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Effect of Three Novel Thiazolidiones on the Development, Reproduction, and Trehalase Activity of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Abstract: *Spodoptera frugiperda* was used to investigate its role as a trehalase inhibitor of three new thiothiazolidone compounds—**6d**, **6e**, and **6f**. The development and reproduction of *S. frugiperda* were investigated to evaluate the potential of these compounds as a novel pesticide for pest control. Compounds **6d**, **6e**, and **6f** were injected into larvae and female pupae. Molecular changes in trehalose and chitin metabolism genes were monitored. Pupal trehalase activity, the female pupal emergence rate, ovarian development, and *SfVg* and *SfVgR* gene expression levels were assessed. The results revealed that all three compounds significantly inhibited trehalase activity in the larvae. The expression of *TRE* was significantly downregulated, and compounds **6d** and **6f** significantly downregulated the expression of *TRE2*. Treated larvae exhibited significantly decreased survival rates and a higher incidence of abnormalities. The egg production and hatching rates were markedly diminished by the inhibitors, and the ovaries displayed blackening and clumping. These compounds exhibit promise as eco-friendly insecticides, but further experiments are required to test their multifaceted capabilities.

Keywords: trehalase inhibitors; gene expression; insect physiology; chitin; piperine compounds

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1. Introduction

Spodoptera frugiperda (Lepidoptera: Noctuidae) is a significant global agricultural pest. It was first discovered in the subtropical and tropical regions of the Americas [1–3]. *S. frugiperda* has deleterious attributes, including a broad host range, a robust reproductive capacity, and migratory prowess [4,5]. Prevention and control measures include chemical pesticides as well as agricultural and biological controls [2,6–8]. These strategies mainly focus on chemical control, which has been important for the management of *S. frugiperda* for decades [9,10]. Developments towards green and sustainable control-technology systems are now required [11–14]. The prolonged and widespread use of insecticides such as pyrethroids and *Bacillus thuringiensis* (Bt) microbial pesticides can engender resistance in pest populations [10,15–17]. This results in a series of "3R" problems—residues, resistance, and resurgence—producing lethal or sublethal effects on beneficial arthropods in the environment [18,19]. There have been notable advances in RNA interference (RNAi) and CRISPR/Cas9-based functional genomic methodologies [20]. Nanocarrier dsRNA delivery systems can effectively penetrate the eggshell and larval body wall of *S. frugiperda*. This

triggers the silence of the target gene in mid–late instar larvae, resulting in sustainable pest management [21,22].

Trehalases (TREs) are crucial enzymes that are recognized for their role in metabolizing trehalose across all organisms as a part of the energy metabolism pathway. The trehalose synthesis pathway is deficient in mammals [23,24], rendering it a significant focal point for pest-management strategies [25,26]. TRE inhibitors possess a structure resembling that of trehalose and include glycosyl and pseudo-glycosyl inhibitors. The basic principle of action is to bind to the active site of trehalase, competitively inhibiting trehalase activity. This has been demonstrated to impact carbohydrate and chitin metabolism in insects [27], controlling insect-life activities. However, it only effectively poisons insects, not mammals [28]. In Jiang's study, novel multichitinase inhibitors with piperine derivatives and similar structures to our new compounds were evaluated for biotoxicity. Consequently, we inferred that the negative impact on beneficial fauna would be small. New trehalase inhibitors have been synthesized based on certain natural trehalase inhibitors, using design methods such as bioelectronic rearrangement, active group combination, glycoside substitution, and modifications to the cyclopentane molecular structure [29–31]. These include validamycin A, trehazolin, and salbostatin, as well as their synthetic compounds [26]. Certain natural alkaloids and their analogs have also been reported to have inhibitory activity against insect or fungal trehalase [32–34]. Tropane alkaloids have been demonstrated to have inhibitory activity against trehalase in *Bombyx mori*, *Spodoptera litura*, and *Fusarium oxysporum* [32]. Ephedrine and its isomers can also significantly inhibit trehalase activity [33,34].

Piperine, alternatively recognized as 1-piperylpiperidine, is a natural amide compound derived from black pepper. Its main structure includes three parts: pepper ring (1,3-benzodioxane) A, bridge chain B, and piperidine ring C [35]. The compound remnants exhibit a certain insecticidal activity after modifying parts B and C. A recent study demonstrated that piperine had inhibitory effects on three chitinases—ChtI, ChtII, and Chi h—in the Asian corn borer *Ostrinia furnacalis* [36,37]. Certain piperine and thiazolidinone compounds have been demonstrated to have an effective inhibitory activity on the trehalase of *S. frugiperda* [29,30]. Piperine compounds may, therefore, be regarded as a potential inhibitor backbone of trehalases. Thiothiazolidinones have been proven to demonstrate agricultural activity against lepidopteran pests [38,39]; they can also target chitinase [40,41]. We designed and synthesized three new thiothiazolidone compounds—**6d**, **6e**, and **6f**—by preserving the original piperine framework and introducing a novel thiothiazolidone structural fragment into the piperidine ring. The biological activity of trehalase and the specific molecular mechanisms regulating insect development and reproduction were explored to examine innovative approaches for the advancement of highly effective biological insecticides. In short, the purpose of our study was accomplished using a series of exploration experiments to preliminarily evaluate the insecticidal potential of **6d**, **6e**, and **6f**.

2. Materials and Methods

2.1. Test Insect Sources and Feeding Methods

S. frugiperda pupae were provided by the Zhejiang Academy of Agricultural Sciences and reared in the laboratory of Hangzhou Normal University (Hangzhou, China). The larvae were fed an artificial diet (Table S1), and the adult insects were nourished using a 10% honey solution in an artificial climate-controlled chamber. The environmental conditions were set as follows: temperature of 26 ± 1 °C, relative humidity of 60 ± 10%, and a photoperiod of 16 L:8 D.

2.2. Microinjection of Larvae and Female Pupae

The three new compounds were injected into *S. frugiperda* larvae on the first day of the third instar and into the female pupae on the first day of pupation, respectively, using a 3.5 Drummond needle and a TransferMan 4r (Eppendorf, Hamburg, Germany) at a concentration of 2×10^{-3} M. The injection site for the larvae was between the second and third thoracic legs. The injection site for female pupae was the junction between the fifth

and sixth abdominal segments. Three novel pepper thiothiazolidones—compounds **6d**, **6e**, and **6f** (the structures are presented in Figure S1)—were provided by the Target-Based Pesticide Discovery Laboratory of China Agricultural University (Table 1, Beijing, China) and dissolved in 2% DMSO. The injection concentration of the compounds was determined using pre-experimental screening in a laboratory. We used validamycin for the screening to obtain an effective trehalase inhibitor with a minimum concentration of 1 g/L [26]. This concentration could reduce the glucose and glycogen contents in *S. frugiperda*. We used our new compounds in subsequent experiments to convert the relative molecular weight. We determined the injection concentration of the new compound to be 2×10^{-3} M. The injection volume was 300 nL. The control group consisted of *S. frugiperda* larvae and female pupae injected with the same volume of 2% DMSO (solvent), also with an injection volume of 300 nL.

Table 1. Names, relative molecular weights, and molecular formulas of trehalase inhibitors.

No.	Code	Amount/mg	Purity	Solvent	MW	Molecular Formula
1	**6d**	10	98%	DMSO	359.3849	$C_{17}H_{10}FNO_3S_2$
2	**6e**	10	98%	DMSO	375.8410	$C_{17}H_{10}ClNO_3S_2$
3	**6f**	10	98%	DMSO	420.2950	$C_{17}H_{10}BrNO_3S_2$

2.3. Determination of Trehalase and Chitinase Activity

Larvae were selected 48 h post-injection to test the trehalase activity. We used three individual insects per group with three biological replicates. Each electropolished (EP) tube contained three larvae, two small sterilized steel balls, and 200 µL PBS. The sample was placed in a tissue crusher and ground at 50 Hz for 2 min before being sonicated for 30 min. We added 800 µL PBS to the sample after crushing. The female pupae were selected 24 h post-injection. We used three female pupae per group with three biological replicates. Female pupae were homogenized in liquid nitrogen [31]. Approximately 0.1 g of tissue and 1 mL of extract were homogenized in an ice bath. The samples were centrifuged at 4 °C and 1000× *g* for 20 min. We used 350 µL of the supernatant at 4 °C and centrifuged it at 20,800× *g* for 60 min. After centrifugation, the supernatant was used to determine the concentrations of soluble trehalase and protein. The precipitates were suspended with 300 µL PBS and used to determine the membrane-bound trehalase and protein concentrations. The trehalase activity was determined using a Trehalase Assay Kit (Solarbio, Beijing, China), and the protein concentration in the sample was assessed using a Bradford protein concentration determination kit (Beyotime, Shanghai, China). We used a spectrophotometer to measure the optical density values of the trehalase samples at 540 nm. The Bradford protein sample's optical density values were measured at 562 nm. Subsequently, the trehalase activity was determined based on the protein concentration as follows: trehalase activity (U/mg prot) = $100 \times y/\mathrm{Cpr}$ (where y is the sample concentration and Cpr is the sample protein concentration).

Chitinase activity in *S. frugiperda* was assessed following the protocol outlined in the chitinase kit (Comin, Suzhou, China). Chitin, when broken down by chitinase, yields N-acetyl glucosamine. This subsequently reacts with DNS (3,5-dinitrosalicylic acid) reagent, resulting in the formation of a brown–red compound with a characteristic absorption peak at 540 nm. Chitinase activity (expressed as mg/h/g fresh weight) was calculated using the formula $3.119 \times (\Delta A + 0.2753)/W$, where A represents the absorbance value, ΔA is the difference between the assay and control absorbance values, and W represents the weight of the sample.

2.4. Developmental and Morphological Analysis of S. frugiperda and Data Analysis

S. frugiperda larvae were observed every 24 h until pupation. The duration of larval development was calculated to observe and calculate the emergence rate from the first-day post-injection. Morphological abnormalities during development were recorded using

photos. The data were compiled and analyzed using Excel. A statistical significance assessment and graphical representation were obtained using SPSS Statistics 20 and GraphPad Prism 9. To test for normality, utilize SPSS by conducting a Shapiro–Wilk test following dataset exploration, and for equality of variance, employ Levene's Test within the ANOVA menu after selecting appropriate variables. The data from the graphs were expressed as the mean ± standard deviation (SD). The significance of differences was analyzed using independent sample *t*-tests (* represents a p-value < 0.05, which was used to indicate significant differences; ** represents a p-value < 0.01, which was used to indicate highly significant differences; and *** represents a p-value < 0.001). We evaluated the statistical significance of the data using IBM SPSS Statistics 20 to assess the data for normality and homogeneity of variance. The differences between the control group and the treatment group were ascertained using a one-way ANOVA or an independent sample *t*-test for comparison. The Duncan method was used in the one-way ANOVA for the post-test; different letters were used to indicate differences between groups ($p < 0.05$). The chi-squared (χ^2) test was used to analyze the effect of inhibitors on the emergence rate.

2.5. Gene Expression Detection

Larvae of *S. frugiperda* were randomly chosen from each experimental group 48 h post-injection, and three larvae were employed as a single biological replicate. Each experimental group underwent three to four biological replicates. The samples were used to detect the expression levels of two types of trehalase (*TRE1* and *TRE2*) (the primer sequences are listed in Table 2). Female insects on the 2nd, 4th, and 6th day after emergence were randomly selected as samples from each group, with the entire abdominal tissues of three female adults used as one biological replicate. The samples were used to quantify the expression of *SfVg* and the receptor *SfVgR*. Total RNA was isolated using a TRIzol kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's guidelines. The quality and concentration of RNA were assessed using agarose gel electrophoresis and a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). Following the RNA extraction, cDNA synthesis was conducted in accordance with the PrimeScript RT reagent Kit instructions, and a gDNA Eraser treatment was incorporated (TaKaRa, Kyoto, Japan). The ribosomal protein L10 (RPL10, GenBank ID: OK319023.1) was employed as an internal reference gene. Unique amplicons from the qPCR were generated, and the relative expression levels were computed using the $2^{-\Delta\Delta CT}$ method [42].

Table 2. qRT-PCR primer sequences of *S. frugiperda*.

Primer Name	Forward Primers (5′–3′)	Reverse Primers (5′–3′)
qRT-RPL10	GACTTGGGTAAGAAGAAG	GATGACATGGAATGGATG
SfTRE1	TCAGATGAAGGTGAACTCGAAGA	GGAATGATGAATCCGTGGGTA
SfTRE2	CTGCTGCTGTCGGAGATGA	TAGGAGGGGAGGCTGTGAT
SfCHS2	GAGTTCACAGTGCGGTTGC	GCCAAAATAGCCCACATCC
SfCht	AAGCGGACAGCAAAGCG	CCAACTCAGGGTCAATAATAAGAAC
SfVg	CGAAGAACCTCAAATACGAAACTGT	TGGTGCTGGAGTGGGTAGATAA
SfVgR	CGACGAGTGCACTGAAGATG	GAGGCGTCAGTATCGGTGTA

2.6. Evaluation of Reproductive Capacity of S. frugiperda

The emerged post-injection female pupae were paired with male adults that had emerged on the same day and placed in a breeding box. Each treatment was repeated for 10–30 pairs. The pre-oviposition period, oviposition period, oviposition quantity, and lifespan of female adults were observed and recorded until their death. We randomly selected 10 pairs of mature egg masses from each group every 24 h and placed them in a feeding box (a total of 10 blocks per group per day) to document the overall hatching rate of the eggs. We also randomly selected 9 female adults from each group on the 2nd, 4th, and 6th day after emergence to dissect their ovaries. These were photographed using a Leica EZ4 HD stereomicroscope (8×) with LAS EZ software. We referred to Zhao's

ovarian development grading criteria to determine the ovarian development status [43]. Adult ovaries can be classified into five stages based on the maturity level, coloration, and quantity of eggs within the ovarian tubules. These stages were the transparent opalescent phase (I), the phase of yolk deposition (II), the phase of egg maturation (III), the peak phase of oviposition (IV), and the terminal phase of oviposition (V).

3. Results

3.1. Survival, Deformity, and Morphological Abnormalities after New Compound Injections

The survival rate of the **6d** larvae group significantly decreased between the third instar (100.0%) and the fifth instar (40.2%) (Figure 1A). Group **6e** demonstrated decreased survival between the third instar (100.0%) and the sixth instar (50.0%) (Figure 1A). A significant number of group **6f** larvae died between the third instar (100.0%) and the fifth instar (39.2%) (Figure 1A). All three compounds caused morphological abnormalities during development, including the melanization of larvae; pupae, as a result of hindered molting; dry and failed pupal shedding; and deformities in pupation. All three compounds were observed to have morphological abnormalities that did not lead to death in a short time (Figure 1B), similar to an absence of the outer epidermis.

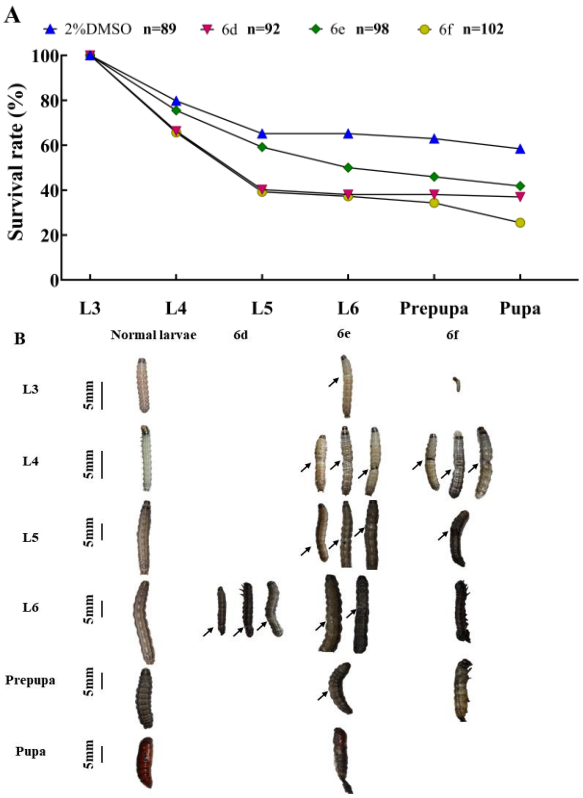

Figure 1. Survival rates (**A**) and morphological abnormalities (**B**) of *S. frugiperda* larvae at different stages after treatment with **6d**, **6e**, and **6f** compounds. The arrow indicates the missing sites of the epidermis. L3: larvae at the third instar; L4: larvae at the fourth instar; L5: larvae at the fifth instar; L6: larvae at the sixth instar.

3.2. Changes in Trehalase Activity and Related Gene Expression Levels in Samples Treated with Thiothiazolidone Compounds

The three new compounds—**6d**, **6e**, and **6f**—significantly inhibited TRE1 and TRE2 activity in the larvae of *S. frugiperda* (Figure 2A,B) compared with the 2% DMSO group whilst significantly downregulating the expression of TRE1 (Figure 2C). Compounds **6d** and **6f** significantly downregulated the expression of TRE2 (Figure 2D).

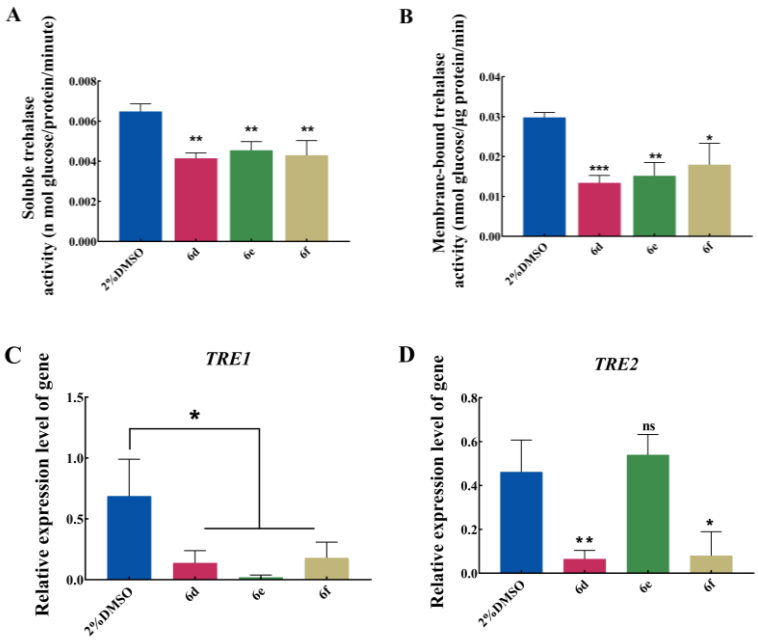

Figure 2. Changes in soluble trehalase activity (**A**), membrane-bound trehalase activity (**B**), and soluble trehalase genes SfTRE1 (**C**) and SfTRE2 (**D**) of membrane-bound trehalase genes after 48 h of treatment with compounds **6d**, **6e**, and **6f**. Values are presented as the mean ± SD. * $p < 0.05$ (significant differences); ** $p < 0.01$ (highly significant differences), ns (no significant differences); *** $p < 0.001$; 2% DMSO: control group; **6d**, **6e**, and **6f**: treatment groups.

3.3. Changes in Chitinase Activity and Chitin Content in Samples Treated with the New Compounds

The chitinase activity in the larvae of the control group of *S. frugiperda* exhibited a significant decrease after 48 h of treatment with **6f**, whereas **6d** and **6e** did not produce significant effects (Figure 3A). In comparison with the control group, only the chitin content of the *S. frugiperda* larvae in the **6e** group exhibited a significant increase. There was no notable alteration in the chitin content between the **6d** and **6f** groups (Figure 3B).

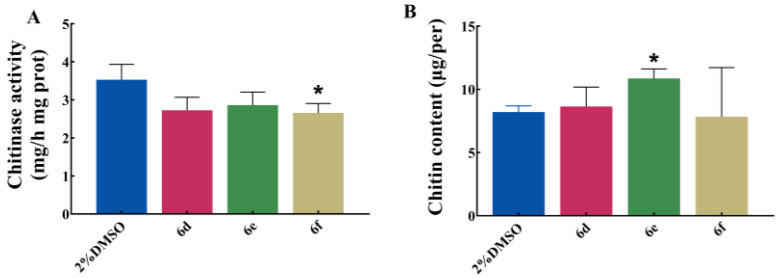

Figure 3. Changes in chitinase activity (**A**) and chitin content (**B**) after 48 h treatment with the new compounds. Values are presented as the mean ± SD. * $p < 0.05$ (significant differences); 2% DMSO: control group; **6d**, **6e**, and **6f**: treatment groups.

3.4. Changes in the Trehalase Activity and Sugar Content of Pupae

The TRE1 activity did not significantly change after the injection of the three compounds at 48 h (Figure 4A). The TRE2 activity was significantly reduced in the **6d** group but not in the **6d** and **6e** groups (Figure 4B). The contents of glucose, trehalose, and glycogen were determined to be without significant changes (Figure 4C–E).

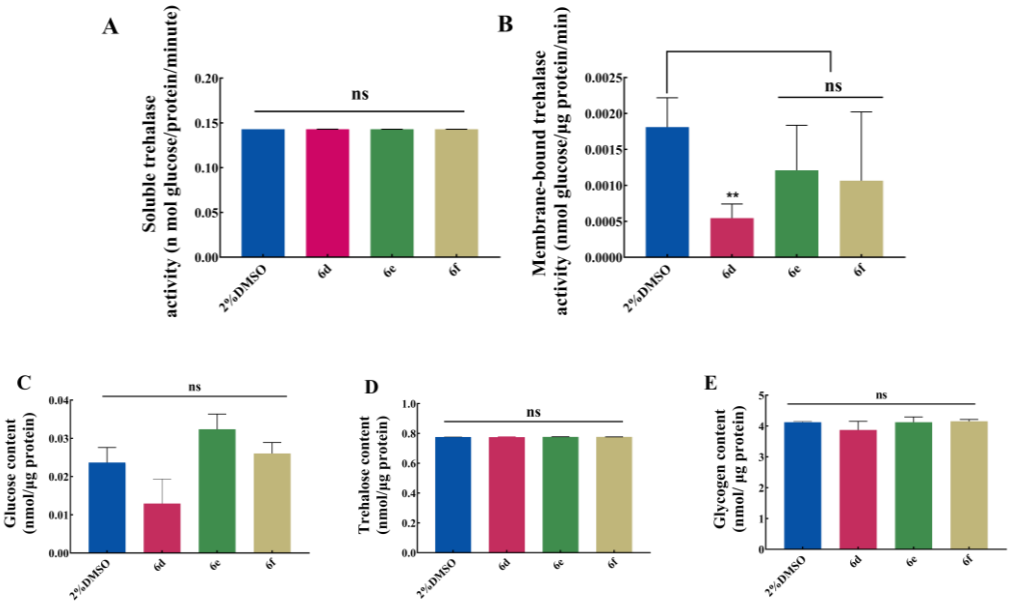

Figure 4. Changes in soluble trehalase activity (**A**) and membrane-bound trehalase activity (**B**) after 48 h. Contents of glucose (**C**), trehalose (**D**), and glycogen (**E**) after 48 h. Values are presented as the mean ± SD. ** $p < 0.01$ (highly significant differences); ns: not significant (independent sample *t*-tests); 2% DMSO: control group; **6d**, **6e**, and **6f**: treatment groups.

3.5. Effect of Injection of Novel Compounds on Adult Female Emergence

The emergence rates of the injected pupae and larvae of the third instar in the **6d**, **6e**, and **6f** groups exhibited no significant reduction in contrast to the control group. The emergence rate in the **6d** group was slightly lower than the control group at 53% (Table 3).

Table 3. Emergence of female *S. frugiperda* adults.

Treatment	Number of Larvae	Number of Pupae	Emergence Rate (%)	Chi-Squared Test
2% DMSO	89	52	71	
6d	92	34	53	df = 1, $p > 0.05$, c2 = 2.958
6e	98	41	76	df = 1, $p > 0.05$, c2 = 0.232
6f	102	26	85	df = 1, $p > 0.05$, c2 = 1.705

The emergence rate was analyzed for significance using the chi-squared test. Each treatment group began with approximately one hundred larvae.

3.6. Effects of Injecting New Compounds on the Lifespan, Pre-Oviposition, and Oviposition Periods of Female Adults

The lifespan of the female adults with regular emergence in the treatment groups notably exceeded that of the control group (Table 4). Only the **6d** group of female moths exhibited a significantly prolonged pre-oviposition period compared with the control group. There was no significant variance in the oviposition period compared with the control group (2% DMSO).

Table 4. Life expectancy, pre-oviposition, and oviposition periods of female *S. frugiperda* adults.

	Pre-Oviposition Period (d)	Oviposition Period (d)	Longevity (d)
2% DMSO	1.9 ± 0.3 b	5.2 ± 1.6 a	8.0 ± 0.8 b
6d	2.7 ± 0.9 a	5.8 ± 1.9 a	10.5 ± 2.4 a
6e	2.5 ± 1.0 ab	5.8 ± 2.0 a	10.8 ± 2.1 a
6f	2.3 ± 0.6 b	5.9 ± 2.2 a	11.2 ± 1.9 a

Values are presented as the mean ± SD. Different letters within the same parameter indicate significant differences ($p < 0.05$, ascertained from an ANOVA followed by a Duncan test). Each treatment group was replicated with 10–30 pairs of moths.

3.7. Effect of Injection of Novel Compounds on Egg Laying and Egg Hatching

The total egg production of single females in the first seven days after the emergence of *S. frugiperda* treated using **6e** and **6f** was significantly reduced compared with the control group (Figure 5A). There was no significant difference in egg production in the **6d** group. There was a significant difference in the hatching rate of eggs laid by female adults treated with the three compounds compared with the control group. The hatching rates of the **6d**, **6e**, and **6f** groups were recorded as 59.2%, 62.8%, and 59.8%, respectively, indicating a notably low percentage in each group (Figure 5B).

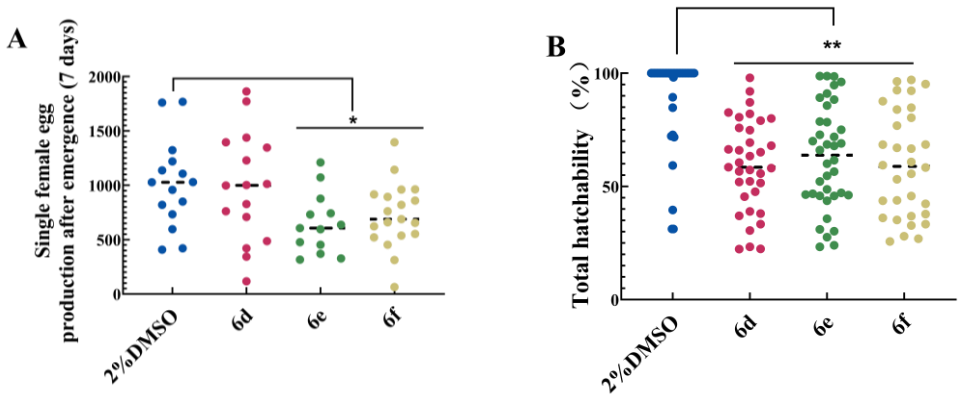

Figure 5. Total egg production (**A**) and total hatching rate of eggs in the first seven days after the emergence of female adult moths (**B**). ** $p < 0.01$; * $p < 0.05$ (*t*-test); 2% DMSO: control group. The dashed lines indicate the mean. Four egg masses, each containing 100 to 200 eggs, were randomly selected from each group over a seven-day period. This resulted in a total of 10 groups. We then recorded the hatching rate of these egg masses.

3.8. Effects of Injection of Novel Compounds on Ovarian Development and SfVg and SfVgR Gene Expression in Female Adults

The expression level of SfVg in female adults was significantly upregulated in the **6e** treatment group on the fourth and sixth day of emergence (Figure 6A). The expression level of SfVgR was significantly downregulated by **6d** on the fourth day of emergence (Figure 6B). We observed that the ovarian grades in each group were predominantly distributed across stages II (yolk deposition period) and III (mature and waiting period) when we dissected the ovaries of female adults on the fourth-day post-eclosion. There were black spots on the ovaries and fallopian tubes of the samples from the **6d**, **6e**, and **6f** groups, and there were blackening and clumping phenomena in the mating sacs (Figure 6C).

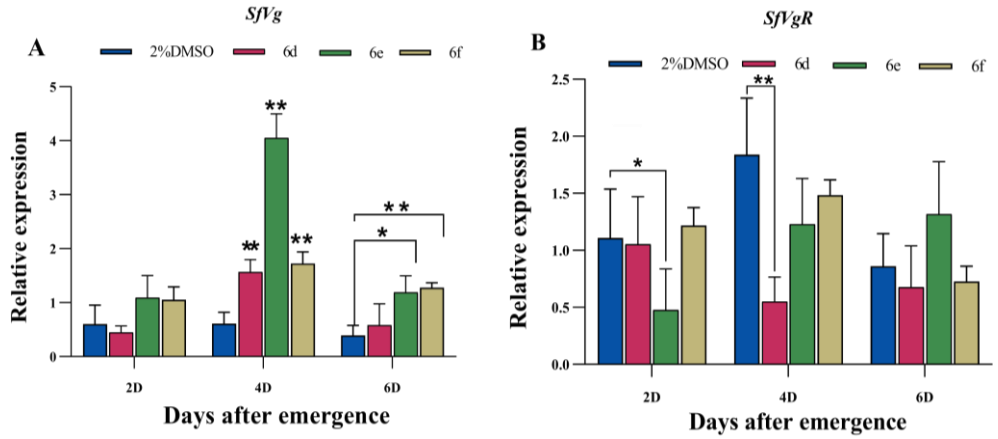

Figure 6. *Cont.*

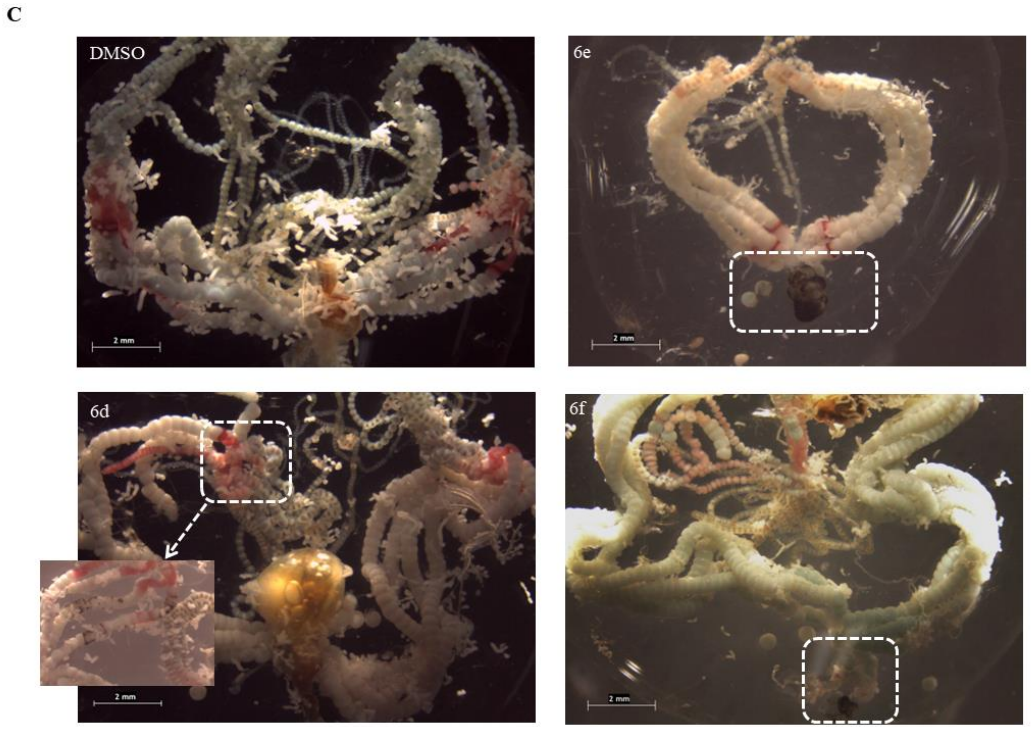

Figure 6. Expression levels of SfVg (**A**) and SfVgR (**B**) were measured on days 2, 4, and 6 post-emergence. Ovaries of female *S. frugiperda* adults on day 4 after emergence (**C**). The dotted boxes indicate where were blackening and clumping phenomena in the mating sacs. Three to five biological replicates were performed for each group. Mean ± SD; *t*-test. ** $p < 0.01$; * $p < 0.05$. Vg: vitellogenin; VgR: vitellogenin receptor; 2D: day 2 after emergence of treated females; 4D: day 4 after emergence of treated females; 6D: day 6 after emergence of treated females.

4. Discussion

The pioneering piperine derivative compounds ZK-I-21 and ZK-PI-4 have demonstrated a noteworthy reduction in TRE2 activity whilst exhibiting no discernible alteration in *TRE2* expression [29]. The three new compounds—**6d**, **6e**, and **6f**—inhibited the trehalase activity in *S. frugiperda* larvae and downregulated the expression of both *TRE1* and *TRE2*, consequently restricting the energy availability in the insects. Silencing the trehalase gene of *Frankliniella occidentalis* also led to significantly downregulated levels of enzymes associated with the energy metabolism pathway [44].

Chitin is a crucial component of various insect structures, including the epidermis, trachea, and peritrophic membrane [45]. Chitin also plays a crucial role in insect molting, growth, and development [46]. Previous studies observed that the emergence failure rate for pupae treated with inhibitors dramatically increased, resulting in morphological abnormalities such as difficulties in wing expansion [31]. Our experimental observations were similar to these outcomes. We noted morphological abnormalities in pupae, encompassing instances of molting failure and irregular pupation. The morphological abnormalities of larvae included blackening, difficulty with molting, and withering. A consistent pattern of developmental disruption was observed. Tellis noted that trehalose levels could affect the size, weight, and metabolic homeostasis of Lepidoptera larvae during larval pupal transitions [47]. We observed that the treatment group could induce a certain abdominal segment contraction in the larvae, similar to morphological abnormalities that did not lead to death in a short time of outer epidermal loss. Previous studies have revealed that parasitic wasps create a suitable nutritional environment for their offspring by upregulating the trehalase activity of host *Spodoptera litura* larvae, thereby positively affecting the normal pupation rate, emergence rate, adult proportion, and offspring size [48]. A decrease in the transcription and translation levels of *TRE* can downregulate the expression of *CHT* and *CHS*, leading to difficulties with molting and the abnormal development of

insects that often results in death [49]. Barbole [50] also observed that an inhibition of chitinase induced transcriptional dysregulation, altering the ecdysteroid-mediated control of *S. frugiperda* development. Inhibiting the expression of the chitinase gene in *S. frugiperda* affected both the degradation of chitin in the old epidermis and the formation of a new epidermis, resulting in an increase in chitin content [7]. Our experiments suggested a close relationship between trehalose metabolism and chitin metabolism. Alterations in enzyme activity and gene expression within the trehalose metabolism pathway consequently influenced chitin metabolism. A disorder in *TRE1* metabolism can lead to a series of morphological abnormalities in insects, including pupation deformity or failure, molting difficulties, wing-spreading difficulties, or wing-shape abnormalities. *TRE2* mainly affects the gene expression of chitin synthase and the chitin content in the mid-gut of larvae [50], thereby presenting a promising avenue for the development of efficacious pest-control interventions.

The study and differentiation of the morphological structures of the larvae and pupae stages of *S. frugiperda* are relatively complete [51]. We observed no significant change in trehalase activity or sugar content in pupae after the injection of the three compounds. This may have been because of the differences in the physiological functions and morphological structures between larvae and pupae in Lepidoptera insects, resulting in different affinities of the three compounds for trehalase in the larvae and pupae. Multiple studies have demonstrated that vitellogenesis can directly affect insect reproduction and is essential when studying reproductive regulation [52–54]. The expression of *SfVg* and *SfVgR* in the female adults of the treatment group in our study exhibited significant upregulation and downregulation, respectively, on the fourth day of eclosion. This alteration in expression levels could lead to the accumulation of vitellogenin in the hemolymph, potentially impacting the subsequent development and oviposition of the ovaries. The main insect vitellogenin (Vg) is transported by the vitellogenin receptor (VgR), providing various nutrients and functional substances for embryonic development [55,56]. Trehalose, a key carbohydrate in the hemolymph, plays a vital role in providing energy for various physiological processes in insects. One such function is the provision of energy for oocytes to absorb Vg through the VgR. The egg production and hatching rates were significantly reduced compared with the control group. This was highly likely related to the transport of sugar during the process of egg formation. Inhibition of the *VgR* expression can cause *Vg* to be deposited in large amounts in the hemolymph. This prevents its smooth entry into the ovaries, ultimately hindering ovarian tube development and significantly reducing egg production [48,57]. The prevention of a normal egg output by blackened and clumped egg and mating sacs in the fallopian tubes may have been a major reason for the decrease in egg production. In conjunction with findings from earlier research [58–60], we believe that trehalase inhibitors limit the energy availability of offspring by inhibiting trehalase or chitinase activity, or they are unable to break through the eggshell because of a lack of chitinase, thereby reducing the egg hatching rate. Experiments on the white-backed planthopper *Sogatella furcifera* [61] and cotton spider *Tetranychus cinnabarinus* [62] demonstrated a close correlation between chitinase activity and insect embryonic development, ovarian development, and egg hatching. A disruption of the trehalose pathway caused delayed ovarian development and egg formation in *Bombyx mori* [63]. Our findings revealed that treatment with inhibitors significantly extended the lifespan of the insects. Brown planthoppers used a similar physiological trade-off between survival and reproduction on amino acid nutrient-deficient media by inhibiting ovarian development owing to limitations in energy resources [64]. This suggests that the prolonged lifespan of *S. frugiperda* was because of energy limitations caused by trade-offs in development and reproduction.

In summary, the three novel pepper thiothiazolidone compounds used in this study—**6d**, **6e**, and **6f**—demonstrated potential insecticidal effects and observable effects in inhibiting the development and reproduction of *S. frugiperda*. By delving deeper into the mechanisms of action and optimizing formulations, future research holds significant promise

in refining the efficacy and environmental compatibility of these compounds. Ultimately, these advances may offer sustainable solutions to mitigate agricultural losses.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy14061315/s1, Figure S1: The structures of compounds **6d**, **6e**, and **6f**, Table S1: Artificial feed ingredients list.

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References

1. Sparks, A.N. A review of the biology of the fall armyworm. *Fla. Entomol.* **1979**, *62*, 82. [CrossRef]
2. Kenis, M.; Benelli, G.; Biondi, A.; Calatayud, P.-A.; Day, R.; Desneux, N.; Harrison, R.D.; Kriticos, D.; Rwomushana, I.; van den Berg, J.; et al. Invasiveness, biology, ecology, and management of the fall armyworm, *Spodoptera frugiperda*. *Entomol. Gen.* **2023**, *43*, 187–241. [CrossRef]
3. Tay, W.T.; Meagher, R.L.; Czepak, C.; Groot, A.T. *Spodoptera frugiperda*: Ecology, evolution, and management options of an invasive species. *Annu. Rev. Entomol.* **2023**, *68*, 299–317. [CrossRef]
4. Guo, J.F.; He, K.L.; Wang, Z.Y. Biological characteristics, trend of fall armyworm *Spodoptera frugiperda*, and the strategy for management of the pest. *Chin. J. Appl. Entomol.* **2019**, *56*, 361–369.
5. Wang, P.; He, P.C.; Hu, L.; Chi, X.L.; Keller, M.A.; Chu, D. Host selection and adaptation of the invasive pest *Spodoptera frugiperda* to indica and japonica rice cultivars. *Entomol. Gen.* **2022**, *42*, 403–411. [CrossRef]
6. Hou, Y.Y.; Ma, Y.; Xu, W.; Desneux, N.; Nkunika, P.O.Y.; Bao, H.P.; Zang, L.S. *Spodoptera frugiperda* egg mass scale thickness modulates *Trichogramma* parasitoid performance. *Entomol. Gen.* **2022**, *42*, 589–596. [CrossRef]
7. Liu, B.; Lu, Y.Y.; Wan, F.H.; Gershenzon, J.; Cheng, D.F. Biological invasion of insects:the roles of microbes. *Entomol. Gen.* **2022**, *42*, 851–861. [CrossRef]
8. Li, T.H.; Wang, S.; Ramirez-Romero, R.; Zang, L.S. Protective scale variation on *Spodoptera* egg masses can potentially support the cost-effective use of *Trichogramma* parasitoids. *Entomol. Gen.* **2023**, *43*, 939–944. [CrossRef]
9. Jia, Z.Q.; Zhan, E.L.; Zhang, S.G.; Wang, Y.; Song, P.P.; Jones, A.K.; Han, Z.J.; Zhao, C.Q. Broflanilide prolongs the development of fall armyworm *Spodoptera frugiperda* by regulating biosynthesis of juvenile hormone. *Entomol. Gen.* **2022**, *42*, 761–769. [CrossRef]
10. Wang, H.H.; Zhao, R.; Zhang, S.; Gao, J.; Xiao, X.; Tian, X.Y.; Liang, P.; Gu, S.H. Monitoring broflanilide resistance and its synergism with metaflumizone and tetraniliprole against fall armyworm, *Spodoptera frugiperda*. *Entomol. Gen.* **2023**, *43*, 535–543. [CrossRef]
11. Wang, L.; Chen, K.W.; Zhong, G.H.; Xian, J.D.; He, X.F.; Lu, Y.Y. Progress for occurrence and management and strategy of the fall armyworm *Spodoptera frugiperda* (Smith.). *J. Environ. Entomol.* **2019**, *41*, 479–487.
12. Wu, K.M. Management strategies of fall armyworm (*Spodoptera frugiperda*) in China. *J. Plant Protcet* **2020**, *46*, 1–5. [CrossRef]
13. Paredes-Sánchez, F.A.; Rivera, G.; Bocanegra-García, V.; Martínez-Padrón, H.Y.; Berrones-Morales, M.; Niño-García, N.; Herrera-Mayorga, V. Advances in control strategies against *Spodoptera frugiperda*. *Rev. Mol.* **2021**, *26*, 5587. [CrossRef] [PubMed]
14. Pavela, R.; Guedes, R.N.C.; Maggi, F.; Desneux, N.; Benelli, G. Essential oil antifeedants against armyworms: Promises and challenges. *Entomol. Gen.* **2023**, *43*, 689–704. [CrossRef]
15. Li, Y.P.; Zhang, S.; Wang, X.J.; Xie, X.P.; Liang, P.; Zhang, L.; Gu, S.H.; Gao, X.W. Current status of insecticide resistance in *Spodoptera frugiperda* and strategies for its chemical control. *J. Plant Prot.* **2019**, *45*, 14–19. [CrossRef]
16. Zhang, Z.Y.; Cheng, W.; Chang, W.C.; Chen, X. Current status of registration and application of pesticides for controlling *Spodoptera frugipterda* in the United States(I): Conventional pesticides. *J. World Pestic.* **2021**, *43*, 18–24+37. [CrossRef]
17. Xu, G.L.; Wang, Z.Y.; Wang, K.; Shu, C.l.; Geng, L.L.; Liao, M.; Zhang, J. Advances in research and application of *Bacillus thuritngiensis* for controlling *Spodoptera frugiperda*. *Chin. J. Biol. Control*, 2023; *Published online ahead*. [CrossRef]

18. Desneux, N.; Decourtye, A.; Delpuech, J.M. The sublethal effects of pesticides on beneficial arthropods. *Annu. Rev. Entomol.* **2007**, *52*, 81–106. [CrossRef] [PubMed]
19. Haddi, K.; Nauen, R.; Benelli, G.; Guedes, R.N.C. Global perspectives on insecticide resistance in agriculture and public health. *Entomol. Gen.* **2023**, *43*, 495–500. [CrossRef]
20. Li, J.J.; Xu, H.M.; Zhao, H.Z.; Pan, M.Z.; Smagghe, G.; Li, Z.Y.; Liu, T.X.; Shi, Y. Regulating role of neuropeptide PTTH releaved in *Spodoptera frugiperda* using RNAi- and CRISPR/Cas9-based functional genomic tools. *Entomol. Gen.* **2023**, *43*, 451–459. [CrossRef]
21. Yan, S.; Yin, M.Z.; Shen, J. Nanoparticle-based nontransformative RNA insecticides for sustainable pest control: Mechanisms, current status and challenges. *Entomol. Gen.* **2023**, *43*, 21–30. [CrossRef]
22. Chao, Z.J.; Ma, Z.Z.; Zhang, Y.H.; Yan, S.; Shen, J. Establishment of star polycation-based RNA interference system in all developmental stages of fall armyworm *Spodoptera frugiperda*. *Entomol. Gen.* **2023**, *43*, 127–137. [CrossRef]
23. Elbein, A.D.; Pan, Y.T.; Pastuszak, I.; Carroll, D. New insights on trehalose: A multifunctional molecule. *J. Glycobiol.* **2003**, *13*, 17R–27R. [CrossRef]
24. Arguelles, J.C. Why can't vertebrates synthesize trehalose? *J. Mol. Evol.* **2014**, *79*, 111–116. [CrossRef]
25. Tang, B.; Zhang, L.; Xiong, X.P.; Wang, H.J.; Wang, S.G. Advances in trehalose metabolism and its regulation of insect chitin synthesis. *J. Sci. Agricul. Sin.* **2018**, *51*, 697–707. [CrossRef]
26. Luo, Y.J.; Chen, Y.; Wang, X.J.; Wang, S.T.; Yang, Y.Y.; Xu, H.X.; Qu, C.; Wu, Y.; Li, C.; Wang, S.G.; et al. Validamycin affects the development and chitin metabolism in *Spodoptera frugiperda* by inhibiting trehalase activity. *Entomol. Gen.* **2022**, *42*, 931–939. [CrossRef]
27. Matassini, C.; Parmeggiani, C.; Cardona, F. New frontiers on human safe insecticides and fungicides: An opinion on trehalase inhibitors. *J. Mol.* **2020**, *25*, 3013. [CrossRef]
28. Anholt, R.R.H.; O'Grady, P.; Wolfner, M.F.; Harbison, S.T. Evolution of reproductive behavior. *J. Genet.* **2020**, *214*, 49–73. [CrossRef] [PubMed]
29. Wang, S.T.; Chen, Y.; Luo, Y.J.; Yang, Y.Y.; Jiang, Z.Y.; Jiang, X.Y.; Zhong, F.; Chen, H.; Xu, H.X.; Wu, Y.; et al. Effect of three novel compounds on trehalose and chitir metabolism and development of *Spodoptera frugiperda*. *J. Sci. Agricul. Sin.* **2022**, *55*, 1568–1578. [CrossRef]
30. Zhong, F.; Yu, L.H.; Jiang, X.Y.; Chen, Y.; Wang, S.; Chao, L.; Jiang, Z.Y.; He, B.E.; Xu, C.; Wang, S.G.; et al. Potential inhibitory effects of compounds ZK-PI-5 and ZK-PI-9 on trehalose and chitin metabolism in *Spodoptera frugiperda* (J. E. Smith). *Front. Physiol.* **2023**, *14*, 1178996. [CrossRef]
31. Jiang, X.Y.; Zhong, F.; Chen, Y.; Shi, D.M.; Chao, L.; Yu, L.H.; He, B.E.; Xu, C.D.; Wu, Y.; Tang, B.; et al. Novel compounds ZK-PI-5 and ZK-PI-9 regulate the reproduction of *Spodoptera frugiperda* (Lepidoptera: Noctuidae), with insecticide potential. *J. Econ. Entomol.* **2023**, *116*, 1850–1861. [CrossRef]
32. Asano, N.; Kato, A.; Kizu, H.; Matsui, K.; Watson, A.A.; Nash, R.J. Calystegine B4, a novel trehalase inhibitor from *Scopolia japonica*. *J. Carbohydr. Res.* **1996**, *293*, 195–204. [CrossRef] [PubMed]
33. Forcella, M.; Cardona, F.; Goti, A.; Parmeggiani, C.; Cipolla, L.; Gregori, M.; Schirone, R.; Fusi, P.; Parenti, P. A membrane-bound trehalase from *Chironomus riparius* larvae: Purification and sensivity to inhibition. *J. Glycobiol.* **2010**, *20*, 1186–1195. [CrossRef]
34. Li, Y.X.; Wang, J.Z.; Kato, A.; Shimadate, Y.; Kise, M.; Jia, Y.M.; Fleet, G.W.J.; Yu, C.Y. Stereocomplementary synthesis of casuarine and its 6-epi-, 7-epi-, and 6,7-diepi-stereoisomers. *Org. Biomol. Chem.* **2021**, *19*, 9410–9420. [CrossRef] [PubMed]
35. Chavarria, D.; Silva, T.; Magalhães e Silva, D.; Remião, F.; Borges, F. Lessons from black pepper: Piperine and derivatives thereof. *J. Expert. Opin. Ther. Pat.* **2016**, *26*, 245–264. [CrossRef] [PubMed]
36. Han, Q.; Wu, N.; Li, H.L.; Zhang, J.Y.; Li, X.; Deng, M.F.; Zhu, K.; Wang, J.E.; Duan, H.X.; Yang, Q. A piperine-based scaffold as a novel starting point to develop inhibitors against the potent molecular target OfChtI. *J. Agric. Food Chem.* **2021**, *69*, 7534–7544. [CrossRef] [PubMed]
37. Jiang, Z.Y.; Shi, D.M.; Li, H.L.; He, D.C.; Zhu, K.; Li, J.Y.; Zi, Y.J.; Xu, Z.J.; Huang, J.X.; Duan, H.X.; et al. Rational design and identification of novel piperine derivatives as multichitinase inhibitors. *J. Agric. Food Chem.* **2022**, *70*, 10326–10336. [CrossRef]
38. Inamori, Y.; Okamoto, Y.; Takegawa, Y.; Tsujibo, H.; Sakagami, Y.; Kumeda, Y.; Shibata, M.; Numata, A. Insecticidal and antifungal activities of aminorhodanine derivatives. *J. Biosci. Biotechnol. Biochem.* **1998**, *62*, 1025–1027. [CrossRef]
39. Pareek, D.; Chaudhary, M.; Pareek, P.K.; Kant, R.; Ojha, K.G.; Pareek, R.; Iraqia, S.M.U.; Pareek, A. Synthesis of some bioactive 4-thiazolidinone derivatives incorporating benzothiazole moiety. *Der Chem. Sin.* **2010**, *1*, 22–35.
40. Yang, H.B.; Liu, T.; Qi, H.T.; Huang, Z.S.; Hao, Z.S.; Ying, J.W.; Yang, Q.; Jian, X.H. Design and synthesis of thiazolylhydrazone derivatives as inhibitors of chitinolytic N-acetyl-βd-hexosaminidase. *J. Bioorg. Med. Chem.* **2018**, *26*, 5420–5426. [CrossRef]
41. Yang, H.; Qi, H.; Hao, Z.; Shao, X.; Liu, T.; Yang, Q.; Qian, X. Thiazolylhydrazone dervatives as inhibitors for insect N-acetyl-β-d-hexosaminidase and chitinase. *J. Chin. Chem. Lett.* **2020**, *31*, 1271–1275. [CrossRef]
42. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **2001**, *25*, 402–408. [CrossRef] [PubMed]
43. Zhao, S.Y.; Yang, X.M.; He, W.; Zhang, H.W.; Jiang, Y.Y.; Wu, K.M. Ovarian development gradation and reproduction potential prediction in Spodoptera frugiperda. *Plant Prot.* **2019**, *45*, 28–34. [CrossRef]
44. Zheng, X.; Yuan, J.; Qian, K.; Tang, Y.; Wang, J.; Zhang, Y.; Feng, J.; Cao, H.; Xu, B.; Zhang, Y.; et al. Identification and RNAi-based function analysis of trehalase family genes in *Frankliniella occidentalis* (Pergande). *Pest. Manag. Sci.* **2024**, *80*, 2839–2850. [CrossRef] [PubMed]

45. Moussian, B. Chitin: Structure, chemistry and biology. *J. Adv. Exp. Med. Biol.* **2019**, *1142*, 5–18. [CrossRef]
46. Bouchebti, S.; Cohen, T.M.; Bodner, L.; Levin, E. Chitin digestion in a eusocial insect: The digestive role of larvae in hornet colonies. *Entomol. Gen.* **2023**, *43*, 491–494. [CrossRef]
47. Tellis, M.B.; Mohite, S.D.; Nair, V.S.; Chaudhari, B.Y.; Ahmed, S.; Kotkar, H.M.; Joshi, R.S. Inhibition of trehalose synthesis in lepidoptera reduces larval fitness. *Adv. Biol.* **2023**, *8*, e2300404. [CrossRef] [PubMed]
48. Song, Y.; Gu, F.; Zhou, W.; Li, P.; Wu, F.; Sheng, S. Parasitoid wasps can manipulate host trehalase to the benefit of their offspring. *Insects* **2022**, *13*, 833. [CrossRef] [PubMed]
49. Lu, K.; Shu, Y.; Zhou, J.; Zhang, X.; Zhang, X.; Chen, M.; Yao, Q.; Zhou, Q.; Zhang, W. Molecular characterization and RNA interference analysis of vitellogenin receptor from *Nilaparvata lugens* (Stal). *J. Insect Physiol.* **2015**, *73*, 20–29. [CrossRef] [PubMed]
50. Barbole, R.S.; Sharma, S.; Patil, Y.; Giri, A.P.; Joshi, R.S. Chitinase inhibition induces transcriptional dysregulation altering ecdysteroid-mediated control of *Spodoptera frugiperda* development. *iScience* **2024**, *27*, 109280. [CrossRef]
51. Yang, Y.J.; Guo, J.W.; Qian, J.N.; Xu, H.X.; Lv, Z.X. Comparison of the morphological characteristics of *Spodcoptera frugiperda* pupae with those of five other lepidopteran pest species. *Chin. J. Appl. Entomol.* **2022**, *59*, 559–567. [CrossRef]
52. Han, S.; Wang, D.; Song, P.; Zhang, S.; He, Y. Molecular characterization of vitellogenin and its receptor in *Spodoptera frugiperda* (J. E. Smith, 1797), and their function in reproduction of female. *Int. J. Mol. Sci* **2022**, *23*, 11972. [CrossRef] [PubMed]
53. Li, J.P.; Deng, H.; Tan, Y.M.; Zheng, T.Y.; Ouyang, X.H. Research progress of insect vitellogenin. *J. Biotechnol.* **2022**, *32*, 114–119. [CrossRef]
54. Yan, X.; Yang, H.; Song, J.H.; Li, R.G.; Zhang, Y.B.; Yang, W.J. Function of vitellogenin receptor gene *TaVgR* in the regulation of reproductive development in *Tuta absoluta* (Lepidoptera: *Ge lechiidae*). *J. Acta Entomol. Sin.* **2022**, *65*, 675–683. [CrossRef]
55. Huang, J.H.; Lee, H.J. RNA interference unveils functions of the hypertrehalosemic horm one on cyclic fluctuation of hemolymph trehalose and oviposition in the virgin female *Blattella germanica*. *J. Insect Physiol.* **2011**, *57*, 858–864. [CrossRef] [PubMed]
56. Cong, L.; Yang, W.J.; Jiang, X.Z.; Niu, J.Z.; Shen, G.M.; Ran, C.; Wang, J.J. The essential role of vitellogenin receptor in ovary development and vitellogenin uptake in *Bactrocera dorsalis* (Hendel). *Int. J. Mol. Sci.* **2015**, *16*, 18368–18383. [CrossRef]
57. Yu, H.Z.; Zhang, Q.; Lu, Z.J.; Deng, M.J. Validamycin treatment significantly inhibits the glycometabolism and chitin synthesis in the common cutworm, *Spodoptera litura*. *J. Insect Sci.* **2022**, *29*, 840–854. [CrossRef]
58. Adrangi, S.; Faramarzi, M.A. From bacteria to human: A journey into the world of chitinases. *Biotechnol.* **2013**, *31*, 1786–1795. [CrossRef]
59. Qu, M.B.; Sun, S.P.; Liu, Y.S.; Deng, X.R.; Yang, J.; Yang, Q. Insect group II chitinase Of ChtII promotes chitin degradation during larva-pupa molting. *Insect Sci.* **2021**, *28*, 692–704. [CrossRef]
60. Zeng, Q.H.; Gong, M.F.; Yang, H.; Chen, N.N.; Lei, Q.; Jin, D.C. Effect of four chitinase genes on the female fecundity in *Sogatella furcifera* (Horváth). *Pest. Manag. Sci.* **2024**, *80*, 1912–1923. [CrossRef]
61. Wang, Z.; Long, G.Y.; Jin, D.C.; Yang, H.; Zhou, C.; Yang, X.B. Knockdown of two trehalase genes by RNA interference is lethal to the White-Backed Planthopper *Sogatella furcifera* (Horváth) (Hemiptera: Delphacidae). *Biomolecules* **2022**, *12*, 1699. [CrossRef] [PubMed]
62. Shen, G.M.; Ma, T.; Chen, X.R.; Chen, L.; Liu, G.M.; Luo, Y.J.; Adang, M.; He, L. Retinoid X receptor 1 is a specific lethal RNAi target disturbing chitin metabolism during hatching of *Tetranychus cinnabarinus*. *Int. J. Biol. Macromol.* **2023**, *245*, 125458. [CrossRef] [PubMed]
63. Katagiri, N.; Ando, O.; Yamashita, O. Reduction of glycogen in eggs of the silkworm, *Bombyx mori*, by use of a trehalase inhibitor, trehazolin, and diapause induction in glycogen-reduced eggs. *J. Insect Physiol.* **1998**, *44*, 1205–1212. [CrossRef]
64. Pan, X.; Lu, K.; Qi, S.; Zhou, Q.; Zhou, Q. The content of amino acids in artificial diet influences the development and reproduction of brown planthopper, *Nilaparvata lugens* (STÅL). *Arch. Insect Biochem. Physiol.* **2014**, *86*, 75–84. [CrossRef] [PubMed]