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# Inhibition of Catalase Isozymes of *Helicoverpa armigera*(Lepidoptera: Noctuidae) by Salicylic acid.

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***Abstract:*** *The cotton bollworm, Helicoverpa armigera is a polyphagous pest and causes oxidative damage to field crops. Salicylic acid is the cell signaling molecule produced in response to insect attack on plants. Catalase plays a major role in the protection of tissues from the toxic effects of $H_2O_2$ and partially reduced oxygen species. Invitroand Invivo effect of salicylic acid was studied on the activities of catalases of Helicoverpa armigera, and tissue distribution of catalase isoforms was also studied. Eight catalase isoforms were found in the gut and whole body preparations whereas single isoforms in the hemolymph. Salicylic acid inhibited over 90 % of catalase activity at 2 mM concentrations in the gel activity staining as well as spectrophotometrically, but catalase I in all the preparations are stable and insensitive to salicylic acid.*

***Key words:****Catalase, Helicoverpa armigera, Inhibition, Salicylic acid*.

## I. Introduction

*Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), is a polyphagous pest attacking more than 200 different plant species including cotton, grain legumes, cereals, vegetables and fruits. Control of *H. armigera* is heavily based on insecticides; as a result pest has developed high levels of resistance to commonly used insecticides [1]. The exposure of chemicals to tissues and cells results in the induction of a reactive oxygen species (ROS) such as $H_2O_2$ and superoxide anion. In general, ROS is harmful to living organisms because ROS tends to give oxidative damages to proteins, nucleic acids, and lipids [2]. In this regard, ROS has been recognized to be related to aging and life span [3-4]. Antioxidant enzymes are typical factors facilitating the scavengery of ROS.Catalase (EC 1.11.1.6, CAT) is one of the antioxidant enzymes involved in regulating the cellular level of active oxygen species. Catalase converts $H_2O_2$ to water and oxygen, hence protecting cells from the damaging effect of $H_2O_2$.

Plants are continuously exposed to the challenge of a variety of herbivores. Plants have evolved constitutive and inducible defense mechanisms against the herbivores. Salicylic acid and jasmonic acid (JA) function as signaling molecules in plants, mediating induced plant responses against herbivory and pathogen infection, leading to the activation of genes mediating host plant resistance to insects [5]. More recently, elevated levels of SA have been found to be associated with herbivore attack in tomato plants [6]. Although the hemolymph is known to generate ROS [7], and thus is not immune to oxidative damage, the hemolymph possesses very low or negligible activities of most antioxidant enzymes SOD, CAT, GST and GR [8]. Most of the enzymes occur at high levels in metabolically active tissues such as fat body, midgut and malphigian tubules. There has been limited study on determining the tissue location of antioxidants in insects [9].The presence of antioxidant enzymes in the digestive lumen, with the exception of catalase is largely unknown and warrants further study.

Salicylic acid (SA) and its analogues have adverse effects on the growth, development, oxidative phosphorylation, mitochondrial dysfunction and accumulation of $H_2O_2$ [10]. SA was shown to be a potent inhibitor of plant and animal catalases [11-12]. Since the level of SA increases during insect and pathogen attack, as well as during exogenous application induces defense mechanism in plants. The present studies were therefore undertaken to determine whether SA could inhibit catalase isozymes of *H. armigera*.

## II. Materials and methods

2.1 Insects

The pests that had developed resistance to various insecticides were supplied by Dr. S.S. Udikeri, Agriculture Research Station, University of Agricultural Sciences, Dharwad, India. The larvae were reared on a chickpea based semi-synthetic diet containing with or without 2 mM SA under laboratory conditions at 27 ± 1 ˚C, 65 ± 5% RH, and 12 h photoperiod. Bioassay was carried out according to out earlier report[10].

2.2 Enzyme extraction

Fourth instar larvae were washed with cold 20mM Tris-HCl buffer pH 7.0. Hemolymph was collected by following the method [13]. The gut contents and the whole body were separately homogenized in 50 mM

Tris-HCl buffer pH 7.0 containing 1mM PMSF and centrifuged for 30 min at 10,000 g. The supernatant was used as enzyme source. Protein concentration was determined by Lowry et al [14] using bovine serum albumin as standard.

2.3 Catalase activity

Catalase catalyzed the decomposition of $H_2O_2$ was monitored by the decrease in the absorbance at 240nmspectrophotometrically over a period of 3 min.One unit of catalase activity was expressed as the amount of enzyme capable of catalyzing the degradation of one micromoles of hydrogen peroxide reduced per minute per milligram of protein, using an extinction coefficient of 39.4 $mM^{-1}$ $cm^{-1}$ [15].

2.4 Native PAGE and activity staining

Equal amount of protein from fat body, midgut and hemolymph were subjected to native PAGE using 8% polyacrylamide gel was carried out by the Davis method [16] under non-reducing conditions. Catalase isoforms were visualized following the modified method [17]. Briefly gels were soaked in 3mM $H_2O_2$ for 10 min at room temperature. Then gels were soaked in staining solution containing 1% potassium ferricyanide and 1% ferric chloride. Catalase isozymes were visualized immediately.

## III. Results and Discussion

In insects, catalases are recognized to be responsible for the scavenging of reactive oxygen species [18] as they are deficient in a selenium dependent glutathione peroxidase, which is another scavenger present in the other organisms [19]. The midgut and whole body preparations shows high activity of 40 and 37 units/mg of protein but very less activity of 8 units/mg with hemolymph preparations. The catalases are widely distributed in various tissues of *H. armigera* larvae and found highest activity in whole body preparation. Among the different tissues, highest activity was detected in the liver extract of the mouse [20]. In in-vitro assay the catalase activity were inhibited by 90% at 2 mM of SA, except for hemolymph catalase (Table 1). Further isoforms of catalases were analyzed by gel activity staining in control and SA treated larvae. Fig 1A shows the tissue distribution of catalase in control larvae and found 8 catalase isoforms in the gut and whole body preparations whereas single isoform in hemolymph. The catalase isoforms 3, 7, 8 of gut and 3, 4, 5 of whole body preparation were in low levels. The larvae fed with 2 mM SA were found to inhibit all the catalases except CAT 1 from all the preparations (Fig 1B). Akbar et al [10] have reported that *H. armigera* larvae fed the diet containing 2 mM SA was found to accumulate high content of $H_20_2$ in the cells and also the larval weight was decreased by around 70%. This further supports our results that SA inhibited catalases of *H. armigera* that led to accumulate $H_20_2$*in vivo,* which might be causing the oxidative damage to the cell. Hence the studies reveal that treatment with SA would be helpful to arrest the growth of *H. armigera* larvae.

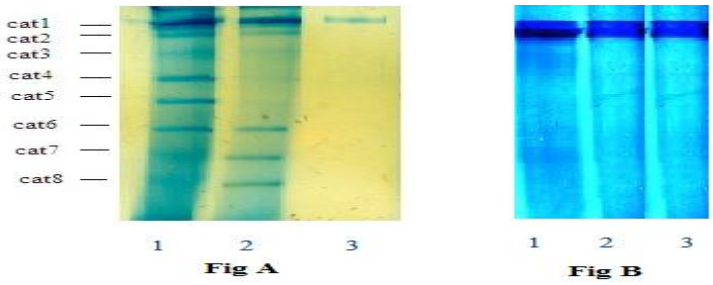
## IV. Figures and Tables

**TABLE 1: Catalase activity in different tissues of *H. armigera.***

| Tissue | Catalase activity (Units/mg) | |
|---|---|---|
| | Control | Treated with SA (2mM) |
| Gut extract | 40.89±2.21 | 5. 45±3.20 |
| Whole body | 37.12± 1.5 | 4.32±2.0 |
| Hemolymph | 8.89±0.92 | 8.89±0.92 |

Values are representation of mean ± SD (n =3)

**Fig 1: Tissue distribution of catalase isozymes. A-control pests, B; Pests treated with 2 mM SA. lane1, gut extract; lane 2, whole body preparation; lane 3, Hemolymph.**



## V. Conclusion

The research reveals the in vitro and in vivo effect of salicylic acid on the catalase isozymes of *Helicoverpa armigera* and shown the application of salicylic acid would be helpful to arrest the growth of *Helicoverpa armigera.*

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