Metabolism and antioxidant activity of SIGSTD1 in *Spodoptera litura* as a detoxification enzyme to pyrethroids

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Glutathione S-transferase (GSTs) are members of multifunction enzymes in organisms and mostly known for their roles in insecticide resistance by conjugation. *Spodoptera litura* (Fabricius) is a voracious agricultural pest widely distributed in the world with high resistance to various insecticides. The function of GSTs in the delta group of *S. litura* is still lacking. Significantly up-regulation of SIGSTD1 was reported in four pyrethroids-resistant populations and a chlorpyrifos-selected population. To further explore its role in pyrethroids and organophosphates resistance, the metabolism and peroxidase activity of SIGSTD1 were studied by heterologous expression, RNAi, and disk diffusion assay. The results showed that $K_m$ and $V_{max}$ for 1-chloro-2,4-dinitrobenzene (CDNB) conjugating activity of SIGSTD1 were 1.68 ± 0.11 mmol L⁻¹ and 76.0 ± 2.7 nmol mg⁻¹ min⁻¹, respectively. Cyhalothrin, beta-cypermethrin, and chlorpyrifos had an obvious inhibitory effect on SIGSTD1 activity, especially for fenvalerate, when using CDNB as substrate. Fenvalerate and cyhalothrin can be metabolized by SIGSTD1 in *E. coli* and in vitro. Also, silencing of SIGSTD1 significantly increased the toxicity of fenvalerate and cyhalothrin, but had no significant effect on the mortality of larvae treated by beta-cypermethrin or chlorpyrifos. SIGSTD1 possesses peroxidase activity using cumene hydroperoxide as a stress inducer. The comprehensive results indicate that SIGSTD1 is involved in fenvalerate and cyhalothrin resistance of *S. litura* by detoxication and antioxidant capacity.

*Spodoptera litura* (Fabricius) is a polyphagous agricultural insect pest widely distributed worldwide. It had high fecundity and a short life cycle, which always resulted in outbreaks. *S. litura* feed on over 150 species of host plants, particularly on economically important crops, causing serious yield and economic losses. The control of *S. litura* still relies on chemical insecticides. As the repeated and indiscrimination application, *S. litura* populations from China, Pakistan, and India were reported to develop high resistance to pyrethroids, organophosphates, and even some new insecticides, such as spinosad and abamectin.

Glutathione S-transferases (GSTs) are an important detoxifying enzyme system dividing into microsomal, mitochondrial, and cytosolic GSTs according to their location in cells. Only microsomal and cytosolic GSTs were reported in insects. Microsomal GSTs in insects are membrane-bound proteins and less reported, while cytosolic GSTs are water-soluble and could be further classified into seven groups, including epsilon, delta, omega, sigma, theta, zeta, and unclassified. GSTs in epsilon and delta groups are unique in insects and mostly reported to contribute to insecticides resistance by metabolism and sequestration. GSTs also participated in organophosphates resistance by catalyzing the conjugation of reduced glutathione (GSH) with insecticides. In *S. litura*, a total of 31 cytosolic GSTs genes have been identified, including 15 epsilon and 4 delta genes. Several GSTs genes from epsilon group were reported to paly roles in insecticides resistance. For example, the expression of SIGSTE1 was up-regulated significantly in four pyrethroid-resistant populations and could be induced by chlorpyrifos. Its recombinant protein showed high binding activity with chlorpyrifos, malathion, phoxim, deltamethrin. The expression of SIGSTE2 and SIGSTE3 in *S. litura* was significantly induced by DDT and herbicide, and the recombinant protein of SIGSTE2 could conjugate with DDT. SIGSTE12 was significantly overexpressed in populations resistant to pyrethroids and organophosphates, and its recombinant protein could

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metabolize phoxim, fenvalerate, cyhalothrin, especially for chlorpyrifos. The expression of SlGSTe9 decreased with chlorpyrifos and phoxim resistance level recession, and its recombinant protein could metabolize chlorpyrifos directly. However, the function of GSTs genes from delta groups of S. litura is still lacking.

In addition to the typical roles in detoxification of insecticides or other xenobiotic compounds, GSTs also showed antioxidant activity to protect organisms from oxidative stress caused by cold, heat, ultraviolet, H2O2, cumene hydroperoxide (CHP), metal, nanoparticles, or insecticides. In S. litura, SlGSTE1, SlGSTE9, SlGSTE12, SlGSTO2 from epsilon and omega clusters have been reported to have antioxidant activity. Our previous study has indicated that SlGSTd1, a GSTs gene from delta cluster, is significantly overexpressed in four field-collected populations (LF, NJ, JD and CZ) resistant to pyrethroids. Zhang et al. also reported the significantly higher expression level of SlGSTd1 in a chlorpyrifos-selected strain than the susceptible strain. Does the overexpression of SlGSTd1 relate with pyrethroids and chlorpyrifos resistance? Heterologously expression and RNAi were adopted to investigate the contribution of SlGSTd1 in insecticides resistance. The findings enriched the knowledge of GSTs gene from delta cluster and also revealed the insecticides resistance mechanism in S. litura.

Results

The expression of SIGSTD1 in E. coli and kinetic properties. To evaluate the role of SIGSTD1 in pyrethroids and chlorpyrifos resistance, SIGSTD1 was heterologously expressed in Escherichia coli. Its recombinant protein was characterized by SDS-PAGE and kinetic properties were determined using 2,4-dinitrochlorobenzene (CDNB) as a standard substrate. The theoretical molecular mass of SIGSTD1 is predicted to be 24.3 kDa by Compute pI/Mw (https://web.expasy.org/compute_pi/), conforming with the indicated band in lane 3 and lane 4 marked with red box in Fig. 1A (between 20 and 26 kDa). The recombinant protein SIGSTD1 showed high catalysis activity to CDNB, and its $K_m$ and $V_{max}$ values were 1.68 ± 0.11 mmol L$^{-1}$ and 76.0 ± 2.7 nmol mg$^{-1}$ min$^{-1}$, respectively (Fig. 1B).

Inhibition of insecticides on the activity of SIGSTD1. To determine the competition binding ability of fenvalerate, beta-cypermethrin, cyhalothrin and chlorpyrifos to SIGSTD1 conjugating activity against CDNB, a set of experiments was carried out using diethyl maleate (DEM, the inhibitor of GSTs) as a positive control. As shown in Fig. 2, the half inhibitory concentrations ($IC_{50}$) value of DEM against SIGSTD1 activity was calculated to be the lowest with 2.1 ± 0.3 μmol L$^{-1}$. The $IC_{50}$ values of fenvalerate, cyhalothrin, beta-cypermethrin and chlorpyrifos to SIGSTD1 activity were 21.6 ± 3.2, 123.8 ± 10.3, 129.3 ± 8.1 and 170.2 ± 15.2 μmol L$^{-1}$, respectively.

In vitro metabolism activity of SIGSTD1 to insecticides. In order to determine the metabolic activity of purified recombinant protein SIGSTD1 to insecticides, the residues of insecticide in the mixture after incubation were detected by ultrahigh-performance liquid chromatography (UPLC). As shown in Table 1, the residual peak area of fenvalerate and cyhalothrin incubated with SIGSTD1 for 3 h decreased significantly compared with that incubated with potassium buffer saline (PBS) or boiled SIGSTD1. While the residual peak area of alphacypermethrin, beta-cypermethrin and chlorpyrifos incubated with SIGSTD1 had no significant reduction compared with PBS or boiled SIGSTD1.
Metabolism activity of SlGSTD1 in E. coli to insecticides. The metabolic activity of SlGSTD1 expressed in E. coli toward insecticides was also evaluated. As shown in Fig. 3A, compared with control, E. coli containing pET-26b(+)/SlGSTd1 showed significant degradation of fenvalerate after incubated for 48, 72 and 96 h. The residual peak area of cyhalothrin incubated with E. coli containing pET-26b(+)/SlGSTd1 was significantly reduced after 72 and 96 h compared with LB liquid medium or pET-26b(+) (Fig. 3B). After incubation with theta-cypermethrin (Fig. 3C), alpha-cypermethrin (Fig. 3D) and chlorpyrifos (Fig. 3E), the residual peak area of E. coli containing pET-26b(+)/SlGSTd1 had no significant change when compared with pET-26b(+) medium.

Silencing of SlGSTd1 increased the susceptibility of S. litura to fenvalerate and cyhalothrin. To validate the involvement of SlGSTd1 in insecticides resistance by in vivo data, RNAi of SlGSTd1 was accomplished by feeding larvae with artificial diet containing dsRNA. As shown in Fig. 4A, the relative expression level of SlGSTd1 decreased significantly after dsSlGSTd1 feeding for 12 and 24 h, when compared to the treatment of dsGFP and H2O, respectively. In addition, compared with dsGFP and H2O, the relative expression level of SlGSTd1 showed no significant change after feeding on dsGFP for 48 h. Bioassay results showed that silencing of SlGSTd1 significantly increased the cumulative mortality after fenvalerate treated for 72 h (Fig. 4B) and cyhalothrin treated for 48, 60 and 72 h (Fig. 4C). The cumulative mortality after treatment with beta-cypermethrin and chlorpyrifos had no significant change after feeding on dsGFP or dsSlGSTd1 (Fig. 4D,E).

Antioxidant activity of SlGSTD1 in E. coli against CHP. To further characterize the antioxidant activity of SlGSTD1, the halo diameter of inhibition zones in LB plates spread with E. coli expressing pET-26b(+)/SlGSTd1 or pET-26b(+) was measured using CHP as a stress inducer. When the concentrations of CHP were 50, 100, 200, and 300 mmol L⁻¹, the inhibition zone halo diameter of E. coli cells expressing pET-26b(+)/SlGSTd1 was significantly lower than that of E. coli cells expressing pET-26b(+), with inhibition rates of 32.0%, 15.5%, 33.9% and 19.5%, respectively (Fig. 5A,B).

Table 1. The metabolism activity of purified recombinant SIGSTD1 to insecticides. Different lowercase letters indicated significant differences analyzed by the student's test (P<0.05).

| Treatment         | Peak area of residual insecticides (mAu s) |
|-------------------|--------------------------------------------|
|                   | Fenvalerate | Cyhalothrin | Alpha-cypermethrin | Theta-cypermethrin | Chlorpyrifos |
| PBS               | 1,646,348 ± 34,091a | 1,065,526 ± 12,562a | 580,381 ± 13,016a | 1,041,216 ± 24,023a | 138,272 ± 8780a |
| Boiled SlGSTD1    | 1,596,852 ± 23,163a | 1,065,982 ± 15,624a | 573,658 ± 19,256a | 1,029,658 ± 30,251a | 135,639 ± 3625a |
| SlGSTD1           | 1,540,917 ± 18,891b | 1,035,990 ± 7368b | 545,015 ± 28,967a | 974,293 ± 55,054a | 137,821 ± 6198a |

Figure 2. The IC₅₀ values of DEM, fenvalerate, cyhalothrin, beta-cypermethrin and chlorpyrifos on enzyme activity of SIGSTD1.

Figure 3. The metabolism activity of SlGSTD1 to insecticides.
Figure 3. The residual peak area of fenvalerate (A), cyhalothrin (B), theta-cypermethrin (C), alpha-cypermethrin (D) and chlorpyrifos (E) metabolized by SIGSTD1 in E. coli. CK indicates the reaction added with LB liquid medium. pET-26b(+) indicates the reaction added with E. coli of empty vector pET-26b(+). pET-26b(+)/SIGSTD1 indicates the reaction added with E. coli of recombinant vector pET-26b(+)/SIGSTD1.

Figure 4. The relative expression of SIGSTD1 and bioassay results of larvae in S. litura after RNAi treatment. (A) The relative expression of SIGSTD1 in S. litura after larvae feeding on dsRNA or H2O. Different lowercase letters indicated significant differences analyzed by ANOVA followed by Tukey’s HSD test (P< 0.05). (B–E) Mortality of larvae treated by fenvalerate (B), cyhalothrin (C), beta-cypermethrin (D), chlorpyrifos (E) after feeding on dsRNA. * Indicated significant differences in larvae mortality between feeding on dsSIGSTD1 and dsGFP (student’s t-test, P< 0.05).
SlGSTd1 or its antioxidant activity, which needs further study. Although the metabolic ability of insecticide can represent the affinity of insecticide to GST enzyme, and this affinity is related to the metabolic ability of insecticide. As shown in Fig. 2, the IC₅₀ value of fenvalerate was lower than that of cyhalothrin, beta-cypermethrin and chlorpyrifos, indicating that fenvalerate had a higher affinity for competitive binding to SlGSTd1.

The significant overexpression of GSTs genes in insecticides-resistant populations is often deduced to play role in insecticides resistance. However, the relationship still needs further validation, and metabolism activity could provide the most direct evidence of gene function. SlGSTd1 is highly likely to be involved in the detoxifying of pyrethroids and chlorpyrifos for its significant overexpression in pyrethroid-resistant populations and a chlorpyrifos-selected strain. Studies have suggested that pyrethroids could be metabolized by insect GSTs. For example, HaGST-8 in Helicoverpa armigera could effectively metabolize cypermethrin in an aqueous solution and in vitro. Additionally, the cumulative mortality of larvae applied by fenvalerate and cyhalothrin was increased significantly after the silencing of SlGSTd1 in S. litura. In conclusion, SlGSTd1 in S. litura was demonstrated to play roles in fenvalerate and cyhalothrin resistance by metabolism activity or its antioxidant activity.

Discussion

As one of the three major detoxification enzymes in the organism, GSTs have been well demonstrated to be involved in the detoxification of both endogenous and xenobiotic compounds, such as insecticides and plant allelochemicals. In this study, SIGSTD1 in S. litura was demonstrated to play roles in fenvalerate and cyhalothrin resistance by metabolism activity or its antioxidant activity.

Cytosolic GSTs are hetero- or homo-dimeric proteins with a molecular weight around 26 kDa. Here, the molecular weight of SIGSTD1 is identified around 26 kDa by SDS-PAGE electrophoresis, similar to its theoretical molecular weight. The kinetic properties of recombinant protein SIGSTD1 were determined using CDNB as a substrate. The results showed that the Kₘ and Vₘₐₓ values of SIGSTD1 were 1.68 ± 0.11 mmol L⁻¹ and 76.0 ± 2.7 nmol min⁻¹ mg⁻¹ (Fig. 1B), indicating that SIGSTD1 was successfully expressed in E. coli. The IC₅₀ value of insecticides on GST activity inhibition can represent the affinity of insecticide to GST enzyme, and this affinity is related to the metabolic ability of insecticide. As shown in Fig. 2, the IC₅₀ value of fenvalerate was lower than that of cyhalothrin, beta-cypermethrin and chlorpyrifos, indicating that fenvalerate had a higher affinity for competitive binding to SIGSTD1.

In conclusion, SlGSTd1 in S. litura can metabolize fenvalerate and cyhalothrin both in E. coli and in vitro. Fenvalerate had a strong affinity to SIGSTD1. The silencing of SIGSTD1 significantly increased the toxicity of fenvalerate and cyhalothrin in S. litura.
Expression and purification of SlGSTD1. Total RNA was extracted from the third instar larvae. The first-strand cDNA was synthesized from 1 μg RNA according to the instructions of FastQuant RT Kit (Tiangen, Beijing, China). The coding sequence of SlGSTD1 was amplified by PCR with primer pairs added with NdeI and XhoI (Table 2). The PCR products were inserted to pET-26b(+) and the expression vector pET-26b(+)/SlGSTd1 was constructed. The constructed expression vector was then transformed into E. coli BL21 (DE3) (Tiangen, Beijing, China) and cultured in LB liquid medium containing kanamycin (50 mg L\(^{-1}\), Tiangen, Beijing, China) at 37 °C. IPTG (Tiangen, Beijing, China) at final concentration of 1 mmol L\(^{-1}\) was added to induce the expression of SlGSTD1. The induced cells were cultured for an additional 3 h at 37 °C, 160 rpm before collection by centrifugation at 10,000 g. The supernatant was collected as crude protein solution.

SDS-PAGE (12.5%) was conducted, and Coomassie Blue R-250 (Tiangen, Beijing, China) was used as a staining solution.

Enzyme kinetics analysis of SlGSTD1. The purified protein was diluted to 1 mg mL\(^{-1}\). The denaturing SDS-PAGE (12.5%) was conducted, and Coomassie Blue R-250 (Tiangen, Beijing, China) was used as a staining solution.

The kinetic parameters of recombinant protein SlGSTD1 were determined using CDNB (J&K, Beijing, China) as a standard substrate. The reaction system consisted of PBS (1 mL, 0.1 mol L\(^{-1}\), pH 7.0), the enzyme preparation (5 μL, 1 mg mL\(^{-1}\)), and freshly prepared GSH (30 μL, 100 μmol mL\(^{-1}\), pH 7.0), NdeI, and XhoI, respectively, to initiate the reaction. Absorbance at 340 nm (\(A_{340}\)) was monitored by a microplate reader (Biotek, USA) within 0–180 s. Each reaction was performed in triplicate with three samples. \(K_m\) and \(V_{max}\) of the protein were obtained according to the Michaelis–Menten equation with SigmaPlot 12.0 (Systat Software, San Jose, CA., URL: https://systatsoftware.com/sigmaplot/).

Enzyme inhibition experiments. The inhibitory effect of fenvalerate (93.4%, Jiangsu Changlong Chemicals Co., Ltd., Jiangsu, China), beta-cypermethrin (95.0%, Beijing Huarrong Biochemical Co., Ltd., Beijing, China), cyhalothrin (98.4%, Beijing Huarrong Biochemical Co., Ltd., Beijing, China), and chlorpyrifos (95.0%, Beijing Huarrong Biochemical Co., Ltd., Beijing, China) on the activity of recombinant protein SlGSTD1 was measured with the method described in Wang et al.\(^{14}\). The assay mixture was consisted of PBS (900 μL, 0.1 mol L\(^{-1}\), pH 7.0), freshly prepared GSH (30 μL, 100 μmol mL\(^{-1}\), pH 7.0), recombinant protein (5 μL, 1 mg mL\(^{-1}\)) and gradient diluted insecticides (10 μL, fenvalerate: 0.3, 0.6, 1.2, 2.4, 4.8, 9.6 mmol L\(^{-1}\), cyhalothrin: 0.55, 1.10, 2.20, 4.40, 8.80, 17.60 mmol L\(^{-1}\), beta-cypermethrin: 1.2, 2.4, 4.8, 9.6, 19.2, 38.4 mmol L\(^{-1}\), chlorpyrifos: 0.35, 0.7, 1.4, 2.8, 5.6, 11.2 mmol L\(^{-1}\)). After the addition of CDNB (50 μL, 50 μmol mL\(^{-1}\)), the mixture was shaken quickly and \(A_{340}\) measured by a spectrophotometer (Tianjin, China). The inhibition rate was used to evaluate the inhibition effect of insecticides on the enzyme activity.
was recorded. The inhibitor of GSTs, DEM (97%, J&K, Beijing, China), was used as positive control. Each reaction was performed in triplicate with three samples. The IC$_{50}$ values were calculated and plotted using SigmaPlot 12.0 (Systat Software, San Jose, CA.).

**Metabolism activity analysis to insecticide.** The metabolism activity of SlGSTD1 towards fenvalerate, beta-cypermethrin, cyhalothrin and chlorpyrifos was determined by UPLC. The procedure was the same as our previous study$^{24}$. For in vitro assay, the purified recombinant protein (180 μL, 1 mg mL$^{-1}$) was mixed with PBS (250 μL, 0.1 mol L$^{-1}$, pH 6.8) and preheated at 37°C, then added with insecticide (20 μL, 500 mg L$^{-1}$). Finally, freshly prepared GSH (50 μL, 100 μmol L$^{-1}$) was added to start the reaction. The reaction system was immediately placed in a water bath and incubated at 37°C for 3 h. The reaction was terminated by adding the same volume of acetonitrile and saturated with sodium chloride to extract the insecticide. After shaken at 200 rpm for 2 h and brief centrifugation at 3000 rpm for 2 min, the supernatant was carefully absorbed and filtered through a 0.22 μm membrane. The extracts were detected by Waters Acquity UPLC system with an Acquity UPLC BEH C18 analytical column (2.1 mm × 100 mm, 1.7 μm). The chromatographic conditions were acetonitrile/water = 80/20 (v/v), flow rate at 0.4 mL min$^{-1}$ and 5 μL injection volume. Fenvalerate, beta-cypermethrin, cyhalothrin, and chlorpyrifos were detected and quantified at 220, 220, 230 and 290 nm, respectively. As a chiral insecticide, beta-cypermethrin could be separated into alpha-cypermethrin and theta-cypermethrin under these detection conditions. The retention time for fenvalerate, alpha-cypermethrin, theta-cypermethrin, cyhalothrin, and chlorpyrifos were 1.86, 1.76, 1.67, 1.69, 1.36 min, respectively. PBS or boiled purified recombinant protein was used as control.

For in E. coli assay, the concentration of pET-26b(+) /SlGSTD1 transformed E. coli in LB liquid medium was diluted to OD$_{600}$ = 1. A total of 2 mL of the E. coli culture was added to a 50 mL LB liquid medium, containing kanamycin (50 mg L$^{-1}$) and insecticide (50 mg L$^{-1}$ for chlorpyrifos or 25 mg L$^{-1}$ for fenvalerate, beta-cypermethrin, cyhalothrin). The mixture was further cultured at 37°C, 120 rpm for 6 h, and then IPTG (1 mmol L$^{-1}$) was added to induce SlGSTD1 expression. The mixture (2 mL) was sampled after cultured for 0, 24, 48, 72 and 96 h. Each treatment was performed in triplicate. The insecticides extraction and detection conditions were the same as the above methods. The control group was treated with LB liquid medium or E. coli transformed with pET-26b(+).

**Silencing of SlGSTD1 by RNAi.** Silencing of SlGSTD1 was conducted with T7 RiboMAX™ Express RNAi System (Promega, Madison, WI). Briefly, DNA fragments of SlGSTD1 appended to a T7 polymerase promoter were amplified with primer pairs dsSlGSTD1F-1, dsSlGSTD1R-1 and dsSlGSTD1R-2 (Table 2). The products were purified and used for dsRNA synthesis. dsRNA of green fluorescent protein (GFP) was also obtained by the methods above and the concentration of dsRNA was determined by NanoDrop 2000 (Thermo Scientific, Foster, CA, USA).

The third instar larvae of NJ population were fed artificial diet containing 3 μg dsRNA after starved for 6 h. The artificial diet containing ddH$_{2}$O or dsGFP under the same conditions was used as control. Quantitative real-time PCR (qRT-PCR) was conducted to determine the dsRNA interference effective after 12, 24, 48 h treatment. The qRT-PCR tests were performed with SuperReal PreMix Plus (SYBR Green, Tiangen, Beijing, China) using a QuantStudio 6 Real-Time PCR System (Applied Biosystems by Life Technologies, Foster, CA, USA). The PCR mixture (20 μL) contained 10 μL of 2× SuperReal PreMix solution, 0.4 μL of 50× ROX reference dye, 1 μL of the cDNA template, 0.6 μL of each primer, and 7.4 μL ddH$_{2}$O. The thermal cycling was set as: 94°C for 3 min, followed by 40 cycles of 95°C for 10 s and 60°C for 32 s. EF1α and RPL10 were used as reference genes to normalize the expression of SlGSTD1. Bioassay was performed by applying 1 μl insecticide at LD$_{20}$ to the thoracic dorsum of larvae by Hamilton syringe after 12 h of treatment with dsRNA or ddH$_{2}$O. Each insecticide treatment consisted of 12 larvae and repeated 3 times. The mortality was checked after insecticides treated for 12, 24, 36, 48, 60, 72 h.

**Antioxidant activity assay.** A disc diffusion assay was conducted in reference to Labade et al.$^{35}$ with slight modification to determine the antioxidant activity of SlGSTD1. The LB liquid medium containing E. coli transformed with pET-26b(+) /SlGSTD1 (OD$_{600}$= 1.0) was distributed on LB agar plates (50 mg L$^{-1}$ for kanamycin, 1 mmol L$^{-1}$ for IPTG) and incubated at 37°C for 1 h. The E. coli solution containing pET-26b(+) was used as control. The filter papers (5 mm diameter) were immersed in CHP (J&K, Beijing, China) at 0, 50, 100, 200, 300 mmol L$^{-1}$ dissolved in acetone. All filter papers were placed on the surface of LB agar plates and incubated at 37°C for 36 h. The diameter of the bacteriostatic zone around the disk was measured. Each concentration was repeated 3 times and the experiment was performed twice.

**Statistical analysis.** The qRT-PCR results were calculated according to the 2$^{-\Delta\Delta Ct}$ method and expressed as mean ± standard deviation (SD). The student's t-test was used to analyze the statistical differences in the cumulative mortality of third instar larvae, and the disc diffusion assay. One-way ANOVA followed by Tukey's HSD test was used to analyze the statistical difference of the metabolism activity of SlGSTD1 in vitro, the expression of SlGSTD1 mediated by RNAi and the metabolism activity of SlGSTD1 in E. coli. SPSS 16.0 (IBM, Chicago, IL, U.S.A., URL:https://www.ibm.com/search?lang=en&cc=us&q=SPSS) was used for statistical analysis and the P values less than 0.05 were considered statistically significant.

**Data availability** All data generated or analyzed during this study are included in this published article.
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**Author contributions**
D.L. and L.X. conceived the experiments and writing the original draft, D.L., L.X., and H.L. conducted the experiments, X.C., and L.Z. analysed the results and review & editing the original draft. All authors reviewed the manuscript.

**Competing interests**
The authors declare no competing interests.

**Additional information**

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