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Concentration of Biogenic Selenium Nanoparticles (SeNPs) on Haemolymph Physiology among Mulberry Silkworm, *Bombyx mori* L. (Lepidoptera : Bombycidae)

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A B S T R A C T

In the present study, "Cconcentration of Biogenic Selenium Nanoparticles (SeNPs) on Haemolymph physiology among mulberry silkworm, *Bombyx mori* L." has been started from Nano Science Division, Department of Zoology, Thiruvalluvar University, Vellore-India, since october-2019. Sericulture, the technique of silk production is an agro-based cottage industry, playing an eminent role in the rural economy of India. It has been identified as a booming multi-faced cottage industry, which aims in uplifting the socio-economic standards of the cultivators. Synthesis of Biogenic selenium nanoparticles on green technique, plant extract of *Prosopis cineraria* leaf was used and characterized by UV, FTIR, XRD and TEM. Let us Supplementation of Biogenic selenium nanoparticles dissolved in double-distilled water and diluted into 500ppm, 1000ppm and 1500ppm concentrations are fortified into *morus alba*. Analysis about concentration of Biogenic selenium nanoparticles on Haemolymph physiology among selected nutritional biomolecules such as total proteins, total protease, total carbohydrates and Total lipids contents in 5th day of 5th instar larval stage. Analysis about the concentration of supplemented biogenic selenium nanoparticles are significantly increased at different level has been enhanced the digestion of ingested nutritional supplementation which in turn reflects on Haemolymph physiology of mulberry silkworm, *Bombyx mori* L.

***Key words*:** Supplementation, Biogenic SeNPs, Haemolymph analysis, *Morus alba, Bombyx mori*

Sericulture, the technique of silk production is an agro-based cottage industry, playing an eminent role in the rural economy of India. It has been identified as a booming multi-faced cottage industry, which aims in uplifting the socio-economic standards of the cultivators. This farming has been one of the main branches of agriculture in Asiatic countries for hundreds of years. World silk production has doubled during the last 30 years in spite of man-made fibers replacing silk for some users. China and India have been the two main producers, together manufacturing more than 50% of the world silk production each year. Silk production plays an important role in the rural economics of these developing nations. One of the objectives of the present investigation is on the biological synthesis of selenium nanoparticles. Biological synthesis was accomplished using green chemistry approach. Greener synthesis of nanomaterials is preferred due to the resultant nano materials stability, cost effective and environmentally friendly nature. It won't require high-energy inputs and moreover limited investigations have been undertaken. Selenium found to be one of the essential trace minerals in living systems [1]. Selenium nanoparticles gain its importance due to their high specific surface area, high surface reactivity [2]. Various modes of synthesis of selenium nanoparticles have been reported. Pulsed laser ablation technology was established by [3]. In metallic nanoparticle synthesis such as biogenic selenium nanoparticles(SeNPs) is a new invention and innovative approach at present era and it is almost very famous on the contribution of physical and biological Science departments on developed countries of United States and developing country of India. Among the different kinds of green technique such as meditional value oriented plants are provide safe and beneficial way to the synthesis of metallic nanoparticle as it is easily available, so there are possibilities for enlarged quantity about creation of Nano products. In metallic nanoparticles Selenium have specific interest due to its ideal properties such as logically high conductivity, green technically high compatibility, basically anti-pathogenic suitability, traditionally anti-cancer acceptability and etc. *Prosopis cineraria* is an evergreen herbal bush like tree plant. The bark, considered anthelmintic, tonic & refrigerant, can be used to treat a variety of other ailments such as asthma, bronchitis, dysentery, skin disorders, leprosy, muscle tremors, piles and wandering of the mind too.

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Ayurveda and other traditional medicine systems have long been used in curing a variety of ailments. Particularly, the bark extract of this tree is used in symptomatic treatment of scorpion and snake bites.The treatments range from alleviating skin ailments to preventing miscarriage and easy deliveries. It has large, dark-ash bark, dark-green leaves, Dome like shape and structure of plant morphology. Major biomolecules such as carbohydrates, lipids, proteins, hormones and chromosomes etc., play an important role in biochemical process underlying growth and development of insects [4]. Metabolism and accumulation of these biomolecules in insect tissues during their development in different stages of life cycle was studied by many workers [5-9]. The concentrations of these biomolecules mainly depend on mulberry leaf quality. Proteins in haemolymph are at higher concentration during development and are useful in silk proteins synthesis. Major biomolecules such as proteins, carbohydrates and lipids play an important role in haemolymph physiological process underlying growth and development of insects [10]. Proteins are known to have remarkable influence on various developmental stages. Protein is necessary for various biological activities during development, metamorphosis and maintenance of various physiological functions in different tissues [11]. Carbohydrates are protecting organisms during adverse conditions and are essential components of energy source for different biological activities in silkworms. Biomolecules such as glycerol, sorbitol acts as thermo protectants synthesize in the tissues and released into haemolymph [12]. Reducing sugars account 5% of total blood sugars in insects. Lipids in the fat body are energy reservoirs and can be mobilised rapidly during moulting, starvation, oogenesis and embryo genesis and used to sustain continues muscular activity [13]. It is a fact that, silkworm requires essential proteins, amino acids, sugars, fatty acids, vitamins, enzymes and micronutrients for its growth and production of silk protein [14]. The present investigation was carried out to evaluate the supplementation of biogenic selenium nanoparticles on mulberry leaves and its concentrations on haemolymph physiology such as micro nutrients of macromolecules contents like total proteins, total carbohydrates and total lipids on disease-free layouts of bivoltine hybrid PM x NB4D2 race of silkworm *B. mori* and using non treated green mulberry leaves as control.

MATERIALS AND METHODS

Biogenic selenium nanoparticles was undertaken with large number of plants having medicinal background were collected from Vellore and neighbouring districts of Tamil Nadu. India. The plants were identified and cleaned with double distilled water and aqueous extract were prepared. The prepared aqueous extracts of different parts of the plants were subjected to identify the extracts having the property to synthesize selenium nanoparticles. The green chemistry principle employed to synthesize selenium nanoparticles is based on the fact that nanomaterials functions are determined by their reductant and capping molecule. The leaves of *Prosopis cineraria* plant were collected from Thiruvalam village, Vellore Dt and then washed thoroughly with double distilled water to remove the dust and dirt particles. The leaves are then grinded under manual mortar with slowly, smoothly and finely collected bulk quantity of extract (Fig 1a-1c).

Fig 1a *Prosopis cineraria* leaves

Fig 1b Gel of *Prosopis cineraria* leaves

Fig 1 bc *Prosopis cineraria* leaves extract

Characterization for green 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 [15]. In the present investigation an attempt was made to identify the influence of green synthesized selenium nanoparticles on some aspects of silkworm physiology. In fortification technique supplementation of biogenic selenium nanoparticles and its influence on assessment of biochemical parameter like haemolymph physiology among mulberry Silkworm (*Bombyx mori* L) based on Non-fortified V_1 mulberry leaves fed larval group of control, 500 ppm level of biogenic selenium nanoparticles fortified V_1 mulberry leaves fed larval group of Treatment-1, 1000 ppm level of biogenic selenium nanoparticles fortified V_1 mulberry leaves fed larval group of Treatment-2 and 1500ppm level of biogenic selenium nanoparticles fortified V_1 mulberry leaves fed larval group of Treatment-3. Interestingly it was observed that the mulberry leaves like *Morus alba* supplemented with green selenium nanoparticles greatly enhance the consumption of the host plant and proper feeding. In general, pronounced growth of silkworm was observed.

Haemolymph collection

Haemolymph was collected at fifth day of fifth instars larvae from the control and tested groups in experimental setup (Fig 2). The first three pair of prolegs was cut and collected haemolymph from silkworm at systematically and it's dropped into pre-chilled glass vials. The collected haemolymph was used immediately for biochemical and enzyme analysis. Collection and preparation of haemolymph juice sample, Protein, carbohydrates and lipid contents was estimated and carried out in 5^{th} day of 5^{th} instar larvae with three renevations. Therefore during first 5^{th} instars, entire larval body was considered. Larvae were selected on 5^{th} day of 5^{th} instar for the analysis. During 5th instar haemolymph were collected at 24 hrs interval on 5^{th} day before moult to spinning.

The biochemical study was done on 5^{th} day of fifth instar larvae dissected out in cold ringer solution. For

haemolymph biochemical analysis of heamolymph physiology, haemolymph was collected by pricking the prolegs of silkworm larvae in small vials precoated with phenylthiourea to prevent the melonization. Then the samples of haemolymph centrifuged immediately at 3000 rpm for 15 minutes for total protein and total carbohydrate estimation samples were prepared in 2N NaoH and for haemolymph total lipid estimation the sample was prepared in Folch's mixture. The enzyme like total protease activity was determined with casein as substrate by a method described by Kunitz.

Fig 2 Fifth day of fifth instar larvae of silkworm

Analysis of total proteins

The protein concentration in the tissue and haemolymph from all the experimental groups were estimated by [16] method. The standard protein solution was prepared by dissolving the 5mg standared protein BSA in 5ml of 2N NaoH. The blank and unknown tubes cantained 0.5 ml of distilled water and homogenate from each group respectively. To each tube add 5ml of Lowry reagent (Reagent, 10% sodium carbonate in 0.1 N NaoH + 1% copper sulphate and 1% sodium potassium tartarate) and kept undisturbed for 15 min. then add 0.5 ml of folin reagent with eaqual amount of water in each test tubes with vigorous shaking and kept undisterbed foe 30 minutes. The colour intensity with blue colour was observed at 660 nm on the spectrophotometer. The standared graphs were plotted with known values of BSA and determine the actual amount of protein from the samples.

Analysis of total protease

The enzyme like total protease activity was determined with casein as substrate by a method described by [17]. Reagents about 0.1M Phosphate buffer, pH 7.6: Add 43 ml of 0.2 mol/1 potassium hydroxide and dilute to 100 ml with distilled water. EDTA: 292.2 mg of EDTA was dissolved in 100ml of distilled water. Casein: 1mg/100ml. Procedure on the assay system consists of 100 micromoles of phosphate, pH 7.6, 5 micro molecules of EDTA, 10 micro molecules of Beta-mercapto ethanol, 10 mg of casein in 2ml of water. The reaction was started by the addition of 1ml of enzyme solution. Incubate at 37°c for 30 minutes. Then the reaction was arrested by the addition of 1 ml of 12% TCA. After standing at room temperature for 30 minutes. The concentration of the TCA soluble peptides was determined in 1 ml of the clear supernatant [16].

Analysis of total carbohydartes

The carbohydrate concentration in haemolymph were estimated by [18] from all the experimental groups. The standared carbohydrate solution was preapared by dissolving 5mg of glucose in 5ml of distilled water. The blank and unknown tubes contained 0.5 ml of distilled water and 0.5 ml of homogenate from each group to which add the 2.5 ml DNSA reagent was added. Then tubes were kept for boiling in water bath for 5 minutes, tubes were cooled immediately added in each tube 2.5 ml of distilled water. The developed colour was measured on spectrophotometer at 540nm. The standard graph of glucose was plotted against the unknown samples and calculates the actual amount of carbohydrate from the samples.

Analysis of total lipids

For lipid estimation homogenates of haemolymph were prepared in Folch's mixture [19]. The folch's mixture was prepared by taking chloroform and methanol in 2:1 ratio. For standard graph known concentration of cholesterol were used for calculation. The blank and unknown tubes contained 0.5 ml of distilled water and homogenate from respective groups. The homogenates were evaporated at 400C in water bath and then added 1 ml of concentrated sulphuric acid. The tubes heated in boiling water bath for 10 minutes. After cooling 2 ml of vanilline reagent were added in each tube. The pink colour developed was measured at 540 nm on spectrophotometer. The standard graph was plotted against unknown samples and calculates the actual amount of lipid in all samples.

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 of concentration of biogenic selenium nanoparticles on haemolymph physiology at disease-free layouts of bivoltine hybrid PM x NB4D2 race of silkworm *B. mori.* Continuous and comprehensive analysis of biochemical parameter like assessment of heamolymph level were performed to find out the significant differences between the contents of biological nutrients of macromolecules such as total protein, total protease, total charbohydrates and total lipids in the haemolymph physiology at the fifth day of fifth instar larvae of silkworm were significant at $p < 0.01$.

RESULTS AND DISCUSSION

Visual observation

Reduction of metal salts into metal nanoparticles by the bio molecules is always accompanied by the color change of reaction medium. In the present study the dark wine color solution of sodium selenite is changed in light red color after drop wise addition of *Prosopis cineraria* leaf extract at 24 hours. As the reduction proceeds, the color of reaction medium is gradually changed to light red color after 24 hours.

Haemolymph physiology

In biochemical assay about analysis of haemolymph contents on the fifth day of fifth instar larvae of bivoltine, crossbreed PM X NB4D2 race of silkworm, *B. mori* (L) on the biogenic selenium nanoparticles treated mulberry leaves with different concentrations of feeding them were found variously reflected in the levels of contents and activities of the total proteins, total protease, total charbohydrates and total lipids in the haemolymph among mulberry silkworm, *Bombyx mori* L (Lepidoptera: Bombicidae). Haemolymph circulates round the body cavity between the various the various organs and transfers the nutrient material [20]. Its various functions

include phagocytosis, storage and intermediate metabolism. Hence it was subjected to biochemical analysis.

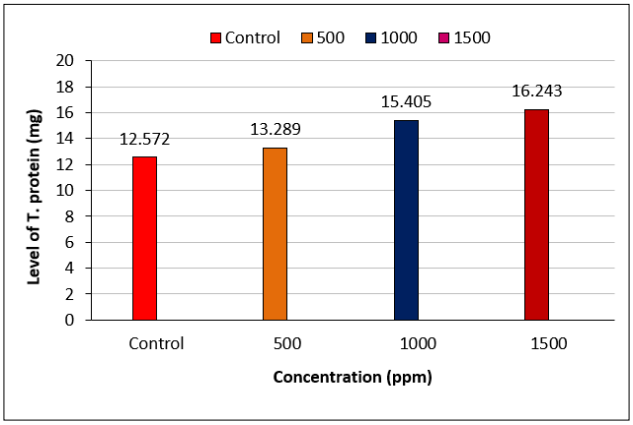
Table 1 Concentration of biogenic selenium nanoparticles on the levels of total protein contents in the haemolymph on the fifth day of fifth instar larvae of mulberry silkworm *Bombyx mori* L

Experimental setup		Mean ± SD mg
Tested groups (BGSNPs Supplementation)	Control	12.572 ± 0.375734
	500ppm	13.289 ± 0.558058
	1000ppm	15.405 ± 0.82701
	1500ppm	16.243 ± 0.709155

BGSNPs - Biogenic Selenium Nanoparticles data represent values of 5 individual observations

Analysis of total proteins

In the assessment about analysis of total protein contents based on supplementation of biogenic selenium nanoparticles and its concentrations of haemolymph on the fifth day of fifth instar larvae of mulberry silkworm, *B. mori*. It is well known fact that protein is intimately connected with all phases of chemical and physical activity of the cells [21]. The results are pointed out that the total protein contents of the tested groups are significantly increased in different ppm levels from simple to complex such as 500ppm (13.289 ± 0.558058), 1000ppm (15.405 ± 0.82701) and 1500ppm (16.243 ± 0.709155) were simply with systematically recorded (Table 1). Finally the gradually increased contents of total proteins activities over the control (12.572 ± 0.375734) were also recorded and represented (Graph 1).



Graph 1 Bar graph showing the concentration of biogenic selenium nanoparticles on the levels of total protein contents in the haemolymph on fifth day of fifth instar larvae of mulberry silkworm, *Bombyx mori* L.

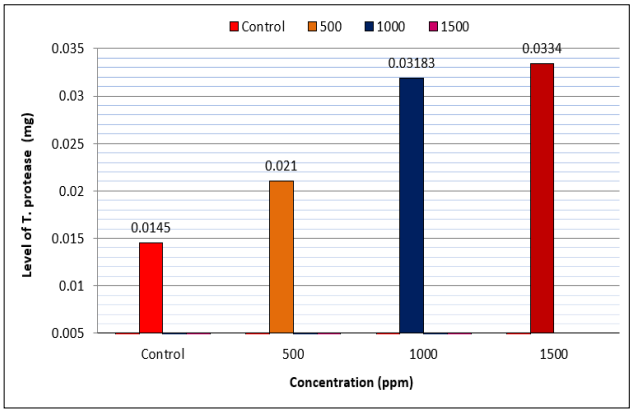
Analysis of total protease

In the evaluation about analysis of total protease contents based on supplementation of biogenic selenium nanoparticles and its concentrations of haemolymph on the fifth day of fifth instar larvae about mulberry silkworm, *B. mori*. (Table 2) shows the enzyme of protease levels in the Haemolymph on silkworm larvae when supplemented with biogenic selenium nanoparticles which indicates the utilization of enzymes for protease metabolism (Fig 2). The results are pointed out that the total protease contents of the tested groups are significantly increased in different ppm levels from simple to complex such as 500ppm (0.021 ± 0.001297), 1000ppm (0.03183 ± 0.002753) and 1500ppm (0.0334 ± 0.003323) were simply with systematically recorded (Table 2). Finally the gradually increased contents of total protease activities over the control (0.0145 ± 0.00408) were also recorded and represented (Graph 2).

Table 2 Concentration of biogenic selenium nanoparticles on the levels of total protease contents in the haemolymph on the fifth day of fifth instar larvae of mulberry silkworm *Bombyx mori* L

Experimental setup		Mean ± SD mg
Tested groups (BGSNPs Supplementation)	Control	0.0145 ± 0.00408
	500ppm	0.021 ± 0.001297
	1000ppm	0.03183 ± 0.002753
	1500ppm	0.0334 ± 0.003323

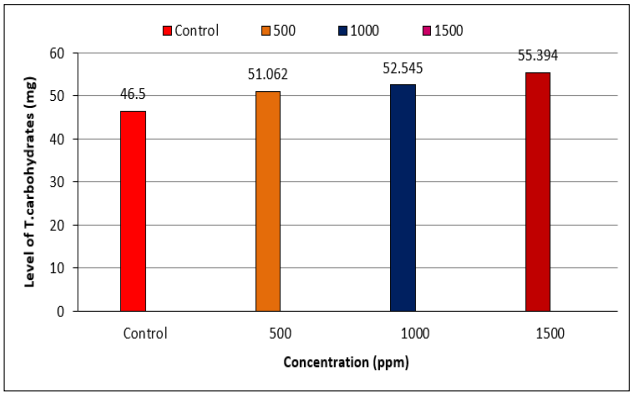
BGSNPs - Biogenic Selenium Nanoparticles data represent values of 5 individual observations



Graph 2 Bar graph showing the concentration of biogenic selenium nanoparticles on the levels of total protease contents in the haemolymph on fifth day of fifth instar larvae of mulberry silkworm, Bombyx *mori* L.

Analysis of total carbohydrates

In the assessment about analysis of total charbohydrate contents based on supplementation of biogenic selenium nanoparticles and its concentrations of haemolymph on the fifth day of fifth instar larvae about mulberry silkworm, *B. mori*. The results are pointed out that the total carbohydrate contents of the tested groups are significantly increased in different ppm levels from simple to complex such as 500ppm (51.062±0.80420), 1000ppm (52.545±0.938806) and 1500ppm (55.394±0.863469) were simply with systematically recorded (Table 3). Finally the gradually increased contents of total carbohydrates activities over the control (46.5±0.900676) were also recorded and represented (Graph 3).



Graph 3 Bar graph showing the concentration of biogeni c selenium nanoparticles on the level of total carbohydrate contents in the haemolymph on fifth day of fifth instar larvae of mulberry silkworm, *Bombyx mori* L.

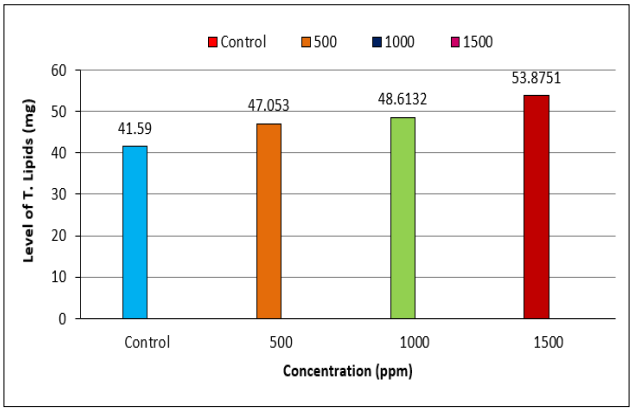
Table 3 Concentration of biogenic selenium nanoparticles on the levels of total carbohydrate contents in the Haemolymph on fifth day of fifth instar larvae of mulberry silkworm *Bombyx mori* L

Experimental setup		Mean ± SD mg
Tested groups (BGSNPs Supplementation)	Control	046.5 ± 0.900676
	500ppm	51.062 ± 0.80420
	1000ppm	52.545 ± 0.938806
	1500ppm	55.394 ± 0.863469

BGSNPs - Biogenic Selenium Nanoparticles data represent values of 5 individual observations

Analysis of total lipids

In the assessment about analysis of total lipid contents based on supplementation of biogenic selenium nanoparticles and its concentration of haemolymph on the fifth day of fifth instar larvae about mulberry silkworm, *B. mori.* The results are pointed out that the total lipid contents of the tested groups are significantly increased in different ppm levels from simple to complex such as 500ppm (47.053±0.719609), 1000ppm (48.6132±0.839954) and 1500ppm (53.8751±0.773271) were simply with systematically recorded (Table 4). Finally the gradually increased contents of total lipids activities over the control (41.59± 0.611509) were also recorded and represented (Graph 4).



Graph 4 Bar graph showing the concentration of biogenic selenium nanoparticles on the level of total lipid contents in the haemolymph on fifth day of fifth instar larvae of silkworm, *Bombyx mori* L.

Table 4 Concentration of biogenic selenium nanoparticles on the level of total lipid contents in the haemolymph on fifth day of fifth instar larvae of mulberry silkworm *Bombyx mori* L

Experimental setup		Mean ± SD mg
Tested groups (BGSNPs Supplementation)	Control	41.59 ± 0.611509
	500ppm	47.053 ± 0.719609
	1000ppm	48.6132 ± 0.839954
	1500ppm	53.8751 ± 0.773271

BGSNPs - Biogenic Selenium Nanoparticles data represent values of 5 individual observations

The field of nanotechnology is one of the most active areas of research in material science and it has been growing at a rapid pace worldwide in the past few years. Nanoparticles exhibit completely new functionalities based on specific characteristics such as size, chemistry, morphology and distribution. Due to the fact nanomaterials have received considerable attention in a wide spectrum area that includes biological science. It is in this content; the idea of investigating the potential application of green synthesized selenium nanoparticles on promoting sericulture has been undertaken. Production of nanoparticles under non-toxic green techniques is of vital importance for biological application. Accordingly in the present investigation leaves of wonder, *Prosopis cineraria* mediated green synthesis has been demonstrated [22]. A large number part of pants of various plants were subjected for bio synthesizing property of selenium nanoparticles. The power of wonder tree, *Prosopis cineraria* reacted with sodium selenite solution and by the end of the reaction (24 hours) a change in colour from brown to brick red of the medium was noted by visual examination. The newly synthesized selenium nanoparticles were characterized using UV-VIS Spectrum, FTIR, XRD, TEM analysis [23].

In this present Analytical study is systematic process about biogenic selenium nanoparticles was established using the wonder tree, *Prosopis cineraria* biomolecules based on a medicinal value-oriented tree plant it is expected that plant extract synthesized for selenium nanoparticles finds promising medicinal uses. Furthermore, this could be a potent candidate for breaking the limitation in the medical field. A green synthetic approach in the present investigation is simple, low cost-effective and eco-friendly. Silkworm (*Bombyx mori* L) is the most beneficial and economically important insect, feeds only mulberry leaf *M. alba* [24]. The active feeding of *B. mori* in its fifth instar larvae has attracted us much to seek into its haemolymph physiology for the search of influences of green synthesized selenium nanoparticles which is responsible to digest a huge amount of *M. alba* leaf in a short time. The contents of total proteins, total carbohydrates and total lipids from mulberry silkworm, *B. mori* lead to the evaluation of different concentrations of ppm level among which like disease-free layouts of bivoltine hybrid PM x NB4D2 strain showed a clear macromolecules production upon screening in Haemolymph [25]. Specifically, concentration of the nutrients of concentration are gradually increased from simple to complex level of ppm like 500ppm, 1000ppm and 1500ppm level, treating the mulberry leaves with biogenic selenium nanoparticles and feeding the fifth instar larvae of mulberry silkworm, *B. mori* absolutely inculcated in the nature of rearing on supplementation. Scientists are made great efforts to success the sequencing project of the whole silkworm Haemolymph physiology which offers sericulture researchers a chance to identify whole nutrients of total proteins, total carbohydrates and total lipid contents using haemolymph analytical method. Proteins in the haemolymph may be broken down to provide a source of amino acids, which are then synthesized in situ in the tissues, by the enzyme protease. The increase in the level of protein in the haemolymph, i.e., the enzyme that is needed for the breakdown of the protein for re-utilization in other metabolic pathways, suggesting greater utilization of the enzyme. Analysis made on the haemolymph of silkworm, *Bombyx mori* L denotes that the Haemolymph protein levels elevated considerably with biogenic selenium nanoparticles supplementation and a corresponding disease in protease enzyme was noted in the haemolymph [26].

CONCLUSION

In Haemolymph physiology confirmed that bivoltine silkworms are superior over disease-free layouts of bivoltine hybridPM x NB4D2 race of silkworm *B. mori* used on analysis of biochemical contents of Haemolymph juice. Nutritional concentration in mulberry silkworm, *B. mori* attributes the goodness that reliably considered being better in

rearing performance and cocoon yield. This concept will be help implement at field level to apply these biogenic selenium nanoparticles fortified mulberry varieties for high yield, healthy silkworms on good biochemical and better commercial cocoon parameters for suitable cocoon and silk productivity.

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