Effect of Juvenile Hormone Analogue, Pyriproxyfen on Antioxidant Enzymes of Greater Wax Moth, *Galleria mellonella* (Lepidoptera: Pyralidae: Galleriinae) Larvae

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Abstract.- Insect growth regulators are mostly used in pest management since they are non toxic to other organisms and have short half life in the environment. Effects of juvenile hormone analogue, pyriproxyfen on catalse (CAT) and superoxide dismustase (SOD) activity of *Galleria mellonella* was investigated. Larvae were exposed to 0.0001; 0.0005; 0.001 and 0.005 mg/ml of pyriproxyfen for 24, 48 and 72 h and CAT and SOD activities were measured spectrophotometrically after treatment. Significant increases in CAT and SOD activity were observed in larvae exposed to 0.0005; 0.001 and 0.005 mg/ml pyriproxyfen for 24, 48 and 72 h. Changes in the activities of SOD and CAT after the juvenile hormone analogue application suggested that exposure to pyriproxyfen induced oxidative stress.

Keywords: Antioxidants, catalase, *Galleria mellonella*, pyriproxyfen, superoxide dismustase.

INTRODUCTION

Insect growth regulators (IGR) act as hormone analogues or anti-hormones and induce a variety of morphogenetic, developmental and reproductive effects in insects (Dhadialla *et al.*, 2005). Insecticides with growth regulating properties may adversely affect insects by regulating or inhibiting specific biochemical pathways or processes essential for insect growth and development. (Tunaz and Uygun, 2004).

Juvenile Hormone Analogues (JHA), such as sesquiterpenoid series of compounds act as insect growth regulators and are presently in use as potential environment friendly pesticides (Awasthi and Sharma, 2012). Some of juvenile hormone analogues damage normal development period in insects by mimicking juvenile hormone and blocking hormone levels (Podoler *et al.*, 1985). The juvenile hormone analogue tested in this study was Pyriproxyfen, 4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether, suppresses embryogenesis, metamorphosis and adult formation by interfering with the hormonal balance of insects (Koehler and Patterson, 1991; Ishaaya and Horowitz, 1992).

Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, and their elimination by protective mechanisms, referred to as antioxidants (El Golli-Bennour and Bacha, 2011; Piner and Üner, 2013). Reactive oxygen species (ROS), such as H_2O_2, superoxide (O_2^-) and the hydroxyl radical (OH^-) are generated in cells by several pathways. Organisms have a variety of detoxifying enzymes, such as superoxide dismutase, catalase, glutathione S-transferase, glutathione peroxidase and glutathione reductase, all of which have been reported to occur in insects to protect against the effects of oxidative stress (Felton and Summers, 1995; Weirich *et al.*, 2002).

Antioxidant enzymes including superoxide dismutase (SOD) play a basic role in oxidative stress. Superoxide dismutase (SOD) catalyzes dismutation of superoxide anion (O_2^-) to form H_2O_2 (McCord and Fridovich, 1969; Khvoshchevskaya *et. al.*, 2005). Another anioxidant enzyme is catalase, one of the antioxidant enzymes and catalyzes the degradation of H_2O_2 to water and oxygen (Switala and Loewen, 2002). CAT is recognized to be solely responsible for the scavenger of ROS in insects. Both SOD and CAT participate in oxygen reduction (Felton and Summers, 1995).

Greater wax moth, *Galleria mellonella* (L.) is a pyralid moth whose larvae feed on combs, wax and honey in beehives. We studied *G. mellonella*

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because it is easily reared under laboratory conditions and is a host species for many parasitoid insects. The IGR pyriproxyfen is an effective molt inhibitor for a wide range of insects. So the aim of this study was to determine the antioxidant activity of *G. mellonella* exposed to 0.0001; 0.0005; 0.001 and 0.005 mg/ml pyriproxyfen over 24, 48 and 72 hours.

MATERIALS AND METHODS

G. mellonella larvae were reared at 25±2°C, 70±5% RH on a diet composed of bran, honey, glycerol, honeycomb and distilled water (Bronksill, 1961). The continuity of the stock culture was ensured by mating the female and male adults insects, and by hatching the eggs.

The last instar larvae were taken from the artificial diet and exposed to pyriproxyfen. Pyriproxyfen (Sigma Chemical Co.) was dissolved in acetone at a storage concentration of 1 mM (3.2 mg/ml). The pyriproxyfen storage solution were diluted to 0.0001, 0.0005, 0.001 and 0.005 mg/ml using acetone. 2 μl of one of the three insecticide concentrations was applied on dorsal thorax of each larvae using a micropipette. Control larvae were treated with acetone only. Each group consisted of 10 larvae and replicated three times (*n*= 10, totally 150 larvae).

Larvae were weighed after 24, 48 and 72 h of treatment and homogenized with 20 (v/w) volumes of phosphate buffer (pH 7.4) using Ultra Turrax. After homogenization, the homogenate were centrifuged at 13,000xg for 20 min at 4°C. Supernatants were used for determining protein, CAT and SOD activities of *G. mellonella* larvae.

Determination of anti-oxidant enzymes activities

CAT activity was determined by the method of Aebi (1984). The reaction mixture was composed of phosphate buffer, supernatant of the treatment concentrations and 3% hydrogen peroxide and read spectrophotometrically at 240 nm. SOD activity was determined spectrophotometrically at 560 nm. by the inhibition of the rate of nitro blue tetrazolium (NBT) reduction with superoxide anion formen in the process of xanthine oxidation by xanthine oxidase (Sun *et al*., 1988). The other procedures were the same with CAT activity assay as described above.

The method of Lowry *et al*. (1951) was used for the total protein determination. Supernatant was added to alkaline copper reagent in eppendorf tubes. After 10 min., 0.5 ml of Folin Ciocalteu's reagent was added to the mixture and eppendorf tubes were shaken thoroughly with Vortex. The tubes were kept 20 min. in room temperature for color development and then were read spectrophotometrically at 750 nm. Bovin serum albumine (BSA) was used as a standard. All absorbances were measured on a Optizen 3220 UV spectrophotometer.

Statistial analysis

Statistical analyses of the data were carried out by variance anayses and student Newman Keuls' procedure using SPSS 16.00 software. Differences between the data were considered significant at the P<0.05 level.

RESULTS

The effect of pyriproxyfen on CAT and SOD activities of seventh instar larvae of *G. mellonella* is given in Table I. Significant increases were observed in larvae exposed to 0.005, 0.001 and 0.005 mg/ml pyriproxyfen for 24 h (171, 156, 123%), at all concentrations after 48 h (413, 602, 935, 995%) and in larvae exposed to 0.0001 mg/ml for 72 h (46%) compared with the control insects (P<0.05) (Table I).

There were also significant increases in SOD activity at all exposure periods compared with the control. The increase in SOD activity was statistically significant in larvae exposed to 0.0005, 0.001 and 0.005 mg/ml (103, 124 and 125%, respectively) concentrations of pyriproxyfen for 24 h, in larvae exposed to 0.005 mg/ml (142%) for 48 h and in larvae exposed to 0.0005, 0.001 and 0.005 mg/ml (125, 251 and 212%, respectively) of the insecticide for 72 h (Table I) (*P*<0.05).

DISCUSSION

Intracellular antioxidant enzymes, such as CAT and SOD eliminate ROS, thereby playing an integral role in oxidative stress defenses of the cell

Table I.- **The effect of pyriproxyfen on CAT and SOD activity of *G. mellonella* larvae. The values are Mean±SEM.**

Pyriproxyfen (mg/ml)	CAT (O.D./mg protein)			SOD (O.D./mg protein)		
	24	48	72	24	48	72
Control	0.20±0.03[a]	0.15±0.01[a]	0.46±0.05[a]	34.98±5.56[a]	45.64±7.19[a]	23.54±0.76[a]
0.0001	0.37±0.05[ab]	0.78±0.06[b]	0.47±0.01[b]	37.07±9.96[a]	47.23±5.90[a]	39.72±4.69[ab]
0.0005	0.54±0.06[b]	1.07±0.18[bc]	0.49±0.03[a]	71.12±11.5[b]	62.08±4.70[a]	53.03±3.18[b]
0.001	0.51±0.06[b]	1.58 ±0.11[c]	0.59±0.03[ab]	78.41±9.08[b]	87.43±12.1[ab]	82.74±4.94[c]
0.005	0.44±0.08[b]	1.67±0.80[c]	0.62±0.02[ab]	78.83±6.08[b]	110.53±16.5[b]	73.65±10.2[c]

* Means in the same column followed by different letters indicate that the difference between control and treated larvae is statistically significant as determined by SNK, between the letters "a-c" the effect of significant ($P<0.05$).

(Bukowska, 2004; Aslanturk *et al*., 2011). Although low levels of ROS are essential for normal physiological signal of transduction pathways that regulate complex processes in cells (Seo *et al*., 2000), accumulation of ROS can damage biological macromolecules such as nucleic acids, proteins, and lipids, leading to cell damage, mutation, and even death (Ryter *et al*., 2007). Antioxidant enzymes may play a key role in cellular adaptation, as SOD converts the superoxide radical into hydrogen peroxide (H_2O_2), which is metabolized by CAT.

Pyriproxyfen is an insect growth regulator which effects only target organisms and mimics juvenile hormone by upsetting hormonal changes in insect body. Hormonal based insecticides lead to disordering metabolism and biochemical activities in insect body by mimicking hormones (Zibaee *et al*., 2011). No previous studies were conducted to elucidate the effect of pyriproxyfen upon the antioxidant system in *G. mellonella*. The result of the present study showed that treatment of seventh instar of *G. mellonella* with pyriproxyfen caused a significant increase in SOD and CAT activities depending on concentrations. This may be due to pyriproxyfen treatments stimulate SOD and CAT to defend the body against the damage caused by excessive amount of oxygen free radicals.

The present data were mostly in accordance with previous studies which showed that some insecticides and IGRs affect antioxidant enzymes in lepidopteran insects (Adamski *et al*., 2003). CAT activity showed a significant increase in *Spodoptera littoralis* larvae exposed to insect growth regulators, buprofezin and pyriproxyfen (Fahmy, 2012). It was also indicated that the influence of methidathion exposure of *Lymantria dispar* larvae showed a

significant increase at SOD and CAT activities (Aslantürk *et al*., 2011). However it was reported that short term (4 days) exposure to fenitrothion decreased, whereas long term exposure (11-18 days) increased CAT and SOD activity compared with control in *Spodoptera exigua* and *Tenebrio molitor* (Adamski *et al*., 2003).

The increased free radicals induced by pyriproxyfen can encounter antioxidant enzymes instead of target molecules to be damaged. The antioxidant defenses enable the body system to restore the prevailing reducing environment and repair the tissue damage (Halliwell and Gutteridge, 1999; Dkhil et al., 2015). The high levels of H_2O_2 inhibit SOD through the formation of excess hydroxyl radicals while the CAT enzyme can be inactivated by the high levels of O_2- (Casano *et al*., 1997; Cakmak, 2000). The activity of these enzymes decreased with increasing exposure times, indicating that the detoxification mechanism was unable to deal with the stress produced by pyriproxyfen (Sowjanya and Padmaja, 2008). However, Hyrsl *et al*. (2007) demonstrated that insect reared with low doses of boric acid increased SOD activity but high doses resulted in decreased SOD activity in larval hemolymph, whereas SOD activity was significantly increased, but CAT activity decreased in the larval fat body.

Beyond this general responses, a significant increase in SOD activity and a concomitant increase in CAT activity were observed in *G. mellonella* larvae in response to oxidative stress. Changes in activities of some antioxidant enzymes in hemolymph can serve as a biomarker for oxidative stress in *G. mellonella* (Lozinskaya *et al*., 2004; Hyrsl *et al*., 2007).

SOD catalyzes the destruction of the superoxide radical and protects oxygen-metabolizing cells against harmful effects of superoxide free radicals. The increase in SOD activity might be considered a contingent response of *G. mellonella* to pyriproxyfen stress. CAT is a hematin-containing enzyme located in peroxisomes and facilitates the removal of hydrogen peroxide (H_2O_2), which is metabolized to molecular oxygen and water. Therefore, the SOD–CAT system provides the first defense against oxygen toxicity. CAT activity is directly regulated by the concentration of H_2O_2 (Fornazier *et al*., 2002; Wu *et al*., 2011). The present results showed that the tendency of CAT was consistent with the changes of SOD under pyriproxyfen stress. Furthermore, CAT activity showed a positive relationship with SOD activity. This indicated that H_2O_2 generated by SOD was removed by the induced activity of CAT (Zhang *et al*., 2007; Wu *et al*., 2011).

This study also demonstrated the role of the relationship between CAT and SOD enzymes, because SOD and CAT together take part in stepwise oxygen reduction (Munday and Winterboume, 1989; Sies, 1991). Since SOD activity was enhanced in experimental groups, this increased SOD activity resulted in an increased H_2O_2 concentration and eventually in a further increase in CAT activity.

Results of the present study showed that pyriproxyfen cause some biochemical changes in *G. mellonella* larvae by reversing hormonal balance. IGRs could cause oxidative stress by effecting nervous system with disordering of endocrine system in insects. Furthermore chemical insecticides that are used to get result in a short periods, could give damage to non-target organisms and environment. Hence the use of insecticides which has an effect only on target pest, will provide positive results without being detrimental to other organisms and environment.

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Conflict of interest
There is no conflict of interest.

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