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GREEN MICRO ALGAL IMPACTS OF *BOMBYX MORI* L. GROWTH REGULATIONS, BIOCHEMICAL ACTIVITIES AND ECONOMIC TRAITS

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ABSTRACT

Enhancing the *Bombyx mori* diet with exogenous nutrients and assessing their effect on larval growth, metabolism and silk production has become the order of traditional research in sericulture. *Chlorella pyrenoidosa* is a single cell green micro alga that contains vitamins, minerals, dietary fiber, nucleic acids, amino acids, enzymes, and other substances. The present study deals with the fortification of mulberry leaves by using plant extract *C. pyrenoidosa* different concentration and fed to III, IV and V instar larvae, once in a day by useful modern technique to increase growth, biochemical contents and economic value of cocoon. The protein, carbohydrate and lipid contents of Fifth instar silk worm were estimated and the data was analysed.

KEYWORDS: Chlorella pyrenoidosa, Algae, B.mori, Mulberry plant, Silkworm.

INTRODUCTION

The silkworm, *B. mori* L. obtains almost all the nutrients required for its growth from the mulberry leaves. Investigations have been carried out on the importance of the various components of mulberry leaves for growth and economic character of silkworm (Krishnaswami et al 1978 and Pillai and Jolly, 1985). Enhancing the silkworm diet (i.e., mulberry leaves) with exogenous nutrients such as vitamins, proteins, carbohydrates, amino acids, minerals, hormones, antibiotics and assessing their effect on larval growth, metabolism and silk production has become the order of traditional research in sericulture (Etebari et al., 2004; Bhattacharya and Kaliwal, 2004, 2005a, 2005b, 2005c, 2005d; Chakrabarty and Kaliwal, 2011).

Silkworm *B. mori* (L) reared on mulberry leaves supplemented with minerals, oral protein supplementation, cereal flours, medicinal extracts, plant growth hormones (Sunder Raj et al., 2000; Singh, 1997) are reported to have beneficial effects on economic parameters.

Previous studies confirmed the effect of many plant extracts on various metabolic activities resulting in accelerated of silk cocoon formation and spinning (Shivakumar, et al.,1995 and Murugan, et al.,1998) and increase in larval, cocoon as well as shell weight (Sridevi, et al.,2003 and Pardesh and Bajad 2014b). Rajasekaragouda et al. (1997) noticed the growth promoting effect of the water and other extracts of the plants such as *Tribulus terrestis* and *Psoralea corylifolia*. Methenol extract of *Pteridium aquillinum* influenced the cocoon, silk gland weight and spinning of *B. mori* (Amirmohammadi et al., 2013).

Spirulina is a rich source of protein and the most powerful and well balanced source of nutrition. One kg of *Spirulina* is said to be equivalent to 1000kg of assorted vegetables (Vijayalakshmi et al., 2004). Kumar et al. (2009) studied the impact of blue green algae (*Spirulina*) on cocoon quantitative parameters (cocoon weight, shell weight, pupal weight, shell percentage and silk filament length) of silkworm.

Chlorella pyrenoidosa is a unicellular green micro alga that grows in fresh water. It has the highest chlorophyll content and also contains high concentrations of certain vitamins, minerals, dietary fiber, nucleic acids, amino acids, enzymes, and other substances (Nigam et al. 2011). Various investigation have shown that *C. pyrenoidosa* is a safe source of protein for consumption and dietary supplementation with *Chlorella* may reduce high blood pressure, lower serum cholesterol and glucose levels, accelerate wound healing, and enhance immune functions.

The present study deals with the fortification of mulberry leaves by using plant extract *C. pyrenoidosa* and feeding to the silkworm is a useful modern technique to increase growth, biochemical contents and economic value of cocoon.

MATERIALS AND METHODS

Bivoltine hybrid DFLs (eggs) were procured from district sericulture office, Konam, Nagercoil, these were reared under standard environmental conditions at temperature of 26+2°C, Relative humidity of 75% RH as per Krishnaswami, 1986. After hatching, the worms were fed with MR_2 variety of mulberry leaves.

C. pyrenoidosa algal extract was procured from Gideon Research Center, Nagercoil.

Systematic Position of *C. pyrenoidosa*:

Kingdom	- Protista
Phylum	- Chlorophyta
Class	- Trebouxiophyceae
Order	- Chlorellales
Family	- Chlorellaceae
Genus	- *Chlorella*
Spices	- *pyrenoidosa*

After the second moult larvae was divided into six groups. Each group consisted of six replicates of 30 Silk worms each. The first group was treated as the control given normal feedings 4-5 times a day and remaining group was considered as the test batch were treated with different concentrations (%) 1,2,3,4 & 5 of *C. pyrenoidosa* algal extract were prepared for the treatment. Fresh *Morus alba* leaves were soaked with different concentration then dried in air for 15 min and fed to III, IV and V instar larvae, once in a day.

The following formula was used to calculate Growth rate (%) ratio of B. *mori*,

$$\text{Growth \%} = \frac{\text{V instar-IV instar)}}{\text{IV instar}} \times 100$$

The following formula was used to calculate and tabulate Silk gland ratio of *B. mori*,

$$\text{Silk gland ratio} = \frac{\text{Silk gland weight}}{\text{Larval weight}} \times 100$$

The ratio of cocoon to shell is assessed for fixing the price. The cocoon shell ratio is expressed in percentage and following formula was used to calculate shell ratio

$$\text{Shell ratio} = \frac{\text{Weight of shell}}{\text{Weight of cocoon}} X100$$

Silk filament length (m) was calculated and tabulated using the following formula,

$$\text{Shell filament length} = \frac{\text{Number of rotations in epprouvette}}{\text{Cocoon shell weight}} X9/8$$

Weight and length of silk filament were calculated and observed for their denier.

$$\text{Denier} = \frac{\text{Weight of silk filament (g)}}{\text{Length of silk filament}} X\ 100$$

The following formula was used to calculate the percentage of fibroin and sericin in cocoon

$$\text{Fibroin percentage} = \frac{\text{Weight of fibroin (mg)}}{\text{Weight of shell (mg)}} X\ 100$$

Sericin percentage = 100-fibroin percentage.

The protein, carbohydrate and lipid contents of Fifth instar silk worm were estimated by the methods of Lowry et al., (1951), Schiefter et al., 1950 and Folch et al. (1957) and the data was analysed by Zar, 1984.

RESULTS

Growth, biochemical constituents and economic traits of *B. mori* larvae fed with *C. pyrenoidosa* green algal extracts are presented in Tables 1-4.

Table 1 shows the growth rate percentage of *B. mori* fed with *C. pyrenoidosa* extract. The maximum growth rate percentage 428.07±31.12 was observed when the larvae fed with 4 per cent of extract. The minimum growth rate percentage 393.93±18.44 was observed when the larvae fed with 5 per cent of extract.

The silk gland ratios of B. mori fed with C. pyrenoidosa extract are presented in Table 2. The maximum growth percentage 19.40±0.43 was observed when the larvae fed with 3 per cent of extract.

The minimum growth percentage 17.45±0.3 was observed when the larvae fed with 2 per cent of extract.

Table 3 shows the Protein, carbohydrate and Lipid content of silk gland of control *B. mori* larvae was 19.40±0.58 mg/g, 15.92±0.88 mg/g and 1.76±0.05 mg/g, respectively. The maximum silk gland protein 29.35±0.53 mg/g was observed in larvae fed with 4 per cent. The highest 20.44±0.53mg/g amount of silk gland carbohydrate was observed with 3 per cent and lowest 15.81±0.52 mg/g with 1 per cent. Lipid content of silk gland increased to 2.05±0.18mg/g with 4 per cent and decreased to 1.33±0.08 mg/g with 1 per cent. The biochemical content of silk gland was inceased by 4 per cent.

Protein, carbohydrate and Lipid content of haemolymph of *B. mori* larvae fed with C. *pyrenoidosa* are presented in Table 3. Protein content of haemolymph increased to 34.45±0.51 mg/ml with 4 per cent and decreased to 24.22±0.58 mg/ml with 1 per cent. In carbohydrate content in haemolymph maximum 16.90±0.34 mg/ml was observed 4 per cent. The maximum 3.11±0.05mg/ml amount of lipid content haemolymph was observed 3 per cent.

Table 3 shows the biochemical content in fat body of larvae fed with *C. pyrenoidosa*. The fat body protein was increased 13.54±0.42 mg/g and decreased 8.43±0.42 mg/g, when the larvae fed with 4 per cent and 1 per cent of extract. The maximum carbohydrate 9.97±0.27 mg/g and lipid 15.44±0.41 mg/g in fat body were observed with 4 and 3 per cent, respectively.

The biochemical content of muscle of *B. mori* larvae fed with *C. pyrenoidosa* is presented in Table 3. The maximum 31.94±1.01 mg/g amount of protein was observed in muscles of *B. mori*, when the larvae fed with 4 per cent. The carbohydrate and lipid content of muscles were 10.32±0.23 mg/g and 3.60±0.09 mg/g increased, when larvae fed with 4 and 2 percent, respectively.

Table 4 shows the maximum cocoon weight, pupal weight, shell weight, shell ratio, filament length, fibroin percentage and denier was 1904±54.12 mg, 1590±48.62 mg, 314±26.74 mg, 16.49±1.52 %, 938.24±29.49 m, 83.10±0.97 % and 3.05±0.06, respectively, When larvae fed with 4 per cent *C. pyrenoidosa* extract.

Table 1

Growth rate % of *B. mori* larvae fed with *Chlorella* pyrenoidosa

Concentration	IV Instar	V Instar	Growth %
Control	416.30±16.19618	1940.00±65.57439	366.01±12.53597
1%	422.40±20.49531	2145.00±54.3139**	407.81±18.67721**
2%	420.70±18.79668	2152.00±80.82388**	411.52±9.46656**
3%	448.30±23.6049*	2261.00 ±52.36888**	404.34±25.99337*
4%	454.10±32.08621*	2398.00±53.45559**	428.07±31.12541**
5%	420.30±28.76524	2076.00 ±80.26519*	393.93±18.44109*

Mean±S.D, *Significant **Highly Significant at P≤0.05, All other deviations aren't significant

Table 2

Silk gland ratio% of *B. mori* larvae fed with *Chlorella* pyrenoidosa

Concentration%	Larva Weight	Silk gland Weight	Silk gland Ratio
Control	1940.00±65.57439	320.10±17.44975	16.50±0.37155
1%	2145.00±54.3139**	413.98±13.79403**	19.30±0.20917**
2%	2152.00±80.82388**	375.52±19.3656**	17.45±0.3**
3%	2261.00±52.36888**	438.63±19.53473**	19.40±0.43157**
4%	2398.00±53.45559**	461.85±18.90559**	19.26±0.36469**
5%	2076.00±80.26519*	371.60±22.78936**	17.90±0.44861**

Mean±S.D, *Significant **Highly Significant at P≤0.05, All other deviations aren't significant

Table 3

Biochemical constituents in Silk gland, Haemolymph, Fat body and Muscles of *B. mori* larvae fed with *Chlorella pyrenoidosa*

Concentration%	Protein			
	silk gland (mg/g)	Haemolymph (mg/ml)	fat body (mg/g)	Muscles (mg/g)
Control	19.40±0.58843	21.74±0.89223	8.76±0.43307	24.65±0.55
1%	20.32±0.46935**	24.22±0.58023**	8.43±0.42727*	24.34±0.67454
2%	24.72±0.53567**	26.48±0.65374**	12.12±0.43717**	29.32±0.75463**
3%	28.05±0.58620**	30.35±0.56**	10.88±0.33276**	31.52±0.8370**
4%	29.35±0.53423**	34.45±0.51562**	13.54±0.42412**	31.94±1.0181**
5%	24.42±0.45373**	31.81±0.519**	11.45±0.3234**	29.81±0.85586**
Carbohydrate				
Control	15.92±0.88622	12.74±0.5186	6.85±0.32894	8.18±0.32711
1%	15.81±0.52658	12.18±0.42446	6.43±0.24234	8.04±0.2858
2%	16.12±0.33620*	13.33±0.56517**	7.86±0.21321**	7.90±0.16768**

3%	20.44±0.53953**	16.86±0.42652**	9.55±0.14720**	9.78±0.24374**
4%	19.84±0.49834**	16.85±0.34823**	9.97±0.27525**	10.32±0.23537**
5%	17.73±0.36077**	15.96±0.40704**	6.53±0.25252*	8.04±0.31083
Lipid				
Control	1.76±0.0563	2.92±0.11225	12.07±0.31937	3.04±0.09618
1%	1.33±0.08192	2.35±0.11516	13.47±0.35663**	3.12±0.07325**
2%	1.58±0.08656**	2.91±0.09325**	12.96±0.23072**	3.60±0.09832**
3%	1.71±0.22312**	3.11±0.05428**	15.44±0.41724**	3.20±0.14721**
4%	2.05±0.18148**	2.95±0.041**	14.97±0.21638**	3.22±0.06845**
5%	1.73±0.14127**	2.72±0.04229**	13.13±0.42247**	2.60±0.07835

Mean±S.D, *Significant **Highly Significant at P≤0.05, All other deviations aren't significant

Table 4

Economic characters of *B. mori* larvae fed with *Chlorella* pyrenoidosa

Parameters	Concentration%					
	Control	1%	2%	3%	4%	5%
Cocoon Weight (mg)	1650±63.14665	1707±64.29663	1773±81.86928*	1892±54.84632**	1904±54.12424**	1767±72.26874*
Pupal Weight (mg)	1391±61.50203	1436±46.22684	1491±62.16206	1587±26.61871**	1585±48.62375**	1486±45.28201*
Shell Weight (mg)	258±25.64176	271±22.4815	282±35.17562	305±28.62842**	319±26.74312**	281±30.1882
Shell Ratio (%)	15.63±0.8665	15.87±1.33697	15.90±1.27755	16.12±1.1238	16.75±1.52732	15.90±0.95565

Filament length (m)	831.55±35.48540	854.83±33.59116	879.41±24.27467*	910.74±35.82487**	938.24±29.49823**	893.40±30.1676*
Fibroin (%)	76.30±1.26026	79.15±1.01245*	80.20±.94983**	81.90±1.07224**	83.10±0.97289**	80.00±1.23466**
Sericin (%)	23.70±1.26099	20.85±1.022445*	19.80±1.14872**	18.10±0.87220**	16.90±0.85277**	20.00±1.12495**
Denier	2.50 ±0.079 06	2.82±0.1804**	2.75±0.07242**	3.00±0.18244**	3.05±0.06**	3.00±0.16814**

Mean±S.D, *Significant **Highly Significant at P≤0.05, All other deviations aren't significant

DISCUSSION

This study was carried out to the effects of *C. pyrenoidosa* algal extract on silkworm growth, economic characters and biochemical changes. IIIrd, IVth and Vth instar larvae, were fed with 4 per cent extract, recorded maximum growth per cent 428.07±31.12 and silk gland ratio 19.40±0.43 per cent, when larvae fed with 3 per cent *C. pyrenoidosa* extract. This finding was confirmed with Hou and Chen (1984). They observed that the supplementation of *S. platensis* protein to *B. mori* produced the better digestion and absorption and convert into body protein.

Protein, Carbohydrate and Lipid play a major role of insects not only in specific transport functions, but also in the development of *B. mori* larvae. Maximum protein content of silkgland, haemolymph, fat body and muscles was 29.35±0.53 mg/g, 34.45±0.51 mg/ml, 13.54±0.42 mg/g and 31.94±1.01 mg/g with 4 per cent, respectively. Supplementation of single cell protein enhanced the protein content of *B. mori* and increased the silkgland weight four times more than control (Mathavan et al., 1984). Datta et al. (2001) stated that the fibroin protein accumulation in the silkgland depending on the stage of development.

Maximum carbohydrates content of silkgland, haemolymph, fat body and muscles was 20.44±0.53 mg/g, 16.90±0.34 mg/ml, 9.97±0.27 mg/g and 10.32±0.23 mg/g with 3, 4, 4 and 4 per cent, respectively. This report agreed Prasad and Upadhyay (2011) studied the influence of magnetization on the glucose content in the tissues of silkworm larvae. Variation in the static magnetic strength significantly influenced the glucose content in the silkgland, fatbody and haemolymph of *B. mori* larvae.

Maximum LIpid content of silkgland, haemolymph, fat body and muscles was 2.05±0.18 mg/g, 3.11±0.05 mg/ml, 15.44±0.41 mg/g and 3.60±0.09 mg/g with 4, 3, 3 and 2 per cent, respectively. Lipid concentration is highly dependent on diet content and nutritional levels, even within species (Satake et al. 2000).

The maximum cocoon weight, pupal weight, shell weight, shell ratio, filament length, fibroin percentage and denier was 1904±54.12 mg, 1590±48.62 mg, 314±26.74 mg, 16.49±1.52 %,

938.24±29.49 m, 83.10±0.97 % and 3.05±0.06, respectively, When larvae fed with 4 per cent algal extract. Kumar et al. (2009) studied the effect of *Spirulina* on cocoon quantitative parameters like cocoon weight, shell weight, pupal weight, shell percentage and silk filament length of silkworm.

The studies have shown that there is a increase in the growth, economic traits, protein, carbohydrate and lipid content of silkgland, haemolymph, fatbody and muscles with the increase in the concentration of *C. pyrenoidosa* than control.

CONCLUSION

Thus, our observations are in agreement with the observations of earlier worker reported above. In the light of above view it is very much clear that there is appreciable improvement in the silk worm growth, silk gland ratio, biochemical contents protein, carbohydrate and lipid content of silkgland, haemolymph, fatbody and muscles and economic traits cocoon weight, pupal weight, shell weight, shell ratio percentage, filament length and denier due to application of *C. pyrenoidosa* in the present study.

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