Biotechnological Applications and Nutritional Supplementation of Ascorbic Acid (Vitamin C) Treated Morus alba (L.) Leaves Fed by Silkworm, Bombyx mori (L.) (Lepidoptera: Bombycidae) in Relation to Silk Production

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Abstract
This study was carried out to determine the cocoon parameters and economic traits of silkworm Bombyx mori (V instar larvae) fed by MR₁ mulberry (Morus alba) leaves and different concentrations of ascorbic acid (Vitamin C) treated MR₂ mulberry leaves in relation to economic parameters like cocoon (length, width and weight), yield of cocoon (%), cocoon shell weight (gm), cocoon shell ratio (%), silk thread length (mts.) and silk thread denier (%). The different concentrations (0.1 %, 0.2 %, 0.4 % and 0.8 %) of prepared ascorbic acid solution were treated to the V instar larval period. In the present study, observed that the cocoon parameters and economic parameters were enhanced by 0.2 % ascorbic acid treated group than control and other treated groups (0.2 %, 0.4 % and 0.8 %). This study was indicated that the ascorbic acid exhibits the presence of certain growth stimulant activity and can be used to increase the silk yield in commercial silkworm rearing.

Key Words: Bombyx mori, Morus alba, Ascorbic Acid, Silk Production, Biotechnology.

Introduction
Sericulture is an age-old land-based practice in India with high employment potential and economic benefits to agrarian families. No doubt, India is the second largest producer of mulberry silk next only to China (Vijayaprakash and Dandin, 2005). The silkworm, Bombyx mori L. (Lepidoptera: Bombycidae), was domesticated more than a thousand years ago. The studies on this had important advances in the last century, mainly on the fields of Nutrition and biotechnology. Corroboration of these new technologies to produce more silk than developed by the end of the nineteenth century. In the nutritional supplementation of ascorbic acid to Bombyx mori feed efficacy to improve the silk producing efficiency with reference to food Biotechnology. Although the currently bred races are much productive, the application of new technologies will promote increases in silk production (Goldsmith et al., 2005). The mulberry silkworm, Bombyx mori L., is a very important economic insect that contributes substantially to the national economy of India and provides gainful occupation to lakhs of people. Besides providing optimal environmental conditions to increase the productivity, the science of breeding is necessary for the production of optimal for the available environments (Raman and Ahmad, 1988). Bombyx mori is monophagous insect that feeds mainly on mulberry leaves. Significant advances in the research of silkworm nutrition began with development of artificial diets (Ito, 1978).

Mulberry (Morus alba L.) is a deep rooted, foliage yielding and fast growing perennial crop grown for its leaf and is the sole food for silk worm (Bombyx mori L.). The growth and development of B. mori are influenced to a greater extent by the nutritional quality of the leaf. As cocoon production is directly dependent on the quality and quantity of leaves. The dietary supplements like protein, vitamins, lipids etc. evincing their specificity at specific dose for various metabolic activities of silkworm (Horie, 1980). Bose et al. (1974), studied on the effect of foliar application of different levels of various micronutrients to mulberry on the quality of bivoltine cocoon and silk. Mulberry leaves are rich in protein and amino acids. It is known that there is high correlation between leaf protein level and production efficiency of cocoon shell, which means cocoon shell weight to the total amount of mulberry leaves consumed by the B. mori (Mechii and Katagiri, 1991). Betasitosterol along with some sterols and watersoluble substance is the main factor which during feeding stimulates the biting action of B. mori (Anonymous, 1976). Therefore, increase in protein level may lead to improvement in productivity of cocoons and silk.

Advances in silkworm nutrition and in the development and use of artificial diets will be of great benefit to the silk industry. The effect of artificial diets with different nutrition on better production of cocoon crops and silk were investigated by many workers like Fukuda and Higuchi, (1963); Yokoyama, (1964); Hamamura, (1964).
The first day of V instar larvae were placed at ambient temperature of 25 ± 2°C and relative humidity of 70 to 80%. The larvae were reared in card board boxes measuring 22 x 15 x 5 cm covered with nylon net and placed in an iron stand with ant wells (Govindan, et al., 1981).

**Selection of the Effective Concentration of Ascorbic Acid**

The Ascorbic acid was diluted to 0.1%, 0.2%, 0.4% and 0.8% concentrations. Fresh MR₂ mulberry leaves were separately soaked with each concentration for 15 minutes and then were dried in air for 10 minutes. The Ascorbic acid treated leaves were used for feeding the V instars larvae of silkworm *B. mori* (Suleman, 1999). The *B. mori* larvae were divided into two groups (Control and Treated). The treated group divided into four sub groups (T₁, T₂, T₃, and T₄) this sub groups were treated with different concentrations of Ascorbic acid (0.1%, 0.2%, 0.4% and 0.8%) efficacy of this concentrations were compared to control group and to find out the feed efficacy of V instar larvae. The control and Ascorbic acid treated MR₂ mulberry (*Morus alpa*) leaves were fed by silkworm *B. mori*, five feedings/per day.

**Experimental Groups**

The V instars of *B. mori* larvae fed with the following MR₂ mulberry leaves. Control group (C) larvae fed with normal mulberry leaves, group T₁ larvae fed with 0.1% Ascorbic acid treated MR₂ mulberry leaves, group T₂ larvae fed with 0.2% Ascorbic acid treated MR₂ mulberry leaves, group T₃ larvae fed with 0.4% Ascorbic acid treated MR₂ mulberry leaves and group T₄ larvae fed with 0.8% Ascorbic acid treated MR₂ mulberry leaves (Rasool, 1995).

**Statistical Analysis**

All the 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 ± standard deviation (SD).

**Materials and Methods**

**Silkworm Rearing**

The first day of V instar of popular Indian bivoltine hybrid (CSRₓCSRₓ) silkworm *B. mori* (Local Bivoltine) race were collected from Silkworm Culture Centre, Salem in Tamilnadu, India. The larvae were reared simultaneously both in control and experimental groups separately on mulberry leaves dipped in Ascorbic acid solution in the laboratory. Proper environmental conditions provided to the silkworms with photoperiod of 12:12 h light and darkness as recommended by Krishnaswami et al. (1973).

**Table 1.** Morphometric data of control and different concentrations of Ascorbic acid treated *Morus alba* leaves fed *Bombyx mori* larvae produced cocoon.

<table>
<thead>
<tr>
<th>Concentrations of Ascorbic acid (Vitamin C)</th>
<th>Cocoon length (cm)</th>
<th>Cocoon width (cm)</th>
<th>Cocoon weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>3.35±0.35</td>
<td>2.10±0.11</td>
<td>1.41±0.09</td>
</tr>
<tr>
<td>Group (T₁) 0.1%</td>
<td>3.36±0.11</td>
<td>2.07±0.08</td>
<td>1.49±0.11</td>
</tr>
<tr>
<td>Group (T₂) 0.2%</td>
<td>3.58±0.17</td>
<td>2.38±0.07</td>
<td>2.07±0.32</td>
</tr>
<tr>
<td>Group (T₃) 0.4%</td>
<td>3.28±0.22</td>
<td>2.09±0.10</td>
<td>1.24±0.17</td>
</tr>
<tr>
<td>Group (T₄) 0.8%</td>
<td>3.27±0.21</td>
<td>2.17±0.14</td>
<td>1.68±0.19</td>
</tr>
</tbody>
</table>

Values are Mean ± Standard Deviation of six observations. Values in the same column with different superscript letters (a, b & c) differs significantly at P<0.05 (DMRT).

**Table 2.** Economic traits of control and different concentrations of Ascorbic acid treated *Morus alba* leaves fed V instar larvae of *Bombyx mori* produced cocoon.

<table>
<thead>
<tr>
<th>Concentrations of Ascorbic acid (Vitamin C)</th>
<th>Yield of Cocoon (%)</th>
<th>Cocoon Shell weight (gm)</th>
<th>Cocoon Shell Ratio (%)</th>
<th>Silk Thread Length (Meters)</th>
<th>Denier (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>81.87±0.77</td>
<td>0.63±0.07</td>
<td>17.08±0.65</td>
<td>919.19±11.76</td>
<td>2.64±0.14</td>
</tr>
<tr>
<td>Group (T₁) 0.1%</td>
<td>80.90±0.18</td>
<td>0.69±0.04</td>
<td>16.59±0.17</td>
<td>923.24±10.39</td>
<td>2.58±0.16</td>
</tr>
<tr>
<td>Group (T₂) 0.2%</td>
<td>85.93±1.05</td>
<td>0.80±0.06</td>
<td>19.84±1.34</td>
<td>965.20±08.73</td>
<td>2.94±0.15</td>
</tr>
<tr>
<td>Group (T₃) 0.4%</td>
<td>80.85±0.90</td>
<td>0.64±0.05</td>
<td>16.50±0.98</td>
<td>917.24±13.87</td>
<td>2.48±0.12</td>
</tr>
<tr>
<td>Group (T₄) 0.8%</td>
<td>80.40±0.74</td>
<td>0.61±0.07</td>
<td>16.91±0.91</td>
<td>926.71±10.41</td>
<td>2.30±0.11</td>
</tr>
</tbody>
</table>

Values are Mean ± Standard Deviation of six observations. Values in the same column with different superscript letters (a, b & c) differs significantly at P<0.05 (DMRT).

Recently, much research has been done on the diet supplementation of mulberry leaves fed to silkworms. These supplemental include vitamins such as ascorbic acid, thiamin, niacin, folic acid, multi-vitamins (Nirwani and Kalivil, 1998; Saha and Khan, 1996; Etebari and Fazilati, 2003; Etebari et al., 2004). Ito, (1961) has recorded relationship of ascorbic acid supplementation and growth of silkworm. The absence of ascorbic acid in the diet of first and second instar larvae postponed growth and development of silkworm. There is enough Vitamin C in mulberry leaves and ascorbic acid content of growing larvae is dependent on amount of this vitamin in diet. Several research demonstrated phagostimulatory effect of ascorbic acid for insects (Ito, 1978; Dobzhenok, 1974). Sengupta et al. (1972) have reported that silk production increased with 1 % ascorbic acid in the diet of silkworm. Etebari et al. (2004) have demonstrated that feeding on mulberry leaves enriched with ascorbic acid at 3% concentration decreased larval weight due to hypervitaminosis. Babu et al. (1992) have observed that the first and second instar larvae reared on 1.5 % ascorbic acid enriched mulberry leaves resulted in higher silk filament length, weight and denier values. Each cocoon is composed of single thread of about 914 meters long. About 3000 cocoons are required to make a pound of silk (Borrer et al., 1981).
P < 0.05 was regarded as statistically significant (Sokal and Rohlf, 1981).

Results

Economic characters like cocoon parameters (length, width and weight) yield of cocoon, cocoon shell weight, cocoon shell ratio, silk thread length and silk filament denier (Silk filament strength) data of control MR2 mulberry leaves and different concentrations of ascorbic acid treated MR2 mulberry leaves fed B. mori larvae produced cocoon and silk filament were presented in Tables 1 and 2. The Morphometric data of mean length, width and weight of the cocoon of B. mori fed with ascorbic acid treated MR2 leaves were found to be more than that of the larvae fed with control MR2 leaves. The length, width and weight of the group ‘C’ larvae produced cocoon were found to be about (3.35 ± 0.35 cm, 2.10 ± 0.11 cm and 1.41 ± 0.09 gm), respectively.

The length, width and weight of the group T1 larvae produced cocoon were observed to be about (3.36 ± 0.11 cm, 2.07 ± 0.08 cm and 1.49 ± 0.11 gm), respectively. The length, width and weight of the group T2 larvae producing cocoon were observed to be about (3.58 ± 0.17 cm, 2.38 ± 0.07 cm and 2.07 ± 0.32 gm), respectively. The length, width and weight of the group T3 larvae produced cocoon were observed to be about (3.28 ± 0.22 cm, 2.09 ± 0.10 cm and 1.24 ± 0.17 gm), respectively (Table 1). The length, width and weight of the group T4 larvae produced cocoon were observed to be about (3.27 ± 0.21 cm, 2.17 ± 0.14 cm and 1.68 ± 0.19 gm), respectively. In these five observations, the 0.2 % (group T1) Ascorbic acid treated larvae produced cocoon length; width and weight were significantly increased than the other four groups (‘C’, T1, T3 and T4).

Table 2 shows that the data of control and ascorbic acid treated MR2 mulberry leaves fed V instar larvae produced cocoons. The yield of cocoon (%) of group ‘C’ larvae (81.87 ± 0.77 %), group T1 larvae (80.90 ± 0.18 %), group T2 (85.93 ± 1.058 %) larvae, group T3 (80.85 ± 0.90 %) and group T4 (80.40 ± 0.74 %), respectively. In these five observations, the 0.2 % (group T3) ascorbic acid treated larvae yield of cocoon (%) was significantly increased than the other four groups (‘C’, T1, T2 and T4). The data shows that the control and ascorbic acid treated MR2 mulberry leaves fed V instar B. mori larvae produced cocoon’s shell weight. The cocoon shell weight (gm) of group ‘C’ larvae (0.63 ± 0.07 gm), group T1 larvae (0.69 ± 0.04 gm), group T2 (0.80 ± 0.06 gm) larvae, group T3 (0.64 ± 0.05 gm) and group T4 (0.61 ± 0.07 gm), respectively. In these five observations, the 0.2 % (group T2) ascorbic acid treated larvae produced cocoon shell weight was significantly increased than the other four groups (‘C’, T1, T3 and T4). The data shows that the control and ascorbic acid treated MR2 mulberry leaves fed V instar B. mori larvae produced cocoon shell ratio. The cocoon shell ratio (%) of group ‘C’ larvae (17.08 ± 0.65 %), group T1 larvae (16.59 ± 0.17 %), group T2 (19.84 ± 1.34 mts.) larvae, group T3 (16.50 ± 0.98 %) and group T4 (16.91 ± 0.91 %), respectively. In these five observations, the 0.2 % (group T2) Ascorbic acid treated larvae silk filament length (meters) was significantly increased than the other four groups (‘C’, T1, T3 and T4). The data shows that the control and ascorbic acid treated MR2 mulberry leaves fed V instar B. mori larvae produced cocoon’s silk thread length. The silk thread length (meters) of group ‘C’ larvae (919.19 ± 11.76 mts.), group T1 larvae (923.24 ± 10.39 mts.), group T2 (965.20 ± 0.87 mts.) larvae, group T3 (917.24 ± 13.87 mts.) and group T4 (926.71 ± 10.41 mts.), respectively. In these five observations, the 0.2 % (group T2) Ascorbic acid treated larvae shell ratio (SR) was significantly increased than the other four groups (‘C’, T1, T2 and T4). The data shows that the control and ascorbic acid treated MR2 mulberry leaves fed V instar B. mori larvae produced cocoon’s silk filament Denier. The silk filament denier of group ‘C’ larvae (2.64 ± 0.14 %), group T1 larvae (2.58 ± 0.16 %), group T2 (2.94 ± 0.15 %) larvae, group T3 (2.48 ± 0.12 %) and group T4 (2.30 ± 0.11 %), respectively. In these five observations, the 0.2 % (group T2) Ascorbic acid treated larvae silk filament length (meters) was significantly increased than the other four groups (‘C’, T1, T3 and T4).

Discussion

Feeding trials conducted by several researchers proved that the level of nutrients in different varieties of mulberry have significant influence on growth and development of silkworm and cocoon production (Bari et al., 1985; Machii and Katagiri, 1990). In general, the present results are in agreement with the observations of earlier workers (Benjamin and Jolly, 1984; Periaswamy and Radhakrishnan, 1985; Ramadevi et al., 1992). Similar observations have also been made related with silk ratio in the cocoons (Pillai et al., 1981), cocoon yield (Giridhar et al., 1991). The total body weight gain on wet weight basis was significantly higher in ascorbic acid treated mulberry leaves followed by control MR2 leaves. Among the mulberry leaves, ascorbic acid treated mulberry leaves has gained maximum cocoon weight and silk trait than the worms fed with control MR2 leaves. The current findings are comparable with the results of Horie, (1978); Chenthilnayaki et al. (2004); Balasundaram et al. (2008); Ponraj Ganesh Prabu et al. (2011); Ganesh Prabu et al. (2012).

Further, it has been revealed from the present study that the weight of cocoon was maximum in silkworm larvae when fed with ascorbic acid treated mulberry leaves than control MR2 leaves. Similar trend was observed by Udupa, (1986); Tayade, (1987), on B. mori in relation to heterosis effect on economic traits of new hybrids. Bose et al. (1995) have reported that succulent mulberry leaves with less fiber and higher mineral contents presumably stimulated the metabolic activities in silkworm, resulting in qualitative improvement of cocoon and silk. From the present observations, it was also evident that consistently better rearing performance was obtained from feeding of leaves of ascorbic acid treated mulberry leaves over control MR2 mulberry leaves. All the parameters governing yield and quality of cocoon were influenced significantly when the leaf was fed by the larvae. This might be attributed due to better quality of ascorbic acid treated mulberry leaves with respect to higher content of protein, carbohydrate and moisture content which ultimately resulted in the production of higher yield and better quality cocoon. Nutrition is known to play an important role on growth, development and overall performance of the cocoon components. Better quality of ascorbic acid treated mulberry leaves therefore, leads to the elevated value of...
growth and yield attributing parameters in the present investigation. Further, future investigation leading towards increasing the yield of control MR1 on improvement of MR2 mulberry leaves with ascorbic acid through cultured manipulation needs to be undertaken to maximize quality yield gap of the commonly grown varieties in zone under the sericulture industry, to be more profitable. The food consumption has a direct relevance on the weight of larvae; cocoon, pupae and shell weight, the independent parameters of consumption and productivity vary depending upon the type of nutrition (Shivakumar, 1995) and silkworm breeds (Ramadevi, et al., 1992). In the present study, the ascorbic acid treated mulberry leaves may have helped the silkworm larvae in a beneficial way, leading to increased conversion and silk synthesis. It is suggested that ascorbic acid treated MR2 mulberry leaves influences the conversion of more food towards cocoon shell content as reported earlier (Radha et al., 1981; Tayade et al., 1988; Govindan et al., 1990). The silk conversion rate is an important factor to be considered while evaluating the economic traits of a silkworm. In this context, providing MR2 mulberry leaves treated with ascorbic acid as a feed for silkworm appears to be beneficial by increasing the silk synthesis. The findings of the present study on feeding leaves with ascorbic acid influenced various economic characters of V instar larvae of B. mori. These results corroborate the earlier findings of Kafian, (1960); Arai and Ito, (1963); Narayanan et al. (1967); Chenthilnayaki et al. (2004); Balasundaram et al. (2008); Ponraj Ganesh Prabu et al. (2011); Ganesh Prabu et al. (2012). In view of the above, the present study has revealed that the nutritional content of mulberry with ascorbic acid for successful larval rearing can be extrapolated on silkworms for better cocoon production. Several researchers demonstrated phagostimulant effect of ascorbic acid for insects (Dobzhhenok, 1974; Ito, 1978). In silk worm, a gustatory stimulating activity has been observed to some extent (Ito, 1961). The heaviest cocoon shell ratio can be obtained by supplementing mulberry leaves with minerals and other nutrients (Mahmood, 1989). The results of Khan et al. (2000), Chenthilnayaki et al. (2004); Balasundaram et al. (2008); Ponraj Ganesh Prabu et al. (2011); Ganesh Prabu et al. (2012) have reported that their study could efficiently be utilized in the feed improvement programme making any project considering cocoon weight, pupal weight and silk ratio as a criteria, considering the importance of silk content in any particular feed. It may be inferred from the present study that the silkworm does respond significantly to MR2 leaf treated with ascorbic acid than control MR2 mulberry leaves resulting in the economic traits such as quality of cocoon, silk ratio, shell weight and denier and silk production.

Conclusion
In the present study, the treatment of ascorbic acid at the concentration of 0.2% may have beneficial effects on the cocoon parameters (length, width and weight) and economic traits like yield of cocoon, shell weight, shell ratio, silk thread length and denier of silk thread and also increased the quantity of silk by enhancing the silk production than control. So, this vitamin supplementation could be prescribed to the farmers to get more quantity of silk.

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