WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 2, 1384-1392. Research Article ISSN 2277– 7105

KINETICS OF CATALASE IN SILKWORM *BOMBYX MORI* L. INFECTED WITH *BEAUVERIA BASSIANA* (BALS) VUILL.

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Article Received on
12 Dec 2014,

Revised on 06 Jan 2015,
Accepted on 31 Jan 2015

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ABSTRACT

Gradual reduction of catalase activity was noticed in the three selected tissues viz., integument (0.56 to 0.10 µ moles of H_2O_2 metabolized/mg protein/min), midgut (0.59 to 0.13 µ moles of H_2O_2 metabolized/mg protein/min) and silkgland (0.50 to 0.12 µ moles of H_2O_2 metabolized/mg protein/min) of inoculated 5th instar silkworm *Bombyx mori* larvae with reference to control in all the three tissues i.e., integument (0.65 to 0.21 µ moles of H_2O_2 metabolized/mg protein/min), midgut (0.66 to 0.32 µ moles of H_2O_2 metabolized/mg protein/min) and silkgland (0.52 to 0.43 µ moles of H_2O_2 metabolized/mg protein/min). The reduction of the enzyme activity may be due to metabolic adjustments of the host organism as a part of the defense mechanism due to invasion of fungal pathogen. The decreased catalase activity in experimental larvae may also envisage the possible damage to peroxisomes in the cell and this might be the probable reason for the decreased catalase activity. Reduced catalase activity may lead to accumulation of H_2O_2 in the *Beauveria bassiana* inoculated silkworms, which may lead to the mortality of the larvae as H_2O_2 is cytotoxic.

KEYWORDS: *Bombyx mori*, *Beauveria bassiana*, Catalase activity, Integument, Midgut, Silkgland.

INTRODUCTION

The success of sericulture industry, primarily depends on the successful harvest of cocoon crops. Perhaps the major problem for sericulture in a tropical country like India is the high incidence of diseases. However, as the rearing practices were refined, the infectious diseases caused by microbes, frequented with potential for the catastrophic loss. Disease development and mortality are ever present phenomena in the silkworms as in other living organisms. The

loss occurs mainly during the final stages of silkworm rearing after considerable energy and money have been expended. The losses, if they occur repeatedly, can dishearten the sericulturists, ultimately the quality of cocoon crops is badly affected and profit levels will reduce drastically. Among the diseases of silkworm, white muscardine caused by *Beauveria bassiana* inflicts heavy economic loss to the sericulturists. All the life stages of the silkworm *viz*., egg, larvae, pupae and moth are found susceptible to the fungal pathogen *Beauveria bassiana*. Out of the total disease occurrence, 10-40% of loss has been accounted for muscardine in India (Janakiraman 1961 and Chandrasekharan and Nataraju 2008). It is obvious that, disease outbreak is a major reason for not achieving the expected vertical improvement in cocoon production.

In insects, catalase is recognized as the key enzyme to be solely responsible for the scavenger of ROS (Felton and summers, 1995). Reactive Oxygen Species (ROS) plays an important role in the innate immunity of the insects (Hao *et al* (2003) and Kumar *et al* (2003). It stimulates signal transduction and mediates different responses such as cell growth and apoptosis. However, during times of stress, ROS levels can increase dramatically. This may result in significant damage to cell structures. Cumulatively, this is known as oxidative stress and this stress can cause disruptions in normal mechanisms of cellular signaling. Catalase (EC 1.11.1.6 CAT) had a strong detoxification effect and it is one of the antioxidant enzymes and catalyzes the degradation of H_2O_2 to water and oxygen (Switala and Loewen 2002). Catalase can catalyze the oxidation of various metabolites and toxins, including formaldehyde, formic acid, phenols, acetaldehyde and alcohols. Alterations of the antioxidant enzyme level under different stress conditions are suggested to serve as indicators of biotic and abiotic stress (Li Lujun *et al* 2005) and catalase induction was proposed as a simple biochemical marker to follow the course of fungal growth (Crespo *et al* (2002) and Pedrini *et al* (2007 and 2009). In view of the significance of catalase in the biological system and scavenging role played by the enzyme, the present investigation has been focused to examine dynamics of catalase activity during the development of fungal pathogen *Beauveria bassiana.*

MATERIALS AND METHODS

PM × CSR2 silkworm strain was selected for the investigation. Silkworms are reared in the laboratory under optimum conditions as suggested by Dandin *et al* (2003). Immediately after fourth moult the heatlhy larvae were selected from the rearing stock and grouped into two

sets. Each group consists of 4 replications with 100 larvae in each replication. One set of larvae was treated with fungal spore suspension with sub lethal concentration (3.25 x 10^6 spores/ ml @ 50 ml/100 worms) and another set of larvae were treated with double distilled water and used as control. Every day, silkworms from both the sets were randomly selected from 1^{st} to 7^{th} day of 5^{th} instar silkworms and dissected in physiological saline solution and collected the three tissues viz., integument, midgut and silkgland to examine the catalase activity. Catalase activity was determined by the method of Chance and Machly (1955).

RESULTS AND DISCUSSION

In the present investigation gradual decline of catalase activity was observed in the selected tissues viz., integument, midgut and silkgland of *Beauveria bassiana* inoculated 5^{th} instar silkworm compared to control. Table -1 to 3 and Graph -1-3 show the results of catalase activity in the three tissues viz., integument, midgut and silkgland of the 5^{th} instar silkworm *Bombyx mori* inoculated with fungal pathogen *Beauveria bassiana.* Decreased trend of catalase activity was recorded in the three tissues i.e., integument, midgut and silkgland of both inoculated and control from 1^{st} day to 7^{th} day of the 5^{th} instar silkworm. However, significant reduction of the enzyme activity was noticed in the inoculated larvae in all the three tissues selected for the study i.e., integument (0.56 to 0.10 µ moles of H_2O_2 metabolized/mg protein/min), midgut (0.59 to 0.13 µ moles of H_2O_2 metabolized/mg protein/min) and silkgland (0.50 to 0.12 µ moles of H_2O_2 metabolized/mg protein/min) compared to control in all the tissues viz., integument (0.65 to 0.21 µ moles of H_2O_2 metabolized/mg protein/min), midgut (0.66 to 0.32 µ moles of H_2O_2 metabolized/mg protein/min) and silkgland (0.52 to 0.43 µ moles of H_2O_2 metabolized/mg protein/min).

The reduction of the enzyme activity may be due to metabolic adjustments of the host organism as a part of the defense mechanism due to invasion of fungal pathogen. The decreased catalase activity in experimental larvae may also envisage the possible damage to peroxisomes in the cell and this might be the probable reason for the decreased catalase activity. Reduced catalase activity may lead to accumulation of H_2O_2 in the *Beauveria bassiana* inoculated silkworms, which may lead to the mortality of the larvae as H_2O_2 is cytotoxic. Similar observations were made by Rajitha and Savithri (2013) in the haemolymph of B*eauveria bassiana* inoculated 5^{th} instar silkworms with reference to healthy worms and the decreased catalase activity in experimental silkworm was attributed to the possible damage to peroxisomes in the cell.

The role of catalase activity in insect defense has been explained by Wu xiaofeng *et al* (1998). Fornazier *et al* (2002) opined that catalase activity was sufficient to cope with an increased concentration of H_2O_2 following treatment with Cd2+ and the cells presented a positive endogenous protective effect. Jagadeeshkumar and Nabizadeh (2010) reported the importance and level of changes in catalse activity in silkworm *Bombyx mori* under thermal stress condition. Madhusudhan *et al* (2012) analysed the catalase activity in both healthy and pebrinized tropical tasar silkworm and reported the gradual decline of catalase activity in both pebrinized and non-pebrinized tasar silkworm where as in pebrinized samples the enzyme activity was declined drastically from 24 hours of post-inoculation. Lowest catalase activity was noticed at 168 hours in inoculated larval tissue, i.e., 3.09 fold decline in catalase activity was noticed in inoculated samples when compared to control. Catalase enzyme can be used as a marker enzyme to know the health status of tropical tasar silkworm.

In contrast, Yamamoto *et al* (2005) reported that irradiation of gamma rays to *Bombyx mori* larvae causes a notable increase in catalase activity in the midgut and fat body. Nabizadeh and Jagadeeshkumar (2010) also reported the highest catalase activity in fat body tissue during thermal stress, because the fat body tissue is the seat of metabolism, active in the synthesis, storage and release of biomolecules to provide clues in relation to energy production, transformation and utilization at cellular and subcellular level during intermediary metabolism in silkworm body. As well, acute thermal stress at 40±1°C, negatively affected rate of food intake, protein synthesis and metabolism during the 5th instar and due to these changes, probably, an intensive decrease was observed in the fat body catalase activity.

Samuels and Paterson (1995) stated that the germination of fungal spores on insect cuticle requires a sequential action of appropriate hydrolytic enzymes. These enzymes could facilitate the early stages of fungal infection. Moreover, some of these catabolic enzymes may be important in the invasion of fungi into the haemocoel or body cavity. During these studies, the effects of catalase activity on germination and virulence were also observed. Shaukat Ali *et al* (2012) reported that conidial germination and virulence was directly related to the rates of catalase production under different growth conditions. A possible reason for such an enzymatic activity pattern can be the repression of hydrogen peroxide. So, catalase production is important during germination and growth for maintaining and control over the ROS produced by germination, mycelia growth and possibly during the invasion process.

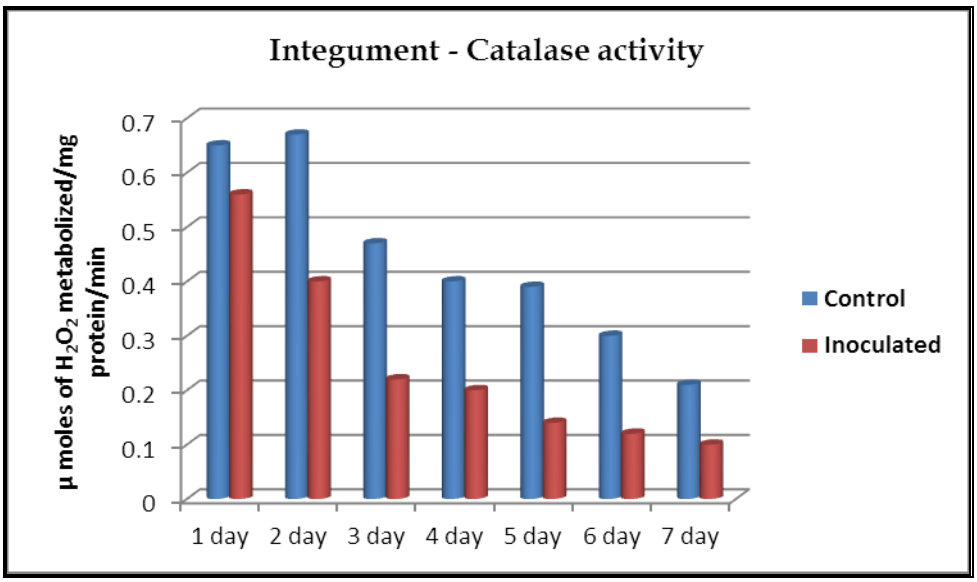
Akbar *et al* (2012) reported that *Helicoverpa armigera* larvae fed with the diet containing 2 mM of salycilic acid (SA) was found to accumulate high content of H_20_2 in the cells and also the larval weight was decreased by around 70 per cent. Salycilic acid inhibited catalases of *Helicoverpa armigera* that led to accumulation of H_20_2 *in vivo*, which might be causing the oxidative damage to the cell, treatment with SA would be helpful to arrest the growth of *Helicoverpa armigera* larvae (Hanumanth Goud *et al* 2012).

The study very clearly shows that reduction of catalase activity in the 5th instar silkworm infected with *Beauveria bassiana*. The decrease in catalase activity may lead to the accumulation of H_2O_2 in the host tissue and cause the mortality of silkworm larvae as H_2O_2 is cytotoxic. Pedrini *et al* (2013) suggested that peroxisomal catalases might be crucial factors for adaptation to oxidative stress generated during fungal growth.

Table – 1 Day to day variations in catalase activity (µ moles of H_2O_2 metabolized/mg protein/min) in the integument of 5th instar silkworm *Bombyx mori* during the progress of fungal pathogen *Beauveria bassiana* compared to control

Treatments	Days of 5th instar						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Control	0.65±0.05	0.67±0.01	0.47±0.03	0.40±0.02	0.39±0.06	0.30±0.01	0.21±0.01
Inoculated	0.56±0.05NS	0.40±0.01****	0.22±0.01****	0.20±0.01****	0.14±0.05*	0.12±0.02****	0.10±0.03*

Mean±Standard Deviation; NS = Not Significant; *P<=0.05, **P<=0.02, ***P<=0.01, ****P<=0.001

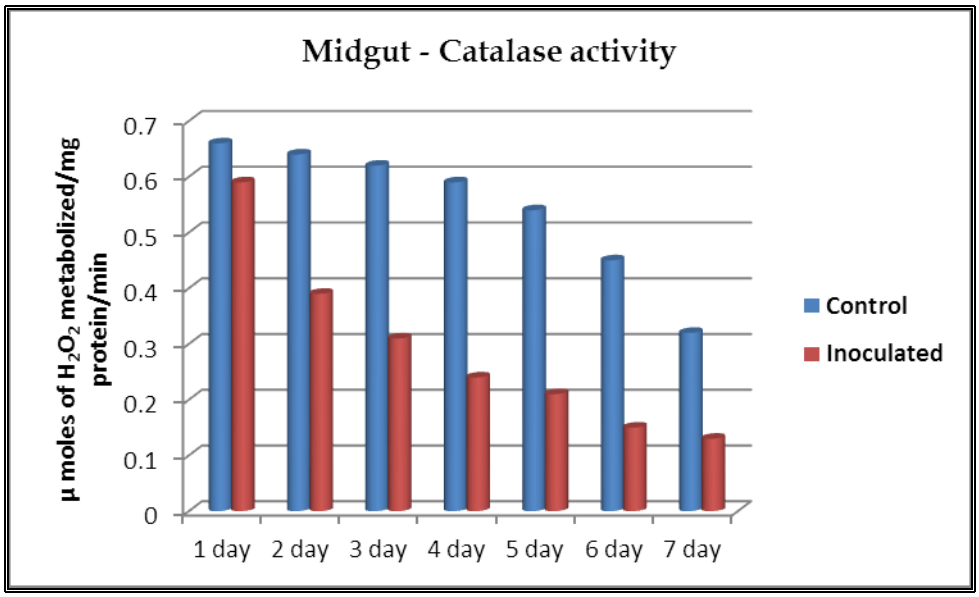


Graph – 1 Histogram showing the day to day variations in catalase activity (µ moles of H_2O_2 metabolized/mg protein/min) in the integument of 5th instar silkworm *Bombyx mori* during the progress of fungal pathogen *Beauveria bassiana* compared to control

Table – 2 Day to day variations in catalase activity (µ moles of H_2O_2 metabolized/mg protein/min) in the midgut of 5th instar silkworm *Bombyx mori* during the progress of fungal pathogen *Beauveria bassiana* compared to control

Treatments	Days of 5th instar						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Control	0.66±0.03	0.64±0.04	0.62±0.02	0.59±0.01	0.54±0.06	0.45±0.06	0.32±0.01
Inoculated	0.59±0.02NS	0.39±0.02****	0.31±0.03****	0.24±0.01****	0.21±0.01****	0.15±0.02***	0.13±0.01****

Mean±Standard Deviation; NS = Not Significant; *P<=0.05, **P<=0.02, ***P<=0.01, ****P<=0.001

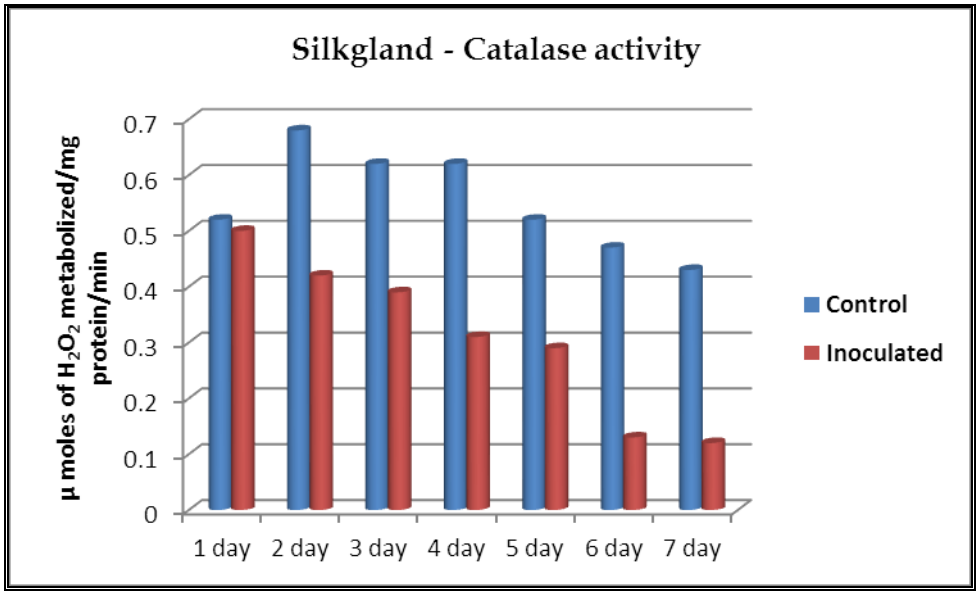


Graph – 2 Histogram showing the day to day variations in catalase activity (µ moles of H_2O_2 metabolized/mg protein/min) in the midgut of 5th instar silkworm *Bombyx mori* during the progress of fungal pathogen *Beauveria bassiana* compared to control

Table - 3 Day to day variations in catalase activity (µ moles of H_2O_2 metabolized/mg protein/min) in the silkgland of 5th instar silkworm *Bombyx mori* during the progress of fungal pathogen *Beauveria bassiana* compared to control

Treatments	Days of 5th instar						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Control	0.52±0.01	0.68±0.02	0.62±0.04	0.62±0.04	0.52±0.02	0.47±0.03	0.43±0.01
Inoculated	0.50±0.05 NS	0.42±0.03 ***	0.39±0.02 ****	0.31±0.02 ***	0.29±0.03 ****	0.13±0.01 ****	0.12±0.02 ****

Mean±Standard Deviation; NS = Not Significant; *P<=0.05, **P<=0.02, ***P<=0.01, ****P<=0.001



Graph – 3 Histogram showing the day to day variations in catalase activity (µ moles of H_2O_2 metabolized/mg protein/min) in the silkgland of 5th instar silkworm *Bombyx mori* during the progress of fungal pathogen *Beauveria bassiana* compared to control.

ACKNOWLEDGEMENTS

The authors are thankful to University Grants Commission (UGC), New Delhi for providing financial assistance under Rajiv Gandhi National Fellowship (RGNF) to carry out the research study.

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