INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

IJPAES

Volume-3, Issue-2, April-June-2013

ISSN 2231-4490

Coden : IJPAES www.ijpaes.com

Received: 02nd Feb-2013 *Revised: 08th Feb-2013* *Accepted: 09th Feb-2013*

Research article

EXTERNAL MORPHOLOGY (CHORION) OF THE EGGS OF MUGA SILKWORM *ANTHERAEA ASSAMENSIS* (HELFER) AFTER TREATED WITH JUVENILE HORMONE -III AND 20 HYDROXYECDYSION

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ABSTRACT: The surface of the chorion of the golden silk producing muga silkworm, *Antheraea assamensis*,Helfer is covered with polygons in network pattern. The number of aeropyles is variable in both treated and untreated eggs. The micropylar pit are surrounded by 17-19 petal shaped primary cells in treated eggs more or less similar in size and shape whereas in the control, their number was reduced to 15-16. Primary cells were surrounded by secondary cells followed by tertiary and quaternary cells.Hatching of the newly hatched larvae takes place through gnawing. Larvae eat their way out through the chorion membrane mostly from the anterior region. Egg buster or spine which aid in hatching are well developed in the newly hatched larvae.

Key words: Aeropyle, chorion, egg, hatching, hormone, ,Muga silkworm

INTRODUCTION

The muga silkworm, *Antheraea assamensis* is restricted mainly to Assam state in the north-eastern part of India.The unique nature of the silk produced by the insect, morphology, behaviour and physiology are quite different from other related species that have found by Dey et al [3] Egg hatching, growth and development processes connected with physiological changes in insect tissues depend on the activity of a number of neuroendrocrine glands which are regulated by three hormones collectively referred as developmental or metamorphosis hormons. Prothoracicotropic hormone (PTTH) induces the thoracic gland to produce another hormone called ecdysone and the action of ecdysteroids on the epidermal cells that is responsible for stimulating the biochemical and morphological events of the molting cycle [15]. The third major effector hormone in insect development is the Juvenile hormone (JH) which plays an important role in insect development and reproduction [19]. Eggs are typically large in size as they contain a great deal of yolk. Egg shell or chorion, a complex extracellular proteinaceous formation has been studied in insects except the muga silkworm following treated with JH-III amd 20 Hydroxyecdysion. Numerous scanning microscopic studies on insect eggs in the external surface structure of the chorion have provided a precise understanding of the mechanism of fertilization and chemical composition of the chorion of some Lepidoptera [5]. The aim of the present study is to carry out SEM investigation on the external morphology of the eggs surface architecture of the muga silkworm, *A. assamensis* to understand its uniqueness after hormonal treatment.The dymnamics of surface features in relation to embryonic development during the time of egg-laying and hatching.

MATERIALS AND METHODS

Insects

Larvae of *A. assamensis* were collected from the rearing farms of Central Muga Eri Research and Training Institute, Jorhat, Assam and reared up to fourth instar on their primary host plant som *Persia bombycina*. Freshly out of 4th moult female larvae were treated with Juvenile hormone (10µg) and 20 hydroxyecdysone (15µg) tropically on head region and reared as usual practice in muga culture.Shortly after emergence, female moths were allowed to copulate with males for 6 hours and oviposition was carried out in dark condition.

Eggs were collected at the same time (10 A.M) on alternate day till hatching.After every collection, eggs were washed thoroughly with distilled water, dried with bloting paper and air. Scanning Electron Microscopy was carried out following standard procedure at 25 ± 0.5ºC.

Observation of Chorion by Bionocular and Scanning Electron Microscope

Eggs were fixed in Neutral buffer formalin solution for 4 to 5 day at 4ºC and observed for overall appearance under a binocular microscope.To observe the surface structure,whole eggs were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH-7.6 for 4h at room temputure.Then eggs were dehydrated in a graed of ethanol series,freeze dried with tert-butyl alcohol,mounted on a stub using double coated adhesive tape,sputter coated with gold and observed for surface morphology with JSM-6360 (Jeol) Scanning Electron Microscope operated at 20KV. Egg dimention (length of major and minor axes and surface area),the unit areas of the polygonal network patterns and size of aeropyles were measured using photograph [7,12].Each value was the average of 50 eggs from eggs batches deposited by 9 moths (10 eggs per batch). For study the mechanisim of eggs hatching,the eggs that are destined to hatch out were observed vigilantly for the activity of the larvae.The hatched eggs along with the larvae inside it were collected for the experiment.

RESULTS

General feature of the Chorionic surface

Aropyles consist of a large number of oval or round spaces or concavities, surrounded by a network pattern mostly hexagonal unit.These network patterns are polygons, which are imprints of the follicular epithelial cells that secrete the chorion proteins. Each polygon has aeropyle at each ridges so that there are six aeropyles in a hexagonal structure, five in pentagonal, seven in heptagonal and so on.But the number of aropyles is more in 20 hydroxyecdysone treated eggs(up to 8 nos).The adjacent chorionic plates oriented in a circular fashion connection with each other through rod like structures (Table.1).The distance between two plates of chorion is maximum in 20hydroxyecdysone 15 µg (6.70±0.17µm) followed by Juvenile hormone III-10 µg (5.41±0.21µm) treatments and in the control (5.30±0.16µm). The length of the major and minor areas were (2.71±0.005 mm) and (2.42±0.01 mm) in 20 hydroxyecdysone, (2.62±0.007mm) and (2.20±0.02mm) in Juvenile hormone -III and (2.5±0.008 mm) and (2.01±0.008mm) in control respectively, while the diameter was (2.406±0.008mm) in 20 hydroxyecdysone, (2.022±0.018mm) in Juvenile hormone -III and (2.06±0.02mm) in the control. Maximum weight of the egg found in 20 hydroxyecdysone treated eggs (6.680±0.13 mg), (6.653±0.22 mg) in Juvenile hormone -III as compared to (5.720±0.15 mg) in the control respectively (Table. 2).

Developmental changes in chorionic architecture

Chorionic surface during development of eggs from laying to hatching reveals significant difference in surface architecture between treated and untreated eggs. At third day,most of the portions of the chorionic surface shows drastic changes in orientation of the plates, characterized by irregularities in their distributions, shrinkage and partial or total loss of demarcating lines of the concavities ,vary uniform in the eggs treated with 20 hydroxyecdysone compared to the control (Fig-4).At fifth days, the orientation changes and chorionic surface shows regular arrangement of chorionic plates in treated eggs but in untreated eggs, there were irregularities in their distributions (Fig-5). The results revealed from the present study that changes in chorionic surface feature takes place during development of eggs.However, the size of the aeropyles was changing as the egg matures. At day 1 of egg development, the aeropyle size was (8.9±0.20 µm), (8.35±0.15 µm), and (7.89±0.17µm), in Untreated, JH-III (Figure. 2), and 20 hydroxyecdysone, respectively. But during the development it increases in 20 hydroxyecdysone treated at day 3 (9.55±0.22 µm), again decreases to (3.7±0.17 µm) at day 5 and it increases up to (9.3±0.16 µm) before hatching (Figure. 7). The size of aeropyle decreases in JH-III and control (6.55±0.27 µm) and (7.95±0.22 µm) respectively before hatching, which shows that in 20 hydroxyecdysone treated eggs hormonal effect was more to take less time in embryo development.(Table. 2).

Table. 1:Changes in chorionic surface architecture from egg laying to hatching

Treatments	Stage of development	Shape of the concavity	Outline of the Concavity	Arrangement of chorionic plate
20E(15µg)	1st day eggs	Round	Demarcating lines are continuous	Oriented regularly surrounding the concavity
JH-III(10µg)		Round	Demarcating lines are not continuous	Oriented regularly surrounding the concavity
Control		Round	Demarcating lines are continuous	Oriented regularly surrounding the concavity
20E(15µg)	3rd day eggs	Roundness disturbed	Loss of demarcating lines	Orientation disturbed
JH-III(10µg)		Roundness disturbed	Loss of demarcating lines	Orientation disturbed
Control		Roundness disturbed	Loss of demarcating lines	Orientation disturbed
20E(15µg)	5th day	Oval and round	Demarcating lines are continuous	Regular orientation
JH-III(10µg)		Oval and round	Demarcating lines are continuous	Regular orientation
Control		Roundness disturbed	Loss of demarcating lines	Orientation disturbed
20E(15µg)	7th day	Round	Demarcating lines are continuous	Orientation
JH-III(10µg)		Oval	Demarcating lines are continuous	Orientation
Control		Oval and round	Loss of demarcating lines	Regular orientation
20E(15µg)	Before hatching	Oval and round	Loss of demarcating lines	Orientation is greatly disturbed
JH-III(10µg)		Oval	Loss of demarcating lines	Orientation is disturbed
Control		Oval and round	Not completely loss of demarcating lines	Orientation disturbed

Table 2. Description of overall measurement of egg of *Antheraea assamensis*

Site	Measurements	Treatments		
		JH-III(10µg)	20E(15µg)	Control
*Length of eggs (chorion surface)	Major axis	2.62±0.007	2.71±0.005	2.5±0.008
	Minor axis	2.20±0.02	2.42±0.01	2.01±0.008
	Diameter of egg surface	2.022±0.018	2.406±0.008	2.06±0.02
*Whole egg	Weight	6.653±0.22	6.680±0.13	5.720±0.15
*Length of chorionic plates	Distance between two,plates	5.41±0.21µm	6.70±0.17µm	5.30±0.16µm
**Aeropyle diameter	Day-1	8.35±0.15	7.89±0.17	8.9±0.20
	Day-3	7.45±0.21	9.55±0.22	3.9±0.20
	Day-5	3.4±0.13	3.7±0.17	4.95±0.18
	Day-7	8.0±0.17	8.8±0.17	9.4±0.16
	Before hatching	6.55±0.27	9.3±0.16	7.95±0.22
*mean ± SD, N= 50; **mean ± SD, N=20.				

The petals surrounding the external micropyle and the rosette pattern made by petals are longitudinal ridges radiate and start from the equator, extended downward .It is irregular in shape without wall and opening canals not distinct in control .The ridges vary prominently in 20 hydroxyecdysone treated eggs than the control (Figure. I).The micropylar pits are surrounded by 17-19 petal shaped primary cells which more or less similar in size and shape but the number of petal shaped primary cells are reduced to 15-16 in the control (Figure. 3). The micropylar pits are almost circular and about 13 µm in width of hormone treated eggs, while it is 10 µm in control.The secondary cells that surrounded the rosette cells which are visible and easy to count. The tertiary and quaternary cells are also polygonal structure. The longitudinal ridges radiated from micropylar area and a few was found above the equator, all of them disappear at the equatorial region (Figure. 8). I t is obvious from the present study that the newly hatched larvae emerge through gnawing. The larvae eat their way out through the chorion membrane mostly from the anterior region but they may emerge also from lateral or any other sites of the egg. Egg buster or spine is well developed in the newly hatched larvae.

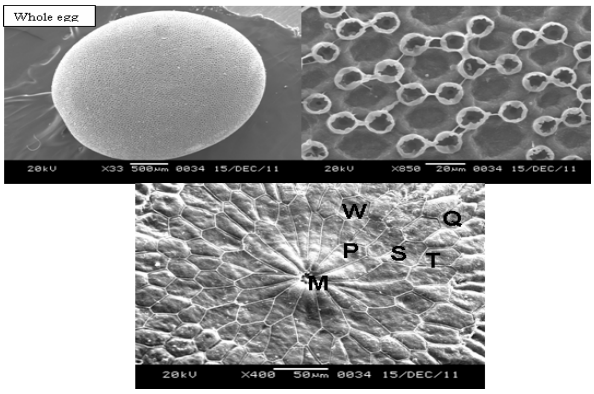

Figure 1. Chorionic morphology at day 1 treated with 20E.M-Micropylar pit, P-Primary cell,S-Secondary cell, T-Tertiary cell, Q-Quaternary cell,W-Cell walls

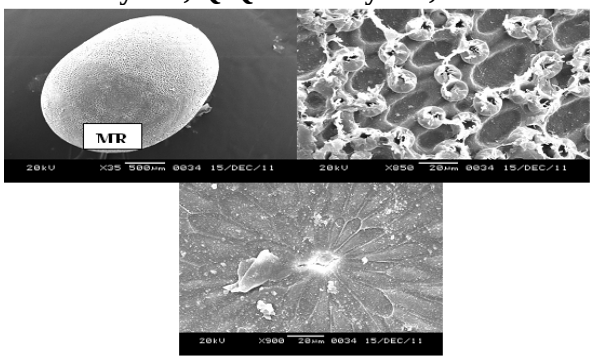

Figure 2. Chorionic morphology at day 1 treated with JH-III, MR-Micropylar region

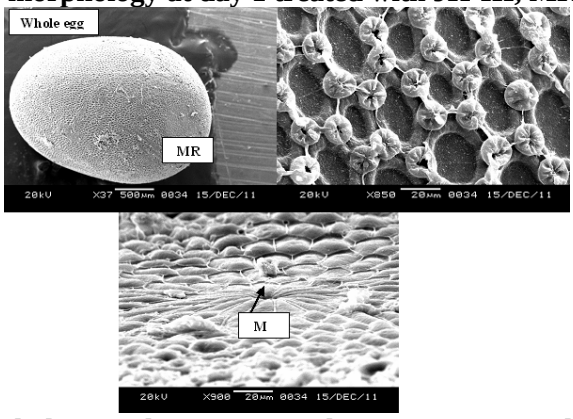

Figure 3.Chorionic morphology at day 1 untreated egg, MR-Micropylar region M-Micropyle

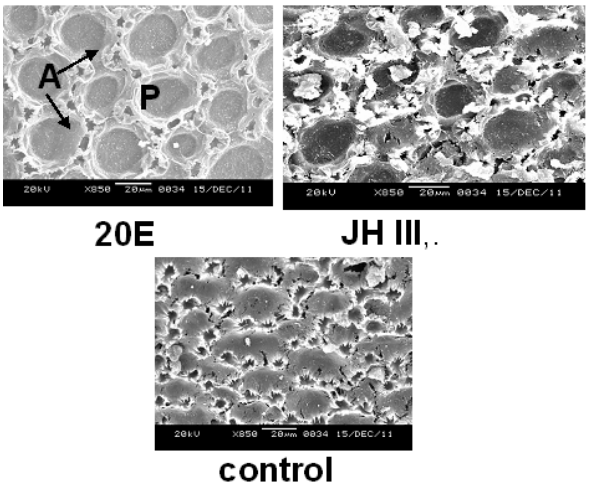

Figure 4.Chorionic morphology at day 3, A-Aropyle,P-Polygons

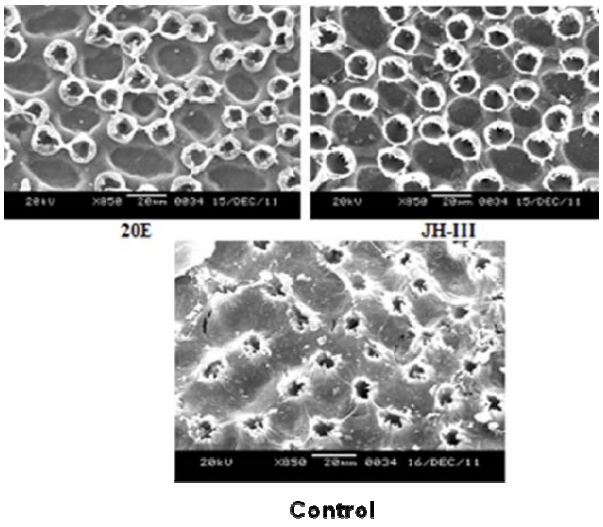

Figure 5. Chorionic morphology at day 5, A-Aropyle, P-Polygons

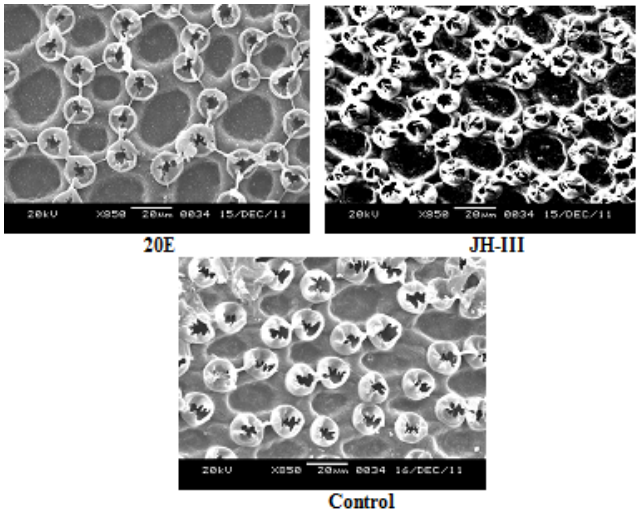

Figure 6. Chorionic morphology at day 7, A-Aropyle, P-Polygons

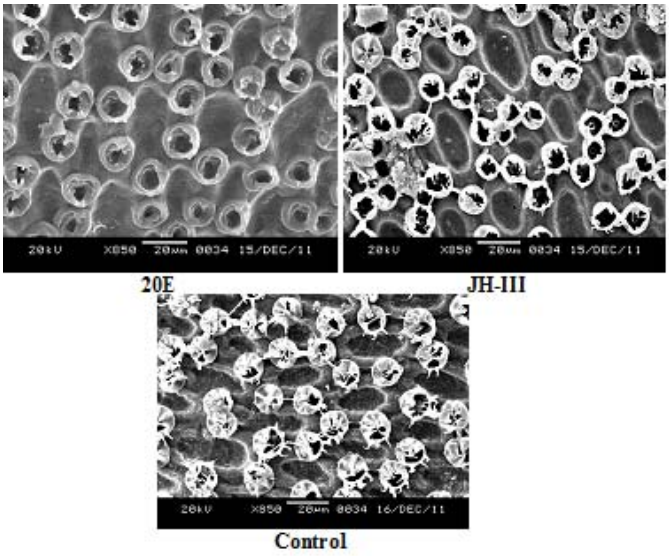

Figure 7. Chorionic morphology of muga silkworm before hatching,A-Aropyle,P-Polygons

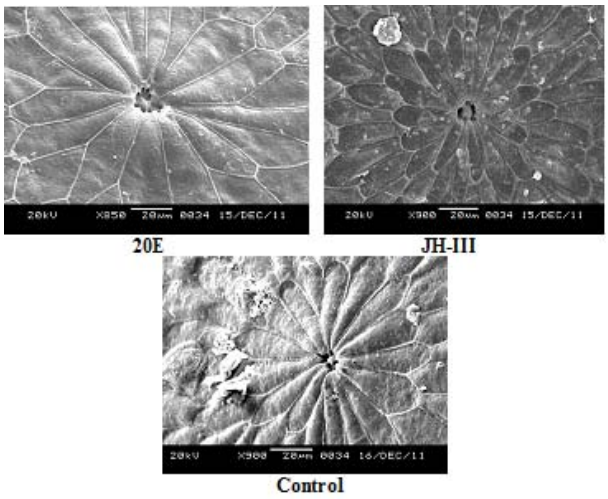

Figure 8. Micropyle morphology of muga silkworm before hatching

DISCUSSION

Althrough studies on surface ultra structure and morphogenesis of chorion have been conducted extensively in several silkmoth egg shell [6] the same has not been investigated in muga silkworm following treated with hormones except one study [3] . The chorion of lepidopteran insects has spatially and morphologically differentiated on surface regions. In the present study, the chorion of *A. assamensis* was observed at the aeropyle crown region. In this respect, the chorion of this species was fundamentally similar to those of *Bombyx mandarina* and *B. mori* [7]. The chorion surface of the mature *A. polyphemus* reveals four clear-cut regions distinguished by differences in cell imprints, i.e., flattened sides, aeropyle crown region, meridional stripe region and micropyle region [14] .Also in *B. mori* [10,17] and *B. mandarina* [7,8] four distinct regions, the posterior and anterior poles, lateral flat sides and ventral (dorsal) edges, are apparent, although there is no aeropyle crown region. The egg size was larger in *A. assamensis* in terms of length of major and minor axis and the area of lateral flat region than those of other lepidopteran insects *viz. B. mori* and *B. mandarina* [7,8] and *Samia ricini* [18].

The petals surrounding the external micropyle and the rosette pattern made by petals as in *Bombyx* [10,11] was also detected in the present study but arrangement and structure are quite different in *A. assamensis*. The polygonal network patterns especially at the lateral flat and marginal regions are regular; there is no significant variation in the unit area of the polygon. At the posterior pole, knobs residing inside the polygonal units are visible prominently. The variability in the size of aeropyles during egg development is difficult to distinguish functionally. However, as they mainly serve for gaseous exchange between the egg and the outside environment, demands for more oxygen uptake by the developing larvae. Too small pore will not permit the entry of adequate supply of oxygen and the eggs of two strains of *Callosobruchus maculatus* with different metabolic rates have differently shaped aeropyles, where only a single aeropyle is present, its size is critically important for gaseous exchange [2]. Again, it is presumed that the decrease may be brought about due to dryness of the egg as the fluid inside continues to dehydrate as a result of development or may be due to its prolonged exposure to dry environment. An interesting case in the eggshell of Hawaiian species *D. grimshawi* which is unusually thick and possibly for that reason has developed numerously but very narrow aeropyles which might function during embryogenesis in dry environment [13]. Thus, the variability in the size of the aeropyles in *A. assamensis* during egg development requires the insect to modify its egg structure according to the demand of the environmental condition.

The study pertaining to hatching of the larvae from the eggs revealed that they gnawed their way out through the chorion membrane in accordance with the process adopted by all larval lepidopteran insects [1] .For the process of hatching, there is rupture of egg either from line of weaknesses or the presence of egg buster or specialized spine or cuticle. The micropylar pits are surrounded by a rosette of petal like primary cells, each of which is out lined by fine walls. According to Downey and Allyn [4] the micropylar region of the chorion often reflects more of the electronic charge back through the micropylar openings. The micropylar pit is about 13 µm in width and circular in hormone treated eggs, is about 10 µm and irregular in shape in the control. In the present study, the overall structure of the egg during the course of development until hatching was observed to change chorionic surface, the minute pores or aeropyles present on the ridges of the entire polygonal network in hormone treated eggs.

ACKNOWLEDGMENT

Authors are grateful to the Department of Science and Technology, New Delhi for the financial assistance and are thankful to the Director CMER&TI, Lahdoigarh for providing necessary facilities and to Head of the Department of Life Sciences, Dibrugarh University for his encouragement.

REFERENCES

[1] Chapman,R.F. 1998. The insects-Structure and function. 4th edition.Cambridge University Press, Cambridge.

[2] Daniel, S.H. and R.H Smith. 1994. Functional anatomy of the egg pore in *Callosobruchus maculatus*, A trade -off between gas exchange and protective functions. Physiol. Entomol. 19,30–38.

[3] Dey, S., R.N.K Hooroo., N.Biswas. and P.S.Dkhar. 2003. Developmental changes in surface architecture in some inorganic components in the muga silk moth (*Antheraea assama*) egg shell (Chorion). Serico. 43,45–54.

[4] Downey, J.C. and A.C Allyn.1981. Chorionic sculpturing in eggs of Lycaenidae.Part I ,Bullatin of Allyn Mus, 61, 1-29.

[5] Hinton, H.E.1981. Biology of insect eggs. Pergamon Press, Oxford.

[6] Hamodrakas, S. J. 1984.Twisted *b*-pleated sheet, the molecular conformation which possibly dictates the formation of the helicoidal architecture of several proteinaceous egg shells, Ins. J. Bio. Macro. 6, 51–53.

[7] Kawaguchi, Y., Y.Banno., K.Roga., T.Kawarabata. and H.Doira.1996a. Comparison of the chorion structure of eggs from *Bombyx mori*, *Bombyx mandarina* (Lepidoptera: Bombycidae) and their first filial generation, Appl. Entomol. Zoo. 31,407–415.

[8] Kawaguchi, Y., M.K. Xu., Y.Banno., K.Roga., T.Kawarabata. and H Doira. 1996b.Egg shell architecture in the wild silkmoth. *Bombyx mandarina*, inhabiting China. Serico. 36,609–618.

[9] Katsuno, S.1984.Surface structure of the chorion in polyploidy silkworm *Bombyx mori.* Journal of Seri. Sci. Japan. 53,97–107.
[10] Kawaguchi, Y., Y.Banno., K.Roga., H.Doira. and H. Fujii.1993. Polygonal patterns of the chorions of giant egg mutant and large eggs induced by 20-hydroxyecdysone in *Bombyx mori*. J. Ins. Physio. 39,437– 443.
[11] Kawaguchi, Y., T.Kusakabe. and K.Roga. 1999. Unique chorion structure of the mottled gray (mgr) egg, an egg-character mutation of *Bombyx mori* (Lepidoptera: Bombycidae). Appl. Entomol. Zoo. 34,359–364.
[12] Kawamura, N. and T.Nakada.1981.Studies on the increase in egg size in tetraploid silkworms induced from a normal and a giant egg strains. Japan J. Gen. 56,249–256.
[13] Margaritis, L.H. 1985.Structure and physiology of the chorion. In Kerkut, G.A., Gilbert, L.I., (Eds) Comp. Ins. Physio. Biochem. Pharmaco. Vol. 1. Oxford, UK: Pergamon Press, 153–230.
[14] Mazur, G.D., J.C. Regier. and F.C. Kafatos.1982. Order and defects in the silkworm chorion, a biological analogue of a cholesteric liquid crystal. In: King, R.C., Akai, H., (Eds) Insect ultrastructure, Vol. 1. Ple. Publi. Corp. New York, 150–185.
[15] Nijhout, H.F. 1994. Insect Hormones. Princeton University Press, Princeton, NJ
[16] Ohtsuki, Y., T. Kanda. and H.Matsumura. 1977a.Surface structure of silkworm (*Bombyx mori*). II. Characteristics of different areas of egg surface, J. Seri. Sci. Japan 46,45–50.
[17] Ohtsuki, Y., T. Kitazawa. and T. Kanda. 1977. In vitro formation of the chorion in the silkworm (*Bombyx mori*). Zoo. Manag. 86,168–173.
[18] Renthlei, C.Z., A. Raghuvaraman., B.Kharbuli. and S. Dey. 2010. Progressive chorion morphology during egg development in Samia ricini (Donovan). Micro.Res.Tech 73, 234–239.
[19] Riddiford, L.M. 1985. Hormone action at the cellular level. In Comprehensive Insect Physiology, Biochemistry and Pharmacology by Kerkut, G. A., Gilbert, L. I., (Eds),Vol. 8, Pergamon Press, New York, 37-85.
[20] Suludere. Z.1988. Studies on the external morphology of the eggs of some Melitaea species (Saturidae:Lepidoptera).Comm. Faci. Sci.Uni. V.6 , 73-84