Pak. J. Bot., 42(6): 3971-3979, 2010.

IMPACT OF TWO MEDICINAL PLANT EXTRACTS ON GLUTATHIONE S-TRANSFERASE ACTIVITY IN THE BODY TISSUES OF *SPODOPTERA EXIGUA* (LEPIDOPTERA: NOCTUIDAE)

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Abstract

The plant extracts from Zygophyllacae and Euphorbiaceae are well known in the human history for medicinal use but their use to control insect pests is still in preliminary stage. In this study the effects of extracts from *Peganum harmala* and *Ricinus communis* on the glutathione S-transferase activity in the fat body as well as midgut tissues of *Spodoptera exigua* are documented at different exposure times. At the sub lethal concentrations (0.24 and0.49 mg ml^{-1}) the highest GST value in fat body tissue (73 nmol CDNB/min/mg protein) was found on the 8^{th} day of combined treatment with ricinine and harmaline while in midgut the highest value (69 nmol CDNB/min/mg protein) was noticed on 6^{th} day after being treated with ricinine individually. At the lethal concentrations (0.37 and 0.71 mg ml^{-1}), the highest GST value (62 nmol CDNB/min/mg protein) was found on the 4^{th} day against combined treatment of ricinine and harmaline in fat bodies. In the midgut the highest GST value (59 nmol CDNB/min/mg protein) was observed on the 2^{nd} day of combined treatment with ricinine and harmaline. The protein values in all the treatments were significantly lower in treated insects as compared to control.

Introduction

Medicinal plants have long been used to cure different diseases in the history of mankind. Scientific experiments on the antimicrobial properties of plant components were first documented during the late 19th century (Zaika, 1975). Their antimicrobial activity has been known for millennia since in China, India, Egypt and Greece different parts of medicinal plants have been used to cure specific ailments. Among these plants, two species have been found to be widely used. The first one is *Peganum harmala,* member of the Zygophyllacae family, known for the exceptional wealth in alkaloids of b-carbolin type as harmaline (4, 9-dihydro-7-methoxy-1-methyl-3H-pyrido [3,4-b]indole) (Siddiqui *et al*., 1987). This plant is common in Northern Africa and frequently used as a traditional medicine (Lamchouri *et al*., 2002). Harmaline and certain other b-carboline have also been reported as normal constituents of human tissues and body fluids (Yu *et al*., 2003). They exhibit a variety of biochemical, psychopharmacological and behavioural effects in humans as well as other animals (Airaksinen & Kari, 1981). The neurotoxicity and neuroprotective effects of harmaline has been discussed in the literature over several years which has been reported to be effective blockers of a putative nH+/K+ in vesicle preparation of the goblet cell apical membrane of *Manduca sexta* as well (Cobuzzi *et al*., 1994; Bonnet *et al*., 2000; Splettstoesser *et al*., 2005; Wieczorek *et al*., 1991) . The second species, *Ricinus communis* belonging to family Euphorbiaceae, have long been known to cure different ailments and for its antimicrobial activity. It has also been found to inhibit the mitochondrial respiratory chain reactions (Ferraz, 1999).

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The allelochemical compounds present in several plants are supposed to play important defensive roles against insects. These compounds can deter insects and other herbivores from feeding and, in some cases, serve as a starting point for the development of novel insecticides. Secondary plant compounds such as pyrethrin, nicotine and rotenone obtained, from Chrysanthemum, Nicotiana and several species of the Leguminosae family respectively, are toxic to insects and have long been used as insecticides (Godfrey, 1994).

The beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae), is a polyphagous herbivore confronted with a diversity of potential host plants. This pest damages numerous kinds of cultivated crops including corn, cotton, beet, tomato, celery, lettuce, cabbage and alfalfa (Moulton *et al*., 2000), responsible for considerable annual economic losses (Jacques *et al*., 2008) around the world and is also a growing problem in China and Pakistan. *S. exigua* has been reported to be resistant to conventional insecticides and a possible reason to this phenomenon can be the higher rates of detoxification inside its body (Shimada *et al*., 2005; Wang *et al*., 2001). In the insect body high detoxification rates mostly occur in the tissues of high metabolic activities, like alimentary tract and fat body of herbivorous insects (Ahmad *et al*., 1991). So keeping in view all this background, present study was designed to investigate the impact of plant chemicals, from two traditional medicinal plants *Peganum harmala* and *Ricinus communis,* on the glutathione S-transferase activity in the midgut and fat body of *S. exigua*.

Material and Method

Insects: The larvae of *S. exigua* Hübner (Lepidoptera: Noctuidae) were reared on semi-synthetic diet (David *et al*., 1975) and kept in growth chambers at 26±2^{o}C with 16L: 8D photoperiod.

Chemicals: Harmaline (Fluka) was purchased from Sigma Aldrich. The crude ricinine obtained by methanolic extraction from *Ricinus communis* leaves prepared in the laboratory, was used for the experiments. Soxhlet extraction procedure was carried out to get solid grains of ricininie. These were mixed with ethanol for crystallization and crude ricinine obtained was used for the experiments.

Insect treatment: Newly moulted 4th instar larvae were separately fed with the artificial diet treated with sub lethal and lethal concentrations 0.24 and 0.37mg ml^{-1} of harmaline, whereas 0.49 and 0.71 mg ml^{-1} of ricinine (Muhammad *et al*., 2009) in 5% methanol in distilled water. Larvae were alternately provided with treated and untreated diet after 24 hrs to avoid the high mortality and to observe the long term effect on glutathione S-transferase activity. Controls were fed with the artificial diet treated only with the carrier. Samples for the enzyme assay were prepared after every 48 hrs (2days) up to 192 hrs (8 days).

Enzyme preparation: Three randomly selected larvae were dissected to remove mid guts and fat bodies for each sample. Mid guts and fat bodies were washed in 1.15% KCl and homogenized in 50 mM phosphate buffer, pH 7.4 and centrifuged at 3000xg for 10 min., at 4^{o}C. The supernatants were used as the enzyme source. The glutathione S-transferase (GST) conjugative activity with glutathione reduced and

1-chloro-2,4-dinitrobenzene as substrate was measured at 340 nm by the procedure of Yu (1982).

Protein concentration: The protein concentrations in homogenates were determined according to Bradford (1976). Bovine serum albumin was used as standard.

Statistical analysis: The enzyme assays were repeated 5 times. Results were analyzed by analysis of variance (ANOVA) and means were compared by Tukey's studentized range test ($p \leq 0.05$). All analysis were performed using SAS 8.01 (Anon., 2000).

Results

Glutathione S-transferase activity at sub lethal concentrations: Data presented in Fig. 2 depicts the changes in glutathione S-transferase activity in the fat bodies of insect over a period of time, treated with sub lethal concentrations of harmaline 0.24 mg ml^{-1} and ricinine 0.49 mg ml^{-1} either individually or in combination (with each other at these concentrations). The value of glutathione S-transferase generally increased with the passage of time from day 2 to day 8, except in case of ricinine where it did fall on the 4^{th} day and hiked again consistently up to 8^{th} day. The lowest value of glutathione S-transferase was found at day 2 (37 μmol/min/mg protein) with harmaline treatment while the highest value was observed at day 8 (73 μmol/min/mg protein) with the combined treatment of ricinine and harmaline.

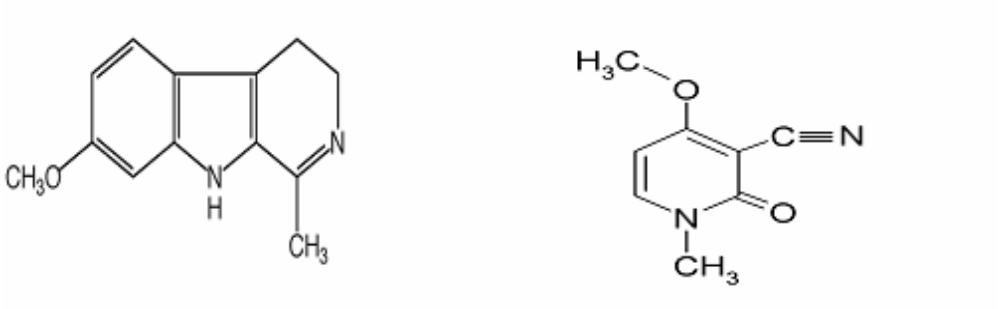
The situation was significantly changed in the midgut, as the value of glutathione S-transferase gradually increased from day 2 to day 6 but a sharp decrease in the values was noted on the day 8 in case of all the treatments. The highest value observed was at day 6 (69 μmol/min/mg protein) after the treatment with ricinine while the lowest value was found at day 2 (42 μmol/min/mg protein) with harmaline (Fig. 3).

Glutathione S-transferase activity at lethal concentrations: At the lethal concentrations of harmaline and ricinine, 0.37 and 0.71 mg ml^{-1} respectively, the value of glutathione S-transferase was increased up to day 4 followed by a sharp decrease except treatment with harmaline as it followed a wavered increase and decrease from day 2 to 8 in fat bodies of insect. The highest and lowest values were found to be in case of combined treatment of ricinine and harmaline (56 and 35 μmol/min/mg protein) at day 4 and 8 respectively (Fig. 4).

In the midgut, the values of glutathione S-transferase insignificantly increased up to day 4 followed by the significant decrease except the combined treatment of ricinine and harmaline where it simply decreased from day 2 to day8. The highest and lowest values of glutathione S-transferase were again found in case of combined treatment of ricinine and harmaline (59 and 33 μmol/min/mg protein) at day 2 and 8 (Fig. 5).

Protein values: Distinct changes in protein values were observed after treatment with ricinine and harmaline at sub lethal and lethal concentrations. At the sub lethal concentrations, 0.24 mg ml^{-1} and 0.49 mg ml^{-1} of harmaline and ricinine the values ended in the increase on the final 8^{th} day of treatment both in fat bodies and midgut (Table 1). The situation was inversed at the lethal concentrations 0.37 and 0.71 mg ml^{-1} of

harmaline and ricinine respectively, as on the final day the sharp decrease in the values was noticed both in fat bodies and midgut tissues. But in all the treatments the protein values were significantly lower than the control treatments (Table 2).



Harmaline Ricinine

Fig. 1. Chemical structures of harmaline and ricinine.

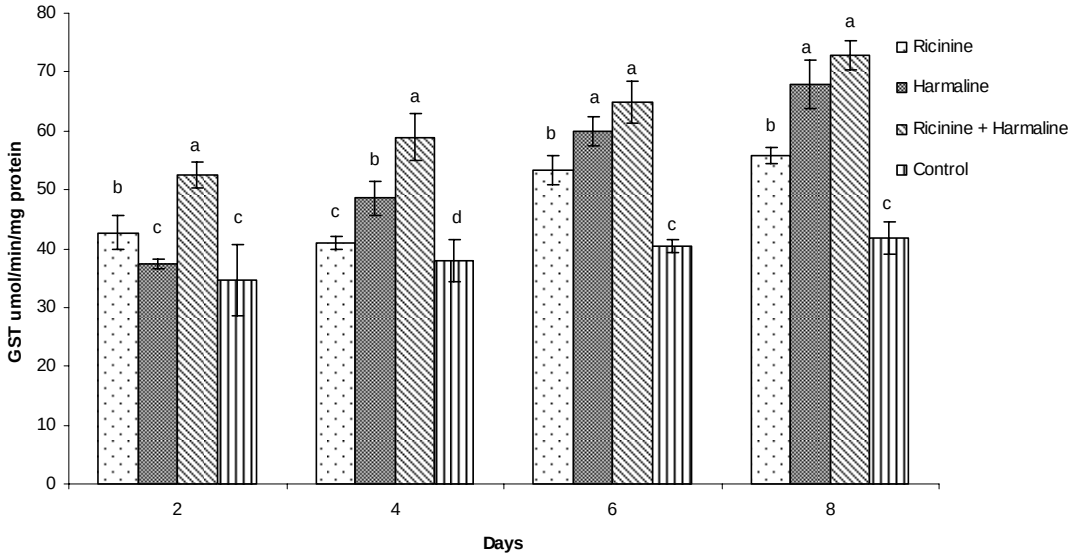


Fig. 2. GST activity (mean± S.E) measured in nmol CDNB/min/mg protein in fat body tissues after being treated at sub lethal concentrations of ricinine and harmaline at different exposure times. Means followed by same letters above bars are not significantly different at based on the Tukey test ($p \leq 0.05$).

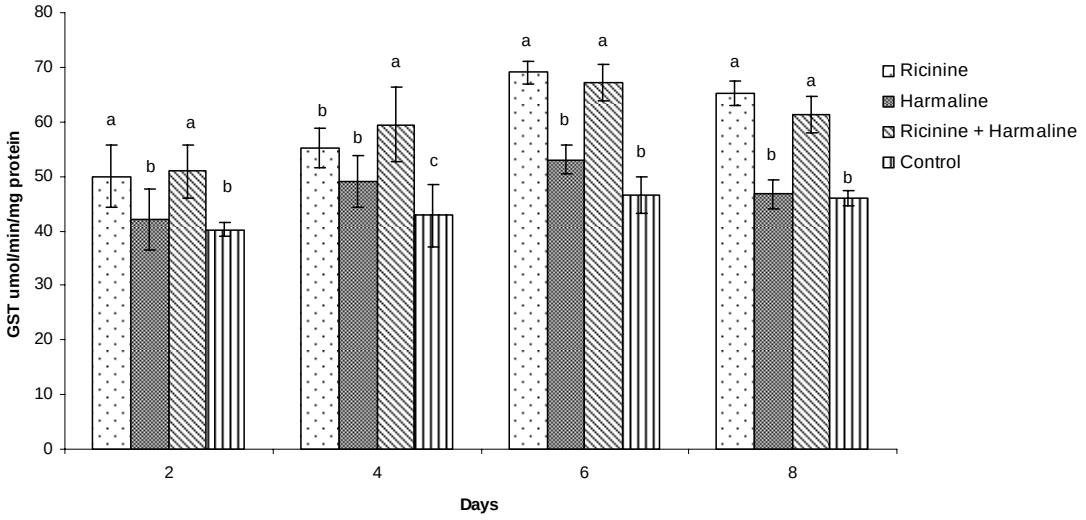


Fig. 3. GST activity (mean± S.E) measured in nmol CDNB/min/mg protein in mid gut tissues after being treated at sub lethal concentrations of ricinine and harmaline at different exposure times. Means followed by same letters above bars are not significantly different at based on the Tukey test ($p \leq 0.05$).

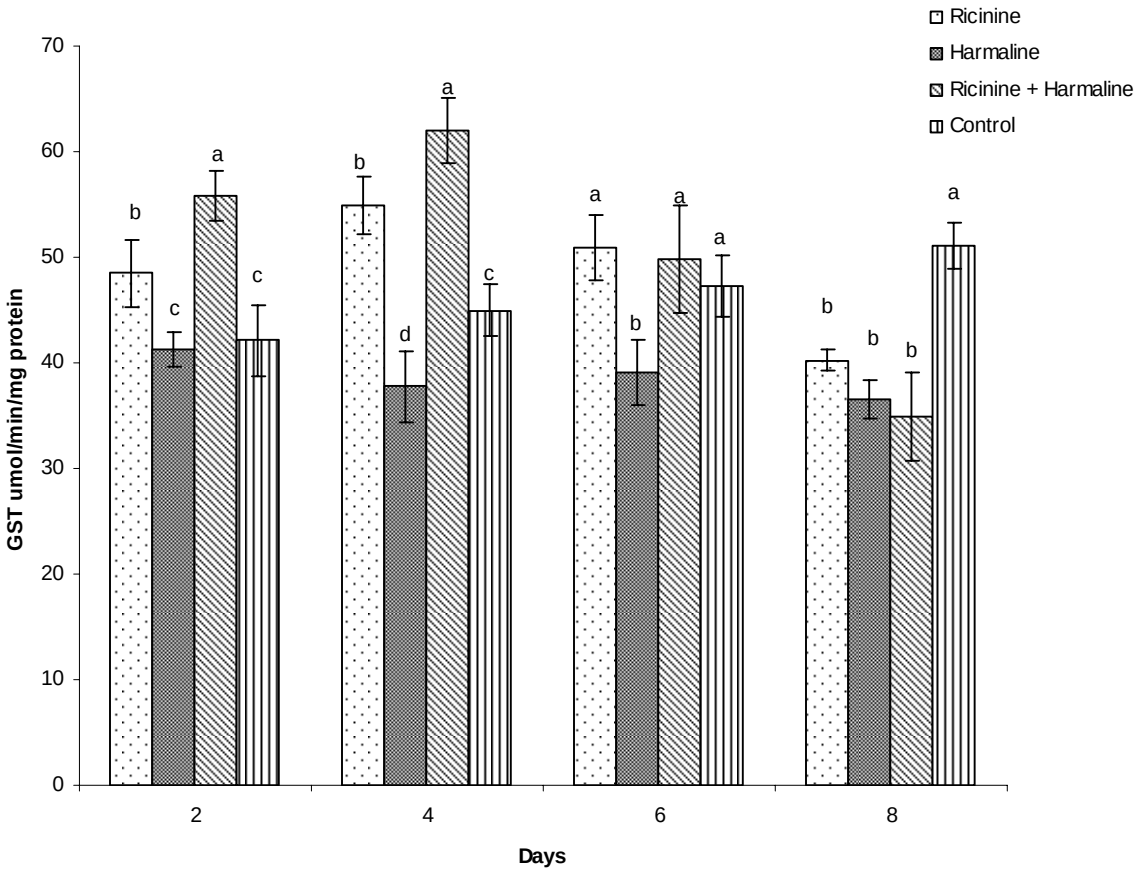


Fig. 4. GST activity (mean± S.E) measured in nmol CDNB/min/mg protein in fat body tissues after being treated at lethal concentrations of ricinine and harmaline at different exposure times. Means followed by same letters above bars are not significantly different based on the Tukey test ($p \leq 0.05$).

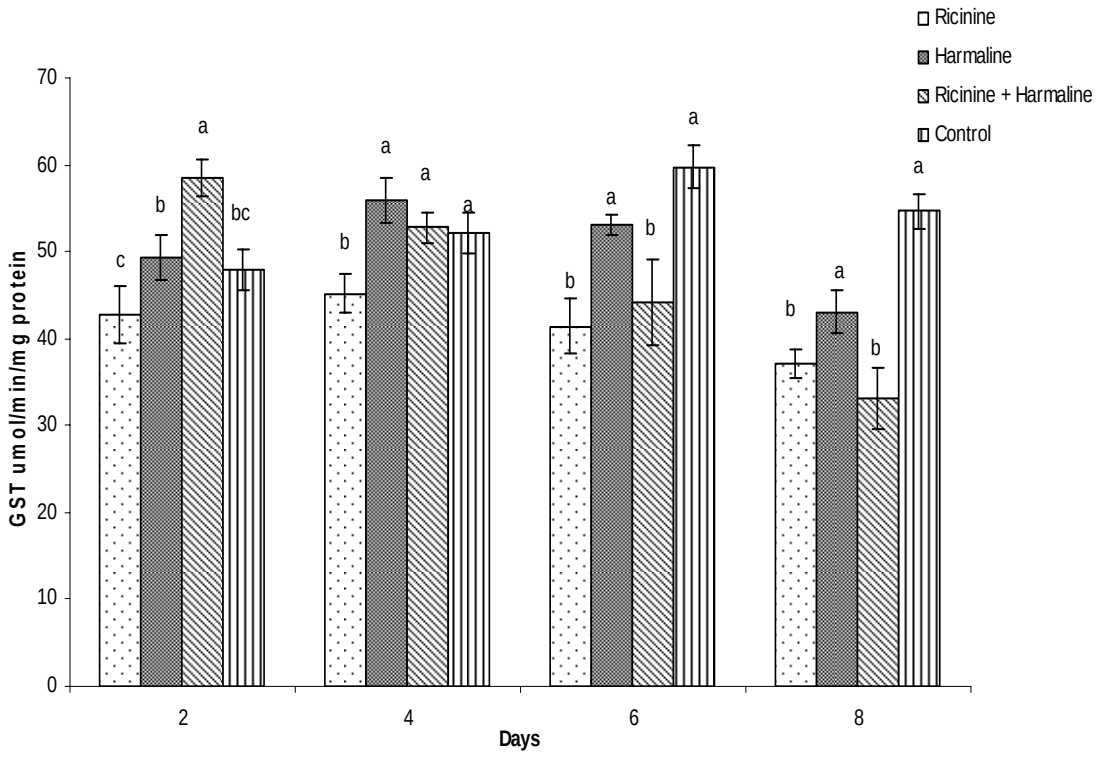


Fig. 5. GST activity (mean± S.E) measured in nmol CDNB/min/mg protein in mid gut tissues after being treated at lethal concentrations of ricinine and harmaline at different exposure times. Means followed by same letters above bars are not significantly different based on the Tukey test ($p \leq 0.05$).

Table 1. Change in protein content in fat body and midgut, after being treated at sub lethal concentrations of ricinine and harmaline (0.24 & 0.49 mg/ml).

Compounds	Protein mg/ g of fat body				Protein mg/ g of Mid gut			
	Day				Day			
	2	4	6	8	2	4	6	8
R	0.29 ± 0.015^{b}	0.33 ± 0.030^{b}	0.35 ± 0.032^{b}	0.4 ± 0.035^{b}	0.31 ± 0.023^{b}	0.28 ± 0.038^{c}	0.36 ± 0.038^{b}	0.39 ± 0.038^{b}
H	0.21 ± 0.027^{c}	0.25 ± 0.032^{c}	0.23 ± 0.020^{c}	0.24 ± 0.020^{d}	0.35 ± 0.038^{ab}	0.38 ± 0.043^{b}	0.32 ± 0.040^{b}	0.31 ± 0.032^{c}
R+H	0.31 ± 0.021^{b}	0.27 ± 0.038^{c}	0.33 ± 0.029^{b}	0.34 ± 0.035^{c}	0.24 ± 0.047^{c}	0.22 ± 0.026^{d}	0.26 ± 0.020^{c}	0.27 ± 0.044^{c}
Control	0.39 ± 0.026^{a}	0.41 ± 0.023^{a}	0.43 ± 0.023^{a}	0.45 ± 0.026^{a}	0.41 ± 0.043^{a}	0.43 ± 0.035^{a}	0.46 ± 0.038^{a}	0.48 ± 0.044^{a}

Means followed by same letters within column are not significantly different based on the Tukey test ($p \leq 0.05$).
R; ricinine
H; harmaline
R+H; ricinine + harmaline (0.24 + 0.49 mg/ml)
Ct; Control

Table 2. Change in protein content in fat body and midgut, after being treated at lethal concentrations of ricinine and harmaline (0.37 & 0.71 mg/ml)

Compounds	Protein mg/ g of fat body				Protein mg/ g of Mid gut			
	Day				Day			
	2	4	6	8	2	4	6	8
R	0.26 ± 0.030^{c}	0.3 ± 0.038^{b}	0.23 ± 0.035^{b}	0.2 ± 0.032^{b}	0.28 ± 0.023^{b}	0.19 ± 0.026^{c}	0.16 ± 0.029^{b}	0.14 ± 0.032^{b}
H	0.23 ± 0.035^{d}	0.22 ± 0.029^{c}	0.19 ± 0.023^{bc}	0.17 ± 0.017^{bc}	0.3 ± 0.032^{b}	0.26 ± 0.038^{b}	0.2 ± 0.030^{b}	0.17 ± 0.018^{b}
R+H	0.32 ± 0.026^{b}	0.24 ± 0.026^{c}	0.17 ± 0.026^{c}	0.13 ± 0.023^{c}	0.25 ± 0.044^{b}	0.29 ± 0.035^{b}	0.2 ± 0.042^{b}	0.17 ± 0.023^{b}
Control	0.43 ± 0.029^{a}	0.45 ± 0.032^{a}	0.47 ± 0.041^{a}	0.49 ± 0.032^{a}	0.46 ± 0.040^{a}	0.47 ± 0.031^{a}	0.51 ± 0.049^{a}	0.52 ± 0.026^{a}

Means followed by same letters within column are not significantly different based on the Tukey test ($p \leq 0.05$).
R; ricinine
H; harmaline
R+H; ricinine + harmaline (0.37 + 0.71 mg/ml)
Ct; Control

Discussion

The compounds from different crop plants are supposed to be inducers or inhibitors of several XME (xenobiotic metabolizing enzymes) in insects (Yu, 1982). Detoxification enzymes like glutathione S-transferase play important role in the metabolism and resistance to insecticides (Yu, 2004) and possess the ability to rapidly change the activity in response to chemical stress, called as enzyme induction. Biochemical processes with participation of glutathione S-transferase (GST) may serve a crucial role in defense system of the insect. The enzyme conjugates wide variety xenobiotics with glutathione and level of glutathione S-transferase expression seems to be a crucial factor in determining the resistance of insects.

Glutathione S-transferase is a group of multifunctional proteins serving several roles in detoxication. The detoxication function of these enzymes may achieve a particular significance in the insect world by contributing to the development of resistance to insecticides by catalyzing their degradation and the high GST activity found in insecticide resistance in insects was associated with increased level of specific mRNA (Yu, 1996).

The present study clearly exhibited that ricinine and harmaline induced detoxification enzyme, glutathione S-transferase in the fat bodies and midgut of the *S. exigua* towards CDNB. Our results showed that the enzyme activity varied at different concentrations, in different tissues of the insect and at different exposure times. The fat bodies of the insect were more sensitive to the ricinine and harmaline, as the highest glutathione S-transferase activity was found in fat bodies after combined treatment with ricinine and harmaline at sub lethal and lethal concentrations. Similar kind of higher glutathione S-transferase values were noticed in fat bodies of *Spodoptera frugiperda* after being treated with triazine herbicides (Yu, 2004).

The relatively lower values of glutathione S-transferase in midgut can be a result of two different reasons at sub lethal and lethal concentrations. At the lower concentrations the decrease in the value near final days could be because of the excretion from the alimentary canal and insects were able to survive. At the higher concentrations of ricinine and harmaline the glutathione S-transferase activity was decreased near the final days, but the decrease in the midgut was sharp as compared to fat bodies. This could have been because; at the lethal concentrations the insect defense system was unable to match the toxicity. The lowered enzyme activities on the final days were also observed by Adamczyk *et al*., (2003); Sowjanya *et al*., 2008 and reported as a possible reason for higher mortality at final days. This idea is powered by the results of our early experiments (Muhammad *et al*., 2009) in which high mortality rates were observed in the final days at lethal concentrations. So glutathione S-transferase enzyme feels to be one of the target sites, of these two botanicals.

The protein values in the treated insects with ricinine and harmaline (either individually or in combination) were significantly lower than the control. The similar findings have been reported by Rharrabe *et al*., (2007) as they observed the significantly lower values of protein in *Plodia interpunctella* after being treated with harmaline.

In short, this study fairly establishes the role of harmaline and ricinine by providing the information about their effect on glutathione S-transferase in insect tissues and also provides the fruitful knowledge of synergistic impact of these two botanicals against *S.*

exigua. These two compounds from Zygophyllacae and Euphorbiaceae also ensure the safer environment and convenient for the humans as they have been reported to be the constituent of human body tissues and easily degradable in the environment as compared to conventional synthetic insecticides. So, they can be a handy tool to develop efficient strategy for the control of this species in the future.

Acknowledgement

We gratefully acknowledge the role of National Science Foundation of People's Republic of China for funding this work under the grant no. 30671387. The authors also thank the support from Foundation for the Author of National Excellent Doctorial Dissertation of P. R. China (FANEDD, NO. 2004061).

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(Received for publication 2 February 2010)