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Effect of Mulberry Cultivars and Magnetic Field on Activity of Protease Enzyme in Midgut of *Bombyx mori* (L.)

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Abstract: Six mulberry cultivars and five rearing methods were evaluated at Department of Entomology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, India. For the study pm x csr₂ race of mulberry silkworm, *Bombyx mori* L was used. The data thus obtained was analysed in fcrd design with cultivar as Factor 'A' and rearing method (magnetic field) as Factor 'B'. The pooled results indicated that silkworm reared on S₁₃ mulberry cultivar for 12 h magnetic field (S₁₃M₁₂) had significant effect on the activity of digestive enzyme Protease in larva of mulberry silkworm and observed maximum enzymatic activity in midgut was 0.111 $\mu\text{M mg}^{-1}$ protein over normal method of rearing on mulberry cultivar V₁ in zero h magnetic field (V₁M₀) in which minimum enzymatic activity in midgut for Protease was 0.0578 $\mu\text{M mg}^{-1}$ protein. Thus, the study indicated that for increasing the digestion and metabolisation and ultimately increasing cocoon production in silkworm larvae they should be reared on S₁₃ cultivar of mulberry in 300 gauss magnetic field for 12 h every day.

Keywords: Mulberry silkworm, Magnetic field, Midgut, Protease

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Introduction

Silk is the most elegant textile fibre in the world with unparalleled grandeur, natural sheen, inherent affinity for dyes, high absorbance, light weight, soft touch, high durability, fineness and is the unique strongest fiber. Hence, called "Queen of Textiles" in the world (Ramana, 1987). Silk is amongst the few commodities having world wide importance. According to western mythology, silk industry is supposed to be originated in China since 2640 B.C. and later spread to the other countries. But Indian mythology gives evidences

about the use of silk garments from the era of Lord Ganesha, where in the silk garments were known as "Dev Vastra" and were the mark of peace, richness, elegance, cleanliness and royalness (Nimbalkar and Aherkar, 2005).

The major silk producing countries in the world are-- China, India, Uzbekistan, Brazil, Japan, Republic of Korea, Thailand, Vietnam, DPR Korea, Iran, etc. Few other countries are also engaged in the production of cocoons and raw silk in negligible quantities-- Kenya, Botswana, Nigeria,

Zambia, Zimbabwe, Bangladesh, Colombia, Egypt, Nepal, Bulgaria, Turkey, Uganda, Malaysia, Romania, Bolivia, etc. The major silk consumers of the world are USA, Italy, Japan, India, France, China, United Kingdom, Switzerland, Germany, UAE, Korea, Viet Nam, etc.

Even though silk has a small percentage of the global textile market, less than 0.2% (the precise global value is difficult to assess, since reliable data on finished silk products is lacking in most importing countries), its production base is spread over 60 countries in the world. While the major producers are in Asia (90% of mulberry production and almost 100% of non-mulberry silk), sericulture industries have been lately established in Brazil, Bulgaria, Egypt and Madagascar as well. Sericulture is labour-intensive. About 1 million workers are employed in the silk sector in China. Silk Industry provides employment to 7.9 million people in India, and 20,000 weaving families in Thailand. China is the world's single biggest producer and chief supplier of silk to the world markets. India is the world's second largest producer. Sericulture can help keeping the rural population employed and to prevent migration to big cities and securing remunerative employment; it requires small investments while providing raw material for textile industries (Anonymous, 2020).

India has produced 35,820 MT of silk against the target of 38,530 MT during 2019-20 achieving 93.0% of the target. The vanya silk (tasar, eri and muga) production grew by 4.5% from 10,124 MT in 2018-19 to 10,581 MT in 2019-20. The production of tasar, eri and muga silks was 3136 MT, 7204 MT and 241 MT, respectively during 2019-20. The export earnings from silk goods was Rs.1,745.65 crore (US\$ 246.67 million) during 2019-20 compared to Rs.2,031.89 crore (US\$ 291.36 million) in 2018-19. The import of raw silk increased by 19.03% from 2,785 MT during 2018-19 to 3,315 MT in 2019-20 (Anonymous, 2019-20).

In India the major silk producing states are

Karnataka, Andhra Pradesh, West Bengal, Tamilnadu and Jammu and Kashmir contributing 65.74, 19.19, 8.92, 4.27 and 0.59 per cent share in silk production, respectively (Anonymous, 2000).

In Maharashtra, Tasar silkworm rearing is practiced from long back in Bhandara, Gadchiroli, Chandrapur and Gondia district and mulberry silkworm rearing has been initiated from 1990's and is slowly becoming popular in this region due to high economic return from this crop. In Vidarbha, sericulture is practiced in Buldhana, Washim, Akola, Amravati, Yeotmal, Wardha and Nagpur district with total area of mulberry plantation upto 575.00 ha and annual production 683.97 kg silk (Anonymous, 2008).

Agriculture is the prime occupation of India in which nowadays sericulture is replacing important crops. According to Ramana (1987), mulberry has replaced sugarcane in Karnataka, fruit crops in Jammu and Kashmir, Jute in West Bengal, grapes in Andhra Pradesh and several other crops including rice and wheat. Similarly, in Vidarbha in days to come mulberry will be competing with cotton cultivation and replacing cotton in Vidarbha.

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 proteins produced by the silkworm is directly derived from the proteins of mulberry leaves (Rangaswami *et al.*, 1976). 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. Patnev and Mankova (1986) observed influences of magnetic field on migratory and exploratory behaviours of bees. Chougale and More (1993) exposed silkworm to electro-magnetic field and observed changes in

biology of silkworm etc.

Increase in the haemocytic cells in haemolymph of mulberry silkworm was observed by Aherkar and Satpute (2005). There was increase in hormone and insulin levels in rat (Udinsteve and Moroz, 1982). Increase in the activity of acid phosphatase in mouse tissue was observed by (Conley *et al.*, 1966).

The previous study conducted with the help of electromagnetic field (Chougale and More 1992, 1993) had positive effect on economic parameters, acid and alkaline phosphatase and silk gland of silkworm. In the present study the permanent magnet were used to study the effect on various economic factor and enzymatic activity. The study indicated that rearing of silkworm in 300 gauss magnetic field for 12 h had positive effect on silkworm.

Magnetic field play important role in increasing the RBC count in human being if exposed. It also plays an important role in disease and disability management in human being Rathi (2003). Keeping this in view, this study was planned to investigate the effect of food and magnetic field on the silkworm to increase the output of silk fiber and to study the effect on enzymatic activity and protein content in the silk gland.

Efforts were 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 mulberry leaf. In the present study a separate view has been evaluated for increasing the disease resistance and enzymatic activity in midgut and haemolymph due to the rare of silk worm larvae in magnetic field with the objectives to study the effect of cultivars and magnetic field on protease activity in midgut of *Bombyx mori*.

Materials and Methods

The experiment was conducted at the Sericulture laboratory and Insect Biotechnology Laboratory,

Department of Entomology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, India.

Material required:

Standards silkworm rearing materials and laboratory cleaning chemicals/reagents were used for silkworm rearing. 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 and the paraffin paper was placed over the magnets. After hatching of the eggs rearing of larvae were under taken as per the scheduled treatments.

Protein and Protease estimation:

Protein estimation was done by the method suggested by Bradford (1976). Protease activity was determined by the method of Sarangi (1986).

Details of experiment:

Design : Factorial Complete Randomized Design (FCRD)

Replication : Four

Silkworm race : PM x CSR₂

No. of larvae per treatment: 200

No. of treatments : 30

Treatment details:

(A) Factor I (Six varieties)		(B) Factor II (Rearing method)
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	

Statistical analysis:

Data obtained were subjected to statistical analysis after appropriate transformation wherever essential (Gomez and Gomez, 1984).

Results and Discussion

Effect of cultivars on protease in midgut (Factor A):

Pooled data of both the trials revealed that all the treatments differed significantly with each other. Significantly highest protease $0.086 \mu\text{M mg}^{-1}$ protein was observed in S_{13} cultivar followed by S_{36} and M_5 where $0.074 \mu\text{M mg}^{-1}$ protein and $0.069 \mu\text{M mg}^{-1}$ protein protease was observed, respectively. Treatment S_{34} and S_{1635} were next best treatments in which $0.065 \mu\text{M mg}^{-1}$ protein and $0.065 \mu\text{M mg}^{-1}$ protein protease in midgut was observed and were at par with each other. Treatment V_1 was least significant and recorded $0.061 \mu\text{M mg}^{-1}$ protein protease in midgut (Table 1).

This study indicated that feeding of larvae on a cultivar has effect on protease enzyme in midgut. Maximum protease in midgut protein was observed due to the feeding of S_{13} cultivar, whereas, it was minimum in V_1 cultivar.

Ruth *et al.* (2019) attempted to identify the most suitable host plants for silkworm rearing under mountainous agro-ecological conditions which can lead to sustainable production of silk in relation to physiological and economic parameters. This confirms the findings of the present study. Xu *et al.* (2019) used iTRAQ proteomic technique to identify and analyze silkworm larval digestive juice proteins. A total of 227 proteins were successfully identified. These were primarily serine protease activity, esterase activity, binding, and serine protease inhibitors, which were mainly involved in the digestion and overcoming the detrimental effects of mulberry leaves. Moreover, 30 genes of the identified proteins were expressed specifically in the midgut. Temporal proteomic analysis of digestive juice revealed developmental dynamic features related to molecular mechanisms of the principal functions of digesting, resisting pathogens, and overruling the inhibitory effects of mulberry leaves protease inhibitors (PIs) with a dynamic strategy, although overruling the inhibitory effects

has not yet been confirmed by previous study. These findings will help address the potential functions of digestive juice in silkworm larvae. This partially confirms the findings of the present study.

Chandana *et al.* (2020) concluded that divulged that the mulberry leaf fortified with niacin at 0.6 % intensify economic parameters of selected bivoltine hybrid silkworm *Bombyx mori*. These findings are in support of the present findings. However, Manjula *et al.* (2011) concluded that supplementation of *Vigna unguiculata* with mulberry leaves at a concentration of 7.5% enhanced the digestion of ingested food which in turn reflects in the quantity of silk produced. These findings are in confirmation with the findings of current study.

Effect of magnetic field on protease in midgut (Factor B):

Pooled results of both the trials indicate that the silkworm larvae reared in magnetic field were significantly superior to non-magnetic field (M_0). Maximum protease $0.087 \mu\text{M mg}^{-1}$ protein was noticed in rearing of silkworm larvae in 12 h magnetic field followed by M_6 , M_{24} and M_3 which exhibited $0.073 \mu\text{M mg}^{-1}$ protein, $0.070 \mu\text{M mg}^{-1}$ protein and $0.065 \mu\text{M mg}^{-1}$ protein protease in midgut, respectively. Whereas, silkworm larvae reared in non-magnetic field (M_0) recorded significantly least protease ($0.056 \mu\text{M mg}^{-1}$ protein) in midgut (Table 1).

Present study indicated that magnetic field had influence on protease in 5th instar silkworm larvae midgut. Highest protease in midgut was observed in rearing of silkworm larvae in 12 h magnetic field over other treatments. Whereas, it was lowest in non-magnetic field treatment.

Shivpuje *et al.* (2016) concluded that magnetization may increase the levels of amino acids followed by accelerated rate of protein synthesis in the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2). Magnetic energy should be utilized as efficiently as possible

Table 1: Effect of cultivars and magnetic field on protease in midgut ($\mu\text{M mg}^{-1}$ protein)

Cultivars	A First trial						B Second trial						C Pooled					
	Interaction						Interaction						Interaction					
	M ₀	M ₃	M ₆	M ₁₂	M ₂₄	Fact A	M ₀	M ₃	M ₆	M ₁₂	M ₂₄	Fact A	M ₀	M ₃	M ₆	M ₁₂	M ₂₄	Fact A
S ₁₆₃₅	0.075	0.071	0.073	0.081	0.081	0.076	0.044	0.044	0.057	0.071	0.055	0.054	0.059	0.058	0.065	0.076	0.068	0.065
M ₅	0.065	0.081	0.086	0.085	0.091	0.082	0.048	0.052	0.053	0.066	0.069	0.057	0.056	0.066	0.069	0.076	0.080	0.069
S ₁₃	0.075	0.082	0.087	0.118	0.085	0.089	0.060	0.082	0.081	0.103	0.091	0.085	0.067	0.082	0.084	0.111	0.088	0.086
S ₃₆	0.048	0.052	0.079	0.114	0.055	0.070	0.056	0.083	0.079	0.083	0.090	0.078	0.052	0.067	0.079	0.099	0.073	0.074
S ₃₄	0.052	0.061	0.083	0.084	0.048	0.065	0.059	0.060	0.070	0.074	0.065	0.065	0.055	0.060	0.076	0.079	0.056	0.065
V ₁	0.050	0.060	0.073	0.078	0.043	0.061	0.042	0.054	0.061	0.084	0.062	0.061	0.046	0.057	0.067	0.081	0.053	0.061
Factor B	0.061	0.068	0.080	0.093	0.067		0.051	0.062	0.067	0.080	0.072		0.056	0.065	0.073	0.087	0.070	

	Fact A	Fact B	Fact A x B		Fact A	Fact B	Fact A x B		Fact A	Fact B	Fact A x B
S.E. (m) $\pm$	0.0005	0.0005	0.0012		0.0003	0.0003	0.0006		0.0003	0.0003	0.0006
C.D. at 5 %	0.001	0.001	0.003		0.001	0.001	0.002		0.001	0.001	0.002

for the progression of growth of larval instars of silkworm, *Bombyx mori* (L) which supports the findings of the present study.

Interaction effect of cultivars and magnetic field on protease in midgut (Factor A x B):

Pooled results of both the trials indicated that, significantly highest protease 0.111 $\mu\text{M mg}^{-1}$ protein content in midgut was recorded in treatment $S_{13}M_{12}$ and was significantly superior over all the treatments (Table 1). Significantly lowest protease 0.046 $\mu\text{M mg}^{-1}$ protein was due to treatment V_1M_0 .

Treatment $S_{36}M_{12}$ and $S_{13}M_{24}$ were the second best treatment recording 0.099 and 0.088 $\mu\text{M mg}^{-1}$ protein protease enzyme and the former treatment was significantly superior to latter one. Both these treatments were also significantly superior to treatments $S_{13}M_6$, $S_{13}M_3$, V_1M_{12} , M_5M_{24} , $S_{34}M_{12}$, $S_{36}M_3$, $S_{34}M_3$, $S_{1635}M_{12}$, M_5M_{12} , $S_{36}M_{24}$, M_5M_6 , $S_{1635}M_{24}$, $S_{36}M_3$, $S_{13}M_0$, V_1M_6 , M_5M_3 and $S_{1635}M_6$ which recorded 0.084 $\mu\text{M mg}^{-1}$ protein, 0.082 $\mu\text{M mg}^{-1}$ protein, 0.081 $\mu\text{M mg}^{-1}$ protein, 0.080 $\mu\text{M mg}^{-1}$ protein, 0.079 $\mu\text{M mg}^{-1}$ protein, 0.079 $\mu\text{M mg}^{-1}$ protein, 0.076 $\mu\text{M mg}^{-1}$ protein, 0.076 $\mu\text{M mg}^{-1}$ protein, 0.076 $\mu\text{M mg}^{-1}$ protein, 0.073 $\mu\text{M mg}^{-1}$ protein, 0.069 $\mu\text{M mg}^{-1}$ protein, 0.068 $\mu\text{M mg}^{-1}$ protein, 0.067 $\mu\text{M mg}^{-1}$ protein, 0.067 $\mu\text{M mg}^{-1}$ protein, 0.067 $\mu\text{M mg}^{-1}$ protein, 0.066 $\mu\text{M mg}^{-1}$ protein and 0.065 $\mu\text{M mg}^{-1}$ protein protease in midgut, respectively. All these treatments were also significantly superior to treatments $S_{34}M_3$, $S_{1635}M_0$, $S_{1635}M_3$, V_1M_3 , M_5M_0 , $S_{34}M_{24}$, $S_{34}M_0$, V_1M_{24} and $S_{36}M_0$ recording 0.060 $\mu\text{M mg}^{-1}$ protein, 0.059 $\mu\text{M mg}^{-1}$ protein, 0.058 $\mu\text{M mg}^{-1}$ protein, 0.057 $\mu\text{M mg}^{-1}$ protein, 0.056 $\mu\text{M mg}^{-1}$ protein, 0.056 $\mu\text{M mg}^{-1}$ protein, 0.055 $\mu\text{M mg}^{-1}$ protein, 0.053 $\mu\text{M mg}^{-1}$ protein and 0.052 $\mu\text{M mg}^{-1}$ protein protease in midgut, respectively.

From the present studies it was concluded that there was significant effect of cultivar and magnetic field on protease in protein of midgut. Similarly, Chundang *et al.* (2020) studied one of the factors and their interaction which had effects on the overall proximate composition and

physicochemical properties ($p < 0.05$), in terms of water absorption index, water solubility, swelling power, pH, relative crystallinity, and FT-IR spectra. Cellulase activity was significantly decreased with larval stage, while a similar pattern was observed for amylase activity, but with relatively low activity in the first stage. The highest trypsin activity was observed in the fourth stage, followed by the second and fifth stages.

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