Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Zoology

Elixir Appl. Zoology 79 (2015) 30304-30308

ISSN: 2229-712X

Impact of botanical extracts on the incidence of major pest (tukra) in mulberry leaves on protein profiles in Silkworm, *Bombyx Mori* L

Mude Jagadish Naik*and A. Samba Naik

Department of Zoology & Aquaculture, Acharya Nagarjuna University, Nagarjunanagar, Guntur, Andhra Pradesh, India.

ARTICLE INFO

Article history:

Received: 3 December 2014;

Received in revised form:

20 January 2015;

Accepted: 5 February 2015;

Keywords

Mealy bug, Plant extracts,

Protein profiles.

ABSTRACT

Mulberry, Morus Alba, (L.)Leaves are the predominant food source for silkworm, Bombyx mori rearing. Pink mealy bug infests the mulberry plants and cause Tukra diseases that leads to qualitative loss of mulberry plantation. Hence a preliminary study on protein profiles by SDS-PAGE was carried out using plant extracts as natural botanicals origin by spraying tukra infested mulberry leaves. The botanical extract sprayed to tukra infested mulberry leaves at earlier infection fed to the silkworms and its impact on protein profiles were assayed in tissues like silkgland, and haemolymph was studied. For the study, good healthy leaves(Control)and plant extracts viz., *Azadirachta indica,Ocimum Sanctum,&parthenium hysterophorus* were sprayed to tukra infested V1 mulberry variety and fed to Silkworm (CSR2 Bivoltine hybrid). The protein a profile has been characterized by the presence of bands when increased in all the tissues when fed with sprayed batch. There was no presence of some bands when fed with tukra fed batch. Foliar sprays of the extracts hold greater promise for control of tukra infested mulberry leaves and did not affect protein content in silkworms. This can sturdily suggest that the natural plant extract sprayed with infested mulberry leaves can be effectively utilized for the silkworm rearing instead of pesticides, insecticides for mulberry sericulturistes.

Introduction

The silkworm, *Bombyx Mori* L. is an important economic insect and also a tool to convert leaf protein into silk. The industrial and commercial use of silk, the historical and economic importance of production and its application in all over the world finely contributed to the silkworm promotion as a powerful laboratory model for the basic research in biology [5]. Due to unfavorable conditions in the environment the pests, insects, bacteria, and fungus plays an important role in agriculture, causing a problem to the farmers. As the farmers are using various pesticides and insecticides to control the diseases in agriculture, but the pests are resistive to that pesticides and multiplying the bugs in the plants and decreasing the productivity. Mulberry foliage is also vulnerable to various pathogens and pests and the pests not only reduce the yield but also alter the biochemical components in mulberry leaves which are obviously nutritionally inferior, it leads to crop failure. This focuses on major pest *i.e.*, of pink mealy bug, *Maconellicoccus hirsutus (Green)* attack the mulberry plantation, but the exact molecular level interaction is yet to understand and involvement of virus was ruled out.

Proteins are the building blocks of organism: haemolymph serves as reservoir for nutrients and metabolites during metamorphosis. The silk is secretive by the silk glands and posses the sericin and fibroin proteins [10].A study on protein concentration of haemolymph in *Bombyx mori* revealed that haemolymph proteins play a major role in metamorphosis. The silk gland proteins are different from the haemolymph proteins. The molecular weight mRNA's and tRNA's and genes [15].Protein quantification by SDS-Page reveal the molecular weight of proteins in the tissues. SDS-PAGE protein profiles on Mulberry silkworm suggested that haemolymph proteins influence the growth and development of silkworm larvae [9].

The silk fiber protein is synthesized by the silk gland cells and stored in the lumen of the silk gland. Subsequently, it is converted in to silk fibers. silk fibroin secreted in the lumen of posterior silk gland of *Bombyxmori* consist of three protein components, (H) chain 350KDA, low (L) chain 26KDA and glycoprotein (P_{25}) 30KDA [2] while three layers of sericin secreted from the midlle silk gland in normal larvae [8].

The fibroin protein is one kind of biological materials used for artificial skin and other medical applications. As a result of its biodegradability. Silk fibroin was evaluated for several biomedical applications [23, 11]. It was based films with a thickness of 10-100 µm were developed for acceleration of wound healing and could be peeled off without damaging the newly formed skin. As such, the application of wound protective membranes made from fibroin was investigated. Silk fibroin, like creatine and collagen, belongs to fibrillar protein. The elements of the supra molecular structure of silk fibers are macro fibrils with a width of up to $6.5x10^5$nm in diameter. Nano fibrils may play an important role in imparting enhanced strength to silks. The molecular weight of natural silk fibroin reaches 370000 Da; fibroin macochain length 150nm; and machrochain diameter, 0.45nm [29, 30].

Different concentrations of botanicals were reported effective in suppression of Tukra i.e. mealy bugs in mulberry [3]. Several natural enemies were recorded from mulberry agro-ecosystem[1]. The changing scenario in mulberry poses newer threats with pests like mealy bugs becoming serious and regular. In the recent years serious damage to mulberry by tukra has been reported in rain fed sericulture tract of Karnataka and Andhrapradesh. The commonly employed chemicals used for

Tele:

E-mail addresses: jagadish100naik@gmail.com

control of tukra are dimethoate, dichlorvos hardly control the disease. Moreover, chemical control of disease leads to environmental pollution as well as bio degradation in soil leads to toxicity[6].There is overwhelming support at the global level to use either biological control to eradicate the disease or to employ plant extracts having potency of controlling or eradicating the disease.plant extracts from variety of plants have been reported to posses the inhibiting of mulberry diseases [4]. The present study explores to assess the plant extract sprayed to tukra infested mulberry leaves fed to silkworm and to analyze the total amount of protein in tissues of silkworm of cross breed CSR2 (Bivoltine hybrid) silkworm.

Materials And Methods

Maintenance of Silkworms

For the present investigation, the popular south Indian cross breeds (CB) silkworms CSR2 of Bivoltine breeds of Mulberry silkworms variety, *Bombyx mori* (L) was used as test materials. The disease free laying (DFLS,) of this cross breed CSR2 (Bivoltine hybrid) were produced under field conditions and brought to the laboratory.

Maintenance of botanical Sprayed tukra infested mulberry leaves:

Mulberry crop was maintained by following standard agronomic practices. Treatments were imposed on 15th day of pruning in each plot, five plants were randomly selected and the population of pink mealy bug was counted. In each plant, population was counted on three leaves (top, middle and bottom). The total number leaves per plant were also counted and the population was expressed as number per leaf. Observations were made just before spraying (pre-treatment count), 3, 5 and 7 days after spraying. The following plant extracts with naturally existing insecticidal properties were selected for preparation of aqueous plant extracts *Azadirachta indica, Ocimum Sanctum, &parthenium hysterophorus.*

Plant materials

The plant leaves of Azadirachta *indica, Ocimum Sanctum, &parthenium hysterophorus* was identified and authenticated by the department of Botany, Nagarjuna University, Guntur. The leaves of plants were collected, washed thoroughly with distilled water and shed dried. The dried leaves were powdered with the help of mechanical device. Further 50 gm powdered, thus obtained was subjected to extraction through soxhlet apparatus with 500 ml methanol solvent for 24 hrs. After 24 hrs, given extract was filtered and filtrate was evaporated completely. Evaporated extract material dissolved in distilled water and diluted to 2.5 % concentration for further experiment. Tukra infected Mulberry leaves at earlier stage were identified and were sprayed with extract concentration to mulberry leaves. Treated leaves of various concentrations were fed to III, IV and V instar larvae, four feeding per day The silkworm larvae fed normal mulberry leaves (Served as control), tukra infected mulberry and extract sprayed were administrated (Served as treated). The feeding was maintained at day of 5^{th} of Vth instar larvae and tissues were used for analysis.

Total protein in Silkworm fed with botanical-Sprayed Mulberry leaves:

A bioassay was conducted to find out the effect of feeding healthy and botanical-Sprayed leaves on silkworm hybrid, CSR2. Leaves were collected from plots from 0, 2, 5, 7, 10, 15 and 20 days after spray and were fed to fifth instar silkworm. The haemolymph was drawn out from the larvae by puncturing the proleg. The haemolymph was collected in small ice cooled test tubes rinsed with phenylthiourea solution (1% w/v). Dissection of Silk gland protein solution containing sericin was adaptod. One of the middle silk gland was removed from a fifth instar larva, washed in water, and cut into three parts at the windings. The gland cells were taken away from each part in water with forceps, and the contents of the silk gland were put into beakers with water, which were shaken gently for 30 min. The supernatant was used as the silk protein solution from the middle silk gland. Mid gut epithelia were dissected from mature larvae, washed with 0.75%Nacl, blotted on a filter paper, frozen in liquied nitrogen, and stored at -80^0C until use as frozen mid gut epithelium.

Experimental Procedure for SDS-PAGE:

The supernatants were mixed with equal volumes of 20% sucrose containing 0.1% SDS, ß-mercaptoethanol and bromophenol blue as the tracking dye. An aliquot of 0.1ml (5µl) of the tissue extract was loaded on to the separating gel directly. The electrode buffer 0.025M Tris and 0.192M glycine was used for [7]method, whereas 0.074 M Tris, 0.1% SDS adjusted to pH 7.8 with concentrated HCl. A constant current of 50 volts for the first 15 minutes followed by 150 volts for the rest of the run was applied to the gel. The current supply was terminated when the tracking dye migrated to a distance of 8 cm from the origin.

Staining and Standardization of Proteins:

A solvent containing 0.25% Coommassie brilliant blue in methanol: water: acetic acid (5:5:1) was used for staining the proteins separated on gel by [7] method. The molecular weight standards used in comparing the variations noticed in the SDS-PAGE were analyzed by molecular weight markers and protein molecular weight standards (22 to 400 KD) in silkgland, Fibronin and Sericin)(10 to 60KD in Midgut)(29-97KD in Haemolymph) from the SIGMA-Chemical company from (USA).

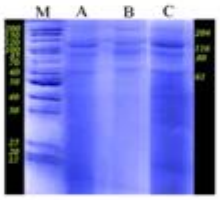
Results And Discussion

The Electrophoretic patterns of proteins at day 2^{nd} and day 6^{th} at Vth instar of CSR2 silkworm were observed in various tissues like haemolymph, silk gland and midgut are presented from plate 1 to plate 5 respectively. The protein patterns observed on SDS-PAGE stained with Coommassie brilliant blue indicated distinct differences in the mobility of some protein bands of silk gland, haemolymph and mid gut.

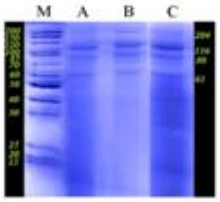
Electrophoretic patterns of silk gland proteins:

An increase in silk gland total protein content during the V^{th} larval instar from day 2^{nd} day4th and day 6^{th} fed with botanical sprayed extracts when compared with normal feeding mulberry (Control). The estimated total protein content at the 5^{th} larval instar was 30.8 mg/g silk gland during day 2 to 72.92 mg/g silk gland at day 6^{th} of the 5^{th} instar (plate1). Generally, the level of total protein showed an increasing trend in the silk gland at the 2^{nd} and 6^{th} day of Vth instar larvae under both control conditions a nd B.sprayed extract fed leaves (plate1). The SDS-PAGE electrophoretic profiles of the silk gland proteins during grown larval instars revealed 17 – 22 bands. At the 2nd day of the 5th larval instar, 17 bands with molecular masses of 58-338 kDa were detected. Four major bands were presented in both control and botanical sprayed fed silkworms when compared to tukra infected leaves fed to silkworms, the bands were less at this day when compared with normal bands four lesser bands at 116 kDa, respectively. As shown in (plate 2) a slight decrease in the number of protein bands was noticed at the 4^{th} day of Vth instar when fed with tukra leves. No obvious difference between control and sprayed leaves fed to silkworm, protein samples in the number of bands (16 bands ranged from 58 to 338 kDa and four major protein subunit bands of 61, 80, 116 and 204 kDa). However, a marked difference in bands intensities, especially

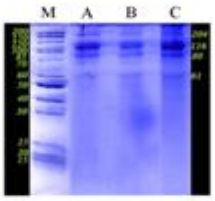
major bands was noticed. It seems that, the Botanical sprayed leaves fed or treatments to silkworm enhanced the synthesis of major bands.



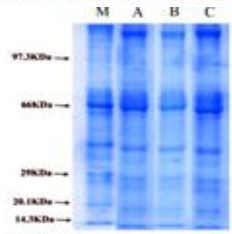
SDS-PAGE pattern of silk gland protein of silk worm *Bombyx mori*. 5th instar, day 2, (M) Standard marker (a) Feeding with healthy mulberry leaves. (b) Feeding of tukra affected mulberry leaves. (c) Feeding of botanical extracts sprayed mulberry leaves.



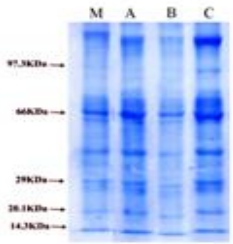
SDS-PAGE pattern of silk gland protein of silk worm Bombyx mori. 5th instar day 4, (M) Standard marker (a) Feeding with healthy mulberry leaves. (b) Feeding of tukra affected mulberry leaves. (c) Feeding of botanical extracts sprayed mulberry leaves.



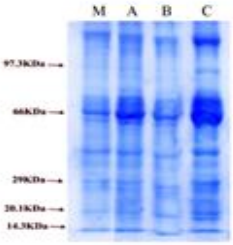
SDS-PAGE pattern of silk gland protein of silk worm *Bombyx mori*. 5th instar day 6, (M) Standard marker (a) Feeding with healthy mulberry leaves. (b) Feeding of tukra affected mulberry leaves. (c) Feeding of botanical extracts sprayed mulberry leaves.



SDS-PAGE pattern of haemolymph protein of silk worm Bombyx mori. 5th instar day 2, (M) Standard marker (a) Feeding with healthy mulberry leaves. (b) Feeding of tukra affected mulberry leaves. (c) Feeding of botanical extracts sprayed mulberry leaves.



SDS-PAGE pattern of haemolymph protein of silk worm Bombyx mori. 5th instar day 4, (M) Standard marker (a) Feeding with healthy mulberry leaves. (b) Feeding of tukra affected mulberry leaves. (c) Feeding of botanical extracts sprayed mulberry leaves.



SDS-PAGE pattern of haemolymph protein of silk worm Bombyx mori. 5th instar day 6, (M) Standard marker (a) Feeding with healthy mulberry leaves. (b) Feeding of tukra affected mulberry leaves. (c) Feeding of botanical extracts sprayed mulberry leaves.

Electrophoretic patterns of haemolymph proteins:

The Haemolymph protein profile of Control and botanical sprayed and tukra infected mulberry reared by silkworm at different days from 2nd to 6th day, haemolymph was drawn for protein patterns. The control groups of haemolymph at 2nd days were seen in to 14bands. The haemolymph proteins observed thick band with molecular weight 105.30KDa, 94KDa, 63.78KDa, 44.45KDa and 29.28KDa. The botanical extract sprayed reared to silkworms, the haemolymph showed a similar bands except the one band which is absent with molecular weight 94KDa compared with control. The plant extracts treated group observed similar bands and was observed that tukra infected fed group the intensity of the bands disappeared. The band with molecular weight 15.66Kda observed in control group and the sprayed fed group the protein bands with molecular weight 63KDa to 38KDa appear thick. The thick bands with molecular weights ranging from 105KDa, 94KDa, 63.78KDa, 57.68KDa, 44.46KDa and 29.28 KDa. SDS-gel indicated a distinct of seven protein bands with several additional bands with poor resolution exhibiting minor variations in the middle region in tukra fed mulberry batch with three protein bands were observed in the haemolymph. Therefore, the protein patterns observed in the haemolymph extract and its secretion are more or less similar with minor variations.

Protein metabolism is considered as most important in the silkworm physiology because of its vital role in the determination of chemical characteristics of silk proteins. Mulberry being a perennial crop and from a sole food to silkworm, *Bombyx mori* L. which is affected by several insects and pests among them in *M. hirsutus* is of late, severe one [27, 28]. As proteins are the important organic constituents of the organs, their role in the compensatory mechanisms of an animal can be expected during acute pests in mulberry when the silkworm feeds.

An increase in silk gland total protein content during the last larval instar was recorded during the present study. The silk gland of silkworms formed the silk gradually during the 5th and at end of 6th larval instars and reached its maximum level just before spinning commences. Maximum level of the protein accumulation during the 5th instar can be correlated with the maximum feeding. Similar findings were reported by [13] who observed that the level of total and soluble proteins recorded an increasing trend in the silk gland tissue from day one to day 6 during the development of 5th instar being 64.54 mg/wet weight on day 6 of total protein comparing to 43.48 mg/wet weight on day 2 of the 5th larval instar when fed with normal and earlier stage of tukra infected mulberry sprayed with botanicals extract with the selected plants like *Azardicta indica, occimum santnum* and *parthenium.* [21]. reported that silkworms produce a large quantity of silk proteins for making the cocoons. The rate of feeding increases at the 4th and 5th larval instars of *B. mori*, the highest amount of silk proteins is synthesized at these stages as reported by [25]. An increase in silk gland total protein content during the 5th larval instar from day 2nd day4th and day 6th fed with botanical sprayed extracts when compared with normal feeding mulberry (Control). The estimated total protein content at the 5th larval instar was 30.8 mg/g silk gland during day 2 to 72.92 mg/g silk gland at day 6th of the 5th instar (Fig. 1). Generally, the level of total protein showed an increasing trend in the silk gland ath the 2nd and 6th day of Vth instar larvae under both control conditions and B.sprayed extract fed leaves (Fig. 1). The SDS-PAGE electrophoretic profiles of the silk gland proteins during grown larval instars revealed 10 – 16 bands. At the 2nd day of the 5th larval instar, 16 bands with molecular

masses of 58-338 kDa were detected. Four major bands were presented in both control and botanical sprayed fed silkworms when compared to tukra infected leaves fed to silkworms, the bands were less at this day when compared with normal bands four lesser bands at 116 kDa, respectively.

The protein components of the silk gland during normal and sprayed fed batch at 2nd and 6th larval instars were detected as about 10 – 16 bands by SDS-PAGE in the present study. No distinguished differences in patterns of both control and sprayed botanicals extract fed batch treatments were observed except increasing in bands intensity toward of the 5th larval instars during day4 and 8. The present data confirm the findings obtained by [20] who found that the rate of protein synthesis during development have markedly increased until the 5th day, while it showed a slight increase between the 3rd day and the 5th day of *B. mori* last larval instar. In the posterior area of the silk gland, proteins with various molecular weights were observed in all stages and increased with the growth of the silk gland [22].

These results may be supported by the findings of [19].who reported that close to the end of the 5th instar the protein increases at stops at the 6th day when fed with good nutritivious mulberry throughout the larval development of silkworm. The level of protein increased through the feeding period and reached the maximum on the final day of the 5th instars. [18].found that maximum level accumulation of protein during 5th instars was similar to that observed for fibroin proteins.

Some of the protein fractions either present or absent. Some of the protein bans increased in their intensity only in some verities. Presence or absence of proteins bands indicates either the non production or utilization or degradation of blood proteins [12]. However, when the studies are concentrated between the races it directly targets the genetic material as they are directly determined by the DNA. Therefore, by studying the silkworm protein with commercial characters, it is possible to have a clear picture of the correlation between them. An understanding of such correlations will help us to identify and exploit the marker molecule during the level of infected mulberry fed to silkworm on its impact of blood protein and silkgland with comprising with normal feed silkworm, *Bombyx mori.*

In the present study, the effect of tukra infection on 2rd and 5th day instar larvae of PMxNB4D2 race haemolymph protein profile in control, inoculated, and plant extracts treated groups showed the variation in protein bands and also the variation in staining intensity on 2nd and 6th day of 5th instars larvae of silkworm. In all groups, the bands observe more in control group than inoculated and plant treated group. The banding pattern was different in all groups of haemolymph sample. During the present study. In haemolymph samples from each group analysed on 3rd and 5th day showed storage protein bands ranges from 63KDa to 38KDa. In control groups the dark stained storage protein bands observe as compared to other groups. On 2nd day the thickness of storage protein bands was less as compared to 6th day haemolymph sample. These changes observed because of alternation in physiological condition. After the infection of tukra fed silkworm the causes and changes in concentration of the haemolymph protein profile in the larvae. After the treatment of botanical extract at earlier tukra the protein bands become normal to control feed and increased in intensity and their number. The tukra fed batch, protein bands number reduced up to 5, 7 and the mealy bug infection was reported by [14]. Investigated electrophoretic haemolymph protein profile of Beaueria inoculated and subsequent treatment of plant extracts in silkworm feed. These results are similar with the present findings. The storage proteins are the major haemolymph proteins in the silk worm larvae playing important role as reservoirs for amino acids that used for development of adult organs [15]. There are two kinds of storage proteins SP-I female specific protein observed in 5th instar.

The levels of the total proteins decreased in the tissues of Silkworm (PMxNB4D2) at day 3 to day 6 of tukra fed leaves and increased the protein profiles when fed with botanical sprayed fed ones. This indicates the deamination of protein synthesis over breakdown during initial stage of pest infection, which is helpful to the animal for developing resistance. It indicates the step wise breakdown of these bimolecular under pest occurring heavily through the diet and no impact of incidence of tukra sprayed by botanical extracts at earlier through the fed mulberry.

References

[1]BandyopadhyayUk, Santha Kumar Mv. Record of natural enemies of mulberry whitefly Dialueropora decempunta and Alleuroclava (Homoptera: Aleyoroididae), West Bengal. *Insect environment* **2007;** (13):2 62-64.

[2] Mondal,MK, Tredy S, Nirmal kumar N. The silk proteins sericin and fibroin in *silkworm, bombyx mori Linn., a review. Caspian J.Env.Sci* **2007(** 5) 63-76

[3] Mukhopadhyay SK, Santha Kumar MV, Das SK. Management of thrips, (Niwa) in mulberry through botanicals. In proceedings of work shop on appropriate technologies for mulberry sericulture held at Berhampore. *India on 17- 18 January*, **2006;(** 1): 48-51

[4]Govindachari TR. Chemical and biological investigation on Azadirachta indicia (neem tree), *J.Current Science.* **1992;(** 63): 117-121.

[5]Ramesbabu KS,RamakrishnaY,HarishkumarreddyG,lakshmi Naidu NV.,Metabolic molecular Mechanism in silkworm larvae during viral infection: review, *African journal of Biotechnology*. **2009;** (8) 899- 907

[6] Purohit MB, Mall LP, and Dubey PS. Residual toxicity of a few herbicides *Journal of Indian Botanical Science.* **1978;(** 57): 305-308.

[7Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage *T.nature.* **1970;** (227):680-685

[8]Akai H, NagashimaT, InoueS, Kobayashi I, TaramuraT. Functional recovery of transgenic silkgland. 20th congress of the international sericultural commission, Bangalore, India, **2005;** (pp: 119): 15-18th December.

[9]Barsagade DD and Tembhare B. Haemolymph aminoacids and protein profile in the tropical tasar silkworm, Antherea mylitta (Drury) (Lepidoptera: Saturniidae) *Entomon* **2004;** (29, 3); 261- 266

[10] Dash R Mukherjee S and Kundu SC. Purification and biochemical characterization of a 70- KDa sericin from tropical tasar silkworm Antherea mylitta. *Int J Bio macromol.* **2006;(** 3);255- 258.

[11] Tsubouchi K, Igarshi Y, Takasu Y, Yamada H. Sericin enhances attachment of cultured human skin fibroblast. *Biosci. Biotechnol. Biochem*, **2005**;(69); 403-405.
[12] Mahesh H B, Thejaswini P H and Honnaiah S. Effect of cytoplasmic polyhydrosis on haemolymph proteins of silkworm *Bombyx mori L. Ann. Entomol* **2000;(** 18,1); 27-32.
[13] Nagajyothi P, Nagalakshamamma K, Phaninathasarma A, Sunitha Y. Effect of ultrasound on biochemical parameters of protein metabolism in the silkgland of fift instar silkworm *Bombyx mori. Sericology***. 2010;(** 5);115-121.
[14] Kadwey M. Studies on the effect of pathogens on some vital organs of tropical tasar silkworm *Anthera mylitta* (D) (Lepidoptera:Saturniidae) Ph.D thesis Rashtrasanth Tukdoji Maharaj, Nagapur University, Nagapur **2009**.
[15] Levenbook in comprehensive insect physiology, Biochemistry and pharmacology, kerkut, G.A. and Gilbert,L.I. (eds). Pergamon press: Oxford. **1985,** 307-346.
[16] Mahmoud S. Feeding effect of different mulberry varieties on silkworm, *Bombyx mori L. Egyptian journal of Applied Science*, **2000**;(15,6): 253-261.
[17] Lakshmikumari B, Anantha narayana SR and Jayaprakash K. Effect of radiation on the activity of digestive enzymes in the silkworm *Bombyx mori L. Sericology* **1997; (**372): 221-228.
[18] Zhong B X, Yu D L, Liang J S, Miao Y G. Comparative analysis of gene expression of enzymes in posterior silk gland cells of silkworm *Bombyx mori* fifth instar larva. *Sericologica.* **2005;**(31):121-128.
[19] Mathavan S, Bhaskaran K, Sironmani A, Pandian T J. Studies on the utilization of single cell protein by the silkworm, *Bombyx mori. Entomol. Experi. Appl* **1984;(** 36): 61-68
[20] Sarangi S K . Studies on the silkgland of *Bombyx mori* L. A comparative analysis of during fifth instar development. *Proc. Anim. Sci* **1985**; (4): 413-419.
[21] Sasaki T, Noda H. Studies on silk fibroin of Bombyx mori directly extracted from the silk gland molecular weight determination in guanidine hydrochloride of urea solution. *Biochim. Biophys. Acta* , **1973**;(310):76-90
[22] Okazaki Y, Yonaiyama S, Nagai T. Analysis of electrophoretic patterns of soluble proteins and tripsin inhibitors associated with the morphological changes of silk gland of *Bombyx mori. J.Seric. Sci.Jpn*, **2005**; (9): 49-67.
[23] Tsubouchi K, Yamada H and Yoko T. Manufacture of high molecular weight sericin by extraction, Jpn Kokai Tokkyo Koho Jap 11092564 A2 (to Norin Suisansho Sanshi Konchu Nogyo Gijutsu Kenkyusho, Japan) 06 April 1999 Heisei, pp 6;Chem Abstr 130**1999**; (22): 301746.
[24] Hisashi H. Varietal differences of leaf protein profiles in mulberry. *Phytochemistyr* **2001;(** 21,7) 1513-1518
[25] Islam S, Haque M A, Qader A R, Khan R. Sericin protein contents in different larval developmental stages in some races of *Bombyx mori L. Pakistan J. Zoo.* **1997**;(29,1): 89-91.
[26] Dhahira Beevi H. Sci. Bull. Fac. Agric. **1991** ;(23): 235-214.
[27] Manjunath D, Ram Kishore K, Sathya Prasad K, Vinod Kumar P, Kumar R K and Datta. Biology of mulberry mealy bug and predatory potential of its biocontrol agent. National Conference on Mulberry Sericulture. **1992** ;(pp. 50).
[28] Tsukda M, Goto Y, and minoura S *j.seric.sci.jpn*., **1990**;(l.59): no.4, pp.325-330.
[29] Gotoh Y, Niimi S, Hayakawa T and Miyashita T. *Biometerials*, **2004**; vol. 25, (no.5):pp.1131-1140