Fortified Meal of Biologically Synthesized Selenium Nanoparticles (SeNPs) on Mulberry Leaves (Morus alba) and its Quantity on the Fat Body among Silkworm, Bombyx mori L.

A. Prabhakaran and G. Singaravelu

Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 12
Issue: 03

Res Jr of Agril Sci (2021) 12: 807–813

CARAS

Res. Jr. of Agril. Sci. (May-Jun 2021) 12(3): 807–813
www.rjas.org

Full Length Research Article

Fortified Meal of Biologically Synthesized Selenium Nanoparticles (SeNPs) on Mulberry Leaves (*Morus alba)* and its Quantity on the Fat Body among Silkworm, *Bombyx mori* L.

A. Prabhakaran*[1] and G. Singaravelu[2]

Received: 19 Dec 2020 | Revised accepted: 08 May 2021 | Published online: 19 May 2021

A B S T R A C T

In the current investigation, "Fortified meal of biologically synthesized selenium nanoparticles (SeNPs) on mulberry leaves (*Morus alba*) and its quantity on the fat body among silkworm, *Bombyx mori* L (Lepidoptera:Bombycidae)" synthesis of green selenium nanoparticles have been started from Nano-science Division, Department of Zoology, Thiruvalluvar University, Vellore-India. The treatment of fortification of mulberry leaves were conducted in a private Sericulture form at Vitchanthangal village, Kanchipuram-Dt, Tamil Nadu, India, since 2019. Silkworm rearing technique for the production of silk at sustainable nature of socio-economic developmental process is based on one man army of the duties and responsibilities of cultivators. Green technique of biologically synthesized selenium nanoparticles made in plant extract of *Prosopis cineraria* leaf was used and characterized by UV, FTIR, XRD and TEM. Multidimensional aspects of selenium nanoparticles have an innovative role on supplementation techniques. Biologically synthesized selenium nanoparticles could be an additional dosage on mulberry leaves (*Morus alba)* with minimum concentrations such as 500ppm, 1000ppm and 1500ppm for increase the fat body tissue level and demonstrated for the estimation of Quantitative level of biochemical assessment like Protein, Glycogen, Reducing sugar, Alanine amino transferase and Aspartate amino transferase level on fat body tissue at fifth day of fifth instar larvae of silkworm, *Bombyx mori* was carried out and its compared to control.

***Key words*:** Biologically synthesized SeNPs, Fat body assessments, *Morus alba*, *Bombyx mori*

Sericulture being technology sensitive, the productivity is dependent on technology up gradation and adoption. Among the various factors which determine the success of sericulture the pathological aspects deserve special attention and is of great economic concern to the silk farmers. Since there is no specific preventive measure for the occurrence and spread of infectious disease other than sanitized breeding, rearing methods, the only commercial practice available today is to discard large stocks of worms in case of infection. Most of the damage caused to sericulture can be attributed directly to silkworm disease rather than to unfavorable weather conditions. In order to make sericulture economically viable prevention of silkworm diseases is prime importance since most of the damage to sericulture can be attributed directly to pathogenic diseases. In the present investigation an attempt was made to identify the influence of biologically synthesized selenium nanoparticles on some aspects of silkworm biology. Further, it has been observed that biogenic Selenium are stable due to natural coating of the organic molecules and do not aggregate with time, whereas external addition of stabilizing agents is required in chemical synthesis. Based on the foregoing facts an attempt was undertaken to synthesis, biocompatible selenium nanoparticles using green chemistry approach.

In its production process, sericulture effects a long chain of interdependent specialized operations which provides means of earning livelihood to a larger section of the rural population [1]. Thus, the income flow is much more continuous and assured in sericulture than in the case of any other cash crop. Sericulture aims at increasing the yield and quality of silk produced which depend on the rearing conditions, sanitation, larval growth rate, size of the silk glands, availability of appropriate quantity of nutrients (Amino acid and other macro and micro nutrients) and efficient protein machinery. Growth of the larval instars in turn depends on the nutrient content of mulberry leaves and their ability to feed, digest, absorb and resynthesize the body protein [2-4]. Several researchers are trying to formulate artificial diet and semisynthetic diet [5-7] for the silkworm, *Bombyx mori* L. Due to variation in soil fertility, management practices, season and also economic status of the farmers, the

* A. Prabhakaran
✉ drprabhass1947@gmail.com
[1-2] Nano-science Division, Department of Zoology, Thiruvalluvar University, Vellore - 632 115, Tamil Nadu, India

leaves produced are not adequately balanced and demands enrichment of the nutrients [8]. The progress of sericulture in the state during the last two decades is revealed by the trends observed in its growth in terms of important parameters like the area under the mulberry, cocoon, production, raw silk production and the generation of employment in the Industry. While technological advantages include the comparatively lesser water requirement for the cultivation of mulberry, on the economic from the sericulture remains to be a highly labour-intensive activity providing vast-scope for both on-farm (mulberry cultivation and silkworm rearing) and off-farm (reeling and weaving of silk yarn) employment. Further, sericulture activities proved economically more profitable than other crops. The number of crops that a sericulturist can raise on a given piece of irrigated land can be anywhere between four and six per year and on a rainfed piece of land the farmer can raise at least two or three crops per year.

MATERIALS AND METHODS

In green technique, biologically synthesized selenium nanoparticles using plant extract of *Prosopis cineraria* leaf has been synthesized, characterized and that the selenium nanoparticles were fortified with mulberry leaves *Morus alba*. For assessment about fat body of silkworm, *Bombyx mori* hybrids: PM x NB_4 D_2 were maintained at hygienic conditions. The treatment of fortification technique was conducted in a private Farmer Sericulture form at Vitchanthangal village, Kanchipuram-Dt, Tamil Nadu, India. during the year 2019 (Pic 1). Biologically synthesized selenium nanoparticles could be an additional dosage on mulberry leaves for increase the fat body tissue level and demonstrated for the estimation of quantitative and qualitative nature of biological macromolecules in fat body was carried out in the organism of fifth day of fifth instar larvae of silkworm, *Bombyx mori* L.

Pic 1 Rearing shed with mulberry (*Morus alba*)

Characterization of biologically synthesized SeNPs

Characterization of Selenium nanoparticles is based on the size, morphology and surface charge, using such advanced microscopic techniques. Properties like surface morphology, size and overall shape are determined by electron microscopy techniques. The *Prosopis cineraria* leaves extract and Na_2SeO_3 solution mixture was then characterized using UV, FTIR, XRD and TEM [9]. In the present investigation an attempt was made to identify the influence of green synthesized selenium nanoparticles on some aspects of silkworm physiology.

Fortified meal of BSSeNPs on Mulberry (Morus alba)

In fortification technique supplementation of biologically synthesized selenium nanoparticles and its quantity on biochemical parameter like fat body physiology among mulberry Silkworm (*Bombyx mori* L) based on non-fortified mulberry leaves, *Morus alba* fed larval group of control, 500 ppm level of biologically synthesized selenium nanoparticles fortified on mulberry leaves fed larval group is Treatment-1, 1000 ppm level of biologically synthesized selenium nanoparticles fortified on mulberry leaves, fed larval group is Treatment-2 and 1500ppm level of biologically synthesized selenium nanoparticles fortified mulberry leaves fed larval group is Treatment-3. Interestingly it was observed that the mulberry leaves like *Morus alba* fortified with green selenium nanoparticles greatly enhance the consumption of the host plant and proper feeding. In general, pronounced growth of silkworm was observed (Pic 2ab).

Pic 2a Fortified mulberry drop to 5th instar larvae

Pic 2b Predation of 5th day of 5th instar larvae *B. mori*

Dissection and separation of fat body

Fat body was separated from dissected organism of fifth day of fifth instars larvae from the control and tested groups in experimental setup about mulberry silkworm, *B. mori*. Ten control and ten experimental larvae were subjected to biochemical analysis. Same procedure was followed for both experimental and control groups. Anaesthetization of silkworm before dissection was done using chloroform, the silkworms were then pinned dorso-ventrally on the dissection board (Pic 3). The body wall of the silkworms was cut open to expose the internal organs. The fat body were carefully dissected, segregated and subjected for the quantitative analysis about assessment of biological macromolecules like Proteins, Glycogen, reducing sugar, Alanine amino transferase and Aspartate amino transferase on fat body tissue at fifth day of fifth instar larvae of silkworm, *Bombyx mori* was carried out and it's compared to the control.

Pic 3 Dissection and separation of fat body

Biochemical assessment on fat body

Biochemical assessment of proteins, carbohydrates and lipids were estimated by using the body tissue like fat body. The fat body tissues were kept in an ice box until further biochemical analysis was done. The exposed tissues were carefully excised and blotted. Then the required quantity of tissues was weighed and used for biochemical studies. The results of the biochemical assessment of tissues were exposed as mean of ten samples. Fifty milligrams of the wet tissue were homogenized with 5ml of 10% tri chloro acetic acid (TCA) and centrifuged for 5 minutes of 4000rpm. The supernatant was used for the estimation of carbohydrate. The precipitate was dissolved in 5ml of 1N sodium hydroxide for the estimation of protein. 3ml of haemolymph collected and diluted with tissue extraction. Fat body being the important tissue which perform the storage function it was subjected for analysis. From the results it is clear that the effect was notable on the assessed parameters of protein, reducing sugars, glycogen, alanine amino transferase and also over the activities of aspartate amino transferase enzyme.

Assessment of proteins in fat body

The total protein contents of the tissue were estimated by the method of [10]. The amino acids containing phenolic hydroxyl group viz; tyrosine and tryptophan react with Folin-Ciocalteu Phenol reagent to give a blue colour due to the reaction of phosphomolybdate, the intensity of the colour if proportional to the concentration of protein. Reagents about 0.1N sodium hydroxide: 0.4 g of sodium hydroxide dissolved in 100ml of distilled water (w/v). Reagent A: 2% sodium carbonate (w/v): 2 g of sodium carbonate dissolved in 100ml of 0.1N sodium hydroxide. Reaent B: 0.5% copper sulphate (w/v): 500 g of copper sulphate dissolved in 100ml of 1.35% sodium potassium tartarate solutions 1.35g of sodium potassium tartarate dissolved in 100ml of distilled water (w/v). This was prepared just before use. Reagent C: Alkaline copper reagent: This was prepared just before use by mixing 50 ml of reagent B. Folin-Ciocalteu phenol reagent (IN): Commercially available 3N reagent diluted to 1N solution with distilled water. Standard: A standard solution of bovine serum albumin (BSA) containing 250mg/ml was prepared in 0.1N sodium hydroxide. Procedure on the dissolved precipitate was made upto 10ml with 1N sodium hydroxide. From this 1 ml was taken and treated with 5 ml of alkaline copper reagent and allowed to stand for 10 minutes at room temperature, then 0.5 ml of Folin-Ciocalteu reagent was added to each tube and shaken well. The colour developed in each test tube was read at 720 nm against a reagent blank in spectronic 21 (Baush and Lamb, USA). The protein content of the sample was calculated using the formula:

$$\frac{\text{OD of sample} \times \text{Conc. of standard}}{\text{OD of standard}}$$

The protein concentration was expressed as mg/g of tissue.

Assessment of Glycogen in fat body

The glycogen content was estimated using anthrone reagent method proposed by [11]. Reagent like 30% Potassium hydroxide: 30gms of potassium hydroxide was dissolved in 100ml of distilled water, 95% ethanol, Anthrone reagent: 0.2 gm anthrone was dissolved in 100 ml of sulphuric acid. Extraction like 100 mg of tissue was weighted accurately and subjected to alkali digestion by treatment with 1ml of 30% potassium in a boiling water bath for 30 minutes, till there was no tissue left undigested. To the glycogen precipitate 3 ml of 95% ethanol was added and the test tubes were kept overnight in a freezer. Glycogen was sedimented by centrifuging at 3000 rpm for 40 minutes. The precipitate was dissolved in warm water, to this ethanol was added and centrifuged again at 3000rpm. The final precipitate was dissolved in 3ml of distilled water and heated for 3 minutes in a boiling water bath. Procedure of the aliquots of tissue were taken to a final volume of 1 ml. A blank with 1 ml of water was also taken. The test tubes were kept in an ice water bath for 2 minutes. 4ml of anthrone reagent was added to each test tubes and the contents were mixed well. All the test tube, the colour of the resulting solution was read at 640 nm. A standard curve was obtained using 20-100 mg of glucose.

Assessment of reducing sugars in fat body

The carbohydrate content of the tissue was estimated by the method of [12]. Sulphuric acid hydrolyses the di and oligosaccharides into mono saccharides and converts the monosaccharides into furfural or furfural derivatives which anthrone reagent. A complex blue colour develops which is proportional to the concentration of carbohydrates. Reagents about Anthrone reagent: 50mg anthrone powder was dissolved in 100ml of 66% sulphuric acid, to this 1g of thiourea was added to stabilize the colour. Standard: 1mg of glucose was dissolved in 10 ml saturated benzoic acid prepare the standard solution. Procedure about assessment of reducing sugars from the extracts prepared for protein and carbohydrate analysis, supernatants were used to find out the amount of carbohydrates present in each sample. 0.5 ml of supernatant was taken and 5ml of anthrone reagent were added to it. This mixture was kept in a boiling water bath for 5 minutes. Then, it was cooled to room temperature in dark, to prevent exposure to light. The colour developed was read at 620 nm against a reagent blank in spectronic-21 [13]. The amount of carbohydrate present in the sample was calculated by using the formula as follows:

$$\frac{\text{OD of sample} \times \text{Conc. of standard}}{\text{OD of standard}}$$

The quantity of carbohydrate is expressed as mg/g of tissue

Assessment of Alanine amino transferase in fat body

Alanine amino transferase activity was determined by the method of [14]. Glutamate pyruvate aminotransferase catalysis the reversible inter conversion between glutamate and alanine and their 2-0×0 analogues. The pyruvate formed after 30 minutes incubation period is measured spectro photometrically by reaction with 2.4 dinitrophenylhydrazine giving a brown-coloured hydrazine after addition of 0.4N sodium hydroxide. Reagents like 0.1 M Phosphate buffer, pH 7.4: 11.3 grams of dry anhydrous disodium hydrogen phosphate and 2.7 grams of anhydrous potassium dehydrogen phosphate is mixed in 1 litre standard flask and made up to the mark with distilled water. Sustrate solution: 29.2 mg of alpha-ketoglutamate and 0.89 grams L-alanine was dissolved in 1N NaOH with stirring of the pH was adjusted to 7.4 and made up to 100ml. This substrate solution contains 2mm of Alpha-ketoglutarate and 100Mm of L-alanine per litre. Standard pyruvate: 22 mg sodium phyruvate was dissolved in 100ml phosphate buffer. This standard contains 2Mm of sodium pyruvate per litre. Colour reagent (DNPH): 19.8 mg of 2.4-dinitrophenyl hydrazine was dissolved in 100ml of 1N HCL and stoved in a brown bath. 0.4N Sodium hydroxide: 16 grams of sodium hydroxide was dissolved in 1 liter of water. Procedure of assessment of alanine amino transferase, From

the enzyme extract 0.2 ml is taken and added to 1ml of buffered substrate for the test. Standard and blank contains 0.2 ml of standard pyruvate and 0.2 ml of distilled water respectively. All the test tubes were incubated at 37°C for 30minutes. Then 1ml of DNPH was added. The test tubes were allowed to stand for 20 minutes at room temperature. 10ml of 0.4N Sodium hydroxide was added to each test tube. After 20 minutes the colour formed was read at 520 nm.

Assessment of Aspartate amino transferase in fat body

Glutamate oxaloacetate amino transferase (GOT) catalysis the reversible interconversion between glutamate and aspartate and their 2.0×0 analogues. The oxaloacetate acid was measured colorimetrically by reaction with 2.4-dinitro phenyl hydrazine giving a brown-coloured hydrazine by addition of 0.4 N NaOH. Reagent like 0.1M Phosphate buffer, pH 7.4. Substrate solution: 29.2 mg Alpha-Ketoglutarate and dissolved in 1N NaOH with stirring. The pH was adjusted to 7.4 with NaOH and made up to 100ml with phosphate buffer 0.2ml, pH 7.4. This substrate contains 2mM of Alpha-Ketoglutarate and 100mM of L-aspartate per litre. Standard Pyruvate: 22mg sodium pyruvate was dissolved in100 ml phosphate buffer. This standard contains 2mM of sodium pyruvate per litre. Colour reagent (DNPH): 19.8 mg of 2,4 – dinitrophenyl hydrazine was dissolved in 100 ml of 1N HCL and stored in a brown bottle. 0.4 Sodium hydroxide: 16 grams of sodium hydroxide was dissolved in 1 litre of water. Procedure on assessment of aspartate amino transferase: From the enzyme extract 0.2 ml was taken and added to 1ml of buffered substrate for the test. Standard and black contains 0.2 ml of standard pyruvate and 0.2 ml of distilled water respectively. All the test tubes were incubated at 37° c for 30 minutes. Then I ml of DNPH was added. The test tubes were allowed to stand for 20 minutes at room temperature. 10 ml of 0.4 N sodium hydroxide was added to each test tube. After 20 minutes the colour formed was read at 520 nm.

Statistical analysis

Statistical Analysis in One-way analysis of variance ANOVA was used to test the significance of differences between mean values of independent observations on fortified meal of biologically synthesized selenium nanoparticles on Morus alba and its quantity on fat body among silkworm *B. mori*. Biochemical analysis like assessment of fat body level were performed to find out the significant differences between the contents of biological nutrients of macromolecules such as Proteins, Glycogen, Reducing sugar, Alanine amino transferase and Aspartate amino transferase in the fat body tissue at fifth day of fifth instar larvae of silkworm, *B. Mori* were significant at $p < 0.01$.

RESULTS AND DISCUSSION

Investigation on fat body tissue

The present investigation gains its importance on the background cited above. Multidimensional aspects of selenium nanoparticles have an innovative role on supplementation of biologically synthesized SeNPs to mulberry silkworm. Fat body being the principal tissue that serves for the deposit of nutrients and energy forming substances it was subjected to biochemical analysis to study the impact of biologically synthesized selenium nanoparticles on its various constituents. In biochemical assessment about analysis of fat body contents on the fifth day of fifth instar larvae of bivoltine, crossbreed PM X NB_4D_2 race of silkworm, *B. mori* (L) on the biologically synthesized selenium nanoparticles treated mulberry leaves with different concentrations of feeding them were found variously reflected in the levels of contents and activities of the proteins, glycogen, redusing sugar, alanine amino transferase and aspartate amino transferase in the fat body tissue among mulberry silkworm, *Bombyx mori* L. (Lepidoptera: Bombicidae).

Table 1 Quantity of biologically synthesized selenium nanoparticles on the levels of protein contents in the fat body on the fifth day of fifth instar larvae of silkworm *Bombyx mori* L.

S. No.	Experimental setup		Mean ± SD mg
1.	Control		185.5064 ± 0.679148
2.	Treatment setup (Fortified meal of BSSeNPs on *Morus alba*)	500ppm	200.136 ± 0.896074
		1000ppm	205.171 ± 0.759298
		1500ppm	206.973 ± 0.74312

BSSeNPs - Biologically synthesized Selenium Nanoparticles data represent values of 5 individual observations

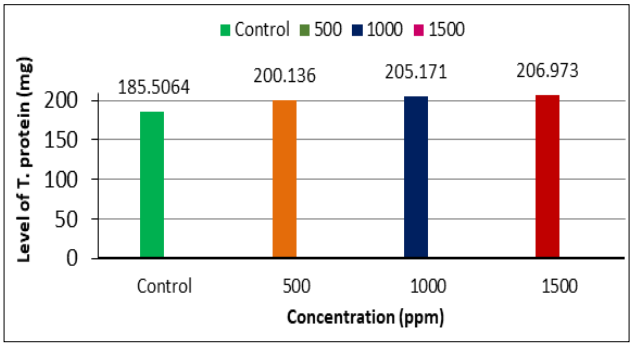

Fig 1 Bar graph showing the quantity of biologically synthesized selenium nanoparticles on the levels of protein contents in the fat body on fifth day of fifth instar larvae of silkworm, *Bombyx mori* L.

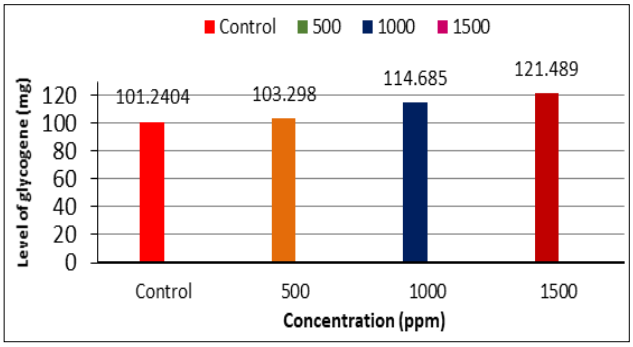

Fig 2 Bar graph showing the quantity of biologically synthesized selenium nanoparticles on the levels of glycogen contents in the fat body on fifth day of fifth instar larvae of silkworm, *Bombyx mori* L.

Proteins level in fat body

Since protein serve as important structural element, their level in the fat body plays a key role in the nutrient supply to the body. The significant increase in the levels of protein indicates the influence of green synthesized selenium nanoparticles fortification of host plant mulberry (Table 1),

thereby helping the larvae meet the nutritional demand both during the larval and the adult stages (Fig 1). Inference on these four observations, 1500 ppm level of green synthesized selenium nanoparticles fortified V_1 mulberry leaves fed larval group of Treatment-3 (206.973 mg) on the protein in the fat body was significantly increased than the other three groups ('C', T_2 and T_3).

Glycogen level in fat body

Glycogen the major storage polysaccharide found in the animal tissues and rightly called as the animal starch, shows significant increase in its levels as a result of the green synthesized selenium nanoparticles fortification. (Table 2) shows, 1500 ppm level of concentration has the highest impact with a mean value of 121.489 mg and 1000 ppm and 500 ppm (114.685 mg, 103.298 mg) also showed a significant increase in the levels, when compare to the control (101.2404). The increase of the glycogen content was significant at $p < 0.001$ level in all the three concentrations. This increase may help the silkworm larvae to meets its nutritional demand during metamorphosis (Fig 2). Inference on these four observations, 1500 ppm level of green synthesized selenium nanoparticles fortified V1 mulberry leaves fed larval group of Treatment-3 (121.489 mg) on the glycogen level in fat body was significantly increased than the other three groups ('C', T_1 and T_2).

Table 2 Quantity of biologically synthesized selenium nanoparticles on the levels of glycogen contents in the fat body on the fifth day of fifth instar larvae of silkworm *Bombyx mori* L.

S. No.	Experimental setup		Mean ± SD mg
1.	Control		101.2404 ± 0.820225
2.	Treatment setup (Fortified meal of BSSeNPs on *Morus alba*)	500ppm	200.136 ± 0.896074
		1000ppm	205.171 ± 0.759298
		1500ppm	206.973 ± 0.74312

BSSeNPs - Biologically synthesized Selenium Nanoparticles data represent values of 5 individual observations

Table 3 Quantity of biologically synthesized selenium nanoparticles on the levels of reducing sugar contents in the fat body on fifth day fifth instar larvae of mulberry silkworm *Bombyx mori* L.

S. No.	Experimental setup		Mean ± SD mg
1.	Control		81.6723 ± 0.825694
2.	Treatment setup (Fortified meal of BSSeNPs on *Morus alba*)	500ppm	200.136 ± 0.896074
		1000ppm	205.171 ± 0.759298
		1500ppm	206.973 ± 0.74312

BSSeNPs - Biologically synthesized Selenium Nanoparticles data represent values of 5 individual observations

Reducing sugar level in fat body

Analysis conducted on levels of reducing sugar in the fat body showed significant increase in the silkworm larvae that were supplemented with the silkworm larvae that were supplemented with the green synthesized selenium nanoparticles when compared with the control groups (Table 3). The mean value of reducing sugar in the control group was 81.6723 mg/gm. The levels increased in the supplemented groups viz; 85.615, 90.3232 and 95.4859 mg/mg with 500 ppm, 1000 ppm and 1500 ppm concentrations (Fig 3). Inference on these four observations, 1500 ppm level of green synthesized selenium nanoparticles fortified V1 mulberry leaves fed larval group of Treatment-3 on the level of reducing sugar in fat body was significantly increased than the other three groups ('C', T_1 and T_2).

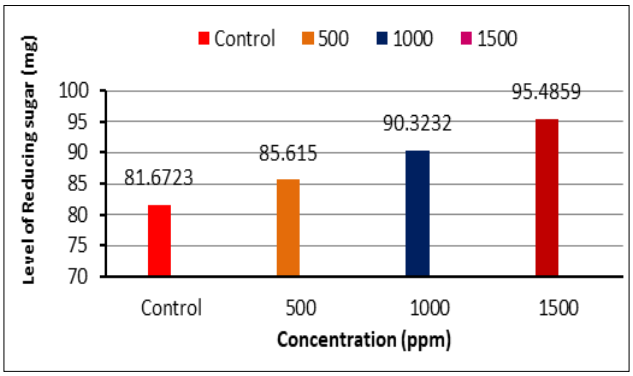

Fig 3 Bar graph showing the quantity of biologically synthesized selenium nanoparticles on the level of reducing sugar contents in the fat body on fifth day of fifth instar larvae of silkworm, *Bombyx mori* L.

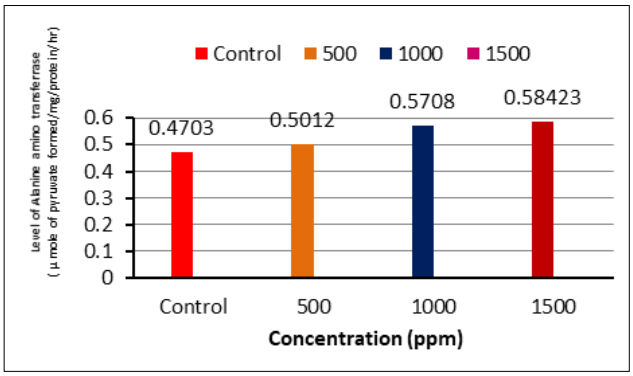

Fig 4 Bar graph showing the quantity of biologically synthesized selenium nanoparticles on the level of alanine amino transferase contents in the fat body on fifth day of fifth instar larvae of silkworm, *Bombyx mori* L.

Alanine amino transferase level in fat body

Data in (Table 4), shows that the levels of enzyme alanine amino transferase increase significantly with the supplementation of green synthesized selenium nanoparticles. However, the increase was significant at $p < 0.2$ level with 500 ppm (0.5012 µmoles of pyruvate formed / mg / protein / hour) and at $p < 0.001$ level with 1000 ppm (0.5708 µmoles of pyruvate formed / mg protein/hour) and 1500 ppm (0.58423

µmoles of pyruvate formed / mg protein hour) (Fig 4). Inference on these four observations, 1500 ppm level of green synthesized selenium nanoparticles fortified V1 mulberry leaves fed larval group of Treatment-3 (0.58423 µmoles of pyruvate formed / mg protein hour) on the alanine amino transferase was significantly increased than the other three groups ('C', T_1 and T_2).

Table 4 Quantity of biologically synthesized selenium nanoparticles on the level of Alanine amino transferase contents in the fat body on fifth day of fifth instar larvae of silkworm *Bombyx mori* L.

S. No.	Experimental setup		Mean ± SD mg
1.	Control		0.4703 ± 0.061361
2.	Treatment setup (Fortified meal of BSSeNPs on *Morus alba*)	500ppm	0.5012 ± 0.008452
		1000ppm	0.5708 ± 0.010796
		1500ppm	0.58423 ± 0.008025

BSSeNPs - Biologically synthesized Selenium Nanoparticles data represent values of 5 individual observations

Aspartate amino transferase level in fat body

The activity level of aspartate amino transferase and alanine amino transferase were determined. Since the activity levels of aspartate amino transferase and alanine amino transferase forms a general index of mobilization of free amino acids respectively, the observed increase of aspartate amino transferase and alanine amino transferase enzyme activities of experimental groups suggests enhanced mobilization of amino acids into glycogen synthesis or silk protein synthesis or both. Similarly, enzyme aspartate amino transferase shows significant increase in its levels (Table 5) with the fortification of green synthesized selenium nanoparticles. This elevated level of the enzymes midgut be helpful in effective transamination process. The levels of elevation was significant at $p < 0.4$ level for 500 ppm (0.3319 µ moles of pyruvate formed / mg protein / hour), $p < 0.5$ level for 1000 ppm (0.3429 µ moles of pyruvate formed / mg / protein / hour) and $p < 0.001$ level for 1500 ppm (0.3514 µ moles of pyruvate formed / mg protein / hour) concentrations (Fig 5). Inference on these four observations, 1500 ppm level of green synthesized selenium nanoparticles fortified V1 mulberry leaves fed larval group of Treatment-3 (0.3514 µ moles of pyruvate formed / mg protein / hour) on the aspartate amino transferase was significantly increased than the other three groups ('C', T_1 and T_2).

Table 5 Quantity of biologically synthesized selenium nanoparticles on the level of Aspartate amino transferase contents in the fat body on fifth day of fifth instar larvae of silkworm *Bombyx mori* L.

S. No.	Experimental setup		Mean ± SD µ moles of pyruvate formed/mg protein/hr
1.	Control		0.336 ± 0.00645
2.	Treatment setup (Fortified meal of BSSeNPs on *Morus alba*)	500ppm	0.3391 ± 0.008188
		1000ppm	0.3429 ± 0.007853
		1500ppm	0.3514 ± 0.006929

BSSeNPs - Biologically synthesized Selenium Nanoparticles data represent values of 5 individual observations

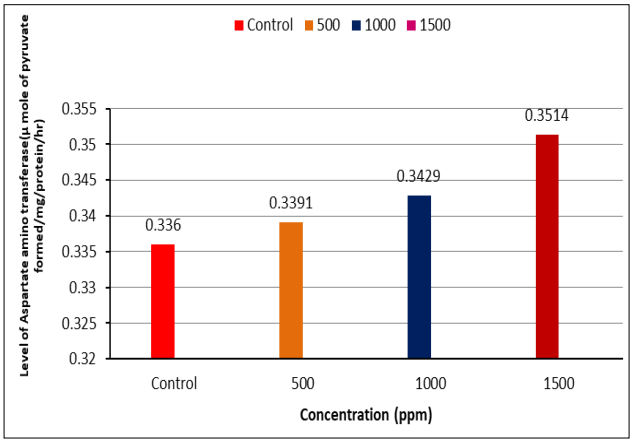

Fig 5 Bar graph showing the quantity of biologically synthesized selenium nanoparticles on the level of Aspartate amino transferase contents in the fat body on fifth day of fifth instar larvae of silkworm, *Bombyx mori* L.

Mulberry silk worm, *Bombyx mori* is a domesticated commercial variety reared in artificial conditions of the cottage. Approach has been made to investigate on some aspects of the identification of supplementary effects on silkworm their digestible rate and their non-stop development for the biometric parameters. Quantitative variations in the concentration of protein, amino acids and carbohydrates in fat body have been observed on all days of the fifth instar larvae of both breeds of the mulberry silkworm. The concentration of total protein in fat body increased progressively during the larval development and reached maximum in the late fifth instar larvae of mulberry silkworm.

The fat body of insects is made up of cells resembling blood cells aggregate to form a rather irregular and diffuse tissue. It serves as a store for food reserves and in some insects for the storage of excretory materials. In a few it becomes modified as a light producing organ. The fat body is of major importance as a centre in which many metabolic processes occur. Protein content the fat body to plays a significant role in the growth and development of the silkworm and has a direct effect on the silk production. The elevated level of protein in the fat body of experimental groups explains the increase in the cocoon weight and shell weight of the experimental groups. [15] observed a similar result when the silkworms were supplemented with vitamin Riboflavin.

Since the activity levels of alanine amino transferase and aspartate amino transferase form a general index of mobilization of free amino acids into gluconeogenesis and oxidation of amino acids respectively, the observed increase of alanine transferase and aspartate amino transferase enzyme activities of experimental groups suggests enhanced

mobilization of amino acid into glycogen synthesis or silk protein synthesis or both [16]. It is a known fact glycogen is the major energy reserve. Some glycogen may occur in most tissues, but commonly the main reserves are present in the fat body. Glycogen synthesized during periods of active feeding are depleted at times of reduced feeding as during a moult over the pupal period or during a period of diapauses.

CONCLUSION

The results of present study shows that the fat body glycogen has significantly increased. This increase in the glycogen content may serve as additional source of energy for pupal adult transformation. The supplementation of green synthesized selenium nanoparticles to the experimental larval will hence definitely help the silkworm to meet the energy demand during its starvational periods, especially for a larva like silkworm, which has to spend an enormous amount of energy in spinning its silk cocoon in starving condition.

Acknowledgment

The work had been supported by Dr. S. Thamaraiselvi., Vice-Chancellor, Thiruvalluvar University, Vellore, India. She has done a great revolutionary action and solved my research barriers and promoting me for my research activities at simply with systematically.

LITERATURE CITED

1. Siddappaji D, Latha CM, Ashoka SR, Basavaraja MG. 2014. Socio- economic development through Sericulture in Karnataka. *IOSR Jr. Humanitis ad Soc. Sci*. 19(10): 24-26.
2. Balamani RSPM, Prince SP, Subburam W, Subburam V. 1995. Effect of zinc on the nutritional indices, economic characters of cocoon and quality of silk of *Bombyx mori* L. *Indian Jr. Sericulture* 34: 69-71.
3. Kavitha S, Sivaprasad S, Saidulla B, Yellamma K. 2012. Effect of zinc chloride and zinc sulphate on the silkworm, *Bombyx mori* growth tissue proteins and economic parameters of sericulture. *The Bioscan* 7(2): 189-195.
4. Waktole and Baskar. 2015. Fortification of mulberry leaves with medicinal botanical plant extracts effect on silkworm, *Bombyx mori* L. (PM×CSR2) (Lepidoptera: Bombycidae) larval growth and cocoon traits. *Journal of Biological Sciences* 15(4): 199-206.
5. Magadum SB, Datta RK, Remadevi OK, Mohan M, Choudhury SK. 1994. Evaluation of semi-synthetic diet for young age silkworm, *Bombyx mori*. L. *Indian Jr. Sericulture* 33(1): 68-69.
6. Miao Y. 2000. Study on the techniques for rearing young silkworm (*Bombyx mori*) using artificial diets. *Acta Sericologica Sinica* 26(2): 91-93.
7. Luciano C, Silvia C, Alessio S, Giovanni S. 2005. Artificial diet rearing system for the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae): effect of vitamin C deprivation on larval growth and cocoon production *Applied Entomology and Zoology* 40(3): 405-412.
8. Sundar Raj S, Chinnaswamy KP, Neelu N. 2000. Soybean to boost cocoon production. *Indian Silk* 39(6): 11.
9. Anu K, Singaravelu G, Murugan K, Benelli G. 2017. Green-synthesis of selenium nanoparticles using garlic cloves (*Allium sativum*): biophysical characterization and cytotoxicity on vero cells. *Jr. Cluster Science* 28: 551-563.
10. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin's reagent. *Jr. Biol. Chemistry* 193: 65-276.
11. Hemavathi B, Bharathi D. 2003. Effect of thyroxine on food utilization by silkworm, *Bombyx mori* L. *Indian Journal of Sericulture* 42: 16-20.
12. Roe JH. 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. *Journal of Biological Chemistry* 153: 373-380.
13. Ito T. 1978. Silkworm Nutrition in the silkworm an important laboratory tool. (Eds) Tazima Y. Ko. Ltd., Tokyo. pp 121-157.
14. Sheeba DV, Padmalatha C, Singh A. 2006. Effect of supplementation of amino acids, leucine and valine on the economic characters of silkworm. *Journal of Zoology* 26: 277-280.
15. Nirwani RB, Kaliwal BB. 1998. Effect of thiamine on commercial traits and biochemical contents of the fat body and haemolymph in the silkworm *Bombyx mori* L. *Sericolgia* 38: 639- 646.
16. Subburathinam KM, Krishnan M. 1998. Role of soyabean protein in silkworm nutrition. *Indian Silk* 37: 9-10.