EFFECT OF CULTIVARS AND MAGNETIC FIELD ON PROTEIN IN LARVAE OF SILKWORM, *BOMBYX MORI* L.

Tambe Vilas Jairam et al.,

**KEYWORDS**

*Bombyx mori*  
Mulberry cultivars  
Magnetic field
INTRODUCTION

Mulberry leaf protein is the chief source for the silkworm to bio-synthesize the silk which is made up of two proteins fibroin and sericin. Nearly 70 per cent of the silk protein produced by the silkworm is directly derived from the proteins of mulberry leaves (Rangaswami et al., 1976). Income is assured in sericulture by taking up remunerative enterprises like mulberry cultivation, silkworm rearing and silk reeling. (Sudhakar et al., 2008) Silkworm consumes 81 per cent of the food in fifth instar where in need of matured leaf is desired (Krishnaswami, 1987). Since larvae consumes maximum food in 5th instar stage the enzymetic activity is studied in the 5th instar of silkworm larvae (Jadhao and Kallapur, 1988). Morphological, physiological and biochemical alterations occurs if living organisms when exposed to magnetic field. Chougale and More (1993) exposed silkworm to electromagnetic field and observed changes in biology of silkworm etc. It is hypothesized that if the cocoon of Bombyx mori are exposed in different magnetic strength, there may by some beneficial effects on the life pattern of silkworm and the productivity of cocoon. (Upadhyay et al., 2010).

Efforts are always made by many workers to increase cocoon yield and cocoon weight by spraying or dipping of mulberry leaf in chemicals and increasing the nutritive values of leaf. But in present study a separate view has been evaluated for increasing the cocoon production along with the objectiveto studythe effect of mulberry cultivars and magnetic field on protein of Bombyx mori L.

MATERIALS AND METHODS

The effect of mulberry cultivars and magnetic field on protein of Bombyx mori L. was studied during 2006 and 2007 in the laboratory, Department of Entomology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola using factorial randomized block design with six cultivars and five magnetic fields. The silkworm larvae fed on S13 mulberry cultivar for 12 h in magnetic field M6 recorded significantly maximum protein 7.062 µg in silk gland, 6.225 µg in midgut and 5.795 µg in haemolymph. Whereas, lowest protein that is 2.303 µg in silk gland, 3.957 µg in midgut and 2.591 µg haemolymph were recorded when the larvae fed with cultivar V1 for zero hour magnetic field. Effect of all other mulberry cultivars and magnetic field on protein of Bombyx mori were statistically significant over V1M0.

ABSTRACT

The experiment on the effect of mulberry cultivars and magnetic field on protein of Bombyx mori L. was studied during 2006 and 2007 in the laboratory, Department of Entomology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola using factorial randomized block design with six cultivars and five magnetic fields. The silkworm larvae fed on S13 mulberry cultivar for 12 h in magnetic field M6 recorded significantly maximum protein 7.062 µg in silk gland, 6.225 µg in midgut and 5.795 µg in haemolymph. Whereas, lowest protein that is 2.303 µg in silk gland, 3.957 µg in midgut and 2.591 µg haemolymph were recorded when the larvae fed with cultivar V1 for zero hour magnetic field. Effect of all other mulberry cultivars and magnetic field on protein of Bombyx mori were statistically significant over V1M0. Efforts are always made by many workers to increase cocoon yield and cocoon weight by spraying or dipping of mulberry leaf in chemicals and increasing the nutritive values of leaf. But in present study a separate view has been evaluated for increasing the cocoon production along with the objectiveto studythe effect of mulberry cultivars and magnetic field on protein of Bombyx mori L.

MATERIALS AND METHODS

The effect of mulberry cultivars and magnetic field on protein of Bombyx mori L. was studied during 2006 and 2007 in the laboratory, Department of Entomology, Dr. PDKV, Akola. The experiment was planed by using factorial randomized block design with six factors A and five factors B. Each treatment was replicated four times. Mulberry leaves of desired six cultivars were harvested from four year old well established and maintained mulberry garden and provided to the silkworm in the laboratory according to the treatment details.

| Treatments Detail |
| Factor A | Factor B (Rearing methods) |
| T<sub>1</sub> | S -1635 | M<sub>0</sub> | Rearing of silkworm in non-magnetic field |
| T<sub>2</sub> | M - 5 | M<sub>1</sub> | Rearing of silkworm in 3 h magnetic field daily |
| T<sub>3</sub> | S-13 | M<sub>2</sub> | Rearing of silkworm in 6 h magnetic field daily |
| T<sub>4</sub> | S-36 | M<sub>3</sub> | Rearing of silkworm in 12 h magnetic field daily |
| T<sub>5</sub> | S -34 | M<sub>4</sub> | Rearing of silkworm in 24 h magnetic field daily |
| T<sub>6</sub> | V<sub>1</sub> | V<sub>1</sub> | Rearing of silkworm in non-magnetic field |

During this period the 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> instar larvae of PM x CSR silkwormrace were reared in magnetic field by tray rearing method as suggested by Ullal and Narsimhanna (1987). Slak lime powder was dusted when the worms were under moult, to provide dry conditions for passing moult. When more than 95 per cent
larvae passed from moult, dusting of (RKO) was undertaken. Magnetic field required for silkworm rearing was prepared by placing the magnets in the tray with north pole facing upward direction and south pole downward as and the paraffin paper was placed over the magnets. After hatching of the eggs rearing of larvae was undertaken as per the scheduled treatments.

### Protein estimation from silk glands

Protein estimation was undertaken by Bradford method using microplate assay (Bradford, 1976). Two larvae of 5th instar larvae of B. mori were randomly selected from each treatment. They were chilled in freezer and were dissected in ice cold sodium phosphate buffer (0.1 M pH 7.0) for separating silk gland. The removed silk gland was kept in 1mL sodium phosphate buffer. Later on silk gland was homogenized separately in mortar and pastel in ice cold condition in sodium phosphate buffer (pH 6.5) containing EDTA and PTU (0.1 mM). The homogenate thus obtained was centrifuged at 15000 rpm for 15 min at 0°C in high speed refrigerated centrifuge. Solid debris and cellular material was discarded, the resultant post mitochondrial supernant obtained was stored at -20°C and was used as protein source.

### Protein estimation from midgut and haemolymph

Two larvae of fifth instar were randomly selected from each treatment and kept in deep freeze at -20°C for about 12 h to avoid the loss of protein and enzyme activity. Later on the midgut was removed along with its content and tissue homogenate with 10 ml of ice cold 0.1 M borate buffer at pH 11 were prepared. The homogenate of tissue was centrifuged in high speed refrigerated centrifuge for 15 minutes at 3000 rpm. The supernatant were used as protein and enzyme source to assess their activity.

Two larvae of fifth instar from fourth moult onward upto spinning of cocoon were randomly selected from each treatment for assessing the protein from haemolymph. The haemolymph was withdrawn from the larvae of each treatment by puncturing at the base of 2nd proleg. The haemolymph so withdrawn was collected into 300ul of neutralized diammonium sulphate (NH4)2SO4 to achieve 40 per cent saturation. The mixture was kept undisturbed for 30 min. A portion of this mixture was retained as whole haemolymph and the rest was centrifuged at 10,000 rpm for 20 min. The supernatant was used as protein source to assess their activity.

### Quantification of protein from silk gland, midgut and haemolymph

The protein of silk gland midgut homogenate and haemolymph of B. mori larvae was quantified by Bradford method (Bradford, 1976) using microplate assay.

The pooled data obtained from two trials of the years 2006 and 2007 were subjected to statistical analysis after appropriate transformation wherever essential (Gomez and Gomez, 1976).

### RESULTS AND DISCUSSION

#### Effect of cultivars on protein in silk gland. (Factor A)

Pooled results of both the trials revealed that, significantly
highest (5.427 µg) protein in silk gland was observed due to rearing of silkworm larvae on S13 cultivar and was significantly superior to S1635 M, S36 and S34 recording 4.429 µg, 4.326 µg, 4.196 µg and 3.812 µg of protein in silk gland, respectively (Table 1). among these treatments. The group of treatments S1635 and M3, M6 and S36R S36 and S34 were at par with each other. Significantly lowest protein in silk gland was observed due to feeding of silkworm larvae on V1 cultivar recording 3.355 µg protein. Similarly Chandgude (2007) when reared PM X CSR race of silkworm on S13 cultivar also recorded maximum protein 513.4 µg in silk gland of silkworm larvae which is in consistency with present findings and supports the present findings.

**Effect of magnetic field on protein in silk gland. (Factor B)**

Data presented in Table 1 revealed that, silkworm larvae reared in the magnetic field were significantly superior over non magnetic field silkworm rearing. Significantly maximum protein (5.170 µg) was observed due to rearing silkworm larvae in 12 h magnetic field. The next superior treatment was M5 which was also at par with M3 recording 4.474 and 4.395 µg protein in silk gland, respectively. Significantly least 3.123 µg protein in silk gland over all the treatments was due to treatment M0. Amongst the magnetic field treatment significantly least protein in silk gland was recorded in the treatment M1 i.e. 4.126 µg protein but it was significantly superior to non magnetic field treatment. Similar results were also noted by (Satpute, 2005) in which maximum protein (609.35 µg) was observed in the silk gland of silkworm reared in 12 h magnetic field.

**Interaction effect of cultivars and magnetic field on protein in silk gland.**

(Factor A x B)

Pooled results of both the trial also indicated that, the silkworm larvae fed on S13 mulberry cultivar for 12 h in magnetic field (S13M12) recorded significantly maximum protein in silk gland i.e. 7.062 µg over all the treatments. Which was also followed by S13M0 which also recorded 5.743 µg protein in silk gland and was significantly more than rest of the treatments (Table 5 & Fig. 5). Significantly least protein in silk gland was observed in the treatment V1M3 2.303 µg which was also at par with S13M6 with 2.445 µg protein. Treatment S13M6 was significantly superior to both these treatment recording 2.880 µg protein in silk gland. Similar results were also reported by Satpute (2005) and Chandgude (2007) where 730.987 and 586.7 µg protein in silk gland was observed due to feeding of silkworm on S13 cultivar for 12 h magnetic field.

**Protein in midgut**

Effect of cultivar on protein in midgut of silkworm larvae (Factor A)

Pooled data of both the trials (Table 1) indicate that all the treatments differ significantly with each other. Maximum protein 5.654 µg was observed when silkworm larvae were reared on S13 cultivar and was significantly superior over all the treatments. The next effective treatment was M3 which exhibited 5.474 µg of protein in midgut of the larvae and was significantly superior to treatments viz., S36R S36 and S635R. Minimum protein was observed (4.159 µg) in midgut of larvae reared on V1 cultivar. The study conducted by Watnabe (1990) reported there is effect of protein content in midgut on protease activity and antiviral activity of the gut juices of silkworm B. mori and observed that protease and antiviral activity depend on amount of protein content in diet.

**Effect of magnetic field on protein in midgut. (Factor B)**

Pooled results of both the trials presented in Table 1 indicate that, significantly highest protein (5.548 µg) was observed in M0 treatment and was significantly superior to all the treatments. Treatment M6 was second in order of merit and recorded 5.262 µg of protein, followed by M3, 5.086 µg and M6, 4.882 µg protein in midgut. The least effective treatment was M0, i.e. non magnetic field treatment in which 4.843 µg of protein was observed in midgut of silkworm larvae. The study indicates that magnetic field has positive effect on the presence of protein in the midgut which plays an important role in metabolic activities in digestion of food material.

**Interaction effect of cultivars and magnetic field on protein in midgut (µg) (Factor A x B)**

Pooled data of both the trials presented in Table 1 indicate that, significantly maximum protein 6.225 µg in midgut was noticed in S13M12 treatment and was significantly superior over all the treatments. Whereas, significantly lowest protein 3.957 µg in midgut was observed in V1M0 treatment. Treatments M3M0, S36M0, S34M12 recording 5.850, 5.826 and 5.808 µg protein were at par with each other and significantly superior to the treatments S36M5 (5.707 µg), M3M12 (5.621 µg), S36M5 (5.582 µg), S13M5 (5.567 µg), M3M0 (5.503 µg), S13M0 (5.452 µg), M0M0 (5.311 µg) and S1635M12 (5.288 µg).

**Protein in haemolymph**

**Effect of cultivars on protein in haemolymph. (Factor A)**

Pooled results of two rearing regarding protein in haemolymph presented in Table 1 indicate that, significantly maximum protein 4.895 µg in haemolymph was observed due to rearing of silkworm larvae on S13 cultivar. Whereas, the minimum protein activity was recorded in M0 cultivar i.e. 3.279 µg. Treatment S13 recorded 3.806 µg protein in haemolymph was second in order of merit and was significantly superior to treatments S36R S635R, V1 showing 3.640µg, 3.552µg and 3.326 µg protein in haemolymph, respectively. The present finds are in consistant with the findings of Satpute (2005) who reported maximum protein in haemolymph (182.69 µg) when the silkworm were reared on seven different cultivars out of which S13 was one which recorded maximum protein in the haemolymph.

**Effect of magnetic field on protein in haemolymph. (Factor B)**

Pooled results of two rearing also gives similar trend as that of earlier trial. Larvae reared in 12 h magnetic field (M12) was most significantly superior over all the treatments exhibited 4.641 µg of protein in haemolymph. However, the next better treatment was M3 in which 4.062 µg protein in haemolymph was observed, followed by M3 (3.565 µg of protein). Least protein in haemolymph was observed in treatment M0 and M6 where 3.237 µg and 3.244 µg protein was observed. However both the treatments were found at par with each other (Table 1). Similar observations were recorded by Satpute (2005) when he reared silkworm on seven different
cultivar and found maximum protein in the haemolymph when reared in the magnetic field upto 12 h i.e. 187.19 µg whereas in present finding it was observed more i.e. 4.641 µg this major difference may be due to the difference of race under study.

**Interaction effect of cultivars and magnetic field on protein in haemolymph (Factor A x B)**

Pooled data of both the trials presented in Table 1 indicate that, significantly highest protein 5.795 µg was observed in the treatment S₁₃M₁₂ and was found significantly superior over all the treatments. Whereas the significantly least protein in haemolymph was observed in treatment V₁M₀ i.e. 2.591 µg the next best group of treatments which recorded maximum protein in haemolymph were S₁₃M₁₂ and S₃₆M₁₂ recording 5.545µg and 5.542 µg protein. In present study, maximum protein in haemolymph was observed due to rearing of silkworm larvae in S₁₃ cultivar for 12 h magnetic field (S 13M12 treatment) and lowest protein (2.591 µg) was observed in V₁M₀ treatment. This indicate that variety and magnetic field treatments has influence on the presence of protein in the haemolymph.

Satpute (2005) also analysed maximum protein (229.79 µg) in haemolymph of silkworm larvae when reared on S₁₃ with 12 hour magnetic field treatment. But the quantity of protein in haemolymph was lower than that of present finding which may be due to difference in race of silkworm under study.

**REFERENCES**


**Chandgude, D. M. 2007.** Effect of mulberry cultivars and magnetic field on economic parameters of Bombyx mori L M.Sc. (Agri.) thesis (unpub.), Dr. PDKV, Akola (M.S.).


**Satpute, R. S. 2005.** Effect of mulberry cultivars and magnetic field on silk gland and silk fiber of Bombyx mori L M.Sc. (Agri.) thesis (unpub.), Dr. PDKV, Akola (M.S.).


