Studies on the Growth Rate of Silkworm *Bombyx mori* (L.) (Lepidoptera: Bombycidae) Fed with Control and Silver Nanoparticles (AgNps) Treated MR2 Mulberry Leaves

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To evaluate the growth rate of larval and pupal parameters of silkworm *Bombyx mori* fed with Silver Nanoparticles (AgNps) treated MR2 mulberry leaves, the following works have been considered. The AgNp was synthesized by chemical reduction method, it was diluted by different concentrations such as 25%, 50%, 75% and 100% (without dilution). Fresh mulberry leaves (*Morus alba* L.) were sprayed by each concentration and were fed to silkworms, from 3rd, 4th and 5th instar, five feedings/day. Group T1 larvae received MR2 mulberry leaves sprayed with distilled water and served as control, group T2, T3, T4 and T5 larvae received 25%, 50%, 75% and 100% AgNps sprayed mulberry leaves, respectively. Silkworm larvae fed on *M. alba* (MR2) leaves sprayed with 25% concentration of AgNps (group T2) was significantly increased the larvae and cocoon length, width and weight as compared to those fed on control (group T1) MR2 mulberry leaves and other groups (T3, T4 and T5). Hence, 25% AgNps dose was fixed as an effective dose. It has been observed from the present study that 25% AgNps treated (group T2) leaves fed by silkworms have enhanced the larval and pupal growth and quantity of silk production than control.

Key words: *Bombyx mori*, *Morus alba*, Silver nanoparticles (AgNps), MR2 Mullbery leaves

Introduction

The silkworm *Bombyx mori* rearing is a traditional industry in Asia and the life of many people is depended on it. Increase of larval growth and cocoon quality and quantity would result better economics for this industry and meet the production needs. Consequently, the enrichment of mulberry leaves by supplementary compounds with the aim of increasing the production of cocoon is a very important aspect. Many investigations have been done on this topic and various reports have been published (Eteberi, 2002; Etebari et al., 2004; Islam et al., 2004). Fortification of mulberry leaves with complementary compounds was found to increase the larval growth and post cocoon characteristics (Etebari, 2002; Etebari and Fazilati, 2003).

Ionic silver has a long history of use in topical medical applications, and it has been shown that ionic silver, in the right quantities, is suitable in treating wounds (Qin et al., 2005; Hermans, 2006; Chopra, 2007; Atiyeh et al., 2007). The US Food and Drug Administration have approved the use of a range of different silver-impregnated wound dressings. Silver nanoparticles are now replacing silver sulfadiazine as an effective agent in the treatment of wounds (Lansdown, 2006; Atiyeh et al., 2007). These Silver nanoparticles are under active research because they posses interesting physical properties differing considerably from that of the bulk phase. It comes from small sizes and high surface/volume ratio (Patel et al., 2005). Metallic silver colloids were first prepared more than a century ago. Ag nanoparticles can be synthesized using various methods: chemical, electrochemical (Vorobyova et al., 1995). The most popular preparation of Ag colloids is chemical reduction of silver salts by Tri sodium citrate. This preparation is simple, but the great care must be exercised to make stable and reproducible colloid. The purity of water and reagents, cleanliness of the
Nutrition plays an important role in improving the growth and development of the silkworm, *B. mori* L. like other organisms. Legay, (1958) has stated that silk production is dependent on the larval nutrition and nutritive value of mulberry leaves plays a very effective role in producing good quality cocoons. Seki and Oshikana, (1959) have observed better growth and development of silk-worm larvae as well as good quality cocoons when fed on nutritionally enriched leaves. Silkworms obtain its entire nutritional requirement from mulberry leaves because this insect is monophagous and can complete the life cycle on mulberry leaves exclusively. Studies of Ito, (1978) have determined that generally vitamins present in the mulberry leaves satisfy minimum needs of silkworm but the amount of vitamins present in mulberry leaves varies on the basis of environmental conditions, usage of fertilizers in field and mulberry varieties and other field practices. Sengupta et al. (1972) have showed that *B. mori* requires specific essential sugars, amino acids, proteins and vitamins for its normal growth, survival and also for the silk gland activity and growth. Akhtar and Asghar, (1972) have found that vitamins and mineral salts played an important role in the nutrition of silkworm. Keeping the importance of vitamins and other compounds like silver nanoparticles on silkworm nutrition are very effective. Mulberry silkworm (*B. mori*) sustains its nutrition from its food plants of *Morus alba*. The nutritional levels of *M. alba* mulberry influence the larval growth of silkworm mulberry leaves treated with some other compounds like silver nanoparticles, which ultimately influence the economic traits such as silk yield, larval and cocoon parameters (Lenth, width and weight). The present study has been aimed to find out the feed efficacy of AgNps treated *M. alba* mulberry leaves with regard to food utilization by larvae and ultimate impact on the cocoon parameters of silkworm so as to spot out the most nutritive one for bivoltine silkworm in Tamilnadu climatic conditions. The work is related to the studies on the growth rate of *B. mori* fed with control and silver nanoparticles treated *M. alba* mulberry leaves are fragmentary. Therefore, this study has been carried out to know the impact of silver nanoparticles on *B. mori*.

Materials and Methods
The eggs of silkworm *B. mori* LNB4, D2 (Local Bivoltine) race 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 5 experimental groups including controls (distilled water control), each group consisting of 6 larvae. The larvae were reared in card board boxes measuring 22×15×5 cms covered with polythene sheet and placed in an iron stand with ant wells. The larvae were subjected to the following treatments. AgNps were synthesized by chemical reduction method. It was diluted in distilled water 25%, 50%, 75% and 100% (without dilution) concentrations. Fresh mulberry leaves were sprayed by each concentration and then dried in air for 10 minutes. The supplementary leaves were fed to silkworms, five feedings / day. Group T1 larvae received mulberry leaves sprayed with distilled water and served as control, group T2 larvae received 25% AgNps sprayed mulberry leaves, group T3 larvae received 50% AgNps sprayed mulberry leaves, group T4 larvae received 75% AgNps sprayed mulberry leaves, group T5 larvae received 100% AgNps sprayed mulberry leaves, respectively and they were maintained up to cocoon. 3rd, 4th and 5th instar larvae length, width and weight, cocoon length, width and weight were determined for all groups.

Preparation of Silver Nanoparticles (AgNps)
Silver nitrate AgNO₃ (Sigma Aldrich, UK) and Trisodium Citrate C₆H₅O₃Na₃ (Sigma Aldrich, UK) of analytical grade purity, were used as starting materials without further purification. The silver colloid was prepared by using chemical reduction method according to the description of Lee and Meisel, (1982). All solutions of reacting materials were prepared in distilled water. In typical experiment, 50 ml of 1·10⁻³ M AgNO3 was heated to boiling. To this solution, 5 ml of 1% Trisodium Citrate was added drop by drop. During the process, solution was mixed vigorously. Solution was heated until color’s change is evident (pale yellow). Then it was removed from the heating element and stirred until cooled at room temperature. Mechanism of reaction could be expressed as follows:

\[
4Ag^+ + C₆H₅O₃Na₃ + 2H₂O \rightarrow 4Ag0 + C₆H₅O₃H₃ + 3Na^+ + H^+ + O₂ ↑
\]

Mulberry (*M. alba*) MR₂ variety
This is one of the varieties of mulberries selected from Jayamkondapattinam sericulture farm. Branches are simple, vertical, grayish leaves are darkly green, unlobed, elliptic, palmate, veined, and leathery/smooth/wrinkled. It has good agronomy characters like high rooting ability (80%).

Mulberry (*M. alba*) MR₂ leaves treated with Silver Nanoparticles (AgNps)
AgNps was prepared by chemical reduction method
according to Lee and Meisel (1982). It was diluted to 25%, 50%, 75% and 100% (without dilution) concentrations. Fresh mulberry leaves were soaked in each concentration for 15 minutes and then were dried in air for 10 minutes. The treated leaves were used for feeding the 3rd, 4th and 5th instar larvae of silkworm <i>Bombyx mori</i>.

**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 means ± SD. P values < 0.05 were regarded as statistically significant.

**Results**

**Larval Parameters**

**Morphometric analysis of 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> instar larvae than control**
Table 1 shows that the Morphometric data of length, width and weight of larval parameters of <i>B. mori</i> fed with control MR<sub>2</sub> leaves and AgNps treated MR<sub>2</sub> leaves in 3<sup>rd</sup> instar larvae of <i>B. mori</i>. The mean length, width and weight of 3<sup>rd</sup> instar larvae of group T<sub>1</sub> were (1.6833 ± 0.14720cm, 0.3500 ± 0.05477cm and 0.1117 ± 0.00753gm), respectively. The mean length, width and weight of 3<sup>rd</sup> instar larvae of group T<sub>2</sub> were (1.9333 ± 0.16330cm, 0.3833 ± 0.04082cm and 0.1233 ± 0.01033gm), respectively. The mean length, width and weight of 3<sup>rd</sup> instar larvae of group T<sub>3</sub> were (1.8500 ± 0.10488cm, 0.3500 ± 0.05477cm and 0.1100 ± 0.00894gm), respectively. The mean length, width and weight of 3<sup>rd</sup> instar larvae of group T<sub>4</sub> were (1.8833 ± 0.14720cm, 0.3500 ± 0.05477cm and 0.1150 ± 0.01049gm), respectively. The mean length, width and weight of 3<sup>rd</sup> instar larvae of group T<sub>5</sub> were (1.8667 ± 0.13663cm, 0.3667 ± 0.05164gm, and 0.1133 ± 0.00816gm).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Length (cm) (Mean ± S.D)</th>
<th>Width (cm) (Mean ± S.D)</th>
<th>Weight (gm) (Mean ± S.D)</th>
</tr>
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<tbody>
<tr>
<td>Control (T&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>1.6833 ± 0.14720&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.3500 ± 0.05477&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1117 ± 0.00753&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>MR&lt;sub&gt;2&lt;/sub&gt; mulberry+25% AgNps (T&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>1.9333 ± 0.16330&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3833 ± 0.04082&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1233 ± 0.01033&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MR&lt;sub&gt;2&lt;/sub&gt; mulberry+50% AgNps (T&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>1.8500 ± 0.10488&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3500 ± 0.05477&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1100 ± 0.00894&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MR&lt;sub&gt;2&lt;/sub&gt; mulberry+75% AgNps (T&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>1.8833 ± 0.14720&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3500 ± 0.05477&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1150 ± 0.01049&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>MR&lt;sub&gt;2&lt;/sub&gt; mulberry+100% AgNps (T&lt;sub&gt;5&lt;/sub&gt;)</td>
<td>1.8667 ± 0.13663&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3667 ± 0.05164&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1133 ± 0.00816&lt;sup&gt;ab&lt;/sup&gt;</td>
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Values are Mean ± S.D of six observations. Values in the same column with different superscript letters (a & b) differs significantly at P<0.05 (DMRT).

Table 1. Morphometric data of various concentrations of AgNps treated with MR<sub>2</sub> mulberry leaves on the 3<sup>rd</sup> instars larvae length, width and weight of <i>Bombyx mori</i>.
instar larvae of group T₃ were (7.0000 ± 0.17889cm, 1.0000 ± 0.14142cm and 3.0950 ± 0.59702gm), respectively. The mean length, width and weight of 5th instar larvae of group T₄ were (6.9833 ± 0.14720cm, 1.0000 ± 0.35809cm, 0.9667 ± 0.35229gm), respectively. In these five observations, 25% AgNps treated 5th instar larvae length, width and weight was significantly increased than the other four groups (T₁, T₂, T₃ and T₄).

**Cocoon parameters**

Table 4 shows the Morphometric data of mean length, width and weight of Bombyx mori.
width and weight of the cocoon of *B. mori* fed with AgNps treated MR₂ leaves were found to be more than that of the larvae fed with control MR₁ leaves. The length, width and weight of the T₁ larvae produced cocoon were found to be about (3.4000 ± 0.3847 cm, 2.1333 ± 0.1211 cm and 1.5117 ± 0.1006 gm), respectively. The length, width and weight of the T₂ larvae produced cocoon were observed to be about (3.6667 ± 0.1633 cm, 2.4000 ± 0.0894 cm and 2.2117 ± 0.3631 gm), respectively. The length, width and weight of the T₃ larvae producing cocoon were observed to be about (6.9833 ± 0.3834 cm, 1.0000 ± 0.0632 cm and 3.2250 ± 0.3522 gm), respectively. The length, width and weight of the T₄ larvae produced cocoon were observed to be about (6.7500 ± 0.3834 cm, 0.9667 ± 0.1366 cm and 2.9550 ± 0.3580 gm), respectively. In these five observations, the 25% AgNps treated larvae produced cocoon length, width and weight were significantly increased than the other four groups (T₁, T₃, T₄ and T₅).

**Discussion**

In the present study, the larval and cocoon length, width and weight were significantly increased in some groups. Many researchers showed that the larval characters improve by different concentrations of complementary compounds such as ascorbic acid, folic acid, thiamin, vitamin B complex etc., (Sarker et al., 1995; Nirwani and Kalival, 1996, 1998; Etaberi et al., 2004; Balasundaram et al., 2008). Muniansy et al. (1995) have showed that multi-vitamins and mineral compounds could increase the food intake, growth and conversion efficiency of silk-worm. In the present study, it has been observed that silk-worms fed by the particular dose of AgNps have enhanced the larval length, width and weight and cocoon characters were concomitantly increased from 3rd to 5th instars, suggested that AgNps which were stimulate silk-worm to feed more amount of nutrients intake than the control. This work is corroborated with Nirwani and Kalival, (1996), suggested that this enhancement in larval and cocoon length, width and weight related to phagostimulation of folic acid. Several authors also reported these effects about ascorbic acid (Dobzhenok, 1974; Ito, 1978; Singh and Reddy, 1981; KI-Karkasy and Idriiss, 1990).

Since most of this multi-vitamin compounds is composed of ascorbic acid, it could be thought that the increase of larval weight is due to an enhancement of feeding activity in treated larvae although the vitamins as cofactors can facilitate the metabolic pathway. Similar findings have also been observed in the present study that AgNps act as vitamins to stimulate the feeding activity in the silkworms. Therefore, AgNps can improve the food digestibility and increase the larval and cocoon length, width and weight.

In this study, cocoon parameters changed in different treatments. Previously, it was reported that enrichment of mulberry leaves by some vitamins could increase the cocoon yield. Nirwani and Kalival, (1996) have determined that folic acid causes a significant increase in economical parameters such as female and male cocoon weight. Evanglista et al. (1997) have also reported that the larval and cocoon length, width and weight increase under multi-vitamin treatment.

The enrichment of mulberry leaves with AgNps increase larval and cocoon length, width and weight increase in these insects was related to metabolisms other than proteins. It is assumed that fortification of diet supports the metabolism of carbohydrates and lipids, in conclusion, AgNps could increase some biological characteristics in silkworm, but this enhancement could economically improve the Sericulture goals.

In the present study, the treatment of AgNps at the concentration of 25% may have beneficial effects on the growth of the silkworm larval and pupal length, width and weight and also increased the quantity of silk production by enhancing the feed efficacy than control. So, this supplementation could be prescribed to the farmers to get more quantity of silk.

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