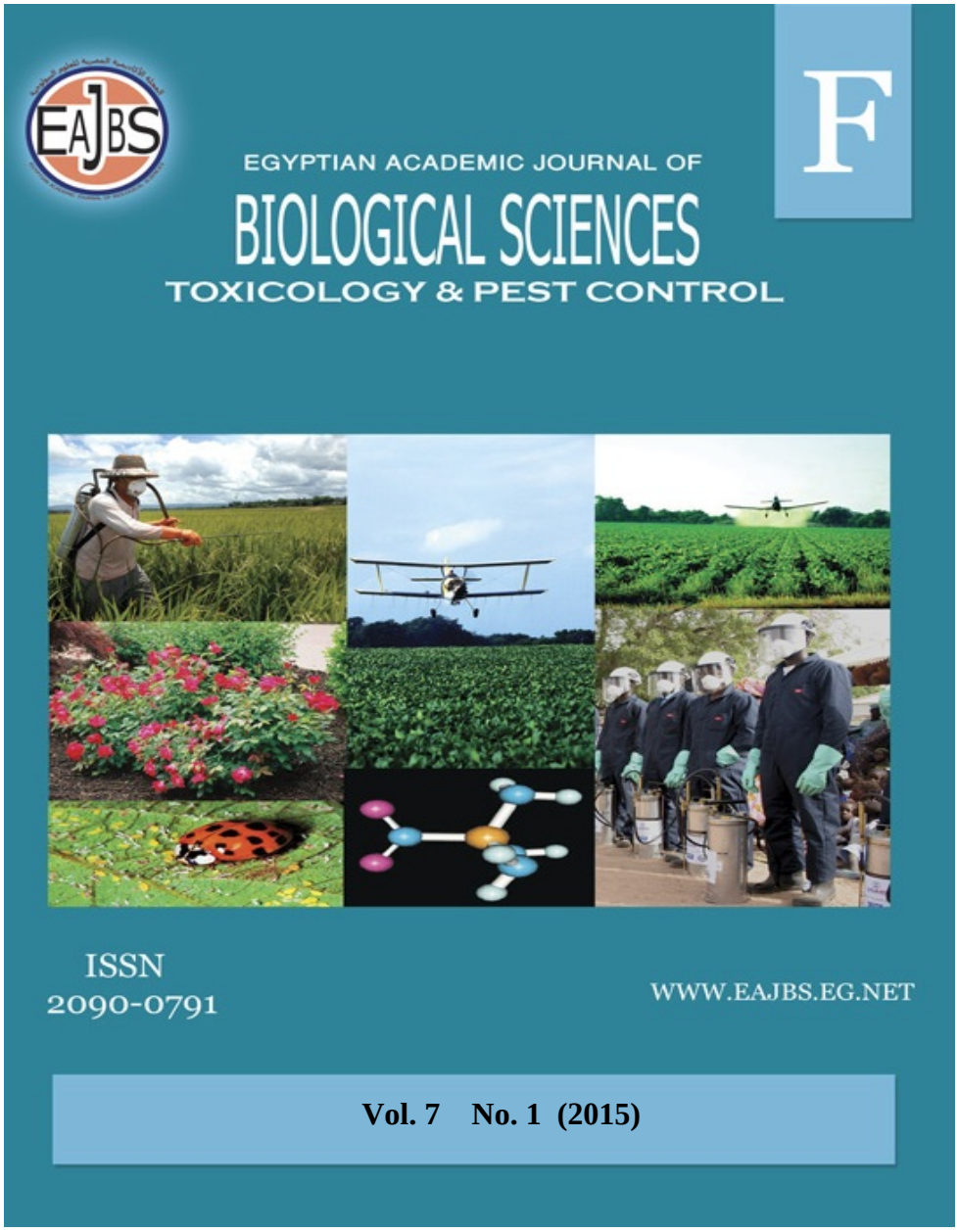

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Oxidative stress effects of abamectin and hematoporphyrin with the antioxidant efficiency in the cotton leaf worm, *Spodoptera littoralis* (Biosd.)

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ABSTRACT

Effects of abamectin and hematoporphyrin (HP), eco-friendly insecticides, on the concentration of the oxidative stress indicator malondialdehyde (MDA) and on the antioxidant enzymes; catalase (CAT), glutathione-*S*-transferase (GST); activity were studied in the 4th instar larvae of *Spodoptera littoralis* (Biosd.). There effects on glutathione (GSH) level were also investigated. MDA content was significantly increased on the 1st day post treatment of both insecticides, but increased significantly only with HP treatment on the 5th day of treatment. Abamectin treatment decreased GSH significantly on the 3rd and 4th days, while HP treatment increased its level on the 1st and 2nd day post treatment. Activities of antioxidant enzymes, CAT and GST, did not show a consistent pattern with respect to abamectin and HP. These results suggest that exposure to abamectin and HP resulted in impaired enzymatic antioxidant defense capacity in 4th instar *S. littoralis* larval tissues.

INTRODUCTION

Oxidative metabolism of cells is a continuous source of reactive oxygen species (ROS). ROS function normally in physiological cell processes at low to moderate concentrations, but at high concentrations oxidative stress occurred due to the imbalance between higher levels of ROS and the cellular antioxidant defense (Ilhan *et al*., 2005).

The effects of oxidative stress are mitigated by an endogenous antioxidant enzyme network (Rindler *et al.*, 2013). In insects, the antioxidant defense system consists of enzymatic such as catalase (CAT), glutathione-*S*-transferase (GST) and superoxide dismutase (SOD) as well as non-enzymatic antioxidants such as glutathione (GSH) and ascorbic acid which can withstand the deleterious effect of ROS (Felton,1995).

The oxidative destruction of lipids acts in a chain reaction to form lipid hydroperoxides (LPO), which can decompose to malondialdehyde (MDA) as an end-product. So, MDA is one of the most frequently used indicators of lipid peroxidation. In other words, LPO may be expressed in the concentration of MDA (Cheeseman, 1993).

GSH constitutes a second line in insect immunity through detoxification and metabolizing of toxins in insect body besides protecting insects from the concomitant oxidative stress (Kumar *et al*., 2003).

GSTs play an important role in the detoxification and metabolism of many xenobiotic and endobiotic compounds (Krishnan and Kodrík, 2006). GST conjugates xenobiotics with reduced glutathione for excretion and they may eliminate organic hydroperoxide from cells and defend cells from potential damage caused from the products of lipid peroxidation (Büyükgüzel *et al*., 2010). Otherwise, CAT has the ability to consume hydrogen peroxide (H_2O_2) at high concentration and quickly converts it to water and oxygen (Kono and Fridorich, 1982; Felton, 1995).

Different classes of pesticides may induce *in vitro* and *in vivo* generation of ROS (Bagchi *et al*., 1995). The damage of most pesticides is represented via the level of lipid peroxidation and GSH depletion, leading to oxidative damage in insect tissues (Ahmad, 1995). Natural pesticides are actually much safer, affect only the target pest, have short residual activity and safer to environment than conventional pesticides (Lyon and Newton, 1999). Abamectin belongs to the family of avermectins, which are macrocyclic lactones produced by a soil actinomycete, *Streptomyces avermitilis* (Burg and Stapley, 1989). It is currently used as a pest control agent in livestock and as an active substance as a nematicide and insecticide for agricultural use (Kolar *et al.,* 2008). Porphyrins, nitrogenous biological pigments (biochromes), can undergo very efficient photo-excitation by sunlight resulting in singlet oxygen generation which is a cytotoxic oxygen derivative (Jori, 1985). The lack of photo-protection in insects confers a special advantage to the photosensitizing compounds as substantially irresistible insecticides (Abdelsalam *et al*., 2014). The insecticidal activities of hematoporphyrin were tested against many insects (Awad *et al*., 2008; Lucantoni *et al*., 2011; Ben Amor *et al*., 1998; Abdelsalam *et al*., 2014). Moreover, hematoporphyrins are stress factors that induce the release of excessive ROS causing severe damage in cells of the treated pest (Ben Amor and Jori, 2000; Abdelsalam *et al*., 2014).

Spodoptera littoralis is one of the most destructive agricultural lepidopterous pests. The host range of it covers over 40 families of economic importance (Salama *et al.,* 1970).

Accordingly, the present study was undertaken in order to evaluate oxidative stress of two eco-friendly insecticides, abamectin and hematoporphyrin (HP), and antioxidant defense in the early 4th larval instar of *S. littoralis.*

MATERIALS AND METHODS

Insects

Stock colony of *S. littoralis* larvae were obtained as 4th instar larvae from Plant Protection Research Institute, Agricultural Research Center. They were maintained under controlled conditions at 28±2ºC, 65 ± 5% RH and 16h light: 8h dark photoperiod.

Insecticides

Abamectin, a commercial formulation of Vertemic® and 1.8% EC, was supplied by Syngenta Agro Services AG, Egypt.

Hematoporphyrin IX (HP) was obtained from Sigma-Aldrich. A stock solution (0.1mol Dm^{-3}) was prepared according to Mascanzoni *et al.* (1987).

Bioassay

Different concentrations of abamectin (5, 15,25,50,75 ppm) and HP (25, 75, 100, 150, 200 ppm) were prepared in distilled water. Bioassays were initially performed using 4th instar larvae of *S. littoralis* using dipping technique of castor oil (*Ricinus communis*) leaves in each concentration for 10 sec then air dried. Control leaves were treated similarly with distilled water only. The dried leaves which were offered to a minimum of 10 larvae per concentration were replicated three times (totally n=30) for 24 h, then they were fed on normal (untreated) leaves. The percentage mortality was determined after 72 h and corrected against control using Abbott's formula (Abbott, 1925). The corrected mortality was subjected to probit analysis (Finney, 1971) and the lethal concentrations were determined (SAS, 1990). The estimated LC_{50} of abamectin was 44.7± 2.15 ppm and that of HP was 112.2 ± 3.76 ppm.

Early 4th instar larvae of the cotton leaf worm, *S. littoralis* were divided into 2 groups, control (fed on castor oil leaves treated previously with distilled water only) and treated group which was subdivided into two divisions; the first one was allowed to feed upon castor leaves treated with LC_{50} abamectin and the second one with LC_{50} of HP, as described above. After 1, 2, 3, 4 and 5 days of treatment, the surviving larvae was collected and used further to be prepared for biochemical analysis.

Biochemical Analysis

Sample preparation

Total larval bodies were homogenized (1gm of tissue in 5 ml of distilled water) using hand glass homogenizer on ice jacket and then centrifuged using Eppendorf refrigerated 5415 (Hamburg, Germany) at 8000 rpm for 15 min at 2°C. The supernatant was kept at –20 °C till use.

Biochemical tests

Determination of enzymes activities

GST activity was determined according to the method of Habig *et al.* (1974). 0.4 ml potassium phosphate buffer (50 mmol/l; pH 6.5), 0.1 ml of supernatant, 1.2 ml water and 0.1 ml CDNB (1-chloro-2,4-dinitrobenzene, 30 mmol/l) were added and incubated in water bath at 37ºC for10 min. After incubation, 0.1 ml of reduced glutathione (30mmol/l) was added. The change in absorbance was measured at 340 nm at one min interval.

CAT activity was measured using Biodiagnostic Kit No. CA 25 17 which is based on the spectrophotometric method described by Aebi (1984). CAT enzyme activity was determined by measuring the rate of H_2O_2 consumption via absorbance at 240 nm.

Determination of lipid peroxidation and glutathione

The assay of GSH levels was performed using Biodiagnostic kit No. GR 25 11 which is based on the spectrophotometric method of Beutler *et al*. (1963). GSH reduced 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) producing a yellow color whose absorbance is measured at 405 nm.

LPO levels were determined by using Biodiagnostic kit. No. MD 25 29 which is based on the spectrophotometric method of Ohkawa *et al*. (1979) in which MDA release served as the index of LPO. MDA was determined by measuring of 2-thiobarbituric acid (TBA) reactive species and absorbance was measured at 534 nm.

Statistical analysis

The data were expressed as means ± SD and separated using Student's t-test. Data were statistically analyzed using one-way analysis of ANOVA within each post-treatment day (SAS,

2009). The difference between means was significant at $p < 0.05$.

RESULTS AND DISCUSSION

The present study is considered as a trial to elucidate the potentiality of two eco-friendly pesticides, abamectin and HP, in causing oxidative stress in the tissues of the 4th larval instar of *S. littoralis*and how these larvae relief and come over such stress via their antioxidant system. Figure (1) shows that treatment of 4th larval instar of *S. littoralis* with LC_{50}abamectin and HP increase significantly in the concentration of MDA in the larval tissues by37.73% and 83.42%, respectively on the1st day post-treatment. While, on the 5th day MDA level increased significantly by 55.89% only in case of HP treatment. At the rest of the tested time, MDA level maintained constant in comparison with the untreated control in both pesticides.

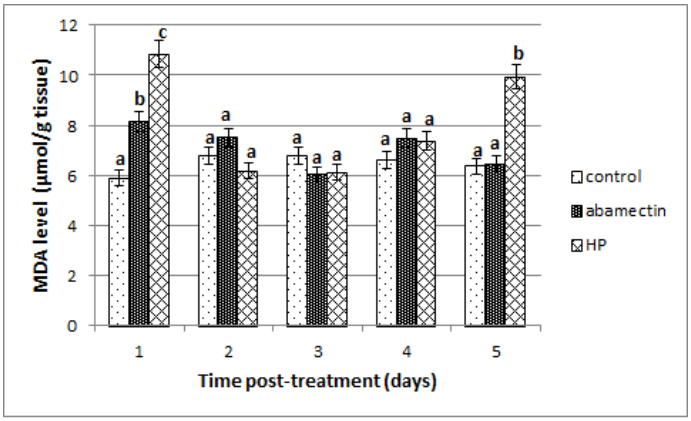

Fig.1: Effect of LC_{50} of abamectin and hematoporphyrin on MDA level in 4th larval instar *S. littoralis* tissue at different time intervals. Columns with different letters within each post-treatment day are significantly different ($P < 0.05$) using one-way ANOVA.

This significant elevation in MDA, its level is a marker of oxidative stress and the antioxidant status in insects, in the present study may result from the accelerated ROS production due to the delayed effect of abamectin and HP (Ben Amor and Jori, 2000). Similar results were reported when *S. littoralis* larvae infected with *Bacillus thuriengensis* (Boctor and Salama, 1983) and treated with pyriproxyfen (Fahmy, 2012). The same trend was detected in the midgut of *Galleria mellonella* after exposure to high dietary concentrations of penicillin (Büyükgüzel and Kalender, 2007).

Figure (2) shows that abamectin treatment (LC_{50}) causes significant decrease in the level of GSH on the 3rd and 4th days post-treatment (up to 15.00 and 24.64 %, respectively). While, at other time intervals abamectin has no effect on GSH level. On the other hand, HP increases significantly GSH level on the 1stand 2nd days after treatment by 60.06 and 30.93 %, respectively, while on the 4th and 5th days of treatment significant decreases by 17.88 and 23.69%, respectively, were recorded.

This observed elevation in GSH concentrations may be due to the fact that it can react non-enzymatically with different ROS and functions directly as a free radical scavenger (Winterbourn and Metodiewa, 1994). Similar trend was found in *Ostrinia nubilalis* larvae, where the higher titer of GSH was attributed to the environmental stress due to pollutants or to the oxidative stress (Jovanovic-Galovic*et al*., 2004). Moreover, it has been reported that the exposure to ROS may raise the GSH content by increasing the GSH synthesis (Rahman *et al.*, 1996). Similar elevation in GSH level was detected in lepidopteran larvae as a physiological response to dietary toxicants (Peric´-Mataruga *et al.*, 1997, Fahmy, 2012).

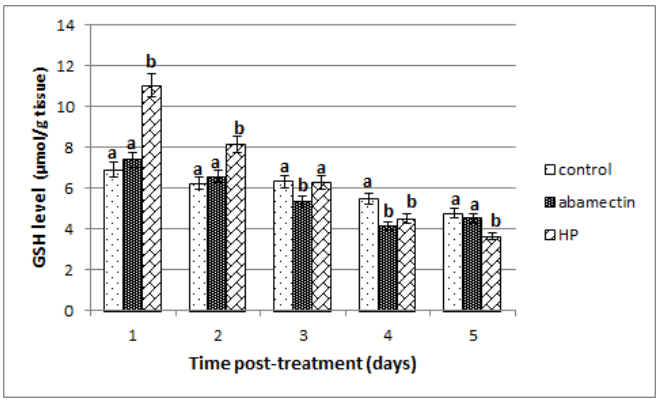

Fig. 2: Effect of LC_{50} of abamectin and hematoporphyrin on GSH level in 4th larval instar *S. littoralis* tissue at different time intervals. Columns with different letters within each post-treatment day are significantly different ($P < 0.05$) using one-way ANOVA.

In fact, it seems that GSH mediated reaction catalyzed by GST (Meister and Anderson, 1983) is one of the important mechanisms that allow insects to survive in a contaminated environment (Poupardina *et al*., 2008).

On the other hand, lower titer of GSH observed during the end of the experiment due to either abamectin or HP treatment in the present study may be due to its consumption in scavenging the generated ROS which may indicate the delayed effect of these pesticides on enhancing oxidative stress (Ilhan *et al*., 2005). Similar results were mentioned in *S. littoralis* treated with pyriproxyfen (Fahmy, 2012) and in *B. thuringiensis* treated *Aedes caspius* larvae (Ahmad, 2011).

Concerning GST activity, Figure (3) shows a significant increase on the 2nd and 3rd day post treatment with abamectin up to 35.15 and 13.92 % followed by a significant decrease at the rest of tested time intervals reaching 16.45 and 16.38 % less than that of control on the 4th and 5th days, respectively. On the other hand, HP treatment caused a highly significant increase by 92.68 % on the 1st day post treatment relative to the untreated ones. This increase was followed by significant decrease down to 73.71, 58.29 and 77.59% on the 3rd, 4th and 5th day of the treatment, respectively.

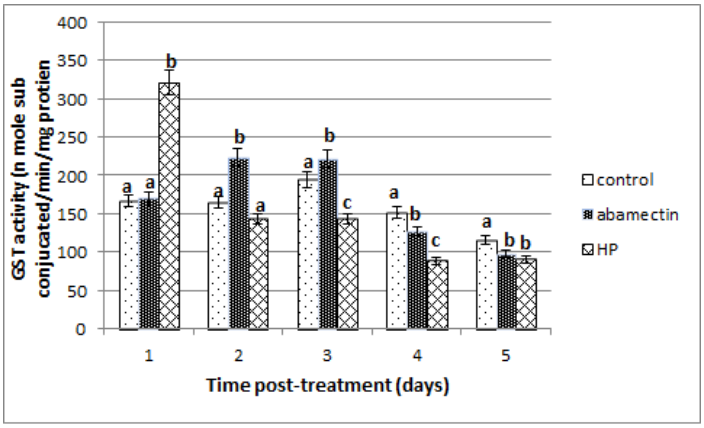

Fig. 3: Effect of LC_{50} of abamectin and hematoporphyrin on GST activity in 4tn larval instar *S. littoralis* tissue at different time intervals. Columns with different letters within each post-treatment day are significantly different ($P < 0.05$) using one-way ANOVA.

These results seem to agree with the results of Fahmy (2012) when *S. littoralis* larvae treated with two IGRs, buprofezin and pyriproxyfen, also Lu and Liu (2008) reported similar results when *Agrotis yplison* treated with celangulin-V. In the 3rd instar of *Parasarcophaga argyrostoma*, the activity of GST was reduced by HP treatment to 39.92%, 46.12%, and 43.44% relative to the controls (Abdelsalam *et al.,* 2014). Also, Memarizadeh *et al.* (2014) reported that GST was significantly decreased compared to the control when *Glyphodes pyloalis* treated with TiO_2-nanoparticles. These findings support the present results on the antioxidant action of abamectin and HP.

On the other hand, the decrease in GST detected in the present experiment may be due its consumption in consuming ROS. GST showed persisted increase in its activity may be to overcome the deleterious effect of accumulating MDA (Fahmy, 2012). Similarly, Mukanganyama *et al*. (2003) reported that DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) decreased GST activity in vitro and in vivo by 33% and 30%, respectively in *Rhopalosiphum padi*.

Activity of CAT in larval tissues of *S. littoralis* in the present work was detected as seen in Figure (4). In case of abamectin treated larvae, CAT was significantly increased compared to the control from the onset of the experiment till the end of the 4th day post-treatment reached its maximum level on the 2nd day (199.3%). On the other hand, HP treatment caused a profound highly significant increase in the activity of CAT, reaching its maximum activity on the 1st and 2nd day of treatment by about 126.99 and 200 %, respectively. On the 5th day, significant decreases were detected in both pesticides as compared to the untreated larvae.

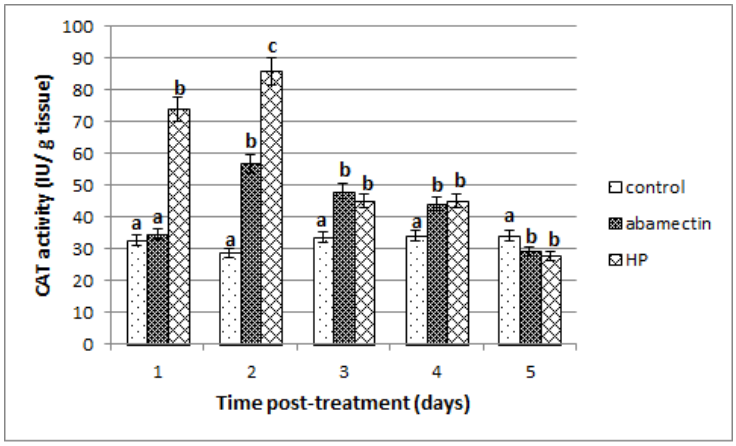

Fig. (4): Effect of LC_{50} of abamectin and hematoporphyrin on CAT activity in 4th larval instar *S. littoralis* tissue at different time intervals. Columns with different letters within each post-treatment day are significantly different ($P < 0.05$) using one-way ANOVA.

Our results are congruent with the results mentioned by Fahmy (2012) in larval tissues of *S. littoralis* when treated with buprofezin and pyriproxyfen, which could be correlated to increased levels of peroxide content (Krishnan and Kodrik, 2006). However, CAT activity in3rd instar larvae, male and female of *P. argyrostoma* was reduced by HP treatment to 31.98, 75.06, and 52.86 %, respectively (Abdelsalam *et al.,* 2014). Also, CAT activity in *G. mellonella* larvae infected with bacteria showed a significant decrease during the whole experimental period (Dubovskiy *et al.*, 2008).

The decrease of CAT activity on the 5th day post-treatment with abamectin and HP, may be due to the fact that CAT is known to be inhibited by the accumulation of superoxide anion during destruction processes (Kono and Fridovich, 1982) which may be caused by both tested pesticides. Porphyrin inactivated CAT through the production of singlet oxygen, which caused amino acid damage (Hirakawa *et al.,* 2013). Our results were supported by Fahmy (2012) who suggested that increased production of free radicals may lead to depletion or inactivation of CAT enzyme. Decreased activity of CAT also was detected due to high level of superoxide radical generation during oxidative stress in the acute stage of bacteriosis in *G. mellonella* (Dubovskiy *et al.*, 2008).

In conclusion, the present study may focus upon the induction of lipid peroxidation due to the treatment of 4th instar *S. littoralis* larvae with the tested eco-friendly pesticides, abamectin and HP, as it caused a significant increase in the MDA level which is an evidence of the induction of oxidative stress. Also, this study indicates an enhancement of the insect antioxidant system for scavenging ROS resulted from oxidative stress of abamectin and HP. Both pesticides worked more or less in a similar manner on the tested biomarkers taken in the present study. Finally, further studies are recommended for more usage of biomarkers explaining exactly the dynamics between oxidative stress exerted of such environmentally save pesticides against that horrible pest *S. littoralis* armed with its potent antioxidant properties.

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