INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-7, Issue-2, April-June-2016 Coden IJABFP-CAS-USA Copyrights@2016

Received: 10th Mar 2016 *Revised: 18th April 2016* *Accepted: 19th April 2016*

Research article

EFFICACY OF MANGROVE PLANT EXTRACTS ON PROTEIN PROFILES IN SILKWORM, *BOMBYX MORI* L

Samba Naik.A[1] * B. Ravi babu[2] and M. Jagadish Naik[1]

[1]Department of Zoology & Aquaculture, Acharya Nagarjuna University, Nagarjunanagar-522 510, Guntur, A.P., India.

[2]Department of Biotechnology, Acharya Nagarjuna University, Nagarjunanagar-522 510, Guntur, A.P., India.

ABSTRACT: Mulberry, Morus Alba, (L.)Leaves are the predominant food source for silkworm, Bombyx mori. 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 tissue like midgut was studied. For the study, healthy leaves (Control) and plant extracts viz., *Azadirachta indica*, *Ocimum sancutum and Parthenium hysterophorus* were sprayed to tukra infested V1 mulberry variety and fed to Silkworm (PMxNB4D2). The protein 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.

Key words: Tukra, Plant extracs, Protein profile, Bombyx mori

*Corresponding author: Samba Naik.A, Department of Zoology & Aquaculture, Acharya Nagarjuna University, Nagarjunanagar-522 510, Guntur, A.P., India E-mail naik.samba@gmail.com

INTRODUCTION

Silkworm, *Bombyx mori*, is a domesticated insect for silk production. It is a lab-reared animal, and its relatively large size allows silkworm to be an important model in molecular genetics and in structural and functional genomics. In addition, the silkworm also is a phytophagous insect and therefore a representative of lepidopteron pest insects. Sustainability sericulture depends upon successful realization of mulberry plantation and rearing of cocoon crop. Various factors like plant diseases and pest affect the mulberry plantation; among these pests are the most dangerous one. Large numbers of chemical pesticides are available for the ruled out these harmful pests. But spray of these toxic chemicals directly or indirectly influence the rearing of silkworm and cocoon productivity. Therefore, routine application of insecticides and pesticides protect the plants from the pests with the short period and however application of toxic chemical prolonged residual effects in mulberry gardens is restricted because of high sensitivity of silkworms (Dandin et al., 2003; Samuthiravelu *et al.*, 2003; Sakthivel et al., 2010; Sambanaik & jagadishnaik, 2012). Some of sucking pests of mulberry viz. pink mealy bug, thrips, spiraling whitefly, etc. have been developed resistance against the available pesticides and because more dangers for mulberry.

Furthermore these chemical pesticides also caused destruction of natural enemies of these pests. Midgut is also a barrier to the foreign substances during food digestion. It has been found that some proteins such as lipase and SP-2 in midgut have antiviral activity against *Bombyx mori* nuclear polyhedrosis virus (BmNPV). Moreover, midgut has early been recognized as one of the important targets for insect control. One successful example is the transgenic crops that produce *B. thuringiensis* crystal *δ*-endotoxins. These toxins bind to their receptors and then form a prepore oligomeric structure through which cell content leaking leads to the death of insect. An alternative method for more specific control of insect is to silence the expression of genes using RNA interference (RNAi) through the midgut.

Reports on Biochemical events in *Bombyx mori* during feeding with contaminated leaves are limited. Vamsheedar *et al*., (2000) reported that impact of mealybug infestation on mulberry leaves on their acceptability as silkworm feed. (Yun – gen miao *et al.,* 2003) reported that biochemical effects of flouride in mulberry leaves inducing toxicity in silkworm haemolymph. Hirayama and Nakamuram (2002) reported the regulation of glutamine metabolism during starvation with effect to development of silkworm larvae. Siddaramaiah and Hegde (1990) studied that the changes in biochemical constituents in silkworm when fed with infected mulberry leaves reducing the chemical constituents like sugars, and amino acids. Zaman *et al*., (1996) reported that silkworms fed on mulberry leaves infected by Tukra (transmitted by *M.hirsutus*) showed negligible decreases in proteins and amino acids and total sugar contents slightly increased.

So far there are limited reports on protein profile of mulberry silkworm in the three tissues Viz., Haemolymph, Silkgland and midgut. But no studies are available comparing the shifts in protein profiles of the haemolymph and Silkgland and midgut in different races of silkworm when fed with Tukra infected leaves and feeding of botanical extracts sprayed in mulberry at different days of the V instar. Hence, in the present investigation a study is made on this line by estimating the protein profiles by (SDS-PAGE) in the silkworm.

MATERIALS AND METHODS

Maintenance of Silkworms

For the present investigation, the popular south Indian cross breeds (CB) silkworms PMxNB4D2 of Bivoltine breeds of Mulberry silkworms variety, *Bombyx mori* (L) was used as test materials. The disease free laying (DFLS,) of this cross breed PMxNB4D2 (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, and 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 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 5th 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, PMxNB4D2. 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 -800C 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 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:

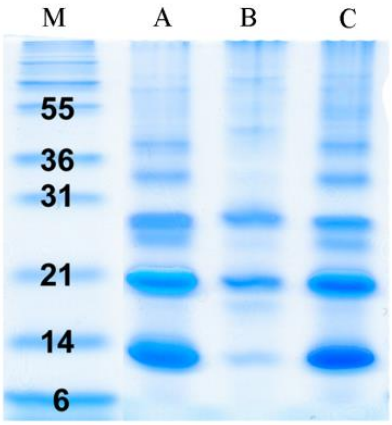
A solvent containing 0.25% Coommassie brilliant blue in methanol: water: acetic acid (5:5:1) was used for staining the proteins separated on gel by [7] method. The molecular weight standards used in comparing the variations noticed in the SDSPAGE were analyzed by molecular weight markers and protein 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

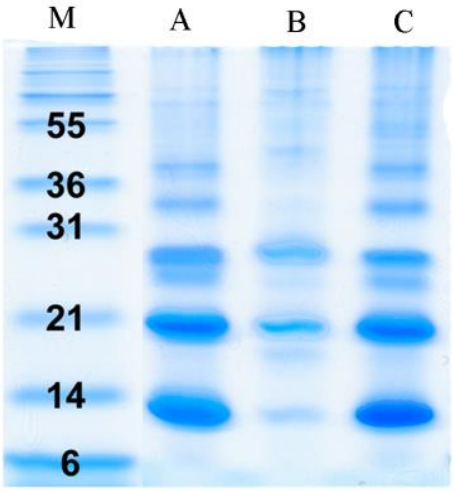
The Electrophoretic patterns of proteins at day 2nd and day 6th at Vth instar of PMxNB4D2 silkworm were observed in midgut are presented from plate 1 to plate 3 respectively. The protein patterns observed on SDS-PAGE stained with Coommassie brilliant blue indicated distinct differences in the mobility of some protein bands of mid gut.

Electrophoretic patterns of midgut proteins:

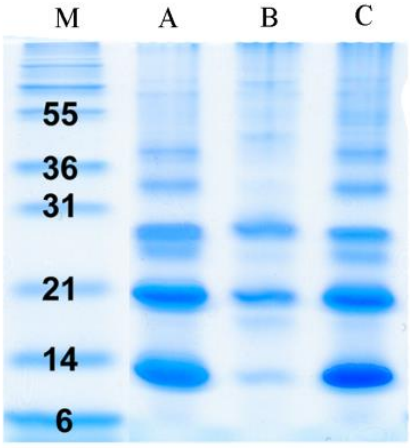
The midgut protein profile of Control, botanical sprayed and tukra infected mulberry reared by silkworm at different days from 2^{nd} to 6^{th} day, midgut was dissected for protein patterns. The control groups of midgut at 2^{nd} days were seen in to 6 bands and the midgut proteins observed thick band with molecular weight 14.0KDa, 21KDa, 26.2KDa, 36KDa. The botanical extract sprayed reared to silkworms, the haemolymph showed a similar bands except the one band which is absent with molecular weight 31 KDa compared with control. The tukra infected mulberry reared batch showed a disappearance of storage protein band from midgut tissue sample on 2^{nd} day of experiment is a decrease in the intensity of protein bands of midgut. The midgut proteins were observed in to 06 bands on 4^{th} and 6^{th} day at 5th instar larvae in normal group. The plant extracts sprayed group observed similar bands with normal group and was observed that tukra infected fed group the intensity of the bands disappeared. The band with molecular weight 31KDa observed in control group and the sprayed fed group the protein bands with molecular weight 14 KDa to 31KDa appear thick. The thick bands with molecular weights ranging from 14KDa, 21KDa, 31KDa, 36KDa, and 55 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 midgut tissue. Therefore, the protein patterns observed in the midgut extract and its secretion are more or less similar with minor variations. The observed gradual enrichment of protein spectrum was probably a result of the consecutive expression of protein in the period of gland growth. Also these results clearly indicated that the most intensive were the protein bands synthesized and accumulated during the last days of the 5th instar which was probably due to the increased synthesis and accumulation of silk with no effect of infection through diet. These results may be supported by the findings of Mathavan *et al.,* (1984) who reported that close to the end of the 5^{th} instar the protein increases at stops at the 6^{th} 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. Zhong *et al.,* (2005) found that maximum level accumulation of protein during 5th instars was similar to that observed for fibroin proteins.



SDS-PAGE pattern of midgut 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 midgut 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 midgut 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 leave

Mahmoud (2000) and Ashour (2005) postulated that *Morus alba* contains comparatively more crude protein, soluble sugar, starch, moisture and fat with no kind of disease during feeding**.** The appearance of particular bands when larvae are fed with tukra infected mulberry a certain variety that disappear with another variety may be explained by varietal differences in crude protein content. The greater utilization of exogenous proteins may be due to high activities of amylase and protease in the haemolymph and midgut tissue, resulting in the production of more silk (LakshmiKumari *et al*., 1997). Thus variable bands might be due to upregulation of digestive (amylase) and oxidizing (succinate dehydrogenase) enzymes to help with no infected mulberry and no impact on silkworm rearing performance with utilize more exogenous food materials ultimately leading to more production by Hisashi (2001).

Quantitative analysis of protein clearly indicated that there is either positive or negative correlation between haemolymph and mid gut proteins with commercial characters. The studies of proteins are paramount importance in the growth and development of organisms. As the haemolymph composition of insects reflects the nature and degree of metabolism of the tissues batched in this fluid, changes in the protein of the haemolymph may show the level of modification in the organism (Mahesha *et al.,* 2000 and Talebi and Subramaya 2011). The silkworm alimentary canal plays an important role in digestion and assimilation of food, it is possible to have a clear picture of protein metabolism by studying haemolymph and mid gut proteins (Saranghi,1985; Nagata and Yashitake , 1989) in their investigations utilizing haemolymph of silkworm, *Bombyx mori* demonstrated that haemolymph proteins which functions as a specific transport media plays a vital role in the growth and development of larvae and it is variable among different breeds. Farshid ghaemi and Mahesha(2012) reported on Studies on haemolymph and midgut tissue proteins with commercial characters by feeding with different mulberry varieties. Our present result also correlates with the results of above workers where in the amount of soluble protein content in the haemolymph and mid gut in PMxNB4D2 race of silkworm fed with control infected tukra fed leaves and selected botanical sprayed extracts to earlier infection of tukra were differently expressed.

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.

CONCLUSION

The botanicals used in crude form reflects its effectiveness, against suppression of mealy bugs was noticed. Based on the results of the study mulberry growers may use botanical extracts instead of chemicals which is used for the suppression of mealy bugs in mulberry fields. Feeding of silkworm with the mulberry leaves treated with botanical extracts showed marked improvement in the silkworms instead of feeding with tukra infected or contaminated mulberry.

REFERENCES

Dandin, S. B., Jayant Jayaswal and Giridhar, K. (2003). *Hand Book of Sericulture Technologies*, Central Silk Board, Bangalore, India, 53 PP**.**

Sakthivel.N, Balakrishna.R, Ravikumar.J, Samuthiravelu.P, Isaiarasu.L and S.M.H. Qadri, **(**2012). Efficacy of botanicals against jassid *Empoasca flavescens* F.(Homoptera: Cicadellidae) on mulberry and their biosafety to naturalenemies. J.Biopest, 5 :246 - 249

Samba naik and Jagadish naik, (2012). Effect of Botanical Extracts against the Tukra (*Maconellicoccushirsutus)* Infested mulberry on Trehalose and Phosphorylase Activity in Silkworm, *Bombyx Mori* L. J.Agriculture and veternery Sci. pp: 50-53.

Samuthiravelu, P., Suresh, A. and Jayaraj, S. (2003). Effects of botanical and biological control tactics against mulberry tukra mealy bug, *Maconellicoccus hirsutus*. Abstract of National Seminar on disease and pest management in sericulture, conducted by Sericulture College, Chintamani, Karnataka on 12-13 September, 2003, 19 P.

Mahesh, H.B., Thejaswini, P.H and Honnaiah, S. (2000). Effect of cytoplasmic polyhydrosis on haemolymph proteins of silkworm *Bombyx mori L.* Ann. Entomol, *18(1):27-32*

Sarangi, S.K., (1985). Studies on the silkgland of *Bombyx mori* L. A comparative analysis of during fifth instar development. Proc. Indian Acad. Sci. Anim. Sci., 94 (4): 413-419.

Mahmoud s (2000). Feeding effect of different mulberry varieties on silkworm, *Bombyx mori L.* Egyptian journal of Applied Science 15(6):253-261.

Ashour A. (2005). Silkworm feeding trials for evaluating the varietal effect of three mulberries leaves on the silkworm growth and cocoon yield quality. Egypt journal of Agricultural Research, 83(3): 1043-1049.

Lakshmi kumara B, Ananthnarayana SR & Jaya prakash K (1997). Effect of radiation on the activity of digestive enzymes in the silkworm *Bombyx mori* L. Sericologia 37(2):221-228.

Hisashi H (2001). Varietal differences of leaf protein profiles in mulberry. Phytochemistry *21(7): 1513-1518*

Mathavan, S., K. Bhaskaran, A. Sironmani, T.J pandian, (1984). Studies on the utilization of single cell protein by the silkworm, *Bombyx mori.* Entomol. Experi. Appl., 36: 61-68

Zhong, B.X., D.L. Yu, J.S. Liang, Y.G. Miao, S.K. Xu, (2005). Comparative analysis of gene expression of enzymes in posterior silk gland cells of silkworm *Bombyx mori* fifth instar larva. Sericologica, *31:121-128.*

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

Zaman, Qader, A., Islam, M.A., barman, S., Alam, A.C., Islam, M.S. (1996). Effect of feeding of tukra affected mulberry leaves on biochemical and economic characters of silkworm, *Bombyx mori* (L.). Pakistan Journal of Zoology, 28(2): 169-171

Vamseedhar, P., Shree, M.P., Subramanyam, M.V.V. (2000). Impact of mealey bug infestation on biochemical constituents of mulberry leaves and acceptability as silkworm feed. Department of sericulture, Bangalore University, Bangalore, India.

Siddaramaiah, A.L. and Hegde, R.K. (1990). Studies on changes in biochemical constituents in silkworm *Bombyx mori* (L.) cercospora infected leaves of mulberry. Mysore J. Agric. Sci. 24: 353-357

ISSN : 0976-4550

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Email : editor.ijabpt@gmail.com

Website: www.ijabpt.com