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Studies on the Growth Rate of Silkworm *Bombyx mori* (L.) (Lepidoptera: Bombycidae) Fed with Control and Silver Nanoparticles (AgNps) Treated MR_2 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 MR_2 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 T_1 larvae received MR_2 mulberry leaves sprayed with distilled water and served as control, group T_2, T_3, T_4 and T_5 larvae received 25%, 50%, 75% and 100% AgNps sprayed mulberry leaves, respectively. Silkworm larvae fed on *M. alba* (MR_2) leaves sprayed with 25% concentration of AgNps (group T_2) was significantly increased the larvae and cocoon length, width and weight as compared to those fed on control (group T_1) MR_2 mulberry leaves and other groups (T_3, T_4 and T_5). Hence, 25% AgNps dose was fixed as an effective dose. It has been observed from the present study that 25% AgNps treated (group T_2) 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), MR_2 Mullbery leaves

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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 glassware are critical parameters. Solution temperature, concentrations of the metal salt and reducing agent, reaction time influences particle size. Controlling size and shape of metal nanoparticles remains a challenge (Bell *et al*., 2001).

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 Oshikane, (1959) have observed better growth and development of silkworm 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 MR_2 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 (Lengh, width and weight). The present study has been aimed to find out the feed efficacy of AgNps treated MR_2 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 MR_2 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^oC 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 $22\times15\times5$ 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 T_1 larvae received mulberry leaves sprayed with distilled water and served as control, group T_2 larvae received 25% AgNps sprayed mulberry leaves, group T_3 larvae received 50% AgNps sprayed mulberry leaves, group T_4 larvae received 75% AgNps sprayed mulberry leaves, group T_5 larvae received 100% AgNps sprayed mulberry leaves, respectively and they were maintained up to cocoon. 3^{rd}, 4^{th} and 5^{th} instar larvae length, width and weight, cocoon length, width and weight were determined for all groups.

Preparation of Silver Nanoparticles (AgNps)

Silver nitrate $AgNO_3$ (Sigma Aldrich, UK) and Trisodium Citrate $C_6H_5O_7Na_3$ (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 $1\cdot10^{-3}$ 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:

$$4Ag^+ + C_6H_5O_7Na_3 + 2H_2O \rightarrow$$

$$4Ag0 + C_6H_5O_7H_3 + 3Na^{+\ +} + H^+ + O_2\uparrow$$

Mulberry (*M. alba*) MR_2 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*) MR_2 leaves treated with Silver Nanoparticles (AgNps)

AgNps was prepared by chemical reduction method

Table 1. Morphometric data of various concentrations of AgNps treated with MR_2 mulberry leaves on the 3rd instars larvae length, width and weight of *Bombyx mori*.

3rd instar larvae			
Groups	Length (cm) (Mean ± S.D)	Width (cm) (Mean ± S.D)	Weight (gm) (Mean ± S.D)
Control (T_1)	1.6833 ± 0.14720^{a}	0.3500 ± 0.05477^{a}	0.1117 ± 0.00753^{ab}
MR_2 mulberry+25% AgNps (T_2)	1.9333 ± 0.16330^{b}	0.3833 ± 0.04082^{a}	0.1233 ± 0.01033^{b}
MR_2 mulberry+50% AgNps (T_3)	1.8500 ± 0.10488^{ab}	0.3500 ± 0.05477^{a}	0.1100 ± 0.00894^{a}
MR_2 mulberry+75% AgNps (T_4)	1.8833 ± 0.14720^{b}	0.3500 ± 0.05477^{a}	0.1150 ± 0.01049^{ab}
MR_2 mulberry+100% AgNps (T_5)	1.8667 ± 0.13663^{b}	0.3667 ± 0.05164^{a}	0.1133 ± 0.00816^{ab}

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).

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 *B. mori*.

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 3rd, 4th and 5th instar larvae than control

Table 1 shows that the Morphometric data of length, width and weight of larval parameters of *B. mori* fed with control MR_2 leaves and AgNps treated MR_2 leaves in 3rd instar larvae of *B. mori.* The mean length, width and weight of 3rd instar larvae of group T_1 were (1.6833 ± 0.14720 cm, 0.3500 ± 0.05477cm and 0.1117 ± 0.00753 gm), respectively. The mean length, width and weight of 3rd instar larvae of group T_2 were (1.9333 ± 0.16330cm, 0.3833 ± 0.04082cm and 0.1233 ± 0.01033gm), respectively. The mean length, width and weight of 3rd instar larvae of group T_3 were (1.8500 ± 0.10488cm, 0.3500 ± 0.05477cm and 0.1100 ± 0.00894gm), respectively. The mean length, width and weight of 3rd instar larvae of group T_4 were (1.8833 ± 0.14720cm, 0.3500 ± 0.05477cm and 0.1150 ± 0.01049gm), respectively. The mean length, width and weight of 3rd instar larvae of group T_5 were (1.8667 ± 0.13663cm, 0.3667 ± 0.05164cm and 0.1133 ± 0.00816gm), respectively. In these five observations, 25% AgNps treated 3rd instar larvae length, width and weight were significantly increased than the other four groups (T_1, T_3, T_4 and T_5).

Table 2 shows that the Morphometric data of length, width and weight of larval parameters of *B. mori* fed with control MR_2 leaves and AgNps treated MR_2 leaves in 4th instar larvae of *B. mori.* The mean length, width and weight of 4th instar larvae of group T_1 were (5.7333 ± 0.24221cm, 0.5333 ± 0.08165cm and 0.4233 ± 0.03445gm), respectively. The mean length, width and weight of 4th instar larvae of group T_2 were (6.0167 ± 0.14720cm, 0.6167 ± 0.07528cm and 0.5450 ± 0.04037gm), respectively. The mean length, width and weight of 4th instar larvae of group T_3 were (5.4500 ± 0.18708cm, 0.6000 ± 0.08944cm and 0.4350 ± 0.03834gm), respectively. The mean length, width and weight of 4th instar larvae of group T_4 were (5.4500 ± 0.20736cm, 0.6500 ± 0.05477cm and 0.4150 ± 0.02739gm), respectively. The mean length, width and weight of 4th instar larvae of group T_5 were (5.4000 ± 0.20976cm, 0.6500 ± 0.05477cm and 0.4150 ± 0.02429gm), respectively. In these five observations, 25% AgNps treated 4th instar larvae length, width and weight were significantly increased than the other four groups (T_1, T_3, T_4 and T_5).

Table 3 shows that the Morphometric data of length, width and weight of larval parameters of *B. mori* fed with control MR_2 leaves and AgNps treated MR_2 leaves in 5th instar larvae of *B. mori.* The mean length, width and weight of 5th instar larvae of group T_1 were (6.7167 ± 0.24833cm, 1.0333 ± 0.12111cm and 2.8350 ± 0.08550gm), respectively. The mean length, width and weight of 5th instar larvae of group T_2 were (7.2500 ± 0.18708cm, 1.1333 ± 0.08165cm and 3.5583 ± 0.23693gm), respectively. The mean length, width and weight of 5th

Table 2. Morphometric data of various concentrations of AgNps treated with MR_2 mulberry leaves on the 4th instars larvae length, width and weight of *Bombyx mori*.

4th instar larvae			
Groups	Length (cm) (Mean ± S.D)	Width (cm) (Mean ± S.D)	Weight (gm) (Mean ± S.D)
Control (T_1)	5.7333 ± 0.24221^{b}	0.5333 ± 0.08165^{a}	0.4233 ± 0.03445^{a}
MR_2 mulberry+25% AgNps (T_2)	6.0167 ± 0.14720^{c}	0.6167 ± 0.07528^{ab}	0.5450 ± 0.04037^{b}
MR_2 mulberry+50% AgNps (T_3)	5.4500 ± 0.18708^{a}	0.6000 ± 0.08944^{ab}	0.4350 ± 0.03834^{a}
MR_2 mulberry+75% AgNps (T_4)	5.4500 ± 0.20736^{a}	0.6500 ± 0.05477^{b}	0.4150 ± 0.02739^{a}
MR_2mulberry+100% AgNps (T_5)	5.4000 ± 0.20976^{a}	0.6500 ± 0.05477^{b}	0.4150 ± 0.02429^{a}

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 3. Morphometric data of various concentrations of AgNps treated with MR_2 mulberry leaves on the 5th instars larvae length,width and weight of *Bombyx mori*.

5th instar larvae			
Groups	Length (cm) (Mean ± S.D)	Width (cm) (Mean ± S.D)	Weight (gm) (Mean ± S.D)
Control (T_1)	6.7167 ± 0.24833^{a}	1.0333 ± 0.12111^{ab}	2.8350 ± 0.08550^{a}
MR_2 mulberry+25% AgNps (T_2)	7.2500 ± 0.18708^{c}	1.1333 ± 0.08165^{b}	3.5583 ± 0.23693^{b}
MR_2 mulberry+50% AgNps (T_3)	7.0000 ± 0.17889^{bc}	1.0000 ± 0.14142^{ab}	3.0950 ± 0.59702^{a}
MR_2 mulberry+75% AgNps (T_4)	6.9833 ± 0.14720^{bc}	1.0000 ± 0.06325^{ab}	3.2250 ± 0.35229^{ab}
MR_2mulberry+100% AgNps (T_5)	6.7500 ± 0.38341^{b}	0.9667 ± 0.13663^{a}	2.9550 ± 0.35809^{a}

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

Table 4. Morphometric data of various concentrations of AgNps treated with MR_2 mulberry leaves on the cocoon length, width and weight of *Bombyx mori*.

Cocoon of *Bombyx mori.*			
Groups	Length (cm) (Mean ± S.D)	Width (cm) (Mean ± S.D)	Weight (gm) (Mean ± S.D)
Control (T_1)	3.4000 ± 0.38471^{ab}	2.1333 ± 0.12111^{a}	1.5117 ± 0.10068^{a}
MR_2 mulberry+25% AgNps (T_2)	3.6667 ± 0.16330^{b}	2.4000 ± 0.08944^{b}	2.2117 ± 0.36318^{b}
MR_2 mulberry+50% AgNps (T_3)	3.4000 ± 0.12649^{ab}	2.0833 ± 0.09832^{a}	1.5500 ± 0.15633^{a}
MR_2 mulberry+75% AgNps (T_4)	3.3167 ± 0.24014^{a}	2.1167 ± 0.11690^{a}	1.3967 ± 0.18151^{a}
MR_2mulberry+100% AgNps (T_5)	3.3000 ± 0.20976^{a}	2.1667 ± 0.15055^{a}	1.5850 ± 0.21961^{a}

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).

instar larvae of group T_3 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 T_4 were (6.9833±0.14720cm, 1.0000±0.06325cm and 3.2250±0.35229gm), respectively. The mean length, width and weight of 5th instar larvae of group T_5 were (6.7500±0.38341cm, 0.9667±0.13663cm and 2.9550±0.35809gm), respectively. In these five observations, 25% AgNps treated 5th instar larvae length, width and weight was significantly increased than the other four groups (T_1, T_3, T_4 and T_5).

Cocoon parameters

Table 4 shows the Morphometric data of mean length, width and weight of the cocoon of *B. mori* fed with AgNps treated MR_2 leaves were found to be more than that of the larvae fed with control MR_2 leaves. The length, width and weight of the T_1 larvae produced cocoon were found to be about (3.4000 ± 0.38471 cm, 2.1333 ± 0.12111 cm and 1.5117 ± 0.10068 gm), respectively. The length, width and weight of the T_2 larvae produced cocoon were observed to be about (3.6667 ± 0.16330 cm, 2.4000 ± 0.08944 cm and 2.2117 ± 0.36318gm), respectively. The length, width and weight of the T_3 larvae producing cocoon were observed to be about (7.0000 ± 0.17889 cm, 1.0000 ± 0.14142 cm and 3.0950 ± 0.59702gm), respectively. The length, width and weight of the T_4 larvae produced cocoon were observed to be about (6.9833 ± 0.14720 cm, 1.0000 ± 0.06325 cm and 3.2250 ± 0.35229gm), respectively. The length, width and weight of the T_5 larvae produced cocoon were observed to be about (6.7500 ± 0.38341 cm, 0.9667 ± 0.13663 cm and 2.9550 ± 0.35809gm), respectively. In these five observations, the 25% AgNps treated larvae produced cocoon length, width and weight were significantly increased than the other four groups (T_1, T_3, T_4 and T_5).

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 Kaliwal, 1996, 1998; Etaberi *et al*., 2004; Balasundaram *et al.*, 2008). Muniandy *et al*., (1995) have showed that multi-vitamins and mineral compounds could increase the food intake, growth and conversion efficiency of silkworm. In the present study, it has been observed that silkworms fed by the particular dose of AgNps have enhanced the larval length, width and weight and cocoon characters were concomitantly increased from 3^{rd} to 5^{th} instars, suggested that AgNps which were stimulate silkworm to feed more amount of nutrients intake than the control. This work is corroborated with Nirwani and Kaliwal, (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 (Dobzshenok, 1974; Ito, 1978; Singh and Reddy, 1981; Kl-Karkasy and Idriss, 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 metabolical 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 Kaliwal, (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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