Research Journal of Agricultural Sciences
An International Journal

Res. Jr. of Agril. Sci. **12**(2): 651–655

P- ISSN: 0976-1675 | **E**- ISSN: 2249-4538

www.rjas.org

Research Paper

A Comparative Study of Fat Body Protein and Commercial Characters of the Silkworm, *Bombyx mori* L. Treated with Juvenile Hormone Analogue

R. S. Umakanth*[1]

Received: 13 Feb 2021 | Revised accepted: 09 Apr 2021 | Published online: 12 Apr 2021

A B S T R A C T

The brain plays vital role in regulating the endocrine system, which serves as an important link between the environment and various physiological activities of the organism. In silkworm *Bombyx mori,* the Juvenile Hormone (JH) is secreted by corpora allatum and JH has a functional role in metamorphosis, reproduction, behaviour, diapause and communication. Proteins are important bio molecules, which play key role in the growth and development as well as silk biosynthesis. The 5th instar silkworm larvae (day three and day five) of two bivoltine hybrids FC_1 and FC_2 and a bivoltine double hybrid $FC_1 \times FC_2$ were used for administration of juvenile hormone analogue (Samrudhi) in three different concentrations 15, 20 and 25 µl and their fat body was collected after 24 h. of treatment along with control and absolute control batches. The results show that a gradual increase in the protein content with the increase in JH concentration in both hybrids and double hybrid under study. The protein content is higher in double hybrid $FC_1 \times FC_2$, followed by the hybrids FC_1 and FC_2 as well as larval duration, cocoon weight, shell weight and filament length exhibited marginal increase.

***Key words*:** Juvenile hormone, Protein, *Bombyx mori*, Fat body, Hybrids

Sericulture is large scale rearing of silkworms for the production of silk for commercial trade. Among several commercial species of silkworm, *Bombyx mori* is the most widely used and intensively studied laboratory model. Sericulture comprises moriculture, silkworm seed production, silkworm rearing, marketing of cocoons, silk reeling, marketing of raw silk and silk weaving. In India, Sericulture industry provides employment to approximately 8 million persons in rural and semi-urban areas. India is the second largest producer of silk in the world, among the four varieties of silk produced, during 2019-20 mulberry silk accounts for 25239 MT (70.46%), Tasar 3136 MT (8.75%), Eri 7204 MT (20.11%) and Muga 241 MT (0.67%) of the total raw silk production of 35820 MT. Proteins are important biological molecules required for growth and development of the silkworm as well as biosynthesis of silk. The proteins get accumulated in various forms as haemolymph protein, fat body protein and silk protein in the silkworm larvae. These proteins are obtained from mulberry leaf, which is the sole food of the silkworm, *B. mori*. The fat body can be found irregularly distributed in the per-visceral space of the abdomen and the thorax, surrounding the organs in the abdomen, dorsal and ventral sinus, closed to the integument, in the head and even in the body appendices. The fat body synthesizes a number of proteins and releases them into the haemolymph during the larval stage [1].

Juvenile hormone (JH) refers to a group of hormones, which ensure growth of the larvae by postponing the maturity. Because of their rigid integument, insects grow during the course of development by successively shedding their old integument and this process is known as moulting. There are four moults during their larval period and juvenile hormones are secreted by a pair of endocrine glands behind the brain called the corpora allatum. [2] succeeded in extracting active juvenile hormone preparation from *Hyalophora cecropia* adults and its purification led to the discovery of juvenile hormone activity. The juvenile hormone (JH) and the 20-hydroxyecdysone (20E) regulate a large number of processes, it is a major circulating hormone which regulates diverse characters like regulation of morphology, reproduction, development and metabolism [3]. Application of the juvenile hormone analogue (JHA) in the late age will prolong the larval stage and inhibit the initiation of larval-pupal differentiation [4]. JH is commercially available in the brand name "samrudhi" used for exogenous application, samruddhi has been found as potent juvenile hormone analogue (JHA) against the silkworm, *Bombyx mori* [5]. The present study was conducted to understand the changes in the protein profiles of the fat body in the silkworm, *Bombyx mori* L. fed with mulberry leaf treated with JH mimic to the 5th instar larvae (day 3 and day 5), in three different concentrations (15, 20 and 25 µl) and to correlate the same with selected commercial characters in treated and control batches.

MATERIALS AND METHODS

*R. S. Umakanth
umakanth@sericulture.uni-mysore.ac.in

[1]Biochemical Genetics Lab, Post-Graduate Department of Studies in Sericulture Science, University of Mysore, Manasa Gangothri, Mysuru - 570 006, Karnataka, India

In the present study, the disease free layings of two bivoltine hybrids FC_1 and FC_2 and one double hybrid $FC_1 \times FC_2$ were procured from Silkworm Seed Production Centre (SSPC), Central Silk Board, Mysuru a unit of National Silkworm Seed Organization were reared following the standard rearing methodology [6]. V_1 mulberry variety cultivated under irrigated condition at Department of Sericulture Science, University of Mysore, Manasa Gangothri, Mysuru was fed to silkworm larvae thrice a day. The data of selected economic traits was recorded during the silkworm rearing for evaluation and correlation of the same with the results of the biochemical studies. The selected economic traits are matured larval weight, total larval duration; cocoon yield, single cocoon weight, single shell weight, shell ratio, filament length and filament weight was recorded. The temperature ranged between 26 - 30°C and the relative humidity was in the range of 55 – 70% during the period of rearing.

Topical (dermal) application method

The larvae after 4th ecdysis were divided into batches of 100 larvae each and all the five batches were maintained in three replicates. The 3rd and 5th day larvae of fifth instar were used for topical application of the juvenile hormone mimic (Samruddhi) by spraying with 15, 20 and 25µl concentrations before feeding the mulberry leaf for all the batches. One batch was maintained as control by spraying distilled water and another batch served as absolute control without any treatment. The treated larvae were fed with untreated V_1 mulberry leaf followed by topical application on day 3 and 5. The fat body was collected by dissecting the treated silkworm larvae (after 24 h. on both day 3 and day 5) in pre-chilled eppendorf tubes was used for quantitative estimation using spectrophotometer. Protein content was estimated through standard procedure of [7] after specified time (24h. after treatment) to understand the impact of the JH on the silkworm body. For accurate quantification, the sample protein was compared with a known amount of a standard protein using Bovine Serum Albumin (BSA). The data obtained for all the batches pertained to amount of protein content present in the fat body was statistically analyzed, correlated and interpreted.

RESULTS AND DISCUSSION

The results obtained for protein content in two bivoltine hybrids FC_1 and FC_2, double hybrid $FC_1 \times FC_2$ of treated, control and absolute control batches by adopting quantitative estimation using spectrophotometer was subjected to statistical analysis using standard deviation and error. This data was used to correlate with the results obtained for protein in the fat body of 5th instar larvae (day 3 and day 5) understudy.

Table 1 Showing the mean values of economic parameters of silkworm rearing (3rd day of 5th instar treated)

Breeds	Treatment µl	Matured larval weight (g)	Larval duration (days)	Cocoon Weight (g)	Shell weight (g)	Shell (%)	Filament length (m)	Filament weight (g)
FC_1	Absolute control	34.85±0.011	22	5.015±0.004	0.989±0.005	19.720±0.108	1073±3.606	0.725±0.004
	Control	34.35±0.008	22	5.023±0.03	1.015±0.004	20.212±0.087	1189±3.055	0.730±0.005
	15	36.78±0.016	22	6.240±0.008	1.134±0.004	20.240±0.052	1348±2.517	0.739±0.002
	20	38.36±0.028	22	6.359±0.003	1.312±0.008	20.763±0.119	1379±6.028	0.742±0.003
	25	38.57±0.005	22	6.722±0.006	1.353±0.004	20.680±0.102	1405±4.509	0.750±0.003
FC_2	Absolute control	35.19±0.003	22	5.126±0.005	1.183±0.003	21.081±0.060	1234±4.583	0.813±0.003
	Control	35.22±0.003	22	5.207±0.007	1.215±0.002	22.351±0.040	1260±2.000	0.816±0.003
	15	38.17±0.033	22	6.217±0.006	1.349±0.004	23.56±0.061	1322±4.509	0.832±0.003
	20	38.95±0.014	22	6.332±0.002	1.427±0.006	24.248±0.085	1388±4.509	0.855±0.006
	25	38.98±0.005	22, 3 h	6.548±0.009	1.487±0.004	24.939±0.054	1433±3.055	0.871±0.002
$FC_1 \times FC_2$	Absolute Control	35.28±0.008	22	5.409±0.008	1.225±0.005	22.647±0.106	1294±2.000	0.816±0.004
	Control	35.34±0.016	22	5.550±0.052	1.236±0.005	22.833±0.090	1356±3.055	0.824±0.057
	15	38.23±0.008	22	6.430±0.004	1.465±0.030	23.560±0.061	1384±3.000	0.841±0.004
	20	39.23±0.004	22	6.594±0.005	1.511±0.026	24.248±0.085	1420±5.000	0.912±0.004
	25	39.25±0.007	22, 6 h	6.817±0.008	1.628±0.066	24.797±0.930	1497±3.606	0.920±0.005

($P<0.05$)

Protein content in fat body

The fat body protein (mg/g.) recorded a slightly higher activity in control over absolute control, while in case of treated batches using the three concentrations the activity shows a gradual increase in batches for all the three concentrations of JH treatments (15 µl, 20 µl and 25 µl). The protein content in the fat body (mg/ml.) is slightly higher in control than absolute control, while a gradual increase was seen in treated batches.

The activity shows a higher trend as the concentration of the JH increases from 15 µl, 20 µl and 25µl in day 3 of 5th instar larvae, the samples were analyzed after 24 h after treatment. The mean values (mg/g) for day 3 larvae with 15 µl recorded were 18.25 in FC_1, 20.73 in FC_2 and 23.13 in $FC_1 \times$

FC_2. The mean values (mg/g) for 20 µl concentration recorded were 18.76 in FC_1, 20.85 in FC_2 and 23.40 in $FC_1 \times FC_2$. The mean values (mg/g) for 25 µl concentration recorded were 19.26 in FC_1, 21.90 in FC_2 and 25.11 in $FC_1 \times FC_2$ after 24 h. of treatment. In case of day 5 treatment, the samples were analyzed after 24 hours after treatment i.e., on 6th day the results obtained are as follows. The mean values (mg/g) for FC_1 recorded was 24.51, 24.81 in FC_2 and 26.17 in $FC_1 \times FC_2$ respectively for 15 µl concentration. While for 20 µl concentration, the recorded mean values (mg/g) is 24.79 for FC_1, 24.92 in FC_2 and 26.43 in $FC_1 \times FC_2$ respectively. The mean values (mg/g) for FC_1 recorded was 25.19, 25.41 in FC_2 and 26.68 in $FC_1 \times FC_2$ respectively for 25 µl concentration. The values of control and absolute control along with the treated batches are presented graphically in (Fig 1-2) respectively. The mean values of selected economic traits of all the four treatments, control and absolute control are presented graphically as (Fig 1-2) ($P < 0.05$).

Table 2 Showing the mean values of economic parameters of silkworm rearing (5th day of 5th instar treated)

Breeds	Treatment µl	Matured larval weight (g)	Larval duration (days)	Cocoon Weight (g)	Shell weight (g)	Shell (%)	Filament length (m)	Filament weight (g)
FC_1	Absolute control	27.19±0.071	23	5.162±0.002	2.023±0.004	39.190±0.082	1232±3.606	0.792±0.004
	Control	27.34±0.166	23	5.557±0.004	2.037±0.005	39.597±0.082	1297±3.055	0.825±0.004
	15	24.77±0.149	23	6.686±0.005	2.230±0.004	39.969±0.070	1370±2.517	0.864±0.004
	20	27.94±1.728	23	6.882±0.004	2.417±0.005	40.165±0.101	1468±6.028	0.935±0.005
	25	26.90±0.606	23	6.909±0.008	2.586±0.003	40.684±0.074	1496±4.509	1.256±0.005
FC_2	Absolute control	32.61±0.062	23	5.225±0.004	2.054±0.003	40.8144±0.055	1297±4.583	0.825±0.004
	Control	32.77±0.005	23	5.309±0.003	2.079±0.005	40.869±0.100	1310±2.000	0.855±0.004
	15	34.27±0.149	23	6.414±0.005	2.415±0.004	40.988±0.512	1331±4.509	1.007±0.003
	20	34.46±1.728	23	6.633±0.004	2.593±0.005	41.018±0.108	1355±4.509	1.152±0.005
	25	38.53±0.034	23, 3 h	6.746±0.003	2.626±0.003	41.436±0.047	1447±3.055	1.195±0.002
$FC_1 \times FC_2$	Absolute Control	31.32±0.008	23	5.505±0.004	2.089±0.051	40.083±0.946	1289±2.000	0.955±0.004
	Control	32.13±0.006	23	5.614±0.002	2.170±0.022	40.970±0.447	1329±3.055	0.998±0.004
	15	33.86±0.002	23	6.725±0.004	2.517±0.005	41.609±0.097	1359±3.000	1.213±0.003
	20	35.88±0.015	23	6.488±0.003	2.665±0.005	41.250±0.071	1467±5.000	1.293±0.004
	25	38.18±0.007	23, 6 h	6.985±0.003	2.780±0.001	41.467±0.023	1520±3.606	1.315±0.004

(P<0.05)

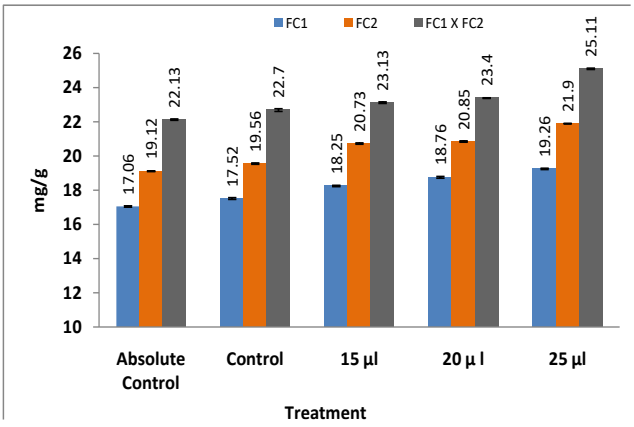

Fig 1 Graph showing the protein content (mg/g) in the larval fat body after 24 h. (5th instar, day 3) treated with JH using three different concentrations

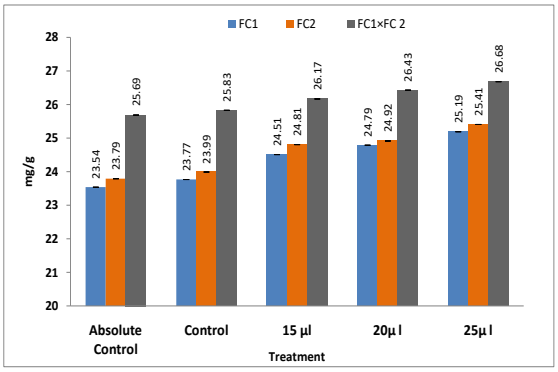

Fig 2 Graph showing the protein content (mg/g) in the larval fat body after 24 h. (5th instar, day 5) treated with JH using three different concentrations

In the silkworm larvae, profound biochemical changes occur in particular, the concentration of proteins and amino acids during the insect metamorphosis. It is a known fact, that factors like weather conditions, diet, feeding habits may affect the enzymatic conditions in the larvae. The endocrine conditions during the fifth larval stage differ from those of other larval instars and are important for larval-pupal metamorphosis. The fat body also synthesizes a number of proteins and releases them into the haemolymph during active feeding (larval) stage [8]. The JH is secreted during the fourth ecdysis and then disappears from the haemolymph during early days of the fifth instar. The ecdysone levels during the

first three days of the last instar are undetectable, but juvenile hormone is still found in the haemolymph [9]. Disappearance of juvenile hormone on day 3, is required for recovery of the prothoracic gland activity for secretion of ecdysone, both ecdysone and juvenile hormone can be detected in the haemolymph [10] after day 5 of the fifth instar larvae. Juvenile hormone analogue causes increase of the haemolymph protein concentrations and this result arises due to preventing sequestration of the storage proteins by the fat body of the *Bombyx mori* [11]. The juvenile hormone controls the silk gland function, prevents their degeneration, and can directly cause an increase in silk production [12]. With this background, many workers used Juvenile hormone mimics in Sericulture to enhance the silk yield, the response of the silkworm in relation to topical administration of the Juvenile hormone mimic depends on the dosage and time [13].

The Juvenile hormone compound was tested and justified in respect to increase the quantity of silk protein, cocoon weight, shell weight which was reported by [14]. The economic characters of the silkworm breeds exhibited a positive response in juvenoid treated breeds without difference in the developmental simultaneity as reported by [15]. The results obtained clearly shows that although Juvenile hormone mimicking compounds influence the silk production positively, it is largely dependent on the dose and time of application as stated earlier [16-17]. In insects, Juvenile hormone exhibited a regulatory effect on protein synthesis. Juvenile hormone and 20E can suppress or induce protein synthesis independently or antagonistically [18-22]. During the fifth instar, in the larvae of *Bombyx mori*, 20E is released in haemolymph from the prothoracic gland on day 3, day 6, and day 9 [23]. Juvenile hormone is secreted during the fourth ecdysis and then subsides from the haemolymph during early days of the fifth instar. Following this, Juvenile hormone titer increases gradually from day 5 until pupation [24].

CONCLUSIONS

Effects of JH mimics on total protein content of fat body were analyzed in the present study. It was observed that the protein content is higher in double hybrid $FC_1 \times FC_2$, followed by hybrids FC_2 and FC_1. There is gradual increase in the protein as the concentration of the JH increases for 15, 20 and 25 µl, which is commonly seen in both the hybrids and in double hybrid higher protein content was recorded. The protein content is higher in control i.e., treated with distilled water than absolute control in both hybrids and the double hybrid under study. The results obtained clearly shows that use of JH mimics in commercial silkworm rearing is beneficial, can prolong the larval period, and adds to the silk content of the cocoon. The economic parameters show a gain in treated batches over control and absolute control. Cocoon weight and shell weight along with filament length has increased as the concentration of JH increased in the treatment. Among the three hybrids under study, FC_1XFC_2 show higher larval weight, FC_2 for shell weight, shell ratio and filament length was observed, FC_1 recorded the least among all the three for all the traits understudy. Larval duration prolonged by one day in batches treated on 5^{th} day in all the breeds under study, while 3 h. and 6 h. increase in FC_2 and $FC_1 \times FC_2$ respectively for treatments of both day 3 and 5 of 5^{th} instar. The results clearly show that spraying of juvenile hormone mimics in commercial silkworm rearing proves to be very beneficial as it prolongs the larval duration and also adds to the silk content in the cocoon due to higher consumption of mulberry leaf contributing to productivity both qualitatively and quantitatively. This in turn gives higher yield per unit area and can also fetch higher price based on the quality of cocoons enhancing the income of the farmers practicing sericulture.

Acknowledgement

The author is thankful to the Chairman, Department of Studies in Sericulture Science, University of Mysore, Manasa Gangothri, Mysuru for the facilities provided to conduct the experiment.

LITERATURE CITED

1. Arrese EL, Soulages JL. 2010. Insect fat body: energy, metabolism and regulation. *Annuals of Review in Entomology* 55: 207-225.
2. Williams CM. 1956. The Juvenile hormone of insects. *Nature* 178: 212-213.
3. Begum NA, Moorthy SM, Venkat S, Nirmal Kumar S, Khadri SMH. 2011. Influence of juvenile hormone analogue, methoprene on the biochemical changes and economic characters of silkworm *Bombyx mori* L. *International Journal of Plant, Animal and Environmental Sciences* 1(3): 171-178.
4. Sakurai S, Okuda M, Ohtaki T. 1989. Juvenile hormone inhibits ecdysone secretion and responsiveness to protharacicotropic hormone in prothoracic glands of *Bombyx mori*. *General and Comparative Endocrinology* 75: 222-230.
5. Kamimura M. 1995. Effects of juvenile hormone analogue, fenoxycarb, on larval growth of the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae). *Appl. Ent. Zoology* 30: 487-489.
6. Dandin SB, Giridhar K. 2010. *Handbook of Sericulture Technologies*. Central Silk Board, Bangalore.
7. Lowry OH, Rosebrough NJ, Farr A, Randall RJ. 1951. Protein measurement with the folin Phenol Reagent. *Jr. Biol. Chemistry* 193: 256-275.
8. Li X, Zhu J, Hui S. 1992. Studies on improving the mounting rate of partitioned cocooning frame by using ecdysone. *Bull. Sericulture* 1: 23-26.
9. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richarson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M. 1998. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92(5): 1-696. doi: 10.1016/s0092-8674(02)09256-5.
10. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M. 1998. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92(4): 573-585. doi: 10.1016/s0092-8674(00)80949-6.
11. Kajiura Z, Yamashita O. 1989. Super growth of silk glands in the larvae of the silkworm, *Bombyx mori*, induced by a juvenile hormone analogue. *Jr. Seric. Sci. Japan* 58: 39-46.

12. Akai H, Kobayashi M. 1971. Induction of prolonged larval instar by the juvenile hormone in *Bombyx mori* L. (Lepidoptera: Bombycidace). *Appl. Entomol. Zoology* 6: 1938-1939.
13. Chowdhury SK, Raju RS, Ogra RK. 1990. Effect of JH analogues on silkworm, *Bombyx mori* L., growth and development of silk gland. *Sericologia* 30: 155-165.
14. Nair KS, Vijayan VA, Nair JS, Trivedi K, Chinya PK. 2000. Influence of a plant based juvenile hormone mimic, ω-formyl longifolene oxime citronellyl ether on silkworm, *Bombyx mori* L. *Sericologia* 40(4): 551-557.
15. Muroga A, Nakajima M, Aomori S, Ozawa Y, Nihmura M. 1975. Utilization of the synthetic juvenile hormone analogue to the silkworm rearing on mulberry leaves. *Jr. Seric. Sci. Japan* 44(4): 267-273.
16. Akai H, Kimura K, Kiuchi M, Shibukawa A. 1985. Increase in silk production by repeated treatments with a Juvenile Hormone Analogue. *Jr. Sericulture Science in Japan* 54: 297-299.
17. Trivedi K, Remadevi OK, Magadum SB, Dutta RK. 1993. Effect of juvenile hormone analogue labomin on the growth and economic character of silkworm *Bombyx mori* L. *Indian Jr. Sericulture* 32: 618-624.
18. Naga Jyothi P, Nagalakshmamma A, Phaninatha Sarma A, Suneetha Y, Siva Prasad S. 2010. Effect of ultrasound on the structural proteins of different tissues of the fifth instar silkworm, *Bombyx mori* L. *Global Journal of Biotech and Biochemistry* 5: 136-140.
19. Riddiford LM. 1985. Hormonal action at the cellular level. *In*: (Eds). Kerkut G.A. and Gilbert L.I. *Comprehensive Insect Physiology Biochemistry and Pharmacology*, Pergamon Press, Oxford. pp 37-84.
20. Ravi Kumar HN, Sarangi SK. 2000. Changes in the concentration of soluble protein and free amino acids during fifth instar development of Eri silkworm, *Philosomia ricini*. In: *Advances in Tropical Sericulture* (Eds) S. B. Dandin, R. K. Mishra, V.P. Gupta and Y.S. Reddy). National Academy of Sericulture Sciences India, Bangalore. pp 433-436.
21. Chandrakala MV, Maribashetty VG. 2009. Application of hormones in silkworm rearing – tapping the potential. *Advances in Mulberry Sericulture.* (Eds). M.C. Devaiah, K.C. Narayanaswamy and V.G. Maribashetty. CVG Publications, Bangalore. pp 345-369.
22. Lix H, Wu XF, Liu JM, Li GL, Miao YG. 2006. Proteomic analysis of the silkworm *Bombyx mori* L. haemolymph during developmental stage. *Jr. Proteome Research* 5: 2809-2814.
23. Chaudhuri A, Sinha AK, Chakraborty D, Ray AK. 1992. Juvenile hormone – Its effect on development and growth of tropical tasar silkworm. *Indian Silk*, Central Silk Board, Bangalore. pp 23-25.
24. Niimi S, Sakurai S. 1997. Development changes in juvenile hormone and juvenile hormone acid titers in the haemolymph and in vitro juvenile hormone synthesis by corpora allata of silkworm, *Bombyx mori* L. *Journal of Insect Physiology* 43: 875-884.