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RESEARCH ARTICLE

STUDIES ON THE QUANTITATIVE PARAMETERS OF SILKWORM *Bombyx mori* (L.) (LEPIDOPTERA: BOMBYCIDAE) FED WITH CONTROL AND SILVER NANPOARTICLES (AGNPS) TREATED V1 MULBERRY LEAVES

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ABSTRACT

The silkworm, *Bombyx mori* being a monophagous insect, derives all the nutrients required for its growth from the mulberry leaves. The quality of silk produced by the silkworm depends on the quality and yield of mulberry leaf as well as environmental conditions. The larval and pupal parameters of silkworm *Bombyx mori* fed with silver nanoparticles (AgNps) treated V1 mulberry leaves, the following works have been considered. The AgNps was synthesized by chemical method, it was diluted by different concentrations such as 25%,50 % and 75% (without dilution) fresh mulberry leaves (*Morus indca L.*) were sprayed by each concentration and were fed to silkworm from 3rd , 4th ,5th instar for four feeding were recommended. Then, group T, larva received V1 mulberry leaves sprayed with distilled water and served as control, group T2, T3, and T4 larvae received 25%, 50% and 75% AgNps sprayed mulberry leaves, respectively. Silkworm larvae fed on *M.indica* (V1) leaves sprayed with 25% concentration of AgNps (group T2) was 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 T1) V1 mulberry leaves and other groups (T3 and T4). 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.

INTRODUCTION

The *Bombyx mori* is essentially monophagous and survives solely on mulberry leaves (*Morus indica*) which play an important role in the nutrition of the silkworms, and in turn cocoon and silk production (Nagaraju, 2002). The nutritional elements of mulberry leaves determine the growth and development of the larvae and cocoon producton (Seidavi *et al*., 2005). It has also been demonstrated that the dietary nutritional management has a direct influence on quality and quantity of silk production in *B.mori* (Murugan *et al*., 1998). The carbon nanotubes have attracted significant scientific interest ranging from nano electronic to biomedical devices because of their unique structure and properties. Nanoparticles posses a very high surface to volume ratio. This can be utilized in areas where high surface areas are critical for success. The principal parameters of nanoparticles are their shape, size and the morphological sub structure of the substances. Silver nanoparticles are silver precursors like silver citrate, silver acetate and silver nitrate. Silver particles size between 1 nm to 100 nm while, frequently described as being 'silver' some are composed of a large percentage of silver oxide due to their large ratio of surface to bulk silver atoms. Nutritional studies in silkworm with respect to food utilization (Ueda, 1982; Horie and Watanabe, 1983), relation between growth, body weight, food digested and ingested, silk gland weight, food consumption and relative rates (Mathavan and Pandian, 1974, Mathavan *et al*., 1987 and Chenthilnayaki, 2004) have been elucidated. The amount and quality of food ingested in the larval phase affect the growth rate, developmental period, body weight and survival rate and also influence the fecundity, longevity. Development of *Bombyx mori* in India and Japan revealed that, food consumption, digestion and food assimilation influence the cocoon production (Naik and Delvi, 1987 and Chenthilnayaki, 2004). The silkworms require certain essential sugars, proteins, amino acids, fatty acids and vitamins for their normal growth and survival. These essential components are necessary for the growth of silk gland and higher production of seed and silk (Ito, 1978). The nutritional levels of V1 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 larval and cocoon parameters and cocoon shell, pupal weight, shell ratio, silk filament length. The present study has been aimed to find out the feed efficacy of AgNps treated V1 mulberry leaves with regard to food utilization by larvae and ultimate impact on the cocoon parameters of silkworm. The work in related to the studies on the quantitative parameters of *B. mori* fed with control and silver nanoparticle treated V1 mulberry leaves (*Morus indica*) are scanty. Therefore, this study has been programmed in the present study to find out the impact of silver nanoparticles on *Bombyx mori***.**

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MATERIALS METHODS

The eggs of silkworm *B. mori* (CSR2 X CSR4) (Local Bivoltine) race were collected from silkworm culture centre at 2nd Agraharam, Salem and Neyveli in Tamilnadu, India. The first day of 3rd instar larvae were placed at ambient temperature of 25 ± 27°C and relative humidity of 70 to 80%. The larvae were reared in card board boxes measuring 22 x15x 5 cms covered with nylon net and placed in an ion stand with ant wells. The larvae were divided into four experimental groups including control (distilled water treatment) and silver nanoparticles treated groups (25%, 50% and 75%) Each group contains 100 larvae. Fresh mulberry leaves were sprayed by each concentration and then dried in air for 10 minutes. The supplementary leaves were fed to silkworm, five feedings/day. Group T1 larvae fed with distilled water cleaned mulberry leaves, it serve as a control, group T2 larvae fed with 25% silver nanoparticles treated mulberry leaves, group T3 larvae fed with 50 % silver nanoparticles treated mulberry leaves and group T4 larvae fed with 75% silver nanoparticles treated mulberry leaves, respectively, and they were maintained up to cocoon 3rd, 4th, 5th instar larvae length widith, weight, cocoon, length, width, weight cocoon shell weight, pupal weight, shell ratio, silk, filament length were determined for all groups.

Preparation of Silver nanoparticles

Silver nitrate AgNO3 and Trisodium citrate C6H5O7Na3 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, 5ml of 1% Trisodium citrate was added drop by drop. During this process, solution was mixed vigorously. Solution was heated until color change is evident (dark brown in colour) the formation of silver nanoparticles in colloid. Then the solution was removed from the heating mantle and stirred until cooled at room temperature.

Mulberry (*Morus indica*) V1 Variety

This is one of the varieties of mulberry plant. It was selected from faculty of Agriculture, Annamalai University, Annamalainagar, Tamilnadu, India. This mulberry plant branches are simple, vertical, grayish leaves are light green, unlobed, elliptic palmately veined, leathery / smooth/wrinkled, it has good agronomic characters like high rooting ability (80%).

Chemical Composition of V1 Mulberry Leaf

Chemical composition of leaf varies with variety and maturity. However on the basis of the analysis carried out at CSR & TI Mysore, the chemical composition of the leaf is as follows.
Moisture : 72.5 – 78.9%
Protein : 24.6%
Minerals : 10-15%
Reducing sugars : 1.2-1.9
Sugar : 16.98%

Mulberry (*M. indica*) V1 leaves treated with Silver Nanoparticles (AgNps)

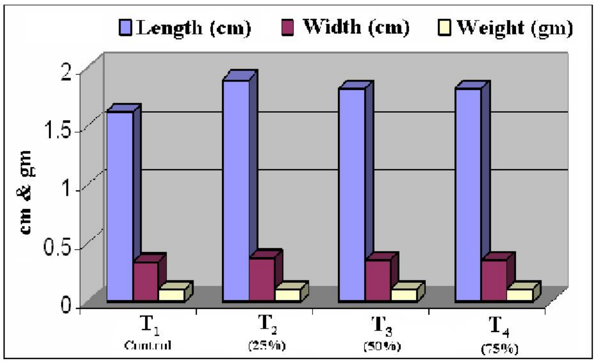
AgNps was prepared by chemical reduction method according to Lee and Meisel (1982). It was diluted to 25%, 50% and 75% without dilution concentrations. Fresh mulberry leaves were soaked in each concentration for 15 minutes and then were dried in air for 10 minutes. The treated leaves were used for feeding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

Statistical Analysis

Data were analyzed by One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). Results were presented as means ± SD p< 0.05 were regarded as statistically significant (Sokal and Rohlf, 1981).

RESULTS AND DISCUSSION

Table 1 shows that the morphometric data of control V1 mulberry leaves and silver nanoparticles treated V1 mulberry leaves fed 3rd instar of *B. mori* larvae length, width, and weight. The mean value of control (group T1) were (1.6201±0.1391cm, 0.3350±0.0447cm and 0.0984±0.0062gm), respectively. The mean value of 25% silver nanoparticles treated group T2 were (1.9024±0.1548cm, 03724±0.0592cm and 0.1147±0.0163gm), respectively. The mean value of 50% silver nanoparticles treated group T3 were (1.8235±0.1467cm, 0.3630±0.0467cm and 0.1038±0.0079gm), respectively. The mean value of 75% silver nanoparticle treated group (T4) were (1.8136± 0.1473cm, 0.3550±0.0497cm and 0.1023±0.0074gm), respectively, in these four observations, 25% silver nano particles (group T2) treated 3rd instar larvae length and weight was significantly increased than control (T1) and other two groups (T3 and T4) (Fig. 1). 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 silver nanoparticles treated V1 mulberry leavers followed by control V1 mulberry leaf. Among the V1 mulberry leaves, AgNps treated V1 mulberry leaves have gained maximum body weight, cocoon weight, and silk trait than the control V1 mulberry leaf.



Graph 1. Morphometric data of control and silver nanoparticles treated III instar larvae of *Bombyx mori*.

Table 1. Morphometric data of various concentrations of AgNps treated with V_1 mulberry leaves on the 3rd instars larvae length, width and weight of *Bombyx mori.*

III instar larvae			
Groups	Length (cm) (Mean ± S.D)	Width (cm) (Mean ± S.D)	Weight(gm) (Mean ± S.D)
Control ($_{T1}$)	1.6201±0.1391^a	0.3350± 0.0447^a	0.0984±0.0062^a
V_1 mulberry + 25% AgNps	1.9024±0.1548^b	0.3724± 0.0592^b	0.1147 ±0.0163^b
V_1 mulberry + 50% AgNps (T_3)	1.8235± 0.1467^{ab}	0.3630± 0.0467^a	0.1038 ± 0.0079^{ab}
V_1 Mulberry + 75% AgNps (T_4)	1.8136 ± 0.1473^{ab}	0.3550± 0.0497^a	0.1023 ±0.0074^{ab}

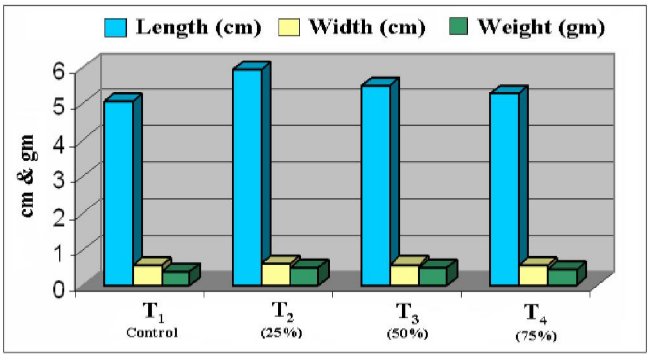
Values are Mean ± S.D of six observations. Values in the same column with different superscript letters (a-b) differ significantly at p < 0.05 (DMRT).

Table 2. Morphometric data of various concentrations of AgNps treated with V_1 mulberry leaves on the 4th instars larvae length, width, weight, of *Bombyx mori.*

IV instar larvae			
Groups	Length (CM) (Mean ± S.D)	Width (CM) (Mean ± S.D)	Weight(gm) (Mean ± S.D)
Control ($_{T1}$)	5.0561±0.1822^a	0.5633± 0.0365^{ab}	0.4133±0.02045^a
V_1 mulberry + 25% AgNps (T_2)	5.9187±0.2760^b	0.6067± 0.0728^b	0.5050 ±0.03137^b
V_1 mulberry + 50% AgNps (T_3)	5.4850± 0.1986^a	0.5901± 0.0577^{ab}	0.4850± 0.02939^a
V_1 Mulberry + 75% AgNps (T_4)	5.2740 ± 0.1978^a	0.5800± 0.0494^{ab}	0.4650 ±0.02734^a

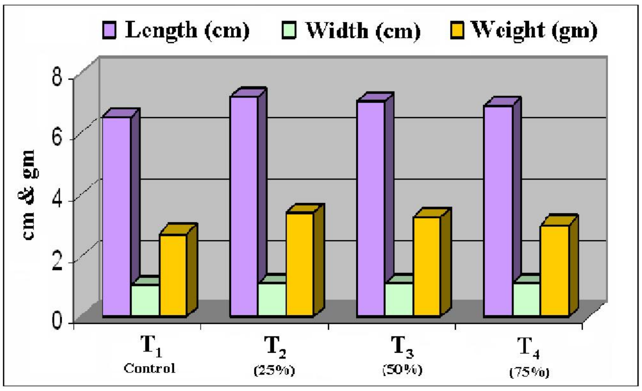
Values are Mean ± S.D of six observations. Values in the same column with different superscript letters (a-b) differ significantly at p< 0.05 (DMRT).

Table 2 shows that the morphometric data of control V1 mulberry leaves and silver nanoparticles treated V1 mulberry leaves fed 4th instar of *B.mori* larvae length, width, and weight. The mean value of control (group T1) were (5.0561±0.1822cm, 0.5633±0.0365cm and 0.4133±0.02045gm), respectively. The mean value of 25% silver nanoparticles treated group (T2) were (5.9187±0.2760cm, 0.6067±0.0728cm and 0.5050±0.03137gm), respectively. The mean value of 50% silvernanaparticles treated group (T3) were (5.4850±0.1986cm, 0.5901±0.0577cm and 0.450±0.02939gm), respectively. The mean value of 75% silver nanoparticles treaded group (T4) were (5.2740±0.1978 cm, 0.5800±0.0494cm and 0.4650±0.02734g), respectively. In these four observations, 25% silver nanoparticles (group T2) treated 4th instar larvae length, width and weight were significantly increased than control (T1) and other two groups (T3 and T4) (Fig. 2). From the present observations, it has also been evident that consistently better rearing performance was obtained from feeding of leaves of silver nanoparticles treated V1 mulberry leaves over another one is control V1 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 AgNps treated mulberry leaves with respect of higher content of protein, carbohydrate and moisture content which ultimately resulted in the production of an higher yield and better quality cocoon.



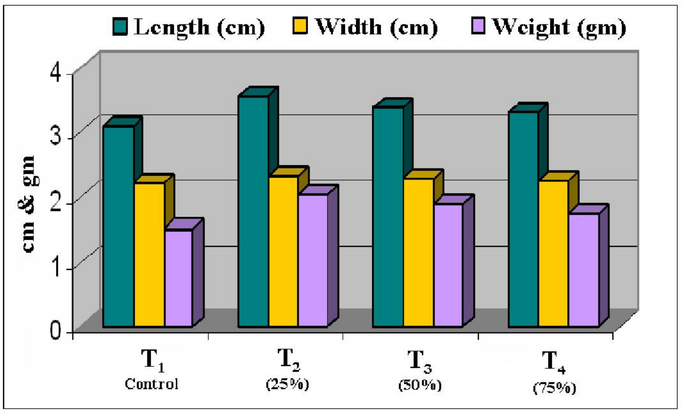
Graph 2. Morphometric data of control and silver nanoparticles treated IV instar larvae of *Bombyx mori*.

Table 3 shows that the morphometric data of control V1 mulberry leaves and silver nanoparticles treated V1Mulberry leaves fed 5th instar of *B. mori* larvae length, width and weight. The mean values of control (group T1) were (6.5187±0.1933cm, 1.063±0.0571cm, and 2.7390±0.1050gm), respectively. The mean value of 25% silver nanoparticles treated group (T2) were (7.1550±0.2857cm, 1.103±0.1216cm and 3.4553±0.2369gm), respectively. The mean value of 50% silver nanoparticles treated group (T3) were (7.0340±0.2389cm, 1.090±0.0914cm and 3.2950±0.1702gm), respectively. The mean value of 75% silver nanoparticles treated group (T4) were (6.8785±0.2020cm, 1.080±0.0732cm and 3.0230±0.1329gm), respectively. In these four observations, 25% silver nanoparticles (group T2) treated 5th instar larvae length, width and weight were significantly increased than control (T1) and other two groups (T3 and T4) (Fig. 3). The current findings are comparable with the results of Centhilnayaki, (2004), Kalivarathan, (2004) and Balasundaram *et al*. (2007 & 2008).



Graph 3. Morphometric data of control and silver nanoparticles treated V instar larvae of *Bombyx mori*.

Table 4 shows the morphometric data of mean length, width and weight of the cocoon of *B. mori* fed with AgNps treated V1 leaves were found to be more than that of the larvae fed with control V1 leaves. The length, width and weight of the T1 larvae produced cocoon were found to be about (3.0910±0.1647cm, 2.2233±0.0811cm and 1.5117± 0.1608gm), respectively.



Graph 4. Morphometric data of control and silver nanoparticles treated *Bombyx mori* larvae Produced cocoon.

Table 3. Morphometric data of various concentrations of AgNps treated with V_1 mulberry leaves on the 5th instars larvae length, width, weight, of *Bombyx Mori*

V instar larvae			
Groups	Length (cm) (Mean ± S.D)	Width (cm)(Mean ± S.D)	Weight (gm) (Mean ± S.D)
Control (T_1)	6.5187±0.1933^{a}	1.063± 0.0571^{ab}	2.7390±0.1050^{a}
V_1 mulberry + 25% AgNps (T_2)	7.1550±0.2857^{b}	1.103± 0.1216^{b}	3.4553 ±0.2369^{b}
V_1 mulberry + 50% AgNps (T_3)	7.0340± 0.2389^{ab}	1.090± 0.0914^{ab}	3.2950± 0.1702^{a}
V_1 Mulberry + 75% AgNps (T_4)	6.8785 ± 0.2020^{ab}	1.080± 0.0732^{ab}	3.0230 ±0.1329^{ab}

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

Table 4. Morphmetric data of various concentrations of AgNps treated with V_1 Mulberry leaves on the cocoon length, width, 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)	3.0910±0.1647^{a}	2.2233± 0.0811^{a}	1.5117±0.1608^{a}
V_1 mulberry + 25% AgNps (T_2)	3.5467±0.2633^{b}	2.3100 ± 0.1144^{b}	2.0217 ±0.3131^{b}
V_1 mulberry + 50% AgNps (T_3)	3.3800± 0.2189^{ab}	2.2833± 0.0982^{a}	1.8860± 0.2533^{ab}
V_1 Mulberry + 75% AgNps (T_4)	3.2957 ± 0.2014^{ab}	2.2667± 0.1910^{a}	1.7567 ±0.2151^{ab}

Value are Mean ± S.D of six observations. Values in the same column with different superscript letters (a-b) differ significantly at $p < 0.05$ (DMRT).

The length, width and weight of the T_2 larvae produced cocoon were found to be about (3.5467± 0.2633cm, 2.3100±0.1144cm, and 2.0217±0.3131gm), respectively. The length, width and weight of the T3 larvae producing cocoon were observed to be about (3.3800±0.2189cm, 2.2833±0.0982cm and 1.8860± 0.2533gm), respectively. The length, width and weight of the T4 larvae produced cocoon were observed to be about (3.2957 ± 0 .2014 cm, 2.2667 ± 0.1910 cm and 1.7567 ± 0.2151 gm), respectively. In these four observations, the 25% AgNps treated larvae produced cocoon length, width, and weight were significantly increased than control (T1) and other two groups (T3 and T4) (Fig.4). 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 (Ramadevi *et al*., 1992).

Table 5. Morphometric data of various concentrations of AgNps treated with V_1 mulberry leaves on the produced cocoon shell and pupal weight

Groups	Cocoon Shell Weight (gm) (Mean ± S.D)	Pupal Weight (gm) (Mean ± S.D)
Control (T_1)	0.3773 ± 0.0146^a	1.1344 ± 0.1256^a
V_1 mulberry + 25% AgNps (T_2)	0.4432 ± 0.0208^b	1.5785 ± 0.0673^b
V_1 mulberry + 50% AgNps (T_3)	0.4180 ± 0.0192^a	1.4680 ± 0.0964^a
V_1 Mulberry + 75% AgNps (T_4)	0.4032 ± 0.0171^a	1.3535 ± 0.0964^a

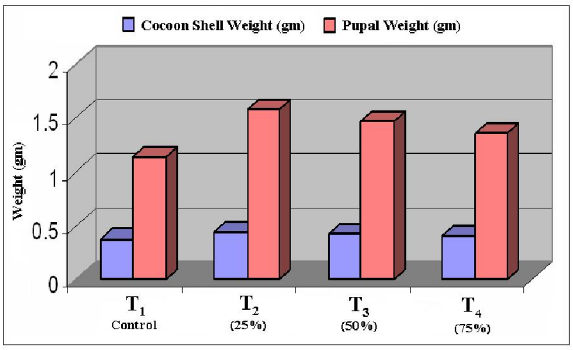
Value are Mean ± S.D of six observations. Values in the same column with different superscript letters (a-b) differ significantly at $p < 0.05$ (DMRT).

Table 6. Morphometric data of various concentrations of AgNps treated with V_1 mulberry leaves on the produced cocoon shell ratio and silk filament length

Groups	Shell Ratio (%)	Silk Filament Length (Meters)
Control (T_1)	13.6743 ± 0.0165^a	794.1977 ± 16.6576^a
V_1 mulberry + 25% AgNps (T_2)	17.1746 ± 0.2475^b	886.5834 ± 24.1885^b
V_1 mulberry + 50% AgNps (T_3)	15.5623 ± 0.2269^{ab}	847.713 ± 21.2572^{ab}
V_1 Mulberry + 75% AgNps (T_4)	14.2564 ± 0.1938^{ab}	823.5134 ± 19.5456^c

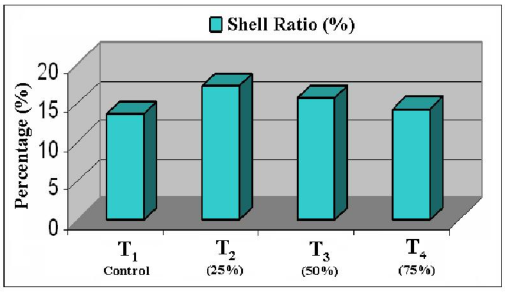
Values are Mean ± S.D of six observations. Values in the same column with different superscript letters (a-c) differ significantly at $p < 0.05$ (DMRT)

Table 5 shows that the morphometric data of control V1 mulberry leaves and AgNps treated V1 mulberry leaves were found to be more than that of the larvae produced, cocoon shell and pupal weight. The mean value of control (T1) were (0.3773±0.0146gm and 1.1344±0.1256gm), respectively. The mean value of 25% silver nanoparticles treated group (T2) were (0.4432± 0.0208gm and 1.5785± 0.0673 gm), respectively. The mean value of 50% AgNps treated group (T3) were (0.4180±0.0192gm and 1.4680 ± 0.0964gm), respectively. The mean value of 75% AgNps treated group (T4) were (0.4032± 0.0171gm and 1.3535± 0.0964gm), respectively. In this four observations, 25% AgNPS (group T2) treated larvae produced cocoon shell and pupal weight was significantly increased than control (T1) and other two groups (T3 and T4) (Fig.5).

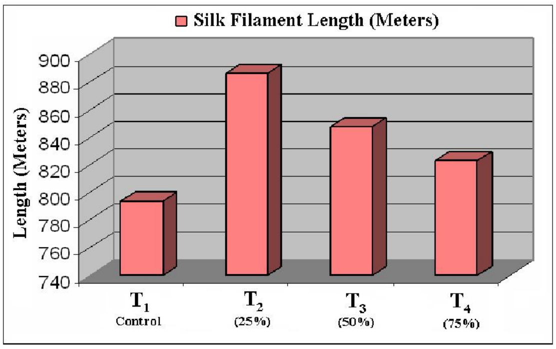


Graph 5. Morphometric data of control and silver nanoparticles treated *Bombyx mori* larvae produced cocoon shell and pupal weight.

The food consumption has a direct consequence on the weight of larvae, cocoon, pupae and shell, the sovereign parameters of using up and output fluctuate depending upon the type of nutrition (Shivakumar, 1995) and silkworm breeds (Ramadevi *et al*., 1992). Table 6 shows that the morphomettric data of control V1 mulberry leaves and AgNps treated V1 mulberry leaves fed *B. mori* larvae produced cocoon shell ratio (%) and silk filament length (Meters). The mean value of control (group T1) were (13.6743± 0.1625% and 794.1977± 16.6576 mtrs), respectively. The mean value of 25% silver nanoparticles treated group (T2) were (17.1746 ± 0.2475 % and 886.5834 ± 24.1885 mtrs), respectively. The mean value of 50% AgNps treated group (T3) were (15.5623± 0.2269% and 847.7134 ± 21.2572 mtrs), respectively. The mean value of 75% AgNps treated group (T4) were (14.2564 ± 0.1938 % and 823.5134 ± 19.5456 mtrs), respectively. In these four observations, 25% AgNps group (T2) treated larvae produced cocoon shell ratio (%) and silk filament length (mtrs) was significantly increased than control (T1) and other two groups (T3 and T4) (Fig.6 & 7). 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 cocoon length, width, weigh and pupal parameters and silk Traits and also increased the quantity of silk production by enhancing the feed efficacy than



Graph 6. Morphometric data of control and silver nanoparticles treated *Bombyx mori* larvae produced cocoon shell ratio



Graph 7. Morphometric data of control and silver nanoparticles treated *Bombyx mori* larvae produced cocoon silk filament length

control so, this supplementation could be prescribed to the farmers to get more quantity of silk.

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