

**EFFECT ON COCOON PARAMETERS AND HISTOLOGICAL CHANGES IN THE  
SILKWORM, *BOMBYX MORI. L* ON EXPOSURE TO AQUEOUS PLANT (MANGROVE)  
EXTRACTS AGAINST TUKRA DISEASE**

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**Abstract:**

Silk is a commercial commodity for India and many other countries. The large-scale production of silk associated with several factors and nutrition remain prime one. The silkworm feed on mulberry leaves and life cycle of worm depends on supply of food and quality as well. There are several diseases affect nutritional values of mulberry leaves in fact leads to diseases. Feeding of silkworm with such poor quality (nutritional values) and infected leaves may affect development of worm and silk production. The emphasis is given to use natural methods and approaches to improve nutritional values of mulberry leaves and infections. In recent studies bug (*Maconellicoccus hirsutus*) is key pathogen for Tukra disease in mulberry leaves and reported one of most common and serious pest problems. The impact of diseased mulberry leaves was studied on silkworm development. The present study has been aimed at investigating various economic parameters of the silkworm cocoon, when fed with aqueous plant extract mulberry leaves with *Sonneratia apetala* and *Avicennia alba*. Further, histological changes in mid gut and silk gland were explored in present study while feeding silkworm with healthy and infected mulberry leaves. The study also provides a detailed histological analysis of while feeding plant extract sprayed mulberry leaves to silkworm. The experimental worms were divided in to three groups there are control group, tukra affected group and extract sprayed fed group. Control group of silkworm larvae was fed with normal mulberry leaves. Cumulatively, the findings of the present study were observed to evaluate the modulatory role of plant extracts with particular reference to the quality and quantity of the silk.

**Keywords:** *Bombyx mori. L*, Plant extracts, cocoons and tukra, histology, mid gut and silk gland

**Introduction**

The major input in the manufacturing of raw silk is cocoons that play an important role in the production and quality of raw silk. In topical countries such as India, cocoons are available throughout the year unlike in temperature countries where the cocoon production is seasonal. Entrepreneurs have to assess the availability of cocoons taking in to consideration the requirement based on different characteristics. The quality of cocoons and its suitability should be ascertained for achieving. Optimum productivity and raw silk quality for an established process line (Madhavappa *et al.*, 2000). The cocoon weight shows a continuous loss of weight from the time of its completion till the emergence of the moth because of loss of moisture. The most important commercial character of cocoon is weight as weight indicates the approximate quantity of raw silk that can be reeled from it that single cocoon weight is very important for the assessment of the quality. The weight of the cocoon depends upon the rate may range from 1g to 3g. The weight of the cocoon is not constant but decreases after the pupa get transformed in to mouth and shell weight another important commercial character shell weight means weight of the silk shell excluding pupa (shell weight =cocoon weight-pupa weight).

The shell ratio ranges from 12 to 15 percent in multivoltine hybrids 16-19% in newly evolved hybrids 19-25% in Japanese reeling cocoons. Filament length of cocoon is a direct measure towards the cost of

the cocoon. This can be measured by reeling silk thread from single cocoon and the filament length varies from race to race with multivoltine races can yield 500mts of silk filament and 700-1000mts from the cocoons of bivoltine races. The thickness of the silk filament is called denier the denier is the important character of the cocoon. The quality of cocoon crop depended on the vigor of silkworm breeds and also influenced by the mulberry leaf quality (Venugopal pillai *et al.*, 1987). According to Bajpeyi *et al.*, (1991) the raw silk production per hectare of land is approximately 52 kg in 40 kg in Japan. Silk is a natural fibrous protein. The protein content of the cell is altered from time to time by their degradation and synthesis and thus operating a dynamic equilibrium in protein environment (Young, 1970). According to Harper *et al.*, (1979) the total protein profile of a tissue may be taken as a diagnostic tool and assessing the physiological status of an animal as a whole. It can be concluded that the silk production is in turn depends on the protein synthesis machinery of silkworm.

The production of good quality of cocoons is also influenced by some chemicals or insecticides which contaminate the mulberry leaves. Kuwana *et al.*, (1967) had studied the effect of several insecticides on the cocoon of silkworm, *Bombyx mori* by means of subcutaneous injection through microsyringe, topical application and oral administration. Significant changes in the commercial characters of cocoon like weight, shell weight, shell ratio in the larval stage of IV&V instars of *Bombyx mori* in relation to botanicals administered on incidence of mealy bugs (Tukra). Keeping in view of this attempts is made in this investigation to study the effect of some promising botanicals against tukra on various cocoon characters of commercial importance like cocoon weight, shell weight and shell ratio.

Histology the study of microanatomy of specific tissues has been successfully employed as a diagnostic tool in medical and veterinary sciences since the first cellular investigations carried out in the nineteenth century (Virchow, 1858). Diagnosis and prediction of physiological consequences in the animal can be obtained through histopathology (Fanta 1997; Silva *et al.*, 1993). Exposure of insects feeding from contaminated leaves cause severe pathological changes at the tissue level and histopathology gives useful data concerning the changes in the insect tissue at the cellular levels. All the tissues and organs in the body of an insect may be potential targets from the toxic effects from pests and pesticides when the insect feeds on mulberry leaves the efficacy of the food required to reach its full potential will be manifested in various ways and degrees (Waldbauer, 1968). Nutritional efficiency in the larval stages significantly influences the resulting pupae. Biochemical, histological and physiological status in the tissues and adult and production of silk particularly in the economically important insects like *B. mori* (Aftab Ahamed *et al.*, 1998).

### **Materials and Methods**

The present investigation was carried out on the PM xNB4D2 hybrid variety of the silkworm, *Bombyx mori*. Since the experiments required continuous maintenance of the test species, silkworms were reared in the laboratory itself in accordance with the procedure (Krishnaswami, 1978). Mulberry crop was maintained by following standard agronomic practices. Treatments were imposed on 15<sup>th</sup> 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 chosen for spray of mealybug infection in mulberry plants. Plants having insecticidal properties like *Sonneratia apetala* and *Avicennia alba* Were taken from the costal line of manginapudi village near Machilipatnam, Guntur, and Andhra Pradesh. The

leaves of plants were collected, washed thoroughly with distilled water the fresh leaves were homogenate with the help of mechanical device. Further 200 gm of crude selected plants were 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 was dissolved in distilled water and diluted to 2.5 % concentration and used for spray at the identified plot with earlier infection of mealy bug in mulberry plants. Botanical extracts sprayed to tukra leaves of various concentrations were fed to third instar larvae with four feeding per day. The feeding was maintained up to the earlier end of cocoon stage of the silkworm.

### **Cocoon parameters**

By the end of seventh day of 5th instar, mounting of the silk worm larvae on the chakra was started. After the cocoons were completely formed the below given economic parameters were analyzed in control and the experimental groups of silk worms. The cocoons from tukra infested mulberry fed batch of Vth instar larvae showed decreased cocoon weight over the control and the changes were found to be statistically significant over the control ( $p < 0.05$ ). Less percent decrease in the botanical sprayed fed larvae the cocoons were observed and were not significant equal to control. The shell weight of the cocoons from the Vth instar *B.mori* L., larvae fed with tukra infested mulberry exhibited lowered cocoon weight from cocoons and more percent depletion of their shell weights and all the changes observed were found to be statistically significant over the control. Less percent decrease in the botanical sprayed fed larvae the cocoons shell weight was observed and were no significant equal to control. The shell ratio of the cocoons from the Vth instar in silkworm, of PMxNB4D2 larvae fed with tukra infested mulberry exhibited lowered cocoon shell from the larvae more percent depletion of their shell weights and all the changes observed were found to be statistically significant over the control. The botanical sprayed fed larvae the shell ratio was equal compared with control were observed and were no significant equal to control.

### **Histology**

The microscopic examinations were carried out for histological changes during infection and feeding of plant extract sprayed mulberry leaves. Silkworm is largely dependent for food on mulberry leaves and in the present study both infected leaves (Tukra diseases) and mangrove plant extract sprayed leaves were used for histological changes. The study involves a high resolution of microscopy of midgut and silk gland during infection with Tukra diseases and feeding of mulberry leaves sprayed with mangrove plant extract. In the control study normal mulberry leaves were fed to PMXNB4D2 hybrid Silkworm, *Bombyx mori*. The microscopic examinations were carried out at 100X and 400X for all major historical parameters including midgut, silk gland.

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). back the cuticle. The fat bodies, free from adhering connective tissues, were carefully taken with the help of forceps and washed with physiological saline (0.9% NaCl). The excess water was removed with the filter paper. The required weight of the tissue was weighed nearest to 0.1mg and used for biochemical analysis. Silk gland protein solution was prepared according to the method of Simizu *et al.*, (2005) 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 residue 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 liquid nitrogen, and stored at  $-80^{\circ}\text{C}$

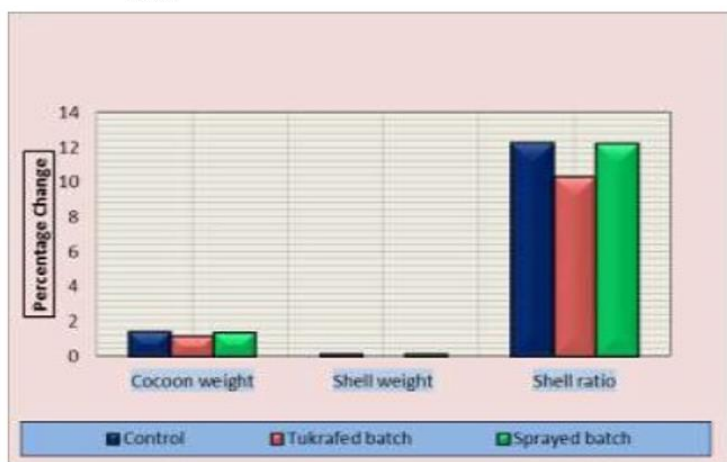
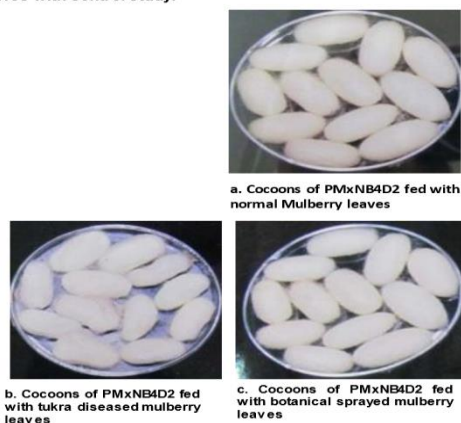
until use as frozen mid gut epithelium. The membrane fraction of mid gut epithelium was prepared by the method of Hinton and Mullock (1997).

## Results

### Cocoon Parameters

Cocoon characters like single cocoon weight, Shell weight, shell ratio, filament length and filament weight in PMxNB4D2 race of silkworms were determined under this study on feeding with tukra and botanicals compared with control by the method as described by Krishnaswami, *et al.*, (1987). After ripening, the silkworms are allowed for spinning of cocoons on bamboo mountages (3'X2') duly covering the moulage were placed horizontally on the rearing stand in tiers to provide uniform environmental conditions. The ideal spinning temperature (260C-270C) and relative humidity (60%-70%) are maintained in the mount age room. The cocoons were harvested on 6th day mounting and the cocoon characters such as cocoon weight, shell weight and shell ratio were studied. The stimulatory capacity of the botanicals against tukra on various cocoon characters contributing to silk yield may be attributed to the synthesis of proteins and nucleic acids in the silkworm. Further the tukra infested mulberry fed batch may affect body cells or regulation of the organs *viz.*, Neurosecretary glands and decreases the biochemical constituents corresponded to increases in cocoon weight and silk output. The increase in cocoon weight and biosynthesis of silk protein (fibroin and sericin) concentration which in turn is related to superior silk quality can be attributed to the increased synthesis of RNA in silkworm.

Morphological changes in *Bombyx mori* cocoons during feeding of Tukra infected and botanical extract sprays mulberry leaves with control study.



Percent change over control in cocoon characters of PMxNB4D2 hybrid silkworm at different days of Vth instar larvae fed with tukra and botanical extracts sprayed mulberry leaves.



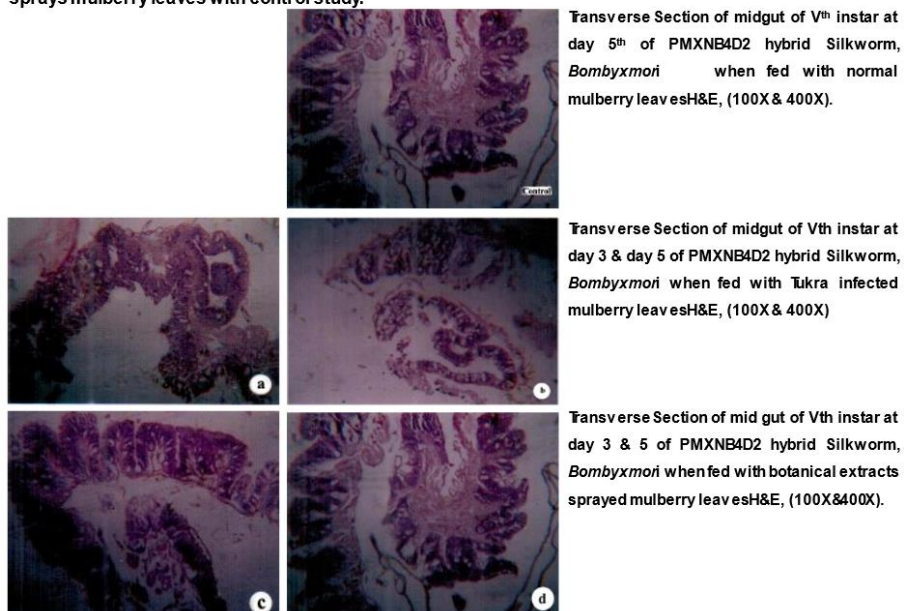
## Histology

Light microscopic sections of the midgut and silk gland of the PMxNB<sub>4</sub>D<sub>2</sub> silkworms, both normal, botanical sprayed and tukra affected chawki leaves fed by the silkworms at fifth instar larva at day 5 were examined. From the plate I it is seen that the normal midgut wall of silk worm consisted of Musculosa, basement membrane, epithelial layer and the peritrophic membrane. The musculosa consisted of outer longitudinal and inner circular muscle fibers. Next to musculosa was the basement membrane followed by the epithelial tissue which was a single layer of columnar and goblet cells. The columnar cells were cylindrical, each containing a large coarse granular nucleus occupying a middle position in the cell. The goblet cells occurred between the columnar cells. The columnar epithelium had striated border (microvilli) covered by the peritrophic membrane. The structure of the midgut wall of silkworms, (Plate I-control and Plate I-C, D) fed by the silkworms with botanical sprayed earlier infestation of mealy bugs mulberry leaves did not show any variation as compared with normal mulberry administration.

## Mid gut histology

The midgut of silkworm fed with tukra infested mulberry at day 3rd examined shows that the midgut epithelial cells slightly sloughed off into the lumen disintegrated slightly and are granular. The peritrophic membrane was not destroyed but the striated borders of microvilli were slightly damaged and the gut lumen filled in with the debris were slightly minimized. In some regions it detached from the epithelium, the circular and longitudinal muscle fibers were slightly degenerated and lost their normal appearance (Plate I-A). In (Plate I -B) the midgut of the silkworm at day 5 fed with tukra affected leaves showed relatively light cell depletion compared with control. Thus, among the normal and botanical sprayed mulberry administrated as feed the tukra fed to silkworms are having the degree of damage in the midgut was seen in the mid gut tissue of the silkworm hybrid.

Histological changes in *Bombyx mori* midgut Vth during feeding of Tukra infected and botanical extract sprays mulberry leaves with control study.

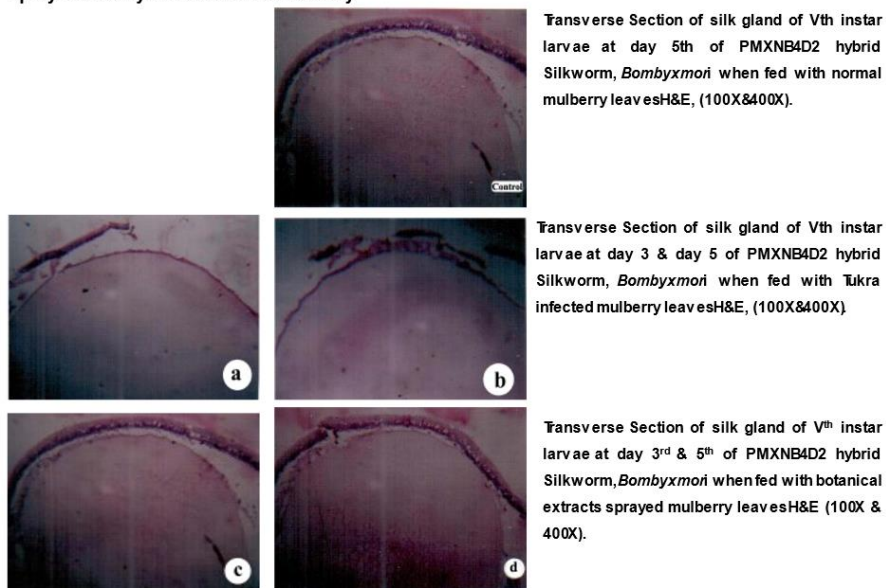


## Silk gland histology

From the Plate II- control it is seen that the silk gland of normal worm had two layers intact embedded in an outer coat. The silk protein consists of about 80% fibroin and about 20% sericin. The number of silk gland cells, however, differs from race to race. In silkworms when fed with normal and botanical sprayed mulberry fed leaves there was no significant damage to the inner and middle layers (Plate II-C)

and normal lacy of epidermal layer and vacuolization in the cells of outer layer was observed (Plate II-D). In the tukra affected leaves fed to silkworms the nuclei of the outer layer of the silk gland were slight by somewhat hypertrophied, vacuolization appeared in the cytoplasm with mild degree of degeneration of cells (Plate II-A) silkworm fed leaves revealed almost similar changes to that of normal fed larvae, but the epithelial layer is larger and completely peeled out and became thin at day 6th of tukra affected leaves when fed to the silkworm (Plate II-B).

Histological changes in *Bombyx mori* Silk gland Vth during feeding of Tukra infected and botanical extract sprays mulberry leaves with control study.



## Discussion

### Effect on Cocoon development

The increase in cocoon weight and biosynthesis of silk protein (fibroin and sericin) concentration which in turn is related to superior silk quality can be attributed to the increased synthesis of RNA in silkworm. The results of the present study clearly demonstrated that the botanical sprayed mulberry fed to silkworm significantly elevated all the parameters related to the economic traits of the cocoons such as the total weight of cocoon, weight of shell and shell ratio. This observation derives support from the research findings of Etebari and Matindoost (2005) who reported that feeding of silk worm on mulberry leaves enriched as equal to normal mulberry feed from fourth instar increased cocoon shell weight at 2.5% concentration while pupal weight. The silk threads of the cocoons of the silkworm, *Bombyx mori* are composed of two major proteins (fibroin and sericin) produced by secretions of the silk glands. The silk gland is divided into three regions: anterior, central and posterior. The posterior silk gland secretes fibroin while sericin a glycoprotein which coats fibroin is secreted by the central silk gland. The fibroin protein is transferred by peristalsis into the central silk gland where it is stored until required for spinning (Shimura, 1993). Mulberry leaves are rich in protein and amino acids and there is a high correlation between leaf protein levels and the production efficiency of the cocoon shell, i.e. the cocoon shell weight relative to the total amount of mulberry leaves consumed by the silkworm (Machii & Katagiri, 1991).

It is therefore possible that an increase in the protein level of mulberry leaves may lead to improvements in cocoon productivity. Mane *et al.*, (1997) opined that some of the commonly available plants with phagostimulant properties should be screened and feed to silkworms along with mulberry leaves, thus

supplementation of phagostimulant may increase the appetite with least wastage of leaves and increase overall productivity. Ito, (1961) and Ito *et al.*, (1964) reported many sterols have the feeding stimulants. Jaypaul *et al.*, (2003) observed treatment with leaf extracts of certain plants on mulberry leaves can increase the productivity of silkworm with limited food leading to economic gain. It is reported (Murugan *et al.*, 1998; Sreedevi *et al.*, 2003) that the medium concentration of the botanicals is highly effective for the improvement of many parameters of silkworm rearing. In the present study also the medium concentration (5%) was found highly effective with less impact of high as well as low concentration of botanicals may be due to the deleterious action of high or low action of lower dosage. Some of the plants used in this study such as *Achyranthes* sp. (Kim *et al.*, 1997), *A. Spinousus* (Jayaprakash rao, 1998) as well as *P. histerophorus* (Hipparagi *et al.*, 2003; Sreedevi *et al.*, 2003) are reported to have positive influence on various quantitative and qualitative characters of silkworm feeding with supplementation of different plant extracts.

### **Effects of diseased versus plant extract sprayed mulberry leaves on Mid Gut histology**

On exposure of the hybrid silkworms at different levels with tukra affected chawki leaves and botanical sprayed mulberry when fed by silkworms were seen on 3rd and 6th day, the structure of the mid gut wall when fed with botanicals treated leaves did not show any variation and tissues, they did not exhibit much variations, because of these symptoms the food conversion efficiency has not decreased in the silkworm. Kumar (1995) reported that an enhanced food conversion by the silkworm resulting the increased body weight in the silkworm feeding with normal mulberry or tukra leaves does not effects on the silkworms. The quality of proteins, carbohydrates available in mulberry leaves, as determined by its composition of amino acids (Ito and Arai, 1965), is of critical importance for growth and development of tissues in an insect (Vanender *et al.*, 1972). The mid gut of silkworms fed with tukra affected chawki leaves showed that epithelial cells are slightly sloughed off into the lumen, became hypertrophed and granular and peritrophic membrane is slightly destroyed and gut lumen filled in with debris from the destroyed cells. Similar observations shows that the metabolic activities in tissues of silkworm shows different changes with lack of nutrition to silkworms or water content is entirely less in mulberry when silkworm feeds and the occurrence of pest largely in the mulberry which sucks the nutritious part in mulberry by pests when the silkworm feeds the metabolic activities will be minimized in the tissues of the silkworms. In most insects the midgut shows no sign of external anatomical differentiation but there may be internal and functional differentiation commonly associated with histological and ultra structural difference (Chapman, 1985). Aruga (1994) reported that the midgut has distinct deep constriction in the posterior region, with lack of energy fuel, compared to the other organs in the silkworm.

### **Effects of diseased versus plant extract sprayed mulberry leaves on Silk gland histology**

The silk gland which is second largest organ in the body which occupies most of the ventro-lateral side of the body. In the botanicals sprayed leaves when fed by silkworm there was no damage in the cells compared to normal silkworm and there was no difference in the epidermal layer of silk gland this sign is due to acceleration of metabolic activity. The inner fibers in and sericin layers however are not affected which indicates that these layers could with stand without infestation of mealy bugs on mulberry at the time of feed to silkworms. The tukra affected leaves when fed with silkworms the damage was restricted only to the outer layer and no marked differences are observed in inner layers depending upon the nutrition and impact of sprayed mulberry upon pests with mulberry feed or nutritionally normal as good healthy leaves taken by silkworm. Aruga *et al.*, (1957) observed formation of inclusion bodies in the middle and posterior positions of the silk gland of silkworms. Masatoshi Kobayashi (1956) observed that during V instar, the mucus like substance in the silk gland is also observable just after first feeding and gradually increases the amount and there after decreases the mucus with lack of nutritive value

present in the mulberry when fed by silkworms. Kaei 1984; Centhilnayaki *et al.*, 2004 and Ganeshprabhu (2012) reported that V1 treated with silver nano particles and various supplements fed to silkworm is having superior enhance to synthesize the secretory materials by silk gland. The mucus like substance is supposed to be released from the gland into the circulating blood from the 3/5 of the V instar larva. Sriharan *et al.*, (1979) showed that at the time of de-saping, mealy bug penetrates their stylet in the tender parts of the plant, and the toxic saliva causes imbalance in the cell metabolism by silkworm.

## **Conclusion**

Silk quality depends on several factors including growth and development of silkworm. Here, diet and quality of diet play an important role in embryonic development of silkworm and silk production as well. There are several risk factors associated with poor quality of silk production. The mulberry leave disease remains a major factor in poor development of silkworm and production of quality silk fiber. There are tremendous growths and development in novel approaches to minimize mulberry leaves infections and disease precisely for Tukra disease. The use of various plant extract seems more effective and eco-friendly approach not only to reduce risk of mulberry leaves disease but also improved quality silk production. Here in present study, we reported mangrove plant extract *Sonneratia apetala* and *Avicennia alba* shown tremendous scope in reducing risk of Tukra disease and improve quality silk production. The extract from *Sonneratia apetala* and *Avicennia alba* had shown improved cocoon physical parameters along with weight. The extract sprayed leaves shown an improve silkworm histology studied for silk gland and mid gut over control studies. As a result, silkworm had shown an enhanced capacity to produce quality silk production.

## **References**

1. Etebari, K., and Matindoost, L., 2005. Application of multivitamins as nutrition the mulberry silkworm, *Bombyx mori*. ANJAC Journal. 17, 47- 53.
2. Harper, H.A., Rodwell, V.M. and Mayer, P.A. 1979. In: Review of physiological chemistry, 17th edition, Longe medical publications, Maruzer Company limited, California.
3. Hipparagi, G. D., Rayar, S. G., and S. S., 2003. Karbhantanal. Field spray for effect of botanicals on mulberry and its impact on silkworm (*Bombyx mori*) growth and development. In: Nutritional Management and quality improvement in Sericulture. (ed.), U.D. Bongale. 415-420. KSSRDI, Thalagattapura, Bangalore.
4. Ito, T. 1961. Sterol requirement of the silkworm *Bombyx mori* L. Nature, 191, 882-883.
5. Ito, T., Kawashima, K., Nakahara, M., Nakanisha, K and Terhara, A., 1964. Feeding stimulants of the silkworm, *Bombyx mori* L. Journal of Insect Physiology.10, 225.
6. Rao, J., 1998. Effect of few botanicals and iodine compounds on the economic traits of eri silkworm *Samia cynthia ricini* Boisduval. M.Sc (Sericulture) Thesis University of Agriculture Sciences, Dharward, 136.
7. Jeypaul, C., Padmalatha, C., Ranjitsingh, A. J. A., Murugesan, A. G. and Dhasarathan, P., 2003. Effects of plant extract on nutritional efficiency in mulberry silkworm *Bombyx mori* L. Indian J. Seric. 42, 128-131.
8. Krishnaswami, S., Narasimhanna, M. N., Suryanaryana, S. K., and Kumaraji, S., 1978. In environmental conditions. In: Sericulture Manual, Vol. 2, Silkworm rearing, F.A.O., 6.
9. Machii, H., and Katagiri K., 1991. Variety differences in nutritive values of mulberry leaves for rearing silkworms. JARQ. 25, 202-208.
10. Mahadevappa, D., Halliyal, V.G., Shankar, A.G., and Bhandiwad, R., 2000. Mulberry silk reeling technology. Oxford and IBH publ. New Delhi. 190-191.



11. Mane, J., Rayer, S. G., Patil, G. M., and Vage, M.K., 1997. Mulberry-A Hobsons Choice. Indian Silk. 36, 38-41.
12. Murugan, K., Jeyabalan, D., Senthil, K.N., Senthil, N.S., Sivaprakasan, N., 1998. Growth promoting effects of plant products on Silk worm. J. Sci. Ind. Res. 57, 740- 745.
13. Sridevi, G., Basker, R. N., and Devaiah, M.C., 2003. Influence of medicinal plant extracts on cocoon parameters of silkworm *Bombyx mori* L. (Abstr.) National Conference on Tropical Sericulture for Global competitiveness. CSRTI, Mysore, 2003.
14. Venugopalapillai, S. 1989. Growth studies in parental stocks of silkworm *Bombyx mori* L. under tropical conditions. *Ind. J. Seri.*, XXVIII, 1989; (1): 53-66.
15. Young, U.R., 1970. In: "Mammalian Protein metabolism." Academic Press, New York, U.S.A, 1970.
16. Sangamithirai, A.V., Sabhanayakam, S., Susithra, N., Ganeshprabhu, N., Mathivanan, V., Hemalatha S., and Elanchezian, C., *International journal of modern research and reviews*. **2014**. 2(1), pp; 20-25.
17. Aruga, H. and Tanaka, S. J., 1968. *Sericult. Sci.*, Japan. 37(5), 441-444.
18. Chapman, R.F., 1985. Structure of the digestive system, in comprehensive insect physiology, Biochemistry and pharmacology. Eds. Kerkut G.A. and Gilbert, L.I., 4: 165-211. Pergamon Press, New York.
19. Fanta, E., 1997. *Ecotoxicol Environ. Sat.* 54, 119-130.
20. Shiva Kumar, C., 1995. Physiological and biochemical studies on nutrition in silkworm, *Bombyx mori* (L.). Ph.D. thesis, Bangalore University, Bangalore, India. **1995**.
21. Mori, T., Hiromu, J. A., and Masatoshi Kobayashi M., 1990. Ultrastructural changes of the fat body in the silkworm during post-embryonic development. Tokyo metropolitan Horticultural High School, Setagaya-Ku, Tokyo; Sericultural Experimental Station, Suginani-Ku, Tokyo.
22. Tanada, Y., Hess, T.R. and Omi, M.E., 1982. *J. Invertebr. Pathol.*, **1982**. 40: 197-204.
23. Umesh Kumar, N.N., Shree, M.P., and Boraiah, G. 1990. *Indian J. Sericul.* 29(1), 93-100.
24. Vail, P.V., Jay, D.L., and Hink. **1973**. *J. Invertebrates. Pathol.*, 22: 231-277.
25. Virchow, R. Diecellular pathologie in brer Bergudung auf physiologische and pathologische gewekhre, Berlin: A. Hireshwald. **1858**