

Variation in Catalase Activity in the Silk Worm, *Bombyx Mori* During, Infection with Bacterial Flacherie

Rashmi Joshi Sawalkar¹ And Raja I. A²

RLT Science College, Akola, Maharashtra, India

Shri Shivaji College, Akola, Maharashtra, India

ABSTRACT

The silkworm is a caterpillar of the domesticated silk moth, Bombyx mori. It is a well known economically significant insect as it is a producer of valuable silk. Bacterial Flacherie is a syndrome associated with bacterial Infection, which is the major cause for the affected production of silk in India. Bacterial Flacherie is caused primarily by Serretiamarcesens, Streptococcus sp, and Staphylococcus sp of bacteria. In the present study healthy and bacterial Flacherie infected mulberry silkworm larvae were collected from the local sericulture units, carried and reared in the laboratory. The analysis for the intensity of catalase activity in both controlled and Flacherie infected larval samples were done. We reported, decrease Catalase activity in larvae suffering from Flacherie in comparison with control ones. The observed variation in Catalase activity can be measured of marker for identification of local mulberry silkworm Crops infected with Flacherie pathogen.

KEY WORDS: Bombyx Mori, Catalase, Flacherie, Serretia, Streptococcus, Staphylococcus.

INTRODUCTION

From last 4,500 years, Silkworm, *Bombyx mori* is known as domesticated insect but like other domesticated animals it is also easily susceptible to a number of diseases, results in great economic loss. Diseases like Grasserie and Flacherie are regular and fluctuate season wise in Maharashtra. It is the high temperature and dry climatic conditions of the region, which are conducive to the occurrence of these infections. In *Bombyx mori*, the worms become infected by both bacteria and viruses resulting in bacterial Flacherie and viral. Both Viral and Bacterial Flacherie are frequent and tend to develop in the hot and humid summer and autumn seasons (Lu Yup-Lian and Liu-Fuan, 1991 Mahesha et.al., 2009: Mahalingam et.al., 2010: Mahesha et.al., 2013: Li et.al., 2018).

The prominent pathogens are Streptococci species and Staphylococci species which are responsible for bacterial Flacherie; along with there are Streptococcus faecalis, Streptococcus faecium as well as Bacillus thuringiensis. The bacteria usually go in through mouth along with the contaminated food into the gut and penetrate mid gut wall and make their way to body tissue and haemolymph. Once attacked the bacteria progressively multiplies in the host system causing specific metabolic changes together with related biochemical alteration in the affected body tissues. Such infections are reported to induce variety of biomolecular and physiological changes in insect tissues (Maratignoni, 1964: Shigematsu, 1969). Earlier studies (Kadoya et.al., 1984: Adolkar, 1990: Aboul-Ela, et al., 1991: Gillespie et al., 1997: Doreswamy, et al., 2004: Manohar Reddy 2004: Mahesha et.al., 2009: Mahalingam et.al., 2010: Mahesha et.al., 2013: Recently, Li et.al., 2018 too reported that infected diseases causing great many effects on biomolecular and physiological functioning of the diseased larvae of silkworm they also emphasized the importance of study of these diseases especially the effects are related to, the biochemical composition of body tissues, fluids and enzyme systems. Among antioxidant enzymes, Catalase (EC 1.11.1.6, CAT) is a ubiquitous antioxidant enzyme catalyses the breakdown of hydrogen peroxide into water and oxygen (Switala and Loewen, 2002).

Several organisms in addition to oxidative stress releases catalase to defend themselves against attacks by hydrogen peroxide which forms the host's immune system. Earlier studies demonstrated that a Catalase-deficient mutant infective organism was more susceptible than its wild-type strain to the oxidative stress promoted by H₂O₂ and immune cell attacks (which involve H₂O₂). Thus it may prove helpful in analysing the activity of Catalase of a pathogen,

ARTICLE INFORMATION:

Corresponding Author Email: rashmisawalkar75@gmail.com

PRINT ISSN: 0974-6455, ONLINE ISSN: 2321-4007

CODEN: BBRCBA

Received: 16th June, 2019 Accepted: 15th July, 2019

Online content available: <http://www.bbrc.in/>

All rights reserved

@Soc. Sci.. Nat. India 2019

Thomson Reuters ISI ESC / Web of Science Clarivate Analytics USA

NAAS Journal Score 2019 (4.38)

Science Journal Impact Factor 2019 (4.196)



and also to get a better knowledge of the basic mechanisms of their pathogenic actions, together with their resistance against oxidative stress. Catalase is known as a sole enzyme only accountable for the scavenger of Reactive Oxygen Species (ROS), playing an important part in the insect's innate immunity system.

Kumar and Nabizadeh et al., 2010 studied the importance and level of changes in catalase activity in silkworm *Bombyx mori* under thermal stress condition. It triggers signal transduction and mediates variety of responses like cell growth and apoptosis. Reports (Felton and Summers, 1995) bring to light the function of Catalase activity in insects defence mechanisms. In the present paper we analysed the intensity of Catalase activity in non infected control silkworms *Bombyx mori* in comparison to infected silkworms *Bombyx mori* and will gain additional understanding about bacterial Flacherie-Silkworm relations.

MATERIAL AND METHODS

Larvae in their early infections with bacterial Flacherie collected from various local sericulture units in Akola district (Maharashtra) and rear on mulberry leaves in laboratory at standard ambient conditions. Infected larvae of 48, 72, 96, 120 and 144 hours after post collection, were homogenised and proceeded for estimation of Catalase enzyme activity by the method of Samuel and Bernard (1950) judge by the decrease if any in absorbance of Catalase enzyme at 240 nm subsequent to the decomposition of H₂O₂. 0.1g larva was extracted in pre-chilled pestle and mortar by using phosphate buffer (0.1M, pH 7.0). The sample was then centrifuged at 4°C at 10,000 rpm for 10 min. The reaction mixture was taken in the spectrophotometer sample cuvette with addition of 40µl of hydrogen peroxide substrate. The reaction was read on spectrophotometer at 240 nm. unit's mg⁻¹ protein⁻¹m¹ was used

for expression of Catalase activity (Havir and Mettale, 1987). Each time three replicates were used. Data were statically analysed for variance (ANOVA).

RESULTS AND DISCUSSION

Sluggish soft bodied larvae were identified as infected with Flacherie and are used to measure Catalase. Sohal et al., (1990): Orr and Sohal, (1992): Dudas and Arking (1995); Seslija et al., (1999): Nicolosi et al., (2013) reported that Catalase activity amplified with age and lowered during growth, in a many of insects. According to Nicolás et al., (1973) peroxisomal Catalase play a role in adaptation to oxidative stress developed during attack with pathogenic fungus. We however reported a lowering trends in infected groups at 24 and 48h hours post larval collection compared to control, whereas in the case of 72nd hours a marked decrease in Catalase activity reported as compared to control groups (Fig. 1 and Table I).

Shobha et al., (2015) too observed significant decreased activities of Catalase from haemolymph of a fungal infected silkworm as compared to control and supported what we reported during infection with pathogenic bacteria, causing Flacherie. Shobha et al., (2015) further documented that reduced Catalase activity may be responsible for gathering of H₂O₂ which is cytotoxic, and thus causing oxidative stress developed during pathogenic growth. The role of Catalase activity in defence mechanisms in insects was well documented by Xiaofeng et al., (1998) and has also been explained by Felton and Summers (1995). Jagadeesh Kumar and Nabizadeh (2010) too found alteration in level of Catalase activity in silkworm *Bombyx mori* L, under stress. Changes in Catalase activities during pathogenic infections as reported by these earlier reports and in the present findings, thus indicated that levels of Catalase enzyme might be used as a marker enzyme to study the stress caused by pathogenic organism in silkworm *Bombyx mori* in the sericulture rearing centres, at local level.

Sr.No	Time Intervals	Control	Infected
1	At the time of collection	3.36 ±0.12c	2.33 ±8.8c
2	48h after collection	3.1 ±5.7d	2.1 ±5.7d
3	72h after collection	2.7 ±8.8e	1.83 ±6.0e
4	96h after collection	2.6 ± 2.0e	1.77 ±1.52ef
5	120h after collection	1.92 ±3.1f	1.61 ±4.0f
6	144h after collection	1.76 ±2.8g	0.57 ±5.5m

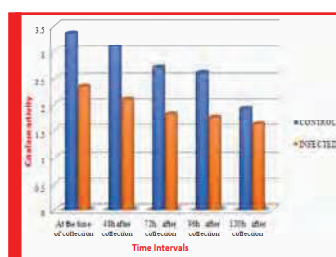


Fig. 1: Graphical variation in Catalase activity during Flacherie infection in Silkworm

REFERENCES

- Aboul-Ela R., Kamel M.Y., Salama H.S., El-Moursy and Abdel-Razek, A (1991). Changes in the biochemistry of the haemolymph of *Plodia interpunctella* after treatment with *Bacillus thuringiensis*. *Journal of Islamic Academy of Sciences* 4 (1): 29-35.
- Adolkar V.V. (1990). Biochemical analysis of disease management in sericulture establishment of the silkworm *Bombyx mori* L (PM x NB18) in Nagpur region of Maharashtra Ph.D. Thesis submitted to the Department of Zoology, Nagpur University Campus, and Nagpur. pp 146.
- Doreswamy C., Govindan R., Devaiah M., C., and Muniswamappa M. V. (2004). Deterioration of cocoon traits of silkworm, *Bombyx mori* L. by the synergistic infection with late larval flacherie pathogens. *Karnataka J. Agric. Sci.*, 17: 345-348.
- Dudas S. P, Arking R. A. (1995). coordinate up regulation of antioxidant gene activities is associated with the delayed onset of senescence in a long lived strain of *Drosophila*. *J Gerontol Biol Sci.*; 50:117-127.
- Felton G W. and Summers C. B. (1995) Antioxidant system in insects. *Arch Insect Biochem Physiol.*; 29:187-197.
- Gillespie J. P., Kanost M. R. and Trenczek T. (1997). Biological

- mediators of insect immunity. *Annu Rev Entomol*, 42: 611-643.
- Havir, E.A. and Mettala, N.A. (1987) Biochemical and developmental characterization of multiple form of catalase in tobacco leaves. *Plant Physiol*. 84:450-455.
- Jagadeesh Kumar, T.S. and Nabizadeh, P. (2010) Thermal Stress Induced Catalase Activity Level in Selected Bivoltine Breeds of Silkworms. *Modern Applied Science*. 4(8):88-95.
- Kadoya T., Yamashita Y. and Kawase S. (1984). Carbohydrate changes in haemolymph and midgut epithelium of the silkworm *Bombyx mori*, during the course of cytoplasmic polyhedrosis. *J. Seric. Sci. Japan*, 53 (4): 352 – 357.
- Li Z, Wang Y, Wang L, Zhou Z. (March 2018). Molecular and biochemical responses in the midgut of the silkworm, *Bombyx mori*, infected with *Nosema bombycis*. *Parasit Vectors*. 6;11(1):147. doi: 10.1186/s13071-018-2755-2.
- Lu Yup-Lian and Liu-Fuan, (1991). Silkworm diseases FAO-UN, FAO Agri Service Bull., 73 (4):1-74.
- Mahalingam C. A., Muruges K. A. and Shanmugam R. (2010). Grasserie Disease Incidence on Silkworm and Development of Botanical Based Management Strategy. *Trends in Biosciences*, 3(2): 212-215.
- Mahesha H. B., Krupa H. P. and Thejaswini P. H. (2009). Effect of nuclear polyhedrosis on some biomolecules of silkworm *Bombyx mori* L., *Indian J. Seric. Sci.*, 48 (2): 126- 132.
- Mahesha H. B., Rahamathulla G. and Thejaswini P. (2013). Studies on Induction of Tolerance against Nuclear Polyhedrosis in Silkworm *Bombyx Mori* L. And it's Biochemical Aspects. *IJBPAS*, 2(7): 1501 – 1512.
- Manohar Reddy B. (2004). Metabolic modulations in various tissues of pre spinning silkworm, *Bombyx mori* (L) Larvae during Grasserie (BmNPV) disease. Ph.D. Thesis, Sri Venkateswara University, Tirupathi, India.
- Martignoni M. E. (1964). Pathophysiology in the insect, *Ann. Rev. Entomol.*, 179 -206.
- Nicolás P, Almudena Ortiz-Urquiza, CarlaHuarte-Bonnet, Shizhu Z. and Nemat O. K. (2013) Targeting of insect epicuticular lipids by the entomopathogenic fungus *Beauveria bassiana*: hydrocarbonoxidation within the context of a host-pathogen interaction. *Frontiers in Microbiology*. 4:1-18.
- Samuel A. Goldblith and Bernard E. (1950) Proctorphotometric Determination of Catalase Activity (From the Department of Food Technology, Massachusetts Institute of Technology, Cambridge) (Downloaded from <http://www.jbc.org/>)
- Seslija D, Blagojevic D, Spasic M and Tucic N. 1999 Activity of superoxide dismutase and catalase in the bean weevil (*Acanthoscelides obtectus*) selected for postponed senescence. *Exp Gerontol.*; 34:185-95.
- Shigematsu, H and Noguchi, A. (1969). Biochemical studies on the multiplication of a nuclear polyhedrosis virus in the silkworm, *Bombyx mori* L. Protein synthesis in the larval tissue after infection, *J. Invertebr. Pathol.* 14 : 301-307.
- Sohal, R.S., Svensson, I. And Brunk, U. T. (1990) *Mech Ageing Dev.*; 53:209-15. 29.
- Nicolosi R. J., Baird M. B., Massie H. R. and Samis H. V. (1973). Senescence in *Drosophila* II. Renewal of catalase activity in flies of different ages. *Exp. Gerontol.* 8:101-108.
- Shobha, R, Venkatappa B. (2015) Biochemical changes of silkworm (*Bombyx mori*) infected with *Aspergillus fumigatus*. (Eds. Viswanath B, Indravathi G), Paramount Publishing House, India. 093-096.
- Switala, J. and Loewen, P. C. (2002) Diversity of properties among catalases. *Arch. Biochem. Biophys.* 401:145-154.
- Xiao Feng, 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.
- Orr, W. C. And Sohal R. S. (1992) The effects of catalase gene over expression on life span and resistance to oxidative stress in transgenic *Drosophila melanogaster*. *Arch Biochem Biophys.*; 297:35-41.