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Impact of two insect growth regulators on the enhancement of oxidative stress and antioxidant efficiency of the cotton leaf worm, *Spodoptera littoralis* (Biosd.)

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ABSTRACT

The present study was conducted for evaluating of the impact of two insect growth regulators (IGRs) namely, Applaud (buprofezin) as a chitin synthesis inhibitor and Admiral (pyriproxyfen) as juvenile hormone analogue (JHA) in the larval body of the cotton leaf worm, *Spodoptera littoralis.* This evaluation was achieved via (1) Estimating the antioxidant system response present in the 4th larval instar of *S. littoralis* through estimating the activity of two enzymes; catalases (CAT) and glutathione-S-transferase (GST), as well as an antioxidant compound; glutathione reduced (GSH). (2) Estimating the accumulated lipid peroxidation in the larval tissues by evaluating the level of Malonaldehyde (MDA) as an indicator for lipid peroxidation. Both tested IGRs used in this study showed more or less similar trend in their mode of action relative to the tested biomarkers in the present work. CAT showed a significant increase in its activity (42.02%) and (139.26%) for buprofezin and pyriproxyfen, respectively. This activity lasts for only one day post treatment then it was inhibited to be very close to that level in normal untreated larvae. This may be due its consumption in scavenging reactive oxygen species (ROS) produced due to significant accumulation of MDA. One the other hand, GST showed persisted increase in its activity especially with buprofezin treated larvae may be to overcome the deleterious effect of accumulating MDA. Similarly, GSH which serves as a free radicals scavenger also showed a significant increase in its level especially due to treatment. The present study which is conducted for the first time, documented the occurrence of lipid peroxidation due to IGRs treatment in the larval tissues in *S. littoralis* larvae which enhanced different antioxidant defensive system to overcome its effect.

Keywords:

INTRODUCTION

Oxidative stress in biological systems is caused by reactive oxygen species (ROS), such as superoxide anion radicals, hydroxyl radicals and hydrogen peroxide, generated during normal oxidative processes in cells and extracellular fluids. In the normal situation, a balance exists between production and elimination of ROS. ROS are highly reactive molecules due to the presence of unpaired valence shell electrons, that indiscriminately interact with essential macromolecules, such as DNA, proteins and lipids specially those in cell membrane, leading to the disturbance of physiological processes (Cnubben *et al.*, 2001).

ROS production can exert adverse effects in different living organisms, although most studies have been conducted in mammalian systems (Livingstone, 2001). The exposure to contaminants can enhance the intracellular formation of ROS, which are able to originate or induce oxidative damage to biological systems (Di Giulio *et al.*, 1989). Under environmental stress, e.g. bacterial infections, xenobiotic exposure,

pathogenesis, ROS levels may increase dramatically, resulting in significant damage to cell structures. This process what is known as oxidative stress (Wang *et al*., 2001).

Herbivorous insects are also challenged by exogenous ROS that are part of the plant defense against them (Krishnan, and Kodrik, 2006) and the pathogens (Doke *et al*., 1996). High ROS concentration impairs the absorption of ingested nutrients and can cause oxidative damage to the midgut cells (Bi and Felton, 1995). Oxidative stress during the viral pathogenesis of insect cell lines has been described previously (Wang *et al.,* 2001). Indeed, oxidative stress is associated with aging and senescence (Arking *et al*., 2000).

Pesticides exert their biological effects via generation of ROS (Sayeed *et al.,* 2003). Bagchi *et al*. (Bagchi *et al.,* 1995). Oxidative stress has been shown to be associated with exposure to several organophosphorous compounds (Hai *et al*., 1997) and different classes of pyrethroids (Main, and Mulla, 1992).ROS cause lipid peroxidation; protein, enzyme, and DNA oxidation; and glutathione (GSH) depletion, leading to oxidative damage in insect tissues (Ahmad, 1995).

A suite of biochemical defense mechanisms called the antioxidant defense system is found in different organisms to prevent cellular damage from ROS. Both antioxidant enzymes such as catalases (CAT), peroxidases (POX) superoxide dismutases (SOD) and glutathione-S-transferase (GST) and nonenzyme antioxidants such as ascorbic acid, glutathione reduced (GSH), ascorbic acid (vitamin C) etc., counteract the deleterious action of ROS and capable of scavenging them (Kale *et al.,* 1999) to prevent cellular and molecular damage (Livingstone, 2001). On the other hand, Lipid peroxidation is one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity of many xenobiotics (Anane and Creppy, 2001). Oxidative stress is defined as an imbalance between higher cellular levels of ROS and the cellular antioxidant defense (Ilhan *et al*., 2005). The status of lipid peroxidation and antioxidants in an organism reflects the dynamic balance between the antioxidants defense and pro-oxidant conditions, which serves as a useful index for assessing the risk of oxidative damage (Vijayavel *et al*., 2006).

Insects can catabolize toxic or other detrimental chemicals that endogenously produce ROS for survival in a chemically unfriendly environment. Insects express a suite of antioxidant enzymes that protect cells from the damaging effects of oxidative stress.

Research on the cellular antioxidative defenses of herbivorous insects has established that many of the same previously mentioned enzymes and compounds are also present in insect cells (Ahmad, 1992; Felton and Summers, 1995).

CAT has been detected in three species of lepidopteran larvae (Felton and Duffey, 1991). It has the ability to be the main scavenger of hydrogen peroxide and quickly converts it to water and oxygen.

GST has a wide range of substrate specificities among antioxidant enzymes involved in detoxification of xenoxenobiotics (Vontas *et al*. 2001).

GSH plays a multifunctional role in antioxidant protection, acting as a cofactor for a number of antioxidant enzymes, and directly scavenging ROS and peroxides (Felton, 1995).

Malonaldehyde (MDA) is one of indicators of lipid peroxidation, and it also reacts with DNA, protein, enzyme, and other biomolecules, leading to oxidative damage (Janero, 1990). Any variability in MDA determinations may arise from variability in nonenzymatic chemical events yielding lipid peroxide products. Some of these products may increase or decrease activities of specific antioxidant enzymes.

Similar variability in activities of various detoxification enzymes in some moths were obtained by various dietary supplements (Hemming and Lindroth, 2000).

On the other hand, insect growth regulators (IGRs) were described as agents that they exert their primary action on insect metabolism, ultimately interfering the processes of molting and metamorphosis of insects, particularly when applied during the sensitive period of insect development (Ishaaya and Horowitz, 1997).

Thus, the current work aimed to quantitatively evaluate the oxidative stress which may be caused via application of two IGRs namely; applaud (buprofezin) as a chitin synthesis inhibitor and admiral (pyriproxyfen) as juvenile hormone analogue (JHA) against the larvae of the cotton leaf worm, *Spodoptera littoralis.* This evaluation will be achieved via the assessment of the activity of two antioxidant enzymes; CAT and GST as well as the levels of the antioxidant; GSH besides lipid peroxide evaluation as a representative to the exerted oxidative stress of these two IGRs.

MATERIALS AND METHODS

Insects:

Early 4th instar larvae of *S. littoralis* were obtained from the cotton leaf worm rearing laboratory, Plant protection research institute, Agricultural research center. They were maintained under crowded conditions at 28±2ºC and 16h light: 8h dark photoperiod.

The tested insect growth regulators:

A-Chitin synthesis inhibitors:-

Buprofezin (Applaud 25% WP): 2-((1,1-dimethylethyl)imino) tetrahydro-3-(1-methylethyl)-5-phenyl-4H-1, 3, 5-thiadiazin-4-one.

B- Juvenile hormone analogue: -

Pyriproxyfen (Admiral 10 % EC): 2-(1-methyl-2-(4- phenoxyphenoxy) ethoxy) pyridine.

Bioassays and treatments:

To determine the proper concentrations of the two IGRs used in this study, bioassays were initially performed using 4th instar larvae of *S. littoralis* by a dispersible concentrate formulations of each IGR diluted with distilled water to adjust the concentration from 10^{-2} to $10,^{-5}$ using dipping technique of clover leaves in each concentration for 10 seconds then air dried. Control leaves were treated similarly using only distilled water. 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. LC_{50} for each IGR was recorded after three days and calculated by using probit analysis (Finney, 1971).

Early 4th instar larvae of the cotton leaf worm, *S. littoralis* were divided into 2 groups, control and treated groups. Larvae of control group were daily fed upon clover leaves treated previously with distilled water only (by the same manner described above) throughout the experimental protocol.

Insects of treated group were subdivided into two divisions; the first one were allowed to feed upon clover leaves treated previously with LC50 (6309.6 ppm) of buprofezin and the second one with LC_{50} (3162.3 ppm) of pyriproxyfen. About six to eight insects were taken daily from each treatment as well as control group to be prepared for biochemical analysis.

Biochemical Analysis:

Sample preparation:

Early 4th larval instar of *S. littoralis* worms were taken to be tested for each IGR on five successive days post treatment. Each day post treatment, larval bodies were homogenized (1gm of tissue in 5 ml of distilled water), using hand glass homogenizer on ice jacket. The body homogenate was centrifuged using Eppendorf refrigerated 5415 (Hamburg, Germany) at 8000 rpm for 15 min. at 2°C.

Biochemical tests:

Determination of enzyme 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 a water bath at 37◦C for10 min. After incubation, 0.1 ml of reduced glutathione (30 mmol/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). Catalase reacts with a known quantity of hydrogen peroxide and the reaction is stopped after 1 min with catalase inhibitor. In the presence of peroxidase, the remaining hydrogen peroxide reacts with 3,5- Dichloro-2- hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with a color intensity inversely proportional to the amount of CAT in the sample. The absorbance was measured at 510 nm.

Determination of lipid peroxidation and glutathione reduced:

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

Lipid peroxidation (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 the malondialdehyde (MDA) release served as the index of LPO. MDA was determined by measuring the thiobarbituric acid reactive species. The absorbance of the resultant pink product was measured at 534nm in a helios alpha thermospectronic (UVA 111615, Cambridge, UK).

Statistical analysis:

The data were expressed as means ± standard error of the mean (S.E.M.). Data were analyzed by Student's t- test. The difference between means was significant at $p < 0.05$. Percentage difference representing the percent of variation with respect to the control was calculated : % difference = (treated mean– control mean/control mean)×100.

RESULTS

Changes in CAT activity in the body tissues of the 4th instar of *S. littoralis* larvae were monitored for five days post treatment by each of the tested IGR; buprofezin and pyriproxyfen.

The data represented in Table (1) show a significant elevation in CAT activity at the onset of the experiment for both tested IGRs. It increased by 42.02 and 139.26% due to buprofezin and pyriproxyfen application after one day post treatment respectively. Later on, CAT activity showed insignificant differences in both tested IGRs in all subsequent time intervals relative to control.

Table 1: Effect of IGRs treatment on catalase activity (U/g tissue) in larvae of the 4th instar of *S. littoralis*.

Insecticide	Days post treatment														
	1st			2nd			3rd			4th			5th		
	Cont.	Treat.	%D	Cont.	Treat.	%D	Cont.	Treat.	%D	Cont.	Treat.	%D	Cont.	Treat.	%D
Buprofezin	a 32.6± 3.05	b 46.3± 4.72	+42.02	a 28.6± 1.15	a 31.5± 3.21	+10.14	a 33.6± 2.65	a 28.42± 2.66	−15.42	a 34.02± 3.85	a 29.4± 1.85	−13.58	a 34± 4.16	a 34± 4.50	0.00
Pyriproxyfen		c 78± 1.52	+139.26		a 33.33± 4.93	+16.54		a 37± 4.26	+10.42		a 35.7± 2.02	+4.94		a 35.3± 2.08	+3.82

Values represent mean±S.E.M.
$p < 0.05$ significant.
A, B and C: different letters mean significant changes between treated measures of the same day.
%D: percentage difference between treated and control.
U: enzyme unit.

Regarding to GST activity shown in Table (2), the present study showed that the insecticide buprofezin caused a significant elevation in the enzyme activity which lasted four days post treatment and reached its maximum activity in the 2nd day up to 109% compared to control. On the other hand, GST had an insignificant decline in its activity by 4.31% on the 5th day post treatment. The same trend was detected in case of the second insecticide; pyriproxyfen as a significant increase was achieved too but for the first three days post treatment then an insignificant decrease was found on both 4th and 5th day after treatment compared control.

Table 2: Effect of IGRs treatment on GST activity (U/g tissue) in larvae of the 4th instar of *S. littoralis*.

Insecticide	Days post treatment														
	1st			2nd			3rd			4th			5th		
	Cont.	Treat.	%D	Cont.	Treat.	%D	Cont.	Treat.	%D	Cont.	Treat.	%D	Cont.	Treat.	%D
Buprofezin	b 166.6± 4.93	a 310± 19.65	+86.07	c 165± 8.32	a 346± 18	+109.70	b 194± 6.02	a 273.6± 12.88	+41.03	b 152± 5.29	a 188± 6.24	+23.68	a 116± 7.37	a 111± 6.42	-4.31
Pyriproxyfen		a 291± 10.14	+74.67		b 293± 7.23	+77.58		b 197± 6.65	+1.55		b 132± 10.40	-13.16		a 110± 5.29	−5.17

Values represent mean±S.E.M. with the number of larvae between parentheses.
$P < 0.05$ significant.
A, B and C: different letters mean significant changes between treated measures of the same day.
%D: percentage difference between treated and control.
U: enzyme unit.

Data presented in Table (3) revealed that a significant increase in the level of GSH was detected on the 1st and 2nd days post treatment with buprofezin up to 55.72 and 73.08 %, respectively compared to control. On the other hand, insignificant quantitative fluctuations were found along the rest of the tested time intervals. Concerning pyriproxyfen application, similar trend was detedcted where a significant elevation in the GSH levels was detected during the first three days post treatment compared to control. This increase ranged from 16.75 up to 175.48% as related to levels of GSH in the untreated larval tissues. Pyriproxyfen caused fluctuations on the 4th and 5th days post treatment with no marked difference with those found in the control.

Table 3: Effect of IGRs treatment on GSH level (mg/g tissue) in larvae of the 4th instar of *S. littoralis*.

Insecticide	Days post treatment														
	1st			2nd			3rd			4th			5th		
	Cont.	Treat.	%D	Cont.	Treat.	%D	Cont.	Treat.	%D	Cont.	Treat.	%D	Cont.	Treat.	%D
Buprofezin	b 6.91± 0.26	a 10.76± 0.43	+55.72	c 6.24± 1.185	b 10.8± 0.99	+73.08	b 6.33± 0.33	b 5.75± 0.292	−9.16	ab 5.48± 0.28	b 4.75± 0.28	−13.32	a 4.77± 0.24	a 4.97± o.24	+4.19
Pyriproxyfen		a 12.75± 1.68	+84.52		a 17.19± 2.40	+175.48		a 7.39± 0.33	+16.75		a 5.92± 0.29	+8.03		b 4± 0.12	−16.14

Values represent mean±S.E.M. with the number of larvae between parentheses.
$P < 0.05$ significant.
A, B and C: different letters mean significant changes between treated measures of the same day.
%D: percentage difference between treated and control.

As shown in Table (4), the level of MDA revealed a significant increase by 91.88 and 16.28 % due to buprofezin application in the 1st and 2nd day, respectively. Again non significant changes were observed during the rest of the studied time intervals till the 5th day post treatment. On the other hand, pyriproxyfen treatment causes a significant elevation in the level of MDA at all the studied intervals except for the 1st day after treatment. The maximum recorded elevation was on 3rd day where it reached 89.23% as compared to the untreated case.

Table 4: Effect of IGRs treatment on MDA level (nmol/g tissue) in larvae of the 4th instar of *S. littoralis*.

Insecticide	Days post treatment														
	1st			2nd			3rd			4th			5th		
	Cont.	Treat.	%D	Cont.	Treat.	%D	Cont.	Treat.	%D	Cont.	Treat.	%D	Cont.	Treat.	%D
Buprofezin	c 5.91± 0. 34	a 11.34± 1.43	+91.88	c 6.79± 0.41	b 8.11± 0.25	+16.28	b 6.78± 0.59	b 6.02± 0.64	−11.21	b 6.61± 0.25	b 7.55± 0.69	+14.22	a 6.37± 0.17	a 6.68± 0.67	+4.87
Pyriproxyfen		c 6.03± 0.21	+2.03		a 9.65± 0.14	+42.12		a 12.83± 0.46	+89.23		a 9.65± 0.31	+45.99		a 7.58± 0.69	+19.00

Values represent mean±S.E.M. with the number of larvae between parentheses.
$P < 0.05$ significant.
A, B and C: different letters mean significant changes between treated measures of the same day.
%D: percentage difference between treated and control.

DISCUSSION

To the best of our knowledge, no previous studies were conducted to elucidate the effect of IGRs upon the antioxidants system in insects. Thus the present study showed a pioneer trial for achieving the potentiality of these compounds in exreting oxidaitive stress in the larval tissues of *S. littoralis.*

The present data showed that treatment of 4th larval instar of *S. littoralis* with the two IGRs; buprofezin and pyriproxyfen caused a significant increase in CAT activity on the 1st day post treatment for both compounds. The subsequent tested time intervals showed insignificant changes in CAT activity relative to the control. CAT is the main scavenger of hydrogen peroxide at high concentration (Kono and Fridorich, 1982).

CAT catalyzes the conversion of hydrogen peroxide to water and molecular oxygen (Dringen and Metabolism, 2000).). Hence, the increase in CAT activity after treatment of both buprofezin and pyriproxyfen treatment could be expected in order to scavenge hydrogen peroxide. Similar results were found in the gut lumens of different Lepidopterans where CAT activity was enhanced in gut contents and tissues due to

plant diet fed larvae which could be correlated to increased levels of peroxide content (Krishnan and Kodrik, 2006). However, CAT activity in *Galleria mellonella* larvae infected with bacteria showed a significant decrease during the whole experimental period (Dubovskiy *et al.*, 2008).

The decrease of CAT activity after the first day post treatment was more or less similar to control, 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 IGRs as the authors suggested that increased production of free radicals may lead to depletion or inactivation of CAT enzyme. CAT in the midgut of *Lymantria dispar* larvae fed on an unfavorable plant has shown similar results (Peric-Mataruga *et al*., 1997). 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).

GSTs are a family of enzymes that catalyze the addition of the tripeptide glutathione to endogenous and xenobiotic substances which have electrophilic functional groups. They play an important role in the detoxication and metabolism of many xenobiotic and endobiotic compounds (Yousef, 2004). The present data obtained during tracing GST activity in *S. littoralis* larval tissues after treatment with both IGRs, buprofezin and pyriproxyfen showed that the former IGR showed a continues significant increase till the fourth day post treatment and by the fifth one, GST activity decreased to be with slight insignificant changes with control. On the other hand the IGR, pyriproxyfen showed a significant elevation in GST activity for two days only after treatment then its activity was inhibited by the third day to be with insignificant differences with untreated larvae.

This finding may prove that GST is involved in the inactivation of toxic lipid peroxidation products accumulated during destructive processes caused by both IGRs. Our reasults are in accordance with those obtained in *G. mellonella* midgut in the early stage of bacteriosis (Dubovskiy *et al.*, 2008). In particular, as other authors have shown that GST may eliminate organic hydroperoxide from cells and defend cells from potential damage from the products of lipid peroxidation (Morrissey and O'Brien, 1980). Similarly, celangulin-V_showed higher induction against *Agrotis yplison* (Lu *et al*., 2008). In contrast, Mukanganyama *et al*. (2003) investigated the effect of DIMBOA (2,4-dihydroxy-7-methoxy- 1,4-benzoxazin-3-one) on the aphid *Rhopalosiphum padi*, and the results showed that DIMBOA decreased GST activity in vitro and in vivo by 33% and 30%, respectively.

GSH in the present work showed a significant elevation in its concentrations in the first two days after treatment with buprofezin while no significant changes were detected in subsequent days post treatment. On the other hand, pyriproxyfen showed an elevation lasts for three days after treatment. By the fourth day, GSH concentration decreased till it reaches insignificant difference with control larvae. On the 5th day post treatment, a significant decrease was detected relative to untreated larvae.

GSH is of importance for protection of cells against oxidative stress and xenobiotics by scavenging free radicals and other ROS. The reduction of peroxide depends on GSH, which in this process is converted to oxidized glutathione (GSSG). In addition, it can act as a substrate in various enzymatic antioxidant defense mechanisms (Dringen, 2000). GSH production is one of the main mechanisms that allow larval stage to survive in this contaminated environment (Poupardina *et al.*, 2008).

Induction of detoxification enzyme activities and elevated GSH level in midguts are also examples of lepidopteran physiological responses to dietary toxicants (Peric´-

Mataruga *et al.,* 1997). Thus observed elevation in GSH concenterations in the present work may be due to the fact that it can react non-enzymatically with different ROS and functions directly as a free radical scavenger (Winterbourn, Metodiewa, 1994).

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) and insecticide- resistance (Prapanthadara *et al.,* 1995). Many studies of insect GSH revealed multiple forms exist in different types of insectssuch as housefly and grass grub (Clark *et al.,* 1985).

These different forms of GSH exhibited varying specificities for different insecticides. Prapanthadara *et al.* (1995) demonstrated that in a DDT-resistant strain of the African mosquito *Anopheles gambiae*, there was an eight-fold increased in DDT treatment due to an increase synthesis of different isoenzymes of glutathione that possessed a greater level of this activity.

Beside playing a central role in the metabolism of insecticides and other xenobiotics (Hemingway *et al*., 2004), GSH constitutes a second line in insect immunity as it plays a role in the detoxification of toxins in insect body, including toxic immune compounds that involve melanin beside protecting insects from the concomitant oxidative stress (Kumar *et al.,* 2003). Moreover, higher titer of GSH in treated larvae (insect name) could be attributed to the environmental stress due to pollutants or to the oxidative stress (Jovanovic-Galovic *et al*., 2004).

On the other hand, lower titer of GSH observed during the end of pyriproxyfen treatment in the present study may be due to its consumption in scavenging the generated ROS which may indicate the delayed effect of pyriproxyfen on enhancing oxidative stress. Similar results were mentioned in *Bacillus thuringiensis* (BT) treated *Aedes caspius* larvae where Bt inhibited induction of the anti-stress factor, the GSH, as an effective larvicidal toxic mechanism, and hence, larvae died within 24 h post-treatment (Ahmad, 2011). Also, The possible reason for the observed rise in the GST level in the tissues of *Penaeus monodon* exposed to fenvelerate might be due to the depletion of GSH as a result of oxidative stress (Vijayavel and Balasubramanian, 2009).

As GSH plays a critical role in cell viability through the regulation of membrane permeability by maintaining sulfhydryl groups in the reduced state. This suggests that the oxidative stress and GSH depletion affects redox status in cell membranes resulting in protein inactivation by oxidation of thiols (Vijayavel and Balasubramanian, 2006). GST is an important enzyme that catalyzes the conjugation of xenobiotics with the help of GH, thereby facilitating their elimination from cell organelles. Moreover, It has been reported that the exposure to ROS may raise the GSH content by increasing the GSH synthesis (Rahman *et al.,* 1996). The reduced form of GSH in larval midguts of *G. mellonella* in response to penicillin toxicity may be oxidized by ROS and organic peroxides to Glutathione disulfide (GSSG). Elevation of GSH and decreased GSSG levels during penicillin exposure may reflect a protective mechanism against cellular injury in midgut caused by antibiotics (Buyukguzel and Kalender, 2007).

The present study expressed lipid peroxidation levels by MDA content. MDA is the major aldehyde metabolite of lipid peroxidation (Paradis *et al.,* 1997). MDA in the present work showed a significant elevation in its concentrations in the first two days after treatment with buprofezin while starting from the third day with no significant changes in its concentrations were detected till the end of the period of the present study. Similar results have been obtained with Bt infection in *S. littoralis* larvae one day post treatment (Boctor and Salama, 1983). On the hand, pyriproxyfen showed an

insignificant change on the first day after treatment but a dramatic increase in its concentrations was found on the subsequent three days i. e. from the second to the fourth day post treatment. By the fifth day, MDA concentration decreased till insignificant difference was obtained relative to control larvae. This may due to again the delayed action of pyriproxyfen. Similar findings were observed where an elevated MDA content as a measure of oxidative challenge in the midgut of *G. mellonella,* may result from the accelerated ROS production and impaired antioxidative protection after exposure to penicillin (Buyukguzel and Kalender, 2007) as the author found increased midgut MDA content in all larval stages exposed to higher dietary concentrations of penicillin.

The oxidative destruction of lipids acts in a chain reaction to form lipid hydroperoxides,which can decompose to MDA as an end-product (Cheeseman, 1993).

The decrease in lipid peroxidation during the last three days in case of treatment with applaud and on the 5th day only post treatment of pyriproxyfen may be due to an increase in antioxidant defense. An increase in the ratio of oxidized to reduced thiols has been demonstrated, together with an increase in lipid peroxidation processes against a background of an increase in cell death (Wang *et al*., 2001). These results demonstrate that enhanced activities of CAT and GST can lead to the elimination of ROS. The increase in GST activity in the larval midgut of different lepidopteran insects on the first day due to viral infection was also observed simultaneously with the increased concentration of MDA (Wang *et al.,* 2001).

In *G. mellonella* larvae. We found that Bt infection resulted in increased activities of GST, MDA and GSH ratio the first day after inoculation. However, CAT activity decreased on the first and following days after bacterial infection by Bt. the authors hypothesized that Bt infection increases the level of oxidative stress in the larval midgut.

However, the data of the present investigation showed that the treatment with both IGRs, caused a significant increase in the MDA level which is an evidence of the induction of oxidative stress. The increase in GST and CAT activities accompanied by a significant increase GSH content may be an attempt to counteract the increase in MDA level as a defense mechanism by cells against free radicals generation (Vijayavel and Balasubramanian, 2009).

Consequently, increased oxidative stress leads to an enhanced regulation of antioxidants, such as CAT and GST and these processes had been mirrored in insect physiological adaptations and resistance. Parallel changes were detected due to particularly protein modification, changes in the synthesis of specific larval gut proteins, and increased oxidative metabolism (Loseva *et al.,* 2001and Candas *et al.,* 2003). In herbivorous insects the situation is exacerbated by the ingestion of pro-oxidant allelochemicals which are eliminated at the cost of increased oxidative stress (Felton and Summers, 1995). Lepidopteran larvae such as *S. littoralis* could maintain gut conditions that enable them to maximize extraction of nutrients from plant tissues while minimizing any deleterious effects of prooxidant secondary plant metabolites. Much of the oxidative stress results from oxidative injury attributed to hydrogen peroxide (Peric´ - Mataruga *et al*., 1997).

Therefore, it could be concluded that, CAT showed a significant increase in its activity for both buprofezin and pyriproxyfen and this activity lasted only for one day post treatment then it was inhibited to be very close to normal untreated level. This may be due its consumption in scavenging reactive oxygen species (ROS) produced due to significant accumulation MDA. One the other hand, GST showed persisted increase in its activity especially with buprofezin treated larvae may be to overcome

the deleterious effect of accumulating MDA. Similarly, GSH which serves as a free radicals scavenger also showed a significant increase in its level especially due to treatment.

Obviously, the treatment of *S. littoralis* larvae with buprofezin and pyriproxyfen in the present study may represent a model for induction of lipid peroxidation and an enhancement of the insect antioxidant system for scavenging ROS redulted due to oxidative stress. Both IGRs used here worked more or less in a similar manner in the tested biomarkers in the present study. However, further studies are recommended for the evaluation of the antioxidant properties of different insect control agents to study different models of oxidative stress.

REFRENCES

Aebi H. (1984). Catalase in vitro. Methods Enzymol., 105: 121-126.

Ahmad, S. (1995). Oxidative stress from environmental pollutants. Arch. Insect Biochem. Physiol. 29: 135-157.

Ahmed, A. M. (2011). Immune and antioxidant defenses in an autogenous *Aedes caspius* mosquito upon infection with *Bacillus thuringiensis* kurstaki. African J. Microbiol. Res. 5(22): 3848-3857.

Anane R and Creppy EE (2001). Lipid peroxidation as pathway of aluminium cytotoxicity in human skin fibroblast cultures: prevention by superoxide dismutase, catalase and vitamins E and C. Hum Exp. Toxicol. 20:477-481.

Arking, R., Burde, V., Graves, K., Hari, R., Feldman, E., Zeevi, A., Soliman, S., Saraiya, A., Buck, S., Vettraino, J., Sathrasala, K. (2000). Identical longevity phenotypes are characterized by different patterns of gene expression and oxidative damage. Exp. Gerontol. 35: 353-373.

Bagchi, D., Bagchi, M., Hassoun E.A., and Stohs, S.J. (1995). In vitro and in vivo generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides, Toxicol. 104: 129-140.

Beutler, E., Duron, O., Kelly, B.M.(1963). Improved method for the determination of blood glutathione. J Lab Clin Med. 61: 882-888.

Bi, J.L., Felton, G.W. (1995). Foliar oxidative stress and insect herbivory: primary compounds, secondary metabolites and reactive oxygen species as components of induced resistance. J. Chem. Ecol. 21:1511-1530.

Boctor, I.Z., Salama, H.S. (1983). Effect of *Bacillus thuringiensis* on the lipid content and compositions of *Spodoptera littoralis* larva. J. Invert. Pathol. 51:381-384.

Buyukguzel, E. and Kalender, Y. (2007). Penicillin-Induced Oxidative Stress: Effects on Antioxidative Response of Midgut Tissues in Instars of *Galleria mellonella*. J. Econ. Entomol. 100(5): 1533-1541.

Candas, M., Loseva, O., Oppert, B., Kosaraju, P. and Bulla, L.A.(2003). Insect resistance to *Bacillus thuringiensis*: alterations in the Indian meal moth larval gut proteome. Mol. Cell. Proteomics. 2, 19–28.

Cheeseman, K. H. (1993). Lipid peroxidation in biological system, *In* B. Halliwell and O. K. Aruoma [eds.], DNA and free radicals. Ellis Horwood, London, United Kingdom. pp. 201-211.

Clark, A.G., Dick, G.L., Martindale, S.M., Smith J.N. (1985). Glutathione transferases from the New Zealand grass grub, *Costelytra zealandica*. Their isolation and characterization and the effect on their activity of endogenous factors. Insect Biochem. 15: 35-44.

Cnubben, H.P., Rietjens, C.M., Wortelboer, H., van Zenden, J. and van Bladeren, P.J..(2001). The interplay of glutathionerelated processes in antioxidant defence, Environ. Toxicol. Pharmacol. 10:141-152.

Di Giulio, R.T., Washburn, P.C., Wenning, R.J., Winston, G.W. and Jewell, C.S. (1989). Biochemical responses in aquatic animals: a review of oxidative stress, Environ. Toxicol. 8: 1103-1123.

Doke, N., Miura, Y., Sanchez, L.M., Park, H. J., Noritake, T., Yoshioka, H. and Kawakita, K. (1996). The oxidative burst protects plants against pathogen attack: mechanism and role as an emergency signal for plant bio-defence. Gene 179: 45-51.

Dringen R. (2000). Metabolism and functions of glutathione in brain. Prog. Neurobiol. 62(6):649-71.

Dubovskiy, I.M., Martemyanov, V.V. , Vorontsova, Y.L. , Rantala, M.J., Gryzanova, E.V. and Glupov, V.V. (2008). Effect of bacterial infection on antioxidant activity and lipid peroxidation in the midgut of *Galleria mellonella* L. larvae (Lepidoptera, Pyralidae). Comp. Biochem. Physiol. 148(1): 1–5.

Felton, G.W. and Duffey, S.S. (1991). Protective action of midgut catalase in lepidopteran larvae against oxidative plant defenses. Journal of Chemical Ecology 17: 1715-1732.

Felton, G. W. 1995. Oxidative stress of vertebrates and invertebrates, pp. 356:434. In Oxidative stress and antioxidant defenses in biology (ed S. Ahmad), Chapman and Hall, New York, pp 356-424.

Finney, D. J. (1971). Probit analysis, 3rd ed., Cambridge University Press, London.

Habig W.H., Pabst M.J. and Jakoby W.B. (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol. Chem. 249(22):7130-7139.

Hai, D.Q. Varga, S.z.I. and Matkovics, M. (1997). Organophosphate effects on antioxidant system of carp (*Cyprinus carpio*) and catfish (*Ictalurus nebulosus*), Comp. Biochem. Physiol. 117: 83–88.

Hemingway J., Hawkes N.J., McCarroll, L. amd Ranson H (2004). The molecular basis of insecticide resistance in mosquitoes. Insect Biochem., Mol. Biol. 34: 653-665.

Hemming, J.D.C., and Lindroth, R.(2000). Effects of phenolic glycosides and protein on gypsy moth (Lepidoptera: Lymantriidae) and forest tent caterpillar (Lepidoptera: Lasiocampidae) performance and detoxication activities. Environ. Entomol. 2: 1108:1115.

Ilhan A, Gurel A, Armutcu F, Kamisli S and Iraz M.(2005). Antiepileptogenic and antioxidant effects of *Nigella sativa* oil against pentylenetetrazolinduced kindling in mice. Neuropharmacol., 49(4):456-464.

Ishaaya, I. and Horowitz, A. R. (1997). Insecticides with novel mode of actions: Overview. In: "Insecticides with novel mode of actions, Mechanisms and application" Eds. By Ishaaya, I. and Degheele, D. , Berlin. pp 1- 39.

Janero, D. R. (1990). Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic. Biol. Med. 9: 515-540.

Jovanovic-Galovic A., Blagojevic D.P., Grubor-Lajsic G., Worland R. and Spasic MB (2004). Role of antioxidant defense during different stages of pre-adult lifecycle in European cornborer (*Ostrinia nubilalis*, Hubn.): diapause and metamorphosis. Arch. Insect Biochem. Physiol., 55: 79-89.

Kale, M., N. Rathore, S. John, and Bhatnagar, D. (1999). Lipid peroxidative damage on pyrethroid exposure and alteration in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species, Toxicol. Lett. 105(3):50-852.

Kono, Y. and Fridovich, I. (1982). Superoxide radical inhibits catalase. J. Biol. Chem. 257: 5751-5753.

Kumar S.(2003). The role of reactive oxygen species on *Plasmodium* melanotic encapsulation in *Anopheles gambiae*. Proc. Natl. Acad. Sci. USA., 100: 14139-14144.

Livingstone, D.R. (2001). Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms, Mar. Pollut. Bull. 42: 656-666.

Loseva, O., Ibrahim, M., Candas, M., Koller, N., Bauer, L.A. and Bulla, L.A. (2001). Changes in protease activity and Cry3Aa toxin binding in Colorado potato beetle: implications for insect resistance to *Bacillus thuringiensis* toxins. Insect Biochem. Mol. Biol. 32: 567-577.

Lu, M. W. and Liu, W, H. (2008). Effect of celangulin V on detoxification enzymes in *Mythimna separata* and *Agrotis ypsilon*. Pest. Biochem. Physiol. 90: 114-118.

Main, L.S. and M.S. Mulla (1992). Effects of pyrethroid insecticides on non-target invertebrates in aquatic ecosystems, J. Agri. Entomol. 9:129-140.

Meister, A. and Anderson M.E.(1983). Glutathione. Annu. Rev. Biochem. 52:711-760.

Morrissey, P., O'Brien, P.J.(1980). Selenium-independent glutathione peroxidase activity in rabbit liver. Can J. Biochem. 58:1012-1017.

Mukanganyama, S., Figueroa, C.C., Hasler, J.A. and Niemeyer, H.M. (2003). The effect of DIMBOA on detoxification enzymes of the aphid *Rhopalosiphum padi* (Homoptera: Aphididae), J.Insect Physiol. 49: 223-229.

Ohkawa H., Ohishi N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 95:351-358.

Paradis V., Mathurin P., Kollinger M., Imbert Bismut F., Charlotte F., Piton A., Opolon P., Holstege, A., Poynard, T. and Bedossa, P. (1997). Journal of Clin. Pathol. 50: 401-406.

Peric´-Mataruga, V., Blagojevic, D., Spasic, M. B., Ivanovic, J., and Jankovic-Hladni, M. (1997). Effect of the host plant on the antioxidative defence in the midgut of *Lymantria dispar* L. caterpillars of different population origins. J. Insect Physiol. 43: 101-106.

Poupardina R., Reynauda S. and Strodeb, C. (2008). Cross-induction of detoxification genes by environmental xenobiotics and insecticides in the mosquito *Aedes aegypti*: Impact on larval tolerance to chemical insecticides. Insect Biochem. Mol. Biol. (38): 540-551.

Prapanthadara L., Promtet, N., Koottathep S., Somboon P., Ketterman A.J. (1995). Isoenzymes of glutathione S-transferase from the mosquito *Anopheles* dirus species B: the purification, partial characterization and interaction with various insecticides. Insect Biochem. Mol. Biol., 30: 395-403.

Rahman I., Bel A., Mulier B., Lawson M.F., Harrison D.J., Macnee W. and Smith, C.A. (1996). Biochem Biophys. Res. Commun. 229: 832-837.

Vijayavel, K. and Balasubramanian, M.P. (2006). Changes in oxygen consumption and respiratory enzymes as stress indicators in an estuarine edible crab *Scylla serrata* exposed to naphthalene, Chemosphere 63(9): 1523-1531.

Vijayavel, K. and Balasubramanian, M.P.(2009). Effect of fenvalerate on oxidative stress biomarkers in the brackish water prawn *Penaeus monodon*. Pest. Biochem. Physiol. 95:113-116.

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

Wang, Y., Oberley, L.W. and Murhammer, D.W. (2001). Evidence of oxidative stress following the viral infection of two Lepidopteran insect cell lines. Free Rad.. Biol. Med. 31:1448–1455.

Winterbourn, C.C. and Metodiewa, D.(1994). The reaction of superoxide with reduced glutathione. Arch. Biochem. Biophys. 314: 284-290.

Yousef, M.I.(2004). Aluminium induced changes in hemato-biochemical parameters, lipid peroxidation and enzyme activities of male rabbits: protective role of ascorbic acid. Toxicol. 199(1):47-57.

ARABIC SUMMARY

تأثير اثنين من منظمات نمو الحشرات على تعزيز الاجهاد التأكسدىوالكفاءه المضاده للاكسده لدودة ورق القطن *سبودوبترا ليتوراليس*

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تمت هذه الدراسه بهدف تقييم تأثيراثنان من منظمات النمو الحشريه وهما الابلود (بابروفيزين)، المثبط لتخليق الكيتين والادميرال (بايريبروكسيفين)، نظير هرمون الشباب لدودة ورق القطن، *سبودوبترا ليتوراليس*. هذا التقييم تم من خلال (1) تقدير استجابة النظام المضاد للأكسده من خلال تقدير نشاط الانزيمين الكاتاليز والجلوتاثاين ترانسفيريس بالاضافه للمركب المضاد للاكسده، الجلوتاثين المختزل. (2) تقدير تراكم الدهون المؤكسده. وقد أظهرت الدراسه ان كلا من منظمى النمو المستخدمين فى الدراسه تشابها فى طريقة عملهما بالنسبه للعلامات الحيويه المستخدمه فى هذه الدراسه. وقد أظهر انزيم الكتاليز زياده ملحوظه وصلت الى 42.02% و139.26% لل(بابروفيزين) و(بايريبروكسيفين) بالترتيب واستمر هذا النشاط لمدة يوم واحد فقط بعد المعامله ثم تم تثبيط نشاطه حتى وصل الى حد مشابه للمجموعه الضابطه الغير معامله . هذا النقص الحادث فى نشاط الكتاليز ربما يكون نتيجة لتثبيطه فى التهام انواع الاكسجين التفاعلى الناتج عن الزياده فى تراكم المالونالدهيد. من ناحية اخرى استمر النشاط الزائد لانزيم الجلوتاثاين ترانسفيريس خصوصا مع البابروفيزين وقد يكون ذلك للتغلب على التاثير المدمر لمركب المالونالدهيد وكذلك الزياده الملحوظه فى للجلوتاثين المختزل بعد المعامله بمنظمى النمو التى ربما تكون لالتهام الشوارد الحره. وثقت هذه الدراسه لاول مره حدوث أكسده للدهون نتيجه استخدام منظمات النمو فى انسجة يرقة *سبودوبترا ليتوراليس* التى عززت من جهاز الدفاع المضاد للاكسده لمعادلة الزياده فى هذه الدهون المؤكسده.