International Journal of Entomology Research
ISSN: 2455-4758; Impact Factor: RJIF 5.24
Received: 20-12-2020; Accepted: 21-01-2021; Published: 02-03-2021
www.entomologyjournals.com
Volume 6; Issue 2; 2021; Page No. 07-11

Effect of mulberry cultivars and magnetic field on activity glutathione-s-transferase activity in midgut of *Bombyx Mori* L.

VJ Tambe, SK Aherkar
Post Graduate Institute, Dr. PDKV Akola, Maharashtra, India

Abstract
Six mulberry cultivars and five rearing methods were evaluated in Department of Entomology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, and Akola during 2006-2007. 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 detoxifying enzyme in larva of mulberry silkworm and observed maximum enzymetic activity in midgut for Glutathione-s-transferase was 8.038 µM mg-1 protein, over normal method of rearing on mulberry cultivar V1 in zero hour magnetic field (V1M0) in which minimum enzymetic activity in midgut for Glutathione-s-transferase was estimated as 3.970 µM mg-1 protein.
Thus, the study indicated that for minimizing the larval mortality rate 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 and GST

Introduction
Silk is the most elegant textile fibre in the world with unparalled 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 worldwide 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) [33]. In the world there are 58 silk producing countries, out of which China is the leading producer of silk, producing 120256 MT of silk followed by India 16329 and Japan 394 MT. Though India is the second largest silk producing country but it is a unique country in the world producing four different type of silk on economical basis i.e. Mulberry silk, Tasar silk, Muga silk and Eri silk. Amongst these four type of silk, major lion share is of Mulberry silk followed by Eri silk, Tasar silk and Muga silk in 91.7, 6.4, 1.4 and
0.5 per cent (Bhaskar, 2007) [12].
In India sericulture is practiced in eleven states, creating employment for 6, and 15,068 peoples, practiced in 57,936 villages of India. In India mulberry is planted on 1,94,463 ha of land producing 1,28,181 tons of silk and earning foreign exchange upto the extent of Rs. 2294.05 lack (Nimbalkar and Aherkar, 2005) [33].
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
Per cent share in silk production, respectively (Anonymous, 2000) [9].
In Maharashtra Tasar silkworm rearing is practice 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 practice 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) [11].
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) [40]. Silkworm consumes 81 per cent of the food in fifth instar where in need of matured leaf is desired (Krishnaswami, 1987) [27]. 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) [35] observed influences of magnetic field on migratory and exploratory behaviours of bees.
Chougale and More (1993) [16] exposed silkworm to electromagnetic field and observed changes in biology of

silkworm etc.

Increase in the haemocytic cells in haemolymp of mulberry silkworm was observed by Aherkar and Satpute (2005) [1]. There was increase in hormone and insulin levels in rat which was observed by (Udinstev and Moroz, 1982) [47]. Increase in the activity of acid phosphatase in mouse tissue was observed by (Conley *et al.* 1966) [17].

The previous study conducted with the help of electromagnetic field (Chougale and More 1992 and 1993) [15, 16] 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 enzymetic 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) [41]. Keeping in view the same points, this study has been planned to study the effect of food and magnetic field on the silkworm to increase the output of silk fiber and to study the effect on enzymetic activity and protein content in the silk gland.

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 mulberry leaf. But in present study a separate view has been evaluated for increasing the disease resistance and enzymetic activity in midgut and haemolymph due to the rare of silk worm larvae in magnetic field on following objective.

Objectives of Study

To study the effect of cultivars magnetic field and on activity of Glutathione-s- transferase in midgut of *B. mori*

The data collected during the study was recorded and subjected to statistical analysis and the results obtained are discussed with available literature.

Material and Methods

The experiment was conducted during 2006 and 2007 in the Sericulture laboratory and Insect Biotechnology Laboratory, Department of Entomology, Dr.

PDKV, Akola. The material used and methods adopted during the course of present investigations are described below

Material Required

Standards silkworm raring 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 is downward and the paraffin paper was placed over the magnets. After hatching of the eggs rearing of larvae was under taken as per the scheduled treatments.

Protein Estimation

A. Phosphate buffer – 0.1 M / lit (pH 7.0)
   Sodium hydroxide – 0.2 M / lit
   Sodium dihydrogen phosphate – 0.2 M/lit
   Distilled water – 100 ml
B. 0.15 N NaCl
C. 1 x Bradford reagent
D. Enzyme stock
E. BSA (Bovine Serum Albumin) for preparing standard of BSA (from Sigma chemicals)

Enzyme Analysis

1. Dissection buffer :
   100 ml Sodium phosphate buffer SPB 0.1 M (pH 7.0)
   1.5 kg KCl
2. Homogenization buffer
   1000 ml to phosphate buffer 0.1 M, pH 6.5, 0.292 g EDTA (Ethylene Diamile Tetra Acetic Acid)
   0.304 g PTU (Phenyl Thio Urea) 1 m M
   0.174 g PMSF (Phenyl Methyl Sulphonyl fluoride 1 m M)
3. Quantification of glutathione – s – transferase

A. Reduced glutathione 50 mM
B. Sodium phosphate buffer (SPB) 0.1 M, pH 6.5 + 1 mM EDTA
C. CDNB (24 dinitro – chlorobenzene 50 mM) in ethanol 50 mM
D. Enzyme stock

Preparation of Midgut Sample for Protein Estimation and Gst Estimation

Two larvae of fifth instar were randomly selected from each treatment and kept in deep freez at -20^0 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 a protein and.

Quantification of Protein from Midgut

The protein of midgut homogenate and haemolymph of *B. mori* larvae was quantified by Bradford method (Bradford, 1976) using microplate assay (Plate 5).

Procedure for Estimation of Protein by Micro-Plate Assay Is As Followed

A. Dye reagent was prepared by diluting 5 Bradford reagent with 4 parts of distilled water.
B. 10 ul of protein sample was taken in microplate well
C. 10 ul of NaCl solution (0.15 N) was added to each well
D. To this 200 ul of 1 Bradford reagent was added and incubated for 15 min at room temperature.
E. Similarly one blank (with no protein) was also maintained
F. The absorbance was measured at 600 nm
G. Each samples were replicated in four times to minimize the error.

Calculations

Absorbance of blank (without protein) is subtracted from absorbance of protein sample and resulting absorbance is multiplied by factor of 6.75 it gives protein per 10 ul sample.

i.e. = O.D. (protein) – O.D. (Blank) x 6.75 protein µl / 10 µl

Quantification of Glutathione-s-transferase activity from midgut

GST estimation was carried out by Kao *et al.* (1989).

Details of experiment
Design: Factorial Complete Randomized Design (FCRD)
Replication: Four
Silkworm race: PM x CSR_2
No. of larvae per treatment: 200
No. of treatments: 30

Treatments detail
Factor I (Six varieties)
T1: S-1635
T2: M-5
T3: S-13
T4: S-36
T5: S-34
T5: S-34

Factor II (Rearing method)
M0: Rearing of silkworm in non-magnetic field
M3: Rearing of silkworm in 3 h magnetic field daily
M6: Rearing of silkworm in 6 h magnetic field daily
M12: Rearing of silkworm in 12 h magnetic field daily
M24: Rearing of silkworm in 24 h magnetic field daily

Statistical analysis
Data obtained were subjected to statistical analysis after appropriate transformation wherever essential (Gomez and Gomez, 1976).

Results and Discussion
Glutathione S-Transferase (GST) in Midgut
Effect of Cultivars on GST in Midgut. (Factor A)
Pooled
Pooled data of both the trials and results presented in Table 8 & Fig. 8 indicate that, in cultivar S13 possessed maximum 6.326 µM mg^{-1} protein GST in midgut of silkworm larvae. It was significantly least (5.394 µM mg-1 protein) GST when the larvae were fed on V1 cultivar and was at par with treatments M5 and S36 in which 5.437 µM mg-1 protein and 5.524 µM mg-1 protein GST was recorded in midgut, respectively. The next best treatments in which maximum GST was observed were S34 and S1635 recording 5.945 µM mg^{-1} protein and 5.834 µM mg^{-1} protein GST in midgut, respectively.
When the silkworm larvae were reared on S13 cultivar maximum protein was observed in midgut which reflected in GST studies of midgut protein, recording maximum GST (6.311 µM mg^{-1} protein) in S13 treatment.But no study could be reviewed in this connection that mulberry cultivar has significant effect on GST of silkworm and hence could not be compared.

Effect of magnetic field on GST midgut (Factor B)
Pooled
Pooled results of two rearing also indicate, similar trend of GST activity in magnetic field and non-magnetic field treatment. Significantly maximum GST
7.458 µM mg^{-1} protein was observed in M12 treatment and was significantly superior over all the treatments (Table 1). Significantly minimum GST 4.120 µM mg^{-1} protein was recorded in non-magnetic field treatment (M0).
Treatment M6 was second in order of merit in which 6.301 µM mg^{-1} protein GST in midgut was observed and was followed by treatment M24 and M3 where 5.784 µM mg-1 protein and 5.053 µM mg-1 protein GST activity was recorded.
There was increase in GST due to rearing of silkworm larvae in 12 h magnetic field than that of 24h, 6h, 3h and 0 h magnetic field. But the results of present findings couldn't be compare due to paucity of literature.

Interaction Effect of Cultivars and Magnetic Field on GST in Midgut. (Factor A x B)
Pooled
Pooled data of both the trials presented in Table 8 & Fig. 8 revealed that, silkworm larvae reared on S13 cultivar for 12 h in magnetic field (i.e. S13M12) treatment was significantly superior over all the treatments and recorded highest 8.038 µM mg-1 protein GST in midgut of silkworm larvae. However, this
Treatment was at par with S1635M12, M5M12, S34M6 recording 7.962 µM mg^{-1} protein, 7.671 µM mg-1 protein, 7.426 µM mg-1 protein GST in midgut respectively. Treatments, S34M12, S36M12, V1M12, S13M6, and S1635M24 observed 7.271
µM mg-1 protein, 6.943 µM mg-1 protein, 6.861 µM mg-1 protein, 6.810 µM mg-1 protein and 6.683 µM mg-1 protein in midgut and was the next group of treatment recording maximum GST in midgut of silk worm larvae found at par with each other.
Treatments S13M24 (6.353 µM mg^{-1} protein), S1635M6 (6.043 µM mg^{-1} protein), S36M6 (5.973 µM mg^{-1} protein), V1M6 (5.932 µM mg^{-1} protein) and S13M3 (5.760 µM mg^{-1} protein) in midgut and were at par with each other. The latter two treatments were also at par with M5M6 (5.623 µM mg^{-1} protein), V1M24 (5.482 µM mg^{-1} protein), S34M24 (5.481 µM mg^{-1} protein), S36M24 (5.400 µM mg^{-1} protein), M5M24 (5.326 µM mg^{-1} protein) and S36M3 (5.272 µM mg^{-1} protein). Significantly least GST in midgut was observed in the treatments S1635M0 and was at par with M5M0, V1M0 and S36M0 recording 3.592 µM mg^{-1} protein, 3.595 µM mg-1 protein, 3.970 µM mg-1 protein and 4.032 µM mg-1 protein GST in midgut of silkworm larvae. However, to this treatment treatments, S34M0, S1635M3, S1635M3, S13M0, V1M3, M5M3, S34M3 were significantly superior in which 4.506 µM mg^{-1} protein, 4.531 µM mg-1 protein, 4.670 µM mg-1 protein, 4.727 µM mg-1 protein, 4.972 µM mg-1 protein and 5.060 µM mg-1 protein GST was observed in the midgut of silkworm larvae.
The study indicates that cultivar and magnetic field both have influence on GST in the protein in midgut of silkworm. If the silkworm were not reared in the magnetic field this was reduction in GST in midgut. Which plays an important role in imparting immunity in the insect and hence along with a good cultivar if the silkworms are reared in the magnetic field there is increase in GST in midgut which automaticaly impart resistance in insect. However this study couldn't be compared with other such type of studies due to paucity of literature.

Table 1: Effect of cultivars and magnetic field on GST in midgut of full grown larvae (µM mg-1 protein)

Cultivars	A First trial						B Second trial						C Pooled					
	Interaction						Interaction						Interaction					
	M0	M3	M6	M12	M24	Fact A	M0	M3	M6	M12	M24	Fact A	M0	M3	M6	M12	M24	Fact A
S1635	4.367	5.392	6.715	7.942	6.640	6.211	3.537	3.670	5.372	7.750	6.727	5.411	3.952	4.531	6.043	7.962	6.683	5.834
M5	4.240	6.475	5.512	8.187	5.495	5.982	2.950	3.470	5.735	7.155	5.157	4.893	3.595	4.972	5.623	7.671	5.326	5.437
S13	4.807	6.470	7.127	8.327	6.590	6.664	4.532	5.050	6.492	7.982	6.117	6.035	4.670	5.760	6.810	8.038	6.353	6.326
S36	3.637	5.640	6.132	6.780	5.297	5.497	4.427	4.905	5.815	7.107	5.502	5.551	4.032	5.272	5.973	6.943	5.400	5.524
S34	4.705	5.310	6.975	7.057	5.350	5.879	4.307	4.810	7.877	7.485	5.572	6.010	4.506	5.060	7.426	7.271	5.461	5.945
V1	4.365	5.060	6.780	6.775	5.555	5.707	3.575	4.395	5.085	6.947	5.410	5.082	3..970	4.727	5.932	6.861	5.482	5.394
Factor B	4.353	5.724	6.540	7.511	5.821		3.888	4.383	6.062	7.404	5.747		4.121	5.053	6.301	7.458	5.784	

Table 2

	Fact A	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) ±	0.131	0.120	0.294	0.132	0.120	0.295	0.108	0.098	0.241
C.D. at 5 %	0.369	0.337	0.825	0.370	0.338	0.828	0.302	0.276	0.676

References

1. Aherkar SK, Satpute RS. Effect of magnetic field and cultivars of Haemocytes of *Bombyx mori* L. Insect environment. 2005; 2(3):100-101.
2. Aherkar SK, Sarnaik DN, Nachane MN. Performance of pure Mysore silkworm race on different cultivars of mulberry. Annual Report of Res. Work, Deptt of Entomology, Dr. PDKV, Akola (M.S), 2003a, 64-67.
3. Aherkar SK, Sarnaik DN, Nachane MN. Effect of magnet on growth and development of silkworm. Annual Report of Res. Work, Deptt of Entomology, Dr. PDKV, Akola (M.S.). 2003b, 67-69.
4. Aherkar SK, Sarnaik DN, Sarode SV. Performance of PM silkworm race on different cultivars of mulberry. Annual Report of Res. Work 2000-02. Univ. Deptt of Entomology, Dr. PDKV, Akola (M.S.). 2002, 53-57.
5. Aherkar SK, Thakare HS, Narkhade SS. Behaviour of Multivoltine pure Mysore, Bivoltine NB18 and its cross in Vidarbha climatic condition. PKV Res. J. 1991; 15(2):108-110.
6. Aherkar SK, Patel JD, Nachane MN, Deshmukh PB, Khambadkar HU. Effect of magnetic field on economic parameters of silkworm. Annual Report of Res. Work, Deptt of Entomology, Dr. PDKV, Akola (M.S.). 2004, 62-65.
7. Akoyundoglou G. Effect of magnetic field on carboxy dimutase. Nature. 1965; 202:452-454.
8. Alexander MP, Ganeshan S. Electromagnetic field induce *in vitro* pollen germination and tube growth. Curr. Sci. 1990; 59:276-277.
9. Anonymous. Compendium of statistics of silk industry, Central silk board, United Mansion, 39, Mahatma Gandhi Road, Bangalore, 2000.
10. Anonymous. Tutichi Lagward Va Reshim Kitak Sangopan – Krishi Sanwadini. Pub by DEE, Dr. PDKV, Akola, 2004, 200-204.
11. Anonymous. Progress Report, Directorate of Sericulture, Nagpur (Maharashtra), 2008.
12. Bhaskar H. Silk testing times. India Trade outlook with knowledge inputs from Dun and Broad Street India. Indian silk. 2007; 46(5):4-7.
13. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem. 1976; 72:248-254.
14. Chandgude DM. Effect of mulberry cultivars and magnetic field on economic parameters of *Bombyx mori* L. M.Sc. (Agri.) thesis (unpub.), Dr. PDKV, Akola (M.S.), 2007.
15. Chougale AK, More NK. Effect of magnetization on the developmental period and cocoon characters of the silkworm *Bombyx mori* L. Ind. J. Seric. 1992; 31(2):115-122.
16. Chougale AK, More NK. Effect of magnetization on acid and alkaline phosphatase in developing silk gland of *B. mori* L. Entomon. 1993; 181:1-5.
17. Conley CC, Mills WJ, Patricia AG. Enzyme activity in macrophages from animals exposed to a very low magnetic field. III Fact Bio-magnetic Symposium. Univ. of synopsis, Chicago, 1966, 13-15.
18. Dary O, Georghiou GP, Parsons E, Pasteur N. Micro-plate adoption of Gomoris assay for quantitative determination of general esterase activity in single insects. J. Econ. Ent. 1990; 83:2187-2192.
19. Devi KN, Rao AD, Hemavathi B, Thyagaraju K. Glutathione s-transferase of *Bombyx mori.* L. Larvae. Ind. J. Seric. 1996; 35(2):114-116.
20. Eguchi M, Iwamoto A, Yamauchi K. Interrelation of proteases from midgut lumen, epithelium and peritrophic membrane of the silkworm, *Bombyx mori* L. Comp. Biochem. Physiol. 1982; 72A:359-363.
21. Eid MAA, El Nakkady AN, Saleh MA. Effect of supplementary amino acids on silk secretion by larvae of *Philosamia ricini* (Boisd.). Ind J. Seric. 1989; 28(2):224- 232.
22. Giridhar K, Shivarami Reddy N. Performance of bivoltine race on new mulberry varieties. Ind. J. Seric. 1991; 30(1):23-29.
23. Gomez KA, Gomez AA. Statistical procedure for Agricultural Research. 2nd edn. New York. Pub. By Wiley inter-science, 1976.
24. Hamano K, Tsuchida K. Effect of dietary level of protein and pyridoxine on growth of 5th instar larvae of silkworm *B. mori* L. J. Seric. Sci. 1989; 58(5):380-386.
25. Haque T, Haque KT, Rahman SM. Performance of some silkworm races feeding on different mulberry cultivars. Bangladesh Bull. Seric. Res. Seridoc. 1990; 7 (1):25-40.
26. Zeng Juan, Cui-Weizheng, Mu-ZhiMei. Effect of crude protein content in linear programming artificial fed on some physiological characteristics of silk worm (*Bombyx mori*). Acta – Sericologicasinica. 2002;

28(3):224-228.
27. Krishnaswami S. New technology of silkworm rearing pub. By Central silk board, Bnaglore, 1987, 1-23.
28. Krishnaswami S. Improved method of rearing young age chawki silkworm. Bull. No. 3 pub. By CSRTI, Mysore, 1986.
29. Miao-Yungen, Bharati D. Effect of some chemicals on cocoon crop production and the activity levels of alkaline phosphatase in the midgut of two different straing of silkworm. *B. Mori* L. Bull of Ind. Acad. of Sericulture. 2001; 5(2):72-75.
30. Mulay IL, Mulay LN. Effect on *Drosophila melanogaster* and S-37 tumor cells, postulates for magnetic field interpretations. In Biological effects of Magnetic fields. M.F. Barnothy (Ed.), Plennum Press, New York, 1964.
31. Nagata M, Yoshitake N. Protein metabolism in the larval development of the silkworm. *Bombyx mori.* L. protein for reutilization at the fourth moult. J. seric. Sci. (Seridoc 5 (3/4) CSRTI, Mysore. 1989; 58(3):221-223.
32. Narsimhana MN. Mannual of silkworm egg production. Central Silk Board. United Manson. 39, M.G. Road Bangalore. 1988, 1-158.
33. Nimbalkar SA, Aherkar S.K. Reshim Udyogachi Mulbhut Tatve Aani Vyavasaikta. Head, Deptt of Entomology, Dr. PDKV, Akola (M.S.). 2005, 35-49.
34. Patil AJ. Effect of mulberry cultivars fortified with natural proteins on *B. mori* L. M.Sc. (Agri.) thesis (Unpub.), Dr. PDKV, Akola (M.S.), 2003.
35. Patnev TP, Mankova MJ. Direct and indirect effects of a constant magnetic field on biological objects. Kosm. Biol. Aviakosm. Med. 1986; 20:73-76.
36. Pawade VN. Effect of cultivars and magnetic field on grainage parameters of *Bombyx mori* L. M.Sc. (Agri.) thesis (Unpub.), Dr. PDKV, Akola (M.S.), 2006.
37. Plummer DT. An introduction to practical biochemistry, 22nd reprint 3rd Edn. Pub. By Tata Mc. Graw Hill Back Company, U.K. 2004, 236.
38. Ponnuvel KM, Vineet Kumar, Babu AM, Datta R. K. Testing of two breeds on resistant to BMNPV (Mistan) and one sensitive (Hosa Mysore) due to changes in level of Alkaline protease. Italian J. of Zoology. 1999; 66(2):121-124.
39. Rajput AR. Effect of some mulberry cultivars on growth and development of pulse Mysore strain of *B. mori* L. M.Sc. (Agri.) thesis (Unpub.). MPKV. Rahuri (M.S.), 1994, 1-68.
40. Rangaswami G, Narsimhana MN, Kasivishwanthan K, Sastri CR, Jolly MS. Introduction in sericulture manual 1. Mulberry cultivation. F.A.O. Bull. 15/1. Reprint by C.S.B. Bangalore, 1976, 1.
41. Rathi P. Miracles of the magnetotherapy, Gayatri Health and Agricultural Research Foundation, Akola (M.S.), 2003. www.gayatri_magneto.com
42. Rathod PK. Impact of feeding mulberry tips to chawki worms on economical traits of *Bombyx mori* L. M.Sc. (Agri.) thesis (Unpub.), Dr. PDKV, Akola (M.S.), 2001.
43. Sarangi SK. Studies on midgut protease activity during fifth instar development of the silkworm *B. mori* L. Entomon. 1986; 11(3):165-169.
44. *B. mori* L. Ind. J. Seric. 29(4):7-11.
45. Sowri DMK, Sarangi SK. Changes in protein and reducing sugar content of the haemolymph during V instar development of *B. mori* L. Bull of Ind. Acad. of Seric, 2002.
46. Tiwari HV. Comparative performance of some improved mulberry cultivars on larval growth and cocoon yield of silkworm, *B. mori* L. M.Sc. (Agri.) thesis (unpub.), Dr. PDKV, Akola (M.S.), 1995.
47. Udinstev NA, Moroz VA. Function of hypophysical adrenal system under the effect of power frequency variable magnetic field of different regimes. Gig. Tr. Prof. Zabol. 1982; 12:54-56.
48. Ullal SR, Narsimhanna. Mulberry leaf, in hand Book of Sericulture, Pub. By Central Silk Board, Banglore. 1987, 7-37.
49. Upadhyay VB, Tripathi SK. Magnetization of eggs influences the reproductive potential of multivoltine mulberry silkworm. *Bombyx mori* L. Seridoc. 2005; 44(2):212-214.
50. Venkatramu BV. Nutrient status of different varieties and its effect on growth and development of *B. mori* L., M.Sc. (Agri.), thesis (unpub.), Univ. of Agril. Sci., Banglore (Seridoc. 1988), 1987.
51. Watanabe H, Nagata M, Wang YX. The effect of protein content of an artificial diet on protease and antiviral activities of the gut juice of the silkworm, *B. mori* L. (Lepidoptera: Bombysdas). Appl. Ento. And Zool. 1990; 25(1):150-151.
52. Xu G, Bull DL. Biochemical properties of esterases in pyrethroid resistant and susceptible strains of the horn fly (Diptera: muscidae). J. Econ. Ento. 1995; 88(5):1186-1191.
53. Young W. Magnetic field and in-situ acetylcholine esterase in the vagal heart system. In: Biological effect of Magnetic Field, Barnothyet (Ed.), Plenum Press. 1969, 79-102.