UV impact on the digestive physiology of *Bombyx mori* L.

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ABSTRACT

MR_2 variety mulberry leaves were sterilized with UV light for 5, 10 and 15 minutes. These leaves were fed to PM x NB_4D_2 hybrid silkworm *Bombyx mori* Linn. life stages and recorded the macromolecular contents (total protein, reducing sugar and glycogen) and digestive enzymes (amylase, invertase and protease) activities. Results revealed feeding of silkworm *B. mori* with UV treated mulberry leaves discloses the enhancement of nutritive value of the host plant thereby increasing the macromolecular contents and digestive enzymes activities.

Keywords: Mulberry, UV treatment, silkworm, macromolecules, digestive enzymes

INTRODUCTION

Sericulture is a highly remunerative agro-based enterprise. India is the unique country in the world to produce all the four types of commercial silks and stands second in the production of mulberry silk (Murugesh *et al.,* 2004) However, disease resistance and other improved traits which can augment productivity and quality are needed to be inculcated to enhance the economic benefits to the sericulture farmers (Nagaraju, 2002). Mulberry leaf, being the only source of nourishment is certainly imperative that the supply of good quality leaf is most important for getting good quality cocoons (Sashindran Nair *et al.*, 2004).

Mulberry silkworm, *Bombyx mori* is susceptible to a number of diseases and also to the attack of pests and parasites. There is no silkworm race at present, which can be deemed as totally resistant to diseases or pest (Nagaraju, 2002). The fungi, bacteria, nematode and viral diseases persist throughout the year. Though most of these diseases appear and cause maximum damage during rainy and winter seasons, there are also few diseases that appear during summer and cause reduction in plant growth (Aleksey Chenko *et al*., 1998).

Helathy fifth instar larvae were used to quantify the In this context Mohamed Sadiq *et al*. (2001) reported that exposure of mulberry leaves to UV light greatly reduced the microbial content of the leaves. In continuation with our previous work (Mohamed Sadiq *et al*., 2001), we studied the impact of UV treated mulberry leaves feeding on the macromolecular profile (total protein, reducing sugar and glycogen) and digestive enzymes like amylase, invertase and protease activities of silkworm *Bombyx mori.*

MATERIAL AND METHODS

Disease free layouts of PM x NB_4D_2 hybrid silkworm *B. mori* were procured from Government Grainage Centre, Krishnagiri and were maintained in the laboratory under controlled hygienic conditions. Freshly hatched larvae (> 3 hrs) were brushed and reared upto fifth instar in an environmental chamber under standard rearing conditions (Krishnaswami, 1978). The larvae were fed with fresh mulberry leaves of MR_2 variety thrice a day. Fresh, healthy and pesticide free mulberry leaves were exposed to UV light in an UV laminar flow (Clean air system; Ultra Violet lamp – Philips, Holland; Wavelength 280-400nm) at three-

Table 1. Total Protein, glycogen and reducing sugar (mg/gm wet weight of the tissue) of *B. mori* fed with UV treated mulberry leaves.

Parameter	Control	UV Exposure time		
		5 min	10 min	15 min
Protein	90.63 ± 0.80	100.76 ± 0.96*	114.10 ± 0.34*	116.38 ± 0.24*
Glycogen	46.10 ± 0.01	49.15 ± 0.02*	50.27± 0.02*	57.07± 0.03*
Reducing Sugar	41.06 ± 0.01	47.00 ± 0.09*	49.49 ± 0.06*	56.40 ± 0.20*

* shows significant difference over control at 5 % level

Table 2. Enzyme activities (micro moles / min /gm tissue) of *B. mor*i fed with UV treated mulberry leaves

Parameter	Control	UV Exposure time		
		5 min	10 min	15 min
Protease	0.61± 0.02	0.92 ± 0.03*	1.27± 0.001*	1.28± 0.005*
Amylase	0.15 ± 0.01	0.23 ± 0.001*	0.24± 0.001*	0.25 ± 0.001*
Invertase	2.48 ± 0.003	2.53± 0.002*	3.08± 0.001*	3.76± 0.001*

* shows significant difference over control at 5 % level

time durations (5 minutes, 10 minutes and 15 minutes) just before feeding. UV treated mulberry leaves was provided from first to fifth instar larvae thrice a day continuously. Each treatment as well as the control was replicated thrice with ten larvae in each category.

Healthy fifth instar larvae were used to quatify the total protein, reducing sugar and glycogen and digestive enzyme activities. Individuals were anaesthetized with cotton pads soaked in chloroform and entire digestive tract dissected out, mid-gut was separated and used for this study. Total protein, glycogen and reducing sugar were carried out according to Lowry *et al.* (1951*)*, Carrol *et al* (1956) and Roe *et al.,* (1955) respectively. The gut was also homogenized in ice-cold phosphate buffer, centrifuged at 5000 rpm and the supernatant was used as the enzyme source. Amylase and invertase and protease analyses were carried out as described by Bernfeld (1955) and Plummer (1988). Data from macromolecular concentrations and enzyme activities were subjected to analysis of variance. The differences between the treatments were also determined by ANOVA and their significance expressed at 5 % level.

RESULTS

The changes in the levels of macromolecules content in the midgut of control and experimental group is presented in table 1. The results indicated that significantly glycogen, reducing sugar, total protein contents of the experimental groups gradually increased from 5 minutes to 15 minutes of UV exposure than the control category. A significant increase of protease, amylase and invertase activities over the control was also recorded (Table 2). Furthermore, as observed for macromolecules these digestive enzymes activities were gradually increased when *B. mori* was fed with mulberry leaves which were exposed to 5 minutes to 15 minutes..

DISCUSSION

Although pesticides contribute a great deal to control diseases and insect pests in sericulture, their use in sericulture causes contamination of mulberry leaves. Feeding silkworms with such contaminated mulberry leaves deteriorated the cocoon quality, reduced the cocoon yield, and reduced the egg production (Mano *et al*., 1992). Feeding infected leaves also leads to adverse effects like prolonged larval periods and poor cocoon formation. The commercial characters of the cocoons were greatly reduced when the larvae were fed by the leaf affected with rust, leaf spot and powdery mildew diseased leaves (Singhvi *et al*., 2002).

Mohamed Sadiq *et al*. (2001) reported that when mulberry leaves was exposed to UV light not only sterilize the leaves but also reduce the unwanted populations of bacteria and fungi present in them. The present investigation strongly supports the enhancement of nutritive value of the host plant due to the UV exposure. It is an established fact that proteins are highly essential for the growth and development of insects. Further, it may also play an essential role in the production of certain hormones and enzymes (Wigglesworth, 1972). Moreover, total protein determines the chemical characteristics features of silk proteins (Shigematsu, 1960).

The results obtained in the present study indicate the enhanced anabolism of protein in the digestive tissue of the silkworm, *B. mori* fed with UV exposed mulberry leaves and may be due to increased availability of amino acids from the UV treated mulberry leaves. It was observed that the level of reducing sugars in the digestive system considerably increased when fed with the mulberry leaves exposed to UV light. From the observations it was also evident that UV exposed mulberry leaves influence the growth of the larvae and increased the concentration of reducing sugars. Mason *et al*. (1990) reported that reducing sugars (pentose and hexose) are derived from assimilation food and are available in the free state. While we expose the mulberry leaves with UV light, these sugars undergoes chemical changes and converted in to available forms to *B. mori*. They were mostly utilized in the body and have a major role in energy production through glycolysis.

The level of total sugar was also changed by hydrolytic enzymes in the gut and haemolymph (Ito and Tanaka, 1959) due to various intermediary metabolic pathways in phosporylation and in the citric acid cycle. It is reasonable to infer that the increased glycogen and reducing sugar content of digestive tissue may be due to increased level of sugars in UV treated host plant mulberry leaves (Mohamed Sadiq *et al*., 2001).

Glycogen being a storage polysaccharide was found to be high in the experimental groups of silkworm *Bombyx mori*. It is significant to correlate to the availability of increased sugars, which may undergo glycogenesis resulting in the more amount of glycogen. To understand the assimilation process of major macromolecules in the silkworm larvae fed with UV exposed leaves, the activities of digestive enzymes like invertase, amylase and protease were analyzed. It was very clear from the results that feeding of larvae with UV light treated mulberry leaves altered the activities of digestive enzymes.

Increase in protease activity may be attributed to the increased concentration of protein. The digestive tissue must be tuned to synthesize more of protease enzyme since the protein content increased significantly than control category. Amylase catalyses the specific hydrolysis of the glycosidic bonds in glycogen (Plummer, 1987), hence the increased amount of glycogen may bring about the increased secretion of digestive enzyme amylase. Invertase catalyses the breakdown of sucrose to glucose and fructose, the increase in the activity of this enzyme in the digestive tissue of the experimental animals may be due to the increased nutritive value of UV treated mulberry leaves. Foregoing facts discloses that UV sterilization of mulberry leaves appears to be more economical and reliable device to be employed in silk industries for quality silk production.

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