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Impact of Biogenic Silver nanoparticles on Physiological Parameters of Silkworm *Bombyx mori* (L.) (Lepidoptera: Bombycidae) In Relation to Feed Efficacy and Economical Traits

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Abstract
Silkworm *Bombyx mori* is an important economic insect and also a tool to convert leaf protein into silk protein. This study was carried out to determine the feed efficacy of silkworm *Bombyx mori* fed by mulberry (*Morus sinensis*) leaves and different concentrations of silver nanoparticles treated mulberry (*Morus sinensis*) leaves in relation to physiological parameters like food consumption (FC), and Economical traits. Biogenic synthesis of silver nanoparticles by using two white rot fungi. The different concentrations (25%, 50%, 75% and 100%) of prepared silver nanoparticles were treated to the larval period. In the present study, it has been observed that the treatment of AgNps at the concentration of G-1 at 50%, GK-14 at 75% may have beneficial effects on the increase of the silkworm larval and pupal length, width, weight and quantity of silk production by enriching the feed effectiveness than control. This study has been indicated that the Silver nanoparticles exhibit the presence of certain growth stimulant activity and can be used to increase the silk yield in commercial silkworm rearing with reference to Sericulture.

Keywords
Bombyx mori, *Morus sinensis*, White rot fungi, Silver Nanoparticles, Feed Efficacy.

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INTRODUCTION:
Sericulture today is practiced in industrially advanced countries such as China, Japan and the U.S.S.R as well as in countries such as India, South Korea, which are now becoming industrialized. In the developing countries, it is essentially a village – based and welfare- oriented industry capable of providing employment to large sections of the population. Although sericulture had been considered for a long time as a subsidiary occupation in rural areas. Recent

technological developments have made it possible to practice sericulture on an intensive scale, producing greater profits than most of the agriculture crops. Silkworm crop loss is directly attributed to occurrence of different diseases caused by microsporidia, bacteria, protozoans, virus, fungi which affect the crop yield and quality. Severe crops loss at farmer's level results in annual loss of 27-35% and 10-15 kg/100 dfls respectively [1]. Among silkworm diseases, flacherie is one of the major bacterial diseases of silkworm, which causes considerable economic damage to the cocoon crop. It is reported that flacherie is caused by different species of bacteria, virus and their mixed infection [2-3].

During silkworm rearing, through different routes the pathogens released by diseased silkworm accumulate and spread in the rearing room, hence disinfection of rearing house and surrounding, use of bed disinfectants and maintenance of hygienic are different preventive measures. In recent times, several chemical bed disinfectants are released. The use of lethal doses of certain compounds present in these bed disinfectants prolonged the incubation period of virus in silkworm caused concern due to their non-biodegradable nature, maximum residual toxicity, ecological incompatibility, adverse effects on human health and non-target organisms. Besides this, the bacteria have presently developed resistance against available antibiotics. Hence, the search for new antibacterial agents having great variety of biodynamic action besides the antimicrobial property [4]. Consequently, the enrichment of mulberry leaves by supplementary compounds with the aim of increasing the production of cocoon is a very important aspect. Fortification of mulberry leaves with complementary compounds was found to increase the larval growth and post cocoon characteristics [5-6]. Nutrition plays a key role in improving the growth and development of the silkworm, *B. mori* L. like other organisms. Legay, (1958) [7] reported that silk production is dependent on the larval nutrition and nutritive value of mulberry leaves which plays a very major role in producing good quality cocoons. Seki and Oshikane (1959) [8] have observed a better growth and development of silkworm larvae as well as good quality cocoons when fed on nutritionally enriched leaves. In recent years, attempts have been made in sericulture with nutrient such as protein, vitamin, carbohydrates, amino acids, vitamins, hormones, and antibiotics and study their impact on the silk production and that has become the order of traditional research in sericulture.

Nanotechnology has attracted a great interest in recent years due to its expected impact on many areas such as energy, medicine, electronics, and space industries. Research in this field has been growing dramatically throughout the world over the last decade [9]. Nanoparticles—particles having one or more dimensions of the order of 100 nm or less—have attracted great attention due to their unusual and fascinating properties, and applications advantageous over their bulk counterparts [10]. Currently, the unique antimicrobial properties of AgNPs have led to their application in areas such as clothing manufacturing, food preservation, and water purification. More importantly, AgNPs are being increasingly utilized in the medical industry due to their antibacterial, antifungal, antiviral, anti-inflammatory principles [11]. Silkworm mortality is mainly caused by bacteria and other pathogenic microorganisms. AgNps have antimicrobial activity against several pathogenic microorganisms.

The aim of the study is mainly concerned on the treatment of silver nanoparticles at different concentrations and to study the beneficial effects on the growth of the silkworm larval, pupal and cocoon length, width and weight.

MATERIAL METHODS:

Reagents and chemicals:

Silver nitrate and all analytical grade chemicals were purchased from Merck Chemicals, Mumbai. Freshly prepared triple distilled water was used throughout the experiment.

Biological synthesis of silver nanoparticles by White rot fungi

The two white rot fungi *Trametes ljubarskyi* (GK 14) KU382503.1 and *Ganoderma enigmaticum* (GK 01) KU870313.1 was used in this study. They were collected from Eturnagaram Forest Warangal District, Telangana, India (18^{0}20'20''N, 80^{0}25'45''E) they were grown and maintained at 32°C on Malt Extract Agar medium (MEA: Malt extract-15 gm, NH_4Cl 1 gm, KH_2PO_4-1 gm, Citric acid 15N per 1000 ml of distilled water). And the cultures were grown in malt extract broth containing glucose 10g/l, malt extract 5g/l. The final pH was adjusted to 6.0 and the flasks were incubated in the orbital shaker at 200 rpm at 32^0 C. After five days of incubation, the mycelium was separated by filtration and supernatant was challenged with equal amount of silver nitrate solution of 1 mM (prepared in deionised water) and incubated in shaker at 200 rpm in dark condition at 32^0 C. Simultaneously, a positive control of silver nitrate solution and deionised water, and a negative control containing only silver nitrate solution were maintained under same conditions [12].

Silkworm rearing

The eggs of popular Indian multivoltine hybrid silkworm *Bomboxy mori* were collected from Sericulture service centre NSPP, Central silk board Vijayawada, Andhra Pradesh India. The eggs were placed at ambient temperature of 25±2º C and relative humidity of 70 to 80% in an incubator for hatching. After hatching, larvae were isolated from stock culture. The larvae were reared in plastic tray boxes measuring 22×15×5 cms covered with nylon net and placed in an iron stand with ant wells. The larvae were reared up to 3rd instar and worms were treated from 3rd instar till maturity.

Selection of the Effective Concentration of Silver nanoparticles

The Silver nanoparticle solution was diluted to 25%, 50%, 75% and 100% concentrations. Fresh M_5 mulberry leaves were separately soaked with each concentration for 15 minutes and then were dried in air for 10 minutes (only for one feed). The Silver nanoparticle treated leaves were used for feeding the 3rd to 5th instars larvae of silkworm *B. mori*. The *B. mori* larvae were divided into two groups (Control and Treated). The treated group divided into four subgroups (T1, T2, T3 and T4) and these subgroups were treated with different concentrations Silver nanoparticle solution (25%, 50%, 75% and 100%). The control and Silver nanoparticles treated M_5 mulberry leaves were fed by silkworm *B. mori* (five feedings/per day) (Fig 1).

Group T_0 larvae received mulberry leaves sprayed with distilled water and served as control. Each treatment was replicated thrice with 100 larvae each, which were allowed to spin cocoons, the cocoons thus harvested were used for calculation of commercial parameters. 3rd, 4th and 5th instar larvae length, width and weight, cocoon length, width and weight were determined for all groups.

RESULTS:

Effective Concentrations of Silver Nanoparticles synthesized from *Ganoderma enigmaticum*

A significant increase in the larval weight was seen in four different concentrations of treatment and it was found relatively more in the concentration of G-1 (50%) (Table 1). The mean body weight of the 5th instar larvae showed a significant increase in 50% concentration when compared with the control. Maximum larval weight was observed in 3rd instar (**1.441±.009**), 4th instar (**5.535±1.04)** and 5th instar (**32.704±1.080)** in 50% concentration followed by 75% concentration (Fig. 2a&b).

Economical traits of Silver Nanoparticles synthesized from *Ganoderma enigmaticum*

In G-1 (50%) concentration, there was significant increase in economic traits when compared with the control. High cocoon yield of 15.90 kgs was recorded in 50% concentration as compared to low cocoon yield of 14.29 kgs in 25% concentration. The average cocoon weight, pupal weight, shell weight and silk ratio percentage of treated and control batches were recorded (Table 2). The commercial traits improved in 50% concentration when compared to low cocoon yield of 14.39 kgs in 25% concentration (Table 1& 2).

Effective Concentration of Silver Nanoparticles synthesized from *Trametes ljubarskyi*

A significant increase in the larval weight was seen in four different concentrations of treatment and it was found relatively more in the concentration of GK-14 (75%). The mean body weight of the 5th instar larvae showed a significant increase in 50% concentration when compared with the control. Maximum larval weight observed in 3rd instar (**1.461±.009**), 4th instar (**5.635±1.04)** and 5th instar (**33.704±1.080)** in 50% concentration, followed by 50% concentration. In control it was recorded as 13.44 kgs (Fig. 3a&b) (Table 3&4).

DISCUSSION:

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 3rd to 5th instars, suggested that AgNps which were stimulate silkworm to feed more amount of nutrients intake than the control. Prabhu *et al.,* (2011) [13] synthesized AgNp by chemical reduction method and treated with AgNps with different concentrations and it was found at that 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.

Ganesh *et al.,* (2012) [6] synthesized silver nanoparticles from the plant extract and reported that the feed efficacy of larvae (5th instar), enhanced by 25% Silver nanoparticles treated group than control and other treated groups (50%, 75% and 100%).

Sujatha *et al.,* (2015) **[14]** have reported that feeding mulberry leaves supplemented with *O. sanctum* extracts increased the weights of larva, pupa and cocoon shell. Ohila and Nuzhat (2016) [15] reported that the 0.5% vitamin C treated group plays a significant role with an increase in growth and better food intake compared to control group and other vitamin C treated groups, and revealed administration of vitamin C stimulate metabolic activity which is used to increase the growth and feeding efficiency with reference to Silkworm rearing. Since most of this multi-vitamin compounds are 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.

CONCLUSION:

In the present study, the treatment of AgNps at the concentration of G-1 at 50%, GK-14 at 75% may have beneficial effects on the increase of the silkworm larval and pupal length, width, weight and quantity of silk production by enriching the feed effectiveness than control. So, this supplementation could be recommended to the farmers to get more quantity of silk. In conclusion, AgNps could increase some biological characteristics in silkworm, but this enhancement could economically improve the Sericulture goals.

Figure: 1 Plantation of M5 Mulberry variety – *Bombyx mori* along with worms in Plastic trays

Figure: 2a

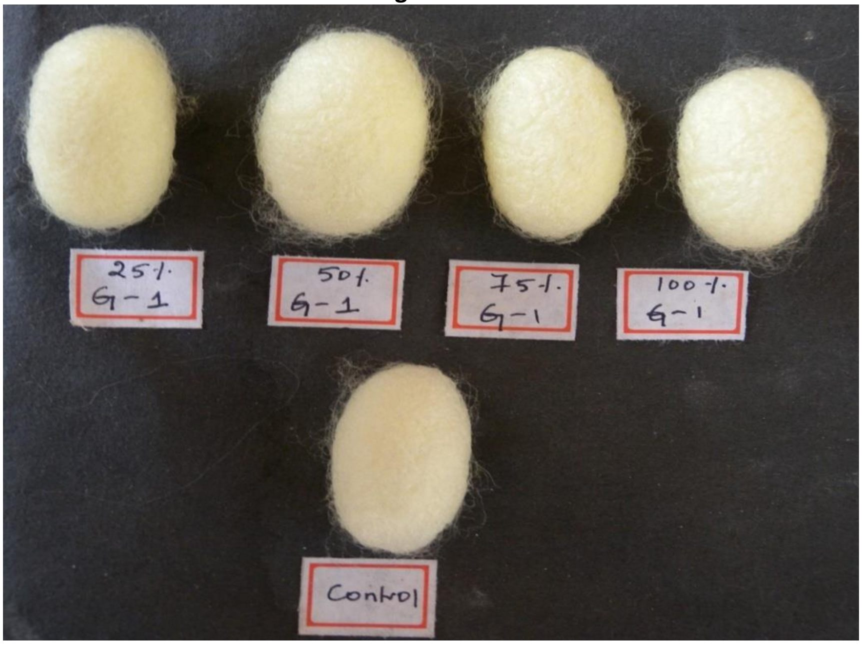

Figure: 2a & 2b Cocoons formed after *G. enigmaticum AgNPs treatment*

Figure: 3a

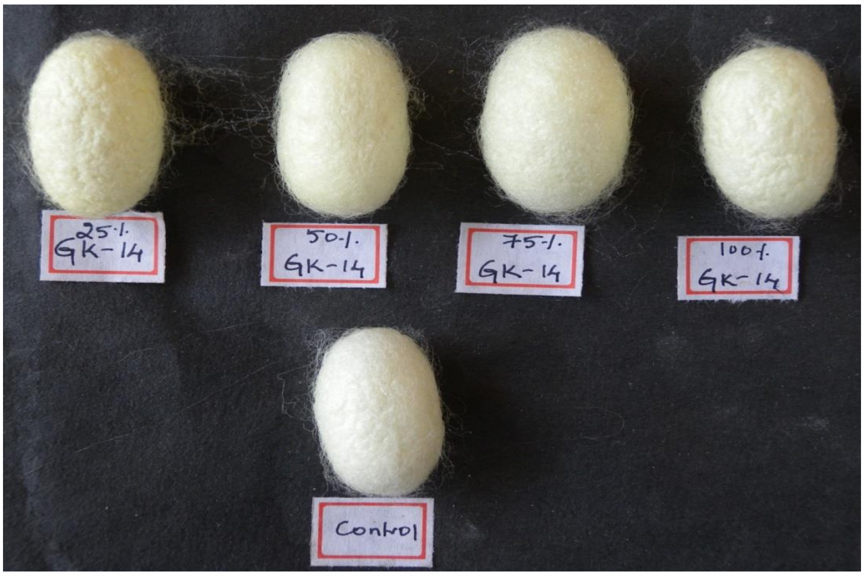

Figure: 3a & 3b Cocoons formed after *T. ljubarskyi AgNPs treatment*

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