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RESEARCH ARTICLE

PESTICIDIAL ORIGIN OF BOTANICAL EXTRACTS WITH INCIDENCE OF TUKRA MULBERRY ON HISTOPATHOLOGY OF SILKWORM, (*BOMBYX MORI L.*)

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A R T I C L E I N F O

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A B S T R A C T

Present study was conducted to find out the effect of various botanical extract on the tissue, cellular and sub cellular level and histopathology of silkworm, findings of the present study gives useful data concerning the changes in the insect. Three plants extract viz *Azadirachta indica*, *Ocimum sanctum* and *Parthenium hysterophorus* were used as experimental while untreated leaves consider as control. These botanicals were sprayed on the tukra (Pink mealy bug) infected mulberry leaves and fed to silkworm. Findings of the study suggested no change in the midgut of the silkworm feed on the botanical sprayed leaves and it was with normal columnar epithelium had striated border (microvillus) covered by the peritrophic membrane. While epithelial cells slightly sloughed midgut was reported in the silkworm fed on the tukra leaves. The outer layers of the nucleolus were reported somewhat hypertrophied and cytoplasm was reported vacuolate with mild degeneration of cell in silkworm fed on the tukra infected leaves. Silkworm fed leaves revealed almost similar changes to that of normal and there was no change in botanical sprayed fed larvae. The impact in tissue of the silkworm when fed with normal and crude botanical extracts against mealy bugs shows normalcy, but in the tukra mulberry leaves fed by silkworms the tissues shows slight degenerative with nutritional impact upon them.

INTRODUCTION

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. So, due to the development of resistance and destruction of natural enemy of mulberry pest, there is a need to develop an ecofriendly IPM approach against these well known mulberry pests. In this context, an effort has been made to investigate the efficacy of some promising botanicals against the major sucking pests of mulberry in the tropical zone of south India in the mulberry ecosystem. Mealy bug *Maconellicoccus hirstus* (Family-Pseudococcidae) presence has been reported in and around Anantapur & Krishna districts of Andhra Pradesh & some places of Tamil Nadu and also reported in outer parts of India, Bangladesh and Indonesia. India and Indonesian species of the mealy bug have been identified as *Maconellicoccus hirstus* (green). The tukra infected mulberry leaves with minute mealy bugs in mulberry garden shows symptoms with curling of apical leaves which the beetles feeds on plant sap and decreases the leaf protein and moisture status during summer seasons(Kumar *et al*.,1997).

Use of botanical extracts for controlling pests and nematodes is becoming appealing because of the growing problem of environmental pollution arising from the use of persistent pesticides (Shivakumar, 1995). There has been de-registration of some hazardous pests and nematicides with increasing pressure on farmers to use non-chemical pest control methods that do not pollute the environment. This emphasis the need for new methods of control such as the use of locally available traditional medicinal plants that are having volatile compounds of various locally available plant extracts in controlling pests has to be study and identify the use of local plant materials for the control of pests.

MATERIALS AND METHODS

Silkworm Rearing

For the study, the popular south Indian cross breeds (CB) silkworm CSR2 of Bivoltine breed were used as test materials. The disease free laying (DFLS) of this cross breed

PMxNB4D2 (Bivoltine hybrid) were maintained under field conditions and brought to the laboratory.

Mulberry Plantation

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 chosen for spray of mealybug infection in mulberry plants.

Preparation of aqueous plant extract

Plants having insecticidal properties like *Azadirachata indica*, *Ocimum sanctum* and *Parthenium hysterophorus* were taken from the department of Botany, University College of sciences, Acharya Nagarjuna University, Guntur, AndhraPradesh. 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.

Microscopic examination used in Silkworm fed with botanical-Sprayed Mulberry leaves

An microscopic examination were carried out to find the effect of feeding healthy and botanical sprayed leaves on rearing silkworm hybrid. Leaves were collected from the experimental plots 0, 2, 5, 7, 10, 15 and 20 days after spray and were fed to second instar larvae. From 3 to 6 days of silkworm tissues were taken for the present study. Morphological changes in silkworm exposed to different percentages of tukra infected and healthy mulberry leaves fed to silkworm and were analyzed and changes in tissue were photographed to observe the external symptoms and the histological sections of midgut and integument were taken. The midgut and integument of fifth instar of day 6 were isolated from normal and experimental batch. They were fixed in Bouin's fluid (75ml saturated aqueous picric acid, 25ml 40% formaldehyde and 5ml glacial acetic acid) for 24 hours. The sections were stained with Harris hemotoxylin (Harris, 1900) and counter stained with eosin, dissolved in 95% alcohol. After dehydration and cleaning, the sections were mounted in Canada balsam. Photomicrographs of the sections preparation were taken using Olympus (PM-6 model) photomicrography equipment.

RESULTS

Microscopic examination was identified in tissues of silkworm fed with botanicals sprayed batch and there was no changes observed in midgut with normal columnar epithelium had striated border (microvillus) covered by the peritrophic membrane Figure1,3(c&d). In the silkworm midgut, fed with tukra infected chawki leaves, epithelial cells slightly sloughed of midgut cells were shown larger i.e. hypertrophied that in the normal cells. Hypertrophied and granular and peritrophic membrane is slightly destroyed and gut lumen filled in with debris from the destroyed cells Figure 1,2(a&b).

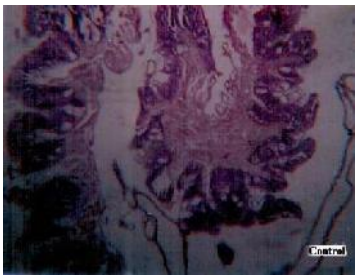

Figure 1 Transverse Section of midgut of Fifth instar at day 5th of Csr2 Silkworm, *Bombyxmor*i when fed with normal mulberry leaves H&E, (100X&400X)

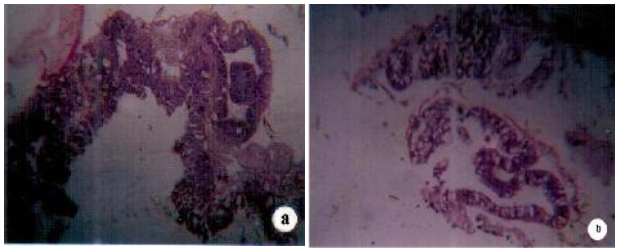

Figure 2 Transverse Section of midgut of Fifth instar at day 3 & day 5 ofCsr2 Silkworm, *Bombyxmor*i when fed with Tukra diseased mulberry leaves H&E, (100X&400X)

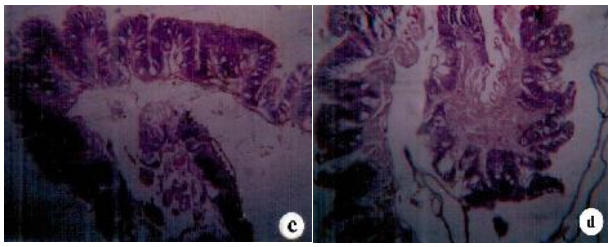

Figure 3 Transverse Section of integument of Fifth instar at day 3 & 5 of Csr2 Silkworm, *Bombyxmor*i when fed with botanical extracts sprayed mulberry leaves H&E, (100X&400X)

In silkworms fed with normal and botanical sprayed mulberry, integument was not showing any significant damage in the exo and endocuticle with an obvious separation from each other in certain areas, and vacuolization in cytoplasm of the epithelial cells (Figure 4,6(c&d).

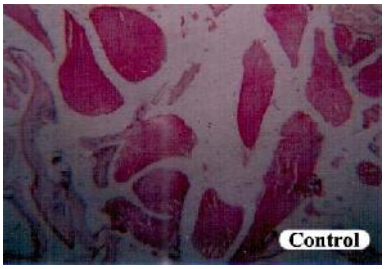

Figure 4 Transverse Section of integument Fifth instar day 5th Csr2 Silkworm, Bombyxmori when fed with normal mulberry leaves H&E, (100X&400X)

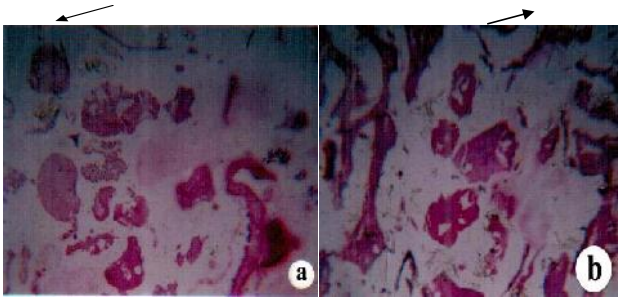

Figure 5 Transverse Section of integument of Fifth instar larvae at day 3 & 5 of Csr2 Silkworm, *Bombyxmor*i when fed with Tukra diseased mulberry leaves H&E, (100X&400X)

In the tukra infected leaves fed to silkworms the nuclei of outer layer cells showed slight hypertrophied; the inclusion bodies were formed in the nuclei and vacuolization appeared in the cytoplasm of the epithelial cells appeared Figure 4, 5(a&b).

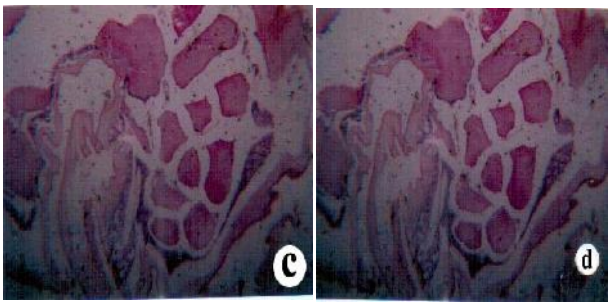

Figure 6 Transverse Section of integument of Fifth instar at day 3 & 5 of Csr2 Silkworm, *Bombyxmor*i when fed with botanical extracts sprayed mulberry leaves H&E, (100X&400X)

DISCUSSION

The silkworm, being a phytophagous insect feeds exclusively on mulberry leaf during its larval stages which account for nearly half of its total life span. The food that is consumed during larval stages utilized for growth as well as accumulation of energy reserves to fuel its metabolisms during non-feeding periods like larval moulting, spinning, pupal and adult stages of silkworm. The mealy bug, *Maconcllicoccus hirsutus* green has been considered the casual agent of tukra the exact nature of the development of tukra is not so far explained. Tukra was earlier believed to be a viral transmitted disease through the mealy bugs (Babu *et al*., 1994). The mealy bugs infected mulberry leaves when fed to silkworm the metabolites of phenylalamine ammonia lyses, showed marked increased in infected leaves when compared with healthy leaves fed to larvae (Muralikumaran & Bhaskhran 1992; Ramarethinam & Sangeetha, 2002; Mukhopadhyay, 2006; Babu *et al*., 2009; Sathyaseelan & Bhaskaran, 2010).

The accurate diagnoses of silkworm based on the external symptoms are not specific and it requires microscopic examination of haemolymph and other tissues of the silkworms when it effects with malnutrition. Histological responses of silkworm at different level of feeding of tukra infected show variations in various physiological and biochemical activities. From the present study it is clear that malnutrition and water deficient leaf shows significant histological changes in different tissues of the hybrid silkworm. However, most of these changes occurred in fat body and some symptoms in the silk gland when fed with tukra normal and botanical extracts administrated on mulberry leaves (or) pest stress to silkworm when insect feeds. On exposure of the hybrid silkworms at different levels with tukra diseased chawki leaves were showing a significant effect on the structure of the midgut wall at 3rd and 6th day while feeding of silkworm with botanicals did not show any variation and tissues they did not exhibit much variation because of these symptoms the food conversion efficiency has not decreased in the silkworm.

Shiva Kumar (1995) reported a significant increase in the food conversion and body weight of the silkworm when feeding with normal mulberry or tukra leaves. The quality of proteins, carbohydrates available in mulberry leaves as determined by its composition of amino acids (Ito & Arai, 1965) is of critical importance for growth and development of tissues in an insect (Vaandrager *et al*., 1989). The midgut of silkworms fed with tukra diseased chawki leaves showed that epithelial cells are slightly sloughed off into the lumen, became hypertrophied 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 that 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.

The integument of insects serves for many purposes that serve as exoskeleton it is a rich sensory depot which perceives stimuli in different forms. The epicuticle selectively permeable to the transport of moulting fluid precursors into the apolysial space and to the return of endocuticular digests into the body proper of the pharate instar. At the same time the pharate epicuticle itself is protected from chemical dissolution by moulting fluid enzymes (Venugopal, 1996). On feeding with sprayed leaves contributed by silkworm there was no change found in the silkworm, the reason could be attributed by more energy fuel taken by silkworms which the silkworms did not show adverse effect or it could not show adaptability.

The bugs might have suck the nuclear sap and moistures containing in leaves this might show a difference in silkworm tissues and digestive system with reference to the body with some characteristic features like inclusion bodies in addition a slight clumping of exo and endo cuticles were observed this may be due to the malnutrition in silkworm feed or the pests occurring largely in the mulberry without nutritive value which shows adverse effect to the integument in the silkworm. Barke and Mcedwen (1959) observed similar changes in the integument of silkworm *B.mori* when fed with contaminated mulberry leaves. Kumar and Chakraborthy (1999) reported that feeding of silkworm with tukra infected

leaves causes significant change in the integument, silkgland, haemolymph tissues of the silkworms. Singh *et al*., (2002) showed that utilization of tukra diseased mulberry leaves for silkworm rearing without affecting the quantitative characters has been discussed.

CONCLUSION

In the present investigation the impact of the crude extract on the silkworms tissues was tested and found that when silkworms fed with normal and crude botanical extracts against mealy bugs shows normalcy, but in the tukra infected mulberry leaves fed by silkworms the tissues shows slight degenerative with nutritional impact upon them.

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