Effect of temperature variation on Acid- and Alkaline phosphates enzyme in developing silk gland of *Bombyx mori* Linn.

S. K. Gupta[1], K. P. Gaur[2] and R. K. Dubey[3]

1- Assistant Professor, Department of Zoology Govt. Degree College, Barakhal, Santkabirnagar-272271 U.P
2- Silkworm Laboratory, Department of Zoology, D.D.U Gorakhpur University, Gorakhpur-273009.U.P
3- Assistant Professor, Department of Zoology, Govt. P.G. College, Chunar Mirzapur- 231304 U.P. (INDIA)

Abstract:

The present study reports to evaluation of effect of temperature on the acid and alkaline phosphates in the silk gland of fifth instars larvae. Variation in temperature significantly ($P<0.01$) influenced the acid phosphates content of silk glands of multivoltine race of *Bombyx mori* and alkaline phosphates content was also considerably. The maximum level of acid phosphates is 5.25µg/mg and alkaline phosphates are 5.83µg/mg was found at 26±1ºC, while the minimum 3.26µg/mg acid phosphates 2.61µg/mg alkaline phosphates values were recorded at 10±1ºC. It appears that in the last phase of fifth instars when the silk glands are ready to synthesize the silk fiber, variation in temperatures alters acid and alkaline phosphates contents in the silk glands of *Bombyx mori.* Thus the 26±1ºC temperature, 12±1 hrs photoperiod and 80±5% RH is most suitable to enhance the activity of acid and alkaline phosphates in tissues and this may be biotechnological tool to boost the sericulture industries. Keeping this view and attempt has been made to study the effect of varying temperature regimes on the acid and alkaline phosphates enzyme activity in the silk gland of larvae of *Bombyx mori* Linn.

Key Words: *Bombyx mori* Larvae, BOD incubator, Rearing Trays, DFLs of egg, Silk gland and acid and alkaline phosphates.

Introduction:

The study on the effect of temperature may provide good understanding of various life processes; therefore a possible ideal ecological model with particular reference to temperature may be formulated for the success of sericulture industry. Being cold blooded body temperature of *Bombyx mori* is variable in accordance with the environmental temperature influencing the developmental process, silk producing potential and biochemical constituent. The secretion of silk is a complex process which involves a chain of enzymatic and biochemical process as a result the level of various chemical constituent like protein, and enzymatic acid and alkaline phosphates may also be influenced due to temperature variation. Thermal acclimation in insect serve as biochemical clock indicating the temperature changes influencing their life cycle and distribution. The ultimate aim of developing sericulture industry for taking heavy production of standard quality of raw silks are making sustainability in World market.

Silkworms require all the ten essential amino acid obtained by mulberry leaves and uses to synthesize silk protein secreted during **(Ramchandra and Rai. 2001).** The acid and alkaline phosphates are from hatching until the spinning stage a steady increase was recorded in the activity of both the enzymes followed with a conspicuous decrease at each molt. During the pupae stage the alkaline phosphates was almost absent, whereas the acid phosphates maintained a high constant value. Increase or decrease of the activity of the enzymes during larval development was reflected in a decrease or increase in the acid soluble phosphorus content. Acid phosphates activity slowly increase from laying of the eggs to hatching of the larvae with a concomitant decrease in the acid

soluble phosphorous. Tissue analysis showed a high concentration of the alkaline enzymes in the intestine but the haemolymph was almost frees both enzymes. Feeding of inorganic phosphate increased the alkaline enzymes in the intestines, whereas glucose had no effect on either of the enzymes in the intestines **(S. Sridhar., et al. 1963).** The present communication reports to the effects of temperature on the quantitative estimation of acid and alkaline phosphates enzymes activity in silk gland of fifth instars larvae and Pupae of *Bombyx mori Linn.*

Materials and Methods:

Seed Cocoon: The seed cocoon (Pupa enclosed in silken case) of multivoltine mulberry silkworm *Bombyx mori nistari,* a native of West Bengal in India, were obtained from the silkworm grain age Behraich, Directorate of Sericulture, Uttar Pradesh, India and were maintained in play-wood trays (23 X 20 X 5 Cm) under the ideal rearing condition **(Krishnaswami, S et .al., 1973)** in the silkworm laboratory. The temperature, photoperiod and relative humidity were maintained in BOD incubator at 26±1ºC, 12±1hrs and 80±5% RH respectively till the emergence of moths from the seed cocoons.

Copulation: Moths have a tendency to pairs immediately after the emergence of silken case with releases Bombycol pheromone by female moth thus they allowed with their mates for copulation. A total of 200 pairs each containing one male and one female from newly emerged moths, were allowed to mate at 26±1ºC, 12±1 hrs light and 80±5% RH condition. After four hours of mating, the paired coupled moths were decoupled manually. The male moths were dies after copulation and female dies after oviposition of eggs.

Oviposition: The gravid females laid eggs on the sheet of paper in dark condition at 26±1ºC temperature and 80±5% RH. After 24 hours of egg laying, the female moths were individually in mortar with pestles and blood smears were examined by microscope under magnification of 15X45 for the detection of bacterial, viral, fungal and protozoan pathogens.

The disease free laying (DFLs) thus prepared were treated with 2% formalin for 15 minutes to increased the adhesiveness of eggs on the paper sheet and surface disinfection. Thereafter, the eggs sheet with eggs laid; was thoroughly washed with running water to remove formalin and eggs were dried in shade. The dried eggs thus obtained were taken for various temperature experimental conditions. To observe the influence of temperature on the acid and alkaline phosphates enzymes in fifth instar larvae and pupae of *Bombyx mori.*

The newly DFLs just after primary processing were kept in static variation of temperature 10ºC, 14 ºC, 18 ºC, 26 ºC, 34 ºC, and 38 ºC chronologically in separate groups to BOD incubator maintained at 26±1 ºC temperature, 80±5% RH and 12±1 hrs photoperiod in a day. The incubation of exposed eggs and further rearing of different stages was performed in the same BOD incubators. Acid and alkaline phosphates activity was determined from the tissues of larvae and pupae developed from variation of temperature of eggs. For the estimation of acid and alkaline phosphates activity, silk gland tissue was taken from the fifth instar larvae of salivary gland. The activity of acid phosphates study as fresh homogenate 0.5ml was added to the reaction mixture containing 2ml citric acid sodium chloride buffer PH-4.4, 2ml double distilled de-mineralized water and 0.5ml disodium, p-nitrophenyl phosphates (Sigma substrate 104R) at a final concentration of 1.43mm. The mixture was incubated for 30 min at 38 ºC. The remainder of the procedure was as proceeds by **(Naqvi, Asharafi and Qadari., 1967).** Preliminary experiments had shown that the rate of reaction was linear with respect to enzyme concentration and limited with respect to time. The activity of alkaline phosphates was measured by method of **(Bergmeyer, H.U., 1963 and modified by Singh and Agrawal., 1989).** Alkaline phosphatase catalyses to the hydrolysis of p-nitrophenol phosphates: in phosphoric acid, and p-nitrophenol. Activity of alkaline phosphate is directly proportion to the quantity of p-nitrophenol produced which gives yellow colour with alkali NaOH due to formation of yellow anions. The optical densities were compared with standard which was prepared by different concentration of p-nitrophenol solution. The enzyme activity was expressed in µg p-nitrophenol liberated hours/mg protein at 38 ºC. Each experiment was repeated five times.

Results:

Acid phosphates; Enzyme activity in the silk gland of fifth instar larvae: - The presented in **Table-1 and Fig-1** clearly indicates that variation in temperature influenced the total content of acid phosphates in the silk gland of *Bombyx mori.* With the increasing the increasing temperature from 10 to 26 ºC the acid phosphate content increase from 3.26µg/mg at 10 ºC, 3.58µg/mg at 14 ºC to the maximum level of 5.25µg/mg at 26 ºC. But further increase in temperature above 26 ºC caused steady decline in total acid phosphate level which reached to the level of 4.18µg/mg of silk gland at 34 ºC. At 38 ºC larvae did not survive after fourth instar stage. One way ANOVA test indicates that variation in rearing temperature has significant (P<0.01) influence on the total acid phosphate content in the silk gland of *Bombyx mori.*

Table-1: - Effect of temperature on total acid phosphate (µg/mg) in the silk gland of *Bombyx mori* larvae.

TEMPERATURE (ºC)						F-ratio n_1- 3 n_2- 8
10	**14**	**18**	**26**	**34**	**38**	
3.26	3.58	3.68	5.25	4.18	N.S.D	**490.00**
±0.028	±0.029	±0.031	±0.037	±0.021		

N.S.D= Not survived

Each value represents mean ±S.D of five replicate.

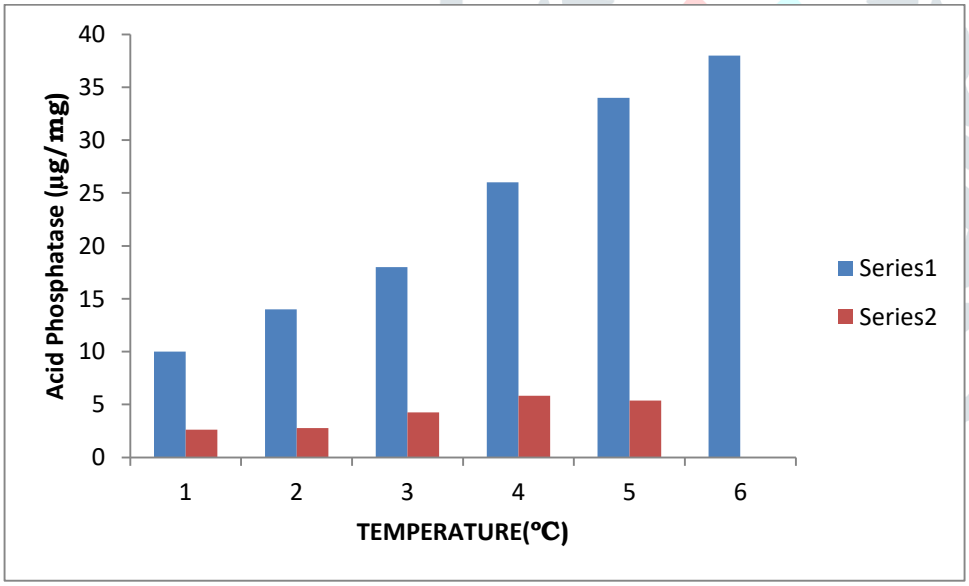

Fig-1: -Effect of temperature on acid phosphate content (µg/mg) in the silk gland of *Bombyx mori larvae.*

Alkaline phosphates; Enzyme activity in the silk gland of fifth instar larvae: - The presented in **Table-2 and Fig-2** clearly indicates that variation in temperature influenced the total content of alkaline phosphates in the silk gland of *Bombyx mori.* With the increasing temperature from 10 to 26ºC the alkaline phosphates content increased from 2.61µg/mg at 10ºC, 2.78µg/mg at 14ºC and 4.25µg/mg at 18ºC to the maximum level 5.83µg/mg at 26ºC. But further increase in temperature above 26ºC caused steady decline in alkaline phosphates level which reached to the level of 5.38µg/mg of silk gland at 34ºC. At 38ºC larvae did not survive after fourth instar stage. One way ANOVA test indicates that variation in rearing temperature has significant (P<0.01) influence on the alkaline phosphate content in the silk gland of *Bombyx mori.*

Table-2: - Effect of temperature on total alkaline phosphate (µg/mg) in the silk gland of *Bombyx mori* larvae.

TEMPERATURE (ºC)						F-ratio n_1- 4 n_2- 10
10	**14**	**18**	**26**	**34**	**38**	
2.61	2.78	4.25	5.83	5.38	N.S.D	**322.20**
±0.014	±0.015	±0.029	±0.035	±0.028		

N.S.D= Not survived

Each value represents mean ±S.D of five replicate.

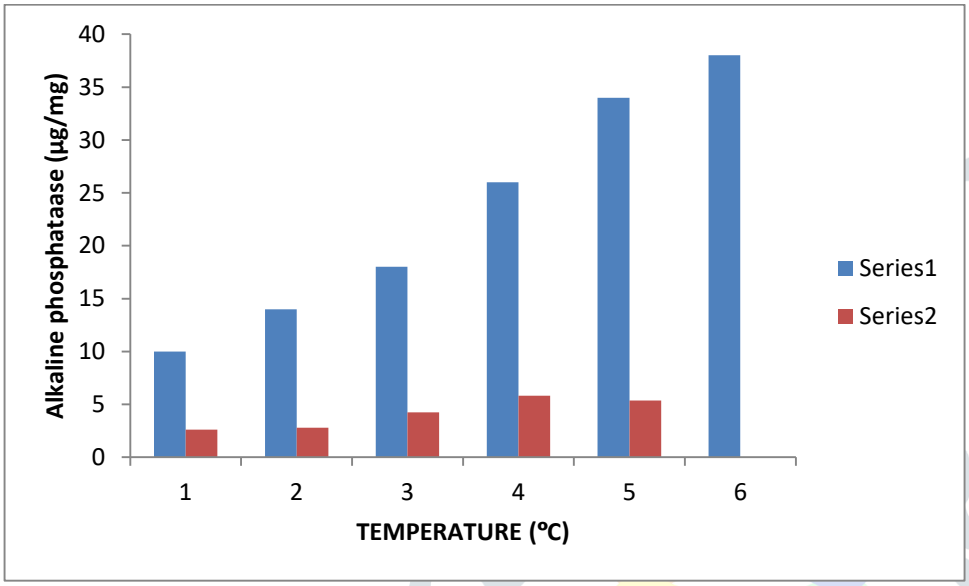

Fig-2: -Effect of temperature on alkaline phosphate content (µg/mg) in the silk gland of *Bombyx mori larvae.*

Discussion:

The acid phosphate was noticed in higher concentration than the alkaline phosphate in the silk gland **(Shridhra, S and J. V. Bhat., 1963)** low magnetic field is responsible for no effect or stimulatory one whereas high magnetic field result in an inhibitory effect **(Mulay, I. L. et al., 1964).** Low magnetic field causes an increase in midgut protease activity in the silkworm while application of higher magnetic field has resulted an inhibition of acetyl cholinesterase activity **(Akoyundoglou, G., 1965).** In present study the activity of acid phosphate is also increased at 26ºC optimum spring temperature while gradually decreased in low and high temperature and at pupal stage the alkaline phosphate enzyme are stop due to stop of feeding and closet the function of metabolic activity. The enzyme activity was greatly inhibited by ferrous chloride sodium fluoride disodium hydrogen phosphate, disodium hydrogen arsenate and cupric chloride and among them ferrous ions caused 100% inhibition. However on the basis of PH for maximum activity 4.4, slight activation by magnesium ion and other values characterized during the present investigation, acid phosphate of silkworm can be placed in category of the scheme of **(Roche. 1950).** The activity of acid phosphate was enhanced while that of the other enzyme got reduced. Acid phosphate occurs in higher concentration than alkaline phosphate in silk glands. The silk glands start the synthesis of silk protein at about 10 day of embryonic life **(Hirano, C. et al, 1967).** They are secreted into the lumen of the gland and the same are spun at the end of each intermoult. The growth of the silk gland cells and the production of silk are stopped at each molting. This continues till the spinning of cocoon begins **(Fitzgerald, L.R., 1950). Mio et al,**

(1989) have supported the above view. There is a heavy accumulation of RNA at the middle of the last instar **(Hirano, C. et al, 1967).** It is also claimed that an increase in the silk production is consequence of an increase in cellular activity **(Eid.et. al, 1989).** Thus at this stage the biosynthetic activity is at their peak and almost all of them are directed towards the silk synthesis. The enhancement in the activity of acid phosphate is optimum at the last instar stage and the same has resulted in an increase in silk proteins and silk production and activity promoted by **(Lim and Zhu, 1985).**

Variation of temperature and exposure of eggs influences to the alkaline phosphates activity in the silk gland of 5th instar larvae of *Bombyx mori.* Alkaline phosphate enzyme is brush boarder enzyme which splits various phosphorus ester bonds at alkaline PH and mediate membrane transport. Enzymatic reaction in the living system is influenced by the variation of temperature and also noticed an enhancement in the metabolic efficiency after application of variate temperature **(Alexander, M. P, 1990).** An activation of some enzyme like carboxymutase and catalos (**Akoyundoglou, G. 1965)** and glutamate dehydrogenase **(Young, W, 1969)** has been obtained by application of magnetic field in in-vitro studies. Variation level of alkaline phosphate activity causes change in commercial traits of silkworm **(kasmaeil, F. G et. al, 2012)** and it involves in active transport glycogen metabolism, secretory activity and synthesis of enzyme participate in protein synthesis **(Geraldine, P. et .al, 1999).** Several factors affect the activity of alkaline phosphate in mid gut of silkworm, such as viral and bacterial infection and administered chemical **(Mio, Y et. al, 1989).** The activity of alkaline phosphate was study during moulting and suggested to either direct or indirect hormoneal control in silkworm **(Karlson, P. et. al, 1959).** Alkaline phosphate actively is gradually increased and become optimum during last instar stage resulting and increased in the silk synthesis due to magnetic exposure larvae **(Chaugle, A. K. et. al, 1993).** The present study also shows an increase in the alkaline phosphate activity due to variation of temperature of eggs in last instar of larvae and pupae of *Bombyx mori.*

Conclusion:

Especially temperature affects the biochemical changes and also affects the cocoon morphology as well as its stiffness and strength, which we attribute to altered spinning behavior and sericin curing time. Biochemical changes affect cocoon coloration, perhaps due to tanning agents. Thus the optimum activity of the acid phosphate and alkaline phosphate during fifth instar stage has a relevance to the process of silk synthesis and its production, particularly during this stage and if the larva is optimum temperature of 26±1ºC there would be a further increase in productivity of silk. Our findings demonstrate environmentally induced quality parameters that must not be ignored when analyzing and deploying silk cocoon, silk filaments or silk derived bio-polymers.

Acknowledgements: The authors thank Prof. Dr.V.B. Upadhyay and Prof. C.P.M. Tripathi Ex. Head Department of zoology, University of Gorakhpur for providing laboratory facilities and linguistic improvement of manuscript, respectively.

References:

[1] Alenxander, M. P and S. Ganeshan, 1990 Electromagnetic field induces in vitro pollen germination and tube growth. Current Science, 59:276-277.

[2] Akoyundoglou, G., 1965. Effect of magnetic field on carboxydimutase. Nature, 202: 452-454.

[3] Bergmeyer, H. U.1963 Methods of enzymatic analysis, Academic Press New York.

[4] Chaugale, A. K. and N. K. More, 1993 Effect of magnetization on acid and alkaline phosphatase in developing silk gland of *Bombyx mori* L.,Entomon, 18(1-2):1-5.

[5] Eid, M., El-Nakkady, A and Saleh, M. 1989 Elongation and amino acid profiles of silk produced by larvae of Philosamia ricini in response to different treatments Indian. J. Seric., 27(2):234-247.

[6] Fitzgerald, L. R., 1950 The alkaline phosphatase of the developing silkworm eggs., J.Exp., Zool., 110: 461-487.

[7] Gerlddine, P., P.S. Bhhawan, J. Kaliamurthy and Z. Zayapragassrazan, 1999 Effect of dichlorointoxicationin fresh water prawn *Macrobranchium malcolmsoni.* Journal Environmental Biologym, 20:141-148.

[8] Hirano, C., and Gilbert, L.I., 1967 Phosphatase phosphohydrolase in fat body of H. cecropia. Comp. Biochem. Physiol., 21:145-149.

[9] Karlson, P. and M. Lususcher, 1959 Pheromones: a New Term for a class of Biologically Active Substance: Nature, 183:55-56.

[10] Kasmaeil, F. G., H. B. Mahesha and P.H. Thejaswini., 2012 Analysis of alkaline phosphatase and its relationship with commercial character of silkworm *Bombyx mori* L. Scholar Research Library, Annals of Biological Research. 3(11):5259-5272.

[11] Krishnaswami, S., M. M, Narasimhanna., S.K, Suryanarayan and K. Raja.,1973 Sericulture mannual2 silkworm rearing F.A.O. Agric. Seroes Bull. Rome, 15(2): 1- 131.

[12] Mulay, I. L. and L. N. Mulay., 1964 Effect on *Drosophila melanogaster* and S-37 tumer cells. Postulate for magnetic field interpretation; in biological effect of magnetic field. M.F. Bombay(ed) Plenum Press, New York.

[13] Mio, Y., L. Jiang and D. Bharti, 1989. Effect of fluoride on; the activity of alkaline phosphatase, adenosine triphosphatase and phosphorylase in midgut of silkworm. Florida Research Report, 38(1): 32-37.

[14] Naqvi, S. N. H. Asharfi, S. H. and Qadari, M.A. H., 1967 Quantitative determination of acid and alkaline phosphatases in different parts of alimentary canal of desert locust, Schistocerca gregaria (Forshal). Pakist. J. Scient. Ind. Res.,10:204-207.

[15] Ramachandra,Y.l., Bali, G and Rai,S.P.,2001 Effect of temperature and relative humidity on spinning behavior of silkworm ., Indian J. Exp. Biol., 39(1):87-89.

[16] Roche, J. 1950 In "the Enzyme". Vol.I. ch.2.pp473-510 (Eds, J.B. Sumner and K. Myrback.) Academic Press, Inc: New York.

[17] Shridhara, S. and J.V. Bhat, 1963 Alkaline and acid phosphatase of silkworm. Journal of Insect Physiology, (:693-701.

[18] Singh, D.K. and R.A Agrawal., 1989 Toxicity of piperanyl butaxide corbaryl synergism on snail *Lymnea accuminata*. International review degesamtem Hydrobilogic, 74:689-699.

[19]Young,W., 1969 Magnetic field and in-situ acetylcholine esterase in vagal heart system; in Biological effect of magnetic field. Barnothyet(ed), pp:79-102. Plenum Press, New York.