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Evaluation of the Contact Toxicity and Physiological Mechanisms of Ginger (*Zingiber officinale*) Shoot Extract and Selected Major Constituent Compounds against *Melanaphis sorghi Theobald*

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Abstract: Botanical pesticides have gradually become accepted for use in the control of agricultural pests. In order to clarify the active compounds of the ginger (*Zingiber officinale*) shoot extract (GSE) and its inhibitory effect on the growth of sorghum aphids (*Melanaphis sorghi*). In this study, LC-MS/MS was used to determine the major active compounds of the GSE, and leaf disc method was used to explore the insecticidal effect of the active compounds of ginger on sorghum aphids and the response mechanism of sorghum aphids. The results showed that phenolic acids were identified as the main active compounds, followed by flavonoids. The aphidicidal activity test using the above compounds found that 6-gingerol, and quercetin-3-O-rutinoside exhibited aphidicidal activity (GSE > quercetin-3-O-rutinoside > 6-gingerol). The growth of sorghum aphid was evaluated by using different concentrations of the GSE. It was found that with the increase of concentration and treatment time, the litter size, longevity and molting of aphids significantly decreased, and the mortality of aphids increased. The enzyme activity of aphids treated with 15 mg·mL^{-1} GSE was determined, and it was found that the GSE could significantly inhibit the activities of pepsin, lipase and α-amylase of aphids, while the activity of superoxide dismutase (SOD) was significantly activated. The activities of peroxidase (POD) and catalase (CAT) increased at first and then decreased. In detoxification enzymes, the carboxylesterase (CarE) activity was significantly activated, the acetylcholinesterase (AChE) activity was significantly inhibited, and the glutathione S-transferase (GST) activity increased at first and then decreased. The above results indicated that the GSE may become a botanical pesticide for aphid control and provide new resources for the development of aphid biological agents.

Keywords: biocontrol; compound analysis; biological activity; botanical pesticide; growth inhibition; enzyme activity

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Citation: Liu, X.; Xi, K.; Wang, Y.; Ma, J.; Huang, X.; Liu, R.; Cai, X.; Zhu, Y.; Yin, J.; Jia, Q.; et al. Evaluation of the Contact Toxicity and Physiological Mechanisms of Ginger (*Zingiber officinale*) Shoot Extract and Selected Major Constituent Compounds against *Melanaphis sorghi Theobald*. *Horticulturae* **2022**, *8*, 944. https://doi.org/10.3390/horticulturae8100944

Academic Editor: Giulia Giunti

Received: 12 September 2022
Accepted: 11 October 2022
Published: 14 October 2022

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1. Introduction

Aphids (Aphidoidea) are worldwide known and spread pests of crops. During the growth and development of aphids, they will suck plant sap, molt, and secrete honeydew. These behaviors not only affect plant photosynthesis, but also lead to the spread of viral diseases in field crops, resulting in a decline of crop quality and even loss of the harvest [1]. The sorghum aphid (*Melanaphis sorghi Theobald*; Hemiptera: Aphididae) is distributed all over the world and is named for its effect on sorghum and corn, and the spread of sorghum red-stripe virus disease [2]. At present, chemical pesticides are typically used as

the primary means to control sorghum aphids, but the excessive use of chemical pesticides can easily lead to pesticide residues, pest resistance, and other problems. For this reason, it is imperative to develop efficient, residue-free, and ecofriendly prevention and control strategies. Botanical insecticides have gradually become of interest to scientists all over the world because of their ecofriendly, residue-free, biodegradable, and cost-effective attributes [3]. Plant extracts and primary active compounds have also been analyzed and explored as natural materials for the production of botanical pesticides. Plant extracts can act as insecticidal, ovicidal, and ovipositional deterrents; feeding deterrents; and growth retardants to pests through acute toxicity and enzyme inhibition [4]. *Adhatoda vasica* leaf extract has strong larvicidal activity against the *Bancroftian filariasis* vector *Culex quinquefasciatus* and dengue vector *Aedes aegypti* [5], and aqueous tobacco root extract exhibits biological activity against grape phylloxera. Egg mortality, nymph mortality, development period, longevity, and female fecundity are significantly affected [6]. Some secondary metabolites with potential insecticidal effects may be present in plant extracts. It is necessary to identify the compounds of plant extracts and determine the main active compounds. For instance, studies have reported that alkaloids in *Chelidonium majus* are the main insecticidal active compounds and have significant insecticidal activity against *Lymantria dispar* [7]. Azadirachtin could potentially control sugarcane aphid infestations in sorghum if applied under favorable environmental conditions [8]. Therefore, botanical insecticides are an important potential method for the green control of aphids, but there are few studies as yet on the antiaphidice compounds of ginger.

Ginger (*Zingiber officinale* Roscoe) is a medicinal spice crop with high edible and economic value. It is widely distributed in central, southeast, and southwest China. Ginger has strong bacteriostatic and insecticidal activity [9]; for instance, ginger extract clearly inhibited *Drosophila melanogaster* mating [10], inhibited the oviposition of whitefly, and repelled the adults of whitefly [11], and has also demonstrated significant toxicity toward *Diaphania hyalinata* larvae, affecting the hatching rate of the pupae [12]. Ginger plant powder can effectively control the parasitic population of alfalfa leaf-cutting wasps, and also shows insecticidal activity against stored grain pests [13,14]. Ginger rhizome volatiles have demonstrated a repellent activity effect on maize weevil [15]. Therefore, ginger has the potential for use as a botanical insecticide, and the insecticidal mechanism of ginger should be evaluated.

Botanical insecticides not only consider their control effect, but also needs to consider the insecticide resistance of pests. The insecticidal mechanism is the key to studying insecticide resistance of pests, among which the relevant enzymes in pests are the important targets. Pepsin, lipase, and amylase are digestive enzymes in insects that promote food digestion and absorb nutrients. Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) are important antioxidant enzymes in insects, as they remove free superoxide radicals and maintain a state of dynamic equilibrium, with the free radicals being maintained at a low level to prevent free radical toxicity [16]. Glutathione S-transferase (GST) and carboxylesterase (CarE) are important detoxification enzymes in insects that can activate the detoxification system and relieve toxicity symptoms [17]. Acetylcholinesterase (AChE) is an important hydrolase in the insect nervous system that can stop nerve impulses by catalyzing the hydrolysis of the neurotransmitter choline [18]. Some studies have shown that some enzymes in pests significantly respond to plant extracts. Zhou et al. [19] found that three types of star anise fruit extracts could inhibit the activities of AChE and GST, and Gao et al. [20] found that isoorientin significantly affected the activities of AChE and POD. These results suggest that the change of enzyme activities in insect pests may be the cause of the death of these pests, and reveal the possible insecticidal mechanism of plant extracts. Hence, studying the activities of these enzymes could contribute significantly to investigations of the mechanisms of action of botanical insecticides.

This study aimed to investigate the compounds of ginger shoot extract (GSE) and its aphidicidal activity, determining the active compounds and the mechanism. The insecticidal active compounds were preliminarily discussed, and the effects of different

concentrations of GSE on the growth and development of sorghum aphids and the activities of nine enzymes were determined. The aim was to clarify the control mechanism of GSE on sorghum aphids, and contribute to the search for new botanical insecticides and theoretical knowledge regarding the ecofriendly control of sorghum aphids.

2. Materials and Methods

2.1. Insects and Plants

The wingless adults of sorghum aphid (*Melanaphis sorghi*) were collected from the experimental field of Tai Hu Farm of Yangtze University in Jingzhou City. They were fed on fresh leaves of beans for more than five generations in climatic chambers (22 ± 2 °C, 65 ± 5% relative humidity, 12L:12D photoperiod). The FengTou variety of ginger from Xuanen County of Enshi City was planted in the greenhouse of Yangtze University. Shoots of ginger were collected when they had 8–10 real leaves.

2.2. Extract of Z. officinale

The shoots of ginger were extracted by a Soxhlet extractor. Anhydrous ethanol was added at the ratio of 1:10, the temperature was adjusted to 80 °C, and after extraction for 6 h, the extract was moved to a round bottom flask, the rotary evaporator was opened, and the temperature was set to 80 °C. The substances, concentrated into paste, were collected and weighed.

2.3. Analysis of the Active Compounds of the Ginger Shoot Extract (GSE)

The active compounds of GSE were identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The methods were those of Chen et al. [21], with slight modification. Together with the standard liquid chromatogram and its standard curve, GSE was qualitatively and quantitatively analyzed. The chromatographic column was an Agilent SB-C18 (1.8 μm, 2.1 mm × 100 mm). Mobile phase A was ultra-pure water (with 0.1% formic acid) and mobile phase B was acetonitrile (with 0.1% formic acid). The elution conditions were as follows: 0.00 min B phase ratio of 5%, followed by a linear increase in B to 95% within 9.00 min, which was maintained at 95% for 1 min, followed by a decrease in B to 5% from 10.00–11.10 min, with B maintained at 5% until 14 min. The column temperature was 40 °C, the injection volume was 4 μL, and the flow rate was 0.35 mL·min^{-1}. The mass spectrometry conditions were as follows: linear ion trap and triple quadrupole (QQQ) scanning were carried out on a triple quadrupole linear ion trap mass spectrometer (Q TRAP) and AB4500 Q TRAP UPLC/MS/MS system, and the positive and negative ion modes were controlled by Analyst 1.6.3 software (AB SCIEX, Inc., Framingham, MA, USA). The operating parameters of electron spray ionization (ESI) were ion source, turbine spray; source temperature 550 °C; ion spray voltage (IS), 5500 V (positive ion mode)/−4500 V (negative ion mode); and the ion source gas I (GS I), gas II (GS II), and curtain gas (CUR) were set to 50, 60, and 25 psi, respectively. The collision-induced ionization parameter was set to high.

2.4. Contact Toxicity of GSE and Its Main Compounds

6-Gingerol and quercetin-3-O-rutinoside, which were the main phenolic acids and flavonoids compounds, were selected for aphidicidal activity analysis. 6-Gingerol, quercetin-3-O-rutinoside and GSE were dissolved in a small amount of methanol, and then 10% ethanol was used to prepare 10 mg·mL^{-1} of solution. The aphidicidal activity test was slightly modified by the impregnation method [22]. The winged aphids were removed from leaves of *Phaseolus vulgaris*, and only 10 wingless aphids were maintained on the leaves. The leaves containing the wingless aphids were dipped in the prepared solution, and removed after 3 s. The bean petiole was then wrapped with absorbent defatted cotton balls and air-dried, and the number of aphids was recorded, following which they were placed into petri dishes (d = 9 cm) and then in an incubator, as per standard insect cultivation

conditions. The control was 10% ethanol. Each treatment was repeated three times, and the number of dead aphids was examined after 12 h and 24 h.

2.5. Aphid Growth Index Assays

The growth index of sorghum aphids was determined by the leaf plate method. GSE was prepared with 10% ethanol in solutions with concentrations of 5 mg·mL^{-1}, 10 mg·mL^{-1}, and 15 mg·mL^{-1}, respectively. A total of 15 wingless aphids (fed for about 5 d) were randomly selected and placed in a petri dish containing slices (diameter of 9 cm and height of 1.5 cm). The petri dishes were then placed in a light incubator (temperature 22 ± 2 °C, relative humidity 65 ± 5%, photoperiod 12 L:12 D). Using 10% ethanol as the control, the leaves of each treatment were soaked in the different solutions for 3–5 s, and each treatment was repeated 3–5 times. The litter size, molting, and the mortality of the aphids were measured on 3 d and 7 d, and the longevity of the aphids was recorded separately.

2.6. Enzyme Assays

The surviving wingless aphids were collected from petri dishes treated with 15 mg·mL^{-1} solution by the leaf plate method and stored under cryopreservation at −80 °C. In this test, the wingless aphids treated with 10% ethanol solution were used as the control (CK), and the enzyme activity was determined at 1, 3, 5, and 7 d.

For the determination of digestive enzyme activity, the samples were removed from the refrigerator at −80 °C. The sample preparation and activity detection of pepsin, lipase, and α-amylase were determined according to the instructions of each enzyme activity detection kit (Nanjing Jiancheng Bioengineering Institute, A080-1-1, A054-1-1, C016-1-1).

The samples used for the determination of SOD, POD, and CAT were added to nine times the volume of normal saline [weight (g): volume (mL) = 1:9]. Each sample was ground and homogenized in an ice bath, and the supernatant was separated after 2500 r ·min^{-1} centrifugation for 10 min, which was the original enzyme solution. The activity of each protective enzyme was determined according to the instructions of the enzyme activity detection kit (Nanjing Jiancheng Bioengineering Institute, A001-1-1, A084-1-1, A007-2-1).

The samples used for the determination of AChE, CarE, and GST were added to nine times the volume of extractive liquid [weight (g): volume (mL) = 1:9]. Each sample was ground and homogenized in an ice bath, and the supernatant was separated after 12,000 r ·min^{-1} centrifugation for 10 min, which was the original enzyme solution. The activity of each detoxification enzyme was determined according to the instructions of the enzyme activity detection kit (Suzhou Grace Biotechnology Co., Ltd., G0907F, G0908F, G0208F).

2.7. Statistical Analysis

The LC-MS/MS results were analyzed by Excel software (Microsoft, Redmond, WA, USA) the relative quantification of each compound was performed using the mean, and the content proportion of each compound was also calculated. Significant differences in mortality, corrected mortality, litter size, molting, longevity, and enzyme activities of *M. sorghi* were analyzed using the one-way analysis of variance test (one-way ANOVA) and Tukey's HSD Multiple comparisons were carried out for different treatments at the same time. The percentage data on mortality and corrected mortality were the arcsine-square root transformed before ANOVA analysis in this study. The mortality and corrected mortality of each treatment group were calculated according to the following formulae: $P_1 = (K/N) \times 100$, where P_1 is the mortality rate, K is the number of dead insects, and N is the total number of treated insects; and $P_2 = (P_i - P_0)/(1 - P_0) \times 100$, where P_2 is the corrected mortality, P_i is the mortality of the treatment group, and P_0 is the mortality of the control group. If the control mortality rate was less than 5%, it did not need to be corrected; if the control mortality rate was between 5% and 20%, it needed to be corrected according to the second formula; and if the control mortality rate was more than 20%, the experiment needed to be redone. The aphid growth index and enzyme activity data are shown as

the mean ± standard error (SE), and were analyzed using the SPSS 20.0 software (IBM Crop., Armonk, NY, USA). Sigma Plot 14.0 (Inpixon Crop., Palo Alto, CA, USA) was used for producing graphs. Before analysis, the original and transformed data were tested for homogeneity and normality of variances with the Kolmogorov—Smirnov and Levene tests, respectively.

3. Results

3.1. Major Constituent Compounds of Ginger Shoot Extract (GSE)

According to LC-MS/MS detection, there were eight compounds in GSE, including phenolic acids, flavonoids, alkaloids, quinones, terpenoids, lignin and coumarins, tannins, and more (Table 1). The highest content was phenolic acids, followed by flavonoids at 41.5% and 27.1%. According to spectral analysis and comparison with standard substances, the most abundant phenolic acid was 10-Paradol at 7.17%, and the most abundant flavonoid was quercetin-3-O-rutinoside at 12.88% (Table 2). Among them, 6-gingerol and quercetin are the main compounds known to be present in GSE, and were detected in the compound analysis.

Table 1. The principal compounds and sub-compounds of ginger shoot extract (GSE) were identified by LC-MS/MS, and their contents were determined by relative quantification.

NO	Class I [a]	Class II [b]	Proportion (%) [c]
1	Phenolic acids	Phenolic acids	41.5%
2	Flavonoids	Chalcones Sinensetin Dihydroflavone Dihydroflavonol Flavonoids Flavonols Flavonoid carbonoside Flavanols Isoflavones	27.1%
3	Alkaloids	Phenolamine Quinoline alkaloids Alkaloids	11.9%
4	Quinones	Plumerane Quinones	2.0%
5	Terpenoids	Sesquiterpenoids Monoterpenoids Ditepenoids Triterpene Terpene	0.9%
6	Lignans and Coumarins	Lignans Coumarins	0.6%
7	Tannins	Tannins	0.5%
8	Others	Others Xanthone	15.5%

[a] 8 principal components determined by LC-MS/MS. [b] Sub-classification under each principal compound. [c] The proportion of each component in the total compounds.

Table 2. The two principal components (phenolic acids and flavonoids) in GSE were deeply explored, and the main properties and contents of their secondary metabolites were analyzed.

	NO.	Compounds	MF [a]	MW [b]	Proportion (%) [c]	CAS Rn [d]
Phenolic acids	1	Protocatechualdehyde	$C_7H_6O_3$	138	4.62	139-85-5
	2	4-Hydroxybenzoic acid	$C_7H_6O_3$	138	6.50	99-96-7
	3	Protocatechuic acid	$C_7H_6O_4$	154	3.61	99-50-3
	4	p-Coumaroylmalic acid	$C_{13}H_{12}O_7$	280	4.77	NA
	5	[6]-Gingerol	$C_{17}H_{26}O_4$	294	0.66	23513-14-6
	6	[8]-Shogaol	$C_{19}H_{28}O_3$	304	5.25	36700-45-5
	7	[8]-Paradol	$C_{19}H_{30}O_3$	306	4.12	27113-23-1
	8	Feruloylmalic acid	$C_{14}H_{14}O_8$	310	11.67	NA
	9	[10]-Paradol	$C_{21}H_{34}O_3$	334	7.17	36700-48-8
	10	[4]-Gingerdiol	$C_{19}H_{28}O_6$	352	4.30	863780-88-5
Flavonoids	1	Pachypodol	$C_{18}H_{16}O_7$	344	5.73	33708-72-4
	2	Ayanin	$C_{18}H_{16}O_7$	344	5.62	572-32-7
	3	Quercetin-4′-O-glucoside	$C_{21}H_{20}O_{12}$	464	6.00	20229-56-5
	4	Quercetin-3-O-glucoside	$C_{21}H_{20}O_{12}$	464	8.96	482-35-9
	5	Quercetin-7-O-glucoside	$C_{21}H_{20}O_{12}$	464	4.97	491-50-9
	6	Hesperetin-5-O-glucoside	$C_{22}H_{24}O_{11}$	464	5.69	69651-80-5
	7	Apigenin-6,8-di-C-glucoside	$C_{27}H_{30}O_{15}$	594	4.15	23666-13-9
	8	Quercetin-3-O-glucoside-7-O-rhamnoside	$C_{27}H_{30}O_{16}$	610	9.62	NA
	9	Quercetin-3-O-rutinoside	$C_{33}H_{40}O_{20}$	756	12.88	55696-57-6

[a] Molecular formula. [b] Molecular weight. [c] The proportion of the content of the compound in its principal component. [d] CAS registry number: Chemical substance digital identification number.

3.2. Aphidicidal Activity Analysis of GSE, 6-Gingerol, and Quercetin-3-O-rutinoside

The GSE, 6-gingerol, and quercetin-3-O-rutinoside exhibited significant aphidicidal activity. After treatment for 12 h and 24 h, the aphidicidal activity of the three substances showed an upward trend, in which the aphidicidal activity of GSE was significantly higher than that of the control, and of 6-gingerol and quercetin-3-O-rutinoside, reaching 39.15% and 66.14%, respectively. Following correction, there was no significant difference between 6-gingerol and the control at 12 h. At 24 h, the aphidicidal activity of 6-gingerol and quercetin-3-O-rutinoside was significantly higher than that of the control, at 23.86% and 37.22%, respectively, and the aphidicidal activity of quercetin-3-O-rutinoside was significantly greater than that of 6-gingerol (Table 3).

Table 3. Mean mortality (±SE) of *M. sorghi* after 12 h and 24 h of treatment with ginger shoot extract, 6-gingerol, and quercetin-3-O-rutinoside.

Treatment ($mg \cdot mL^{-1}$)	12 h		24 h	
	Mortality (%) [a]	Corrected Mortality (%) [b]	Mortality (%)	Corrected Mortality (%)
GSE	39.15 ± 3.40 a	35.01 ± 4.22 a	66.14 ± 2.71 a	56.79 ± 0.00 a
6-gingerol	12.28 ± 6.14 b	0.00 ± 0.00 b	33.00 ± 3.66 bc	23.86 ± 2.71 c
quercetin-3-O-rutinoside	28.78 ± 2.21 ab	23.86 ± 2.71 a	45.00 ± 3.33 b	37.22 ± 2.01 b
CK [c]	12.28 ± 6.14 b	0.00 ± 0.00 b	21.14 ± 2.71 c	0.00 ± 0.00 d
F	7.59	49.19	37.63	199.784
df	3, 8	3, 8	3, 8	3, 8
p	0.01	<0.0001	<0.0001	<0.0001

[a] Mortality of sorghum aphid by contact. [b] Correcting the mortality by a correction formula. [c] CK is 10% ethanol. The percentage data were transformed to meet normality assumptions and significant differences determined by using arcsine-square root transformation. Values within a column followed by same letter are not significantly different by Tukey's HSD Multiple Comparison ($\alpha = 0.05$).

3.3. Effect of GSE on the Growth Index of M. sorghi

Compared with the control, there were significant differences in the litter size, longevity, molting number, and mortality of the sorghum aphids treated with GSE. After 3 and 7 d

of treatment with GSE, the litter size of the aphids was significantly lower than that of the control ($F = 57.41$, $df = 11$, $p < 0.05$; $F = 83.67$, $df = 11$, $p < 0.05$). The litter size under 15 mg·mL^{-1} at 3 d was only 7.00, which was 0.38 times that of the control, while those of 5 mg·mL^{-1} and 10 mg·mL^{-1} were 0.80 and 0.71 times higher than that of the control, respectively. There was no significant difference between the two groups. Compared with the control, the longevity of the aphids treated with GSE was significantly shortened to 7.80 d at 15 mg·mL^{-1} concentration, which was 0.49 times that of the control, while at 5 mg·mL^{-1} and 10 mg·mL^{-1}, it was 0.62 times and 0.59 times that of the control, respectively ($F = 13.14$, $df = 11$, $p < 0.05$). However, there was no significant difference between the three groups (Table 4).

Table 4. Mean litter size and longevity (±SE) of *M. sorghi* after treatment with different concentrations of GSE, and the data of litter sizes were taken from 3 d and 7 d treatment.

Extract Concentration (mg·mL^{-1})	3 d		7 d		Longevity (d)	Ratio
	Litter Size	Ratio [a]	Litter Size	Ratio		
5	14.67 ± 0.33 b	0.80	50.33 ± 2.03 b	0.83	9.80 ± 0.42 b	0.62
10	13.00 ± 0.58 b	0.71	44.00 ± 1.53 b	0.72	9.40 ± 0.60 b	0.59
15	7.00 ± 0.58 c	0.38	26.67 ± 1.76 c	0.44	7.80 ± 1.10 b	0.49
CK [b]	18.33 ± 0.88 a	1.00	61.00 ± 0.57 a	1.00	15.93 ± 1.46 a	1.00
F	57.41	-	83.67	-	13.14	-
df	3, 8	-	3, 8	-	3, 8	-
p	<0.0001	-	<0.0001	-	0.002	-

[a] Taking the control as the datum point, the ratio under different concentration treatments was calculated. [b] CK is 10% ethanol. Values within a column followed by same letter are not significantly different by Tukey's HSD Multiple Comparison ($\alpha = 0.05$).

GSE had a significant effect on the molting and mortality of the aphids, and the effect increased with the increase in concentration and treatment duration. After 3 d of treatment with concentrations of 10 mg·mL^{-1} and 15 mg·mL^{-1} GSE, the molting of the aphids was significantly lower than that of the control ($F = 6.22$, $df = 11$, $p < 0.05$). After 7 d of treatment, the molting of the aphids at the three concentrations was only 0.65, 0.33, and 0.17 times higher than that of the control, respectively, and the molting significantly decreased with the increase in concentration ($F = 401.83$, $df = 11$, $p < 0.05$). Compared with the control, the mortality and corrected mortality increased with the increase in concentration. When the GSE concentration was 5 mg·mL^{-1}, the mortality of the aphids showed no significant difference compared with the control, and the corrected mortality of the sorghum aphid was only 12.83% at 3 d. When the concentration was 10 mg·mL^{-1}, the mortality was 22.73% and 41.23%, respectively. When the concentration reached 15 mg·mL^{-1}, the corrected mortality was 46.49% and 58.02% respectively, exhibiting higher insecticidal activity. The results showed that GSE could not only significantly affect the litter size and longevity of aphids, but could also significantly inhibit the molting number of the aphids, inhibit their growth and development, and increase their mortality (Table 5).

Table 5. Mean molting and mortality (±SE) of *M. sorghi* after 3 d and 7 d of treatment with different concentrations of GSE.

Extract Concentration (mg·mL^{-1})	3 d		7 d		3 d		7 d	
	Molting	Ratio [a]	Molting	Ratio	Mortality (%) [b]	Corrected Mortality (%) [c]	Mortality (%)	Corrected Mortality (%)
5	1.33 ± 0.33 ab	0.57	25.00 ± 0.58 b	0.65	12.83 ± 0.09 c	12.83 ± 0.09 c	31.52 ± 1.10 c	22.43 ± 0.95 c
10	1.00 ± 0.00 b	0.43	12.67 ± 0.88 c	0.33	22.73 ± 0.27 b	22.73 ± 0.27 b	41.23 ± 1.88 b	33.60 ± 1.76 b
15	0.67 ± 0.33 b	0.29	6.67 ± 0.33 d	0.17	46.49 ± 1.34 a	46.49 ± 1.34 a	67.02 ± 1.14 a	58.02 ± 0.67 a
CK [d]	2.33 ± 0.33 a	1.00	38.67 ± 0.88 a	1.00	0.00 ± 0.00 c	0.00 ± 0.00 c	20.92 ± 0.48 d	0.00 ± 0.00 d
F	6.22	-	401.83	-	821.99	821.99	247.15	522.36
df	3, 8	-	3, 8	-	3, 8	3, 8	3, 8	3, 8
p	0.017	-	<0.0001	-	<0.0001	<0.0001	<0.0001	<0.0001

[a] Taking the control as the datum point, the proportion under different concentration treatments was calculated. [b] Mortality of sorghum aphid nymph. [c] Correcting the Mortality by a correction formula. [d] CK is 10% ethanol. The percentage data were transformed to meet normality assumptions and significant differences determined by using arcsine-square root transformation. Values within a column followed by same letter are not significantly different by Tukey's HSD Multiple Comparison (α = 0.05).

3.4. Effects on the Enzyme Activity of M. sorghi

GSE significantly inhibited the activities of pepsin, lipase, and α-amylase in the sorghum aphids. Under treatment with 15 mg·mL^{-1} of GSE, the pepsin activity in the sorghum aphids significantly decreased at 1 d and 7 d, but there was no significant difference between the pepsin activity and control at 3 d and 5 d (Figure 1A). Compared with the control, the lipase activity in the sorghum aphids significantly decreased at 1 d, 5 d, and 7 d, and the lipase activity at 5 d was 3.39 U·mg^{-1}, while that of the control was 13.55 U·mg^{-1}. The lipase activity decreased by 75% (Figure 1B). In addition, the activity of α-amylase in the sorghum aphids was significantly lower than that of the control at 7 d, and the activity of α-amylase in the sorghum aphids was 0.57, 0.7, and 0.49 times higher than that of the control at 3 d, 5 d, and 9 d, respectively. The amylase activity at 1 d was only 0.06 U·mg^{-1}, which decreased by 89% compared with the control (Figure 1C). It is evident that active compounds were present in GSE that could inhibit the digestive enzymes of the sorghum aphid, and the inhibitory effect on α-amylase was higher than that of pepsin and lipase.

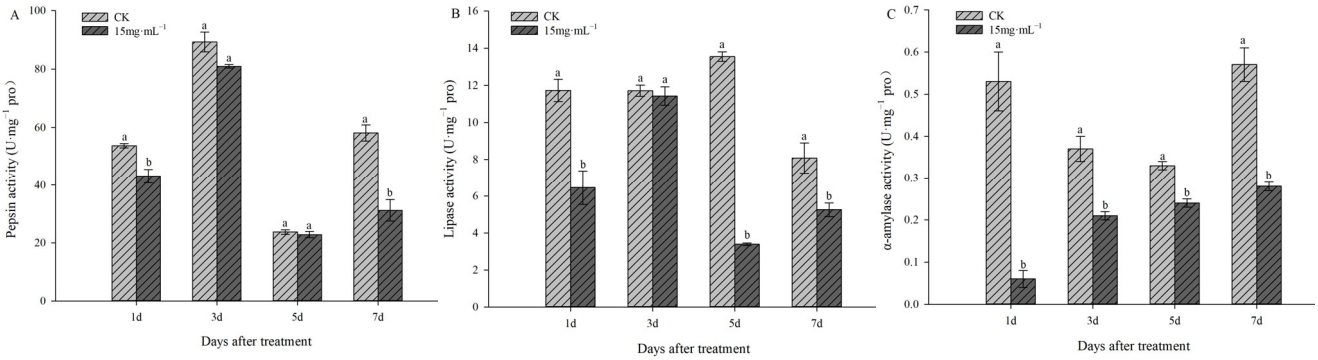


Figure 1. The impact of 15 mg·mL^{-1} GSE on pepsin (**A**), lipase (**B**), and α-amylase (**C**) of *M. sorghi* across successive treatments. CK, 10% ethanol. Data are mean ± SE. Different letters (a, b) for the same time in each figure indicate significant differences (α = 0.05).

There were significant differences in the activities of the three protective enzymes in the sorghum aphids treated with GSE. Compared with the control, sorghum aphids showed significantly activated SOD activity at 7 d under treatment with 15 mg·mL^{-1} GSE. The enzyme activity decreased with time, and the enzyme activity was the highest at 3 d by 1.26 times (Figure 2A). After treatment for 1 d and 3 d, the POD activity in vivo was 1.30 times and 1.39 times higher than that of the control at 0.43 U·mg^{-1} and 0.43 U·mg^{-1}, respectively. When the treatment duration was extended to 5 d and 7 d, there was no significant difference in POD activity between the treatment and control (Figure 2B). The CAT activity in the sorghum aphids was significantly activated by GSE at 1 d and 3 d by

2.68 and 1.51 times higher than that of the control, respectively. Over time, the enzyme activity of the treatment group began to decrease gradually, and there was no significant difference between the treatment and the control at 5 d and 7 d (Figure 2C). The results showed that the synergistic action of the three protective enzymes in the sorghum aphids could reduce the effect of toxic substances under treatment with GSE.

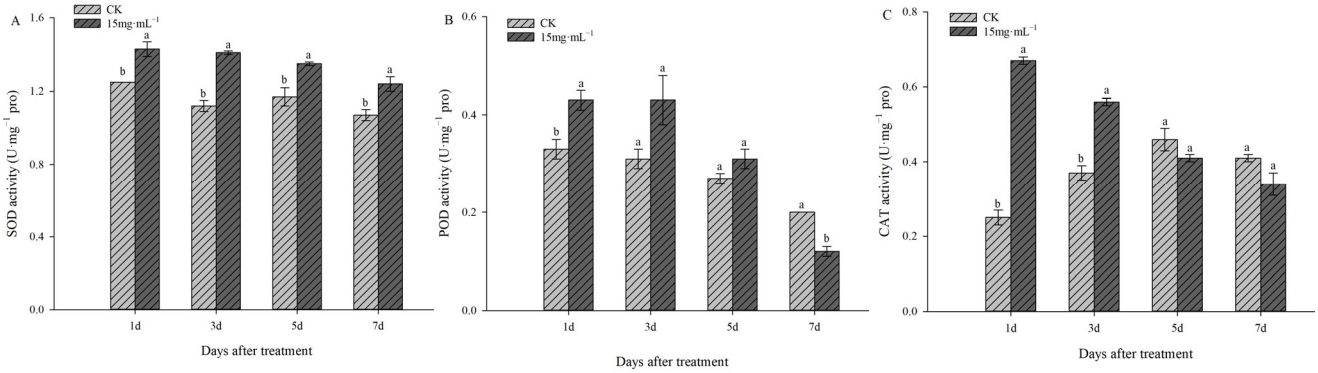


Figure 2. The impact of 15 mg·mL^{-1} GSE on superoxide dismutase (SOD) activity (**A**), peroxidase (POD) activity (**B**), and catalase (CAT) activity (**C**) of *M. sorghi* across successive treatments. CK, 10% ethanol. Data are mean ± SE. Different letters (a, b) for the same time in each figure indicate significant differences (α = 0.05).

There were significant differences in the activities of the three detoxification enzymes in the sorghum aphids treated with GSE. The GST activity of the 15 mg·mL^{-1} GSE was 0.52 U·mg^{-1} at 1 d, which was 1.58 times that of the control. At 3 d and 5 d, the activity of GST in vivo was not significantly different from that of the control, but on day 7, the activity of GST in the sorghum aphids had significantly decreased to 0.11 times that of the control (Figure 3A). Compared with the control, the AChE activity in sorghum aphids treated with GSE significantly decreased. The enzyme activities of the sorghum aphids at 7 d were 0.03 U·mg^{-1}, 0.02 U·mg^{-1}, 0.03 U·mg^{-1}, and 0.02 U·mg^{-1}, respectively, making them 0.02, 0.01, 0.03, and 0.03 times higher than the control, and the differences were significant (Figure 3B). In addition, the CarE activity in the sorghum aphids was significantly higher than that of the control at 7 d, and over time, the enzyme activity increased first and then decreased. The enzyme activities of the treatment and control showed the greatest difference at 5 d of 2.23 times (Figure 3C). This indicated that some toxic substances in the GSE inhibited the activity of detoxification enzymes in the sorghum aphids, thus damaging the nervous system and tissues.

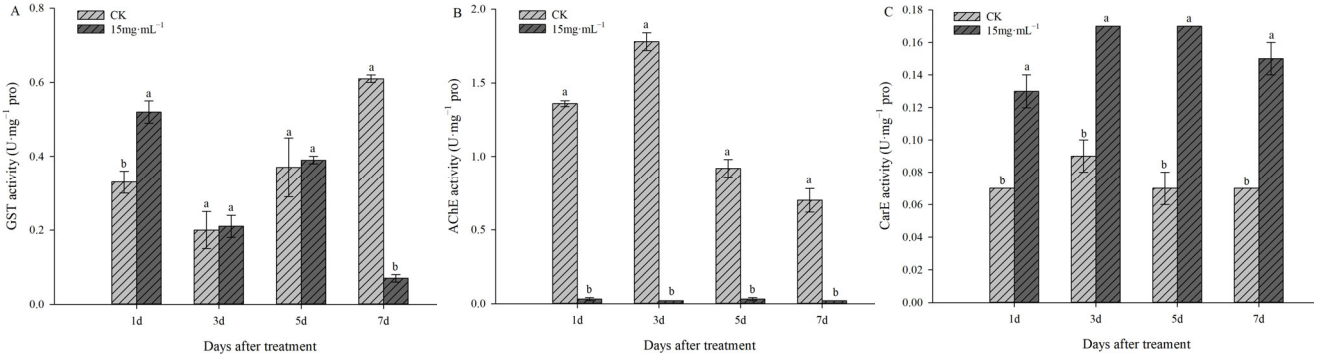


Figure 3. The impact of 15 mg·mL^{-1} GSE on glutathione S-transferase (GST) activity (**A**), acetylcholinesterase (AChE) activity (**B**), and carboxylesterase (CarE) activity (**C**) of *M. sorghi* across successive treatments. CK, 10% ethanol. Data are mean ± SE. Different letters (a, b) for the same time in each figure indicate significant differences (α = 0.05).

4. Discussion

To explore the insecticidal effect of ginger stems and leaves, the main compounds of GSE were identified in this study. The results showed that the content of phenolic acids in GSE was the highest, reaching 41.5%, followed by flavonoids (27.1%) and alkaloids (11.9%). Phenolic acids are benzene ring compounds containing oxhydryl, which have a unique aromatic smell. Some studies have shown that phenolic acids had insecticidal effect; for instance, ferulic acid and coumaric acid can affect the ovipositing selection of *Pieris rapae* [23], tannic acid can prolong the growth period of grain aphids and reduce their daily fecundity [24], and salicin significantly reduced the feeding function and survival rate of gypsy moths [25]. These studies indicated that phenolic acids have insecticidal effects. Because of the high proportion of phenolic acids in GSE, it could be inferred that it may also have this effect. Flavonoids have important physiological and biochemical effects because of their unique chemical structure, and alkaloids have natural toxicity or strong physiological effects on organisms. Studies have shown that flavonoids and alkaloids are natural insecticidal active compounds in plants. For example, 15 flavonoids isolated from *Eupatorium adenophorum* all had an inhibitory effect on acetylcholinesterase in *Spodoptera litura*, resulting in inhibition of its growth [26]. 3-deoxyanthocyanidin flavonoids in sorghum had significant aphidicidal activity [27], and sanguinarine had strong insecticidal activity against gypsy moth [28]. These results indicated that flavonoids and alkaloids also have insecticidal effects, and the content of these two substances was also high in GSE, so we speculated that flavonoids and alkaloids in GSE might also have insecticidal effects.

To further determine the insecticidal effects of phenolic acids and flavonoids in GSE, we selected 6-gingerol and quercetin-3-O-rutinoside for aphicidal activities determination. The results showed that 6-gingerol and quercetin-3-O-rutinoside had significant aphidicidal activity. Similarly, previous studies have shown that 6-gingerol has significant contact toxicity to both *Nilaparvata lugens* and *Spodoptera* spp. [29,30], and quercetin has significant contact toxicity to Aedes aegypti larvae [31], while the insecticidal activity of quercetin-3-O-rutinoside has not been studied. Our results indicated that the main compounds of GSE had aphidicidal activities against sorghum aphids. However, since field experiments or greenhouse experiments were not carried out in this study, the efficacy stability of the main compounds need to be further studied.

Prior to this study, the effect of GSE on aphid populations and their progeny was not known. In this study, GSE significantly reduced the litter size and shortened the longevity of the aphids. This was similar to the results reported by Shivali et al. [32], who found that chrysin, a secondary metabolite of plants, can significantly inhibit the growth and fecundity of *Zeugodacus cucurbitae*, indicating that GSE may contain active compounds with similar effects. In addition, GSE inhibited the molting of nymph aphids, making them grow slowly and increasing their mortality. Similarly, Meriam et al. [33] reported that *Pergularia tomentosa* extract significantly increased the mortality of *Locusta migratoria* nymphs, and Xin et al. [34] reported that sulfoxaflor affected the development and increased the mortality of *Sitobion avenae* and *Rhopalosiphum padi* progeny. It is speculated that GSE may contain active compounds that inhibit the growth of nymph aphids, leading to mass death. According to aphid digestive enzyme tests, the activities of protease, lipase, and α-amylase were inhibited. Similarly, Chen et al. [35] found that carvacrol can inhibit digestive enzyme activities in *Lymantria dispar*. GSE may also contain effective compounds to inhibit the digestive enzymes of nymph aphids. The above results indicated that GSE may cause malnutrition by inhibiting the digestive system of aphids, thereby inhibiting their growth and causing their death.

Relevant enzymes in insects are important factors in studying the mechanism of insecticide resistance [36]. This study explored the aphidicidal physiological mechanism of GSE, and found that GSE can significantly promote the activities of SOD and CarE, similarly to the finding that the essential oils of *Santolina chamaecyparissus* and *Tagetes patula* significantly activated SOD activity in aphids, and chlorantraniliprole significantly activated CarE activity in *Plutella xylostella* [37,38]. The results indicated that GSE could

activate the internal defense system of sorghum aphids, remove excessive reactive oxygen species in the body, and keep it in homeostasis. SOD and CarE were sensitive to GSE. The activities of POD, CAT, and GST were activated first and then inhibited by GSE. The results showed that when sorghum aphids were exposed to toxic substances, the activities of POD, CAT and GST were significantly activated, but with the increase of exposure time, the toxic substances further accumulated, resulting in the decrease of POD, CAT, and GST activities, and eventually death of the aphids [39]. This study also found that GSE can significantly inhibit AChE activity, which can cause acetylcholine receptor overexcitation and lead to nervous system disorder, achieving an insecticidal effect. Similarly, *Parthenium argentatum* extract significantly inhibited the activity of AChE in *Spodoptera frugiperda* [40], indicating that GSE may contain active compounds with similar effects. These results indicated that GSE contains substances that are toxic to sorghum aphids, causing a disturbance in the aphids' defense system in vivo, leading to aphid death.

5. Conclusions

In summary, the main active compounds of GSE demonstrated contact toxicity against sorghum aphids. According to growth experiments and enzyme activity experiments, GSE may also inhibit the growth and development of sorghum aphids by inhibiting digestive enzymes and affecting protective enzymes and detoxification enzymes, thus leading to the death of the aphids. Field test research on the insecticidal active compounds of ginger now needs to be carried out urgently. Combined with this research, it could provide a theoretical basis for the development of new botanical aphidicides.

Author Contributions: Conceptualization, X.L. and K.X.; methodology, X.L., Q.J. and Y.L.; formal analysis, X.L., Y.W. and J.M.; data curation, X.L. and K.X.; writing—original draft preparation, X.L.; writing—review and editing, X.H., Y.Z. and J.Y.; supervision, Q.J. and Y.L.; funding acquisition, J.M., R.L., X.C. and Y.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by Key Research and Development program of Hubei province, under grant no. 2022BBA0061; the National Natural Science Foundation of Hubei Province, under grant no. 2021CBF512; and Condiment industry system major special projects of Chongqing, under grant no. 2021-2022-07.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding authors.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Maurice, H.; Bernard, C.; Evelyne, T.; Jean, C.S. Encyclop' Aphid: A website on aphids and their natural enemies. *Entomol. Gen.* **2019**, *40*, 97–101.
2. Nibouche, S.; Costet, L.; Medina, R.F.; Holt, J.R.; Sadeyen, J.; Zoogones, A.S.; Brown, P.; Blackman, R.L. Morphometric and molecular discrimination of the sugarcane aphid, *Melanaphis sacchari*, (Zehntner, 1897) and the sorghum aphid *Melanaphis sorghi* (Theobald, 1904). *PLoS ONE* **2021**, *16*, e0241881. [CrossRef] [PubMed]
3. Selvaraj, S.R.; Sengottayan, S.N.; Kannan, R.; Rajamanickam, C.; Annamalai, T.; Prabhakaran, V.S.; Athirstam, P.; Edward, S.E.; Venkatraman, P. Toxicity of *Alangium salvifolium* Wang chemical constituents against the tobacco cutworm *Spodoptera litura* Fab. *Pestic. Biochem. Phys.* **2016**, *126*, 92–101.
4. Senthil, N.S.; Man, Y.C.; Chae, H.P.; Hong, Y.S. Food consumption, utilization, and detoxification enzyme activity of the rice leaffolder larvae after treatment with *Dysoxylum triterpenes*. *Pestic. Biochem. Phys.* **2006**, *88*, 260–267. [CrossRef]
5. Thanigaivel, A.; Chandrasekaran, R.; Revathi, K.; Nisha, S.; Sathish, N.S.; Kirubakaran, S.A.; Senthil, N.S. Larvicidal efficacy of *Adhatoda vasica* (L.) Nees against the bancroftian filariasis vector *Culex quinquefasciatus* Say and dengue vector *Aedes aegypti* L. in in vitro condition. *Parasitol. Res.* **2012**, *110*, 1993–1999. [CrossRef] [PubMed]
6. Wang, Z.Y.; Su, J.P.; Liu, W.W.; Guo, Y.Y. Effects of intercropping vines with tobacco and root extracts of tobacco on grape phylloxera, *Daktulosphaira vitifoliae* Fitch. *J. Integr. Agric.* **2015**, *14*, 1367–1375. [CrossRef]

7. Zou, C.S.; Lv, C.H.; Wang, Y.J.; Cao, C.W.; Zhang, G.C. Larvicidal activity and insecticidal mechanism of *Chelidonium majus* on *Lymantria Dispar*. *Pestic. Biochem. Phys.* **2017**, *142*, 123–132. [CrossRef]
8. Calvin, W.; Beuzelin, J.M.; Liburd, O.E.; Branham, M.A.; Simon, L.J. Effects of biological insecticides on the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), in sorghum. *Crop Prot.* **2020**, *142*, 105528. [CrossRef]
9. Manjree, A.; Suresh, W.; Swaran, D.; Bhupinder, P.S.K. Insect growth inhibition, antifeedant and antifungal activity of compounds isolated/derived from *Zingiber officinale* Roscoe (ginger) rhizomes. *Pest Manag. Sci.* **2001**, *57*, 289–300.
10. Jerome, N.; Nancy, D.E. Attraction of *Ceratitis capitata* (Diptera: Tephritidae) sterile males to essential oils: The importance of linalool. *Environ. Entomol.* **2018**, *47*, 1287–1292.
11. Zhang, W.; Heather, J.M.; David, J.S. Repellency of ginger oil to *Bemisia argentifolii* (Homoptera: Aleyrodidae) on tomato. *J. Econ. Entomol.* **2004**, *97*, 1310–1318. [CrossRef] [PubMed]
12. Moreira, S.I.; Alvarenga, S.M.; Souza, T.W.; Dos, S.A.; Serrão, J.E.; José, V.Z.A.; Frederico, W.C.; Cola, Z.J.; Sigueyuki, S.C. Toxicity of essential oils to *Diaphania hyalinata* (Lepidoptera: Crambidae) and selectivity to its parasitoid *Trichospilus pupivorus* (Hymenoptera: Eulophidae). *J. Econ. Entomol.* **2020**, *113*, 2399–2406. [CrossRef] [PubMed]
13. Mikhaela, O.; Nora, C.; Hanna, D.; Wayne, G.; Danica, B. Insecticidal activity of plant powders against the parasitoid, *Pteromalus venustus*, and its host, the alfalfa leafcutting bee. *Insects* **2020**, *11*, 359.
14. Faheem, A.; Naeem, I.; Syed, M.Z.; Muhammad, K.Q.; Qamar, S.; Khalid, A.K.; Hamed, A.G.; Mohammad, J.A.; Waqar, J.; Muhammad, A.; et al. Comparative insecticidal activity of different plant materials from six common plant species against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Saudi J. Biol. Sci.* **2019**, *26*, 1804–1808.
15. Ukeh, D.A.; Birkett, M.A.; Bruce, T.J.A.; Allan, E.J.; Pickett, J.A.; Luntz, A.J.M. Behavioural responses of the maize weevil, *Sitophilus zeamais*, to host (stored-grain) and non-host plant volatiles. *Pest Manag. Sci.* **2010**, *66*, 44–50. [CrossRef]
16. Colinet, D.; Cazes, D.; Belghazi, M.; Gatti, J.; Poirié, M. Extracellular superoxide dismutase in insects: Characterization, function, and interspecific variation in parasitoid wasp venom. *J. Biol. Chem.* **2011**, *286*, 40110–40121. [CrossRef]
17. Felipe, A.D.; Ana, C.P.G.; Hugo, D.P.; Renata, S.G.; Carolina, R.O.; Raquel, L.L.O.; Marta, C.; Carla, R.P.; Didac, S.; Marco, M.; et al. Identification of a selenium-dependent glutathione peroxidase in the blood-sucking insect *Rhodnius prolixus*. *Insect Biochem. Molec.* **2016**, *69*, 105–114.
18. Guo, D.H.; Luo, J.P.; Zhou, Y.N.; Xiao, H.M.; He, K.; Yin, C.L.; Xu, J.H.; Li, F. ACE: An efficient and sensitive tool to detect insecticide resistance-associated mutations in insect acetylcholinesterase from RNA-Seq data. *BMC Bioinformatics* **2017**, *18*, 330. [CrossRef]
19. Zhou, B.G.; Wang, S.; Dou, T.T.; Liu, S.; Li, M.Y.; Hua, R.M.; Li, S.G.; Lin, H.F. Aphicidal activity of *Illicium verum* fruit extracts and their effects on the acetylcholinesterase and glutathione S-transferases activities in *Myzus persicae* (Hemiptera: Aphididae). *J. Insect Sci.* **2016**, *16*, 1–7. [CrossRef]
20. Gao, Q.; Shi, Y.H.; Liao, M.; Xiao, J.J.; Li, X.X.; Zhou, L.J.; Liu, C.W.; Liu, P.; Cao, H.Q. Laboratory and field evaluation of the aphidicidal activity of moso bamboo (*Phyllostachys pubescens*) leaf extract and identification of the active components. *Pest Manag. Sci.* **2019**, *75*, 3167–3174. [CrossRef]
21. Chen, W.; Gong, L.; Guo, Z.L.; Wang, W.S.; Zhang, H.Y.; Liu, X.Q.; Yu, S.B.; Xiong, L.Z.; Luo, J. A novel integrated method for large-scale detection, identification, and quantification of widely targeted metabolites: Application in the study of rice metabolomics. *Mol. Plant* **2013**, *6*, 1769–1780. [CrossRef]
22. Oyagbemi, T.O.; Ashafa, A.; Adejinmi, J.O.; Oguntibeju, O.O. Preliminary investigation of acaricidal activity of leaf extract of *Nicotiana tabacum* on dog tick *Rhipicephalus sanguineus*. *Vet. World* **2019**, *12*, 1624–1629. [CrossRef]
23. Kristen, S.W.; Jessica, L.B.; Mary, E.L.; Amanda, J.L.R. Effects of host plant phenolic acids and nutrient status on oviposition and feeding of the cabbage white butterfly, *Pieris rapae*. *BIOS* **2014**, *85*, 95–101.
24. Grzegorz, C.; Bogumił, L.; Paweł, C.; Hubert, S.; Henryk, M.; Robert, K.; Cezary, S. Effect of phenolic acids from black currant, sour cherry and walnut on grain aphid (*Sitobion Avenae* F.) development. *Crop Prot.* **2012**, *35*, 71–77.
25. Ma, J.Y.; Sun, L.L.; Zhao, H.Y.; Wang, Z.Y.; Zou, L.; Cao, C.W. Functional identification and characterization of GST genes in the Asian gypsy moth in response to poplar secondary metabolites. *Pestic. Biochem. Phys.* **2021**, *176*, 104860. [CrossRef] [PubMed]
26. Li, M.Y.; Gao, X.; Lan, M.X.; Liao, X.B.; Su, F.W.; Fan, L.M.; Zhao, Y.H.; Hao, X.J.; Wu, G.X.; Ding, X. Inhibitory activities of flavonoids from *Eupatorium adenophorum* against acetylcholinesterase. *Pestic. Biochem. Phys.* **2020**, *170*, 104701. [CrossRef] [PubMed]
27. Kariyat, R.R.; Gaffoor, I.; Sattar, S.; Dixon, C.W.; Frock, N.; Moen, J.; De, M.C.M.; Mescher, M.C.; Thompson, G.A.; Chopra, S. Sorghum 3-deoxyanthocyanidin flavonoids confer resistance against corn leaf aphid. *J. Chem. Ecol.* **2019**, *45*, 502–514. [CrossRef]
28. Zou, C.S.; Wang, Y.J.; Zou, H.; Ding, N.; Geng, N.N.; Cao, C.W.; Zhang, G.C. Sanguinarine in *Chelidonium majus* induced antifeeding and larval lethality by suppressing food intake and digestive enzymes in *Lymantria dispar*. *Pestic. Biochem. Phys.* **2018**, *153*, 9–16. [CrossRef]
29. Noor, H.M.S.; Norhidayah, A.; Rahayu, M.A.; Adilah, A.; Nur, H.I. Botanical insecticide of chili and ginger extract on *Nilaparvata lugens*, brown planthopper. *Mat. Sci. Eng.* **2020**, *932*, 012001.
30. Keosaeng, K.; Songoen, W.; Yooboon, T.; Bullangpoti, V.; Pluempanupat, W. Insecticidal activity of isolated gingerols and shogaols from *Zingiber officinale* Roscoe rhizomes against *Spodoptera spp.* (Lepidoptera: Noctuidae). *Nat. Prod. Res.* **2022**, *36*, 1–6. [CrossRef]

31. Leticie, Z.S.P.; Jonatas, L.D.; Ricardo, M.A.F.; Anna, E.M.F.M.O.; Rodrigo, A.S.C.; Silvia, M.M.F.; José, C.T.C.; Caio, P.F.; Raimundo, N.P.S.; Raquel, S.A. Nanosuspension of quercetin: Preparation, characterization and effects against *Aedes aegypti* larvae. *Rev. Bras. Farmacogn.* **2018**, *28*, 618–625.
32. Shivali, P.; Sumit, S.; Satwinder, K.S. Inhibitory effect of chrysin on growth, development and oviposition behaviour of melon fruit fly, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae). *Phytoparasitica* **2022**, *50*, 151–162.
33. Meriam, M.; Khemais, A.; Amel, B.H.; Iteb, B.; Mouna, M.; Fatma, A.; Monia, B.H.K. Physiological, histopathological and cellular immune effects of *Pergularia tomentosa* extract on *Locusta migratoria* nymphs. *J. Integr. Agric.* **2019**, *18*, 2823–2834.
34. Xin, J.J.; Yu, W.X.; Yi, X.Q.; Gao, J.P.; Gao, X.W.; Zeng, X.P. Sublethal effects of sulfoxaflor on the fitness of two species of wheat aphids, *Sitobion avenae* (F.) and *Rhopalosiphum padi* (L.). *J. Integr. Agric.* **2019**, *18*, 1613–1623. [CrossRef]
35. Chen, Y.Z.; Zhang, B.W.; Yang, J.; Zou, C.S.; Li, T.; Zhang, G.C.; Chen, G.S. Detoxification, antioxidant, and digestive enzyme activities and gene expression analysis of *Lymantria dispar* larvae under carvacrol. *J. Asia Pac. Entomol.* **2020**, *24*, 208–216. [CrossRef]
36. Karatolos, N.; Williamson, M.S.; Denholm, I.; Gorman, K.; Constant, R.; Nauen, R. Resistance to spiromesifen in *Trialeurodes vaporariorum* is associated with a single amino acid replacement in its target enzyme acetylcoenzyme A carboxylase. *Insect Mol. Biol.* **2012**, *21*, 327–334. [CrossRef]
37. Czerniewicz, P.; Chrzanowski, G. The effect of *Santolina chamaecyparissus* and *Tagetes patula* essential oils on biochemical markers of oxidative stress in aphids. *Insects* **2021**, *12*, 360. [CrossRef]
38. Hu, Z.D.; Feng, X.; Lin, Q.S.; Chen, H.Y.; Li, Z.Y.; Yin, F.; Liang, P.; Gao, X.W. Biochemical mechanism of chlorantraniliprole resistance in the diamondback moth, *Plutella xylostella* Linnaeus. *J. Integr. Agric.* **2014**, *13*, 2452–2459. [CrossRef]
39. Matsumura, T.; Matsumoto, H.; Hayakawa, Y. Heat stress hardening of oriental armyworms is induced by a transient elevation of reactive oxygen species during sublethal stress. *Arch. Insect Biochem.* **2017**, *96*, e21421. [CrossRef]
40. Carlos, L.C.; Mariano, M.V.; Jose, S.C.; Juan, R.S.; Eduardo, A. Insect growth regulatory activity of some extracts and compounds from *Parthenium argentatum* on fall armyworm *Spodoptera frugiperda*. *Z. Naturforsch. C* **2015**, *56*, 95–105.