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**Research Article**

# EFFECTS OF *SPIRULINA PLATENSIS* MEDIATED $TIO_2$NPS ON BIOCHEMICAL ANALYSIS (*BOMBYX MORI.* L)

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## ABSTRACT

The treatment comprised *S. platensis* mediated $TiO_2$NPs at different concentrations of 25, 50, 75, 100, 125 and 150ppm was diluted from the stock solution. The Double Hybrid silkworm (CSR6 ×CSR26) × (CSR2 × CSR 27) were fed with mulberry leaves treated with *S. platensis* mediated $TiO_2$NPs.The application of mulberry leaves treated with *S. platensis* mediated $TiO_2$NPs influenced silkworm economic parameters. The *S. platensis* mediated $TiO_2$NPs at the concentration of 50 ppm treated mulberry leaves fed to silkworm which recorded increased Total haemolymph protein (8.62 and 8.63 mg/ml), Haemolymph carbohydrates(14.96 and 14.97 mg/ml) and Haemolymph lipids(19.99 and 20.00 mg/g)as compared to control.

**Keywords:** *Spirulina*, $TiO_2$Nps, Silkworm Larvae, Biochemical Parameters.

## INTRODUCTION

Sericulture is one of the most remarkable industries, which includes the exploitation of silk fibre from silkworms such as mulberry, eri, tasar and muga. Among these four types of silkworms, mulberry silkworm (*Bombyx mori* L.) is a major type that feeds exclusively on mulberry leaves (*Morus alba* L.). Silkworm obtains required nutrients entirely from mulberry leaves because mulberry silkworm is monophagous in nature. Generally, vitamins and other essential nutrients present in the mulberry leaves fulfils the minimum needs of silkworms but the amount of nutrients present in the mulberry leaves diverged on the basis of environmental conditions, usage of fertilizers, mulberry varieties and other cultivation practices. *B. mori* takes essential sugars, amino acids, proteins and vitamins for its normal growth and development. Recently many researchers have made attempts to increase raw silk production in various ways like silkworm hybridization, usage of artificial diet and application of phyto juvenoids. Breeding of silkworm races has been a key strategy to improve silk production, little improvement in silk production has been achieved to date. As a result, the development of sericulture economy has not progressed well, pointing to the need of new ways for improvement of silk production (Ni *et al.*, 2015).

Application of nanotechnology in sericulture for improving the silk yield made a great avenue in the last decade. Nanotechnology deals with the most advanced applications in multidisciplinary fields including targeted drug delivery, molecular diagnosis and electronic imaging (Shankar *et al.*, 2014). Recently nanotechnology created a greater impact in agriculture and allied sciences including sericulture. Many researchers were made various attempts in increasing silk production, midgut flora assessment and enhancement of reproduction ability in silkworms through nanotechnology (Kumar *et al.*, 2012; Shyed and Ahmad, 2013). In sericulture among this usage of various nanoparticles for different purposes, increase the silk production is gaining momentum. Nano particles are synthesized by several methods of which green synthesis of nano particles through plants, microorganisms and algae. Which is considered environmentally safe, clean, efficient and profitable. In the past few years, the synthesis of nanoparticles using cyanobacteria has become an active research field. Cyanobacteria are a diverse group of photoautotrophic prokaryotes that exist in wide range of

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ecosystems possessed sustainable resources for various bioactive metabolic products and has high therapeutical application, one such is *Spirulina platensis*. The blue-green algae *S. platensis* contain various minerals, 18 amino acids and vitamins. In sericulture, *S. platensis* mediated NPs act as an activation of tissue metabolism and seems to be an essential factor for promotion of biological parameters of silk gland of silkworm larvae (Thangapandiya and Dharanipriya, 2019).

## MATERIALS AND METHODS

### Chawki worms

Chawki worms of the Double Hybrid (CSR 6 × CSR 26) × (CSR2 × CSR 27) were obtained from the G.S. Chawki rearing centre, Dasanaickenpalayam, Mettubavi, Kinathukadavu, Coimbatore district.

### Disinfection

Prior to the commencement of silkworm rearing program, the rearing room and the appliances were disinfected with 2.5 per cent Sanitech (stabilized chlorine dioxide) + 0.3% slaked lime solution.

### Bioassay on silkworm

The late age silkworms from 1$^{st}$ day of 3$^{rd}$ instar to last day of 5$^{th}$ instar were used for experimental purpose. A total of 120 worms were reared for each treatment (40 worms per replication). During the rearing process, the following observations were obtained.

### Biochemical analysis

### Haemolymph protein (mg/ml)

Each replication had three larvae removed in order to gather the haemolymph. By cutting off one of the fifth instar larva thoracic legs and bending the body to reveal the sternum where the leg was severed, haemolymph samples were taken. This guaranteed appropriate haemolymph outflow and reduced the possibility of internal organ destruction. To avoid sample melanization, the haemolymph from each treatment was collected in 1.5 ml Eppendorf tubes with a few phenyl-thiourea (PTU) crystals (Mahmoud 1988). After that the samples were centrifuged for 10 minutes at 10,000 rpm. The supernatant was removed and kept at -20°C for analysis. According to Lowry *et al.* (1951) the estimation of total haemolymph protein was performed. The recovered haemolymph was diluted with 0.5ml of distilled water. Alkaline copper reagent (5 ml) was added to the 0.5 ml aliquot of this solution. After waiting for 10 minutes, 0.5 ml of Folin Ciocalteu's reagent was added, carefully mixed, and then the colour was allowed to develop for 20 minutes. On the UV spectrophotometer 650 nm, readings were taken. The reference employed was Bovine Serum Albumin (BSA). Milligrams per millilitre were used to denote the mean.

### Haemolymph carbohydrate (mg/ml)

The carbohydrate content of the haemolymph was estimated using the anthropone technique of Schiefter *et al.* (1950). Two millilitres of 10% trichloroacetic acid and eight millilitres of distilled water were added to one millilitre of the sample. Centrifugation was performed on the collected haemolymph at 4000 rpm for 15 minutes. After centrifugation, 4 ml of freshly made anthrone reagent were added to 1 ml of the supernatant. The tubes were placed in a boiling water bath for 10 minutes with aluminium foil covering them. The colour intensity was measured on the spectrophotometer at 620 nm after cooling at room temperature. The reference standard was glucose. The carbohydrate content in the haemolymph is measured as mg/ml.

### Haemolymph lipids (mg/g)

The method of Folch *et al.* (1957) was used for lipid estimation, using chloroform methanol mixture (2:1). Haemolymph and fat body samples were homogenized with appropriate volume of chloroform: methanol mixture (1:10). The homogenate was then quantitatively transferred to a 50 ml separating funnel and then similar volume of chloroform was added. The two solvents were partitioned by the addition of 0.2 volume of water. After the funnel was shaken, the mixture was allowed to stand overnight. The lower chloroform layer containing lipid was drawn off. The lipid sample was kept in vacuum desiccators until constant weight obtained. The lipid of fat body was expressed as mg/g.

## RESULTS AND DISCUSSION

Effects of *S. platensis* mediated $TiO_2$NPs on the biochemical analysis of silkworm haemolymph were studied and results were obtained in crop I and crop II (Table 1). Total haemolymph protein (mg/ml) of *S. platensis* mediated $TiO_2$NPs showed that the highest amount of haemolymph protein (8.62 mg/ml) was recorded at 50 ppm in crop I followed by $TiO_2$ alone (8.21 mg/ml). Whereas, 25 ppm (8.04 mg/ml), 75 ppm (8.07 mg/ml) and *Spirulina* alone (8.10 mg/ml) were found to be on par. The least amount of total haemolymph protein was noticed at 150 ppm (5.62 mg/ml). Similar observations were also made in crop II, the maximum amount of total haemolymph protein (8.63 mg/ml) was observed in 50 ppm of *S. platensis* mediated $TiO_2$NPs treated silkworms compared to the distilled water spray (7.83 mg/ml) and absolute control (7.69 mg/ml) (Table 1).

The least amount of total haemolymph protein (5.64 mg/ml) was noted at 150 ppm. The highest amount of haemolymph carbohydrates (mg/ml) were recorded at *S. platensis* mediated $TiO_2$NPs at the concentration of 50ppm (14.96 mg/ml) in crop I followed by 75 ppm (14.70 mg/ml) and 25 ppm (14.37 mg/ml). Whereas, *S. platensis* alone (14.14 mg/ml) and $TiO_2$alone (14.10 mg/ml) were found to be on par. Lowest amount of haemolymph carbohydrates were recorded at 150 ppm (10.02 mg/ml).

Similar observations were observed in crop II, the maximum amount of haemolymph carbohydrates (14.97 mg/ml) was noted in 50 ppm treated silkworms compared to absolute control (13.99 mg/ml) (Table 6). The lowest value of haemolymph carbohydrates (10.03 mg/ml) was noticed at 150 ppm (Table. 1).The haemolymph lipids (mg/g) were observed in a crop I. The maximum amount of haemolymph lipids (19.99 mg/g) was noted in 50 ppm treated silkworms compared to absolute control (19.05 mg/g) followed by 75 ppm (19.56 mg/g), 25 ppm (19.37 mg/g), *Spirulina* alone (19.26 mg/g), $TiO_2$ alone (19.17 mg/g), distilled water spray (19.06 mg/g). The minimum amount of haemolymph lipids (18.00 mg/g) was observed at 150 ppm. Similar observations were made in crop II (Table 1).

**Table 1.** Effect of *S. platensis* mediated $TiO_2$NPs on biochemical analysis of haemolymph.

| | Treatments | Total haemolymph protein (mg/ml) | | Haemolymph carbohydrates (mg/ml) | | Haemolymph lipids (mg/g) | |
|---|---|---|---|---|---|---|---|
| | | Crop I | Crop II | Crop I | Crop II | Crop I | Crop II |
| $T_1$ | *S. p* $TiO_2$NPs 25 ppm | $8.04^{c}$ | $8.05^{d}$ | $14.37^{c}$ | $14.38^{c}$ | $19.37^{c}$ | $19.38^{c}$ |
| $T_2$ | *S. p* $TiO_2$NPs 50 ppm | $8.62^{a}$ | $8.63^{a}$ | $14.96^{a}$ | $14.97^{a}$ | $19.99^{a}$ | $20.00^{a}$ |
| $T_3$ | *S. p* $TiO_2$NPs 75 ppm | $8.07^{c}$ | $8.08^{cd}$ | $14.70^{b}$ | $14.71^{b}$ | $19.56^{b}$ | $19.57^{b}$ |
| $T_4$ | *S. p* $TiO_2$NPs 100 ppm | $5.96^{f}$ | $5.97^{g}$ | $10.56^{f}$ | $10.57^{h}$ | $18.26^{g}$ | $18.27^{g}$ |
| $T_5$ | *S. p* $TiO_2$NPs 125 ppm | $5.81^{g}$ | $5.82^{h}$ | $10.15^{g}$ | $10.16^{i}$ | $18.03^{h}$ | $18.04^{h}$ |
| $T_6$ | *S. p* $TiO_2$NPs 150 ppm | $5.62^{h}$ | $5.64^{i}$ | $10.02^{h}$ | $10.03^{j}$ | $18.00^{i}$ | $18.01^{i}$ |
| $T_7$ | *Spirulina* alone | $8.10^{c}$ | $8.12^{c}$ | $14.14^{d}$ | $14.15^{d}$ | $19.26^{d}$ | $19.27^{d}$ |
| $T_8$ | TiO2 alone | $8.21^{b}$ | $8.22^{b}$ | $14.10^{d}$ | $14.10^{e}$ | $19.17^{e}$ | $19.18^{e}$ |
| $T_9$ | Distilled water spray | $7.82^{d}$ | $7.83^{e}$ | $14.02^{e}$ | $14.03^{f}$ | $19.06^{f}$ | $19.07^{f}$ |
| $T_{10}$ | Absolute control | $7.68^{e}$ | $7.69^{f}$ | $13.97^{e}$ | $13.99^{g}$ | $19.05^{f}$ | $19.06^{f}$ |
| | SE(d) | 0.04 | 0.03 | 0.03 | 0.02 | 0.01 | 0.01 |
| | CD (0.05) | 0.07** | 0.06** | 0.6** | 0.03** | 0.02** | 0.01** |

Means followed by different superscript letters are significantly different at $p \leq 0.05$.
Means followed by same superscript alphabets letters are on par with each other.
**Highly significant.

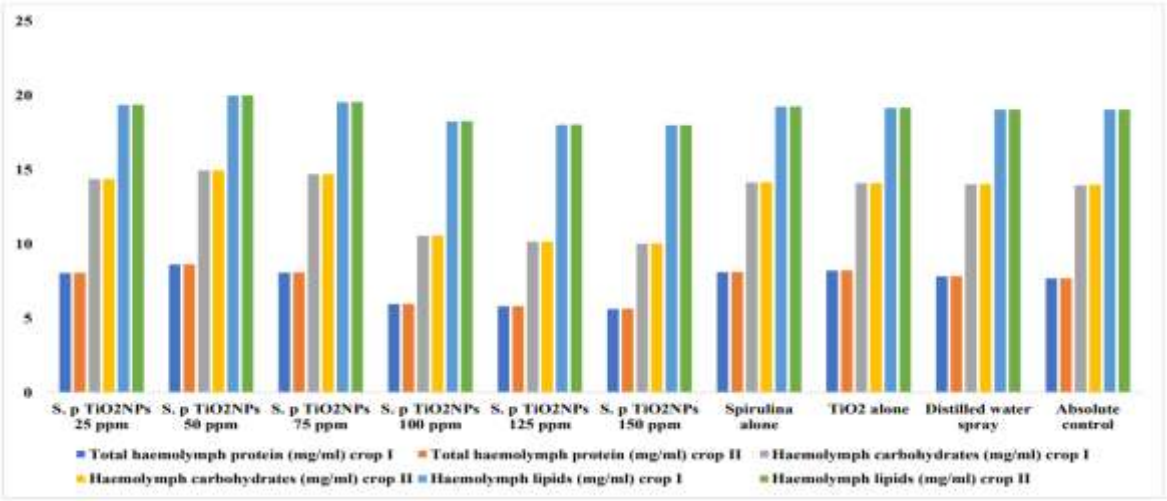


**Figure 1**. Effect of *S. platensis* mediated $TiO_2$NPs on biochemical analysis.

The total haemolymph protein, haemolymph carbohydrates, haemolymph lipids were significantly increased due to the feeding the larvae with *S. platensis* mediated $TiO_2$NPs at concentration of 50 ppm treated mulberry leaves (8.62 mg/ml and 8.63 mg/ml, 14.96 mg/ml and 14.97 mg/ml, 19.99 ml/g and 20.00 mg/g) as compared to control (7.68 mg/ml and 7.69 mg/ml, 13.97 mg/ml and 13.99 mg/ml, 19.05 ml/g and 19.06 mg/g) (Figure 1).This result

corroborates with the findings of Soliman *et al.* (2021) who observed that higher amount of total haemolymph protein at 0.05 percentage of *Spirulina* treated mulberry leaves. Similar, findings have been reported by Rani *et al.* (2011) who recorded that supplementation of mulberry leaves with probiotics like *S. cerevisiae* and *Spirulina* increased the haemolymph protein and amino acids.

## CONCLUSION

The study explored the impact of *S. platensis* mediated $TiO_2$NPs at various concentrations on the economic parameters of the Double Hybrid silkworm (CSR6 × CSR26) × (CSR2 × CSR 27). The treatment involved the dilution of $TiO_2$NPs from a stock solution, and mulberry leaves treated with these nanoparticles were used as feed for the silkworms. The results revealed that the concentration of 50 ppm of *S. platensis* mediated $TiO_2$NPs had a positive influence on silkworm economic parameters. Specifically, silkworms fed with mulberry leaves treated with 50 ppm $TiO_2$NPs exhibited increased levels of Total haemolymph protein (8.62 and 8.63 mg/ml), Haemolymph carbohydrates (14.96 and 14.97 mg/ml), and Haemolymph lipids (19.99 and 20.00 mg/g) compared to the control group. These findings suggest that the application of *S. platensis* mediated $TiO_2$NPs at an optimal concentration of 50 ppm can enhance the nutritional content in the silkworm haemolymph. This information may have implications for sericulture practices, potentially improving the economic yield of silkworms in terms of silk production and quality. Further research and exploration are warranted to better understand the mechanisms underlying these effects and to ensure the safety and sustainability of such treatments in sericulture.

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