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Induction of Glutathione Antioxidant System of 5th Larval Instar and Adult of Hermetia Illucens as A Result of Injection Malathion Treatment

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Abstract

The relative levels of glutathione antioxidant system in gut tissues of 5th larval instar and males of *Hermetia illucens* insect which injected with different concentration of malathion (0, 0.005,0.01, 0.015 and 0.02 %) were studied. The activity of glutathione peroxidase (GPx) in gut homogenate tissues increase gradually from 0 % concentration till 0.015 % malathion concentration in 5th instar larval insect. Also, the results reveled that there was a significant increase about 15 and 40 % increase at 0.01 % concentration in both 5th instar, and male adult insect respectively, with respect to 0% injected malathion. The highest values of Gluthione S-transferase antioxidant enzyme activity occurred at 0.005 and 0.02 % injected malathion with respect to 0% concentration in both 5th larval instar, and male adult of *H. illucens* insect, respectively.

Keywords: Hermetia Illucens; Glutathione Antioxidant; Oxidative Stress; Malathion

Introduction

To The Black Soldier Fly (BSF) is a cosmopolitan fly and tropical species with environmental potential impact for the processing of various organic waste and byproducts [1, 2]. However, under various types of environmental stresses, e.g. Under relatively low or high temperatures, oxidative damage can occur in organisms and lead to oxidative stress [3]. The antioxidant defense is primarily constituted by the enzymatic actions of Glutathione S-transferase (GST), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GR) and ascorbate peroxidase. In all the tissues of the insects and the digestive tract, the non-enzymatic and enzymatic antioxidants are used to defend the oxidative stress. The antioxidant enzymes of *Drosophila* are reduced glutathione (GSH), glutathione reductase (GR), Glutathione S-transferase (GST), catalase (CAT), superoxide dismutase (SOD) disulfide reductase and methionine sulfoxide reductase (MSR) [4, 5].

The reactive Oxygen species (ROS) produce from organisms exposure to the different xenobiotics and pollutants. The cell membrane lipid peroxidation and oxidative stress is resulted from increases ROS formation after insecticide fipronil treatment [6, 7, 8]. Therefore, this study aims to investigate antioxidant system of *Hermetia illucens* as a result of organophosphates insecticide (malathion) treatment.

Materials and Methods

Black solider fly, *Hermetia illucens* were gained from Entomology Department, Faculty of Science, Cairo University, with its kitchen waste food was supplied from household within Giza government, Egypt. Insects were divided into larval group and adult group each group set was divided into 5 subgroups which represented to 0, 0.005, 0.01, 0.015, 0.02 % malathion concentration *injection* application with a time of 30 min post injection for circulation. For each experimental subgroup, 50 insects pool of 5th instar and adult of *H. illucens* were dissected to isolate gut tissues for further analysis and were stored at −20 °C until use.

The gut tissue extracted from each experimental subgroup were homogenized using mortar in ice-cold PBS pH=7.0 and centrifuged at 1000 rpm and the supernatant was ready to be used. The activity of glutathione peroxide (GPx) was determined according to with slight modifications. The following reagents mixture was 0.1 ml EDTA, 0.1 ml sodium azide, 0.1 ml reduced glutathione (GSH), 0.1 ml H_2O_2, and 1.5 ml of the supernatant, then the mixture was incubated for 10 minutes at 37 °C, and 1 ml of 10% trichloroacetic acid was added and centrifuged at 1730 g for 5 minutes at room temperature. The supernatant was mixed with 1.5 ml Na_2HPO_4 (pH 7.5) and 0.3 ml 5, 5´-Diothio bis-2-nitrobenzoic acid (DTNB) just before measuring the absorbance at 420 nm. The activity of GPx was expressed as OD/mg protein/min.

The activity of GR was determined according [9] with the following minor modifications. The reaction mixture contained 1750 µl oxidized glutathione (GSSG), 175 µl potassium phosphate buffer (50 mM, pH =7.5), 875 µl DTNB, 175 µl NADPH, and 350 µl supernatant. The absorbance was measured at 420nm, and the GR activity was expressed as OD/mg protein/min.

The activity of GST was determined according to method of [10] with minor modification. The reaction mixture contained 0.1 ml of supernatant, and 0.9 ml of the following mixture (882 µl PBS pH 7.0, 9 µl CDNB and 9 µl GSH). The absorbance was measured at 340 nm. G-S-T activity was expressed in OD/mg protein/min. All protein concentrations from all samples were measured according to [11], with bovine serum albumin as a standard.

Statistical analysis of all glutathione system sample analysis were done as a parametric test, which was performed using a one-way analysis of variance (ANOVA, Tukey's-b test, $p < 0.05$), and were presented as mean ± SE. Correlations between the concentration of malathion and the glutathione antioxidant system results (GPx, GR, and G-S-T) were performed based on Pearson's regression analysis using multiple regression models. Hierarchical Cluster Analysis (HACA) based on agglomerative statistics using Ward's Method was calculated for all glutathione antioxidant system. The goal of HACA is to find possible clusters or groups among the observational units, based on level of similarities and differentiations [12]. At each stage, the average similarity of the cluster is measured. While, the difference between each case within a cluster and that average similarity is calculated. Principle component analysis (PCA) is a data analysis technique that used as pattern seeker of data by using algorithm concept. All statistical analyses were performed using IBM SPSS Statistics for Windows (Version 17.0. Armonk, NY: IBM Corp.).

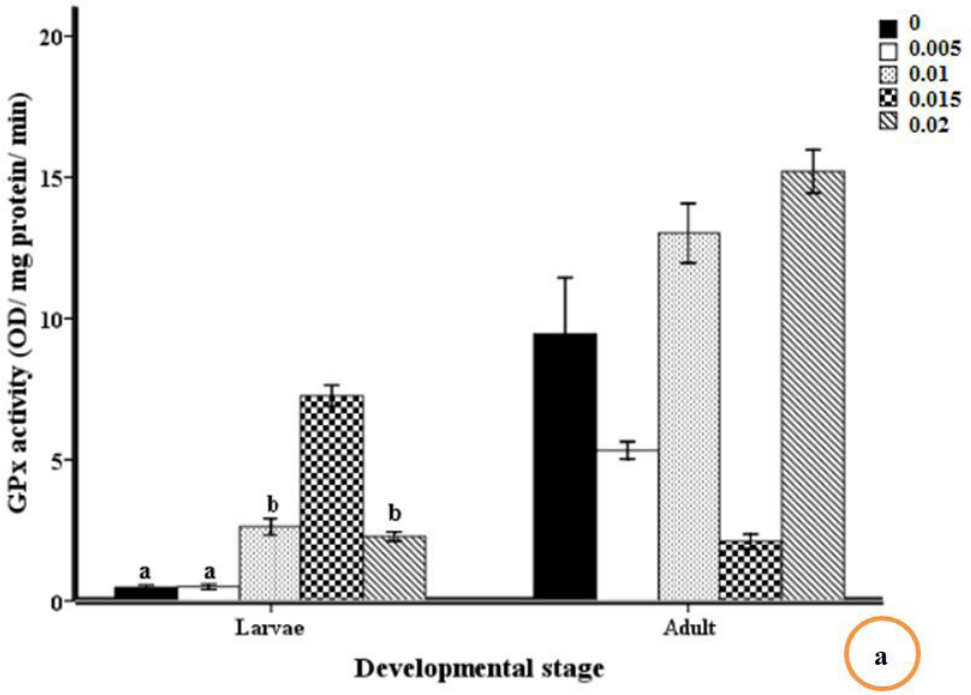
Results and Discussion

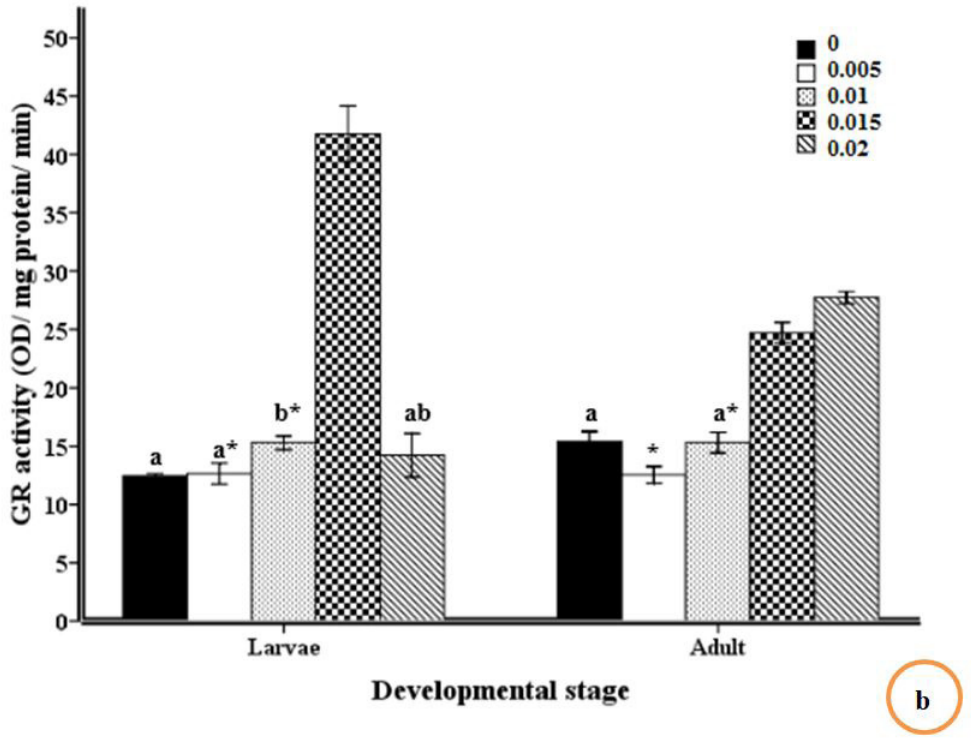
The relative levels of glutathione antioxidant system in 5th larval instar and male's of *Hermetia illucens* insect which injected with different concentration of malathion (0, 0.005,0.01, 0.015 and 0.02 %) were shown in Fig 1a-c. The activity of glutathione peroxidase (GPx) in gut homogenate tissues increase gradually from 0 %

concentration till 0.015 % malathion concentration in 5th instar larval insect. Also, the results reveled that there was a significant increase about 15, 40 % increase at 0.01 % concentration in both 5th instar and male adult insect respectively, with respect to 0% injected malathion (Fig. 1a).

[13] showed that various concentrations of boric acid (BA) led to increase of Gluthione S-transferase (GST) activity. Within various organs exposed to BA and borax, the levels of antioxidant enzymes differ [14].

The results showed that, glutathione reductase antioxidant enzymes (Fig. 1b) were not significantly difference between 0, 0.005, 0.01 and 0.02 % of injected malathion into 5th larval instar of *H. illucens.* However, there was a significant increase among 0, 0.015, and 0.02% of injected malathion into male adults *H. illucens.* The highest values of GST antioxidant enzyme activity occurred at 0.02, and 0.005 % injected malathion with respect to 0% concentration in both 5th larval instar, and male adult of *H. illucens* insect, respectively. (Figure 1c).





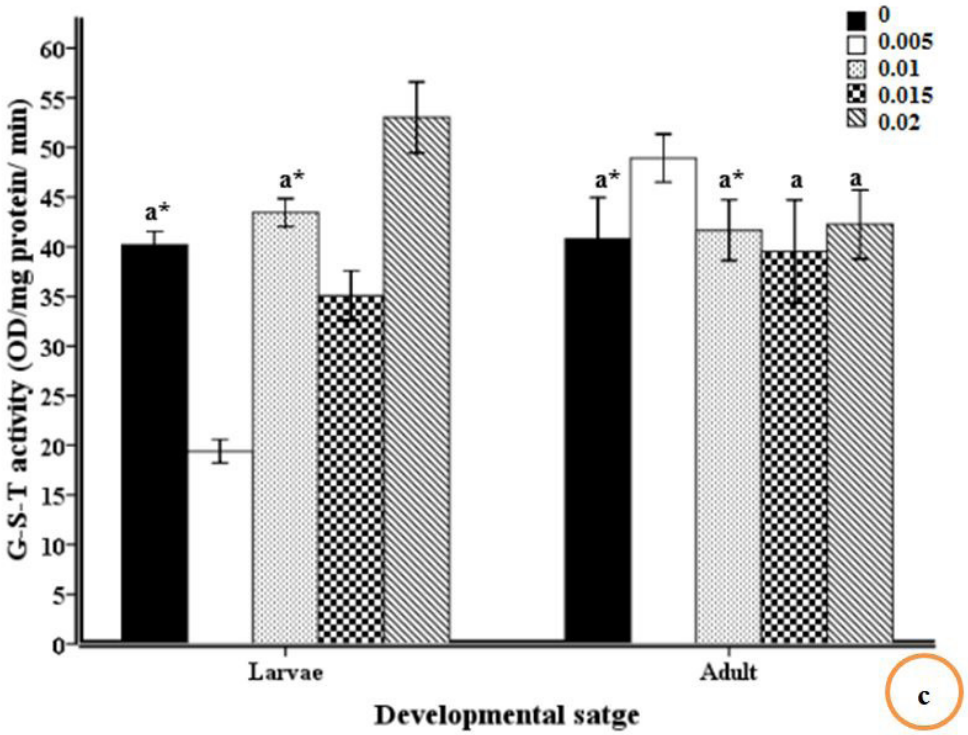


Figure 1: Activity of glutathione antioxidant system, which included glutathione peroxidase (GPx) (a), glutathione reductase (GR) (b), and glutathione-s-transferase (G-S-T) (c) expressed as mean, and standard error (Mean ± SE) of each enzyme activity, obtained from gut homogenates of 5th larval instar and adult of Hermetia illucens injected with different concentration of malathion (0, 0.005,0.01, 0.015, and 0.02) %.

The stress caused by xenobiotics is affected with values of the peroxidation and enzymatic activity parameters [15]. Indoxacarb affected the activities of the total amount of free SH groups, GPx and GST while chlorantraniliprole significantly affected the activities of GST, SOD, CAT and the total amount of free SH groups, as well as chlorantraniliprole+lambda cyhalothrin affected the activities of the total amount of free SH groups, CAT and GST [16]

The principal component analysis (PCA) were showed in Fig. 2. This results related to 5th instar larval, and male adult stage of glutathione antioxidant system (glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-s-transferase (G-S-T) of *Hermetia illucens* injected with different concentration of malathion (0, 0.005,0.01, 0.015 and 0.02) %. The analysis variance-covariance relation showed that a high variability in terms of glutathione antioxidant system regarding to concentration of injected malathion. The first component demonstrated that was a significance relationship, also eigen value tends to be depend on 2 variables which were classified into first component and second component.

A cluster analysis using Ward's method revealed slightly dissimilar patterns for 5th instar larval , and male adult insect, however, the similar general tendency (Fig 3a-b). The glutathione enzymatic response (GPx, GR, and G-S-T) was had a separate loop in case of 0, 0.005, 0.01, 0.02 % of malathion in the gut homogenates of 5th larval insects. However, in gut male's insect, the glutathione antioxidant response, had a great similarity between 0.015, and 0.02 % injected malathion concentration, also it had a separated cluster between 0, 0.005, 0.01 % injected malathion (Figure 3b).

Assessment of the overall relationship among concentration of malathion injected (0, 0.005, 0.01, 0.015 and 0.02) % of malathion and mean activity of glutathione antioxidant system (GPx, GR, and G-S-T) in gut tissues of 5th instar larval and male adult of H. illucens were performed. The tested samples revealed a positive correlation (from moderate to strong relationship) in both experimental developmental stage and in both experimental tests with a linear equation for prediction with a wide range of chi-square from negative to positive value (Table 1).

[17, 18] stated that mosquitoes adapted a mechanism that protects them from immune-related oxidative stressor via the antioxidant GSH). On the other hand, they recorded significant elevation of GST minimized the resulting cellular damage as antioxidative response [19].

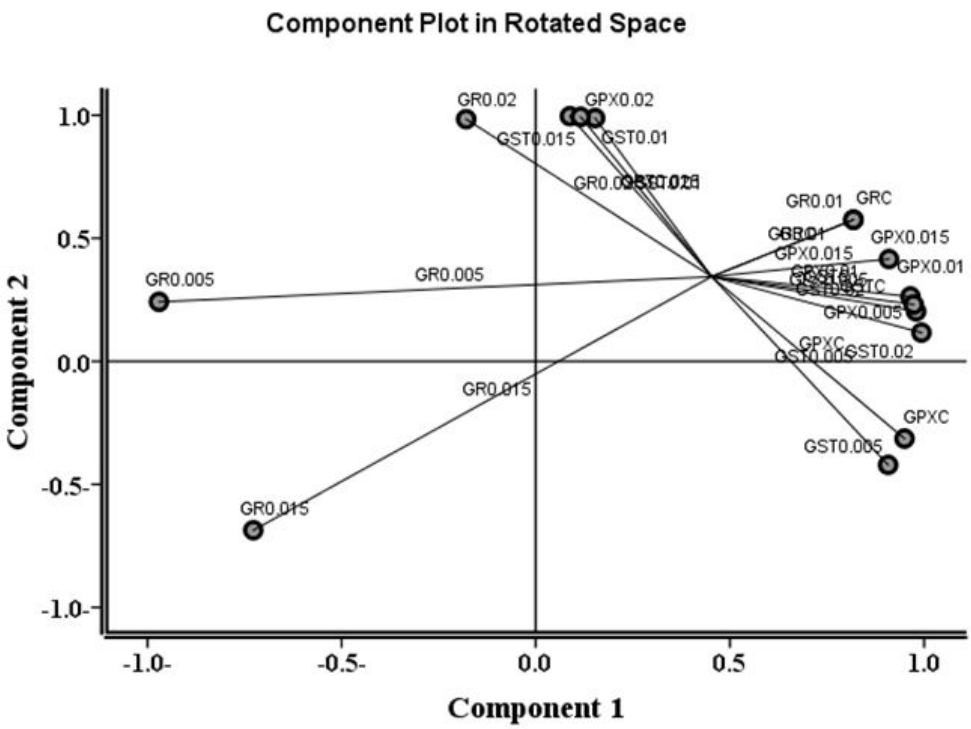


Figure 2. Principal component analysis (PCA) of 5th instar larval, and male adult stage of glutathione antioxidant system (glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-s-transferase (G-S-T) of *Hermetia illucens* injected with different concentration of malathion (0, 0.005,0.01, 0.015, and 0.02) %.

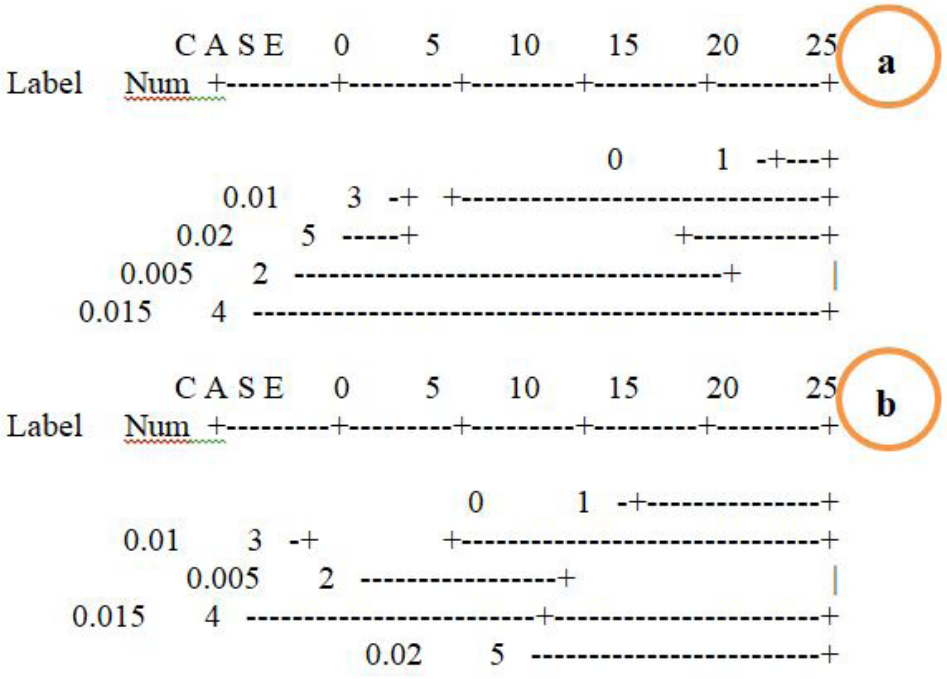


Figure 3: Dendrogram of the cluster analysis (using Ward′ s Method) applied for glutathione antioxidant system (glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-s-transferase (G-S-T) in 5th larval instar (a), and male adult (b), of *Hermetia illucens* injected with different concentration of malathion (0, 0.005,0.01, 0.015 and 0.02) %.

[20] Mean values marked with same small letters are not significantly different among control and different treatment sample with malathion. * denote no significant differences among 5th larval instar and male adult in each case separately (ANOVA test Tukeys, $p > 0.05$).

Glutathione antioxidant system	Developmental stage	r	Equation	Type of equation	R^2
GPx	5th larval instar	0.54*	Y= 244X	Linear Equation for Prediction	0.33
	Adult	0.24	Y= 656X		-0.71
GR	5th larval instar	0.41	Y=1502X		-0.25
	Adult	0.87**	Y=1521X		-0.53
G-S-T	5th larval instar	0.48	Y=2774X		-2.35
	Adult	-0.44	Y=2798X		-29.7

Table 1: Pearson's correlation coefficient among glutathione antioxidant system (glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-s-transferase (G-S-T) (which expressed as OD/ mg protein/ min, samples obtained from gut tissues of 5th larval instar and male adult of *Hermetia illucens* injected with different concentration of malathion (0, 0.005,0.01, 0.015 and 0.02) %.

References

1. St-Hilaire SK, Cranfill MA, Mcguire EE Mosley and JK Tomberlin et al. (2007) Fish offal recycling by the Black Soldier Fly produces a foodstuff high in omega-3 fatty acids. J World Aquacult Soc 38: 309-13.

2. Marshall SA, NE Woodley and M Hauser (2015) The historical spread of the lack soldier fly, Hermetia illucens (L.) (Diptera, Stratiomyidae, Hermetiinae) and its establishment in Canada. J ent Soc Ont 146: 51-4.

3. Lopez-Martinez G, Elnitsky MA, Benoit JB, Lee RE Jr and Denlinger DL (2008) High resistance to oxidative damage in the Antarctic midge Belgica antarctica and developmentally linked expression of genes encoding superoxide dismutase, catalase and heat shock proteins. Insect Biochem. Mol Biol 38: 796-804.

4. Missirlis F, Rahlfs S, Dimopoulos N, Bauer H, Becker K, et al. (2003) A putative glutathione peroxidase of Drosophila encodes thioredoxin peroxidase that provides resistance against oxidative stress but fails to complement a lack of catalase activity. J Biol Chem 384: 463-72.

5. Radyuk SN, Sohal RS and Orr WC (2003) Thioredoxin peroxidases can foster cytoprotection or cell death in response to different stressors: over- and underexpression of thioredoxin peroxidase in Drosophila cells. Biochem J 371: 743-52.

6. Ojha A, Yaduvanshi SK and Srivastava N (2011) Effect of combined exposure of commonly used organophosphate pesticides on lipid peroxidation and antioxidant enzymes in rat tissues. Pestic Biochem Physiol 99: 148-56.

7. Ki YW, Lee JE, Park JH, Shin IC and Koh HC (2012) Reactive oxygen species and mitogen-activated protein kinase induce apoptotic death of SH-SY5Y cells in response to fipronil. Toxicol Lett 211: 18-28.

8.Margarido TC, Felício AA, De Cerqueira, Rossa-Feres D and De Almeida EA (2013) Biochemical biomarkers in Scinax fuscovarius tadpoles exposed to a commercial formulation of the pesticide fipronil. Mar Environ Res 91: 61-7.

9. Carlberg I and Mannervik B (1985) Glutathione reductase assay. Methods Enzymol., 113: 484-95.

10. Seyyedi MA, Farahnak A, Jalali M and Rokni MB (2005) Study on Glutathione -S-Transferase (GST) Inhibition Assay by Triclabendazole. I: Protoscoleces (Hydatid Cyst; Echinococcus granulosus) and Sheep Liver Tissue. Irani J of Public Health 34: 38-46.

11. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72: 248-54.

12. Azam I, Afsheen S, Zia A, Javed M, Saeed R, et al. (2015) Evaluating insects as bioindicators of heavy metal contamination and accumulation near industrial area of Gujrat, Pakistan. BioMed Res. Int 1-11.

13. Habes D, Kilani-Morakchi S, Aribi N, Farine JP, Soltani N (2001) Toxicity of boric acid to Blattella germanica (Dictyoptera: Blattellidae) and analysis of residues in several organs. Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol Wet., 66: 525-34.

14. Ince S, Kucukkurt I, Ibrahim Hakki, Cigerci IH, Fidan AF et al. (2010) The effects of dietary boric acid and borax supplementation on lipid peroxidation, antioxidant activity, and DNA damage in rats. J. Trace Elem. Exp. Med 24: 161-4.

15. Büyükgüzel E, Büyükgüzel K, Snela M, Erdem M, Radtke K, et al. (2013) Effect of boric acid on antioxidant enzyme activity, lipid peroxidation, and ultrastructure of midgut and fat body of Galleria mellonella. Cell Biol. Toxicol., 29:117-29.

16. Franeta F, Mirčić D, Todorovic D, Milovac Z, Granica N, Obradović S, et al. (2018) Effects of different insecticides on the antioxidative defense system of the European Corn Borer (Ostrinia nubilalis Hübner) (Lepidoptera: Crambidae) larvae. Arch Biol Sci 70: 765-73

17. Erden-Inal M, Sunal E and Kanbak EG (2002) Age-related changes in the glutathione redox system. Cell. Biochem. Funct., 20: 61-6.

18. Christensen BM, LI J, Cheng C and Nappi AJ (2005) Melanization immune responses in mosquito vectors. Trends Parasitol., 21: 193-9

19. Ahmed AM (2012) Lipid Peroxidation and Oxidative Protein Products as Biomarkers of Oxidative Stress in the Autogenous Mosquito, Aedes caspius, Upon Infection with the Mosquitocidal Bacterium, Bacillus thuringiensis kurstaki. Pakistan J. Zool 44: 525-36.

20. Hafeman DG, Sunde RA and Hoekstra WG (1974) Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. The J Nutr 104: 580-87.