

Effects of pyriproxyfen exposure on damage to midgut and related gene expressions in the *Bombyx mori* silkworm

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ABSTRACT: Silkworm is an important economic insect and very sensitive to pesticides. The pollution of pyriproxyfen leads to the detriment of the growth and cocooning of the silkworm, which seriously affects the development of sericulture. Results showed that at 24 h and 48 h after pyriproxyfen exposure, the relative expressions of digestive enzyme genes, oxidative phosphorylation genes, and antioxidant enzyme genes were reduced, suggesting the inhibition of pyriproxyfen on digestion and absorption, energy metabolism, and antioxidation in the midgut of a silkworm. However, the activities of detoxification enzymes and the expressions of detoxification-related genes were elevated in the midgut of silkworm after 24 h and 48 h of pyriproxyfen exposure, indicating the enhanced resistance of silkworm to pyriproxyfen. In general, this study revealed the silkworm's midgut response to pyriproxyfen and provided an important reference to understand the metabolic mechanism of pyriproxyfen in this insect.

KEYWORDS: pyriproxyfen, digestion and absorption, antioxidation, detoxification, silkworm

INTRODUCTION

Bombyx mori, or silkworm is an important economic insect and vulnerable to adverse environmental effects, including virus infection and pesticide poisoning, due to its long-term indoor isolation and poor stress resistance [1, 2]. As a chitin synthesis inhibitor of alkoxy pyridine juvenile hormone [3], pyriproxyfen can prevent insects from forming new epidermis when molting, thus hindering metamorphosis or even causing deforming and death of the insects [4, 5]. Pyriproxyfen is one of the most widely used insecticides at present because of its low toxicity and high efficiency. However, the phenomenon of non-cocooning of *Bombyx mori* in major silkworm areas in China appeared in recent years, which was attributed to the intake of mulberry leaves polluted by pyriproxyfen pesticides, suggesting the sensitivity of *Bombyx mori* to pyriproxyfen.

The midgut is the digestive organ of silkworms, and it is mainly responsible for the digestion and absorption of nutrients. Meanwhile, the midgut is the first barrier against foreign substances, such as pathogens or pesticides, that pass through the midgut before entering other tissues and organs.

Previous studies have shown that trace amounts of pesticides can cause damages to the midgut of silkworm and affect its digestion and absorption functions, as well as the physiological and biochemical indexes of the body [6]. For instance, low-dose pesticides cause the accumulation of reactive oxygen species (ROS) and the decrease in antioxidant enzyme activity of silkworms [7]. The increased activity of detoxification enzymes of silkworm indicated its stress response to external adverse factors and its improvement of environmental adaptability [6, 8, 9].

In the present study, we aimed to investigate the damages of pyriproxyfen exposure on the midgut and to evaluate the relative expression levels of genes associated with various signaling pathways and the detoxification process. The elucidation of the responses of silkworms to pyriproxyfen will provide a conclusive value to prevent pesticide pollution in silkworms.

MATERIALS AND METHODS

Insect strains and chemicals

The silkworm strain Jingsong was preserved in Sericultural Research Institute, Chinese Academy

of Agricultural Sciences. The larvae were reared with mulberry leaves at 28 ± 1 °C with 85% relative humidity at the age from the 1st instar to the 3rd instar, and at 25 ± 1 °C with 70% relative humidity at the age from the 4th instar to the 5th instar [10].

Pyriproxyfen (analytical grade) was purchased from Sigma-Aldrich Company (Shanghai, China): IUPAC chemical name: 4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether, 95% purity. All the other general chemical reagents were purchased from Sangon Biotech (Shanghai) Co., Ltd.

Sample preparation

Pyriproxyfen was dissolved in acetone. To ensure the survival rate of the silkworm, the dose of pyriproxyfen was far lower than the recommended concentration in the field [11]. The stock solution was diluted with ddH₂O to a final concentration of 0.001 mg/l as a working solution [12]. Mulberry leaves were treated by an immersion method. Briefly, mulberry leaves were soaked in the working solution for 1 min and then dried naturally.

The silkworm larvae were fed normally with fresh mulberry leaves from the 1st instar to the 3rd day of the 5th instar. On the 4th day of the 5th instar, the larvae were divided into two groups: a control group and a treatment group. Each group consisted of three individual replicates of 40 larvae with a total of 120 larvae; and among them, two sub-groups of 60 larvae were used for statistical analysis and anatomical materials, respectively. In the control group, the larvae were fed with mulberry leaves treated with ddH₂O from the 4th day until mounting. In the treatment group, the larvae were fed with mulberry leaves treated with 0.001 mg/l pyriproxyfen until molting. After treatments, silkworms from both groups were dissected at 24 h intervals and the midgut tissues were collected.

Histopathological evaluation of midgut

Three midguts were randomly selected from each group and fixed in 4% formalin, and then embedded in paraffin. Sections (5 μm) were sliced from the paraffin blocks, fixed on the flakes, stained with hematoxylin-eosin, and then observed under optical microscopy (Nikon U-III Multipoint Sensor System, USA).

Measurement of detoxification enzyme activities

At 24 h and 48 h after pyriproxyfen exposure, 100 mg of midguts from the control and the treatment groups were isolated and separately placed in the grinding tubes. Subsequently, 1 ml of cell

lysis buffer and 10 μl of PMSF were added into each tube. When the tissues were fully ground and lysed, the samples were centrifuged, and the supernatants containing proteins were saved and stored at -80 °C until further processing. P450, GST, and CarE enzyme assay kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China). The detoxification enzyme activity was measured following the manufacturer's instructions, and the total protein levels were determined by the Bradford method.

Isolation of total RNA and RT-PCR

At 0, 24, 48, 72, and 96 h after pyriproxyfen exposure, the midguts of larvae were quickly dissected and collected. TRIzol reagent was used to extract total RNA from the midguts, followed by the DNase treatment to remove potential genomic DNA contamination. First-strand cDNA was synthesized with M-MLV Reverse Transcriptase and oligo (dT) primer following the manufacturer's instructions. All reagents were from Takara, Dalian, China except the primer was from Sangon, Shanghai, China. The quality of RNA was assessed by formaldehyde agarose gel electrophoresis, followed by spectrophotometric quantification.

Quantitative real-time PCR (qRT-PCR) analysis

The primers used for qRT-PCR (Table S1) were designed using primer (6.0) software and synthesized by Shanghai Biotechnology Co., Ltd. SYBR Prime Script™ RT-PCR kit was used for the determination on ABI Prism 7300 Fluorescence Quantitative PCR Instrument (Applied Biosystems, USA). The specific experimental steps were in accordance with the instructions, and the reaction system was 20 μl. The reaction procedure was: denaturation at 95 °C for 1 min; followed by 40 cycles of: 15 s at 95 °C and 31 s at 60 °C. All the control and treatment samples were measured, and the data were expressed by the mean \pm standard error of three independent experiments.

RESULTS

The pathological evaluation of midgut

Via microscopic observation of tissue sections, the pathological changes of the silkworm midgut at the 4th day of the 5th instar were further examined after being poisoned with 0.001 g/l pyriproxyfen. As shown in Fig. 1, in the control group, the midgut structure of the larvae was intact, indicating the good condition for cell growth. Compared with the

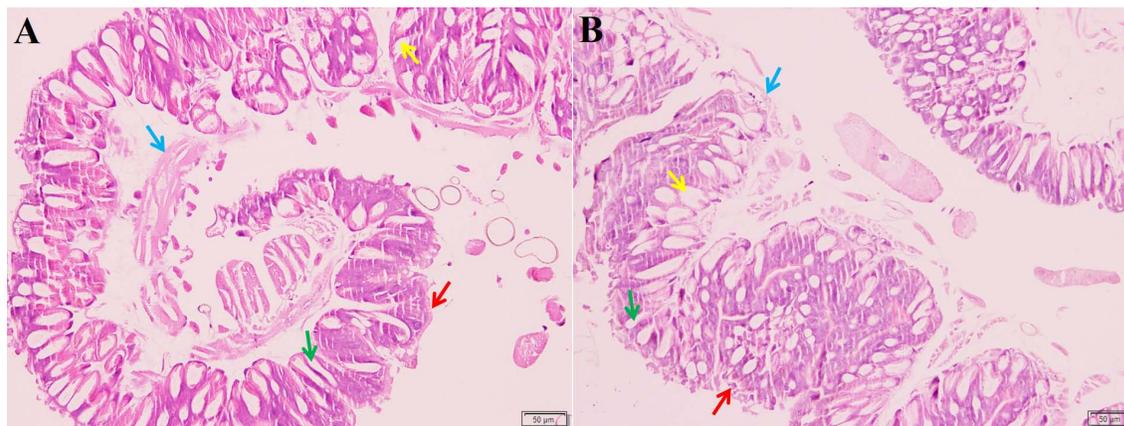


Fig. 1 Histopathology of the midgut in the 5th instar larvae of silkworm: (A) midgut from the control group; (B) midgut from the treatment group. The different arrow colours indicate: red, muscle layer; yellow, cylindrical cells; green, goblet cells; and blue, peritrophic matrix.

midgut wrapped by the outer muscle layer of the control group (Fig. 1A), the midgut muscle layer of the pyriproxyfen-fed larvae almost disappeared and shattered (Fig. 1B). In addition, the cylindrical cells and the goblet cells in the control group were intact (Fig. 1A), but the cells in the treatment group were severely damaged with blurred structure (Fig. 1B), which may be caused by the rupture of the cell membrane and the outflow of cell contents.

Owing to the protection of the peritrophic matrix of the midgut on the intestinal wall, serious lesions could be caused when the peritrophic matrix is attacked by foreign substances. Similarly, our study showed that the peritrophic matrix of the control group was relatively intact (Fig. 1A), while the peritrophic matrix of the treatment group was basically destroyed (Fig. 1B).

Changes in the expression of genes related to digestion after pyriproxyfen exposure

With various types of secreted enzymes, the midgut of the silkworm serves as the main organ of food digestion and nutrition absorption. As shown in Fig. 2, the overall expression level of genes related to digestion and absorption in the treatment group was lower than that in the control group. Specifically, the expression levels of α -amylase, Trypsin-like protease, and Lipase 1 in the treatment group were significantly decreased at 24 h (0.27, 0.32, and 0.94 fold, respectively) compared with the control group. At 48 h, the levels of α -amylase, Trypsin-like protease, and Lipase 1 in the treatment group were 0.98, 0.07, and 0.84 fold as much as that in the control group.

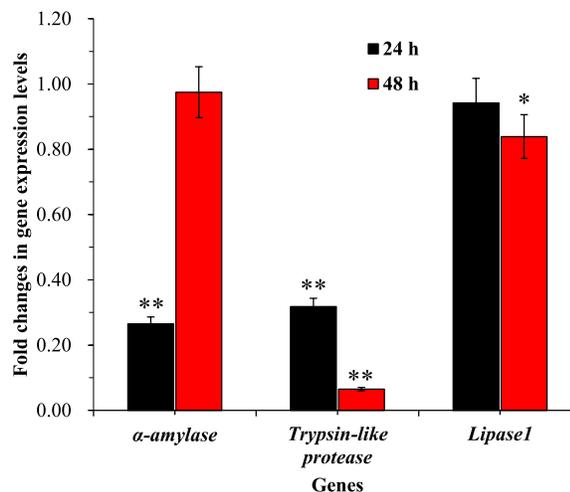


Fig. 2 Effects of pyriproxyfen exposure at 24 h and 48 h on expression levels of genes related to digestion and absorption in the midgut. Abscissa represents different genes, and ordinate represents fold changes in gene expression levels. The results are shown as the mean \pm S.E. Asterisks denote significant differences between the treatment and the control groups, as indicated by * $p \leq 0.05$, and ** $p \leq 0.01$.

Changes in the expression of genes related to oxidative phosphorylation after pyriproxyfen exposure

Oxidative phosphorylation is a process in which energy released by organic matter is used to produce ATP during oxidative degradation in the body. The expression levels of genes related to oxidative phosphorylation were all decreased after pyriproxyfen

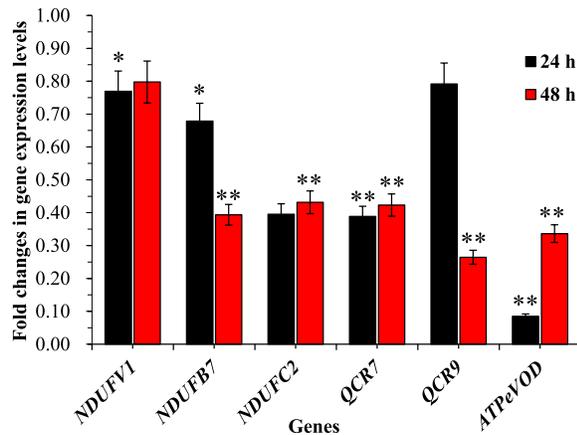


Fig. 3 Effects of pyriproxyfen exposure at 24 h and 48 h on expression levels of genes related to oxidative phosphorylation in the midgut. Abscissa represents different genes, and ordinate represents fold changes in gene expression levels. The results are shown as the mean \pm S.E. Asterisks denote significant differences between the treatment and the control groups, as indicated by * $p \leq 0.05$, and ** $p \leq 0.01$.

exposure. As shown in Fig. 3, the expression levels of *NDUFV 1*, *NDUFB 7*, *NDUFC 2*, *QCR 7*, *QCR 9*, and *ATPeVOD* in the treatment group were 0.77, 0.68, 0.40, 0.39, 0.79, and 0.09 times of those in the control group at 24 h after treatment, respectively; and the levels were 0.80, 0.39, 0.43, 0.42, 0.27, and 0.34 times at 48 h, respectively.

Changes in the expression of genes related to antioxidation after pyriproxyfen exposure

SOD, *GST*, *GPX*, and *CAT* are antioxidant-related genes in the midgut of silkworm. The expression levels of these genes were detected after pyriproxyfen exposure. As seen in Fig. 4, at 24 h after pyriproxyfen exposure, the expression levels of *SOD*, *GST*, *GPX*, and *CAT* genes in the treatment group were 0.92, 0.97, 0.99, and 0.31 times of those in the control group, respectively; and the level at 48 h after the exposure were 0.89, 0.61, 0.69, and 0.30 times of those in the control group, respectively. Hence, pyriproxyfen exposure decreased the expression levels of *SOD*, *GST*, *GPX*, and *CAT* with significant alteration at 48 h.

The effects of pyriproxyfen exposure on the expression of detoxification-related genes

The detoxification ability of detoxifying enzymes in silkworm reflects its resistance to the pesticide to some extent. In this study, qRT-PCR was

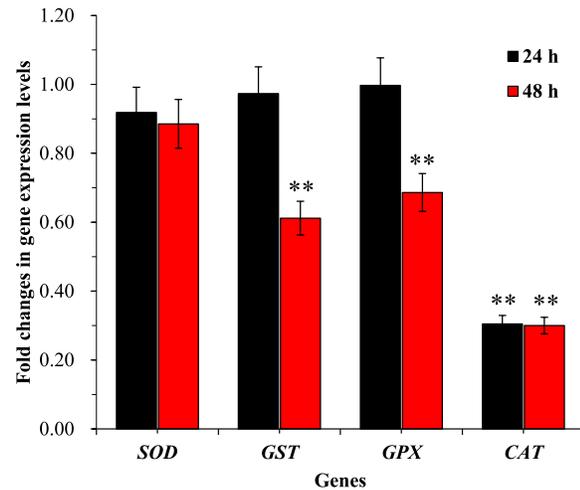


Fig. 4 Effects of pyriproxyfen exposure at 24 h and 48 h on expression levels of genes related to antioxidation in the midgut. Abscissa represents different genes, and ordinate represents fold changes in gene expression levels. The results are shown as the mean \pm S.E. Asterisks denote significant differences between the treatment and the control groups, as indicated by * $p \leq 0.05$, and ** $p \leq 0.01$.

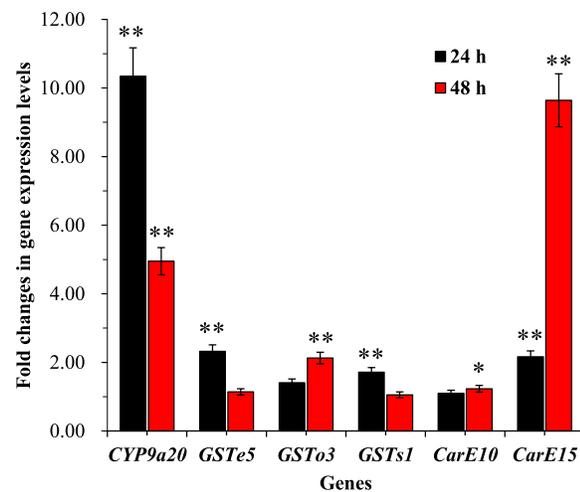


Fig. 5 Effects of pyriproxyfen exposure at 24 h and 48 h on the expression levels of detoxification enzymes-related genes in the midgut. Abscissa represents different genes, and ordinate represents fold changes in gene expression levels. The results are shown as the mean \pm S.E. Asterisks denote significant differences between the treatment and the control groups, as indicated by * $p \leq 0.05$, and ** $p \leq 0.01$.

used to determine the relative expression of some detoxification-related genes in the midgut of silkworm after pyriproxyfen exposure. It can be seen

that the relative expressions of these genes were increased significantly at both 24 h and 48 h. Compared with the control group, the expression levels of *CYP9a20*, *GSTe5*, *GSTo3*, *GSTs1*, *CarE10*, and *CarE15* were significantly increased at 24 h by 10.35, 2.33, 1.40, 1.71, 1.10, and 2.16 fold, respectively; and the levels were 4.95, 1.14, 2.13, 1.06, 1.23, and 9.64 times of those in the control group at 48 h, respectively (Fig. 5).

The activity of detoxification enzymes in the midgut

To examine the effects of pyriproxyfen exposure on detoxification enzymes in the midgut, the activities of the main enzymes including P450, GST, and CarE were determined after pyriproxyfen treatment (Table 1). Compared with the control group, the activities of P450 enzymes were significantly increased at 48, 72, and 96 h in the treatment group ($p \leq 0.05$). At 24, 48, 72, and 96 h after treatment, the activities of GST enzymes were significantly increased to 2.01, 1.61, 2.07, and 2.73 fold of those of the control group ($p \leq 0.01$), respectively. In addition, the CarE enzyme activity showed little changes at 24, 48, and 72 h after treatment, but it was increased by 1.19 fold at 96 h compared with the control group ($p \leq 0.05$).

DISCUSSION

Silkworm is easily influenced by diseases and hostile environments, which directly affect the yield and the quality of cocoons. The midgut is the main organ of food digestion and nutrient absorption with multiple secreted enzymes. Histopathology results in our study showed that the muscle layer, the cylindrical cells, the goblet cells, and the peritrophic matrix of the midgut were all damaged after pyriproxyfen exposure. It has been known that the cylindrical cells of the midgut are the main components of the epithelial layer that secrete digestive juice and absorb nutrients, while the goblet cells only secrete digestive juice. The peritrophic matrix is an important barrier to protect the midgut from poison [13]. When the silkworm is attacked by viruses and other exogenous substances, the peritrophic matrix will cause serious lesions, thus affecting the normal digestion and absorption of the silkworm. Accordingly, we speculated that the damage to the muscle layer, the cylindrical cells, the goblet cells, and the peritrophic matrix of the midgut could be one of the main reasons for the inhibition of digestion, absorption, and growth of silkworm after pyriproxyfen exposure.

Carbohydrate is the main energy source of life activities. As the catalyst of carbohydrate hydrolysis, amylase plays an important role in carbohydrate metabolism [14]. The starch and dextrin in mulberry leaves are broken down into glucose, the energy source of its life activities and material basis, under the action of α -amylase secreted by the midgut cells. Owing to this important role in carbohydrate metabolism, α -amylase is one of the most pivotal digestive enzymes in silkworms [15]. Our study showed that the relative expression of α -amylase in the treatment group was lower than that in the control group, suggesting the impacts of pyriproxyfen on the secretion of α -amylase by the midgut cells of silkworm and the metabolism of carbohydrates (Fig. 2). In the midgut of *Lepidoptera* larvae, trypsin participates in proteolysis that promotes the cleavage of the peptide chain [15–18], which is particularly essential for digestion. We also showed that the expression of *Trypsin-like protease* in the treatment group decreased compared with the control group, suggesting the regulation of protein hydrolysis and the digestion of the midgut. In 2003, Ponnuvel et al isolated *Lipase 1* from the digestive fluid of silkworm for the first time [19]. Given the specific expression of the *Lipase 1* gene in the midgut of the silkworm, it has been suggested that *Lipase 1* has the basic function of hydrolysis in the digestive tract of the silkworm, and plays an important role in the regulation of physiological function [20]. In this study, the relative expression of *Lipase 1* in the treatment group decreased significantly, indicating the effects of pyriproxyfen exposure on the digestion of nutrients and the regulation of physiological functions.

ROS is a byproduct of normal oxygen metabolism, which plays an important role in cell signal transduction. While maintaining the normal metabolism of the body, excessive ROS could damage the body to a certain degree [21, 22]. The oxidative phosphorylated electron transfer chain (ETC) in mitochondria is considered to be one of the main sources of ROS [23]. By detecting the isolated mitochondria, two ROS forming sites were located in complexes I and III [24]. NADH dehydrogenase (*NDUFC1*, *NDUFC2*, and *NDUFB7*) and cytochrome oxidase (*QCR7*, *QCR8*, and *QCR9*) are complexes I and III in the oxidative phosphorylation pathway, respectively [25, 26]. We found that the relative expressions of *NDUFC1*, *NDUFC2*, *NDUFB7*, *QCR7*, *QCR8*, and *QCR9* decreased at mRNA level after 24 h and 48 h of pyriproxyfen exposure. Therefore, we speculated that pyriproxyfen can inhibit the

Table 1 Detoxification enzyme activities in the midgut of *B. mori* exposed to pyriproxyfen.

Exposed time (h)	Enzyme activity (U/g prot)					
	P450		GST		CarE	
	Control	Pyriproxyfen	Control	Pyriproxyfen	Control	Pyriproxyfen
0	24.25 ± 1.15	24.71 ± 1.24	155.88 ± 8.74	165.06 ± 9.12	0.74 ± 0.03	0.75 ± 0.04
24	24.34 ± 1.65	25.52 ± 1.32	159.20 ± 9.89	320.45 ± 18.61**	0.76 ± 0.07	0.77 ± 0.02
48	24.47 ± 0.92	27.38 ± 1.66*	204.29 ± 10.72	329.07 ± 19.08**	0.76 ± 0.10	0.74 ± 0.08
72	24.49 ± 0.89	29.11 ± 1.58*	188.57 ± 9.55	390.01 ± 21.39**	0.73 ± 0.08	0.75 ± 0.05
96	26.08 ± 1.33	29.59 ± 1.90*	148.64 ± 7.39	405.72 ± 25.84**	0.81 ± 0.12	0.96 ± 0.14*

The experiments were performed in three replicates. The significance of differences is indicated by * $p \leq 0.05$, and ** $p \leq 0.01$.

activity of ETC complexes I and III and destroy the ETC, leading to the accumulation of ROS and subsequently causing a certain degree of damage to the body. Furthermore, we found that the expression of *ATPeVOD* at mRNA level decreased significantly at 24 h and 48 h after pyriproxyfen exposure. Because of the participation of ATPase in energy conversion [27], we speculated that digestive and absorption disorders in the midgut of silkworms could be associated with the inhibition of pyriproxyfen on ATP synthesis and energy metabolism.

When the active oxygen metabolism in an organism is under extreme environmental stresses such as bacteria, viruses, high temperature, low temperature, ultraviolet radiation, SO_2 in the air, etc., this imbalance will lead to the production of excessive ROS [28]. Particularly, the loss of ROS metabolism balance leads to damages of the oxidative and the immune systems. Therefore, the elimination of excessive reactive oxygen radicals in higher organisms is essential for them to resist the adverse environment and to improve their immunity. In the long-term evolution process, in order to avoid oxidative damages, organisms have formed a relatively complete protection system, the antioxidant system, to maintain the redox balance. They can adjust their metabolism in accordance with environmental changes to achieve the accordant heritability changes. Silkworm, as an important economic insect and a representative of *Lepidoptera*, is no exception [29]. In the present study, we found that the relative expressions of *SOD*, *CAT*, *GST*, and *GPX* in the treatment group at 24 h and 48 h were lower than those in the control group. As an important antioxidant enzyme, superoxide dismutase (*SOD*) is the only enzyme that directly metabolizes superoxide ROS [30]. *SOD* is a member of the antioxidant system for ROS removal. It con-

verts oxygen anion to H_2O_2 , which is then removed by *CAT* [31]. *CAT* plays an important role in cell senescence and apoptosis, and its role in metabolism has attracted more attention, especially in the physiological functions of insects. Many studies have found that *CAT* enhances the resistance of insects. *GST* can remove H_2O_2 and organic peroxides. *GPX* can use reduced glutathione or AOS (activated oxygen species) as an electron donor to catalyze the reduction of H_2O_2 or organic peroxide to water and corresponding alcohol [32]. In the present study, we found that the relative expressions of *SOD*, *CAT*, *GST* and *GPX* in the treatment group at 24 h and 48 h were lower than those in the control group. We thus speculated that the antioxidant defense system in the midgut of the silkworm could be destroyed by pyriproxyfen, which will cause oxidative damage and disruption of immune system.

In order to survive, organisms must have certain physiological, biochemical, and even behavioral adaptabilities [33]. The main and common adaptive mechanism of detoxification in organisms is the decomposition of metabolites or heterologous substances through the detoxifying enzyme system [34]. The insect detoxifying enzyme system is rather heterogeneous, which can metabolize a large number of endogenous or exogenous substrates [35]. The change of detoxification-related genes in insects at the transcription level leads to the change of the enzyme activities, thus changing the metabolic detoxification ability of the insect to pesticides. In this study, the activities of detoxifying enzymes and the expression levels of the related genes were measured. The results showed that the expression level of *CYP9a20* was significantly up-regulated at 24 h and 48 h. Consistently, P450 enzyme activity was also increased compared with the control group, indicating its critical role in the midgut detoxification. In addition, at 24 h and 48 h,

the transcription levels of *GSTe5*, *GSTo3*, and *GSTs1* were up-regulated. This trend of gene expression was in line with the significantly increased activity of the GST enzyme, which also played a major role in the midgut detoxification process after pyriproxyfen exposure. In addition, the transcription levels of *CarE10* and *CarE15* increased at 24 h and 48 h after pyriproxyfen exposure, while the enzyme activity of CarE increased significantly only at 96 h after exposure. In general, the exposure of pyriproxyfen can significantly affect the activity of detoxifying enzymes and the expression of related genes in the midgut of the silkworm, indicating its considerable role in the midgut detoxification of silkworm.

CONCLUSION

Low dose of pyriproxyfen at 0.001 mg/l is toxic to silkworm, inducing the inhibition of digestive absorption, energy metabolism, and antioxidant enzyme systems in the insect midgut. In addition, we found that the activity of detoxifying enzymes and the expression of the related genes increased, suggesting the enhanced detoxification ability of the silkworm midgut by pyriproxyfen, as well as the resistance of silkworm to pyriproxyfen. In general, our study revealed the response of the silkworm midgut to pyriproxyfen, providing a conclusive reference to understand the insect's metabolic mechanism.

Appendix A: Supplementary data

Supplementary data associated with this article can be found at <http://dx.doi.org/10.2306/scienceasia1513-1874.2021.108>.

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Appendix A. Supplementary data

Table S1 Primer sequences for qRT-PCR.

Gene name	Accession No.	Primer sequence (5'-3')	Produce size (bp)
<i>Actin3</i>	NM_001126254.1	F: CGGCTACTCGTTCACTACC R: CCGTCGGGAAGTTCGTAAG	147
<i>α-amylase</i>	NM_001173153.1	F: ATCCTGCTGTTCCCTACGGC R: TGTCAGGGCAGCAGTTGTAG	178
<i>Trypsin-like protease</i>	NM_001046885.1	F: GGTCCATCAAGTGAGCCGAT R: AATGACCACTACCGAGCCAC	212
<i>Lipase1</i>	XM_004932287.2	F: ACTTCTTCGTTCTCCCTCGC R: CGGGTATGACGAAAGCGTTG	167
<i>NDUFV1</i>	XM_004934174.2	F: ATGGAGGTTGAAGGGTGCTC R: ACGATCCAGTCGGTTCCTTTC	168
<i>NDUFB7</i>	XM_004926400.2	F: GCTTCTTATCGATGCGCCC R: GCGGACGTTCCCGTTCAAAT	178
<i>NDUFC2</i>	XM_004926501.2	F: TACGTTCCGGTATTGGCACAGG R: TTGGATAACCGCTGAACACTGG	167
<i>QCR7</i>	NM_001045492.1	F: GGGCCTACAATCTTTCGGGA R: AAGTCTGCGGAGTGCTTCAG	169
<i>QCR8</i>	XM_004927834.2	F: CCCGCCCTTCATCATGGAT R: ATCAGCAGGGTCTTCCGTG	167
<i>QCR9</i>	XM_004928783.2	F: GACCAGTTTCGAGCTTTGACG R: AGACCACACGGACATTTTGA	170
<i>ATPeVOD</i>	NM_001046964.1	F: CCGAGTTGAACGGGCGTAAA R: AAGCCCTCCAAATACCCAGC	167
<i>SOD</i>	NM_001043619.1	F: TCCTCAAGCACCTACACC R: GATAGCCAATGATACACG	216
<i>GST</i>	NM_001043509.2	F: TTGAACCCTCAACACACGGT R: GCCTTTGGCGTACTTGTTC	102
<i>GPX</i>	NM_001043534.1	F: CTGGTCAAGAGCCTGGCAATCC R: GCCGTGGCGTTCAACTGGAA	221
<i>CAT</i>	NM_001043447.1	F: AAACGGTGGGCAAGAATGGT R: TCGTTCTGGAATACGCTCCC	92
<i>CYP9a20</i>	EF421989	F: CTCGGATTCTTCCACCAG R: TGTGGACAGACGATGACTTGG	144
<i>GSTe5</i>	NM_001114992.1	F: GCGGCGAGAAAGAAATACG R: CTGATGGAAGTAACGCAGCAA	139
<i>GSTb3</i>	NM_001046970.1	F: CTCCGACACTGTCAATGAGGA R: CTCGAACCAAGGCCATATCAT	145
<i>GSTs1</i>	NM_001043612.1	F: GACATGGGGTGATTTTCGTG R: AGCCTTCACTTTGGGCTGT	136
<i>CarE10</i>	EU523535	F: ACAGAGTGCTGGAGGAAC R: CAGATTGGCTGACGATTA	129
<i>CarE15</i>	EU727141.1	F: ACTTCGCGTGAATTTTCGT R: TATTTGCCTACCGCCTCT	142