

# **A Correlational Study in Changes of Haemolymph Protein Concentration In Certain Bivoltine Silkworm Races of Bombyxmori.Lduring 5<sup>th</sup> Instar Development**

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**ABSTRACT :** Haemolymph protein of the fifth instar larvae of four Bivoltine races of Bombyxmori. L was studied. GEN-3 race showed best performance in haemolymph protein content as well as certain economic characters like cocoon weight (CW), Shell weight (SW) and Shell Ratio Percentage (SR%). Basing upon the above characters the efficiency of the silkworm races was considered. The present study showed that there is a direct relationship established between haemolymph protein with body weight, cocoon weight, shell weight (SW) and shell ratio percentage (SR %). The above characters were important for the selection for hybridization as well as commercial rearing.

**KEYWORDS:-**Haemolymph protein, Bivoltine, Cocoon weight, Shell weight, Shell ratio percentage (SR%), Rearing performance.

## **I. INTRODUCTION**

Haemolymph protein is an important component for the metamorphosis of silkworm. The chemical composition of haemolymph is highly variable among the diverse species and at different developmental stages of the some species (Florkin and Jeuniaux, 1974). It constitutes a variety of nitrogenous substances such as amino acids, proteins, amines, ammonia and peptides. Proteins are among the most complex of all known chemical compounds and also the most characteristic of living organisms. Major changes in protein content occur during development (Agosin, M. 1978; Wyatt, G. 1960). A characteristic feature of insect haemolymph is its high amino acid content. Sometimes it is considered as a taxonomic character for the insect (Florkin, M. 1959).

## **II. RELATED WORK**

Some important proteins, growth hormone such as juvenile hormones are transported through haemolymph lipoprotein in silkworm and locusts (Chino *et. al.*, 1977, 1981 and 1982). Plasma proteins are synthesized in the fat body cell and then secreted into the haemolymph periodically during post embryonic development and metamorphosis. (Tojo *et al.*, 1980, Tomino, 1985, Sakai *et.al.* 1988, Mori *et. al.* 1991a,b, Kishimoto *et.al* 1999).

The haemolymph protein plays an important role in the production of silk in *Bombyxmori* (Denuce, 1958 and Fukuda *et. al.* 1958). The significant increment of protein in the 5<sup>th</sup> instar larvae might be due to synthesis of fibroin protein (Maekawa H and Suzuki Y. 1980) as well as certain stage specific proteins. Considering the above importance of haemolymph in *Bombyxmori.L* the present work was undertaken to understand the variability among races for future rearing programmes.

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Basing on this piece of work many work has been done on the basis of the protein of silk gland in this context, but my piece of work is a bit different that is a correlational study on the concentration of the haemolymph protein of certain bivoltine larvae of the silkworm *Bombyx mori*. L during 5<sup>th</sup> instar development.

It also includes which factors are able to do the concentration change in subsequent varieties of bi and multivoltine also.

### III. MATERIAL AND METHODS

Four Bivoltine races of *Bombyx mori*.L namely GEN-3, BCON-1, DUN-22 and BCON-4 races were collected from different breeding stations of Central Silk Board. For the present work silkworms have been collected from Central Sericulture Research and Training Institute (CSRTI), Berhampore (wb). The races were reared and maintained by adopting the **Krishnaswami**(1978) method. Fifth instar larvae were weighted and sacrificed from the 1<sup>st</sup> day onwards till cocoon formation. The haemolymph was collected in a clean vile containing few thiourea-granules by pricking the abdominal leg just before the last leg. For the estimation of total protein in the haemolymph of silkworm **Lowry's method** (1951) was adopted with a little modification. Besides the cocoon weight, the shell weight and shell ratio percentage were calculated. All the data were subjected to ANOVA using standard statistical procedure and the relationships between two continuous variables was computed. The data's were also subjected to correlation coefficient.

### IV. OBSERVATION

The protein content in the haemolymph increased significantly to its peak on 6<sup>th</sup> day in all the races (Table-1). Beside haemolymph protein progressive significant increments in the body weight were also observed in all the races is a common feature. The present data's showed a significant variation in the quantity of the haemolymph protein was also recorded as in Table-1. The observed data showed strong degrees of positive Correlation between the haemolymph protein and the body weight. However a significant correlation was also exists between the cocoon weight and shell weight.

**Table-1 : Comparison in the Concentration of Protein (mg/ml.) in the Haemolymph of some Bivoltine Silk worm Races, *Bombyx mori* L during 5<sup>th</sup> instar development**

Vth Instar Duration	BIVOLTINE RACES			
	GEN-3 MEAN ± S.E.M	BCON-1 MEAN ± S.E.M	DUN-22 MEAN ± S.E.M	BCON-4 MEAN ± S.E.M
1 <sup>st</sup> Day	9.027 ± 0.005	8.215 ± 0.004	7.911 ± 0.043	7.201 ± 0.008
2 <sup>nd</sup> Day	9.23 ± 0.006	9.027 ± 0.012	8.317 ± 0.018	8.012 ± 0.009
3 <sup>rd</sup> Day	20.083 ± 0.046	15.113 ± 0.015	15.52 ± 0.018	14.808 ± 0.034
4 <sup>th</sup> Day	25.053 ± 0.022	29.617 ± 0.013	29.211 ± 0.014	28.229 ± 0.024
5 <sup>th</sup> Day	27.284 ± 0.004	32.356 ± 0.043	31.139 ± 0.005	30.53 ± 0.016
6 <sup>th</sup> Day	39.76 ± 0.007	38.137 ± 0.032	37.427 ± 0.008	36.514 ± 0.025



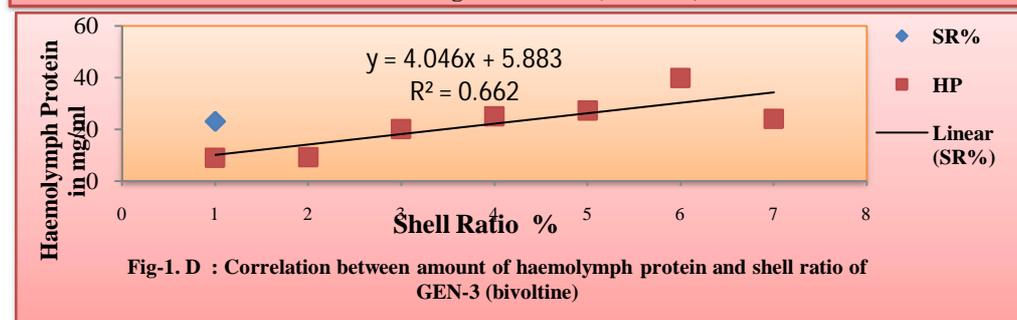
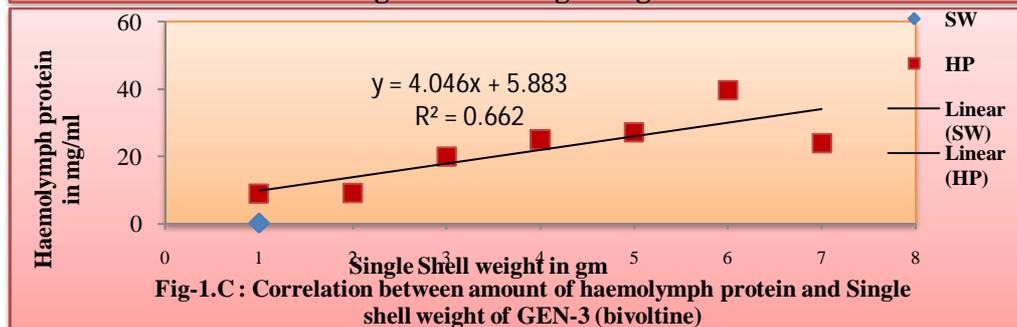
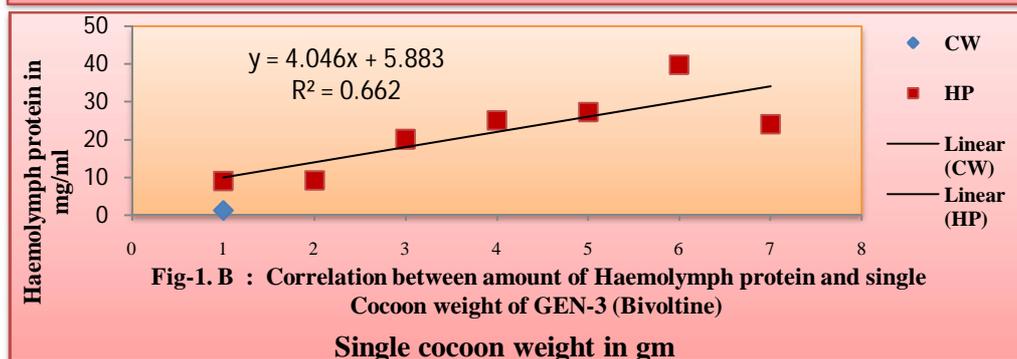
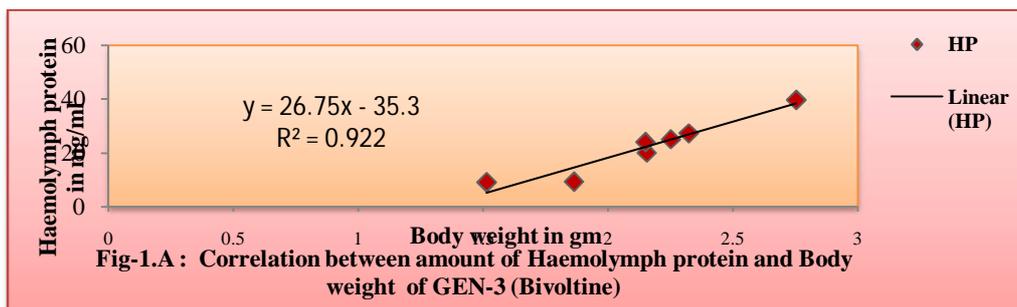
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occurs during the larval feeding stage. The mature larvae contain high protein level in haemolymph. The storage proteins synthesized during the feeding stage of last instar larvae are utilized as an energy and amino acid source for protein synthesis during metamorphosis. (Thompson, J. 1975; Wyatt H. G. and Pan, M., 1978).



During metamorphosis the haemolymph protein of insects indicates a quantitative differences ( Laufer, 1960 ; Buckmannet al., 1966 ; Chippendale and Beck, 1966 ; Pantelouris and downer, 1969 ; Colin, 1969). The specific

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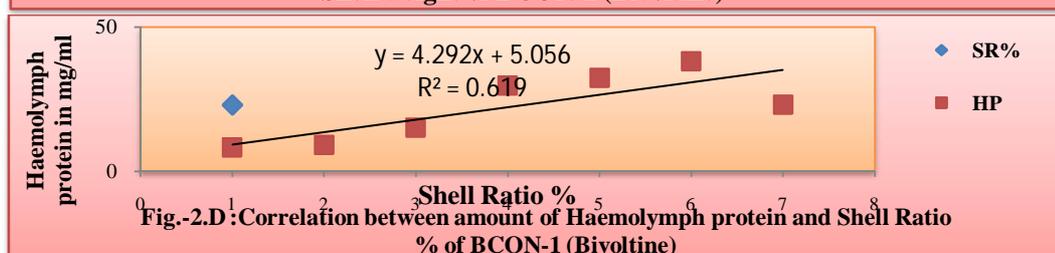
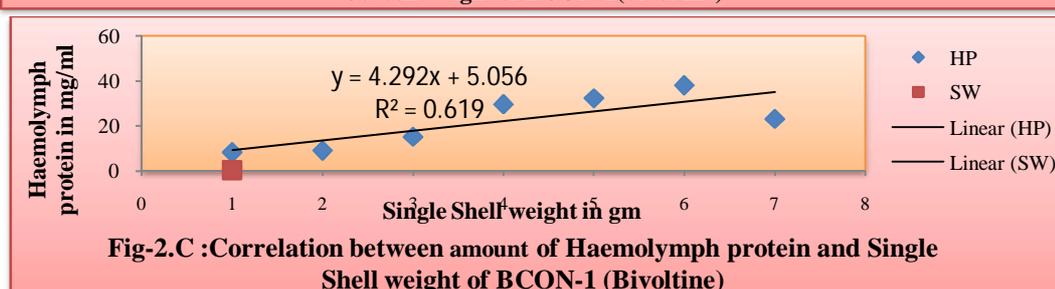
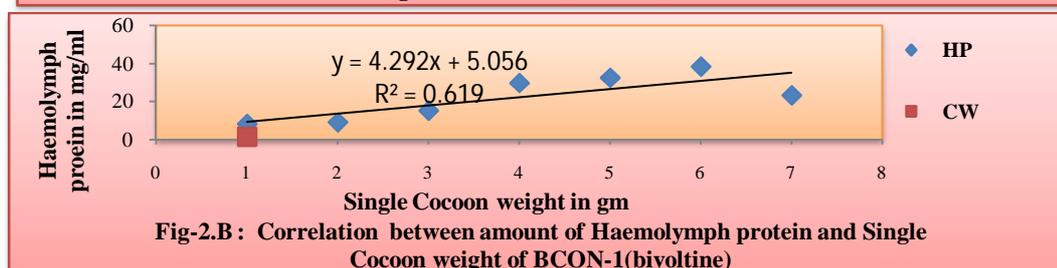
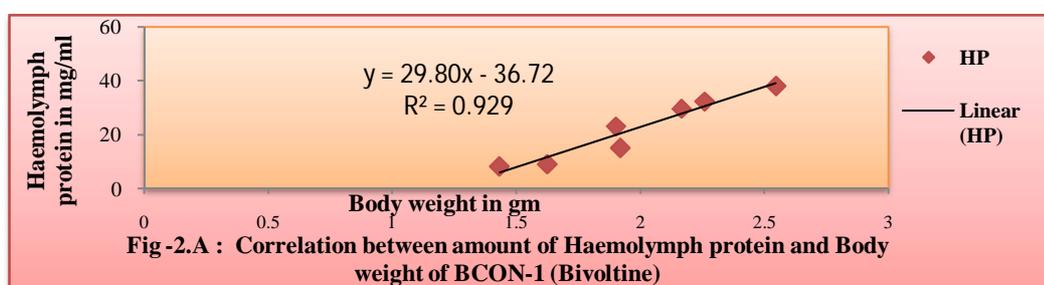
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haemolymph protein are sequestered in the fat body (Chippendale, G., 1973b, Thomposon, J. 1975; R. Dean *et al.*,1985). Such type of *Bombyxmori.L* races may be said as better nutrigenetic trait in silkworm. The silkworm *Bombyxmori.L* feeds exclusively on the mulberry foliage for its nutrition and the larvae obtain its nutrients from mulberry leaves to build up body, sustain life, spin cocoons and egg production. Such nutritional requirement in food consumption has direct impact on the overall genetic traits such as larval and cocoon weight, amount of silk production pupation and reproductive trait.

The present data on Haemolymph protein as in Table-1, showed a significant variations among different bivoltine (Table-2) races of silk worm and different days also.



Such variation in the quantity of Haemolymph protein among the races might be due to their nutritional requirement in different races of *Bombyxmori.L*. Such variation probably reflect the balance between the synthesis, storage, transport and degradation of structural and functional protein during ontogeny as well as a response to particular ecological and physiological condition.(Florquin and Jeuniuax, 1974).

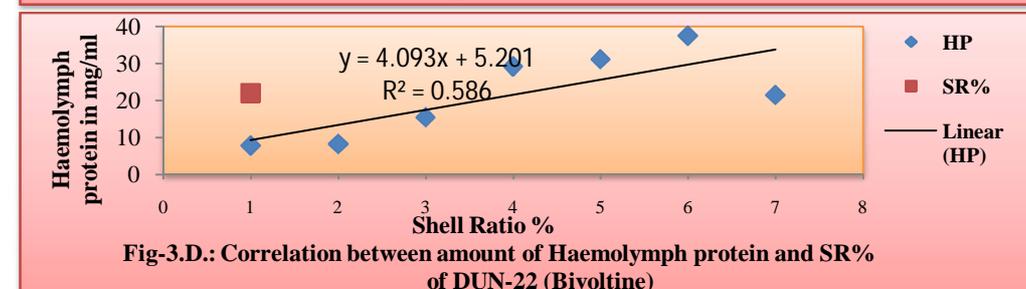
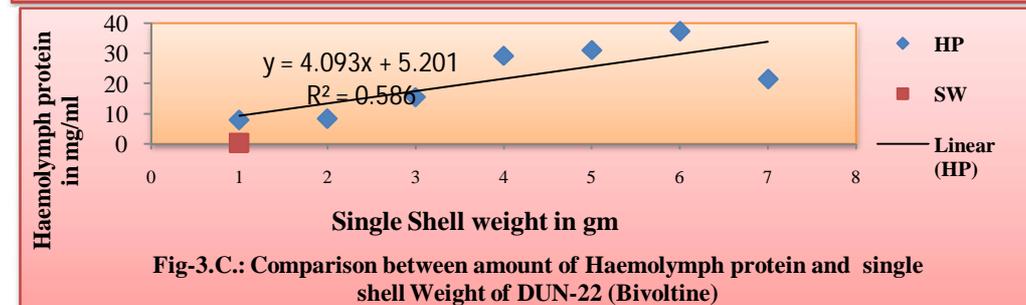
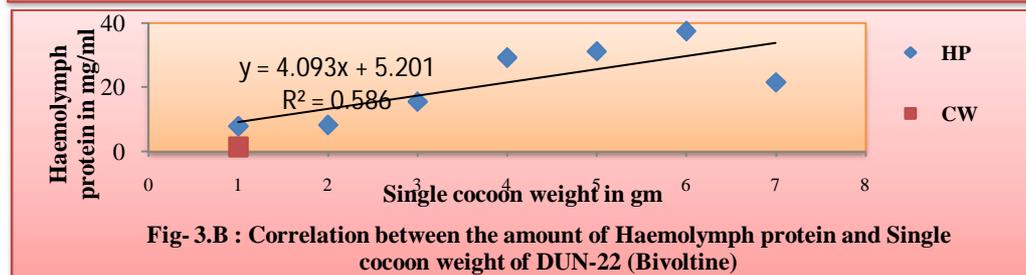
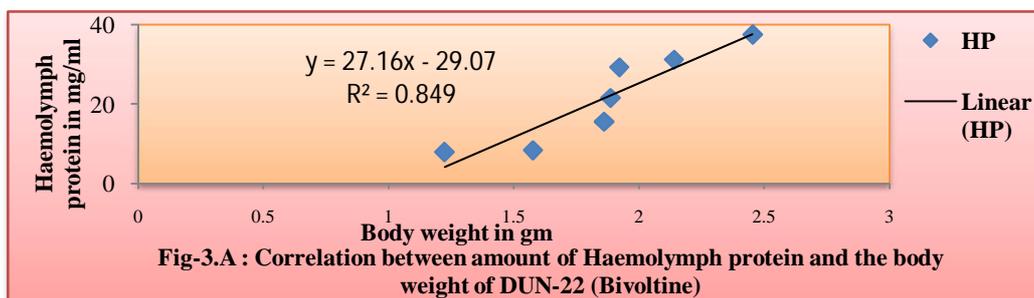
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The highest recorded value in the concentration of haemolymph protein in GEN-3, may be due to active synthesis of protein and their release in to the haemolymph (Nuguta and Kobayashi, 1990) have shown an increase in the protein structure during feeding phase. The increased value of protein content in bivoltine and multivoltine races might be due to higher feeding activity of bivoltine races (Ramadeviet. *al* 1992) than multivoltine races.



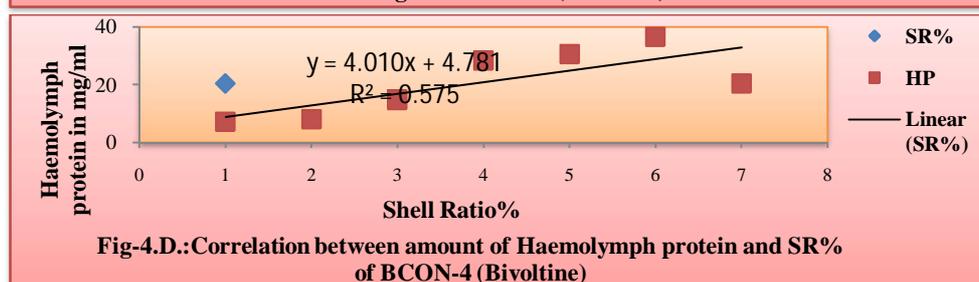
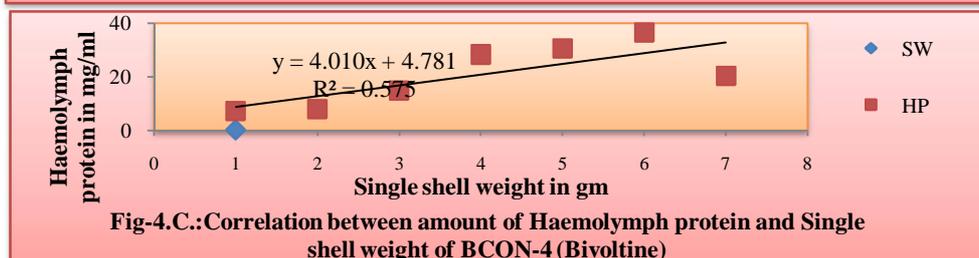
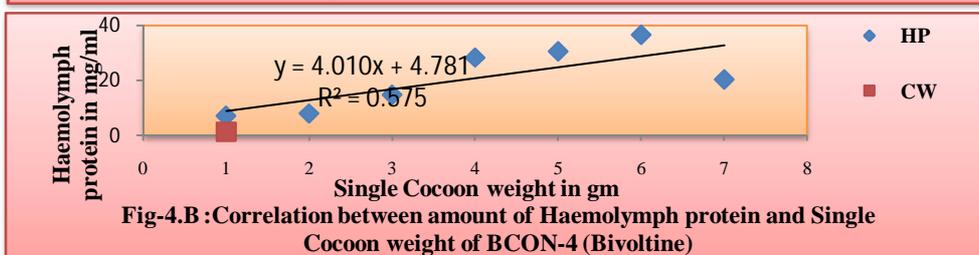
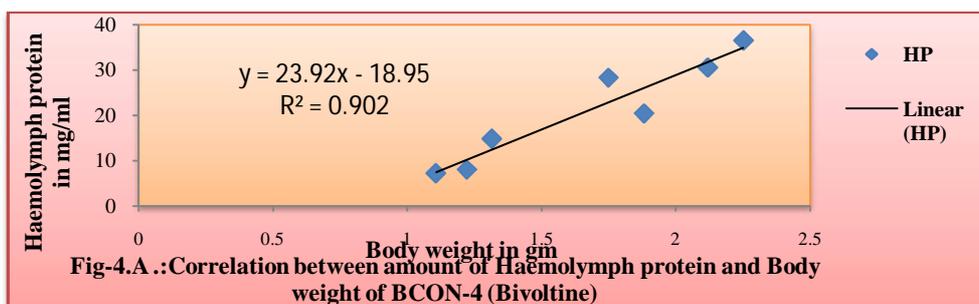
The present results clearly indicated that the total haemolymph protein has positive correlation with certain selected commercial characters like cocoon weight and shell weight.

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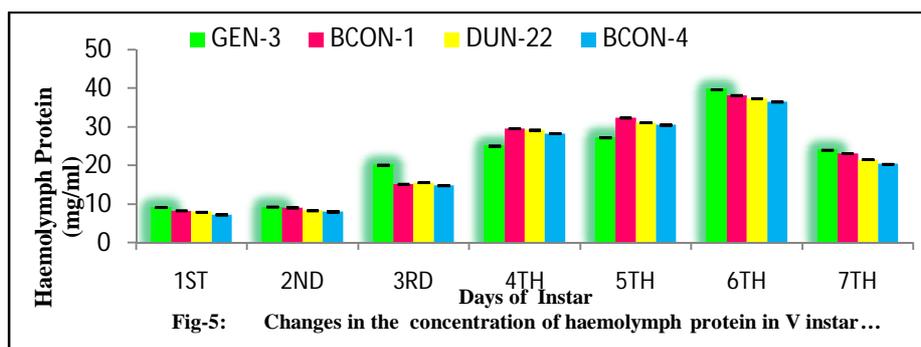
Haemolymph protein increased to its peak on the 6<sup>th</sup> day of 5<sup>th</sup> instar period as in the Table: - 1, is due to accumulation of many stage specific proteins. It has been reported in *Bombyxmori.L* that the 30K protein (Izumi et.al. 1981) and the major haemolymph protein (Plantevin et. al.1987) appeared during the last instar development. In *Bombyxmori.L* a group of structurally related proteins, termed 30K accumulated in the haemolymph of the last instar larvae. The 30K proteins are mainly synthesized in fat bodies after juvenile hormone disappearing from the third (3<sup>rd</sup>) day of the 5<sup>th</sup> instar, and then mainly discharging to haemolymph. It is presumed, therefore that the 30K proteins may be the source of amino acids for the synthesis of other functional proteins during embryogenesis (Zhong, B. X.,1999). According to few authors metabolism of 30K proteins in a pre-requisite for the embryos normal development (Zhong B.X. et.al. 2005). These proteins also have properties like anti apoptotic and defence beside embryogenesis.

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Correlation studies (Table:-2) performed to establish the relationship between haemolymph protein, body weight, shell weight and SR%.

The degree of association or relationship between two variables is measured by correlation coefficient ( $r$ ). The correlation coefficient may be positive or negative. Positive correlation indicates that the two variables are varying in the same direction. If one variable increases, the other variable is also increase. In negative correlation the two variables vary in opposite direction. If one variable increases other variable decreases. Correlation between different pairs of quantitative characters has been studied as in Table:-2. The data revealed high significant ( $p < 0.001$  level) positive value between haemolymph protein concentration and body weight and also between haemolymph protein, Cocoon weight, Shell Weight and SR% as in Fig-1.A up to 4.D.

## VI. CONCLUSION

The present work on haemolymph protein showed an understanding of larval development during fifth instar larval period. The work is aimed to assess variations among bivoltine races basing on the amount of haemolymph protein through some light to know the genetic variability among races. It also helpful to setup hybridization for selection processes and breed the best.

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