

A Study on the effect of Silver nano particles synthesized using *Rosa rubiginosa* plant extract on the growth parameters of Silkworm *Bombyx mori* L

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Abstract:

Sericulture plays a major role in rural employment, poverty alleviation and earning foreign exchange. A lot of entrepreneurial opportunities are available in various areas of sericulture. The quality of mulberry leaves fed to the silk worms is considered to be the prime factor for good cocoon and crop production. To find out the food efficacy of silkworm larvae fed with silver nanoparticles of *Rosa rubiginosa* treated with mulberry leaves. The silver nanoparticles were synthesized by chemical reduction method. The fifth instar larvae were selected for our study. Fifth instar of silkworm larvae, *Bombyx mori* L. were fed on mulberry leaves fortified with *Rosa rubiginosa* silver nanoparticle extract at certain concentrations. The impact of the food efficacy factors such as, Food Consumption, Food Assimilation, Approximate Food Digestibility, Food Consumption Index and Coefficient of Food Utilization were examined. Different results were obtained depending upon on the various concentrations used. Almost in all the experimental concentrations of *Rosa rubiginosa* silver nanoparticle increased feed efficacy than control without any supplementation. And there is a little elevation observed in 1% concentration. Thus, supplementation of mulberry leaves with *Rosa rubiginosa* silver nanoparticle enhance feed efficacy, subsequently, enhancing the commercial qualities of cocoon. Thus it may be beneficial to the farmers and entrepreneurs in silk production.

Key Words: *Bombyx mori*, silver nanoparticles, phytochemicals, mulberry leaves, *Rosa rubiginosa*.

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1. Introduction

Sericulture or farming is the rearing of silkworm for the production of raw silk. Although there are several commercial species of silkworm, *Bombyx mori* is the most widely used and intensively studied silkworm. In integrated farming system sericulture is an important component, which is an agro-based rural industry with tremendous potential for employment generation in rural areas. Sericulture industry provided employment to approximately 8.51 million persons in rural and semi urban areas in India during 2016-2017. Of these, a sizeable number of workers belong to the economically weaker sections of society, including women indulged in this activity. This potential is par excellence and no other industry generates this kind of employment, especially in rural areas, hence, Sericulture is used as a tool for rural reconstruction [1].

The quality of leaves provided to the worms for feeding has been considered as the prime factor governing the production of good crop cocoon. Mulberry (*Morus* species) leaf is the sole food and source of nutrition for the silkworm, *Bombyx mori* due to the presence of morin [2]. The leaves of superior quality enhance the chance of reaping good cocoon crop [3]. Diseases in silkworm and mulberry plants caused by pathogens reduce the quality and quantity of silk production which in turn affects national economy [4, 5]. The fortification of mulberry leaves with supplementary nutrient and feeding silkworm is a useful modern technique increased the economic value of cocoon [6]. Recently much research has been done on the diet supplementation of mulberry leaves fed to silkworms. These supplementations include vitamins such as ascorbic acid, thiamin, niacin, folic acid, multi-vitamins and vitamin C [7-11]. The enrichment of mulberry leaves with nutrient supplementation such as pre and probiotics, antibiotics, vitamins, amino acids are one of the strategies by which cocoon and silk productivity can be increased and the quality can be enhanced [12, 13]. Nutritional requirement in food consumption have direct impact on the overall genetic traits such as larval and cocoon weight, amount of silk production, pupation and reproductive traits [14]. Silkworm nutrition refers to the substances required by silkworm for its growth and metabolic functions and obtained from ingested food of mulberry/artificial diet and remaining other nutritional components are being synthesized itself through various biochemical pathways including proteinaceous silk fiber of commercial interest [15-17].

The present study is an attempt to evaluate the influence of silver nanoparticles synthesized *Rosa rubiginosa* plant extract treated mulberry leaves on the food efficacy factors such as, Food Consumption, Food Assimilation , Approximate Food Digestibility, Food Consumption Index and Coefficient of Food Utilization.

2. Materials and Methods

2.1. Experimental Design

The present study was carried out in the silkworm rearing centre at Sadakathullah Appa College, Rahmathnagar, Palayamkottai, Tirunelveli. Egg cards of silkworm *Bombyx mori*(L× CSR2) double hybrid were obtained from Government sericulture farm at Nannagaram, Tenkasi.

Silkworms reared under standard conditions at 26-28°C. The fresh mulberry leaves were collected from the sericulture garden at Sadakathullah Appa College, Tirunelveli.

The silkworms were isolated from the stock before the experiment and reared separately in rearing racks. Each tray has 50 larvae and a measured quantity of leaf was provided. The young caterpillars were fed with the tender portion of the mulberry leaves. The caterpillars were maintained properly, carefully observed and monitored for their general good growth. Third, fourth and fifth instar larvae were taken for the present study. Every day the trays were cleaned to remove faecal materials and unfed leaves to maintain a hygienic condition.

2.2. Preparation of Plant Extract

Fresh petals of *Rosa rubiginosa*, free from diseases were collected from Karungulam village, washed thoroughly 2-3 times with tap water and once in sterile water. The Collected materials were dried in shadow places at a room temperature (28 ± 2 °C) for 5 days and powdered to be used for synthesis of Silver nanoparticles.

2.3. Preparation of Crude Extract

5g petals powder of *Rosa rubiginosa* was taken in a separate sterile conical flask. 100ml of distilled water was poured and the mixer was kept in incubator for 48 hours in room temperature. After incubation, the

solution is subjected to centrifuge at 6000 rpm for 10mins. Then the supernatant solution was collected from the centrifuge tube and kept for evaporation (to sediment the particles) until it get fully evaporated.

2.4. Synthesis of silver nanoparticles

After effective evaporation, the settle powdered was collected. The powdered material was made into 0.5ppm, 1ppm, 1.5ppm Concentration and mixed with 100ml of distilled water was taken in 3 sterile conical flask. 1mm Silver nitrate solution was taken and introduced into each conical flask and mixed well. The conical flask containing silver nitrate solution was kept in a magnetic stirrer. Then 20ml of various concentrated sample solution was taken and it was added drop by drop into the silver nitrate solution. This process continued until the colour change from green to brown. The obtained solution is kept for evaporation at room temperature or in an incubator. After evaporation the product will be obtained as powder. Then the powder is washed twice with distilled water which is used for further processes.

2.5. Experimental Groups

The fifth instar larvae of *Bombyx mori* were fed with the following mulberry leaves. Control group (C) larvae fed with normal mulberry leaves, groups 1,2, 3 and 4 received 0.5%, 1%, 1.5% and 2% of silver nanoparticles synthesized *Rosa rubiginosa* plant extract treated mulberry leaves respectively.

2.6. Characterisation of Silver Nanoparticles

2.6a. UV- Visible Spectroscopy Analysis

Silver nanoparticles are reduced by silver ion solutions with *Rosa rubiginosa* petal extract may be easily absorbed by UV-Visible Spectroscopy. The absorption Spectra of petals extract quantities and metal concentrations were measured using 200-800 range.

It uses light in the visible and adjacent (near UV and near – infrared (NTR) ranges. The absorption in the visible range directly. In the region of the electromagnetic spectrum, molecules undergo electronic transitions [18].

2.6b. Fourier Transform Infrared Spectroscopy

Synthesised Silver nanoparticles were measured by using Firkin Elmer Spectrum One FTIR with 4000-400cm of Spectral range in KBr Pellet. FTIR and Analysis was used for the characterization of the extract and the resulting nanoparticles, powder samples for the FTIR was prepared similarly as for powder diffraction measurements. The FTIR Spectra of *Rosa rubiginosa* extract taken before and after synthesis of nanoparticles were analyzed which discussed the possible biomolecules in the *Rosa rubiginosa* extract responsible for the reduction of ions and also the capping agents, so their responsibility for the stability of the biogenic nanoparticles solution [18].

2.7. Energetics studies

Food efficacy factors such as, Food Consumption, Food Assimilation, Approximate Food Digestibility, Food Consumption Index and Coefficient of Food Utilization were analysed.

3. Statistical analysis

All the experiments were carried out carefully and the data were recorded analyzed statistically. Results were presented as mean \pm standard deviation (SD). $P < 0.05$ was regarded as statistically significant [19].

4. Results

Feed efficacy characters like Food Consumption (FC), Food Assimilation (FA), Approximate Digestibility (AD), Food Consumption Index (FCI) and Co-efficient of Food Utilization (CFU) data of fifth instar larvae of *Bombyx mori* fed with control mulberry leaves and different concentrations of Silver nanoparticles treated mulberry leaves were presented in Table 1, Fig 1 and 2 .

Table 1, Fig 1 showed that the food consumption (FC) data of fifth instar larvae of *Bombyx mori* fed with control and Silver nanoparticles treated mulberry leaves. The food consumption of group 'C' larvae was found to be 46.9 ± 1.01 g, group T₁ showed 40.78 ± 1.06 g, group T₂ 47.52 ± 0.79 g, group T₃ 43.79 ± 0.29 g and group T₄ 43.41 ± 0.88 g, respectively. It was observed that in 1% (group T₂) Silver nanoparticles treated larvae, the food consumption was significantly increased than the other four groups.

The food assimilation of fifth instar larvae of *Bombyx mori* fed with control and Silver nanoparticles treated mulberry leaves were given in Table 1, Fig 1. The food utilization of control group was $45.4000 \pm$

0.1511g, in groups T₁, T₂ T₃ and T₄ were 40.09 ±0.62g, 46.28±0.39g, 41.76±1.09g and 42.18±1.19g respectively. Group T₂ showed a high elevation in food consumption in fifth instar larvae.

Approximate food digestibility (%) in control group was found to be 84.90 ±0.35. It was noted that it was 83.03±0.12 in T₁ group, 90.0±1.21 in T₂ group, 84.62±0.18 T₃ group and 83.09±0.34 T₄ group. A significant increase in the approximate food digestibility was observed in the group which received 1% of silver nanoparticles synthesized plant extract.

The food consumption index (FCI) of fifth instar larvae of *Bombyx mori* fed with control and Silver nanoparticles treated mulberry leaves were given in Table 1, Fig 2. It was found to be 36.71±1.02% in control group and 34.23 ±1.48%, 40.62±0.97%, 34.19±1.78%, 32.19±1.39% in the groups which received the extract at 0.5%, 1%, 1.5% and 2% respectively. Group T₂ showed a significant increase in food consumption index than the other extract treated groups.

The co-efficient of food utilization (%) of group C was 85.02±0.67. A significant elevation was observed in the groups T₁ (84.12±0.35), T₂ (88.71±0.81). It was observed 80.65±0.91 in group T₃ and 82.36±0.49 in group T₄. It was found to be maximum in the group which received the 1% of extract treated with silver nanoparticles.

Table 1: Food Efficacy of fifth instar larvae of *Bombyx mori* fed with control and different concentrations of silver nano extract of *Rosa rubiginosa*

Experimental groups and Concentrations	Food consumption (gm)	Food assimilation(gm)	Approximate food Digestibility (%)	Food consumption index (%)	Coefficient of food utilization(%)
Control(C)	46.9±1.01	42.17±1.09	84.90 ±0.35	36.71±1.02	85.02±0.67
AgNps (T ₁) 0.5%	40.78±1.06	40.09 ±0.62	83.03±0.12	34.23 ±1.48	84.12±0.35
AgNps (T ₂) 1%	47.52±0.79	46.28±0.39	90.0±1.21	40.62±0.97	88.71±0.81
AgNps (T ₃)1.5%	43.79±0.29	41.76±1.09	84.62±0.18	34.19±1.78	80.65±0.91
AgNps (T ₄) 2%	43.41±0.88	42.18±1.19	83.09±0.34	32.19±1.39	82.36±0.49

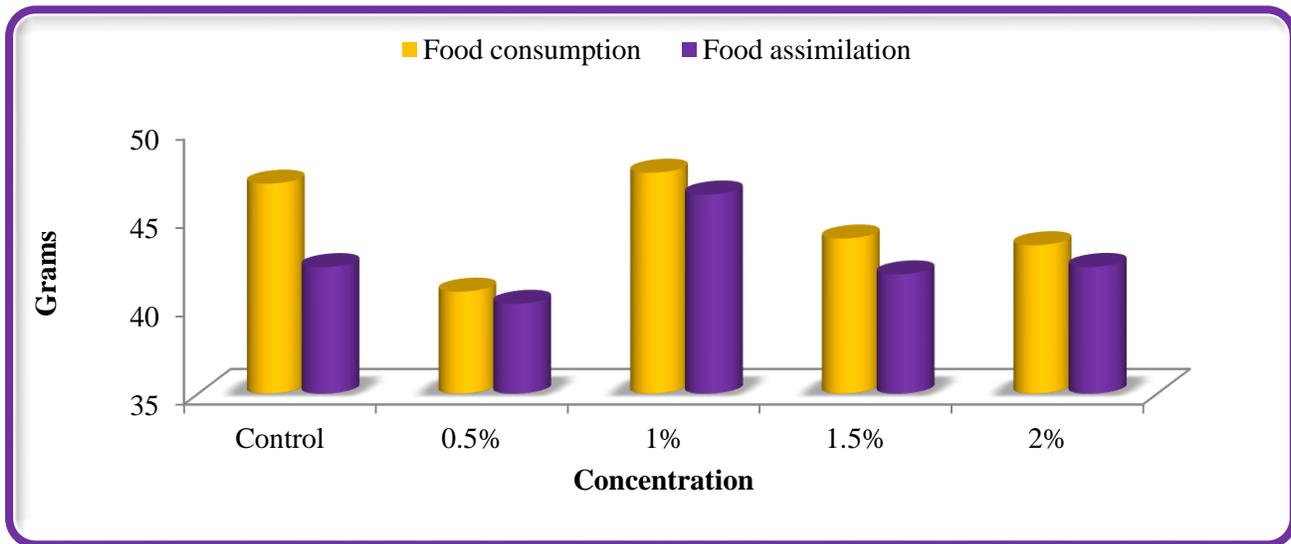


Fig 1. Showing the result of silver nanoparticles treated *Rosa rubiginosa* extract on food consumption and assimilation of fifth instar larvae

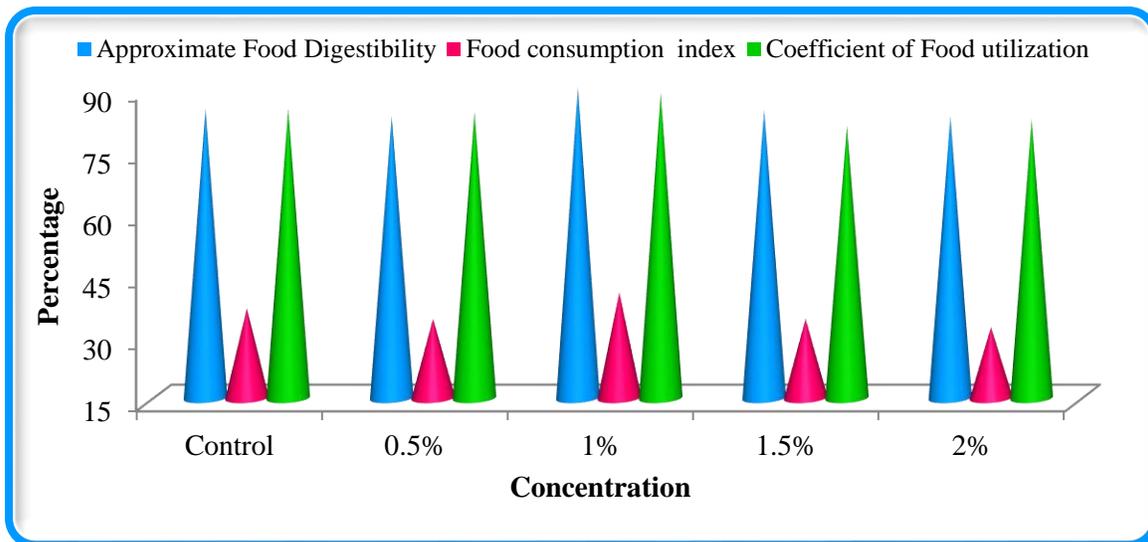


Fig 2. Showing the effect of silver nano particles treated *Rosa rubiginosa* extract on Approximate food Digestibility, Food consumption index and Coefficient of food utilization of fifth instar larvae

5. Discussion

From the present observations, it is proved that the different concentrations of *Rosa rubiginosa* silver nanoparticle increased the feed efficacy of silkworm. It was reported that the nutrient supplemented mulberry leaves increased the growth of larvae [20].

It was revealed that the increased cocoon weight of the silkworm larvae was the result, when mulberry leaves were fortified by some medicinal plant leaves [21, 22]. The present study results in agreement with the observations of previous work done [23] and reporting that the mineral supplemented mulberry leaves increases the feed efficacy. Previous report suggested that there was a significant increase in larval growth when they fed by different mulberry varieties with varying nutrients [24, 25].

6. Conclusion

Silver nanoparticles have become the focus of much research interest due to their wide variety of applications in various fields. They could increase some biological characteristics in silkworm and this enhancement could economically improve the Sericulture needs of today.

From the present work, it is evident that better rearing performance is gained from feeding silver nano treated mulberry leaves. All the energy parameters controlling the production of quality silk were inclined considerably by the nutritive enhancement of larvae with silver nanoparticles of rose petals. So, this supplementation could be prescribed to the farmers to get more quantity of silk. Thus, this green synthesis method with crude extract is alternative to chemical method, since it is cheap, pollutant free and eco-friendly but needs an elaborate study with pure culture.

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