INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

Short Communication

IMPACT OF PEBRINE INFECTION ON CATALASE ACTIVITY IN TROPICAL TASAR SILKWORM (*ANTHERAEA MYLITTA* D.)

Madhusudhan, K.N., Iresh-Kumar, Nungshi-Devi, C., Singh, G.P., Sinha, A.K., Kirankumar, K.P. and Prasad, B.C.
Silkworm Pathology Section, Central Tasar Research and Training Institute, Piska Nagri, Ranchi, Jharkhand, INDIA.

ABSTRACT
Pebrine disease infection to tropical tasar silkworm is the main reason for the decreased production of tasar silk in India. The understanding of the impact of pebrine infection on catalase activity will provide basic information about tasar-pebrine interaction. The tropical tasar silkworm larvae were pebrinised experimentally by oral feeding. The non-pebrinised and pebrinised larval samples were harvested at different time intervals. The samples were analyzed for the level of catalase activity in both pebrinised and non-pebrinised larval samples. Results revealed that, decrease catalase activity was observed in pebrinised larval samples in comparison with control ones. The variation in catalase activity can be considered as marker for identification of pebrine infection in tropical tasar silkworm.

KEYWORDS: *Antheraea mylitta*, Pebrine, *Nosema mylitta*, Catalase.

INTRODUCTION

Tasar culture is being practiced by the poor tribal people of middle and north eastern states of India. The production of tasar silk is low because of the occurrence of diseases. Tropical tasar silkworm (*Antherea mylitta* D.) is affected by different diseases, *viz*., pebrine, virosis, bacteriosis and mycosis. Among these diseases, pebrine disease which is caused by *Nosema mylitta* has been considered as major threat for the production of tasar silkworm. Pebrine disease causes yield loss upto 20-25% (Sahay *et al.,* 2000). Insects defend themselves against pathogens using different innate immune defenses (Lemaitre and Hoffman, 2007). During the process of active infection, the primary source of exogenous oxidative stress for pathogenic bacteria is attack by host phagocytic cells. Phagocytes utilize the cytotoxic effects of many of the reactive oxygen species, such as superoxide, hydrogen peroxide, and the highly toxic hydroxyl radical. These reactive oxygen species can damage the nucleic acids, proteins, and cell membranes of pathogens. On the other hand, pathogens have effective enzymatic pathways of oxidant inactivation, including those catalyzed by superoxide dismutase (SOD), catalase/peroxidase, and glutathione in combination with glutathione peroxidase and glutathione reductase. Reactive Oxygen Species (ROS) plays an important role in the innate immunity system of the insects. It stimulates signal transduction and mediates different responses such as cell growth and apoptosis. Superoxide dismutase (SOD), catalase, xanthine oxidase, peroxidase, monoamine oxidase, oxidase, glutathione reductase, vitamin E, vitamin C, and glutathione are considered as good markers of oxidative stress (Iiyama *et al.,* 2007).

Living organisms require mechanisms regulating reactive oxygen species (ROS) such as hydrogen peroxide and superoxide anion. Catalase (EC 1.11.1.6, CAT) is one of the antioxidant enzymes and catalyzes the degradation of H_2O_2 to water and oxygen (Switala & Loewen, 2002). In insects, CAT is recognized as the key enzyme to be solely responsible for the scavenger of ROS. Early reports highlighted role of catalase activity in defense mechanisms in insects (Felton & Summers, 1995). The objective of the present study was to quantify the level of catalase activity in healthy (non-pebrinised) and pebrine infected tasar silkworms.

MATERIALS AND METHODS

Nosema mylitta infected tropical tasar silkworms (*Antheraea mylitta* D) were collected from the rearing plots of Central Tasar Research and Training Institute, Ranchi and they were maintained in the laboratory conditions. The infected fifth instar larvae were homogenized and centrifuged. Spores were purified on a discontinuous percoll gradient (25, 50, 75, and 100% v/v) by centrifugation at 10000 rpm for 10 min. The pellets were rinsed and stored at 4^{o}C for later use. The newly hatched larvae were fed with pebrinised leaves. The larvae were harvested at different time intervals. The harvested larvae were kept in -20^{o}C for further experimentation.

Catalase activity estimation

Catalase (CAT) activity was assayed by recording the decrease in absorbance of catalase enzyme at 240 nm following the decomposition of hydrogen peroxide. By using phosphate buffer (0.1M, pH 7.0), larva (0.1g) was extracted in pre-chilled pestle and mortar. The extracted sample was centrifuged at 4^{o}C at 10,000rpm for 10min. The protein content in the supernatant was estimated by using Lowry's method (Lowry *et al*., 1951). The reaction mixture containing 3 ml of Phosphate buffer along with 40µl crude extract was taken in the spectrophotometer sample cuvette and 40µl of H_2O_2 substrate was added. The reaction was measured spectrophotometrically at 240nm. Catalase activity was expressed as units mg^{-1} protein^{-1} min^{-}

[1](Havir and Mettale, 1987). Each measurement was considered with three replicates.

Data analysis

Data generated from the quantification of catalase was analyzed by using the statistical computer application package SPSS 10.0. The data generated were the average of three replicates. Data were subjected to analysis of variance (ANOVA) and the means were compared for significance using Duncan's new Multiple Range Test (DMRT; $p<0.05$) (Duncan, 1955).

RESULTS AND DISCUSSION

The temporal change in catalase activity was analyzed in both healthy (non-pebrinised) and pebrinised larvae of tropical tasar silkworm. In non-pebrinised larval samples the gradual decline of catalase activity was noticed where as in case of pebrinised samples the activity declined drastically from 24hpi. Lowest activity was noticed at 168h in inoculated larval tissue. At 168hpi, 3.09-fold decline in catalase activity was noticed in inoculated samples when compared with control ones (Table 1).

TABLE 1. Temporal changes of catalase activity in healthy and pebrine infected tasar silkworm larvae

Time intervals	Healthy	Infected
0h	3.76 ± 8.8^{a}	3.53 ± 0.14^{a}
24h	3.53 ± 8.8^{b}	2.53 ± 8.8^{b}
48h	3.36 ± 0.12^{c}	2.33 ± 8.8^{c}
72h	3.1 ± 5.7^{d}	2.1 ± 5.7^{d}
96h	2.7 ± 8.8^{e}	1.83 ± 6.0^{e}
120h	2.6 ± 2.0^{e}	1.77 ± 1.52^{ef}
144h	1.92 ± 3.1^{f}	1.61 ± 4.0^{f}
168h	1.76 ± 2.8^{g}	0.57 ± 5.5^{m}
192h	1.61 ± 4.1^{h}	1.09 ± 2.5^{ghi}
216h	1.47 ± 2.1^{hi}	1.21 ± 4.1^{g}
240h	1.35 ± 2.0^{i}	0.95 ± 2.51^{ij}
264h	1.09 ± 5.1^{j}	0.99 ± 1.8^{hij}
288h	1.04 ± 1.8^{j}	0.75 ± 1.4^{kl}
312h	1.1 ± 3.6^{j}	0.82 ± 2.6^{jk}
336h	1.18 ± 3.3^{j}	0.88 ± 4.4^{jk}
360h	1.15 ± 1.5^{j}	1.14 ± 1.3^{gh}
384h	1.15 ± 2.9^{j}	1.15 ± 2.9^{gh}
408h	1.09 ± 3.3^{j}	0.88 ± 1.7^{jk}
432h	1.04 ± 3.9^{j}	0.61 ± 3.7^{lm}

Different time intervals

Every value represents the mean of three replicates with standard error and values with the different letters are significantly different according to Duncan's Multiple Range Test (P= 0.05).

The role of catalase activity in insect defense has been explained by Xiaofeng *et al.* (1998). Recently, Jagadeeshkumar and Nabizadeh (2010) reported the importance and level of changes in catalase activity in silkworm *Bombyx mori L.* under stress condition. Inoculation with the pebrine spores induced decreased catalase activity when compared to the non-pebrinised silkworms. Based on the results of the present study, catalase enzyme can be used as marker enzyme to know the health status of tropical tasar silkworm larvae.

REFERENCES

Duncan, D.B. (1955) Multiple range and multiple F tests. Biometrics 11:1-42.

Felton, G. W. and Summers, C. B. (1995) Antioxidant system in insects. Arch. Insect. *Biochem. Physiol*. 29: 187-197.

Havir, E.A. and Mettale, N.A. (1987) Biochemical and developmental characterization of multiple form of catalase in tobacco leaves. *Plant Physiol*. 84:450-455.

Iiyama, K., Chieda, Y., Lee, J.M., Kusakabe, T., Yasunaga-Aoki, C. and Shimizu, S. (2007) Effect of Superoxide Dismutase Gene Inactivation on Virulence of Pseudomonas aeruginosa PAO1 toward the Silkworm, *Bombyx mori*. Applied and Environmental Microbiology. 73(5): 1569-1575.

Jagadeesh Kumar, T.S. and Nabizadeh, P. (2010) Thermal Stress Induced Catalase Activity Level in Selected BivoltineBreeds of Mulberry Silkworm *Bombyx mori* L. Modern Applied Science. 4(8):88-95.

Lemaitre, B. and Hoffmann, J. (2007) The Host Defense of *Drosophila melanogaster. Annual Review of Immunology***.** 25: 697-743.

Lowry, O.H., Roserbrough, N.J., Farr, A.L. and Randal, R. J. (1951) Protein measurement with the folin-phenol reagent. *J. Biol. Chem*. 193: 265-275.

Sahay, D. N., Roy, D. K. and Sahay, A. (2000) Diseases of tropical tasar silkworm, *Antheraea mylitta* D., Symptoms and control measures, In: *Lessons on Tropical Tasa*r. Ed. By K. Thangavelu, Central Tasar Research and training Institute, Piska Nagri, Ranchi, pp 104.

Switala, J. and Loewen, P. C. (2002) Diversity of properties among catalases. *Arch. Biochem. Biophys*. 401: 145-154.

Xiaofeng, W., Junliang, X. and Weizheng, C. (1998) The activity of catalase in haemolymph of the silkworm *Bombyx mori* (L) and its relation to larval resistance. *Acta Entomologica Sinica*. 98-02.