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**ORIGINAL ARTICLE**

**STUDIES ON THE QUANTITATIVE PARAMETERS OF SILKWORM *BOMBYX MORI* (L.) (LEPIDOPTERA: BOMBYCIDAE) FED WITH CONTROL AND *SPIRULINA* TREATED MR<sub>2</sub> MULBERRY LEAVES**

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**ABSTRACT**

Sericulture is one of the most important cottage industries, which involves the utilization of mulberry trees and rearing of silkworm on commercial basis to produce silk. The larval and pupal parameters of silkworm *Bombyx mori* fed with *Spirulina* treated MR<sub>2</sub> mulberry leaves, the following works have been considered. The *Spirulina* in different concentrations such as 100ppm, 200ppm and 300ppm fresh mulberry leaves of (*Morus alba* L.) aqueous extract by each concentration and were fed to silkworms from 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> instar for five feedings were recommended. Then, group T<sub>1</sub> larvae received MR<sub>2</sub> mulberry leaves soaked with distilled water and served as control, group T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>, larvae received 100ppm, 200ppm and 300ppm *Spirulina* treated mulberry leaves, respectively. Silkworm larvae fed on MR<sub>2</sub> leaves soaked with 300ppm concentration of *Spirulina* (group T<sub>4</sub>) significantly increased the larvae and cocoon length, width and weight, cocoon shell weight, pupal weight, shell ratio and silk filament length as compared to those fed on control (group T<sub>1</sub>) MR<sub>2</sub> mulberry leaves and other group (T<sub>2</sub> and T<sub>3</sub>). It has been observed from the present study that 300ppm treated (group T<sub>4</sub>) leaves fed by silkworm have enhanced the larval and pupal growth and quantity of silk production than control.

**Keywords:** Silkworm, *spirulina*, cocoon, Mulberry leaves

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**1. INTRODUCTION**

The domesticated silkworm *Bombyx mori* has been the target of intensive scientific study. Since the dawn of human civilization, the silkworm has been used as a source of silk for producing exquisite textiles and dress materials. Nutrition is considered as a major influence on silkworm rearing. Better cocoon production has been found to be directly related by evolving successful rearing techniques. The quality of leaf has a greater influence on the amount of food ingested. In addition, a quality under feeding is always linked with quantitative under feeding (Sudo *et al.*, 1981; Chentilnayaki, 2004; Kalivarathan, 2004 and Balasundaram *et al.*, 2007 & 2008). The local yield of cocoon is almost half that of the countries having advanced sericulture technology (Khawaja, 1989). Monophagous feeding habit of silkworm requires improvement in the diet to enhance the silk production. Ahmad, (1983) has found that different combinations of nutrients gave better larval growth and silk production.

*Spirulina* is a blue-green algae. *Spirulina* is a photosynthetic, filamentous, spiral-shaped, multicellular microalgae. It contains

18 amino acids and vital vitamins *biotin*, *tocopherol*, *thiamine*, *riboflavin*, *niacin*, *folic acid*, *pyridoxoic acid*, *beta-carotene* and *vitamin B<sub>12</sub>* etc. The nutrients which are very easy to digest protein, carbohydrate, 50 different minerals and trace minerals, *beta-carotene*, *chlorophyll* and many other nutrients found in *Spirulina*.

Mulberry leaf supplemented with *Spirulina* as a feed to *Bombyx mori* (L.) orally found to be effective in enhancing the larval and cocoon characters (Venkataramana, 2003). The names of cyanobacteria and green blue algae (cyanophyceae), are considered comfortable terms. The first one refers to the phylogenetic/taxonomic relationship, while the second represents the ecological/biological correlation (Castenholz and Waterbury, 1989). The various researches have been carried out on the diet of supplementation of mulberry leaves fed to silkworms. These supplementations include vitamins such as ascorbic acid, thiamine and multivitamin (Eteberiet *al.*, 2004).

The presence of vitamin is appropriate for growth of larvae and the reproduction in many insects (Ishii, 1971;

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Yazan1972; Baker1975; Ritter and Johnson,1991; Levison,1992; Ozalp and Emer 1992; Chang and Li, 2004 ).The dietary supplements like protein, vitamins, lipids etc, evincing their specificity at specific dose for various metabolic activities of silkworm (Horie, 1980). Nutritional study on silkworm is an essential prerequisite for its proper commercial exploitation. Nutrition of silkworm in sole factors which almost individually augment quality and quantity of silk (Laskar and Datta, 2000).

## 2.MATERIALS ANDMETHODS

### Silkworm rearing method

The eggs of popular Indian bivoltine hybrid (CSR<sub>2</sub> ×CSR<sub>4</sub>) silkworm *B. mori* were collected from farmers training centre at Jayankondapattinam, Tamilnadu, India. The eggs were placed at ambient temperature of 25±2°C and relative humidity of 70 to 80% in an incubator for hatching. After hatching, larvae were isolated from stock culture. The larvae were divided into four experimental groups including control (distilled water treatment), each group containing 100 larvae. The larvae were reared in cardboard boxes measuring 22×15×5cms covered with nylon net and placed in an iron stand with ant wells. The control and treated MR<sub>2</sub> mulberry leaves were fed to silkworms, five feedings/day. They were maintained up to cocoon stage.

### Preparation of *Spirulina* solution

*Spirulina* powder was purchased from pharmaceutical company at Chidambaram and to prepare the experimental dose for 100ppm, 200ppm, 300ppm concentration,respectively.

### Mulberry (*Morus alba* L.) MR<sub>2</sub> leaves treated with *Spirulina*

*Spirulina* was dissolved in distilled water and diluted into 100ppm, 200ppm and 300ppm concentrations, respectively. Fresh MR<sub>2</sub> Mulberry leaves were soaked in this two concentrations for 15 minutes and then were dried in air for 10 minutes. The treated leaves were used for feeding(five feeding/day) the 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> instars larvae of silkworm, *B.mori* they were maintained up to cocoon stage.

### Experimental groups

There are four experimental groups. 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> instars of *B. mori* larvae were fed with the following treated MR<sub>2</sub> mulberry leaves. Group T<sub>1</sub> larvae fed with distilled water treated mulberry leaves, it act as a control, group T<sub>2</sub> larvae fed with 100ppm *Spirulina* treated mulberry leaves, group T<sub>3</sub> larvae fed with 200ppm *Spirulina* treated mulberry leaves, group T<sub>4</sub> larvae fed with 300ppm *Spirulina* treated mulberry leaves. They were maintained up to cocoon stage.

### Statistical Analysis

Data were analyzed by one-way Analysis of Variance (ANOVA) followed by Duncan's Multiple range test (DMRT) using a commercially available Statistics Software Package (SPSS® for Windows, V.16.0, Chicago, USA). Results were presented as mean±S.D P values <0.05 were regarded as statistically significant.

## 3.RESULTS

### Larval Parameters

Table 1 shows that morphometric data of control MR<sub>2</sub> mulberry leaves and *Spirulina* treated MR<sub>2</sub> mulberry leaves fed 3<sup>rd</sup> instar of *B. mori* larvae length, width and weight. The mean value of control (group T<sub>1</sub>) were (1.7301±0.1281cm, 0.4460±0.0558cm and 0.0884±0.0073gm), respectively. The mean value of 100ppm *Spirulina* treated (group T<sub>2</sub>) were(1.8334±0.1578cm, 0.4520±0.0658cm and 0.1047±0.0168gm), respectively. The mean value of 200ppm *Spirulina* treated (group T<sub>3</sub>) were(1.8434±0.1658cm, 0.4824±0.0682cm and 0.1058±0.0274gm), respectively. The mean value of 300ppm *Spirulina* treated (group T<sub>4</sub>) were (1.9246±0.1782cm,0.4950±0.0797cm and 0.1158±0.0283gm), respectively. In these four observations, 300ppm *Spirulina* (group T<sub>4</sub>) treated 3<sup>rd</sup> instar larvae length, width and weight was significantly increased than control (T<sub>1</sub>) and other two groups (T<sub>2</sub> and T<sub>3</sub>).

Fig.1. Morphometric data of control and *Spirulina* treated III instar larvae of *Bombyx mori*

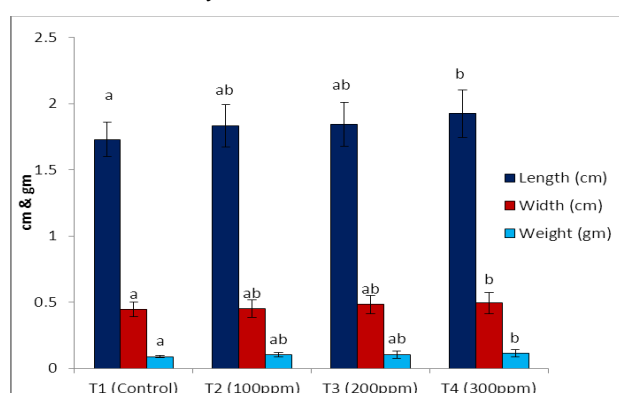


Fig.2. Morphometric data of control and *spirulina* treated IV instar larvae of *Bombyx mori*.

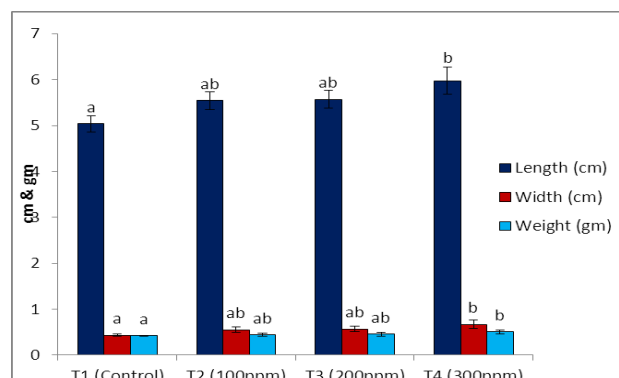


Fig.3. Morphometric data of control and *spirulina* treated V instar larvae of *Bombyx mori*.

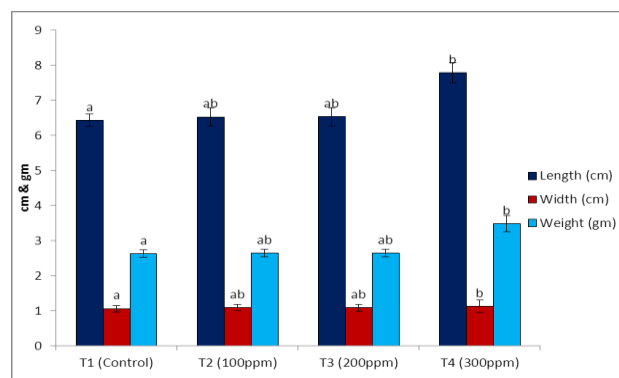


Table 2 shows that the morphometric data of control MR<sub>2</sub> mulberry leaves and *Spirulina* treated MR<sub>2</sub> mulberry leaves fed 4<sup>th</sup> instar of *B.mori* larvae length, width and weight. The mean value of control (group T<sub>1</sub>) were (5.0372±0.1723cm, 0.4322±0.0278cm and 0.4233±0.01048gm), respectively. The mean value of 100ppm *spirulina* treated (group T<sub>2</sub>) were (5.5450±0.1945cm, 0.5523±0.0610cm and 0.4468±0.03637gm), respectively. The mean value of 200ppm *Spirulina* treated (group T<sub>3</sub>) were(5.5687±0.1965cm, 0.5667±0.0623cm and 0.4530±0.03733gm), respectively. The mean value of 300ppm *Spirulina* treated(group T<sub>4</sub>) were (5.9730±0.2987cm, 0.6700±0.0892cm and 0.5038±0.03783gm), respectively. In these four observations, 300ppm *Spirulina* (group T<sub>4</sub>) treated 4<sup>th</sup> instar larvae length, width and weight were significantly increased than control (T<sub>1</sub>) and other two groups (T<sub>2</sub> and T<sub>3</sub>).

Fig.4. Morphometric data of control and *spirulina* treated *Bombyx mori* larvae produced cocoon

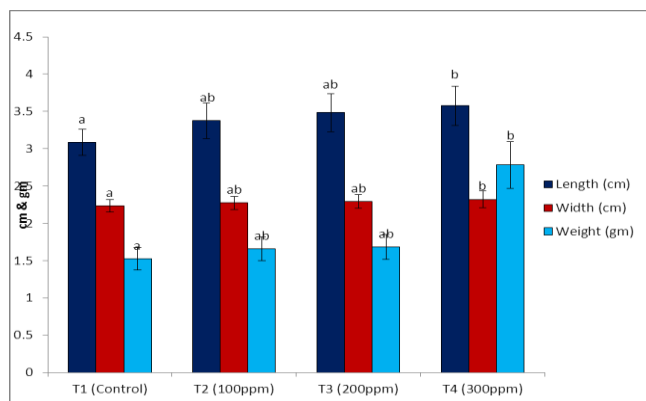


Fig.5. Morphometric data of control and *spirulina* treated *Bombyx mori* larvae produced cocoon shell and pupal weight.

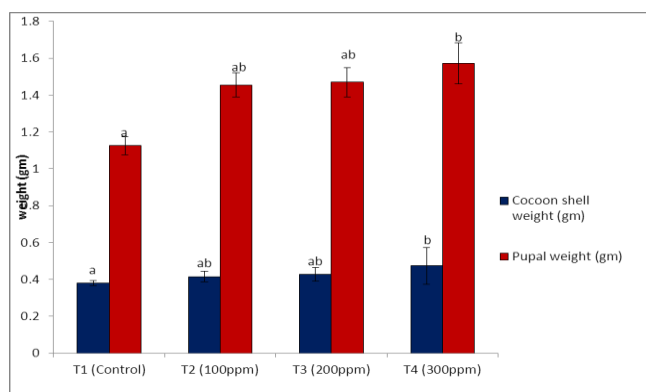


Fig.6. Morphometric data of control and *spirulina* treated *Bombyx mori* larvae produced cocoon shell ratio and silk filament length

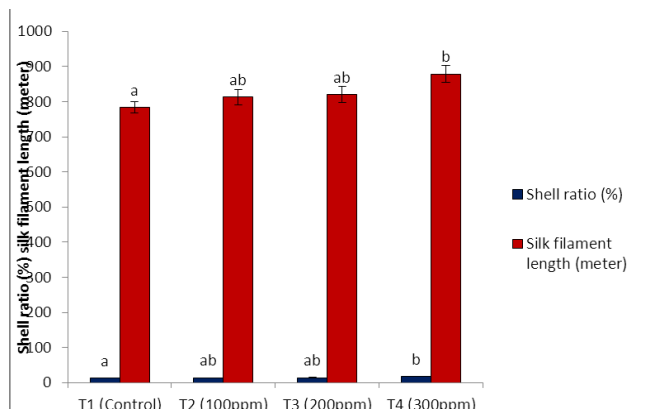


Table 3 shows that the morphometric data of control MR<sub>2</sub> mulberry leaves and *Spirulina* treated MR<sub>2</sub> mulberry leaves fed 5<sup>th</sup> instar of *B.mori* larvae length, width and weight. The mean value of control (group T<sub>1</sub>) were (6.4283±0.1838cm, 1.058±0.0873cm and 2.6270±0.1040gm), respectively. The mean value of 100ppm *Spirulina* treated (group T<sub>2</sub>) were (6.5240±0.2528 cm, 1.083±0.0932cm and 2.6350±0.1068gm), respectively. The mean value of 200ppm *Spirulina* treated (group T<sub>3</sub>) were (6.5280±0.2653cm, 1.087±0.0982cm and 2.6447±0.1075gm) (6.5240±0.2528cm, 1.083±0.0932cm and 2.6350±0.1068gm), respectively. The mean value of 300ppm *Spirulina* treated (group T<sub>4</sub>) were (7.7883±0.2853cm, 1.123±0.1829cm and 3.4753±0.2334gm), respectively. In these four observations, 300ppm *Spirulina* (group T<sub>4</sub>) treated 5<sup>th</sup> instar larvae length, width and weight were significantly increased than control (T<sub>1</sub>) and other two groups (T<sub>2</sub> and T<sub>3</sub>).

### Cocoon Parameters

Table 4 shows the morphometric data of mean length, width and weight of the cocoon of *B.mori* fed with *Spirulina* treated MR<sub>2</sub> leaves were found to be more than that of the larvae fed with control MR<sub>2</sub> leaves. The length, width and weight of the T<sub>1</sub> larvae produced cocoon were found to be about (3.0820±0.1757cm, 2.2346±0.0815cm and 1.5250±0.1583gm), respectively. The length, width and weight of the T<sub>2</sub> larvae produced cocoon were found to be about(3.3750±0.2389cm, 2.2723±0.0885cm and 1.6550±0.1523gm), respectively. The length, width and weight of the T<sub>3</sub> larvae produced cocoon were found to be about (3.4850±0.2534cm, 2.2910±0.0920cm and 1.6815±0.1685gm), respectively. The length, width and weight of the T<sub>4</sub> larvae produced cocoon were found to be about (3.5768±0.2643cm, 2.3200±0.1138cm and 2.7812±0.3145gm), respectively. In these four observations, 300ppm *Spirulina* (group T<sub>4</sub>) treated 5<sup>th</sup> instar larvae length, width and weight were significantly increased than control (T<sub>1</sub>) and other two groups (T<sub>2</sub> and T<sub>3</sub>).

### Pupal Parameters

Table 5 shows the morphometric data of control MR<sub>2</sub> mulberry leaves and *Spirulina* treated MR<sub>2</sub> mulberry leaves were found to be more than that of larvae produced, cocoon shell and pupal weight. The mean value of control(T<sub>1</sub>) were (0.3798±0.0135gm and 1.1250±0.0486gm), respectively. The mean value of 100ppm *Spirulina* treated group(T<sub>2</sub>) were(0.4150±0.0280gm and 1.4550±0.0654gm), respectively. The mean value of 200ppm *Spirulina* treated group(T<sub>3</sub>) were(0.4272±0.0358gm and 1.4688±0.0782gm), respectively. The mean value of 300ppm *Spirulina* treated group (T<sub>4</sub>) were (0.4732±0.1235gm and 1.5725±0.1122gm), respectively. In these four observations, 300ppm *Spirulina* (group T<sub>4</sub>) treated larvae produced cocoon shell and pupal weight was significantly increased than control (T<sub>1</sub>) and other two groups (T<sub>2</sub> and T<sub>3</sub>).

### Silk Traits

Table 6 shows that the morphometric data of control MR<sub>2</sub> mulberry leaves and *Spirulina* treated MR<sub>2</sub> mulberry leaves fed *B.mori* larvae produced cocoon shell ratio(%) and silk filament length (meters). The mean value of control (group T<sub>1</sub>) were (13.4340±0.1730% and 783.1865±15.5688mtrs),

respectively. The mean value of 100ppm *Spirulina* treated group ( $T_2$ ) were (14.2352±0.2372% and 812.4224±22.1520mtrs), respectively. The mean value of 200ppm *Spirulina* treated group ( $T_3$ ) were (14.2432±0.2578% and 820.4321±23.1765mtrs), respectively. The mean value of 300ppm *Spirulina* treated group ( $T_4$ ) were (17.1664±0.2657% and 878.5354±24.1985mtrs), respectively. In these four observations, 300ppm *Spirulina* (group  $T_4$ ) treated larvae produced cocoon shell ratio (%) and silk filament length(mtrs) was significantly increased than control ( $T_1$ ) and other two groups ( $T_2$  and  $T_3$ ).

#### 4.DISCUSSION

In the present study, the larval and cocoon length, width and weight were significantly increased in some groups. The total body weight gain on wet weight basis was significantly higher in *Spirulina* treated MR<sub>2</sub> mulberry leaves followed by control MR<sub>2</sub> mulberry leaf. Among the MR<sub>2</sub> mulberry leaves, *Spirulina* treated MR<sub>2</sub> mulberry leaves has gained maximum body weight, cocoon weight and silk trait than the control MR<sub>2</sub> mulberry leaf. The current finding are comparable with the results of Centhilnayaki,(2004), Kalivarathan, (2004), Balasundaram *et al*(2007 & 2008)and Ganeshprabhu(2012).

From the present observations, it has also been evident that consistently better rearing performance was obtained from feeding of leaves of *Spirulina* treated MR<sub>2</sub> mulberry leaves over another one is control MR<sub>2</sub> mulberry leaves. All the parameters governing yield and quality of cocoon were influenced significantly, where the leaf was fed by the larvae. This might be attributed due to better quality of *Spirulina* treated mulberry leaves with respect to higher content of protein, carbohydrate and moisture content which ultimately resulted in the production of an higher and better quality cocoon. The food consumption has a direct relevance on the weight of larvae, cocoon, pupae and shell, the independent parameters of consumption and productivity vary depending upon the type of nutrition (Shivakumar, 1995) and silkworm breeds (Ramadeviet *al*, 1992).

The weight of 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> instar larvae were found to be increased when the worms fed with *Spirulina* treated mulberry leaves followed by, MR<sub>2</sub> leaf. The feeding efficacy of different mulberry feed varieties such as MR<sub>2</sub> and *Spirulina* treated mulberry leaves on larval growth and development was also reported (Puttaraja, 2000 and Centhilnayaki, 2004). In the present study, the treatment of *Spirulina* at the concentration of 300ppm may have beneficial effects on the growth of the silkworm larval and cocoon length, width, weight and pupal parameters and silk traits and also increased the quantity of silk production by enhancing the feed efficacy than control. So, this supplementation to be prescribed to the farmers to get more quantity of silk.

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