Available online at www.journalijmrr.com

INTERNATIONAL JOURNAL OF MODERN RESEARCH AND REVIEWS

IJMRR

ISSN: 2347-8314

International Journal of Modern Research and Reviews

Volume 2, Issue 2, pp 102-106 February,2014

ORIGINAL ARTICLE

STUDIES ON HISTOLOGICAL CHANGES IN FAT BODY AND SILK GLAND OF SILKWORM *BOMBYX MORI* (L.) (LEPIDOPTERA: BOMBYCIDAE) FED WITH MR_2 MULBERRY LEAVES AND VEGETABLE DYE TREATED MULBERRY LEAVES

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Article History: *Received 2nd Jan,2014, Accepted 5 thFeb,2014,Published 25th Feb,2014*

ABSTRACT

The domesticated silkworm, *Bombyx mori* has been the target of intensive scientific study. Since the dawn of human civilization, the silkworm has been used as a source of silk for producing exquisite textile and dress materials. Because it is industrial importance 'serio' is a latin word meaning silk. Silk "the queen of fibers" is admired by people the world over the silk products are always in great demand. Silk is a fine, lustrous, resilient, elastic and strong proteinaceious fiber. It has been the most loved material for fabrics silk, keeping in this view, the present study is aimed to find out the histological changes in the fat body, and silk gland of silkworm fed with treated vegetable dye on mulberry MR_2 leaves. The present result revealed that characteristic histological changes in the V instars of silkworm, fat body and silk gland of silkworm fed with treated vegetable dye than control silkworm. Silkworm fat body consisted of nucleus, cytoplasmic vacuole and granular substances. In control, silk gland showed columnor epithelium with nucleus, vacuoles and the lumen contained secretory substances, whereas in vegetable dye treated MR_2 mulberry leaves fed silkworm in the V instar fat body and silk gland showed spectacular changes such as swollen nucleus, with more vacuoles in the fat body, whereas, the silk gland showed shrunken nucleus, vacuole in the epithelium, lumen contained less substances suggested that the mobilization of to secretory substance from V instar posterior silk gland to middle silk gland which enhance and to store more amount of secretory substance for spinning.

Keywords: *Bombyx mori*, MR_2 mulberry leaf variety, silk gland fat body

1.INTRODUCTION

Silk worms are lepidopteran insects. The larvae are caterpillars which at the end of the larval stage, spin a cocoon of silk, and transform into pupae and finally into an adult moths, sericulture is both an art and science of raising silkworms for silk production. Indian silk has enthralled fashion watches and all categories of consumers across the world with its vast repertoire of motifs, techniques and brilliant hues, India's traditional and culture bound domestic market and an amazing diversity of silk garments that reflect 'geographic specificity' gave India the status of being the second largest producer of raw silk after China. Fat body serve as the precursor for metabolism in other tissues. The fat body consists of either loosely aggregated or compact masses of mesodermal cells enclosed in a membranous sheath. It is freely suspended in the haemocoel and is surrounded by the haemolymph. Mostly, the fat body tissues are found in the abdominal region. They occur in groups of lobes below the integument around the digestive tract and reproductive organs of the insect. In female insects, the amount of fat body is higher than that of the male insects. The fat body plays a vital role in homeostatic maintenance of the insect. It also serve as a storage organ for the nutrients.

The silk gland in also a kind of the dermal glands. Silk gland in *Bombyx mori* is a protein factory specialized in silk production during the larval growth and development structure situated on the ventrolateal sides of the body from the 4^{th} to 8^{th} segment. The silk proteins are synthesized in the silk glands larvae posses a pair of silk glands which are long tubular structures located parallel to the gut, extending nearly throughout the entire length of the larvae, but much longer, being coiled in the posterior part, anatomically and functionally the silk glands are divided into anterior, middle and posterior silk glands. The silk fibre protein, fibroin is synthesized in cells of the posterior silk gland, and secreted into the lumen and transported to the middle silk gland for storage, glue proteins called sericins are synthesized and

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secreted in the middle silk glands and coat the fibroin accumulating there. The two anterior silk glands, which serve as ducts, coverage near the oral cavity, forming a scleroid structure, the spinneret, through which the silk is extruded as fibre. The structure, development, secretory activity and degeneration of the silk glands and the biochemical characterization of the silk proteins have been thoroughly studied in *Bombyx mori* and some temperature wild silkworms (Prudhomme *et al.,* 1985; Sehnal and Akai, 1990 and Centhilnayaki, 2004). The silk glands, particularly middle silk gland (MSG) and posterior silk gland (PSG) undergo degeneration during pupation. The anterior silk gland (ASG) is non-secretory while the MSG and PSG are secretory parts of the silk gland. They show a remarkable cyclical activity (Barsagade and Tembhare, 20000 and Chenthilnayaki, 2004). The process of histologis in posterior silk gland cells of the silkworm during metamorphosis from larvae to pupa have been studied by Matsumura *et al.* (1968). The gland cells to Lyonet's gland which is accessory to the silk gland in the silkworm larva, is characterized by the presence of complicated canaliculi bearing microvilli on their inner surface, large numbers of mitochondria and remarkable convoluted basal plasma membrane (Yoshio Wakv and Ken-Ichi Sumimoto, 1974). The silk gland in the V instar of silk producing lepidopteran larvae are known to pass through four consecutive phase (i.e.) growth, secretory, regression and degenerative phase and are revealed in *Bombyx mori* (Toshiro *et al.*, 1976 and Sehnal *et al.* 1983; Sehnal and Akai, 1983; Chenthilnayaki, 2004, Balasundaram, 2008 and Ganeshprabu, 2012).

Nature has gifted us more than 500 dye-yielding plant species. The natural dyes can be taken from various vegetable sources like flowers, stem or wood, roots, bark, etc. as well as animal sources and mineral sources, natural colours can also be bright as chemical colours. The natural colour sooths and touches the soul. Extracts from the flowers, fruits and leaves of plants and trees are used in the dyeing process. Each plant gives its own unique colour and each dye can produce a range of tones.

2.MATERIALS AND METHODS

Silkwork V instar of *Bombyx mori* LN B_4, D_2 (Local Bivoltine) were collected from silkworm culture centre at 2nd Agraharam, Salem and Neyveli in Tamil Nadu and they were maintained up to cocoon.

The larvae transported from Salem and Neyveli were tranfereed to bamboo baskets of size 26 cm diameter and 5 cm height as described by Govindan *et al.* (1981). The bamboo baskets were covered with paraffin paper and placed in an iron stand with ant wells. The larvae were reared simultaneously both in control and experimental groups separately on mulberry leaves dipped in vegetable dye solution in the laboratory. The V instar larvae placed at ambient temperature of 25 ± 27°C and relative humidity of 70 to 80%. The larvae were reared in card board and boxes measuring 22 × 15 × 5 cms covered with nylon net and placed in an iron stand with ant wells. The control and vegetable dye treated MR_2 mulberry (*Morus alba*) leaves were fed to silkworm *Bombyx mori*.

Preparation of vegetable dye

Vegetable dye powder were purchased from vegetable dye manufactures in Tamil Nadu, India and to prepare the experimental dose for 1 gram concentration of 1% solution.

Mulberry (*M. alba*) MR_2 variety

This is one of the variety of mulberries selected from Jayankondapattinam Sericulture Farm, Branches are simple vertical, grayish leaves are darkly green, unlobed, elliptic, palmate veined and leathers/smooth/wrinkled. It has good agronomy characters like rooting ability.

Mulberry (*Monus alba* L.) MR_2 leaves treated with vegetable dye

Vegetable dye were diluted to add 100 ml distilled water 1 g, 2 g, 4 g, 8g indigo dye concentration, respectively contents of 1%, 2%, 4% and 8% respectively. Fresh MR_2 mulberry leaves were soaked in this concentration for 15 minutes and then were dried in air for 10 minutes. The treated leaves were used for feeding (five feeding/day) the 3rd, 4th and 5th instars larvae of silkworm, *B. mori* they were maintained upto to cocoon stage. It has been observed that 1% concentration of vegetable dye (Indigo) taken as an optimum dye noted all the morphometric changes economic and biological changes last, confirmed 1% vegetable dye has been chosen as an optimum the enhance silk production.

Experimental group

There are 5 experimental groups 3rd, 4th and 5th instars of *B. mori* larvae fed with the following treated MR_2 mulberry leaves control groups C larvae fed with distilled water treated mulberry leaves. It serve as a control group. T_1 larvae fed with 1 gram indigo 2 g, indigo 4 g, indigo 8 g, indigo dye larvae fed silkworm. They were maintained upto cocoon stage.

Preparation of tissue sampling for histological study

Control and vegetable dye treated MR_2 mulberry leaves fed V instar *Bombyx mori* larvae fat body and silk gland (anterior, middle and posterior) were dissected in insect Ringer's solution (Ephurussi and Beadle, 1936). Dissected fat body and silk gland were fixed by immersion in Bovin's solution or 10% formalin in separate sterilized sample bottles or vials.

Preparation of permanent histological slides

After 24 h of fixation, the fat body and silk gland tissues were processed for dehydration using ascending grades of alcohol. The tissues were gross stained in 70% aqueous eosin to facilitate orientation during embedding. The tissues after dehydration in absolute alcohol and acetone were cleared in xylol and finally embedded in paraffin wax (58–62°C). Sections were cut at 6 μ thickness were deparaffinized using ascending grades of alcohol and stained with haematoxylin and counter stained with aqueous eosin for microscopical observation and microphotographs were taken (Gurr, 1958).

3.RESULTS

The transverse section of the control fat body this V instar appeared to be dirty white mass of tissue. The cytoplasm which stained less intensely stained with eosin exhibited granular organizations. The occurrence of large sized vacuoles was a characteristic feature of this stage. The nucleus seems to be an irregularly shaped. These changes indicate that the fat body cells were in a stage of less synthetic and secretory activity during this period, suggesting the mobilization of more amounts of substances from this fat body which appeared to be sequestered probably in to the silk gland for spinning purpose (Fig. 1a).

Fig. 1. Histological section of control V instar *Bombyx mori* larvae

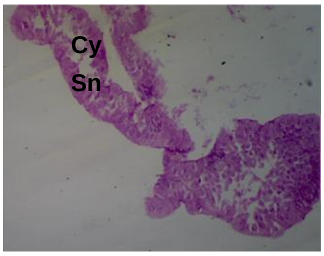

Fig.1a. Histological section of V instar *Bombyx mori* larvae fat body (Control) (× ca 40): Cy-Cytoplasm; Sn- Shrunken nucleus

Fig.1b. Histological section of V instar *Bombyx mori* larvae anterior silk gland (Control) (× ca 40): L-Lumen; Ss-Secretory substance

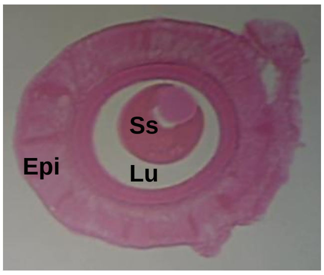

Fig.1c. Histological section of V instar *Bombyx mori* larvae middle silk gland (Control) (× ca 40) : L-Lumen; Ss- Secretory substance; Epi- Epithelium

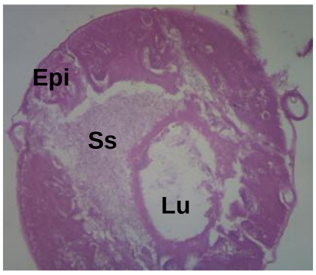

Fig.1d. Histological section of V instar *Bombyx mori* larvae posterior silk gland (Control) (× ca 40): L-Lumen; Ss- Secretory substance; Epi-Epithelium

The silk gland has three regions, anterior, middle, and posterior. The anterior silk gland of V instar larvae exhibited certain remarkable histological changes than the previous stage such as less packed epithelial layer with shrunken nuclei and less cytoplasmic inclusions and more vacuoles in the cytoplasm, indicating very less synthetic and secretory activity. The lumen contained more amounts of secretory substances, suggesting its less utilization of these substances for the act of spinning (Fig. 1b). The middle silk gland of V instar larvae showed certain histological architecture such as an occurrence of a thick disorganized, shrunken and thin epithelial layer with less cytoplasmic inclusions, indicating less synthetic and secretory activity by these cells. The lumen contained globular and gelations colloidal secretory substances. Comparatively the secretory substances in the middle silk gland of V instar larvae were more than the IV instar larvae, in this stage, the lumen was completely filled with secretory substances, indicating both the secretory substances of MSG and PSG considered as storage organ (Fig. 1c). The posterior silk gland was composed of less packed epithelium with large and longitudinal nuclei. It has less packed chromatin materials with more vacuoles in the cytoplasm. The secretory droplets were well evident in the cell as well as in the lumen of PSG in the V instar larvae fed with MR_2 mulberry leaf (Fig. 1d).

The natural dye treated silkworm exhibited remarkable histological changes on the fat body and silk gland. Such as the occurrence of vacuoles in the cytoplasm and less granular substances, indicating the mobilization of nutrient materials for the enhancement of synthetic and secretory activity of the silk gland. The shrunken nuclei in the fat body cells fed to more secretory than the control activity (Fig. 2a). The anterior silk gland of the V instar larvae of *Bombyx mori* exhibited certain histological architecture such as an occurrence of an outer thick epithelial layer cells were every active due to the presence of swollen nuclei, indicating an higher synthetic and secretory activity (Fig. 2b). The lumen contained secretory substances which were seems to be homogenous and globular in nature and more compare to control. The middle silk gland of the V instar larvae of *Bombyx mori* showed certain histological changes such as the presence of an outer thin and faint epithelium. The epithelial cells became degenerated and the lumen was completely filled with secretory substances, indicating the dual function of MSG, both secretory and storage of fibroin and sericin from PSG and MSG, respectively (Fig. 2c). The posterior silk gland of this worm showed certain remarkable changes in the structure of the gland. The outer-epithelium was thick, in each cell contained intact cytoplasm with shrunken nuclei indicating the less synthetic and secretory activity. The lumen consisted of less amount of secretory substances then the lumen of the same age group of worms when fed with MR_2 leaves, indicating that vegetable dye seems to stimulate the secretory and synthetic activity of the silk gland. The secretory substances of the lumen consisted of two types, one was homogenous and the other one was globular in appearance where probably the synthesis of fibroin protein from the PSG (Fig. 2d).

Fig. 2. Histological section of treated V instar *Bombyx mori* larvae

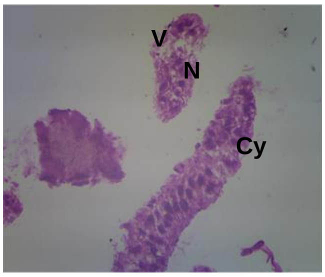

Fig. 2a. Histological section of V instar *Bombyx mori* larvae fat body (Treated) (× ca 40):V- Vacuole;N-Nucleus;Cy-Cytoplasm

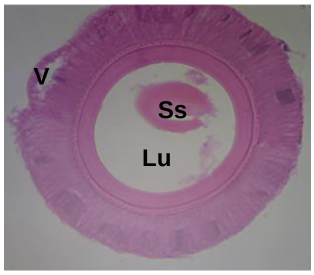

Fig.2b. Histological section of V instar *Bombyx mori* larvae anterior silk gland (Treated) (× ca 40) V-Vacuole; Ss-Secretory substance; Epi- Epithelium

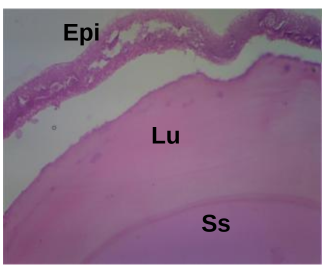

Fig.2c. Histological section of V instar *Bombyx mori* larvae middle silk gland (Treated) (× ca 40): L-Lumen; Ss- Secretory substance; Epi- Epithelium

Fig.2d.Histological section of V instar *Bombyx mori* larvae posterior silk gland (Treated) (× ca 40): L-Lumen; Ss- Secretory substance; Epi-Epithelium; V-Vacuole

In the present observation, it has been shown for *Bombyx mori* that it's fat body undergoes marked histological changes during the period from V instar before spinning. The granular materials identified in the cytoplasm of the cells of fat body with small and large sized cytoplasmic vacuoles seems to increase concomitantly in the V instar and also the cytoplasm and nuclei stained very feebly subsequently in V instar larvae of *Bombyx mori* fed with MR_2, and natural dye treated mulberry leaves. Further, the granular materials representing the nutrient substances seem to have reduced their concentration in the cytoplasm of fat body cells during the act of spinning. The volume of the nucleus is also found to have reduced significantly. It is evident from the present study that the synthetic and secretory activity of the fat body become concomitantly decreased in accordance with the utilization of these nutrient substances for the act of spinning by this silkworm, *B. mori* in V instar larvae. Further, the secretory activity by the silk glands have been found to have increased concomitantly in V instar larvae of *Bombyx mori* when fed with MR_2 natural dye treated mulberry leaves than control in relation to the act of spinning.

4.DISCUSSION

The structure and secretory activity of the silk gland in *Bombyx mori* fed with MR_2 and natural dye treated mulberry leaves have been thoroughly investigated during the present study. Differentiation of silk glands in to three regions, anterior (ASG), middle (MSG) and posterior (PSG) as the sericin secretory and fibroin secretory regions, respectively has been noticed in *Bombyx mori* and other silkworm also (Sehnal and Akai, 1990, Barsagade and Tembhare, 2000, Centhilnayaki, 2004, Centhilnayaki *et al.,* 2004). The maximum growth of silk gland further, that the lumen contains more amount of secretory substances in V instar of silkworm fed with natural dye treated mulberry leaves than the silkworm fed with MR_2 leaf. These changes may be attributed due to an intense secretory activity in the epithelial cells of silk gland rather than MR_2 fed silkworms. Similar changes have also been reported earlier by Akai (1984), Barsagade and Tembhare (2000), Centhilnayaki (2004), Centhilnayaki *et al.* (2004), Balasundaram (2008) and Ganeshprabhu (2012). From the above results, it may be inferred that the MR_2 leaf treated with natural dye is comparatively superior than the MR_2 leaf for sericulture industry to enhance the synthesis of more amount of secretory materials by the silk glands of *Bombyx mori* and also influences the larval and economic characters such as silk production and the nature of silk trait. This MR_2 leaf treated with natural dye must be recommended to the formers to get more performance both in economic characters and morphometric characters . Silk gland secretion and silk production is comparatively more than MR_2 mulberry leaf. From the present study, it is inferred that MR_2 mulberry leaf treated with natural dye is superior to control MR_2, mulberry leaf.

5.CONCLUSION

The present work is a study on the use of natural dye. Natural dye produce bright and fast colours. Natural dyes can proved special aesthetic qualities which, combined with the ethical significance of a product that is environmentally friendly. Natural colours can also be as bright as chemical colours, the natural colour sooths and touches the soul. The present work is a study on the use of natural dye for the determination of secretory activity of fat body ad silk gland ability of the most commercially important silkworm. Therefore, more future comprehensive works are very much solicited in this line with highest concentrations on this species is required. Studies on histological changes in fat body and silk gland of silkworm *Bombyx mori* may be suggested that the MR_2, leaf variety with natural dye is comparatively superior than the MR_2, leaf variety alone for sericulture industry to enhance the synthesis of more amount of secretory materials by the silk gland of *Bombyx mori* and also influences the larval and economic characters such as superior cocoon and silk production. Further, from this present investigation, it is also suggested that an extensive study may be carried out in the silkworm, *Bombyx mori* which is a cost effective, economically feasible, ecologically viable and rich silk producing for the individual as well as to the society and also, the nation as a whole by adopting these techniques and produce good quality and higher production of silk. This vegetable dye (Indigo) has been recommended to the farmers to get more (yield) of silk which is very useful in the field of **sericulture Industry.**

6.ACKNOWLEDGEMENT

The authors are grateful to authorities of Annamalai University, Annamalai Nagar. The help rendered by Dr. (Mrs.) Selvisabhanayakam, (UGC-SAP sponsored), Department of Zoology, Annamalai University, Annamalai Nagar is duly acknowledged.

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