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Photo Periodically Induced Changes in Haemolymph Protein of Selected Mulberry Silkworm Races /Breeds

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Abstract: In the present study, the changes in haemolymph protein in different days of larval development during 5th instar. The popular pure races of the silkworm namely, *C.nichi*, Pure Mysore (Multivoltine) and NB₄D₂ (Bivoltine) races were selected for the imposed longer photoperiodic schedules. The haemolymph protein was increased during different days of larval growth period in 5th instar larvae of *C. nichi*. At 28^oC, the haemolymph protein showed a consistent increase in their level for the first five days and negative change or lesser increase on 6th and 7th day. Pure Mysore also showed similar pattern of changes in haemolymph protein though the actual level of increase in different photoperiod and temperatures. Pure Mysore had revealed haemolymph protein than *C. nichi* at any stage of growth and also an increase in haemolymph was observed till 6th day after which a fall in haemolymph protein was observed. In NB₄D₂, the haemolymph protein was higher in control than Pure Mysore. The level of increase was observed in haemolymph protein of NB₄D₂ were relatively higher at 15^oC than the other two races and more so at 28^oC especially in mid growth period of 5th age. In longer photoperiods, the combined effect of photoperiod and temperature was more evident at 15^oC in longer photoperiods in *C. nichi*. The haemolymph protein showed higher increase in all photoperiods at all temperature, the increase observed being more at 15^oC and much higher at 28^oC. The combined effect of photoperiod and temperature in Pure Mysore, longer photoperiods tend to stimulate greater increase in haemolymph protein, the increases becoming relatively higher from 2nd day onwards and higher temperature producing greater increase in haemolymph protein. NB₄D₂ responded in a similar manner as *C.nichi* and Pure Mysore, the haemolymph protein showing greater increases in control than other two multivoltine races. The level of haemolymph protein were comparatively more and significant in NB₄D₂ than *C. nichi* and Pure Mysore. Longer photoperiod showed a relatively more at both the temperature regimes till day of spinning but a significant fall in the haemolymph protein was observed during spinning activity.

Keywords: Haemolymph protein, photoperiod, silkworm breeds, temperature.

I. INTRODUCTION

Life on the earth is obviously exposed to encounter an extraordinary variety and ranges of seasonal conditions. Insects are not an exception to it. Insects are adopted to such conditions to tide over the unfavorable environment by entering into state of suspended animation (quiescence) called diapause (Wheeler, 1893). The life cycle of insects includes four different stages of development such as egg, larva, pupa and imago. The diapause may be confined to any one stage of development and occurrence of diapause is related to the stages of insect facing unfavorable environment. Diapause, which is characterized by a number of morphological, behavioral, physiological and biochemical features (Beck, 1968 and Lees, 1968), is a widespread form of dormancy among insects and acarines. Its onset, maintenance and termination, the post-diapause development and the subsequent growth, development and reproduction represents distinct phenological episodes in the life history of an insect species.

Embryonic diapause in the silkworm is maternally determined (Toyoma, 1902) and anticipated a generation before commencement of embryonic diapause development. The preparation for commencement of embryonic diapause begins in the embryonic, larval and pupal stages of mother moth destined to lay diapause eggs. The biochemical changes in the haemolymph and tissues like midgut, fat body reflect the pattern of preparation in phase for laying diapausing eggs. There are fragmentary reports on the changes in the biomolecules in the diapausing eggs and different developmental stages in the life cycle. Chandrashekar and Geethabali (1986) reported deposition of more glycogen content in diapausing eggs of bivoltine races which serves as a sustained source of energy for prolonged embryonic stage in diapausing eggs.

Yamashita *et al.* (1975) reported that, the higher glycogen content in diapausing eggs is due to mobilization of fat body glycogen as haemolymph trehalose which is taken up by ovarian trehalase and glucose formed in the ovarian capsule is transformed to ovarian glycogen and deposited in the diapausing eggs. Irie and Yamashita (1980) observed that yolk protein supports 60% of the embryonic development and contributed by haemolymph vitellin.

Hence, in the present study, protein changes in the haemolymph of larval development during 5th instar were estimated to understand the rate of synthesis of the silkworm, *B. mori*. L.

II. MATERIALS AND METHODS

The popular pure races of silkworm namely, *C.nichi*, Pure Mysore (Multivoltine) and NB₄D₂ (Bivoltine) races were selected for the present study. The stocks of above mentioned races were maintained under standard laboratory conditions for utilization of selected age of the silkworm. The healthy silkworm larvae are selected for observational studies in haemolymph of the above said races.

Maintenance of silkworm races: Two disease free layings of three races of the silkworm namely *C.nichi*, Pure Mysore and NB₄D₂ each about 350 – 500 eggs were obtained from Germplasm Bank, Department of Studies in Sericultural Science, Manasagangotri, Mysore. The eggs were incubated at 25±1°C temperature and 80 – 85% relative humidity for about 10 – 12 days till their hatching. Wooden trays of 90 x 70 x 10 cm size were washed with water, dried and disinfected with 2 – 4% formalin solution and covered with paraffin paper of the same size. The silkworm layings were black boxed at blue egg stage in order to obtain uniform hatching and the hatched larvae on 10th day were brushed into labeled wooden trays. The silkworm larvae were reared under standard rearing conditions on four feeding schedules (Krishnaswami, 1978). Mulberry leaf of M₅ variety was obtained from the garden of Sericulture Department and selected leaves chopped into suitable size according to stage of larvae fed to the silkworm. The silkworm bed was cleaned after II moult and thereafter every day. Healthy silkworms were maintained till day of commencement of spinning. The ripened worms were allowed to spin the cocoons on bamboo mountages. The cocoons were harvested on 5th day of spinning and selection of cocoons was made on following day and preserved in wooden trays. The moths emerged on 10th to 12th day were allowed for pairing for about 4 hours. The depaired females were retained in the cellules on craft brown paper for oviposition. The egg sheets were disinfected by dipping in 2% formalin solution and dried after washing in running tap water at room temperature. The female moths, after oviposition were subjected to pebrine detection by mother moth examination. The layings of NB₄D₂ breed were acid treated within 24 h of oviposition to prevent diapause development, and to continue the embryonic development. The layings are then exposed to different combinations of photoperiod and temperature to favour diapause development in *C.nichi* and PM and to prevent it in NB₄D₂ breed

Characterstic features of selected silkworm races / breeds

C. nichi: *C.nichi* is basically bivoltine race imported from Japan and introduced in Karnataka during early part of 20th century. It has acclimatized to local environment, due to continuous rearing in tropical climatic condition. It is behaving like multivoltine race and less productive. This race is characterized by slender larvae, spinning dumbbell shaped white cocoons and laying non-diapausing egg. The larval duration is shorter and it will take 22-23 days to spin the cocoon, each cocoon weighing about 0.9 to 1.0 g. The shell weight ranges from 0.10 to 0.15 g with shell ratio of 10-11%.

Pure Mysore: Pure Mysore is a multivoltine race indigenous to southern states of India and its origin is traced to China (Charsley, 1982). Ever since introduction into erstwhile Mysore State in the late 18th century, PM has acclimatized to local environment (high temperature and low humidity) poor hygiene and leaf quality. It is used as female component in the preparation of commercial hybrid layings in Karnataka, Tamil Nadu and Andhra Pradesh states. The race is characterized by plain larvae of slender size, spinning greenish yellow spindle shaped cocoons. It lays non-diapausing eggs and hence it could be reared in all seasons of the year. The larval period is longer and it will take 28 – 30 days to spin the cocoon. The single cocoon weight ranges from 1.0 to 1.10 g having 18 – 20% of floss with a shell ratio of 10 – 12%.

NB₄D₂: NB₄D₂ is a tropical bivoltine race and is an inbred line derived from Japanese double cross hybrid (Kokko x Seihaku) x (N124 x J124). This race was evolved in 1971 at CSR & TI, Mysore (Krishnaswami, 1978) and currently in use because of its better viability traits as a pure race and combining ability with PM. This race is characterized by long and plain larvae, spinning dumbell shaped white cocoons. The total larval duration is 23 – 25 days. The eggs are of diapause type, each cocoon weighing about 1.8 to 2.0 g. The shell weight ranges from 0.38 to 0.40 g with shell ratio of 16 to 18% and floss content of about 4%.

Schedules of Photoperiod and Temperature regimes

The experiment was designed and conducted to identify the sensitive stages in life cycle to environmental stimuli by exposing all the stages of development to selected constant temperature and light (L) and dark (D) conditions. The eggs and early larval stages were then exposed to selected LD cycle and temperature conditions as follows. The silkworm larvae from the same genetic stocks are divided into two batches, with enough number of larvae for experimentation. The first batch of eggs and early larval stages of all the races namely *C.nichi*, PM and NB₄D₂ were reared under normal rearing condition as control and the second batch of eggs and early larval

stages were exposed to a constant high temperature of 15 and 25 $\pm$ 1°C and long day length schedules of LD 12:12, 14:10, 16:8 and 18:6 h of LD cycle at 80% relative humidity. Rearing of the silkworm in both the experiments from 3rd instar onwards was done till spinning under natural photoperiod of LD 12:12 at 25 $\pm$ 1°C and 80% relative good. Cocoons were harvested and layings were prepared and nature of eggs (non-diapausing or diapausing) was observed.

III. RESULTS

Table: 1. Changes in haemolymph protein (mg/ml) during 5th instar in *C.nichi* exposed to long photoperiod and temperature regimes.

Days	LPP	15 ^o c		28 ^o c	
		Mean $\pm$ SD	% Change	Mean $\pm$ SD	% Change
1	12:12	19.33 $\pm$ 0.45	+12.96	19.77 $\pm$ 0.61	+15.57
	14:10	19.66 $\pm$ 0.13	+14.92	20.44 $\pm$ 0.71	+19.46
	16:8	20.33 $\pm$ 0.50	+18.81	22.10 $\pm$ 0.80	+29.19
	18:6	19.47 $\pm$ 1.16	+13.84	21.85 $\pm$ 0.49	+27.72
2	12:12	22.13 $\pm$ 1.38	+9.53	23.11 $\pm$ 2.03	+14.38
	14:10	23.36 $\pm$ 1.57	+15.65	27.22 $\pm$ 1.31	+34.75
	16:8	24.59 $\pm$ 0.69	+21.69	27.11 $\pm$ 1.15	+34.17
	18:6	24.59 $\pm$ 0.55	+21.71	25.21 $\pm$ 1.16	+24.77
3	12:12	33.24 $\pm$ 2.46	+26.41	35.36 $\pm$ 1.48	+34.46
	14:10	35.39 $\pm$ 1.54	+34.57	34.63 $\pm$ 3.01	+31.70
	16:8	36.14 $\pm$ 0.12	+37.42	38.13 $\pm$ 0.14	+44.97
	18:6	35.42 $\pm$ 0.50	+34.68	37.21 $\pm$ 0.31	+41.49
4	12:12	42.99 $\pm$ 0.24	+43.72	43.64 $\pm$ 0.53	+45.89
	14:10	40.35 $\pm$ 2.55	+34.91	42.25 $\pm$ 1.33	+41.25
	16:8	43.85 $\pm$ 0.51	+46.61	45.76 $\pm$ 1.49	+52.99
	18:6	42.32 $\pm$ 1.46	+41.47	43.82 $\pm$ 0.54	+46.49
5	12:12	46.97 $\pm$ 2.23	+41.87	49.07 $\pm$ 0.04	+48.21
	14:10	46.83 $\pm$ 2.55	+41.46	48.26 $\pm$ 0.96	+45.76
	16:8	50.07 $\pm$ 1.10	+51.22	52.50 $\pm$ 1.88	+58.56
	18:6	49.84 $\pm$ 0.78	+50.53	51.25 $\pm$ 0.28	+54.81
6	12:12	51.35 $\pm$ 0.98	+29.46	52.89 $\pm$ 1.06	+33.33
	14:10	40.43 $\pm$ 0.47	+1.92	50.01 $\pm$ 0.02	+26.08
	16:8	41.59 $\pm$ 0.96	+4.86	43.34 $\pm$ 1.88	+9.26
	18:6	40.13 $\pm$ 0.05	+1.17	42.65 $\pm$ 0.72	+7.52
7	12:12	39.65 $\pm$ 5.18	+25.34	42.70 $\pm$ 1.45	+34.97
	14:10	Spinning			
	16:8	Spinning			
	18:6	Spinning			

(Each value is the mean of four individual observations)

Fig.1a: Per cent changes in haemolymph protein of *C.nichi* at 15^oC and LPP schedules

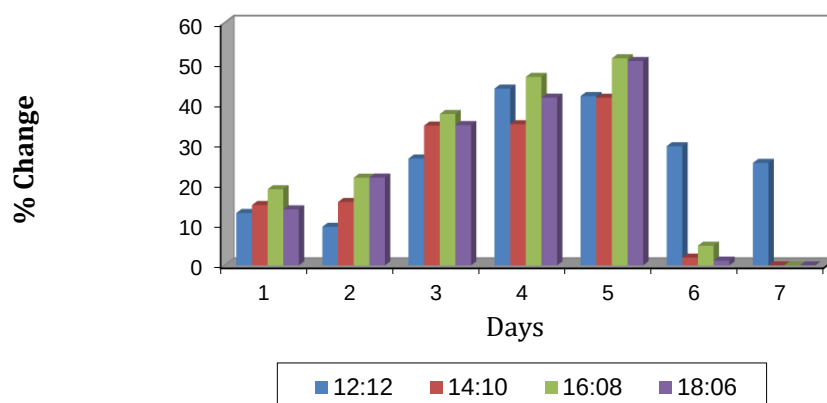


Fig.1b: Per cent changes in haemolymph protein of *C.nichi* at 28°C and LPP schedules

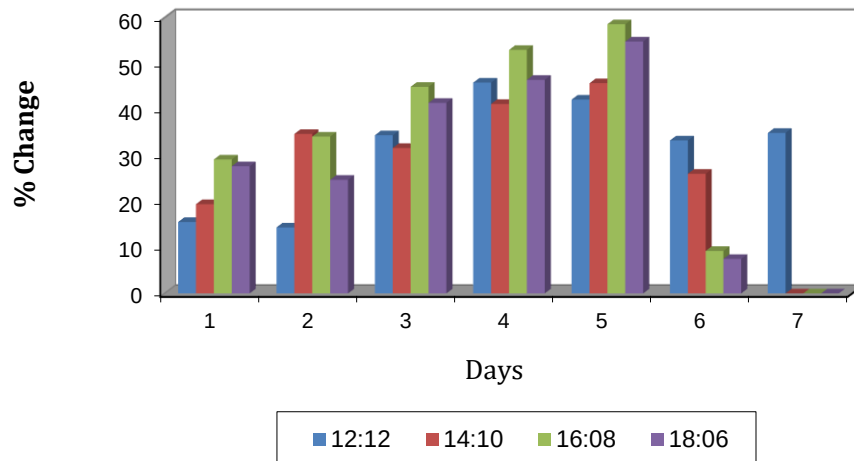


Table: 2. Changes in haemolymph protein (mg/ml) during 5th instar in Pure Mysore exposed to selected long photoperiod and temperature regimes
(Each value is the mean of four individual observations)

Days	LPP	15°C		28°C	
		Mean $\pm$ SD	% Change	Mean $\pm$ SD	% Change
1	12:12	20.54 $\pm$ 0.57	+4.82	24.10 $\pm$ 0.09	+22.94
	14:10	23.18 $\pm$ 0.19	+18.28	23.70 $\pm$ 0.46	+20.96
	16:8	20.84 $\pm$ 0.98	+6.33	22.10 $\pm$ 0.02	+12.74
	18:6	21.60 $\pm$ 0.62	+10.21	24.17 $\pm$ 0.65	+23.29
2	12:12	30.75 $\pm$ 0.51	+37.81	30.70 $\pm$ 0.42	+37.60
	14:10	30.38 $\pm$ 0.21	+36.15	31.25 $\pm$ 0.28	+40.07
	16:8	28.34 $\pm$ 0.51	+27.04	32.00 $\pm$ 1.23	+43.43
	18:6	31.12 $\pm$ 0.07	+39.48	32.27 $\pm$ 0.81	+44.64
3	12:12	41.13 $\pm$ 0.81	+49.92	39.78 $\pm$ 0.94	+45.02
	14:10	40.81 $\pm$ 0.46	+48.76	43.77 $\pm$ 2.45	+59.96
	16:8	36.41 $\pm$ 0.47	+32.75	43.61 $\pm$ 0.93	+59.98
	18:6	42.73 $\pm$ 0.70	+55.78	45.27 $\pm$ 0.26	+65.04
4	12:12	45.08 $\pm$ 0.08	+30.20	45.99 $\pm$ 0.70	+32.84
	14:10	18.42 $\pm$ 0.26	+39.85	48.95 $\pm$ 0.22	+41.39
	16:8	47.34 $\pm$ 0.20	+36.72	51.35 $\pm$ 0.49	+48.32
	18:6	49.58 $\pm$ 1.06	+43.20	51.74 $\pm$ 1.09	+49.45
5	12:12	50.41 $\pm$ 0.54	+29.02	50.62 $\pm$ 0.58	+29.52
	14:10	51.34 $\pm$ 0.51	+31.38	56.47 $\pm$ 1.93	+44.49
	16:8	52.39 $\pm$ 0.43	+34.07	54.34 $\pm$ 0.46	+39.04
	18:6	53.03 $\pm$ 0.66	+35.71	55.71 $\pm$ 1.07	+42.55
6	12:12	56.11 $\pm$ 0.11	+30.46	58.29 $\pm$ 0.25	+35.55
	14:10	57.63 $\pm$ 1.75	+34.01	60.63 $\pm$ 0.55	+41.00
	16:8	57.65 $\pm$ 0.56	+34.05	62.00 $\pm$ 0.22	+44.18
	18:6	58.22 $\pm$ 0.29	+35.38	63.29 $\pm$ 0.49	+47.18
7	12:12	48.88 $\pm$ 0.52	+21.84	48.54 $\pm$ 0.58	+21.01
	14:10	45.77 $\pm$ 2.76	+14.09	49.52 $\pm$ 0.53	+23.46
	16:8	47.15 $\pm$ 2.00	+17.54	50.19 $\pm$ 0.09	+25.13
	18:6	46.87 $\pm$ 1.28	+16.84	53.47 $\pm$ 3.18	+33.30

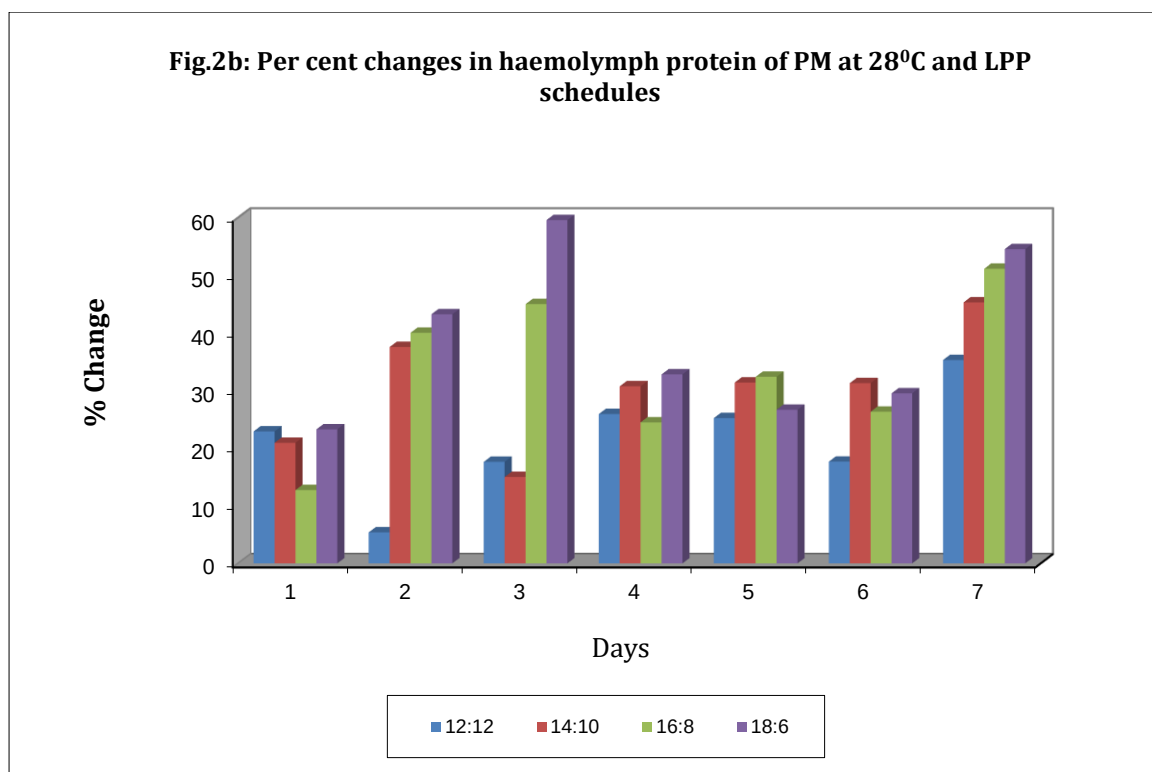
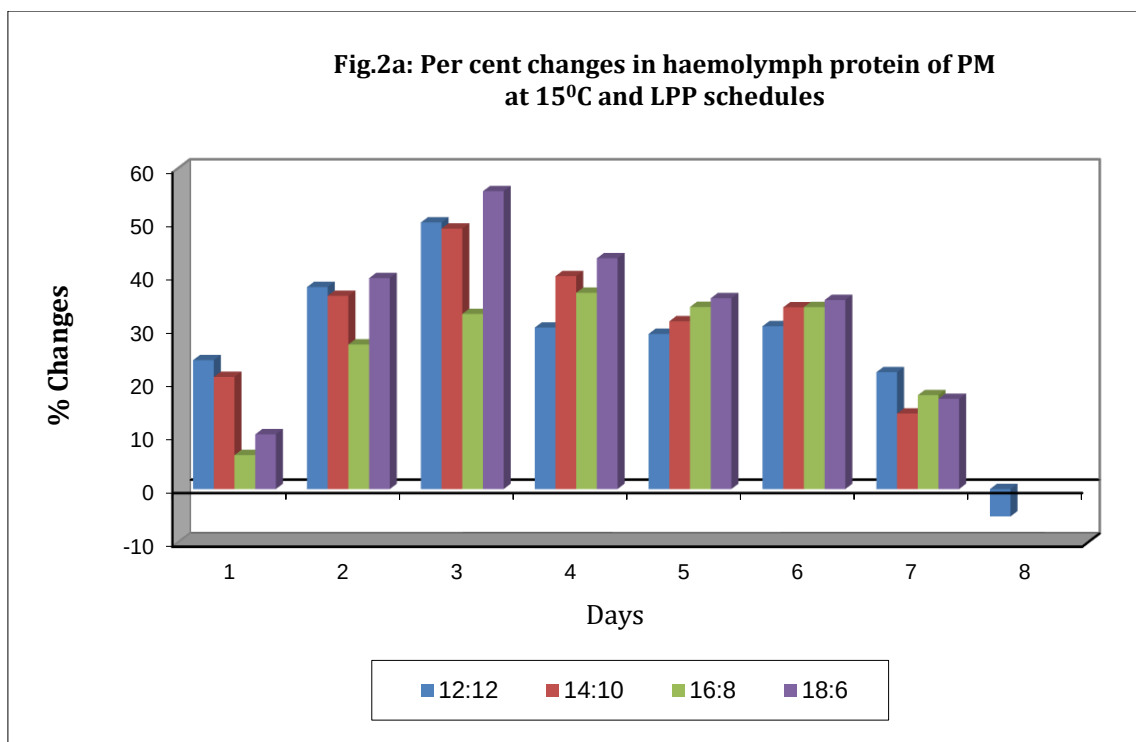


Table: 3. Changes in haemolymph protein level (mg/ml) during 5th instar in NB₄D₂ exposed to long photoperiod and temperature regimes (Each value is the mean of four individual observations)

Days	LPP	15°C		28°C	
		Mean ± SD	% Change	Mean ± SD	% Change
1	12:12	25.04±0.05	+4.04	25.04±0.05	+1.46
	14:10	24.16±0.09	+0.38	24.16±0.09	+0.54

	16:8 18:6	27.84±0.55 25.95±1.45	+15.68 +7.81	27.84±0.55 25.95±1.45	+4.30 -4.14
2	12:12 14:10 16:8 18:6	39.06±0.77 38.31±0.53 40.34±0.46 40.04±1.38	+29.85 +27.33 +34.09 +33.09	39.06±0.77 38.31±0.53 40.34±0.46 40.04±1.38	+28.50 +35.61 +34.16 +21.11
3	12:12 14:10 16:8 18:6	58.01±1.41 52.11±0.00 58.10±0.02 52.55±1.72	+68.44 +51.29 +68.68 +52.58	58.01±1.41 52.11±0.00 58.10±0.02 52.55±1.72	+46.87 +54.96 +69.81 +52.49
4	12:12 14:10 16:8 18:6	61.72±1.10 58.84±0.49 63.31±0.24 62.85±2.61	+53.86 +46.67 +57.82 +56.66	61.72±1.10 58.84±0.49 63.31±0.24 62.85±2.61	+46.18 +67.07 +59.32 +47.58
5	12:12 14:10 16:8 18:6	68.41±0.48 68.90±0.38 70.81±0.54 66.24±0.26	+42.52 +43.55 +47.51 +38.01	68.41±0.48 68.90±0.38 70.81±0.54 66.24±0.26	+38.50 +46.97 +46.78 +32.61
6	12:12 14:10 16:8 18:6	55.08±2.76 57.70±0.93 53.67±2.39 54.57±0.43	+3.70 +8.63 +1.04 +2.74	55.08±2.76 57.70±0.93 53.67±2.39 54.57±0.43	+2.79 +13.25 +4.47 +2.79
7	12:12 14:10 16:8 18:6	48.27±3.50 48.63±0.49 40.40±0.50 51.14±0.12	+27.00 +27.94 +6.28 +34.53	48.27±3.50 48.63±0.49 40.40±0.50 51.14±0.12	+32.58 +40.16 +16.74 +14.46

Fig. 3a: Per cent changes in haemolymph protein of NB₄D₂ at 15°C and LPP schedules

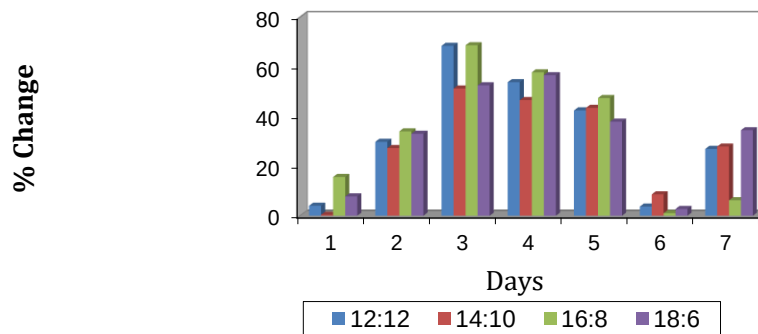
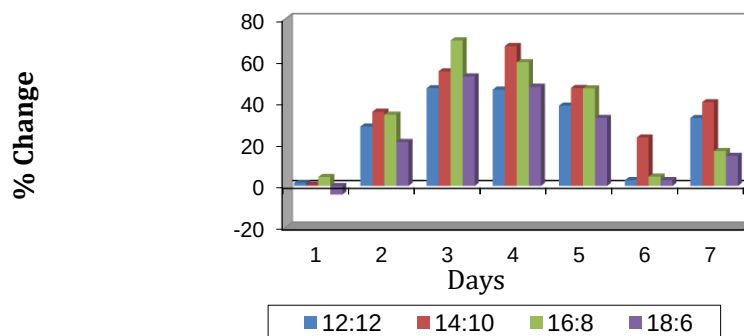


Fig. 3b: Per cent changes in haemolymph protein of NB₄D₂ at 28°C and LPP schedules



IV. DISCUSSION

Insect phenology, particularly with reference to embryonic diapause in the mulberry silkworm, *Bombyx mori* L. plays a significant role in the racial distribution and productivity. A number of studies have been conducted on seasonal progression of diapause and its maintenance and termination (Tauber and Tauber, 1973a), but pre-diapause development leading to a series of vital physiological, biochemical and endocrinological changes affecting the productivity traits of the silkworm and the environmental stimuli that are important for induction, maintenance and termination of embryonic diapause have received much less attention.

The photoperiod and temperature are the two physical factors, which vary predictably during different seasonal progression. In many insects, short photoperiod and low temperature results in embryonic diapause, whereas, in silkworm, long photoperiod and high temperature during embryonic and early stages of larval development induced embryonic diapause in the following life cycle (Kogure, 1933). In the present investigation, the change in the response of three selected silkworm races to different combinations of long photoperiods and low and high temperatures were studied, in order to investigate into the effects of photoperiod and temperature in different combinations on the progression of diapause development by observing the physiological and biochemical changes through the larval stages before the onset or avoidance of embryonic diapause development. four long photoperiods namely LD12:12, 14:10, 16:08 and 18:6 and temperatures at 15°C and 28°C were selected for study, based on the results obtained with relative effects of photoperiod and temperatures. Kulkarni (1997) also studied the influence of photoperiod and temperature stimuli inducing diapause development in Pure Mysore.

The silkworms are exposed to long photoperiods with low or high temperature and treatment is limited to the most sensitive stages of life cycle of insect, namely embryonic development and young age silkworms. The physiological changes induced by the combination of photoperiod and temperature leading to diapause or non-diapause development were observed in the 5th instar stage by which time the destiny of the nature of eggs to be laid could have been decided as ovarian development is completed before emergence. The diapausing tendency is best observed when the two environmental stimuli complement with each other like long photoperiod and higher temperature. In the case of long photoperiod and low temperature again the influence of low temperature predominates nullifying the diapausing tendency of long photoperiod.

In combination of longer photoperiod and higher temperature regimes increase in haemolymph protein than the other combinations of photoperiod and temperature and on some days of larval development a reciprocal relationship exists suggesting interconvert ability of fuel reserves to meet the needs of growth and development in silkworm larvae. Simex and Kodrik (1986) also reported that the route of mobilization of fat body glycogen into ovarian glycogen. The fat body glycogen is converted into trehalose and released into haemolymph. The haemolymph trehalose is taken up by ovary as glucose by ovarian membrane bound to Trehalase. The glucose is then incorporated into egg glycogen and deposited into oocyte. Similarly the haemolymph protein also needs to be mobilized for the utilization of silk gland protein due to imposed photoperiodic induction under controlled condition can modulate level of increased trend in the protein synthesis revealed cellular and sub cellular events of biological status of an organism in the expression and manifestation of the silk production in the mulberry silkworm utilized in the present experimentation were discussed.

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