In vitro Inhibition of Glutathione S-transferases by Several Insecticides and Allelochemicals in Cotton Bollworm, *Helicoverpa armigera* Hübner[1]

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J. Entomol. Sci. 42(2): 296-305 (April 2007)

Abstract Glutathione S-transferases are a group of enzymes catalyzing the conjugation of reduced glutathione (GSH) with a wide range of xenobiotics bearing electrophilic sites. In insects, GSTs are involved in resistance to insecticides and allelochemicals. In vitro inhibitory effects of several insecticides and allelochemicals on glutathione S-transferases activity in the cotton bollworm, *Helicoverpa armigera* Hübner, were studied. All organophosphate, carbamate and pyrethroid insecticides tested demonstrated moderate or low inhibiting GST activity toward 1-chlorine-2, 4-ditrobenzo (CDNB). Three allelochemicals (quercetin, tannic acid and rutin) were the most potent inhibitors of the enzymes among all compounds tested. Tannic acid was competitive with CDNB, quercetin was noncompetitive, and rutin was neither competitive nor noncompetitive.

Key Words glutathione S-transferase, *Helicoverpa armigera,* allelochemicals, insecticides, inhibition

Glutathione S-transferases (GSTs, EC2.5.1.18) are a group of enzymes that catalyze the conjugation of reduced glutathione (GSH) with a wide range of toxicants bearing electrophilic sites (Habig et al. 1974). Conjugation increases the solubility of target molecules, thus facilitating their excretion of the molecules from organism. GSTs have been implicated in the development of resistance of cells and organisms toward drugs, insecticides, herbicides and antibiotics, and hence have been the subject of intense research (Wilce et al. 1995). In insects, it has been suggested that increased expression of GSTs contributes to resistance to a wide variety of insecticides and plant allelochemicals (Das et al. 1984, Reidy et al. 1990, Ku et al. 1994, Yu 1999, Kostaropoulos et al. 2001a).

Cotton bollworm, *Helicoverpa armigera* Hübner, is an economically important pest of cotton and other crops worldwide, and insecticides are widely used for its control. This pest has developed resistance to almost all classes of insecticides, such as pyrethroids (McCaffery et al. 1989, Martin et al. 2002), organophosphates (Wu et al. 1997, Ahmad et al. 1999) and carbamates (Ahmad et al. 2001). Elevated GST activity has been reported from resistant strains of cotton bollworm to organophosphates (McCaffery et al. 1997, Tang et al. 2000), carbamates (Ahmad et al. 2001, Buès et al. 2005), endosulphan (Rajurkar et al. 2003), and pyrethroids (Martin et al. 2002). In our

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laboratory, we previously studied the induction of GSTs in *H. armigera* by some allelochemicals and its relationship with insecticide resistance (Dong et al. 1998a, b, Gao et al. 1997, Gao et al. 1999, Chen et al. 2003). To better understand the interaction of GSTs with insecticides and allelochemicals, we studied herein their in vitro inhibitory effects on 1-chlorine-2, 4-ditrobenzo (CDNB)-conjugating activity of GSTs in the cotton bollworm. Another purpose of this study is to find some potential GST inhibitors which might be used as synergists to enhance the toxicity of insecticides.

Materials and Methods

Chemicals. 1-chloro-2, 4-dinitrobenzene (CDNB, ≥99%) and reduced glutathione (GSH, ≥99%) and tannic acid (>99%) were purchased from Sigma Chemical Co. (St. Louis, MO), phenylmethylsulfonyl (PMSF, >99%) from Merk (Darmstadt, Germany), dithiothreitol (DTT, 99%) from Promega (Madison, WI), rutin (97%) and quercetin (99%) from Beijing Chemical Co. (Beijing, China), and from Shanghai chemical stock (Shanghai, China). All other chemicals were of analytical grade and purchased from commercial sources.

The insecticide phoxim (99.0%) was purchased from Tianjin Pesticide Industry, methyl-parathion (93.0%) and parathion (98.0%) from Jiangsu Chemical and Pesticide Group, malathion (92.3%) from Liaoning Huludao Pesticide Industry, methomyl (99.0%) from Shandong Jining Experimental Chemical Industry, deltamethrin (98.0%) from Zhejiang Wei'erda Chemical LTD, and alphamethrin (95.2%) from Tianjin Longdeng Chemical Industry. Allelochemicals and insecticides were dissolved in absolute ethanol.

Insects. The cotton bollworms were initially collected from Handan, Hebei province, China, and then reared on artificial diet (Liang et al. 1999) without insecticide selection for more than 60 generations. They were maintained at 26 ± 1°C, 75% RH and a 16:8 LD photoperiod.

Preparation of cytosolic fractions. Sixth-instar larvae of *H. armigera* were dissected and their midguts and fat bodies removed and placed on ice. Peritrophic membranes associated midgut contents were removed. The midguts were washed in ice-cold KCl (1.15% W/V). Midguts and fat bodies were homogenized separately in sodium phosphate buffer (pH 6.5, 0.1 M) containing EDTA (1 mM), PMSF (1 mM, dissolved in absolute alcohol) and DTT (1 mM). The homogenate was centrifuged at 10,000 g for 20 min at 4°C, and the supernatant was filtered through 3 layers of cellulose filter paper grade 3 (Whatman, Middlesex, UK). The filtrate was centrifuged at 105,000 g for 60 min at 4°C. The lipid layer of the supernatant fraction was removed by filtration with filter paper.

Assay for glutathione S-transferase activity. The glutathione S-transferase activity using CDNB as the substrate was assayed spectrophotometrically according to Habig et al. (1976). The assay mixture contained 1 mM CDNB and 1 mM GSH. The assay was initiated by the addition of 20 µL enzyme; the absorbance at 340 nm was monitored for 1 min. Controls without enzyme always accompanied each assay. Activity was calculated with an extinction coefficient of 9.6 mM/cm for CDNB. Enzyme activity was expressed as nmol/min at 25°C, and the specific activity as nmol/min/mg protein using an extinction coefficient of 9.6 $mm^{-1}cm^{-1}$. The method of Bradford (1976), with BSA as a standard, was used for protein quantitation.

Inhibition of insecticides and allelochemicals on GSTs. Inhibition of CDNB-conjugating activity of GSTs was determined in assays containing 1 mM CDNB and

1 mM GSH and various insecticides (0.1 mM and/or 0.5 mM) or allelochemicals (0.1 mM) as inhibitors. All assays, including controls, contained 0.6% ethanol. All assays were run in triplicate.

Dose-dependent inhibition of CDNB-conjugation was studied with fixed concentrations of 1 mM CDNB and 1 mM GSH by adding to the incubating reaction mixtures 10 µL of allelochemicals dissolved in absolute ethanol at various concentrations. The I_{50} values, concentrations of inhibitors required to reduce the reaction rate by 50%, were determined by linear regression of the average percent activity on the log of the inhibitor concentration (Neal and Berenbaum 1989). The experiment was performed in triplicate for each inhibitor.

Kinetics of GST inhibition by allelochemicals. Kinetics of GST inhibition by tannic acid, quercetin and rutin were determined in assays containing various concentrations of CDNB and a fixed concentration of each inhibitor. The GSH concentration was kept constant at 1 mM. The results were presented on double-reciprocal Lineweaver-Burk plots. The inhibitory constant, K_i, the equilibrium constant for dissociation of the enzyme-inhibitor complex, was determined from plots of reciprocal velocity versus reciprocal concentration (Lineweaver-Burk plots) (Dixon and Webb 1979). The Michaelis constant, K_m, and the maximum velocity, V_{max}, also were determined from these plots.

Results

The inhibition of GST activity toward CDNB by several insecticides and allelochemicals is shown in Table 1. Three allelochemicals (tannic acid, quercetin and rutin) were the most potent inhibitors tested, inhibiting more than 75% of GST activity at a final concentration of 0.1 mM. The inhibitory effects of insecticides tested were much lower than these three allelochemicals. They produced <40% inhibition of GST activity at a concentration of 0.1 mM whereas pyrethroids were noninhibitory at this concentration. When the concentration of insecticides increased to 0.5 mM, the inhibition rates were all increased accordingly. Among the insecticides tested, organophosphates and one carbamate were moderate inhibitory and two pyrethroids were the least inhibitory.

The sensitivities of GSTs to the allelochemicals (tannic acid, quercetin and rutin) were also investigated and are expressed as I_{50} values. Tannic acid, quercetin and rutin inhibited GST activity to CDNB in vitro in a dose-dependent manner (Fig. 1). The I_{50} values of these three allelochemicals for GST activity in midguts and fat bodies are summarized in Table 2, with I_{50} values ranging from 1.18 µM to 13.8 µM, tannic acid being the most potent inhibitor. Tannic acid inhibited GST activity in the midguts and in the fat bodies to a similar degree, but quercetin and rutin inhibited to different degrees. This suggests that glutathione S-transferases in the two tissues are qualitatively different in isozyme composition and thus different in sensitivity to quercetin and rutin.

Kinetic analyze of GST inhibition by tannic acid, quercetin and rutin are shown in Fig. 2 and Table 3. Tannic acid inhibited GST activity in the midguts in a competitive manner to CDNB with K_i of 6.25×10^{-4} mM. The V_{max} values (833.3 nmol/mg pro./min) for GST toward CDNB was not significantly changed after inhibition by tannic acid. Quercetin was noncompetitive with CDNB with K_i of 9.38×10^{-4} mM. The K_m value for GST toward CDNB was 0.33 mM, and was not significantly changed by quercetin. Unlike tannic acid and quercetin, rutin showed neither typical competitive

Table 1. Inhibition of GSTs by several insecticides and allelochemicals in *H. armigera* larvae

Inhibitors	Tissues	% Inhibition (means ± SD) 0.1 mM	0.5 mM
Organophosphate			
Parathion	Midgut	25.03 ± 1.50	79.05 ± 1.51
	Fat body	24.49 ± 3.62	85.69 ± 6.23
Methyl-parathion	Midgut	28.66 ± 0.98	46.17 ± 4.04
	Fat body	22.15 ± 5.76	44.56 ± 1.77
Phoxim	Midgut	33.36 ± 2.77	80.73 ± 7.02
	Fat body	22.42 ± 1.17	80.27 ± 1.53
Malathion	Midgut	28.71 ± 0.57	49.34 ± 1.05
	Fat body	30.28 ± 3.06	33.43 ± 1.72
Carbamate			
Methomyl	Midgut	25.97 ± 1.05	42.75 ± 3.87
	Fat body	24.27 ± 3.79	43.5 ± 4.93
Pyrethriod			
Alphamethrin	Midgut	<5	29.48 ± 4.78
	Fat body	<5	29.77 ± 0.53
Deltamethrin	Midgut	6.72 ± 0.04	13.59 ± 3.02
	Fat body	<5	14.64 ± 6.10
Allelochemical			
Quercetin	Midgut	84.59 ± 2.42	
	Fat body	88.12 ± 7.06	
Tannic acid	Midgut	95.61 ± 5.10	
	Fat body	93.83 ± 3.63	
Rutin	Midgut	75.48 ± 2.52	
	Fat body	77.28 ± 6.46	

nor noncompetitive inhibition to CDNB, with K_i of 9.72×10^{-4} mM. The V_{max} value was decreased and K_m value was increased by rutin.

Discussion

In insects, GSTs are implicated in resistance to a variety of insecticides including organophosphates, organochlorines and cyclodienes, plus allelochemicals and other xenobiotics (Prapanthadara et al. 2000, Kostaropoulos et al. 2001a). More recently,

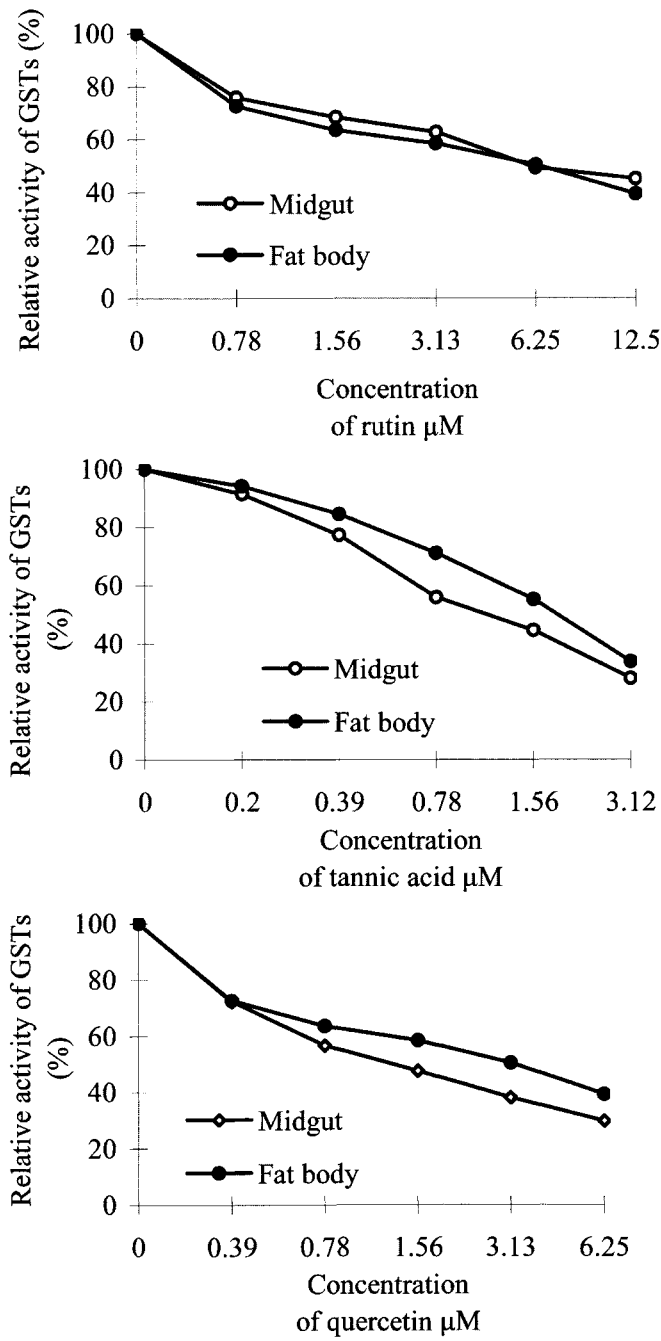

Fig. 1. *In vitro* inhibition of GSTs toward CDNB by three allelochemicals in *H. armigera* larvae. Note differences in scales.

Table 2. I_{50} values of allelochemicals for GST activity toward CDNB in *H. armigera* larvae

Allelochemicals	I_{50} μM (means ± SD)	
	Midguts	Fat bodies
Tannic acid	1.18 ± 0.3 a A	1.77 ± 0.5 a A
Quercetin	2.21 ± 0.62 a A	13.8 ± 6.22 b B
Rutin	7.34 ± 1.00 b A	5.46 ± 0.32 c B

The data were analyzed using analysis of variance (ANOVA). The difference is significant if $P < 0.05$.
Means within a column followed by the same small letter are not significantly different.
Means within a row followed by the same capital letter are not significantly different.

GST involvement in pyrethroids resistance in insects has been reported and its mechanisms have been suggested (Vontas et al. 2001, Kostaropoulos et al. 2001b).

In vitro inhibition studies are useful for understanding the biotransformation of exogenerous compounds mediated by GSTs. There are several reports on the *in vitro* effects of insecticides on GST activity in insects. For example, recombinant GST in *Anopheles dirus* was inhibited by several organochlorine and organophosphorous insecticides, but not by carbamates (Prapanthadara et al. 1998). Methyl-parathione and malathion competitively inhibited GST CDNB conjugating activity in *Tenebrio molitor* L. (Kostaropoulos et al. 2001b). Although it was generally accepted that pyrethroids were not the substrate of glutathione S-transferase, it has been reported that they bind with GST or the active site of the enzyme and, thus, competitively inhibited GST activity toward CDNB (Kostaropoulos et al. 2001a).

In the present study, we found that *in vitro* all insecticides tested inhibited GST activity from the midguts and fat bodies of cotton bollworm at the concentration of 0.5 mM. Organophosphorous and carbamate insecticides were moderately inhibitory to GST activity but pyrethroids were less so. All showed dose-dependent inhibtory effects. At 0.1 mM, pyrethroids (alphamethrin and deltamethrin) were noninhibitory, and all the other insecticides showed even less inhibition. Compared with previous reports, the inhibitory effects of insecticides studied here were very low. For example, Kostaropoulos et al. (2001a, b) showed that methyl-parathion, malathion and deltamethrin (at 1-20 μM) competitively inhibited CDNB conjugation activity of GST in *T. molitor*. In the preliminary experiment, we determined the inhibitory effects of all the insecticides at the concentration of 10 μM, and no apparent inhibitory effects (≤5%) were found. Prapanthadara et al. (2000) also reported that insecticides they tested had no inhibitory effects on one isoenzyme GST 1-6 in *Anophele dirus*. These results suggest that isoenzymes of GSTs may affect in susceptibility to insecticides.

In our laboratory, we have also studied the metabolism of insecticides by GSTs in *H. armigera*, demonstrating apparent metabolism of one organophosphate (methyl-parathion) and one carbamate (methomyl), but no metabolism of one pyrethroid (deltamethrin) (unpubli. data). Metabolism and inhibition studies suggest that organophosphates and carbamates are important substrates for GSTs, and pyrethroids can bind with GSTs but are not metabolized by these enzymes. Results of in vitro inhibition studies are consistent with the reports that GSTs in *H. armigera* play a role in

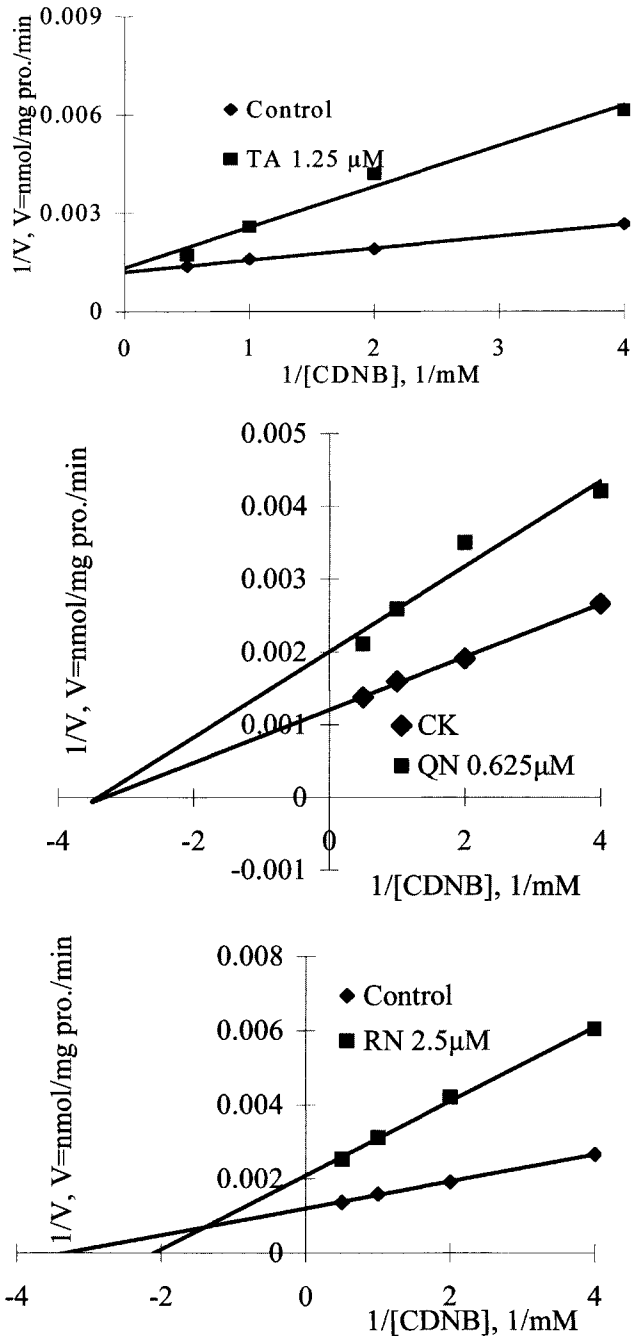

Fig. 2. Kinetics of GST inhibition by three allelochemicals in *H. armigera* larvae QN: quercetin; TA: tannic acid and RN: rutin.

Table 3. Inhibition kinetics parameters of GSTs by three allelochemicals in *H. armigera* larvae

Allelochemicals	K_i mM	K_m mM	V_{max} nmol/mg pro./min
Control	—	0.33	833.3
Tannic acid	6.25×10^{-4}	0.92	769.2
Quercetin	9.38×10^{-4}	0.3	500
Rutin	9.72×10^{-4}	0.48	496.2

The values were determined by Lineweaver-Burk plots. The GSH concentration was kept constant at 1 mM.

resistance to organophosphates, carbamates and even pyrethroids. Therefore, *in vitro* inhibition is a very useful method for studying the metabolism of xenobiotics catalyzed by GSTs and the GST involvement in resistance.

There are several reports that naturally-occurring polyphenols are potent inhibitors of GST activity in insects (Wadleigh and Yu 1987, 1988, Yu and Abo-Elghar 2000). Tannic acid, quercetin and rutin are plant phenols and potent inhibition was observed in our study. Our kinetic analyze indicated that tannic acid competitively inhibited CDNB conjugation activity of GST in the cotton bollworm, in contrast to that reported in the fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Yu and Abo-Elghar 2000) and in rat liver (Zhang and Das 1994). The noncompetitive inhibition by quercetin and the competitive inhibition by tannic acid that we observed herein also differed from results of Lee (1991) and Yu and Abo-Elghar (2000), respectively. These results indicate that inhibitors have different reaction modes to different isoenzymes. Even for isoenzymes very similar in structure, inhibition mechanisms may differ significantly (Jirajaroenrat et al. 2001).

Yu and Abo-Elghar (2000) suggested that some allelochemicals found in certain foliage might serve as synergists by interfering with GST-mediated detoxification in phytophagous insects. In this study, we found that tannic acid was the most effective inhibitor of GSTs in *H. armigera*. The high *in vitro* inhibitory potency of tannic acid for GSTs have been reported in insects (Yu and Abo-Elghar 2000) as well as in mammals (Zhang and Das 1994, Gyam et al. 2004). However, the application of tannic acid as synergist of insecticides is scarely reported, which needs more study on its *in vivo* effects on GSTs and/or other degradation enzymes and its interaction with insecticides *in vivo*. Previously, we reported that tannic acid *in vivo* had some inhibitory effects on GSTs activity in *H. armigera* and strains feeds on tannic acid showed more susceptibility to deltamethrin (Chen et al. 2003). These results showed that tannic acid had the potential of serving as a synergist for enhancing the toxicity of insecticides in cotton bollworm. However, its application as a synergist in insect control requires further investigation.

Acknowledgments

This research was supported by National Basic Research Program of China (Contract No. 2006CB10200) and National Natural Science Foundation of China (Contract No. 30530530, 30471153 and 30170621).

References Cited

Ahmad, M., M. I. Arif and Z. Ahmad. 1999. Patterns of resistance to organophosphate insecticides in field populations of *Helicoverpa armigera* in Pakistan. Pestic. Sci. 55: 626-632.

2001. Resistance to carbamate insecticides in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. Crop Prot. 20: 427-432.

Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein–dye binding. Ann. Biochem. 72: 248-254.

Buès, R., J. C. Bouvier and L. Boudinhon. 2005. Insecticide resistance and mechanisms of resistance to selected strains of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in the south of France. Crop Prot. 24: 814-820.

Chen, F.-J., X.-W. Gao, M.-Q. Lei and B.-Z. Zheng. 2003. Effect of tannic acid on glutathione S-transferases in cotton bollworm *Helicoverpa armigera.* Entomol. Sin. 46: 684-690.

Das, M., D. R. Bickers and H. Mukhtar. 1984. Plant phenols as *in vitro* inhibitors of glutathione S-transferase(s). Biochem. Biophys. Res. Commun. 120: 427-433.

Dixon, M. and E. C. Webb. 1979. Enzyme inhibition and activation, pp. 332-467. *In* M. Dixon and E. C. Webb (eds.), Enzymes. Academic Press, New York, NY.

Dong, X.-L., X.-W. Gao and B.-Z. Zheng. 1998a. Effects of plant allelochemicals on the glutathione S- transferse and acetylcholinesterase in *Helicoverpa armigera.* Acta Phytophy. Sin. 25: 72-78.

1998b. The effects of plant allelochemicals on the insecticide tolerance in cotton bollworm *Helicoverpa armigera* Hübner. Acta Entom. Sin. 43: 111-116.

Gao, X.-W., X.-L. Dong, B.-Z. Zheng and Q. Chen. 1997. Glutathione S-transferases (GSTs) in cotton bollworms: induction by insecticides and plant allelochemicals and metabolism of insecticides. Acta Entom. Sin. 40: 122-127.

1999. Induction of carboxylesterase, glutathione S-transferase and acetylcholinesterase by quercetin in *Helicoverpa armigera.* Chin. J. Pestic. Sci. 1: 56-60.

Gyam, M. A., I. I. Ohtani, E. Shinno and Y. Aniya. 2004. Inhibition of glutathione S-transferases by thonningianin A,isolated from the African medicinal herb, *Thonningia sanguinea, in vitro.* Food Chem. Toxicol. 42: 1401-1408.

Habig, W. H., M. J. Pabst and W. B. Jakoby. 1974. Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249: 7130-7139.

1976. Glutathione S-transferase AA from rat liver. Arch. Biochem. Biophys. 175: 710-716.

Jirajaroenrat, K., S. Pongjaroenkit, C. Krittanai, L. Prapanthadara and A. J. Ketterman. 2001. Heterologous expression and characterization of alternatively spliced glutathione S-transferases from a single Anopheles gene. Insect Biochem. Mol. Biol. 31: 867-875.

Kostaropoulos, I., A. I. Papadopoulos, A. Metaxakis, E. Boukouvala and E. Papadopoulou-Mourkidou. 2001a. Glutathione S-transferase in the defense against pyrethroid insecticides. Insect Mol. Biol. 31: 313-319.

2001b. The role of glutathione S-transferases in the detoxification of some organophosphorus insecticides in larvae and pupae of the yellow mealworm, *Tenebrio molitor* (Coleoptera: Tenebrionidae). Pest Manag. Sci. 57: 501-508.

Ku, C.-C., F.-M. Chiang, C.-Y. Hsin, Y.-E. Yao and C.-N. Sun. 1994. Glutathione transferase isozymes involved in insecticide resistance of diamondback moth larvae. Pestic. Biochem. Physiol. 50: 191-197.

Lee, K. 1991. Glutathione S-transferase activities in phytophagous insects: induction and inhibition by plant phototoxins and phenols. Insect Biochem. 21: 353-361.

Liang, G.-M., W.-J. Tang and Y.-Y. Guo. 1999. The technological improvement of artificial diet of cotton bollworm. Plant Prot. 25: 16-18.

Martin, T., F. Chandre, O. G. Ochou, M. Vaissayre and D. Fournier. 2002. Pyrethroid resistance mechanisms in the cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae) from West Africa. Pestic. Biochem. Physiol. 74: 17-26.

McCaffery, A. R., A. B. S. King, A. J. Walker and H. El-Nayir. 1989. Resistance to synthetic

pyrethroids in the bollworm, *Heliothis armigera* from Andhra Pradesh, India. Pestic. Sci. 27: 65-76.

McCaffery, A. R., D. J. Head, J.-G. Tan, A. A. Dubbledam, V. R. Subramaniam and A. Callaghan. 1997. Nerve in sensitivity resistance to pyrethroids in heliothine Lepidoptera. Pestic. Sci. 51: 315-320.

Neal, J. and M. Berenbaum. 1989. Decreased sensitivity of mixed-function oxidases from *Papilio polyxenes* to inhibitors from host plants. J. Chem. Ecol. 15: 439-446.

Prapanthadara, L., H. Hanson, P. Somboon and J. Hemingway. 1998. Cloning, expression and characterization of insect class I glutathione S-transferase from *Anopheles dirus* species B. Insect Biochem. Mol. Biol. 28: 321-329.

Prapanthadara, L., N. Promter, S. Koottathep, P. Somboon and A. J. Ketterman. 2000. Isoenzymes of glutathione S-transferases from the mosquito *Anopheles dirus* species B: the purification, partial characterization and interaction with various insecticides. Insect Biochem. Mol. Biol. 30: 395-403.

Rajurkar, R. B., Z. H. Khan and G. T. Gujar. 2003. Studies on levels of glutathione S-transferase, its isolation and purification from *Helicoverpa armigera*. http://www.iisc.ernet.in/currsci/nov102003/1355.pdf

Reidy, G. F., H. A. Rose, S. Visetson and M. Murray. 1990. Increased glutathione S-transferase activity and glutathione content in an insecticide-resistant strain of *Tribolium castaneum* (Herbst). Pestic. Biochem. Physiol. 36: 269-276.

Tang, F., Y.-D. Yue and R.-M. Hua. 2000. The relationships among MFO, glutathione S-transferases, and phoxim resistance in *Helicoverpa armigera*. Pestic. Biochem. Physiol. 68: 96-101.

Vontas, J. G., G. J. Small and J. Hemingway. 2001. Glutathione S-transferases as antioxidant defence agents confer pyrethroid resistance in *Nilaparvata lugens*. Biochem. J. 357: 65-72.

Wadleigh, R. W. and S. J. Yu. 1987. Glutathione transferase activity of fall armyworm larvae toward a, β-unsaturated carbonyl allelochemicals and its induction by allelochemicals. Insect Biochem. 17: 759-764.

1988. Detoxification of iso-thiocyanate allelochemicals by glutathione S-transferase in three lepidopterous species. J. Chem. Ecol. 14: 1279-1288.

Wilce, M. C. J., P. G. Board, S. C. Feil and M. W. Parker. 1995. Crystal structure of a theta-class glutathione transferase. EMBO J. 14: 2133-2143.

Wu, K.-M., G.-M. Liang and Y.-Y. Guo. 1997. Phoxim resistance in *Helicoverpa armigera* in China. J. Econ. Entomol. 90: 68-72.

Yu, S. J. 1999. Induction of new glutathione S–transferase isozymes by allelochemicals in the fall armyworm. Pestic. Biochem. Physiol. 63: 163-171.

Yu, S. J. and G. E. Abo-Elghar. 2000. Allelochemicals as inhibitors of glutathione S-transferases in the fall armyworm. Pestic. Biochem. Physiol. 68: 173-183.

Zhang, K. and N. P. Das. 1994. Inhibitory effects of plant polyphenols on rat liver glutathione S-transferases. Biochem. Pharmacol. 47: 2063-2068.