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Vasanth V
Research Scholar, Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, Tamil Nadu, India

Ramamoorthy R
Research Scholar, Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, Tamil Nadu, India

Arasakumar E
Research Scholar, Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, Tamil Nadu, India

Ranjith Kumar S
Research Scholar, Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, Tamil Nadu, India

Nilav Ranjan Bora
Research Scholar, Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, Tamil Nadu, India

Gajjala Nitish
Research Scholar, Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, Tamil Nadu, India

Nandha Kumar R
Research Scholar, Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, Tamil Nadu, India

Moulidharshan R
Research Scholar, Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, Tamil Nadu, India

Corresponding Author:
Vasanth V
Research Scholar, Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, Tamil Nadu, India

Evaluation of *Spirulina* mediated TiO_2NPs on silkworm economic traits

Vasanth V, Ramamoorthy R, Arasakumar E, Ranjith Kumar S, Nilav Ranjan Bora, Gajjala Nitish, Nandha Kumar R and Moulidharshan R

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Abstract
The treatment comprised *S. platensis* mediated TiO_2NPs at different concentrations of 25, 50, 75, 100, 125 and 150 ppm 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_2NPs. The application of mulberry leaves treated with *S. platensis* mediated TiO_2NPs influenced silkworm economic parameters. The *S. platensis* mediated TiO_2NPs at the concentration of 50 ppm treated mulberry leaves fed to silkworm which recorded increased mature larval weight (4.34 g), cocoon weight (2.24 g), shell ratio (26.26%), silk filament length (1455 m), filament weight (0.36 g), denier (2.22) and reduced renditta (5.17 kg) as compared to control.

Keywords: *Spirulina*, TiO_2Nps, silkworm larvae, economic 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 obtain 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 phytojuvenoids. 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) [20].

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) [21]. 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) [9, 17].

In sericulture among these usage of various nanoparticles for different purposes, increase the silk production is gaining momentum. Nanoparticles are synthesized by several methods of which green synthesis of nanoparticles 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 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* contains 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) [3].

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 1st day of 3rd instar to last day of 5th 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.

Larval characters

Larval weight (g)

The weight of the larvae was measured on the 5th day of the V instar. The weight of ten randomly selected larvae from each treatment and replication was taken and weighed individually with an electronic balance, with the mean was expressed in gram.

Larval duration (hr)

The larval duration of fifth instar was recorded and mean was expressed in hour.

Effective rate of rearing (ERR)

The total number of cocoons formed in each replication was recorded on the third day of spinning, and ERR was calculated by dividing the number of cocoons spun by the total number of worms reared and expressed in percentage.

$$\mathrm{ERR} = \frac{\text{No. of cocoons harvested}}{\text{No. of larvae used}} \times 100$$

Mortality

According to Joseph *et al*. (2010) [10], mortality was calculated using the following formula and expressed in the percentage.

$$\text{Mortality (\%)} = \frac{\text{No. of dead larvae}}{\text{No. of larvae used}} \times 100$$

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 2015) [11].

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) [10] 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) [12]. 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) [5] 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.

Consumption and digestion indices

The following indices were computed by gravimetric method (Waldbauer, 1968) [18].

Calculation of ingested food

Weight of fresh food ingested = Weight of fresh leaves offered to larvae – Weight of fresh remnants

$$\text{Weight of fresh remnants} = \frac{\text{Fresh weight of control}}{\text{Oven dry weight of control}} \times \text{Weight of dry remnants}$$

Calculation of digested food

Weight of fresh food digested = Weight of fresh food ingested – Weight of fresh excreta produced.

Consumption index (CI)

Consumption index explains in a nut shell about the rate at which nutrients enter into digestive system. It is expressed as consumption per mean larval body weight per day.

$$CI = \frac{F}{TA}$$

Where,
F = Fresh weight of food eaten
T = Duration of feeding (Days)
A = Mean fresh weight of larvae during feeding period

$$\text{Mean weight of larvae} = \frac{\text{Intial larval body weight} + \text{Final body weight in each instar}}{2}$$

Growth rate (GR)

Growth rate (g/g/d) explains how much of fresh matter increased in the body of the larvae per day per gram of body weight.

$$GR = \frac{G}{TA}$$

Where,
G = Fresh weight gain of larvae during feeding period
T = Duration of feeding (Days)
A = Mean fresh of dry weight of larvae during feeding period

$$\text{Weight gain of larvae} = \frac{\text{Weight of larvae before moult} - \text{Weight of larvae after moult/hatch}}{\text{Number of larvae taken per sample}}$$

Approximate digestibility

Approximate digestibility (AD) was calculated by the formula,

$$AD\ (\%) = \frac{\text{Weight of food ingested} - \text{Weight of faeces}}{\text{Weight of food ingested}} \times 100$$

Efficiency of conservation of ingested food (ECI)

Efficiency of conservation of ingested food (%) to body substance (ECI) was calculated by the formula,

$$ECI = \frac{\text{Weight gained}}{\text{Weight of food ingested}} \times 100$$

(or)

$$ECI = \frac{GR}{CI} \times 100$$

Efficiency of conservation of digested food (EDI)

Efficiency of conservation of digested food (%) to body substance (ECD) was calculated by the formula,

$$ECD = \frac{\text{Weight gained}}{\text{Weight of food ingested} - \text{Weight of faeces}} \times 100$$

Silk gland weight

Each silkworm (3 worms per replication) was dissected separately under aspectic condition on the 5th day of 5th instar worms, the silk gland was collected on a pre-weighed glass slide, and their weights were recorded using an electronic balance. This mean was expressed in milligram.

Silk productivity

The following formula was used to determine the silk productivity (Iyengar *et al*., 1983) [7].

$$\text{Silk productivity (cg/day)} = \frac{\text{Weight of silk filament (cg)}}{\text{V instar larval duration (days)}}$$

Cocoon characters

Single cocoon weight

On the fifth day of spinning, ten cocoons were randomly selected per replication and individually weighed using an electronic balance and the mean was expressed in gram.

Shell weight

The shell weight was recorded from 10 randomly selected cocoons by cutting open the cocoons from each replication and mean were expressed in gram.

Shell percentage

The shell percentage was determined by using the following formula. The mean is expressed in per cent.

$$\text{Shell percentage} = \frac{\text{Shell weight (g)}}{\text{Cocoon weight (g)}} \times 100$$

Yield / 10,000 larvae

Cocoon yield/10,000 larvae (by number)

$$\text{Cocoon yield/10,000 larvae} = \frac{\text{No. of cocoons harvested}}{\text{No. of larvae retained in fifth instar}} \times 10{,}000$$

Cocoon yield/10,000 larvae (by weight)

$$\text{Cocoon yield/10,000 larvae} = \frac{\text{Weight of cocoons harvested}}{\text{No. of larvae retained in fifth instar}} \times 10{,}000$$

Reeling parameters

After complete spinning (5 days), 10 cocoons were randomly selected from each replication and reeled using the single cocoon reeler (Epprouvette). The reeling parameters are as follows.

Silk filament length

Five cocoons from each replication were reeled on Epprouvette and the filament length was determined by the formula and expressed as meters.

L = R x 1.125

Where,
L = Total length of the silk filament
R = Number of revolutions recorded
1.125 = Circumference of Epprouvette in m.

Silk filament weight
Silk obtained from the cocoon was weighed, dried at hot air oven at 80 °C to arrive at constant moist content at 65% and expressed as gram.

Denier
The thickness of silk was calculated by using the following formula,

$$\text{Denier} = \frac{\text{Weight of the silk filament (g)}}{\text{Lenght of the silk filament (m)}} \times 9000$$

Renditta
Renditta was calculated by using the following formula,

$$\text{Renditta} = \frac{10{,}000}{\text{Shell ratio} \times \text{Raw silk recovery (70\%)}} \times 9000$$

Results
Effects of *S. platensis* mediated TiO_2NPs on silkworm economic traits
S. platensis mediated TiO_2NPs at different concentrations of 25, 50.75,100,125 and 150 ppm were prepared from the stock solution and fed to silkworms through leaves and the following parameters were recorded for validation (Plate. 13, 14 and 15).

Effect of *S. platensis* mediated TiO_2NPs on larval characters
Larval weight (g)
S. platensis mediated TiO_2NPs showed that a significant difference was observed between different treatments. The maximum larval weight of 4.34 g and 4.30 g were recorded at 50 ppm in crops I and II respectively. The next better treatment was 75 ppm (3.83 g and 3.81 g) which were found to be on par with *Spirulina* alone (3.82 g and 3.80 g) and TiO_2 alone (3.81 g and 3.79 g). The minimum larval weight (2.45 g and 2.43 g) was recorded at 150 ppm (Table 1).

Larval duration (hrs)
The significant difference in larval duration (hrs) was observed between the treatments of *S. platensis* mediated TiO_2NPs in crops I and II. The minimum larval duration (203 hrs and 202 hrs) was recorded at 50 ppm followed by 75 ppm (205 hrs and 204 hrs) which were on par with 25 ppm (207 hrs and 205 hrs), *Spirulina* alone (206 hrs and 207 hrs) and TiO_2 alone (209 hrs and 210 hrs). The maximum larval duration (235 hrs and 234 hrs) was observed at 150 ppm (Table 1).

Table 1: Effect of *S. platensis* mediated TiO_2NPs on larval weight (g) and larval duration (hrs)

	Treatments	Larval weight (g) 5th Instar		Larval duration (hrs) 5th Instar	
		Crop I	Crop II	Crop I	Crop II
T_1	*S. p* TiO_2NPs 25 ppm	3.65^c	3.67^c	207^b	205^b
T_2	*S. p* TiO_2NPs 50 ppm	4.34^a	4.30^a	203^a	202^a
T_3	*S. p* TiO_2NPs 75 ppm	3.83^b	3.81^b	205^b	204^b
T_4	*S. p* TiO_2NPs 100 ppm	2.94^f	2.95^f	234^d	232^d
T_5	*S. p* TiO_2NPs 125 ppm	2.63^g	2.64^g	235^d	233^d
T_6	*S. p* TiO_2NPs 150 ppm	2.45^h	2.43^h	235^d	234^d
T_7	*Spirulina* alone	3.82^b	3.80^b	206^b	207^b
T_8	TiO2 alone	3.81^b	3.79^b	209^b	210^b
T_9	Distilled water spray	3.41^d	3.42^d	224^c	226^c
T_{10}	Absolute control	3.21^e	3.25^e	226^c	228^c
	SE(d)	0.14	0.13	10.23	10.23
	CD (0.05)	0.30**	0.29**	22.12**	22.12**

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.

Effective rate of rearing (%)
The statistically superior effective rate of rearing was observed at 50 ppm (92.22%) followed by 75 ppm (82.22%) and 25 ppm (78.89%) which was found to be on par. The least effective rate of rearing was recorded at 150 ppm (51.11%) in crop I.
Similar observations were also made in crop II. The highest effective rate of rearing was noticed at 50 ppm (93.33%) and the lowest value of effective rate of rearing was recorded in 150 ppm (50.00%) (Table. 2).

Larval mortality (%)
The lowest larval mortality was observed in crop I (8.89%) and crop II (7.78%) at 50 ppm concentration of *S. platensis* mediated TiO_2NPs followed by 75 ppm (17.78% and 16.67%) and 25 ppm (21.11% and 20.00%). The maximum larval mortality had present at 150 ppm (48.89% and 50.00%). Whereas, control showed 22. 22 and 23.33 per cent of larval mortality in respect to crop I and II (Table. 2).

Table 2: Effect of *S. platensis* mediated TiO_2NPs on effective rate of rearing (%) and larval mortality (%)

	Treatments	Effective rate of rearing (%)		Larval mortality (%)	
		Crop I	Crop II	Crop I	Crop II
T_1	*S. p* TiO_2NPs 25 ppm	78.89^{bc}	80.00^{bc}	21.11^{ef}	20.00^{ef}
T_2	*S. p* TiO_2NPs 50 ppm	92.22^a	93.33^a	8.89^g	7.78^g
T_3	*S. p* TiO_2NPs 75 ppm	82.22^b	83.33^b	17.78^f	16.67^f

T_4	*S. p* TiO_2NPs 100 ppm	65.56^{e}	66.67^{e}	34.44^{c}	33.33^{c}
T_5	*S. p* TiO_2NPs 125 ppm	55.56^{f}	56.67^{f}	44.44^{b}	43.33^{b}
T_6	*S. p* TiO_2NPs 150 ppm	51.11^{g}	50.00^{g}	48.89^{a}	50.00^{a}
T_7	*Spirulina* alone	75.56^{cd}	76.67^{cd}	24.44^{de}	23.33^{de}
T_8	TiO2 alone	78.89^{c}	80.00^{bc}	21.11^{ef}	20.00^{ef}
T_9	Distilled water spray	72.22^{d}	73.33^{d}	27.78^{d}	26.67^{d}
T_{10}	Absolute control	77.78^{bcd}	76.67^{c}	22.22^{de}	23.33^{e}
	SE(d)	2.72	1.99	2.63	1.79
	CD (0.05)	5.68**	4.14**	5.48**	3.74**

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.

Effect of *S. platensis* mediated TiO_2NPs on biochemical analysis

Total haemolymph protein (mg/ml)

Effects of *S. platensis* mediated TiO_2NPs on the biochemical analysis of silkworm haemolymph were studied and results were obtained in crop I and crop II (Table 6).

Total haemolymph protein (mg/ml) of *S. platensis* mediated TiO_2NPs 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_2NPs treated silkworms compared to the distilled water spray (7.83 mg/ml) and absolute control (7.69 mg/ml) (Table 6). The least amount of total haemolymph protein (5.64 mg/ml) was noted at 150 ppm.

Haemolymph carbohydrates (mg/ml): The highest amount of haemolymph carbohydrates (mg/ml) were recorded at *S. platensis* mediated TiO_2NPs at the concentration of 50 ppm (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. 6).

Haemolymph lipids (mg/g)

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 6).

Table 6: Effect of *S. platensis* mediated TiO_2NPs 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_2NPs 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_2NPs 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_2NPs 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_2NPs 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_2NPs 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_2NPs 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.

Effect of *S. platensis* mediated TiO_2NPs on nutritional indices

Consumption index: The significant difference in consumption index was observed between the treatments of *S. platensis* mediated TiO_2NPs in crops I and II. Similar results were obtained in both crops. In the third instar, the maximum consumption index was recorded at 50 ppm (0.53 and 0.55), followed by 75 ppm (0.48 and 0.48) which was on par with 25 ppm (0.40 and 0.39). The lowest value was recorded at 150 ppm (0.24 and 0.25).

In the fourth instar, the highest consumption index was recorded at 50 ppm (0.64 and 0.65), followed by 75 ppm (0.62 and 0.62) which was on par with 25 ppm (0.61 and 0.60). The lowest value was recorded at 150 ppm (0.55 and 0.54).

In the fifth instar, the maximum consumption index was recorded at 50 ppm (1.69 and 1.71), followed by 75 ppm (1.68 and 1.69) which was on par with 25 ppm (1.64 and 1.63). The lowest value was recorded at 150 ppm (1.60 and 1.62) (Table 7).

Table 7: Effect of *S. platensis* mediated TiO_2NPs on consumption index

	Treatments	Consumption index (Crop I)				Consumption index (Crop II)			
		Instar				Instar			
		3rd	4th	5th	Mean	3rd	4th	5th	Mean
T_1	*S. p* TiO_2NPs 25 ppm	0.40^{bc}	0.61^{bc}	1.64^{b}	0.88	0.39^{bc}	0.60^{bc}	1.63^{bc}	0.87
T_2	*S. p* TiO_2NPs 50 ppm	0.53^{a}	0.64^{a}	1.69^{a}	0.95	0.55^{a}	0.65^{a}	1.71^{a}	0.97
T_3	*S. p* TiO_2NPs 75 ppm	0.48^{ab}	0.62^{b}	1.68^{a}	0.93	0.48^{ab}	0.62^{b}	1.69^{a}	0.93
T_4	*S. p* TiO_2NPs 100 ppm	0.29^{cd}	0.58^{e}	1.62^{cd}	0.83	0.33^{cd}	0.56^{de}	1.63^{bc}	0.84
T_5	*S. p* TiO_2NPs 125 ppm	0.25^{d}	0.55^{f}	1.62^{cd}	0.81	0.25^{d}	0.54^{ef}	1.62^{bc}	0.80
T_6	*S. p* TiO_2NPs 150 ppm	0.24^{d}	0.54^{f}	1.60^{d}	0.79	0.25^{d}	0.53^{f}	1.62^{c}	0.80
T_7	*Spirulina* alone	0.38^{bc}	0.62^{b}	1.63^{bc}	0.88	0.39^{bc}	0.61^{bc}	1.63^{bc}	0.88
T_8	TiO2 alone	0.39^{bc}	0.61^{bc}	1.61^{d}	0.87	0.40^{bc}	0.60^{bc}	1.61^{c}	0.87
T_9	Distilled water spray	0.39^{bc}	0.60^{cd}	1.64^{b}	0.88	0.39^{bc}	0.59^{c}	1.63^{bc}	0.87
T_{10}	Absolute control	0.38^{bc}	0.59^{de}	1.64^{b}	0.87	0.38^{c}	0.58^{cd}	1.64^{b}	0.87
	SE(d)	0.05	0.01	0.01		0.04	0.01	0.01	
	CD (0.05)	0.11**	0.01**	0.02**		0.09**	0.02**	0.02**	

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.

Growth rate (g/g/day)

The growth rate was recorded in crops I and crop II, In the third instar, the highest growth rate was recorded at 50 ppm (0.43 g/g/day and 0.45 g/g/day), followed by 75 ppm (0.41 g/g/day and 0.43 g/g/day) which was on par with 25 ppm (0.40 g/g/day and 0.42 g/g/day). The lowest value was recorded at 150 ppm (0.35 g/g/day and 0.34 g/g/day).
In the fourth instar, the maximum growth rate was recorded at 50 ppm (0.36 g/g/day and 0.37 g/g/day). The next statistical superior treatment was followed by 75 ppm (0.35 g/g/day and 0.36 g/g/day) which was on par with 25 ppm (0.33 g/g/day and 0.35 g/g/day). The lowest value was recorded at 150 ppm (0.30 g/g/day and 0.31 g/g/day) in treated batches.
In the fifth instar, the highest value of growth rate was recorded at 50 ppm (0.18 g/g/day and 0.20 g/g/day). The next better treatment was followed by 75 ppm (0.17 g/g/day and 0.19 g/g/day) which was on par with 25 ppm (0.15 g/g/day and 0.17 g/g/day). The lowest value was recorded at 150 ppm (0.13 g/g/day and 0.16 g/g/day).

Table 8: Effect of *S. platensis* mediated TiO_2NPs on growth rate

	Treatments	Growth rate (g/g/day) (Crop I)				Growth rate (g/g/day) (Crop II)			
		Instar				Instar			
		3rd	4th	5th	Mean	3rd	4th	5th	Mean
T_1	*S. p* TiO_2NPs 25 ppm	0.40^{cd}	0.33^{b}	0.15^{c}	0.29	0.42^{bc}	0.35^{ab}	0.17^{cde}	0.31
T_2	*S. p* TiO_2NPs 50 ppm	0.43^{a}	0.36^{a}	0.18^{a}	0.32	0.45^{a}	0.37^{a}	0.20^{a}	0.34
T_3	*S. p* TiO_2NPs 75 ppm	0.41^{b}	0.35^{a}	0.17^{b}	0.31	0.43^{ab}	0.36^{a}	0.19^{ab}	0.33
T_4	*S. p* TiO_2NPs 100 ppm	0.37^{g}	0.31^{de}	0.15^{c}	0.28	0.39^{cd}	0.31^{bcd}	0.18^{bcd}	0.29
T_5	*S. p* TiO_2NPs 125 ppm	0.36^{h}	0.29^{f}	0.14^{d}	0.26	0.37^{de}	0.32^{cd}	0.17^{de}	0.29
T_6	*S. p* TiO_2NPs 150 ppm	0.35^{i}	0.30^{ef}	0.13^{d}	0.26	0.34^{e}	0.31^{d}	0.16^{e}	0.27
T_7	*Spirulina* alone	0.41^{bc}	0.32^{cd}	0.16^{bc}	0.30	0.40^{c}	0.34^{abc}	0.18^{bc}	0.31
T_8	TiO2 alone	0.40^{de}	0.33^{bc}	0.16^{bc}	0.30	0.41^{bc}	0.35^{a}	0.17^{bc}	0.31
T_9	Distilled water spray	0.39^{e}	0.31^{de}	0.17^{b}	0.29	0.41^{bc}	0.33^{bcd}	0.19^{ab}	0.31
T_{10}	Absolute control	0.38^{f}	0.30^{ef}	0.16^{bc}	0.28	0.40^{cd}	0.31^{d}	0.18^{ab}	0.30
	SE(d)	0.005	0.006	0.005		0.01	0.01	0.007	
	CD (0.05)	0.01**	0.01**	0.01**		0.02**	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.

Efficiency of conversion of ingested food (ECI) (%)

The Efficiency of conversion of ingested food was observed in crops I and II. In the three instar like III, IV and V, higher ECI was recorded at 50 ppm (25.66% and 25.95%, 64.86% and 64.90%, 29.89% and 29.90%) followed by 75 ppm (25.23% and 25.26%, 64.21% and 64.33%, 29.34% and29.37%) and the lower ECI was recorded at 150 ppm (22.55% and 22.57%, 59.96% and 59.99%, 26.79% and 26.81%) compared to absolute control (23.04% and 23.13%, 62.06% and 62.15%, 27.82% and 27.92%) (Table 9).

Table 9: Effect of *S. platensis* mediated TiO_2NPs on efficiency of conversion of ingested food (%)

	Treatments	Efficiency of conversion of ingested food (ECI) (%) (Crop I)				Efficiency of conversion of ingested food (ECI) (%) (Crop II)			
		Instar				Instar			
		3rd	4th	5th	Mean	3rd	4th	5th	Mean
T_1	*S. p* TiO_2NPs 25 ppm	24.76^{bc}	63.55^{c}	29.04^{b}	**39.12**	24.80^{c}	63.65^{c}	29.06^{c}	**39.17**
T_2	*S. p* TiO_2NPs 50 ppm	25.66^{a}	64.86^{a}	29.89^{a}	40.14	25.95^{a}	64.90^{a}	29.90^{a}	40.25
T_3	*S. p* TiO_2NPs 75 ppm	25.23^{ab}	64.21^{b}	29.34^{b}	39.59	25.26^{b}	64.33^{b}	29.37^{b}	39.65
T_4	*S. p* TiO_2NPs 100 ppm	$23.28d^{ef}$	61.54^{f}	27.30^{e}	37.37	23.36^{de}	61.62^{e}	27.36^{f}	37.45

T_5	*S. p* TiO_2NPs 125 ppm	22.84^{fg}	61.24^{f}	27.11^{ef}	37.06	22.91^{fg}	61.35^{e}	27.13^{f}	37.13
T_6	*S. p* TiO_2NPs 150 ppm	22.55^{g}	59.96^{g}	26.79^{f}	36.43	22.57^{g}	59.99^{f}	26.81^{g}	36.46
T_7	*Spirulina* alone	24.44^{c}	63.30^{c}	28.71^{c}	38.82	24.50^{c}	63.41^{c}	28.74^{d}	38.88
T_8	TiO2 alone	23.66^{d}	63.34^{c}	28.62^{c}	38.54	23.70^{d}	63.42^{c}	28.69^{d}	38.60
T_9	Distilled water spray	23.47^{de}	62.36^{d}	27.95^{d}	37.93	23.60^{d}	62.38^{d}	27.96^{e}	37.98
T_{10}	Absolute control	23.04^{efg}	62.06^{d}	27.82^{d}	37.64	23.13ef	62.15^{d}	27.92^{e}	37.73
	SE (d)	0.27	0.27	0.16		0.18	0.21	0.12	
	CD (0.05)	0.56**	0.57**	0.32**		0.38**	0.44**	0.26**	

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.

Efficiency of conversion of digested food (ECD) (%)

In the present study efficiency of conversion of ingested food was observed in crops I and II. In the third instar, the highest ECD was recorded at 50 ppm (29.83% and 29.86%). This was followed by 75 ppm (29.38% and 29.31%) and 25 ppm (29.02% and 29.03%). The lowest value of ECD was recorded at 150 ppm (27.50% and 27.49%).

In the fourth instar, maximum ECD was recorded at 50 ppm (75.93% and 75.95%) followed by 75 ppm (75.32% and 75.35%) and 25 ppm (75.01% and 75.02%). The lowest value of ECD was recorded at 150 ppm (73.83% and 73.84%).

In the fifth instar, the highest ECD was recorded at 50 ppm (44.93% and 44.95%) followed by 75 ppm (44.41% and 44.42%) and 25 ppm (44.16% and 44.17%). The lowest value of ECD was recorded at 150 ppm (42.62% and 42.63%) (Table 10).

Table 10: Effect of *S. platensis* mediated TiO_2NPs on efficiency of conversion of digested food (%)

	Treatments	Efficiency of conversion of digested food (ECD) (%) (Crop I)				Efficiency of conversion of digested food (ECD) (%) (Crop II)			
		Instar				Instar			
		3rd	4th	5th	Mean	3rd	4th	5th	Mean
T_1	*S. p* TiO_2NPs 25 ppm	29.02^{c}	75.01^{c}	44.16^{c}	49.40	29.03^{c}	75.02^{c}	44.17^{c}	49.41
T_2	*S. p* TiO_2NPs 50 ppm	29.83^{a}	75.93^{a}	44.93^{a}	50.23	29.86^{a}	75.95^{a}	44.95^{a}	50.25
T_3	*S. p* TiO_2NPs 75 ppm	29.38^{b}	75.32^{b}	44.41^{b}	49.70	29.39^{b}	75.35^{b}	44.42^{b}	49.72
T_4	*S. p* TiO_2NPs 100 ppm	28.01^{f}	73.99^{g}	42.96^{f}	48.32	28.03^{g}	74.01^{h}	42.96^{g}	48.33
T_5	*S. p* TiO_2NPs 125 ppm	27.89^{f}	73.93^{g}	42.86^{g}	48.23	27.87^{h}	73.93^{i}	42.87^{h}	48.22
T_6	*S. p* TiO_2NPs 150 ppm	27.50^{g}	73.83^{h}	42.62^{h}	47.98	27.49^{i}	73.84^{j}	42.63^{i}	47.99
T_7	*Spirulina* alone	28.92^{c}	74.87^{d}	43.96^{d}	49.25	28.93^{d}	74.88^{d}	43.97^{d}	49.26
T_8	TiO2 alone	28.86^{c}	74.78^{d}	43.97^{d}	49.20	28.87^{d}	74.79^{e}	43.99^{d}	49.22
T_9	Distilled water spray	28.53^{d}	74.45^{e}	43.90^{d}	48.96	28.56^{e}	74.46^{f}	43.92^{e}	48.98
T_{10}	Absolute control	28.21^{e}	74.15^{f}	43.20^{e}	48.52	28.31^{f}	74.16^{g}	43.24^{f}	48.57
	SE(d)	0.08	0.04	0.03		0.04	0.03	0.02	
	CD (0.05)	0.16**	0.09**	0.07**		0.09**	0.06**	0.04**	

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.

Approximate digestability (AD)

The Approximate digestability (AD) was observed in crops I and II. In the third instar, the highest value of AD was recorded at 50 ppm (84.70% and 84.69%), followed by 75 ppm (84.57% and 84.54%), 25 ppm (84.31% and 84.31%) and the lowest value of AD was recorded at 150 ppm (82.07% and 82.06%).

In the fourth instar, maximum AD was recorded at 50 ppm (84.99% and 85.00%) followed by 75 ppm (84.87% and 84.88%), 25 ppm (84.44% and 84.45%) and the minimum value of AD was recorded at 150 ppm (82.12% and 82.14%).

In the fifth instar, the highest value of AD was recorded at 50 ppm (68.33% and 68.67%) followed by 75 ppm (67.55% and 67.57%), 25 ppm (67.13% and 67.15%) and the lowest value of AD was recorded at 150 ppm (65.34% and 65.35%) (Table 11).

Table 11: Effect of *S. platensis* mediated TiO_2NPs on approximate digestibility (%)

	Treatments	Approximate digestibility (AD) (%) (Crop I)				Approximate digestibility (AD) (%) (Crop II)			
		Instar				Instar			
		3rd	4th	5th	Mean	3rd	4th	5th	Mean
T_1	*S. p* TiO_2NPs 25 ppm	84.31^{c}	84.44^{c}	67.13^{c}	78.63	84.31^{c}	84.45^{c}	67.15^{c}	78.64
T_2	*S. p* TiO_2NPs 50 ppm	84.70^{a}	84.99^{a}	68.33^{a}	79.34	84.69^{a}	85.00^{a}	68.67^{a}	79.45
T_3	*S. p* TiO_2NPs 75 ppm	84.57^{b}	84.87^{b}	67.55^{b}	79.00	84.54^{b}	84.88^{b}	67.57^{b}	79.00
T_4	*S. p* TiO_2NPs 100 ppm	82.92^{g}	82.97^{h}	65.99^{f}	77.29	82.88^{h}	82.98^{h}	66.00^{f}	77.29
T_5	*S. p* TiO_2NPs 125 ppm	82.48^{h}	82.56^{i}	65.88^{f}	76.97	82.46^{i}	82.57^{i}	65.88^{f}	76.97
T_6	*S. p* TiO_2NPs 150 ppm	82.07^{h}	82.12^{i}	65.34^{g}	76.51	82.06^{j}	82.14^{j}	65.35^{g}	76.52
T_7	*Spirulina* alone	83.24^{e}	84.00^{d}	66.88^{cd}	78.04	83.22^{e}	84.00^{d}	66.88^{cd}	78.03
T_8	TiO2 alone	83.44^{d}	83.76^{e}	66.76^{d}	77.99	83.42^{d}	83.76^{e}	66.77^{d}	77.98
T_9	Distilled water spray	83.03^{f}	83.13^{f}	66.33^{e}	77.50	83.01^{f}	83.14^{f}	66.34^{e}	77.50

T_{10}	Absolute control	82.98^{fg}	83.01^{g}	66.20^{f}	77.40	82.96^{g}	83.02^{g}	66.20^{f}	77.39
	SE (d)	0.03	0.01	0.16		0.02	0.01	0.16	0.03
	CD (0.05)	0.06**	0.02**	0.32**		0.04**	0.02**	0.32**	0.04**

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.

Silk gland weight (mg)

In crop I, silkworms were reared in mulberry leaves treated with *S. platensis* mediated TiO_2NPs which recorded the statistically superior silk gland weight of 790.31 mg at the concentration of 50 ppm followed by 75 ppm (782.28 mg), 25 ppm (776.61 mg), *Spirulina* alone (775.04 mg), TiO_2 alone (774.17 mg), distilled water spray (686.29 mg), absolute control (684.95 mg), 100 ppm (660.02 mg) and 125 ppm (640.15 mg). The 150 ppm showed that the statistically inferior silk gland weight (640.15 mg).

In crop II, silk gland weight of 790.70 mg was observed at maximum in 50 ppm treatment which was found to be statistically superior over all other treatments. The lowest silk gland weight of 630.79 mg was recorded at 150 ppm concentration (Table 12).

Silk productivity (cg/ day)

Statistically superior silk productivity of 9.411 cg day^{-1} and 9.778 cg day^{-1} was registered at the treatment of 50 ppm in crop I and II respectively followed by 75 ppm (8.289 cg day^{-1} and 8.833 cg day $^{-1}$). The statistically inferior silk productivity of 3.100 cg day^{-1}and 3.122 cg day^{-1} was noticed at 150 ppm (Table.12).

Table 12: Effect of *S. platensis* mediated TiO_2NPs on silk gland weight and silk productivity

	Treatments	Silk gland weight (mg)		Silk productivity (cg day^{-1})	
		Crop 1	Crop 1I	Crop 1	Crop 1I
T_1	*S. p* TiO_2NPs 25 ppm	776.61^{c}	776.98^{c}	6.322^{d}	4.178^{f}
T_2	*S. p* TiO_2NPs 50 ppm	790.31^{a}	790.70^{a}	9.411^{a}	9.778^{a}
T_3	*S. p* TiO_2NPs 75 ppm	782.28^{b}	782.33^{b}	8.289^{b}	8.833^{b}
T_4	*S. p* TiO_2NPs 100 ppm	660.02^{h}	660.61^{g}	4.478^{f}	3.889^{f}
T_5	*S. p* TiO_2NPs 125 ppm	640.15^{i}	640.63^{h}	3.411^{g}	3.333^{g}
T_6	*S. p* TiO_2NPs 150 ppm	630.40^{j}	630.79^{i}	3.100^{h}	3.122^{h}
T_7	*Spirulina* alone	775.04^{d}	775.06^{d}	6.911^{c}	7.533^{c}
T_8	TiO2 alone	774.17^{e}	774.46^{d}	5.900^{e}	6.600^{e}
T_9	Distilled water spray	686.29^{f}	686.67^{e}	6.000^{e}	7.011^{d}
T_{10}	Absolute control	684.95^{g}	685.07^{f}	5.811^{e}	6.878^{de}
	SE (d)	0.34	0.46	0.10	0.18
	CD (0.05)	0.70**	0.96**	0.20**	0.39**

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.

Effects of synthesised *S. platensis* mediated TiO_2NPs on cocoon parameters

Single cocoon weight (g)

The highest cocoon weight was recorded on 50 ppm (2.24 g). This was followed by 75 ppm (2.09 g), *Spirulina* alone (2.03g), distilled water spray (2.01g), absolute control (2.00g), TiO_2 alone (2.01g and 25 ppm (1.95 g) which were found to be on par with each other. The lowest cocoon weight was recorded on 125 ppm (1.25 g) and 150 ppm (1.14g).

Similar observations were made in crop II. The maximum cocoon weight was recorded on 50 ppm (2.16 g). This was followed by 75 ppm (2.09 g), *Spirulina* alone (2.03g), distilled water spray (2.01g), absolute control (2.00g), TiO_2 alone (2.01g) and 25 ppm (1.95 g), which were found to be on par with each other. The lowest cocoon weight was recorde on 125 ppm (1.25 g) and 150 ppm (1.14g) (Table 13).

Pupa weight (g)

Pupa weight was observed in both crops I and II. Both crops recorded the highest pupa weight at 50 ppm (0.91g and 0.90g). The next best treatment was 75 ppm (0.87 g and 0.85 g) followed by 25 ppm (0.79 g and 0.78 g) and the lowest pupa weight was recorded at 150 ppm (0.69g and 0.68 g) in both crops I and II respectively (Table 13).

Table 13: Effect of *S. platensis* mediated TiO_2NPs on cocoon weight (g) and pupa weight (%)

	Treatments	Cocoon weight (g)		Pupa weight (g)	
		Crop I	Crop II	Crop I	Crop II
T_1	*S. p* TiO_2NPs 25 ppm	1.95^{c}	2.00^{cd}	0.79^{bcd}	0.78^{c}
T_2	*S. p* TiO_2NPs 50 ppm	2.24^{a}	2.16^{a}	0.91^{a}	0.90^{a}
T_3	*S. p* TiO_2NPs 75 ppm	2.09^{b}	2.09^{b}	0.87^{b}	0.85^{b}
T_4	*S. p* TiO_2NPs 100 ppm	1.21^{de}	1.48^{e}	0.77^{cd}	0.78^{c}
T_5	*S. p* TiO_2NPs 125 ppm	1.25^{d}	1.45^{e}	0.73^{de}	0.71^{d}
T_6	*S. p* TiO_2NPs 150 ppm	1.14^{e}	1.42^{e}	0.69^{e}	0.68^{e}
T_7	*Spirulina* alone	2.03^{bc}	2.03^{c}	0.80^{bc}	0.77^{c}
T_8	TiO2 alone	1.98^{c}	1.98^{d}	0.79^{cd}	0.77^{c}
T_9	Distilled water spray	2.01^{bc}	2.01^{cd}	0.82^{bc}	0.77^{c}
T_{10}	Absolute control	2.00^{bc}	2.00^{cd}	0.77^{cd}	0.73^{d}

	SE(d)	0.05	0.02	0.03	0.01
	CD (0.05)	0.1**	0.05**	0.07**	0.02**

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.

Shell weight (g)

The results of shell weight were observed in crop I. These different treatments were highly significant to each other. The maximum shell weight was recorded at 50 ppm (0.59 g). In this experiment next better treatment was 75 ppm (0.53g), followed by *Spirulina* alone (0.45g), TiO_2 alone (0.40g), distilled water spray (0.42g) and absolute control (0.41g) which were found to be on par with each other. The lowest shell weight had recorded at 150 ppm (0.19 g).
Similar results of shell weight were observed in crop II. These different treatments were highly significant to each other. The maximum shell weight had recorded at 50 ppm (0.54 g). In this experiment next best treatment was 75 ppm (0.48g), followed by 25 ppm (0.38 g), *Spirulina* alone (0.38g), TiO_2 alone at (0.37), distilled water spray (0.37 g), absolute control (0.37g) which were found to be on par with each other. The lowest shell weight was recorded at 150 ppm at (0.17 g) (Table 14).

Shell ratio (g)

The shell ratio of the cocoon was significant among the different treatments. The highest value of shell ratio of 26.16 g and 24.86 g was recorded at 50 ppm in I and II crops respectively. The lowest value of shell ratio was recorded at 150 ppm (14.96g and 10.95g) (Table 14).

Table 14: Effect of *S. platensis* mediated TiO_2NPs on shell weight (g) and shell ratio (%)

	Treatments	**Shell weight (g)**		**Shell ratio (%)**	
		Crop I	**Crop II**	**Crop I**	**Crop II**
T_1	*S. p* TiO_2NPs 25 ppm	0.25^{e}	0.38^{c}	21.10^{bc}	19.20^{c}
T_2	*S. p* TiO_2NPs 50 ppm	0.59^{a}	0.54^{a}	26.16^{a}	24.86^{a}
T_3	*S. p* TiO_2NPs 75 ppm	0.53^{b}	0.48^{b}	25.16^{a}	22.97^{b}
T_4	*S. p* TiO_2NPs 100 ppm	0.23^{e}	0.25^{d}	19.24^{cd}	14.71^{d}
T_5	*S. p* TiO_2NPs 125 ppm	0.20^{f}	0.19^{e}	17.62^{d}	11.79^{e}
T_6	*S. p* TiO_2NPs 150 ppm	0.19^{f}	0.17^{e}	14.96^{e}	10.95^{e}
T_7	*Spirulina* alone	0.45^{c}	0.38^{c}	22.31^{b}	18.84^{c}
T_8	TiO2 alone	0.40^{d}	0.37^{c}	20.20^{bc}	18.86^{c}
T_9	Distilled water spray	0.42^{d}	0.37^{c}	21.03^{bc}	18.58^{c}
T_{10}	Absolute control	0.41^{d}	0.36^{c}	21.13^{bc}	18.14^{c}
	SE (d)	0.01	0.01	1.03	0.69
	CD (0.05)	0.02**	0.02**	2.15**	1.43**

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.

Effect of *S. platensis* mediated TiO_2NPs on cocoon yield characters

Cocoon yield/10,000 larvae (by number)

The cocoon yield by numbers was observed in crop I. A significant with among all these different treatments. The maximum number was recorded at 50 ppm (9222.22), followed by 75 ppm (8222.22) which were on par with 25 ppm (7888.89) and TiO2 alone (7888.89), Spirulina alone (7555.56) which were on par with absolute control (7777.78) and distilled water spray (7222.22), 100 ppm (6555.56), 125 ppm (5555.56). The lowest number was recorded at 150 ppm (5111.11).
Similar observations were recorded in crop II. The maximum number was recorded at 50 ppm (9333.33), followed by 75 ppm (8333.33) which were on par with 25 ppm (8000.00) and TiO2 alone (8000.00), Spirulina alone (7666.67) which were on par with absolute control (7666.67) and distilled water spray (7333.33), 100 ppm (6666.67), 125 ppm (5666.67). The lowest number was recorded at 150 ppm (5000.00) (Table 15).

Cocoon yield/10,000 larvae (by weight) (g)

The maximum weight of cocoon yield was recorded at 50 ppm (20696 g) in crop I and II respectively, followed by 75 ppm (17212.22 g), 25 ppm (15622.22 g) which were on par with TiO2 alone (7888.89 g) and *Spirulina* alone (15.366.67 g), *Spirulina* alone (15.366.67 g) which were on par with absolute control (15582.22 g) and distilled water spray (15366.67 g), 100 ppm (7951.11 g), 125 ppm (6388.89 g) which were on par with 150 ppm (6315.56 g). The lowest weight was recorded at 150 ppm (6315.56 g).
Similar observations were recorded in crop II. The maximum weight was recorded at 50 ppm (20154.44 g). This was followed by 75 ppm (17417.78 g), 25 ppm (15974.44 g) which were on par with TiO2 alone (15841.11 g), *Spirulina* alone (15588.89 g) and control (15360 g), *Spirulina* alone (15588.89 g) which were on par with TiO2 alone (15841.11 g), absolute control (15360 g) and distilled water spray (14763.33 g), 100 ppm (11685.56 g), 125 ppm (8972.22 g) which was on par with 150 ppm (7933.33 g). The lowest weight was recorded at 150 ppm (7933.33 g) (Table 15).

Table 15: Effect of *S. platensis* mediated TiO_2NPs on cocoon yield

	Treatments	Cocoon yield/10, 000 larvae (by number)		Cocoon yield/10,000 larvae (by weight) (g)	
		Crop I	Crop II	Crop I	Crop II
T_1	*S. p* TiO_2NPs 25 ppm	7888.89^{b}	8000.00^{bc}	15622.22^{c}	15974.44^{c}
T_2	*S. p* TiO_2NPs 50 ppm	9222.22^{a}	9333.33^{a}	20696.67^{a}	20154.44^{a}
T_3	*S. p* TiO_2NPs 75 ppm	8222.22^{b}	8333.33^{b}	17212.22^{b}	17417.78^{b}
T_4	*S. p* TiO_2NPs 100 ppm	6555.56^{e}	6666.67^{e}	7951.11^{e}	11685.56^{e}
T_5	*S. p* TiO_2NPs 125 ppm	5555.56^{f}	5666.67^{f}	6388.89^{f}	8972.22^{f}
T_6	*S. p* TiO_2NPs 150 ppm	5111.11^{g}	5000.00^{g}	6315.56^{f}	7933.33^{f}
T_7	*Spirulina* alone	7555.56^{cd}	7666.67^{cd}	15366.67^{cd}	15588.89^{cd}
T_8	TiO2 alone	7888.89^{bc}	8000.00^{bc}	15621.11^{c}	15841.11^{cd}
T_9	Distilled water spray	7222.22^{d}	7333.33^{d}	14541.11^{d}	14763.33^{d}
T_{10}	Absolute control	7777.78^{cd}	7666.67^{c}	15582.22^{cd}	15360.00^{cd}
	SE (d)	272.17	198.76	500.01	536.80
	CD (0.05)	567.73**	414.61**	1043.01**	1119.75**

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.

Effect of *S. platensis* mediated TiO_2NPs on silk filament characters

Silk filament weight (g)

The results of silk filament weight were recorded during crop I. The maximum silk filament weight was observed at 50 ppm (0.35 g) followed by 75 ppm (0.33 g), 25 ppm (0.29 g) which was on par with the distilled water spray (0.29 g), 100 ppm (0.28 g) which were on par with absolute control (0.27 g) and *Spirulina* alone (0.22 g), 125 ppm (0.19 g) on par with TiO_2 alone (0.20 g). The least value of silk filament weight was recorded at 150 ppm (0.17 g).
Similar observations were made in crop II. The maximum silk filament weight was observed at 50 ppm (0.37 g). the better treatment was followed by 75 ppm (0.33 g), 25 ppm (0.28 g) which was on par with the distilled water spray (0.29 g), 100 ppm (0.26 g) which were on par with absolute control (0.27 g) and *Spirulina* alone (0.22 g), 125 ppm (0.20 g) on par with TiO_2 alone (0.22 g). The lowest value of silk filament weight was recorded at 150 ppm (0.17 g) (Table 16).

Silk filament length (m)

In crop I the highest silk filament length was recorded 50 ppm (1455 m). This was followed by 75 ppm (1356 m), distilled water spray (1300 m) which was on par with absolute control (1282 m), absolute control (1282 m) which was on par with TiO_2 alone (1255 m), TiO_2 alone (1255 m) which was on par with *Spirulina* alone (1260 g), 25 ppm (1220), 100 ppm (1006 m) which was on par with 125 ppm (973m), 125 ppm (973 m) which was on par with 150 ppm (953 g). The lowest value was recorded at 150 ppm (953 g).
In crop II. The highest value was recorded at 50 ppm (1447 m), followed by 25 ppm, 75 ppm and all other treatments. Except for maximum and minimum values, all were on par with each other. The minimum value was recorded at 150 ppm (957 m) (Table 16).

Table 16: Effect of *S. platensis* mediated TiO_2NPs on silk filament parameters

	Treatments	Silk filament weight (g)		Silk filament length (m)	
		Crop I	Crop II	Crop I	Crop II
T_1	*S. p* TiO_2NPs 25 ppm	0.29^{c}	0.28^{c}	1220^{e}	1237^{c}
T_2	*S. p* TiO_2NPs 50 ppm	0.35^{a}	0.37^{a}	1455^{a}	1447^{a}
T_3	*S. p* TiO_2NPs 75 ppm	0.33^{b}	0.33^{b}	1356^{b}	1318^{b}
T_4	*S. p* TiO_2NPs 100 ppm	0.28^{d}	0.26^{d}	1006^{f}	1061^{d}
T_5	*S. p* TiO_2NPs 125 ppm	0.19^{f}	0.20^{f}	973^{fg}	977^{e}
T_6	*S. p* TiO_2NPs 150 ppm	0.17^{g}	0.17^{g}	953^{g}	957^{e}
T_7	*Spirulina* alone	0.22^{e}	0.23^{e}	1260^{d}	1265^{c}
T_8	TiO2 alone	0.20^{f}	0.22^{f}	1255^{de}	1243^{c}
T_9	Distilled water spray	0.29^{c}	0.29^{c}	1300^{c}	1286^{bc}
T_{10}	Absolute control	0.27^{d}	0.27^{d}	1282^{cd}	1287^{bc}
	SE(d)	0.01	0.01	17.92	25.28
	CD (0.05)	0.01**	0.01**	37.38**	52.73**

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.

Denier

In crop I the highest value was recorded at 50 ppm (2.16), The next best treatment was 25 ppm (2.16) followed by 75 ppm (1.99) which was on par with distilled water spray (2.03) and absolute control (1.94), *Spirulina* alone (1.79) which was on par with TiO_2 alone (1.76), TiO_2 alone (1.76) which was on par with 100 ppm (1.64) and 125 ppm (1.63). The lowest value was recorded at 150 ppm (1.52).
In crop II. The highest value was recorded at 50 ppm (2.28), followed by 75 ppm (2.21), 25 ppm (2.08) and the lowest value was recorded at 150 ppm (1.57) (Table 17).

Renditta (kg)

The renditta were recorded in crop I. The minimum value was recorded at 50 ppm (4.98 kg), followed by 75 ppm (5.96 kg) which was on par with 25 ppm (5.80 kg),

Spirulina alone (6.24 kgs), distilled water spray (6.43 kg) which was on par with 100 ppm (6.41 kg), absolute control (7.29 kg), 125 ppm (7.89 kg), TiO_2 alone (8.31 kg). The lowest value was recorded at 150 ppm (9.46 kg).
In crop II. The minimum value was recorded at 50 ppm (5.36 kg), followed by 75 ppm (6.04 kg), distilled water spray (7.17 kg) which was on par with 25 ppm (7.23 kg), 25 ppm (7.23 kg) which was on par with *Spirulina* alone (8.24 kg) and absolute control (8.28 kg), absolute control (8.28 kg) which was on par with TiO_2 alone (8.49 kg), 100 ppm (9.40 kg), 125 ppm (10.78 kg). The lowest value recorded at 150 ppm (11.63 kg) (Table 17).

Table 17: Effect of *S. platensis* mediated TiO_2NPs on reeling parameters

	Treatments	Denier		Renditta (Kg)	
		Crop I	Crop II	Crop I	Crop II
T_1	*S. p* TiO_2NPs 25 ppm	2.16^{a}	2.08^{bc}	5.80^{b}	7.23^{bc}
T_2	*S. p* TiO_2NPs 50 ppm	2.16^{a}	2.28^{a}	4.98^{a}	5.36^{a}
T_3	*S. p* TiO_2NPs 75 ppm	1.99^{b}	2.21^{ab}	5.96^{b}	6.04^{a}
T_4	*S. p* TiO_2NPs 100 ppm	1.64^{de}	1.64^{ef}	6.41^{d}	9.40^{e}
T_5	*S. p* TiO_2NPs 125 ppm	1.63^{de}	1.64^{ef}	7.89^{f}	10.78^{f}
T_6	*S. p* TiO_2NPs 150 ppm	1.52^{f}	1.57^{f}	9.46^{h}	11.63^{g}
T_7	*Spirulina* alone	1.79^{c}	1.81^{de}	6.24^{c}	8.24^{bc}
T_8	TiO2 alone	1.76^{cd}	1.79^{d}	8.31^{g}	8.49^{de}
T_9	Distilled water spray	2.03^{b}	2.06^{bc}	6.43^{d}	7.17^{b}
T_{10}	Absolute control	1.94^{b}	2.05^{bc}	7.29^{e}	8.28^{cd}
	SE (d)	0.06	0.09	0.08	0.52
	CD (0.05)	0.13**	0.18**	0.17**	1.1**

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.

Discussion

Effect of *S. platensis* mediated TiO_2NPs on larval characters

The larval weight and larval duration were significantly increased due to the feeding the larvae with *S. platensis* mediated TiO_2NPs at concentration of 50 ppm treated mulberry leaves as compared to control. This result corroborates with the findings of Dharanipriya and Thangapandiya (2019) [3] who observed that more mature larval weight when fed with *S. platensis* mediated AgNPs treated mulberry leaves. Due to the stimulation of AgNPs and spirulina from the third to fifth instars feeding more amount of nutrients intake. Similar, observation was also made by Soliman (2021) [16] who reported that larval weight was increased to be 1.98 g during 5th instar, which is fed with 0.05 per cent of spirulina extract enriched mulberry leaves.

The *S. platensis* mediated TiO_2NPs the concentration 50 ppm treated mulberry leaves when fed to silkworm had registered significantly higher effective rate of rearing 92.22 per cent and 93.33 per cent compared to control 77.78 per cent and 76.67

per cent, lower mortality 8.89 percent and 7.78 per cent respectively in I and II crops. This might be due to increased macronutrient content present in *S. platensis* mediated TiO_2NPs treated mulberry leaves fed to silkworms had higher larval weight and effective rate of rearing of silkworm to control. The present result falls in line with Govindaraju *et al.* (2011) [6] who observed that higher effective rate of rearing and lower mortality rate. Similar trend was observed by Alipanah *et al.* (2021) [1] who reported that TiO_2NPs treated mulberry leaves when fed to silkworm larvae resulted in higher ERR and lower mortality rate over control.

Effect of *S. platensis* mediated TiO_2NPs 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_2NPs 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). This result corroborates with the findings of Soliman *et al.* (2021) [16] 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) [14] who recorded that supplementation of mulberry leaves with probiotics like *S. cerevisiae* and *Spirulina* increased the haemolymph protein and amino acids.

Effect of *S. platensis* mediated TiO_2NPs on nutritional indices

The *S. platensis* mediated TiO_2NPs the concentration 50 ppm treated mulberry leaves when fed to silkworm had registered significantly higher consumption index (CI) (0.95 and 0.97), growth rate (GR) (0.32 g/g/day and 0.34 g/g/day), efficiency of conversion of ingested food (ECI) (40.14% and 40.25%), efficiency of conversion of digested food (ECD) (50.23% and 50.25%), approximate digestibility (79.34% and 79.45%) respectively in I and II crops. Similar, trend was observed by Alipanah *et al.* (2021) [1] who reported that TiO_2NPs treated mulberry leaves when fed to silkworm larvae resulted in higher CI, GR, ECI, ECD and AD over control. The present result falls in line with who observed that higher nutrition metabolism through effects of TiO_2NPs.

Effect of *S. platensis* mediated TiO_2NPs on silk gland parameters

Noticeable differences were recorded with regard to silk gland weight and silk productivity in *S. platensis* mediated TiO2NPs at the concentration of 50 ppm as compared to that of control. Significantly highest silk gland weight (782.28 mg and 790.70 mg) and silk productivity (9.411 cgday^{-1} and 9.778 cgday^{-1}) were observed in *S. platensis* mediated

TiO2NPs concentration of 50 ppm followed by 75 ppm which might be due to *Spirulina* mediated activation of tissue metabolism seems to be an essential factor for the promotion of biological parameters of silk gland of silkworm larvae (Kumar and Balasubramanian, 2014) [13]. These results are in parity with the findings of Chai *et al.* (2015) [2] who reported that TiO_2NPs treated mulberry leaves and feeding these leaves to silkworm increased the silk gland weight and silk productivity. The present result also made agreement with Dharanipriya and Thangapandiya (2019) [3] who registered the silk gland weight (870 mg).

Effect of *S. platensis* mediated TiO_2NPs on cocoon parameters

Application of *S. platensis* mediated TiO_2NPs formulation had significantly influenced the economic traits of cocoon. The results on the economic traits of cocoon *viz*., single cocoon weight, pupa weight, shell weight and shell ratio were found to be superior in *S. platensis* mediated TiO_2NPs in the concentration of 50 ppm (2.24g and 2.16g, 0.91g and 0.90, 0.59g and 0.54g, 26.16% and 24.86%). This might be due to presence of higher crude protein and soluble protein content in *S. platensis* mediated TiO_2NPs treated mulberry leaves. The present result falls in line with Dharanipriya and Thangapandiyan (2019) [3] who reported the significantly increased single cocoon weight (1.84 g), shell weight (0.332 g) and shell ratio (24.77%) due to feeding the larvae with *S. platensis* mediated TiO_2NPs treated mulberry leaves. It was strengthened by the Soliman *et al.* (2021) [16] who observed the positive effect on single cocoon weight, shell weight and shell ratio due to the fortification of *Spirulina* treated mulberry leaves. The higher amount of protein content present in the *Spirulina* which leads to increase the protein assimilation in silkworm ultimately cocoon weight and shell weight were increased.

The present result corroborates with Esaivani *et al.* (2014) [4] who stated that pupal weight, cocoon weight, shell weight, shell ratio and silk traits of *Bombyx mori* were significantly increased by *Saccharomyces cerevisiae* with *Spirulina* probiotic. The reason might be which increased the enzymatic activity of silkworms. Similar, observation was observed by Alipanah *et al.* (2021) [1] who reared the silkworms which fed with TiO_2NPs and noticed cocoon weight (1.89 g), shell weight (0.414 g) and shell ratio (22.02%).

Effect of *S. platensis* mediated TiO_2NPs on cocoon yield parameters

Significantly higher cocoon yield per 10000 larvae was registered in the larvae fed with *S. platensis* mediated TiO_2NPs at the concentration of 50 ppm, followed by 75 ppm and 25 ppm compared to control. This might be due to increased ERR in the *S. platensis* mediated TiO_2NPs at the concentration of 50 ppm treated mulberry leaves. The present study falls in line with Govindaraju *et al.* (2011) [6] who reported an increased cocoon yield was noticed. Chai *et al*. (2015) [2] also reported that the application of TiO_2NPs treated mulberry leaves enhanced the cocoon yield.

Effect of *S. platensis* mediated TiO_2NPs on silk filament parameters

Significantly higher filament length and filament weight of 1445 m and 1447 m, and 0.35 g and 0.37g in I and II crops respectively were significantly higher in the cocoons spun by larvae treated with *S. platensis* mediated TiO_2NPs at the concentration of 50 ppm. The present result corroborates with findings of Ni *et al.* (2014) [13] filament length and silk weight were significantly increased by TiO_2NPs treated mulberry leaves. Zhang *et al.* (2014) [19] who reported that TiO2 NPs can enhance the food conversion efficiency of silkworms and which will in turn increases the quality and yield of cocoon and silk filament.

The silk filament denier of 2.16 and 2.28 respectively was recorded in the cocoons spun by fed with *S. platensis* mediated TiO_2Nps at the concentration of 50 ppm treated mulberry leaves in I and II crops. This might be due to presence of protein rich sources in *S. platensis* mediated TiO_2Nps treated mulberry leaves which enhance silk protein synthesis and would reflects in longer filament length and higher silk denier. It is also supported by Ni *et al.* (2015) [20] who reported that significantly increased denier of 2.17 due to TiO_2Nps treated mulberry leaves.

Conclusion

Larvae fed mulberry leaves treated with *S. platensis* mediated TiO_2NPs at the concentration of 50 ppm recorded on larval weight (4.34 g), larval duration (203 hr), ERR (92.22%) and larval mortality (8.89%) compared to control. Silk gland parameters *viz*., silk gland weight and silk productivity were significantly superior in larvae fed with leaves obtained from *S. platensis* mediated TiO_2NPs (50 ppm) treated mulberry leaves. The fortification of mulberry leaves with *S. platensis* mediated TiO_2NPs at the concentration of 50 ppm treated mulberry leaves also significantly enhanced the cocoon weight (2.24 g), shell weight (0.57 g), shell ratio (26.16%) and cocoon yield/10000 larvae (by number and by weight) (9222.22 Nos and 20696 g). The reeling parameters *viz*., filament length (1455 m), filament weight (0.36 g) and renditta (5.36 kg) were also found to be superior due to *S. platensis* mediated TiO_2NPs at the concentration of 50 ppm.

References

1. Alipanah M, Feizi H, Nasiri A, Sarjamei F. Effects of Dioxide Titanium nanoparticles (TiO2) on growth and production characteristics of silkworms; c2021.
2. Chai L, Shao H, Hu X, Zhang Y. Reinforced and ultraviolet resistant silks from silkworms fed with titanium dioxide nanoparticles. ACS Sustainable Chemistry & Engineering. 2015;3(10):2551-2557.
3. Dharanipriya R, Thangapandiya. Comparative study of nutritional and economical parameters of silkworm (*Bombyx mori*) treated with silver nanoparticles and Spirulina. The Journal of Basic and Applied Zoology. 2019;80(1):1-12.
4. Esaivani C, Vasanthi K, Bharathi R, Chairman K. Impact of probiotic Saccharomyces cerevisiae on the enzymatic profile and the economic parameters of Silkworm *Bombyx mori* L. Adv. Biol. Biomed. 2014;1:1-7.
5. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J biol Chem. 1957;226(1):497-509.
6. Govindaraju K, Tamilselvan S, Kiruthiga V, Singaravelu G. Silvernanotherapy on the viral borne disease of silkworm *Bombyx mori* L. Journal of Nanoparticle Research. 2011;13(12):6377-6388.

7. Iyengar M, Jolly M, Datta R, Subramanian R. Relative silk productivity of different silkworm breeds and its use breeding index. Natl. Semi. Silk Res. & Dev., CSB, Bangalore; c1983. p. 10-13.
8. Joseph I, Edwin Chellaiah D, AJA Ranjit Singh. Studies on the influence of Beauveria bassiana on survival and gut flora of groundnut caterpillar, Spodoptera litura Fab. Journal of Biopesticides. 2010;3(3):553.
9. Kumar P, Govindaraju M, Senthamilselvi S, Premkumar K. Photocatalytic degradation of methyl orange dye using silver (Ag) nanoparticles synthesized from *Ulva lactuca*. Colloids and Surfaces B: Biointerfaces. 2012;103:658-661.
10. Lowry OH. Protein measurement with the Folin phenol reagent. J biol Chem. 1951;193:265-275.
11. Mahmoud D, Salem D, Mo'men S, Barakat E, Salama M. Purification and characterization of phenoloxidase from immunized haemolymph of Schistocerca gregaria. African Journal of Biotechnology. 2015;14(44):3027-3036.
12. Schefter RP, Olsen KD, Gaffey TA. Cervical lymphangioma in the adult. Otolaryngology-Head and Neck Surgery. 1950;93(1):65-69.
13. Ni M, Li F, Tian J, Hu J, Zhang H, Xu K, *et al*. Effects of titanium dioxide nanoparticles on the synthesis of fibroin in silkworm (*Bombyx mori* L.). Biological trace element research. 2014;166(2):225-235.
14. Rani GA, Padmalatha C, Raj RS, Singh AR. Impact of supplementation of amway protein on the economic characters and energy budget of silkworm *Bombyx mori* L. Asian Journal of Animal Sciences. 2011;5(3):190-195.
15. Sankar R, Rizwana K, Shivashangari KS, Ravikumar V. Ultra-rapid photocatalytic activity of Azadirachta indica engineered colloidal titanium dioxide nanoparticles. Applied Nanoscience. 2015;5(6):731-736.
16. Soliman AM. The Impact of Fortification of Mulberry Leaves with the Yeast Saccharomyces cerevisiae and the Blue Green Algae Spirulina platensis on some Quantitative Parameters of Silkworm *Bombyx mori* (L.). Journal of Plant Protection and Pathology. 2021;12(1):55-59.
17. Shyed H, Ahmad M. Green synthesis of titanium dioxide (TiO_2) nanoparticles by using Mentha arvensis leaves extract and its antimicrobial properties. Inorganic and Nano-Metal Chemistry. 2013;50(10):1032-1038.
18. Waldbauer GP. The consumption and utilization of food by insects. In Advances in insect physiology, 229-288. Elsevier; c1968.
19. Zhang H, Ni M, Li F, Xu K, Wang B, Hong F, *et al*. Effects of feeding silkworm with nanoparticulate anatase TiO2 (TiO2 NPs) on its feed efficiency. Biological trace element research. 2014;159(1):224-232.
20. Ni X, Wong ZJ, Mrejen M, Wang Y, Zhang X. An ultrathin invisibility skin cloak for visible light. Science. 2015 Sep 18;349(6254):1310-4.
21. Shankar S, Shanker U. Arsenic contamination of groundwater: A review of sources, prevalence, health risks, and strategies for mitigation. The scientific world Journal. 2014 Oct 14;2014.
22. Kumar RS, Balasubramanian P, Govindaraj P, Krishnaveni T. Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of *Coriandrum sativum* L. roots (Coriander). Journal of Pharmacognosy and Phytochemistry. 2014;2(6):74-8.