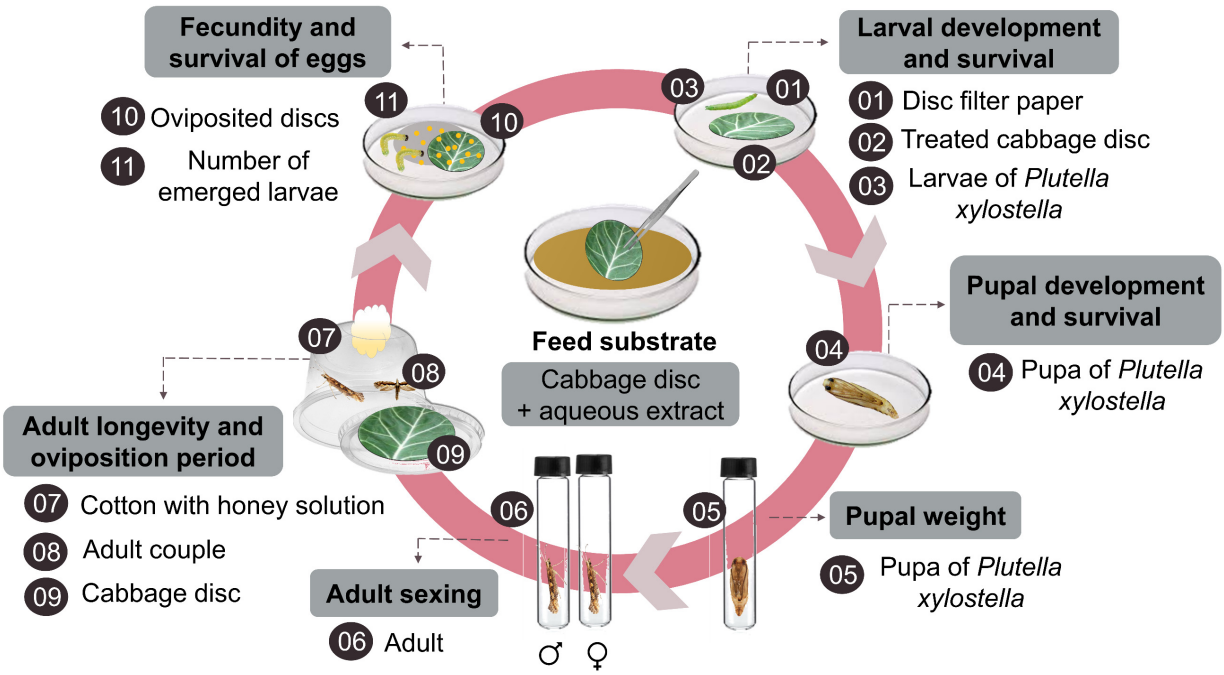

Figure 1. Schematic representation of methodology used to assess lethal and sublethal effect of *Simarouba* sp. aqueous extract against *P. xylostella*.

The experiment for the bioactivity assay of AE-S on *P. xylostella* was a completely randomized design, with seven treatments (six concentrations and a control) each consisting of 50 larvae. Each individual was considered a replicate. In the pupal and adult stages, the number of replicates was dictated by the number of individuals that survived the larval stage.

All the variables were analyzed with a generalized linear model. The best model for the data of the weight and duration of larval and pupal stages variables was a model with a gamma distribution with the inverse link function. Mortality and rate of development showed binomial and Gaussian distribution, respectively. The goodness of fit of the models was assessed using half-normal plots with a simulated envelope using R.

As mean development did not show normal distribution, we estimated the rate of development using the following equation:

$$r(T) = 1.0/e\{ \mid \sum \ln (di) \mid n\}$$

where r(T) is development rate, di is individual observations of development time (days), and n is number of observations [64].

2.7. Rearing of C. elegans

The selective toxicity of different concentrations of AE-S was evaluated in vivo in the nematode *C. elegans* from the wild strain N_2. The nematodes were incubated in Petri dishes with nematode growth medium (NGM) agar, at a temperature of 20 °C. The individuals were fed with *Escherichia coli* OP50-1 bacteria added to the Petri dish, and to synchronize nematodes for the bioassay, sodium hypochlorite (2%) and sodium hydroxide (5 M) were used in pregnant hermaphrodites. The eggs were incubated at 20 °C for 48 h to obtain L4 phase nematodes.

2.8. Toxicity Assessment

To perform the toxicity experiment, the methodology of Dengg and Meel [65] was used. In a 96-well plate, 10 to 20 nematodes in phase L4 that were incubated with AE-S at concentrations of 10, 5, 1, 0.1, 0.05, and 0.01%, solubilized in M9 minimal medium, were added and maintained at a constant temperature of 20 °C (Figure 2). In the control treatment, nematodes in the L4 phase were incubated in M9 minimal medium. Nematodes survival assessment was performed after 24 and 48 h of incubation, and nematodes were considered dead when they did not show any movement when touched repeatedly with the micro spatula. A Motic SMZ-140 and W10X/23 stereo microscope was used for the survival evaluation.

Two independent experiments were performed in triplicate (10 to 20 nematodes per well). The data were submitted to analysis of variance (ANOVA) and the mean values were compared by the Dunnett test ($p < 0.05$) using the GraphPad Prisma 5 program. The data are represented as average ± standard error of the mean (EEM).

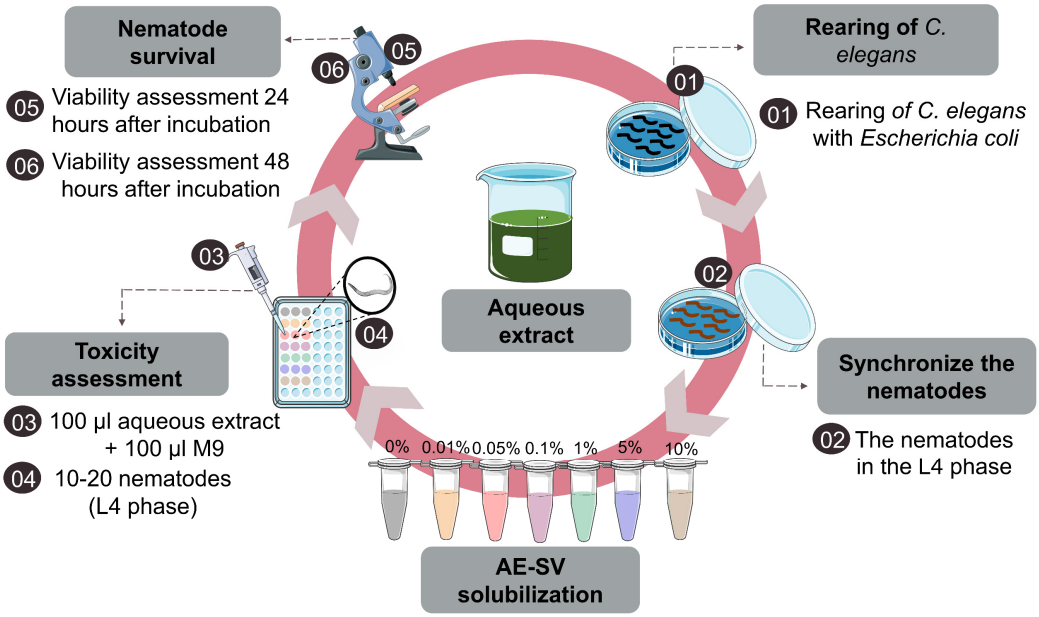

Figure 2. Schematic representation of methodology used to assess toxicity.

3. Results

3.1. Chemical Prolife

The chemical analysis of AE-S showed phenol at the highest concentration (935.08 ± 0.58 mg GAE/g extract), followed by flavonoids (623.42 ± 2.44 mg QE/g extract), flavanol (444.56 ± 6.22 mg QE/g extract), and condensed tannins (36.18 ± 10.25 mg CAE/g extract).

The HPLC-MS/MS chromatogram reported flavanone (1) (Rt = 6.56 min), kaempferol (2) (Rt = 7.22 min), 4,5-dimethoxycanthin-6-one (3) (Rt = 8.73 min), 11-acetylamarolide (4) (Rt = 9.57 min), ailanthinone (5) (Rt = 10.09 min), and glaucarubinone (6) (Rt = 10.58 min) (Figure 3, Table 1).

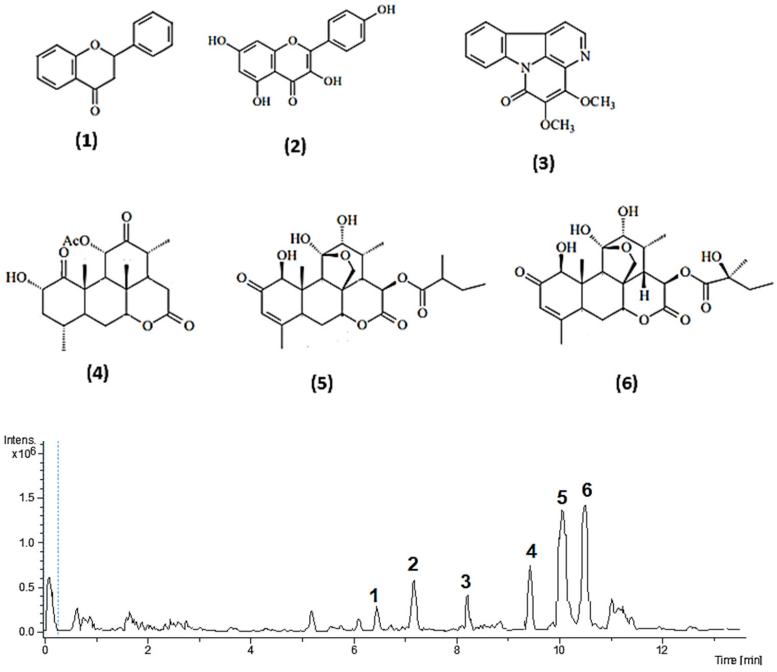

Figure 3. HPLC-MS chromatogram of aqueous extract of *Simarouba* sp. (AE-S) leaves obtained in positive mode: flavanone (**1**), kaempferol (**2**), 4,5-dimethoxycanthin-6-one (**3**), 4. 11-acetylamarolide (**4**), ailanthinone (**5**), glaucarubinone (**6**).

Table 1. The main compounds observed by HPLC-MS/MS in positive mode of the aqueous extract (AE-S) leaves.

Peak	Compound	RT (min)	[M+H] *m/z*	Molecular Formula	MS/MS [M+H] Fragments
(1)	Flavanone	6.56	224.0837	$C_{15}H_{12}O_2$	223, 195
(2)	Kaempferol	7.22	286.2432	$C_{15}H_{10}O_6$	286, 165, 153, 137, 99
(3)	4,5-Dimethoxycanthin-6-one	8.73	280.0848	$C_{16}H_{12}N_2O_3$	265, 251, 221, 237
(4)	11-Acetylamarolide	9.57	406.1991	$C_{22}H_{30}O_7$	378, 318, 274, 214
(5)	Ailanthinone	10.09	495.5009	$C_{25}H_{34}O_9$	478, 345, 301, 104
(6)	Glaucarubinone	10.58	494.2151	$C_{25}H_{34}O_{10}$	493, 375, 345, 301, 117

3.2. Bioactivity of AE-S

All concentrations of *Simarouba* sp. significantly interfered on the survival rate of *P. xylostella*. The median lethal time for *P. xylostella* was estimated at 96 h under all treatments tested with AE-S (Figure 4).

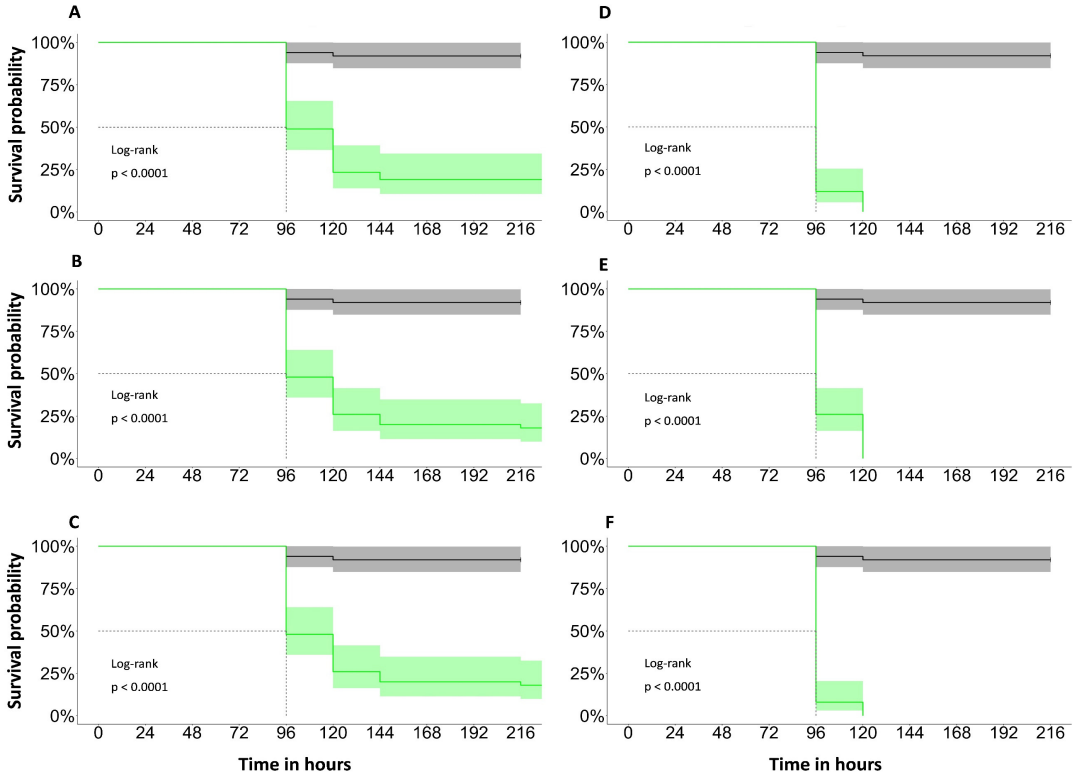

Figure 4. Survival rate of *Plutella xylostella* larvae after exposure to aqueous extract of *Simarouba* sp. at different concentrations: (**A**) 0.01%; (**B**) 0.05%; (**C**) 0.10%; (**D**) 1.00%; (**E**) 5.00%; (**F**) 10.00%.

Concentrations of the aqueous extract of 0.01, 0.05, and 0.1% caused longer duration, until they died or reached the pupal stage (Figure 4A–C), in the larval stage than the control, with a mortality rate of higher than 70% after 120 h of exposure. After 144 h, the rate of action of these aqueous extract concentrations on larvae decreased, causing longer development in the larval stage for some survivor individuals.

The highest percentage of mortality occurred at AE-S concentrations of 1, 5, and 10% (Figure 4D–F). On the first day of the bioassay, larvae took a test bite and continued feeding. In the following days, food consumption was reduced until larval death. In total, >50% of the population had died after 96 h of exposure to the aqueous extract, and within 120 h, 100% of individuals were dead. Owing to the total (100%) larval mortality rate, we could not evaluate the parameters related to pupal development (Table S1) and adult reproduction in

larvae treated with 1, 5, and 10% AE-S (Table S2). On the other hand, for the control group all larvae remained alive after 216 h, reaching the pupal stage.

The healthy larvae from the control treatment had a greenish coloration (Figure 5A), whereas those treated with 0.1 and 0.05% AE-S had dark spots at the ends of their bodies (Figure 5B,C), and some that were treated with 10% AE-S presented with a petrified appearance, dark coloration, and an extremely strong odor (Figure 5D). Notably, larvae treated with AE-S in concentrations of 0.01, 1, and 5% showed the same characteristics as those treated with the other concentrations, except larvae treated with 10% showed a "petrified" aspect.

Figure 5. Larvae and adult *Plutella xylostella*. (**A**) Healthy *P. xylostella* larvae. (**B**–**D**) Larvae treated with 0.05%, 0.1%, and 10% AE-S, respectively. (**E**,**F**) Adult *P. xylostella* emerged from larvae treated with 0.05% AE-S.

When we compare the larval development duration expressed in days there was no difference between the AE-S concentrations, with exception to the control in relation to AE-S concentrations of 0.01, 0.05 and 0.10% (Table 2), we could not quantify this parameter at the AE-S concentrations equal or over than 1% due to no larval survival. We analyzed the data obtained from surviving individuals from the larval stage that reached the pupal stage. We observed an average reduction of approximately 33% in pupal biomass developed from larvae fed with cabbage discs treated with 0.01% AE-S in relation to the control group (Table S1). We could not evaluate the adult phase parameters for larvae fed with 0.05% AE-S due to the emergence of deformed adults (Figure 5E,F).

Table 2. Larval development and larval mortality (%) of *Plutella xylostella* pupae exposed to aqueous extract of *Simarouba* sp.at different concentrations.

Concentration (%)	Larval Development (Days)	Larval Mortality (%)
0.00	6.54 ± 0.24 a	08.00 ± 03.87 b
0.01	5.06 ± 0.27 b	82.00 ± 05.54 a
0.05	4.92 ± 0.66 b	92.00 ± 03.87 a
0.10	4.48 ± 0.41 b	96.00 ± 02.79 a
1.00	-	100.00 ± 0.00 *
5.00	-	100.00 ± 0.00 *
10.00	-	100.00 ± 0.00 *
F/χ^2 and *P value*	F = 19.87; P (>F) < 0.0001	χ^2= 719.95; χ^2 (>F) < 0.0001

Means followed by different letters within a column differed from each other at the 5% significance level. * Data not considered in the analysis because of no variability.

3.3. Toxicity of AE-S in C. elegans

When analyzing the toxicity of AE-S in *C. elegans*, we observed that lowest concentrations (0.1, 0.05, and 0.01%) did not induce mortality, with a viability rate of 80% or more, similar to the control (Figure 6A,B).

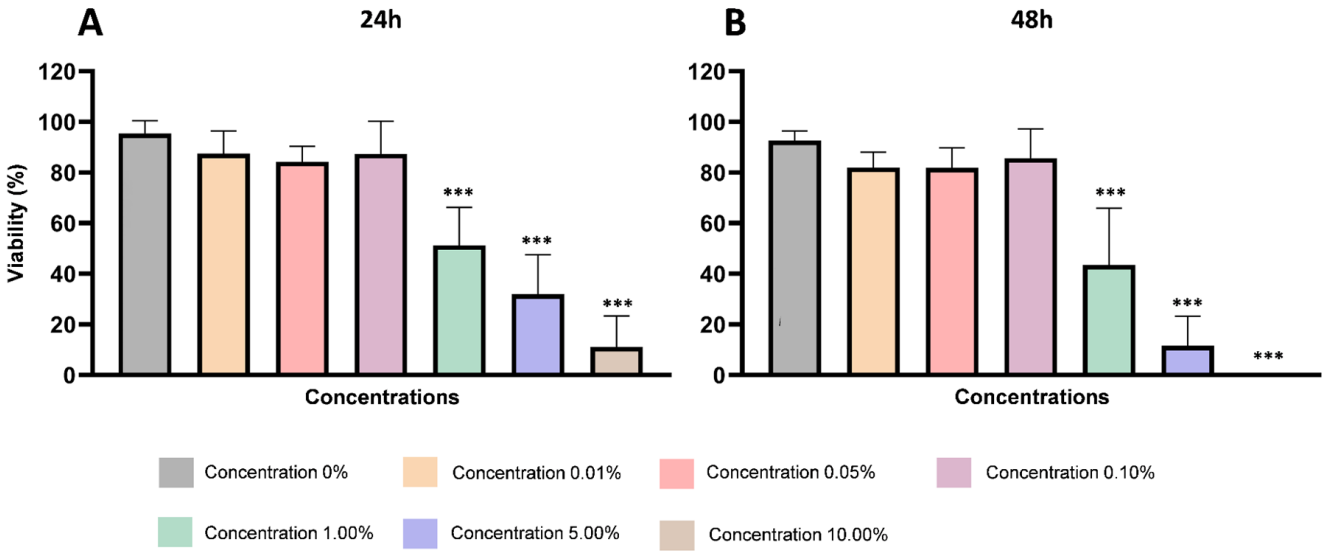

Figure 6. Viability (%) of nematodes (*C. elegans*) treated with different concentrations of *Simarouba* sp. aqueous extract (AE-S) after (**A**) 24 h and (**B**) 48 h of incubation. *** Statistically significant results ($p < 0.001$) when treated group was compared with control group (concentration 0%).

Thus, these concentrations did not exhibit toxic action and can be considered selective. However, 1, 5, and 10% concentrations showed toxicity to the non-target biological model; for example, 1 and 5% AE-S killed approximately 55% within 24 h of incubation and >80% of the population after 48 h. In addition, 10% AE-S killed 100% of individuals within 48 h of incubation (Figure 6A,B).

4. Discussion

The present study is the first to describe the action of AE-S in the biology of *P. xylostella* and its toxicity against *C. elegans*. We found that only 96 h of incubation with the highest concentrations of *Simarouba* sp. extract was enough to promote 50% mortality of *P. xylostella*. The lowest concentrations accelerated pupation in those individuals that survived during the larval stage. These surviving individuals had reduced weight gain wich provoked early metamorphosis into the pupal stage, as well as having adults with reduced size, which made mating hard and resulted in fewer fertile females [66]. Moreover, because of the need to degrade allelochemicals within the extract, the insect reallocates resources that would otherwise be used to gain weight in the larval stage; thus there is a great energy expenditure toward degrading toxic compounds and low conversion rates of ingested nutrients [67], resulting in low pupal biomass as found at AE-S concentrations of 0.01%. Extracts in higher concentrations tended to modify feeding behavior, either by inhibiting feeding or negatively affecting the biology after ingestion. This may be because higher concentrations mean a greater number of secondary compounds will be extracted, consequently increasing their effects on the insect [68]. This result is of great relevance in the field, as the diamondback moth causes a great deal of damage during the larval stage [69]. Therefore, having a pest control method that causes development acceleration and mortality in a significant proportion of the population (>97%) in the larval stage could lead to a reduction in crop losses and a parallel reduction in the cost of pest control [70]. Similar results were observed by Ferreira et al. [26] with species of *Ludwigia*, where the antibiosis effect of *Ludwigia* extracts

was evidenced by the mortality of the individuals in the larval phase and the reduced pupal weight, fecundity, number of newly emerged larvae and percentage of surviving eggs.

Low pupal biomass may reflect the difficulty of converting food into biomass [71,72], and thus the quality and quantity of feeding. Additionally, sublethal effects during the developmental stage may cause complications in subsequent life cycle stages [63,73,74], as observed in the results obtained in the pupal and adult phases in the adult survivors (Tables S1 and S2). Therefore, pupal biomass can be considered an essential factor in adult reproduction that directly affects the quantity of eggs produced by females [68]. Recent studies have shown that reduced pupal biomass is correlated with reduced fecundity [21,26,27,75]. Thus, the decreased pupal biomass may affect the size of adults, resulting in smaller moths, and this size difference may interfere with mating, consequently reducing the total number of eggs produced per female [21,27,66].

To determine the potential impact on the environment, the toxicity of the extract was evaluated in a non-target organism, the free-living nematode *C. elegans*. The lowest concentrations of *Simarouba* sp. extract did not cause mortality in *C. elegans*, with viability of 80% or more, which was similar to the control, therefore these concentrations did not exhibit toxic action and can be considered selective. The *Simarouba* sp. compounds may have feeding deterrent properties, inhibiting peristalsis in addition to restricting hatching [76], therefore higher concentrations of the extract are not recommended.

Low concentrations of *Simarouba* sp. aqueous extract (0.1, 0.01, and 0.05%) negatively affected the development of *P. xylostella*, which means they are potentially effective against this target organism, showing no difference compared to the control in the selective toxicity test, i.e., they were not toxic to *C. elegans*. However, the highest concentrations showed high mortality rates for both *P. xylostella* and *C. elegans*.

Previous studies described the petrified and dark appearance of lepidopteran larvae when they come in contact with a biotic control agent. For instance, *Oxydia vesulia* larvae (Cramer, 1779; Lepidoptera: Geometridae), when exposed to *Bacillus thuringiensis* var. kurstaki (Bacillaceae), presented symptoms similar to those found in this study, such as reduced movement and feeding as well as dark spots all over the body [77]. In *Helicoverpa zea* larvae (Bodie, 1850; Lepidoptera: Noctuidae), intestinal paralysis and total paralysis were observed after contact with *B. thuringiensis*, with lethal effects three days after contamination [78]. In *P. xylostella* larvae treated with aqueous extract of *Alibertia sessilis* (Vell.), K. Schumand (Rubiaceae), and *Alibertia intermedia* (Mart.) (Rubiaceae), mortality occurred during the larval stage, with dark coloration and petrified appearance, in addition to reduced pupal biomass [21].

Owing to the presence of many compounds in aqueous extracts of *Simarouba* sp., particularly quassinoids and glaucarubinone, which exert direct effects on various biological characteristics of pests (such as those observed in *P. xylostella*), an increasing number of studies have investigated the desirable characteristics of *S. versicolor* for insect pest management. Many quassinoids are responsible for changes in feeding behavior and regulation of insect growth [79,80]. Thus, the changes in development observed in this study are another indication of the insecticidal potential of *Simarouba* sp. Changes in feeding behavior and growth regulation have been verified in *Spodoptera litura* (Fabricius, 1775; Lepidoptera, Noctuidae) [80], in addition to insecticidal activity against *Tetranychus urticae* (Koch, 1836; Acari, Tetranychidae), *Myzus persicae* (Sulzer, 1776; Hemiptera, Aphididae), and *Meloidogyne incognita* (Kofoid and White, 1919; Nematoda, Heteroderidae) [79].

Reports indicate that quassinoids potently inhibit feeding through the function of bitter taste [81,82], which was observed in this study; individuals remained in the larval stage for an additional 3 to 4 days, and many did not reach the pupal stage due to food reduction/restriction, plant toxicity, and/or food conversion. In other insect species, quassinoids are reported to slow lepidopteran pupation, even when applied at low concentrations, showing that the combination of food restriction and toxicity can delay development [82,83], resulting in growth regulation [80] with overall slowed development. In third instar larvae of *Locusta migratoria migratorioides* (Reiche and Fairmaire; Orthoptera: Acrididae),

quassinoids induced antifeeding activity [84], which has also been observed in other insect pests [85].

The analysis of *Simarouba* sp. showed variations in the contents of phenolic and polyphenolic compounds, highlighting a high concentration of total phenolics followed by flavonoids and flavanol, which is in agreement with the LC-MS/MS results for flavanone and kaempferol (Figure 3, Table 1). Phenolic and polyphenolic compounds make up a major group of phytochemicals in plants; flavonoids (polyphenolic substances) present a wide range of biological activities, including insecticide effects [86–89]. Flavonoids are responsible for the reduced growth of larvae and pupal survival [90], impaired feeding, digestion inhibition and the release of free radicals [91]. Thus, we can partially associate the effectiveness of AE-S in inducing mortality in *P. xylostella* by the presence of these compounds. In this context, future studies addressing the chemical fractionation and isolation of active molecules are of great importance, and these are the next steps of our research group.

Combining quassinoids with other compounds, such as kaempferol, further increases the potential of the plant, since their presence can alter the palatability of plants and decrease their nutritional value; they can produce free radicals in insects, thus decreasing digestibility or even function as toxins. Additionally, they can cause rupture of the midgut epithelial membrane or disturbance to its metabolism [91]. They can also cause increased mortality and sublethal effects in larvae [92], reduced oviposition [93], and decreased pupal survival, body weight, fecundity [21,94], and mortality.

Botanical insecticides are considered alternative pest control tools because they are safe with respect to biodiversity and the health of humans and the environment, they have no cumulative effect [95,96], and they biodegrade easily [97]. The recent survey conducted by Padial et al. [98], with aqueous extract of *Miconia albicans* (Sw.) Triana (Melastomataceae), observed that *P. xylostella* larvae treated with this extract showed reduction in larval duration and survival, fertility and repellency of females, both by oviposition and by feeding.

In the trials performed in this study, aqueous extract of *Simarouba* sp. has shown to be effective for population control of *P. xylostella*, with low toxicity to the non-target organism *C. elegans*. Additionally, it is highly accessible and inexpensive. However, the extract should be used carefully. The results presented here are preliminary, but represent indispensable tests on natural enemies and pollinators.

5. Conclusions

Here, we show for the first time that the extract of *Simarouba* sp. leaves at all concentrations is effective in inducing mortality in *P. xylostella*, and that it contains flavonoids, alkaloids, and quassinoids. Among all tested treatments, the 0.1% concentration stood out and could be explored in future field studies as an alternative control method. The extract with a concentration of 0.1% causes larval mortality, is non-toxic to the nematode *C. elegans*, and a low concentration requires less vegetal matter.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su15107759/s1, Table S1: Larval and pupal development (days) and biomass (g) of Plutella xylostella pupae exposed to aqueous extract of Simarouba sp. at different concentrations; Table S2: Longevity of females and males (days), fecundity, egg survival and oviposition period of *Plutella xylostella* pupae exposed to aqueous extract of *Simarouba* sp. at different concentrations.

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References

1. Roy, S.; Handique, G.; Muraleedharan, N.; Dashora, K.; Roy, S.M.; Mukhopadhyay, A.; Babu, A.A. Use of plant extracts for tea pest management in India. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 4831–4844. [CrossRef] [PubMed]
2. Nkechi, E.F.; Ejike, O.G.; Ihuoma, N.J.; Maria-goretti, O.C.; Francis, U.; Godwin, N.; Njokuocha, R. Effects of aqueous and oil leaf extracts of *Pterocarpus santalinoides* on the maize weevil, *Sitophilus zeamais*, pest of stored maize grains. *Afr. J. Agric. Res.* **2018**, *13*, 617–626. [CrossRef]
3. Sande, D.; Mullen, J.; Wetzstein, M.; Houston, J. Environmental impacts from pesticide use: A case study of soil fumigation in Florida tomato production. *Int. J. Environ. Res. Public Health* **2011**, *12*, 4649–4661. [CrossRef]
4. Campos, A.P.; Boiça Junior, A.L. Lagartas de *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) submetidas a diferentes concentrações de óleo de nim. *Rev. Bras. Milho Sorgo* **2012**, *11*, 137–144. [CrossRef]
5. Oliveira, F.A.L.D.; Silva, R.O.; Oliveira, N.R.X.D.; Andrade, G.S.; Pereira, F.F.; Zanuncio, J.C.; Coutinho, C.R.; Pastori, P.L. Reproduction of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) with different densities and parasitism periods in *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) pupae. *Folia Biol.* **2018**, *43*, 103–110. [CrossRef]
6. Corrêa, J.C.R.; Salgado, H.R.N. Atividade inseticida das plantas e aplicações: Revisão. *Rev. Bras. Plantas Med.* **2011**, *13*, 500–506. [CrossRef]
7. Bandeira, G.N.C.; Camara, A.G.; Moraes, M.M.; Barros, R.; Muhammad, S.; Akhtar, Y. Insecticidal activity of *Muntingia calabura* extracts against larvae and pupae of diamondback, *Plutella xylostella* (Lepidoptera, Plutellidae). *J. King Saud Univ. Sci.* **2013**, *25*, 83–89. [CrossRef]
8. Alves, D.S.; Oliveira, D.F.; Santos Júnior, H.M.; Carvalho, D.A.; Santos, M.A.I.; Arvalho, H.W.P. Plant extracts as an alternative to control *Leucoptera coffeella* (Guérin-èneville) (Lepidoptera: Lyonetiidae). *Neotrop. Entomol.* **2011**, *40*, 123–128. [CrossRef]
9. Shabana, Y.M.; Abdalla, M.E.; Shahin, A.A.; El-Sawy, M.M.; Draz, I.S.; Youssif, A.W. Efficacy of plant extracts in controlling wheat leaf rust disease caused by *Puccinia triticina* Egypt. *J. Basic Appl. Sci.* **2017**, *1*, 67–73. [CrossRef]
10. Capinera, J.L. *Encyclopedia of Entomology*, 2nd ed.; Springer: Gainesville, FL, USA, 2008; p. 5223.
11. APRD Arthropod Pesticide Resistance Database. *Plutella xylostella*. Available online: https://www.pesticideresistance.org/ (accessed on 10 March 2022).
12. Zalucki, M.P.; Shabbir, A.; Silva, R.; Adamson, D.; Shu-Sheng, L.; Furlong, M.J. Estimating the economic cost of one of the world's major insect pests, *Plutella xylostella* (Lepidoptera:Plutellidae): Just how long is a piece of string? *J. Econ. Entomol.* **2012**, *105*, 1115–1129. [CrossRef]
13. Shakeel, M.; Farooq, M.; Nasim, W.; Akram, W.; Khan, F.Z.A.; Jaleel, W.; Zhu, X.; Yin, H.; Li, S.; Fahad, S.; et al. Environment polluting conventional chemical control compared to an environmentally friendly IPM approach for control of diamondback moth, *Plutella xylostella* (L.), in China: A review. *Environ. Sci. Pollut. Res. Int.* **2017**, *24*, 14537–14550. [CrossRef]
14. Aliste, M.; Garrido, I.; Flores, P.; Hellín, P.; Pérez-Lucas, G.; Navarro, S.; Fenoll, J. Photocatalytic degradation four insecticides and their main generated transformation products in soil and pepper crop irrigated with reclaimed agro-wastewater under natural sunlight. *J. Hazard. Mater.* **2021**, *414*, 125603. [CrossRef] [PubMed]
15. Kim, S.I.; Roh, J.Y.; Kim, D.H.; Lee, H.S.; Ahn, Y.J. Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis*. *J. Stored Prod. Res.* **2003**, *39*, 293–303. [CrossRef]
16. Menezes, E.L.A. *Inseticidas Botânicos: Seus Princípios Ativos, Modo de Ação e Uso Agrícola*, 1st ed.; Seropédica: Rio de Janeiro, Brazil, 2005; pp. 4–20.
17. Sola, P.; Mvumi, B.M.; Ogendo, J.O.; Mponda, O.; Kamanula, J.F.; Nyirenda, S.P.; Belmain, S.R.; Stevenson, P.C. Botanical pesticide production, trade and regulatory mechanisms in sub-Saharan Africa: Making a case for plant-based pesticidal products. *Food Secur.* **2014**, *6*, 369–384. [CrossRef]

18. Pavela, R. History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects—A review history, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects—A review. *Plant Prot. Sci.* **2016**, *52*, 229–241. [CrossRef]
19. Krinski, D.; Massaroli, A.; Machado, M. Potencial inseticida de plantas da família Annonaceae. *Rev. Bras. Frutic.* **2014**, *36*, 225–242. [CrossRef]
20. Hikal, W.M.; Baeshen, R.S.; Said-Al Ahl, H.A. Botanical insecticide as simple extractives for pest control. *Cogent Biol.* **2017**, *3*, 1–16. [CrossRef]
21. Peres, L.L.S.; Sobreiro, A.I.; Couto, I.F.S.; Silva, R.M.; Pereira, F.F.; Heredia-Vieira, S.C.; Cardoso, C.A.L.; Mauad, M.; Scalon, S.P.Q.; Verza, S.S.; et al. Chemical compounds and bioactivity of aqueous extracts of *Alibertia* spp. in the control of *Plutella xylostella* L. (Lepidoptera: Plutellidae). *Insects* **2017**, *8*, 125. [CrossRef]
22. Souza, S.A.; Padial, I.M.P.M.; Couto, I.F.S.; da Silva, M.M.M.; de Carvalho, E.M.; Mussury, R.M. Extrato aquoso de *Campomanesia adamantium* (Myrtaceae) (Cambess.) O. Berg afeta o desenvolvimento de traça-das-crucíferas. In *Meio Ambiente: Impacto do Convívio Entre Vegetação, Animais e Homens 2*, 2nd ed.; Teófilo, T., Silva, T., Silva, F., Eds.; Atena Editora: Ponta Grossa, Brazil, 2020; pp. 34–44.
23. Couto, I.F.S.; Silva, S.V.; Valente, F.I.; Araújo, B.S.; Souza, S.A.; Mauad, M.; Scalon, S.P.Q.; Mussury, R.M. Botanical extracts of the Brazilian savannah affect feeding and oviposition of *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae). *J. Agric. Sci.* **2019**, *11*, 322–333. [CrossRef]
24. Couto, I.F.S.; Souza, S.A.; Valente, F.I.; Silva, R.M.; Scalon, S.P.Q.; Ferreira, F.F.; Silva, S.V.; de Carvalho, E.M.; Mussury, R.M. Changes in the biological characteristics of *Plutella xylostella* using ethanolic plant extracts. *Gesunde Pflanz.* **2020**, *72*, 383–391. [CrossRef]
25. Silva, R.M.; Santos, L.P.; Silva, G.B.; Miranda, L.O.; Fioratti, C.A.G.; Scalon, S.P.Q.; Mauad, M.; Mussury, R.M. *Alibertia* spp. (Rubiaceae) extracts interfere with the development and reproduction of *Plutella xylostella* L. (Lepidoptera: Plutellidae). *Gesunde Pflanz.* **2020**, *72*, 351–360. [CrossRef]
26. Ferreira, E.A.; de Souza, S.A.; Domingues, A.; da Silva, M.M.M.; Padial, I.M.P.M.; de Carvalho, E.M.; Cardoso, C.A.L.; da Silva, S.V.; Mussury, R.M. Phytochemical screening and bioactivity of *Ludwigia* spp. in the control of *Plutella xylostella* (Lepidoptera: Plutellidae). *Insects* **2020**, *11*, 596. [CrossRef] [PubMed]
27. Padial, I.M.P.M.; Matiasso, A.S.; Souza, S.A.; Mussury, R.M. Efeito de extratos vegetais de *Styrax camporum* Pohl. sobre a oviposição de *Plutella xylostella* (L., 1758) (Lepidoptera: Plutellidae). *Braz. J. Develop.* **2020**, *6*, 67038–67055. [CrossRef]
28. Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological effects of essential oils—A review. *Food Chem. Toxicol.* **2008**, *46*, 446–475. [CrossRef] [PubMed]
29. Joseph, B.; Sujatha, S. Insight of botanical based biopesticides against economically important pest. *Int. J. Pharm. Life Sci.* **2012**, *11*, 2138–2148.
30. Lengai, G.M.W.; Muthomi, J.W.; Mbega, E.R. Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. *Sci. Afr.* **2020**, *7*, e00239. [CrossRef]
31. Penteado, S.R. *Defensivos Agrícolas Naturais*, 3rd ed.; Grafimagem: Campinas, SP, Brazil, 1999; p. 95.
32. Castillo-Sánchez, L.E.; Jiménez-Osornio, J.J.; Delgado-Herrera, M.A.; Candelaria-Martínez, B.; Sandoval-Gío, J.J. Effects of the hexanic extract of neem *Azadirachta indica* against adult whitefly *Bemisia tabaci*. *J. Entomol. Zool. Stud.* **2015**, *3*, 95–99.
33. Rocha, A.N.; de Souza, S.A.; Fioratti, C.A.G.; Mauad, J.R.C.; Mauad, M.; Mussury, R.M. *Tradescantia pallida* (Commelinaceae) Promotes Reductions in *Plutella xylostella* (Lepidoptera: Plutellidae) Populations. *Agronomy* **2022**, *12*, 2646. [CrossRef]
34. Soković, M.; Glamočlija, J.; Marin, P.D.; Brkić, D.; Van Griensven, L.J.L.D. Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. *Molecules* **2010**, *15*, 7532–7546. [CrossRef]
35. Alencar, G.V.; Mendonça, E.S.; Oliveira, T.S.; Jucksch, I.; Cecon, P.R. Percepção ambiental e uso do solo por agricultores de sistemas orgânicos e convencionais na Chapada da Ibiapaba, Ceará. *Rev. Econ. Sociol. Rural* **2013**, *51*, 217–236. [CrossRef]
36. de Mendonça, F.A.C.; da Silva, K.F.S.; dos Santos, K.K.; Ribeiro Júnior, K.A.L.; Sant'Ana, A.E.G. Activities of some Brazilian plants against larvae of the mosquito *Aedes aegypti*. *Fitoterapia* **2005**, *76*, 629–636. [CrossRef] [PubMed]
37. Coelho, A.A.M.; Paula, J.E.; Espíndola, L.S. Insecticidal activity of cerrado plant extracts on *Rhodnius milesi* (Carcavallo, Rocha, Galvão & Jurberg) (Hemiptera: Reduviidae), under laboratory conditions. *Neotrop. Entomol.* **2006**, *35*, 133–138. [CrossRef] [PubMed]
38. Simote, S.Y. Estudo Fitoquímico de *Helietta puberula* (Rutaceae), *Simarouba versicolor* (Simaroubaceae) e Busca de um Processo de Microencapsulação de Compostos Ativos Visando o Controle de Formigas Cortadeiras. Ph.D. Thesis, Universidade Federal de São Carlos, São Carlos, SP, Brazil, 2006.
39. Peñaflor, M.F.G.V.; Almeida, R.N.A.; Simote, S.Y.; Yamane, E.; Bueno, O.; Hebling, M.J.A.; Fernandes, J.B.; Vieira, P.C.; da Silva, M.F.G.F.; Pagnocca, F. Toxicity of substances isolated from *Simarouba versicolor* St. Hil. (Simaroubaceae) to the leaf-cutting ant *Atta sexdens* L. (Hymenoptera: Formicidae) and the symbiotic fungus *Leucoagaricus gongylophorus* (Singer) Möller. *Bioassay* **2009**, *4*, 1–7. [CrossRef]
40. Barbosa, L.F.; Braz-Filho, R.; Vieira, I.J.C. Chemical constituents of plants from the genus *Simaba* (Simaroubaceae). *Chem. Biodivers.* **2011**, *8*, 2163–2178. [CrossRef] [PubMed]
41. Saraiva, R.C.G.; Pinto, A.C.; Nunomura, S.M.; Pohlit, A.M. Triterpenos e alcaloide tipo cantinona dos galhos de *Simaba polyphylla* (Cavalcante) W. W. Thomas (Simaroubaceae). *Quim. Nova* **2006**, *29*, 264–268. [CrossRef]

42. Collins, J.J.; Evason, K.; Kornfeld, K. Pharmacology of delayed aging and extended lifespan of *Caenorhabditis elegans*. *Exp. Gerontol.* **2006**, *41*, 1032–1039. [CrossRef]
43. Cheng, S.C.; Li, W.H.; Shi, Y.C.; Yen, P.L.; Lin, H.Y.; Liao, V.H.C.; Chang, S.T. Antioxidant activity and delayed aging effects of hot water extract from *Chamaecyparis obtusa* var. *formosana* leaves. *J. Agric. Food Chem.* **2014**, *62*, 4159–4165. [CrossRef]
44. Duangjan, C.; Rangsinth, P.; Gu, X.; Zhang, S.; Wink, M.; Tencomnao, T. *Glochidion zeylanicum* leaf extracts exhibit lifespan extending and oxidative stress resistance properties in *Caenorhabditis elegans* via DAF-16/FoxO and SKN-1/Nrf-2 signaling pathways. *Phytomedicine* **2019**, *64*, 153061. [CrossRef]
45. Queirós, L.; Pereira, J.L.; Gonçalves, F.J.M.; Pacheco, M.; Aschner, M.; Pereira, P. *Caenorhabditis elegans* as a tool for environmental risk assessment: Emerging and promising applications for a "nobelized worm". *Crit. Rev. Toxicol.* **2019**, *49*, 411–429. [CrossRef]
46. Leelaja, B.C.; Rajini, P.S. Biochemical and physiological responses in *Caenorhabditis elegans* exposed to sublethal concentrations of the organophosphorus insecticide, monocrotophos. *Ecotoxicol. Environ. Saf.* **2013**, *94*, 8–13. [CrossRef]
47. Zeng, R.; Yu, X.; Tan, X.; Ye, S.; Ding, Z. Deltamethrin affects the expression of voltage-gated calcium channel α1 subunits and the locomotion, egg-laying, foraging behavior of *Caenorhabditis elegans*. *Pestic. Biochem. Physiol.* **2017**, *138*, 84–90. [CrossRef] [PubMed]
48. Cole, R.D.; Anderson, G.L.; Williams, P.L. The nematode *Caenorhabditis elegans* as a model of organophosphate-induced mammalian neurotoxicity. *Toxicol. Appl. Pharmacol.* **2004**, *194*, 248–256. [CrossRef]
49. Bongers, T.; Ferris, H. Nematode community structure as a bioindicator in environmental monitoring. *Trends Ecol. Evol.* **1999**, *14*, 224–228. [CrossRef]
50. Hope, I.A. Background of *Caenorhabditis elegans*. In C. elegans*: A Practical Approach*, 1st ed.; Hope, I.A., Ed.; Oxford Univiversity Pressione: Oxford, UK, 1999; pp. 1–15.
51. Khweek, A.A.; Amer, A.O. Factors mediating environmental biofilm formation by *Legionella pneumophila*. *Front. Cell. Infect. Microbiol.* **2018**, *8*, 38. [CrossRef]
52. Beare, M.H. Fungal and bacterial pathways of organic matter decomposition and nitrogen mineralization in arable soil. In *Soil Ecology in Sustainable Agricultural Systems*; Brussaard, L., Ferrara-Cerrato, R., Eds.; Lewis Publishers: Boca Raton, FL, USA, 1997; pp. 37–70.
53. Parodi, D.A.; Jasmine, S.; Yichang, C.; Patrick, A. Reproductive toxicity and meiotic dysfunction following exposure to the pesticides Maneb, Diazinon and Fenarimol. *Toxicol. Res.* **2015**, *4*, 645–654. [CrossRef]
54. Jacques, M.T.; Bornhorst, J.; Soares, M.V.; Schwerdtle, T.; Garcia, S.; Ávila, D.S. Reprotoxicity of glyphosate-based formulation in *Caenorhabditis elegans* is not due to the active ingredient only. *Environ. Pollut.* **2019**, *252*, 1854–1862. [CrossRef]
55. ASTM International. Standard Guide for Conducting Laboratory Soil Toxicity Tests with the Nematode Caenorhabditis elegans E 2172–01. Available online: https://www.astm.org/Standards/E2172.htm (accessed on 15 February 2021).
56. *ISO 10872*; Water Quality—Determination of the Toxic Effect of Sediment and Soil Samples on Growth, Fertility and Reproduction of *Caenorhabditis elegans* (Nematoda). ISO: Geneva, Switzerland, 2010. Available online: https://www.iso.org/standard/46253.html (accessed on 10 February 2021).
57. Nass, R.; Hamza, I. The nematode *C. elegans* as an animal model to explore toxicology in vivo: Solid and axenic growth culture conditions and compound exposure parameters. *Curr. Protoc. Toxicol.* **2007**, *31*. Unit1.9. [CrossRef] [PubMed]
58. Tralau, T.; Riebeling, C.; Pirow, R.; Oelgeschläger, M.; Seiler, A.; Liebsch, M.; Luch, A. Wind of change challenges toxicological regulators. *Environ. Health Perspect.* **2012**, *120*, 1489–1494. [CrossRef]
59. Hunt, P.R. The *C. elegans* model in toxicity testing. *J. Appl. Toxicol.* **2017**, *37*, 50–59. [CrossRef]
60. Lin, J.Y.; Tang, C.Y. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chem.* **2007**, *101*, 140–147. [CrossRef]
61. Broadhurst, R.B.; Jones, W.T. Analysis of condensed tannins using acidified vanillin. *J. Sci Food Agric.* **1978**, *29*, 788–794. [CrossRef]
62. Brenton, A.G.; Godfrey, A.R.J. Accurate mass measurement: Terminology and treatment of data. *J. Am. Soc. Mass Spectrom.* **2010**, *21*, 1821–1835. [CrossRef] [PubMed]
63. Barros, R.; Thuler, R.T.; Pereira, F.F. Técnica de criação de *Plutella xylostella* (L., 1758) (Lepidoptera: Yponomeutidae). In *Técnicas de Criação de Pragas de Importância Agrícola, em Dietas Naturais*, 1st ed.; Pratissoli, D., Ed.; Edufes: Vitória, Brazil, 2012; pp. 65–84.
64. Logan, J.A.; Wollkind, D.T.; Hoyt, J.C.; Tanigoshi, L.K. An analytic model for description of temperature dependent rate phenomena in arthropods. *Environ. Entomol.* **1976**, *5*, 1130–1140. [CrossRef]
65. Dengg, M.; van Meel, J.C.A. *Caenorhabditis elegans* as model system for rapid toxicity assessment of pharmaceutical compounds. *J. Pharmacol. Toxicol. Methods* **2004**, *50*, 209–214. [CrossRef] [PubMed]
66. Rodriguez, H.C.; Vendramim, J.D. Toxicidad de extractos acuosos de Meliaceae en *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Manag. Integr. Plagas* **1996**, *42*, 14–22.
67. Tanzubil, P.B.; McCaffery, A.R. Effects of Azadirachtin on reproduction in the African armyworm (*Spodoptera exempta*). *Entomol. Exp. Appl.* **1990**, *57*, 115–121. [CrossRef]
68. Costa, E.L.; Silva, N.R.F.P.; Fiúza, L.M. Efeitos, aplicações e limitações de extratos de plantas inseticidas. *Acta Biol. Leopoldensia* **2004**, *26*, 173–185.
69. Reddy, G.V.P.; Tabone, E.; Smith, M.T. Mediation of the host selection and oviposition behavior in the diamondback moth *Plutella xylostella* and its predator *Chrysoperla carnea* by chemical cues from cole crops. *Biol. Control* **2004**, *29*, 270–277. [CrossRef]

70. Monnerat, R.G.; Leal-Bertioli, S.C.M.; Bertioli, D.J.; Butt, T.M.; Bordat, D. Caracterização de populações geograficamente distintas da traça-das- crucíferas por susceptibilidade ao *Bacillus thuringiensis* Berliner e RAPD-PCR. *Hortic. Bras.* **2004**, *22*, 607–609. [CrossRef]
71. Maroneze, D.M.; Gallegos, D.M.N. Efeito de extrato aquoso de *Melia azedarach* no desenvolvimento das fases imatura e reprodutiva de *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae). *Semin. Cienc. Agrar.* **2009**, *30*, 537–550. [CrossRef]
72. Souza, S.A.; Ferreira, E.A.; Silva, M.M.M.; Padial, I.M.P.M.; Melo, A.M.M.F.; Mussury, R.M. Extrato aquoso de *Tabernaemontana solanifolia* a. Dc. (Apocynaceae) é uma alternativa efetiva no controle de Traça-das-crucíferas. In *Ciências Aplicadas: Princípios Fundamentais*, 1st ed.; Ulhôa, J.L.R., Saraiva-Bonatto, E.C., Sousa, A.R., Barbosa, F.C., Gontijo, C.E.O., Pires, P.F., Eds.; Editora Conhecimento Livre: Piracanjuba, Brazil, 2020; pp. 123–140. [CrossRef]
73. Bisht, K.; Verma, S.; Singh, N.N. Comparison of enhanced artificial diets for mass rearing of *Helicoverpa armigera* (Hubner) under laboratory conditions. *J. Entomol. Zool. Stud.* **2018**, *6*, 2551–2553.
74. Wang, P.; Furlong, M.J.; Walsh, T.K.; Zalucki, M.P. Moving to keep fit: Feeding behavior and movement of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on artificial diet with different protein: Carbohydrate ratios. *J. Insect Sci.* **2019**, *19*, 20. [CrossRef]
75. Silva, P.R.C.; Camaroti, J.R.S.L.; Almeida, W.A.; Ferreira, E.C.B.; Paiva, P.M.G.; Barros, R.; Napoleão, T.H.; Pontual, E.V. *Schinus terebinthifolia* leaf extract is a larvicidal, pupicidal, and oviposition deterring agent against *Plutella xylostella*. *S. Afr. J. Bot.* **2019**, *127*, 124–128. [CrossRef]
76. Wuyts, N.; Swennen, R.; de Waele, D. Effects of plant phenylpropanoid pathway products and selected terpenoids and alkaloids on the behaviour of the plant-parasitic nematodes *Radopholus similis*, *Pratylenchus penetrans* and *Meloidogyne incognita*. *Nematology* **2006**, *8*, 89–101. [CrossRef]
77. Gonçalves, L.; Spindola, C.B.; Almeida, F. *Bacillus thuringiensis* var. *kurstaki* (Bacillaceae): Potencial no controle, no desenvolvimento e reprodução de *Oxydia vesulia* (Geometridae), em laboratório. *Acta Biol. Parana.* **2008**, *37*, 147–163. [CrossRef]
78. Pingel, R.L.; Lewis, L.C. Field application of *Bacillus thuringiensis* and *Anagrapha falcifera* multiple nucleopolyhedrovirus against the corn Earworm, (Lepidoptera: Noctuidae). *J. Econ. Entomol.* **1997**, *90*, 1195–1199. [CrossRef]
79. Latif, Z.; Craven, L.; Hartley, T.G.; Kemp, B.R.; Potter, J.; Rice, M.J.; Waigh, R.D.; Waterman, P.G. An insecticidal quassinoid from the new australian species *Quassia* sp. aff. bidwillii. *Biochem. Syst. Ecol.* **2000**, *28*, 183–184. [CrossRef]
80. Govindachari, T.R.; Krishna Kumari, G.N.; Gopalakrishnan, G.; Suresh, G.; Wesley, S.D.; Sreelatha, T. Insect antifeedant and growth regulating activities of quassinoids from *Samadera indica*. *Fitoterapia* **2001**, *72*, 568–571. [CrossRef]
81. Okano, M.; Fukamiya, N.; Lee, K.H. Bioactive quassinoids. *Stud. Nat. Prod. Chem.* **2000**, *23*, 285–333. [CrossRef]
82. Braga, F.C.; Castilho, R.O. Potencialidade do cerrado como fonte de substâncias bioativas e de espécies medicinais para o desenvolvimento de fitoterápicos. In *Farmacogn. Coletânea Científica*; Souza, G.H.B., Mello, J.C.P., Lopes, N.P., Eds.; UFOP: Ouro Preto, MG, Brazil, 2012; pp. 295–318.
83. Lidert, Z.; Wing, K.; Polonsky, U.; Imakura, Y.; Okano, M.; Tani, S.; Lin, Y.M.; Kiyokawa, H.; Lee, K.H. Insect antifeedant and growth inhibitory activity of forty-six quassinoids on two species of agricultural pests. *J. Nat. Prod.* **1987**, *50*, 442–448. [CrossRef]
84. Odjo, A.; Piart, J.; Polonsky, J.; Roth, M. Etude de l'effet insecticide de deux quassainoides sur des larves de *Locusta migratoria magratoroides* R et F (Orthoptera, Acrididae). *Comptes Rendus Sci. Paris* **1981**, *293*, 241–244.
85. Klocke, J.A.; Arisawa, M.; Handa, S.S.; Kinghorn, A.D.; Cordell, G.A.; Farnsworth, N.R. Growth inhibitory, insecticidal and antifeedant effects of some antileukemic and cytotoxic quassinoids on two species of agricultural pests. *Experientia* **1985**, *41*, 379–382. [CrossRef]
86. Napal, G.N.D.; Palacios, S.M. Bioinsecticidal effect of the flavonoids pinocembrin and quercetin against *Spodoptera frugiperda*. *J. Pest Sci.* **2015**, *88*, 629–635. [CrossRef]
87. Hay, W.T.; Behle, R.W.; Berhow, M.A.; Miller, A.C.; Selling, G.W. Biopesticide synergy when combining plant flavonoids and entomopathogenic baculovirus. *Sci. Rep.* **2020**, *10*, 6806. [CrossRef] [PubMed]
88. Sarria, A.L.F.; Matos, A.P.; Volante, A.C.; Bernardo, A.R.; Cunha, G.O.S.; Fernandes, J.B.; Forim, M.R.; Vieira, P.C.; Silva, M.F.G.F. Insecticidal activity of copper (II) complexes with flavanone derivatives. *Nat. Prod. Res.* **2021**, *36*, 1342–1345. [CrossRef] [PubMed]
89. Stamp, N.E.; Skrobola, C.M. Failure to avoid rutin diets results in altered food utilization and reduced growth rate of *Manduca sexta* larvae. *Entomol. Exp. Appl.* **1993**, *68*, 127–142. [CrossRef]
90. Silva, T.R.F.B.; Almeida, A.C.S.; Moura, T.L.; Silva, A.R.; Freitas, S.S.; Jesus, F.G. Effect of the flavonoid rutin on the biology of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Acta Sci. Agron.* **2016**, *38*, 165–170. [CrossRef]
91. Appel, H.M. Phenolics in ecological interactions: The importance of oxidation. *J. Chem. Ecol.* **1993**, *19*, 1521–1552. [CrossRef]
92. Pandey, A.; Misra, P.; Chandrashekar, K.; Trivedi, P.K. Development of *AtMYB12*-expressing transgenic tobacco callus culture for production of rutin with biopesticidal potential. *Plant Cell Rep.* **2012**, *31*, 1867–1876. [CrossRef]
93. Salunke, B.K.; Kotkar, H.M.; Mendki, O.S.; Upasani, S.M.; Maheshwari, V.L. Efficacy of flavonoids in controlling *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae), a post-harvest pest of grain legumes. *Crop Prot.* **2005**, *24*, 888–893. [CrossRef]
94. Gazzoni, D.L.; Hulsmeyer, A.; Hoffman-Campo, C.B. Efeito de diferentes doses de rutina e de quercetina na biologia de *Anticarsia gemmatalis*. *Pesqui. Agropecu. Bras.* **1997**, *32*, 673–681.
95. Talukder, F.A.; Islam, M.S.; Hossain, M.S.; Rahman, M.A.; Alam, M.N. Toxicity effects of botanicals and synthetic insecticides on *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.). *Bangladesh J. Environ. Sci.* **2004**, *10*, 365–371.
96. Garcez, W.S.; Garcez, F.R.; Silva, L.M.G.E.; Sarmento, U.C. Substâncias de origem vegetal com atividade larvicida contra *Aedes aegypti*. *Rev. Virtual Química* **2013**, *5*, 363–393. [CrossRef]

97. Neeraj, G.S.; Kumar, A.; Ram, S.; Kumar, V. Evaluation of nematicidal activity of ethanolic extracts of medicinal plants to *Meloidogyne incognita* (kofoid and white) chitwood under lab conditions. *Int. J. Pure Appl. Biosci.* **2017**, *1*, 827–831. [CrossRef]
98. Padial, I.M.P.M.; de Souza, S.A.; Malaquias, J.B.; Cardoso, C.A.L.; da Silva Pachú, J.K.; Fioratti, C.A.G.; Mussury, R.M. Leaf Extracts of *Miconia albicans* (Sw.) Triana (Melastomataceae) Prevent the Feeding and Oviposition of *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae). *Agronomy* **2023**, *13*, 890. [CrossRef]