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

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KEYWORDS

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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. Punjabrao Deshmukh Krishi Vidyapeeth, Akola using factorial randomized block design with six cultivars and five magnetic fields.The silkworm larvae fed on S_{13} mulberry cultivar for 12 h in magnetic field M_{12}recorded significantly maximum protein7.062 μg in silk gland, 6.225 μg in midgutand 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 V_1 for zero hour magnetic field. Effect of all other mulberry cultivars and magnetic field on proteinof *Bombyx mori* were statistically significant over V_1M_0.

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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. PDKV, Akola.The experiment was planed by using factorial randomized block design with six factors A and five factorsB. 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_1	S -1635	M_0	Rearing of silkworm in non-magnetic field
T_2	M- 5	M_3	Rearing of silkworm in 3 h magnetic field daily
T_3	S-13	M_6	Rearing of silkworm in 6 h magnetic field daily
T_4	S-36	M_{12}	Rearing of silkworm in 12 h magnetic field daily
T_5	S -34	M_{24}	Rearing of silkworm in 24 h magnetic field daily
T_6	V_1		

During this period the 3rd, 4th and 5th instar larvae ofPM x CSR_2silkwormrace 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 estimatation 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 freezerand 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 0^0C in high speed refrigerated centrifuge. Solid debris and cellular material was discarded, the resultant post mitochondrial supernant obtained was stored at -20^0C and was used as protein source.

Protein estimationfrom midgut and haemolymph

Two larvae of fifth instar were randomly selected from each treatment and kept in deep freeze at -20^0C 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 an 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 2ndproleg. The haemolymph so withdrawn was collected into 300ul of neutralized diammoniumsulphate $(NH4)_2\ SO_4$ to achieve 40 per cent saturation. The mixture was kept undisturbed for 30 min. A portion of this mixture was retained as a whole haemolymph and the rest was centrifuged at 10,000 rpm for 20 min. The supernatant was used as an 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

Table 1: Pooled effect of cultivars and magnetic field on protein in silk gland, midgut and haemolymph of full grown larvae

Cultivars	Protein in silk gland(μg)					Protein in mid gut(μg)					Protein in haemolymph(μg)				
	Interaction				Fact A	Interaction				Fact A	Interaction				Fact A
	M_0	M_3	M_6	M_{12}	M_{24}		M_0	M_3	M_6	M_{12}	M_{24}		M_0	M_3	M_6	M_{12}	M_{24}	
S_{1635}	3.312	4.252	4.756	5.241	4.586	4.429	4.451	5.057	5.101	5.288	4.593	4.898	3.302	3.683	3.741	3.948	3.086	3.552
M_5	3.496	3.997	4.278	5.178	4.682	4.326	5.311	5.503	5.621	5.850	5.086	5.474	2.903	3.193	3.468	3.997	2.833	3.279
S_{13}	4.212	5.131	5.743	7.062	4.988	5.427	5.452	5.567	5.826	6.225	5.202	5.654	4.462	4.932	5.25	5.795	3.761	4.895
S_{36}	2.880	4.052	4.635	5.020	4.392	4.196	5.100	5.156	5.707	5.808	5.226	5.399	3.201	3.466	3.811	5.542	3.012	3.806
S_{34}	2.445	3.702	4.033	4.800	4.078	3.812	4.790	5.220	5.220	5.582	4.990	5.160	2.962	3.125	4.058	5.545	3.508	3.640
V_1	2.303	3.620	3.401	3.722	3.641	3.355	3.957	4.013	4.096	4.537	4.193	4.159	2.591	2.988	3.767	4.018	3.263	3.326
Factor B	3.123	4.126	4.474	5.170	4.395		4.843	5.086	5.262	5.548	4.882		3.237	3.565	4.062	4.641	3.244	

Protein in silk gland				Protein in mid gut			Protein in haemolymph		
	FactA	Fact B	Fact Ax B	Fact A	Fact B	Fact A x B	Fact A	Fact B	Fact A x B
'F' test	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
S.E. (m) ±	0.066	0.060	0.148	0.007	0.006	0.016	0.013	0.011	0.029
C.D. at 5%	0.186	0.169	0.415	0.020	0.018	0.046	0.036	0.033	0.081

highest (5.427 μg) protein in silk gland was observed due to rearing of silkworm larvae on S_{13} cultivar and was significantly superior to S_{1635}, M_5, S_{36} and S_{34} recording 4.429μg, 4.326μg, 4.196μg and 3.812 μg of protein in silk gland,respectively (Table 1). among these treatments. The group of treatments S_{1635} and M_5; M_5 and S_{36}; $S_{36}$and S_{34} were at par with each other. Significantly lowest protein in silk gland was observed due to feeding of silkworm larvae on V_1 cultivar recording 3.355 μg protein.Similarly Chandgude (2007) when reared PM X CSR_2race of silkworm on S_{13} 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 1revealed 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 M_6 which was also at par with M_{24} 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 M_0. Amongst the magnetic field treatment significantly least protein in silk gland was recorded in the treatment M_3 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 indicate that, the silkworm larvae fed on S_{13} mulberry cultivar for 12 h in magnetic field ($S_{13}M_{12}$) recorded significantly maximum protein in silk gland i.e. 7.062 μg over all the treatments. Which was also followed by $S_{13}M_6$ 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 V_1M_0 2.303 μg which was also at par with S_{34} M_0 with 2.445 μg protein. Treatment $S_{36}M_0$ 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 S_{13} cultivar for 12 h magnetic field.

Protein in midgut

Effect of cultivar on protein in midugt 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 silk worm larvae were reared on S_{13} cultivar and was significantly superior over all the treatments. The next effective treatment was M_5 which exhibited 5.474 μg of protein in midgut of the larvae and was significantly superior to treatments viz., S_{36}, S_{34} and S_{1635}. Minimum protein was observed (4.159 μg) in midgut of larvae reared on V_1 cultivar. The study conducted by Watnabe (1990) reported there is effect of protein content in midgut on proteinase activity and antiviral activity of the gut juices of silkworm *B. mori* and observed that proteinase 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 M_{12} treatment and was significantly superior to all the treatments. Treatment M_6 was second in order of merit and recorded 5.262 μg of protein, followed by M_3 5.086 μg and M_{24} 4.882 μg protein in midgut. The least effective treatment was M_0 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 $S_{13}M_{12}$ treatment and was significantly superior over all the treatments. Whereas, significantlylowest protein 3.957 μg in midgut was observed in V_1M_0 treatment. Treatments M_5M_{12}, $S_{13}M_6$, $S_{36}M_{12}$ recording 5.850, 5.826 and 5.808 μg protein were at par with each other and significantly superior to the treatments $S_{36}M_6$ (5.707 μg), M_5M_6 (5.621 μg), $S_{34}M_{12}$ (5.582 μg),$S_{13}M_3$ (5.567 μg), M_5M_3 (5.503 μg), $S_{13}M_0$ (5.452 μg), M_5M_0 (5.311 μg) and $S_{1635}M_{12}$ (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 S_{13} cultivar. Whereas, the minimum protein activity was recorded in M_5 cultivar i.e. 3.279 μg. Treatment S_{36} recorded 3.806 μg protein in haemolymph was second in order of merit and was significantly superior to treatments S_{34}, $S_{1635,}$ V_1 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 S_{13} 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 (M_{12}) was most significantly superior over all the treatments exhibited 4.641 μg of protein in haemolymph. However, the next better treatment was M_6in which 4.062 μgpritein in haemolymph was observed, followed by M_3(3.565 μg of protein). Least protein in haemolymph was observed in treatment M_0 and M_{24} where 3.237μg 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 $S_{13}M_{12}$ and was found significantly superior over all the treatments. Whereas the significantly least protein in haemolymph was observed in treatment V_1M_0 i.e. 2.591 μg the next best group of treatments which recorded maximum protein in haemolymph were $S_{34}M_{12}$ and $S_{36}M_{12}$ recording 5.545μg and 5.542 μg protein. In present study, maximum protein in haemolymph was observed due to rearing of silkworm larvae in S_{13} cultivar for 12 h magnetic field ($S_{13}M_{12}$ treatment) and lowest protein (2.591 μg) was observed in V_1M_0 treatment. Thisindicate 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 $S_{13}$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.

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