Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **3 (2):** 479-486 (2015)

INTERNATIONAL JOURNAL OF PURE & APPLIED BIOSCIENCE

Research Article

OPEN ACCESS

Determination of minimum effective dose of nutrilite for optimal growth, metabolism and silk production in the silkworm, *Bombyx mori*

Sivaprasad Srinivasamurthy[1]*, Thulasi Neerugatti[2]
[1, 2]Department of Zoology, Smt. N.P.S. Government College for Women, Chittoor- 517001(A.P.), INDIA
*Corresponding Author E-mail: sivaprasadzoology@gmail.com

ABSTRACT

The sericultural productivity could be effectively modulated by enriching the silkworm diet with exogenous protein-rich nutrients like the Amway nutrilite. The minimum effective dose (MED) of nutrilite that promotes optimal larval growth, activates metabolism and improves silk production in Bombyx mori has been determined, by a step- down process starting from higher dose (5%) to a lower dose (1%) together with a parallel zero-dose control. The MED for larval growth was determined by analyzing changes in the body weight and that of metabolism and silk production by assaying protein levels of the silk gland, fat body and haemolymph. The findings were meaningfully interpreted in terms of compound periodical growth rates. The study demonstrated that the MED of nutrilite differs from tissue to tissue. Nutrilite at 1% dosage level (1 g in 100 ml/100 worms) evoked greater response in the larval body growth and optimal protein synthesis in the silk gland and fat body. Hence, 1% dose of nutrilite in distilled water is recommended as the MED for positive improvements in the larval growth, metabolism and silk production in the mulberry silkworm, Bombyx mori.

Keywords*: Bombyx mori, Growth, Minimum effective dose, Nutrilite, Proteins*

INTRODUCTION

Nutrition is the single most factor that contributes to the growth and development in *B.mori*[1-2]. A variety of nutrients, minerals, antibiotics, vitamins, hormones and other exogenous modulators were successfully applied in sericulture with a view to stimulate growth, metabolism and silk production in *B. mori* [3-11]. Most of these nutritional-enrichment studies were based largely on indiscriminate application of commercial and expensive nutrients. The application of AMWAY nutrilite has not been explored in the field of sericulture so far. The present study intends to determine the minimum effective dose (MED) of Amway nutrilite and to recommend that dose for testing its nutritive role on *Bombyx mori*, with special reference to larval growth, metabolism, silk production and economic traits of sericulture.

MATERIAL AND METHODS

The present investigation was carried out on Pure Mysore x CSR_2 hybrid strain of *Bombyx mori* reared under 28°C and 85% RH as per Krishnaswami[12]. The silkworm larvae were fed with M_5 variety of mulberry leaves at 6 AM, 10 AM, 2 PM, 6 PM and 10 PM, under normal 12 hr light and 12 hr dark conditions. After the third moult, the fourth instar larvae were divided into five batches (one control and four experimental) of 100 worms each. The worms of the control batch were fed with normal mulberry leaves and designated the zero-dose control (ZDC) and those of the experimental batches were fed with nutrilite-enriched mulberry leaves once in a day at 6.00 PM, while normal feeding pattern continued at other timings in the day.

Before feeding, the nutrilite-enriched mulberry leaves were prepared by dipping them in different concentrations (viz., 5%, 3%, 2% and 1%) of the nutrient and dried under cool weather conditions.

The minimum effective dose (MED) of nutrilite was determined by step-down process, starting from a higher concentration of 5% to a lower concentration of 1% along with the zero-dose control (ZDC)[13-15]. The MED of nutrilite for larval growth was determined by recording the body weight of 25 randomly selected silkworms in an electronic balance (ELICO; MODEL BL-22OH) during fourth and fifth instar stages and the same was expressed in grams. The total protein content was estimated in 1% homogenates of silk gland (SG) and fat body (FB) and 1:9 diluted haemolymph (HL) in distilled water by the method of Lowry *et al*[16]., and the same was expressed in mg protein /gram wet weight of tissue (or) mg/ml of haemolymph. The experimental data were statistically analyzed online by using Graphpad (www.graph.pad.com/quickcalcs/indexctm) and percent change (www.percent-change.com)/index/php) packages and meaningfully interpreted in terms of computing compound periodical growth rates (CPGR) as per Sivaprasad[17].

RESULTS AND DISCUSSION

Minimum effective dose (MED) is the lowest dosage level that yields a response greater than that of the zero-dose control. In growth related studies involving treatment conditions, it is used as a powerful tool for tracing the dose-response relationship[18-19]. The Amway nutrilite, which is a prime source of soya protein (80%) and 9 essential amino acids, could play a vital role in the larval growth and development of silkworm and could potentially improve the economic traits of sericulture[6]. Its impact in this area could vary as a function of its dosage in the diet and the stage of development of silkworm and the physiological condition of the tissues under study. Hence, it is essential to determine its minimum effective dose for different economic parameters of sericulture. The findings of the present investigation are expected to achieve this objective (Tables 1and 2; Figures 1and 2).

Nutrilite versus larval body growth

During the fourth instar development, the larval body weight of ZDC batch showed an overall growth rate (OGR) of ~168.9% and a compound periodical growth rate (CPGR) of 39.03% (Table.1A). When the silkworm larvae were fed with 5% nutrilite the growth rates remained un-affected and the larvae maintained growth trends at par with those of ZDC. However, the nutrilite evoked positive response in larval growth rates at three other dosages (i.e., 3%, 2% and 1%). As shown in table 2A, the larval body weight recorded an OGR of ~181% and a CPGR of 41.16% under the influence of nutrilite uniformly at these three dosages (Table 1A and Fig. 2). The impact of nutrilite on fifth instar larvae is quite different. It evoked varying levels of positive response at different dosages that culminated in significant elevations in both OGR and CPGR values. As shown in table 1B, the larval body weight of the ZDC batch recorded an OGR of ~206% and a CPGR of 20.52%. When fed with nutrilite-enriched mulberry diet, the OGR and CPGR values were elevated respectively by~243% and 22.78% at 5% dosage level, ~247% and 23.03% at 3% level, ~253% and 23.41% at 2% level and 266% and 24.14% at 1% level (Table 1B and Fig.1).

The study highlights that the growth rates show instar specificity in the silkworm. The silkworm grows steadily at a faster rate in fourth instar (CPGR: 39.03%) compared to fifth instar (CPGR: 20. 52%). Contrarily, the impact of nutrilite on larval growth was almost negligible in fourth instar but more pronounced in fifth instar. While, the nutrient caused no change in its growth rates at 5% dosage level, it affected same levels of elevation in growth rates at 3%, 2% and 1% levels during fourth instar development. At these three doses, the nutrilite caused elevation in fourth instar larval growth rates by 12.5 additional percentile points (181.3-168.8) in OGR and 2.13 percentile points (41.16 – 39.03) in CPGR (Table 1A). On the other hand, the nutrient enhanced the fifth instar growth rates in a liner fashion with an inverse relationship; the lower the dosage of nutrilite the greater will be the enhancement in growth rates. For instance, the nutrient boosted the OGRs and CPGRs respectively by 36.2 (242.6-206.4) and 2.26 (22.78–20.52) additional percentile points at 5% level, 40.4 (246.8-206.4) and 2.51 (23.03–20.52) additional percentile points at 3% level, 46.8 (253.2-206.4) and 2.89 (23.41–20.52) additional percentile points at 2% level and maximally by 59.6 (266.0-206.4) and 3.62 (24.14 – 20.52) additional percentile points at 1% level (Table 1B and Fig.1).

Thus, the effect of nutrilite is specific and directed towards boosting the growth rate in the silkworm larvae during fifth instar development. Selectively, the nutrilite stimulates growth in slow growing fifth instar worms, but doesn't affect that in fast-growing fourth instar worms. Though, the reason for the growth stimulating effect is not clear, it is attributable to the phago-stimulant activity of nutrients, it contains[20]. Thus, the potency of nutrilite has been demonstrated at 1% dosage level (i.e., 1 g of nutrilite in 100ml/100 worms). Hence, this dose is strongly recommended as the minimum effective dose (MED) for larval growth in *B. mori*.

Nutrilite versus tissue proteins

The analysis of tissue-based proteins under treatment conditions could be considered as an index of metabolic rate in *B. mori* [15]. Obviously, the MED of nutrilite that positively impacts the larval metabolism in the silkworm could be determined by analyzing the protein profiles of SG, FB and HL.

Silk gland proteins (SGP): The silk gland is the major site of silk protein synthesis. Apart from two silk proteins (fibroin and sericin), it synthesizes and stores 91 other proteins involved in metabolism, immunity, heat-shock mechanism, cytoskeleton formation, protease inhibition, transport and transcription[21-26]. The positive growth trends observed in the larval body weight were reflected in the protein profiles of silk gland (SG). In the ZDC batch, the SGP levels recorded an elevation of ~320% in OGR and 104.99% in CPGR (Table 2A).When the larvae were fed with 5% nutrilite-fortified mulberry leaves, the SGP levels recorded an OGR of ~409 % and a CPGR of 125.58%. At the 3% concentration of nutrilite, the SGP levels recorded an elevation of ~410% and in CPGR levels by 125.93%. At 2% dosage level, the nutrilite caused an elevation in SGP levels by 430% and a CPGR of 130.66%. At a minimal level of 1% dosage, the nutrilite caused maximal elevation of ~460% in SGP levels with a CPGR of 136.70% (Table. 2A and Fig.2). Thus, under the influence of nutrilite, the OGR and CPGR of SGP were elevated respectively by 88. 7 (408.9-320.2) and 20.59 (125.58-104.99) additional percentile points at 5% dosage level, 90.1 (410.3-320.2) and 20.94 (125.93-104.99) additional percentile points at 3% dosage level, 109.8 (430.0-320.2) and 25.67 (130.66-104.99) additional percentile points at 2% dosage level and maximally by 140.1 (460.3-320.2) 31.71 (136.70-104.99) additional percentile points at 1% dosage level (Table 2B; Fig.2). Though, the reasons for their changes have not been examined in the current study, the positive impact of nutrilite on the protein content of silk gland is attributable to its mineral and vitamin composition[27-29].

Fat Body Proteins (FBP): The insect fat body represents the major site of protein synthesis and amino acid metabolism. Functionally, it is analogous to the liver and adipose tissue of higher vertebrates[30]. In *B. mori*, the FB synthesizes and stores over 177 proteins implicated in larval growth and development[31-32]. The study demonstrated that nutrilite caused more promising impact on FBP levels at lower concentrations during fifth instar larval development (Table. 2B and Fig.2). In the ZDC batch, the FBP levels were elevated significantly, showing an OGR of ~58% and a CPGR of 25.83%. At 5% concentration of nutrilite, the protein levels were doubled with an OGR of ~102% and a CPGR of ~42.02%. At 3% concentration, the FBP levels projected an OGR of ~114% and a CPGR of 46.41%. At 2% dosage level, they showed an OGR of ~107% and a CPGR of 43.45% and at 1% level an OGR of ~117% and a CPGR of 47.47% (Table. 2B and Fig.2). Thus, under the influence of nutrilite, the OGR and CPGR of FBP were elevated respectively by 43. 6 (101.9-58.3) and 16.24 (42.07-25.83) additional percentile points at 5% dosage level, 56.1 (114.4-58.3) and 20.59 (46.41-25.83) additional percentile points at 3% dosage level, 48.3 (106.6-58.3) and 17.62 (43.45-25.83) additional percentile points at 2% dosage level and by 59.2 (117.5-58.3) and 21.64 (47.47-25.83) additional percentile points at 1% dosage level (Table: 2B; Fig: 2). Thus, the present study highlights the positive impact of nutrilite on FBP levels at its MED of 1% in distilled water.

Haemolymph Proteins (HLP): The HL plays a dual role of transportation and storage. It stores and transports about 241 to 298 proteins involved in metamorphosis, ecdysis, chitin and haemocyte formation, growth of silk gland and reproductive organs[33-35].

The analysis of growth trends in ZDC batch indicates that the total protein levels in the circulating medium of haemolymph have shown an OGR of ~28.2% and a CPGR of 13.24% during fifth instar development. Under the influence of nutrilite, the HLP levels recorded 32% elevation in OGR and 14.88% rise in CPGR at 5% dosage level. Likewise, the HLP levels grew by 37.9% in OGR and 17.43% in CPGR at 3% level, 39% in OGR and 17.9% in CPGR at 2% level and 37.8% in OGR and 17.37% in CPGR at 1% level (Table 2C). Thus, the HLP levels were positively modulated by nutrilite at all dosages examined, but more significantly at 2% level. At this concentration the CPGR of HLP was elevated maximally by10.8 additional percentile points in OGR (39.0-28.2) and 4.66 percentile points (17.90-13.24) in CPGR. Whereas, it caused comparatively lower elevations in HLP levels at three other doses. For instance, it caused just 3.8 additional percentile points in OGR (32.0-28.2) and 1.64 percentile points (14.88- 13.24) in CPGR at 5% dosage level, 9.7 additional percentile points in OGR (37.9-28.2) and 4.19 percentile points in CPGR (17.43-13.24) at 3% dosage level, 10.8 percentile points in OGR (39.0-28.2) and 4.66 (17.9-13.24) percentile points in CPGR. However, at 1% dosage level, the nutrilite caused an elevation of 9.6 (37.8-28.2) percentile points in OGR and 4.13 (17.37-13.24) percentile points in CPGR. It is likely that the nutrilite-treated mulberry leaves could stimulate somatic growth in the larval body during metamorphosis by selectively altering the biochemical composition of haemolymph by mobilizing hormone molecules like bombyxin [4, 35-36]. This could be one potential area for further research in this field.

The study demonstrates two points. Firstly, the MED of nutrilite differs from tissue to tissue. It is effective at 1% dosage level in the silk gland and fat body and at 2% dosage level in haemolymph. Secondly, its tissue-based impact on protein profiles indicate that the nutrilite modulates metabolism either by de novo protein synthesis or by mobilizing proteins from other tissues as suggested in our previous study[15]. The growth trends in their levels indicate that both the mechanisms are likely in *B. mori*. The differential dose requirements of nutrilite by different tissues, further substantiates that the protein pool in the silk gland is reinforced by inputs from the fat body- derived haemolymph proteins. The prevalence of higher proteins in the silk gland, more particularly in the fat body, coupled with their declining trends in the circulating medium of haemolymph at 1% dosage level, indicates that proteins are not only synthesized in the silk gland, but also mobilized from the reserve pool of the fat body. More importantly, the decline level of proteins in haemolymph under the influence of 1% nutrilite is probably due to transportation of proteins from the fat body to silk gland through the haemolymph[37].

Table 1: Effect of different concentrations (5%, 3%, 2%, 1%) of nutrilite on the body weight of *Bombyx mori* during fourth (A) and fifth instar (B) larval stages

Day	Statistical tool	Zero dose control	Experimental (Concentration of nutrilite)			
			5%	3%	2%	1%
(A) Growth in fourth instar larval body weight (mg/g)						
Day-1	Mean S.D	0.16±0.009	0.16±0.009	0.16±0.009	0.16±0.009	0.16±0.009
Day-4	Mean S.D	0.43±0.01*	0.43±0.01*	0.45±0.007*	0.45±0.004*	0.45±0.01*
OGR (%)		168.8	168.8	181.3	181.3	181.3
CPGR (%)		39.03	39.03	41.16	41.16	41.16
(B) Growth in fifth instar larval body weight (mg/g)						
Day-1	Mean S.D	0.47±0.007	0.47±0.007	0.47±0.007	0.47±0.007	0.47±0.007
Day-7	Mean S.D	1.44±0.01*	1.61±0.01*	1.63±0.003*	1.66±0.007*	1.72±0.004*
OGR (%)		206.4	242.6	246.8	253.2	266.0
CPGR (%)		20.52%	22.78%	23.03%	23.41%	24.14%

* Statistically significant; **statistically not significant

Each value represents the mean body weight of 25 worms ± standard deviation of four individual observations (P value < 0.001). The overall growth rates (OGRs) were calculated taking the zero dose control as the base value and compound periodical growth rates (CPGR) on the basis of first and last day values as per Sivaprasad[17].

Table 2: Effect of different concentrations (5%, 3%, 2%, 1%) of nutrilite on total protein levels of silk gland (A), fat body (B) and haemolymph (C) in *Bombyx mori* during fifth instar larval development

Day	Statistical tool	Zero dose control	Experimental (Concentration of nutrilite)			
			5%	3%	2%	1%
A. Growth in silk gland proteins (mg/g)						
Day-1	Mean S.D	16.67±0.10	16.67±0.10	16.67±0.10	16.67±0.10	16.67±0.10
Day-7	Mean S.D	70.05±0.04*	84.83±1.22*	85.09±2.92*	88.69±1.44*	93.40±1.58*
OGR (%)		320.2	408.9	410.3	430.0	460.3
CPGR (%)		104.99%	125.58	125.93	130.66	136.70
B. Growth in fat body proteins (mg/g)						
Day-1	Mean SD	25.92±1.25	25.92±1.25	25.92±1.25	25.92±1.25	25.92±1.25
Day-7	Mean SD	41.04±0.03*	52.32±1.74*	55.56±0.61	53.54±1.34*	56.37±1.28*
OGR%		58.3	101.9	114.4	106.6	117.5
CPGR (%)		25.83%	42.07%	46.41%	43.45%	47.47%
C. Growth in haemolymph proteins (mg/ml)						
Day-1	Mean SD	6.41±0.18	6.41±0.18	6.41±0.18	6.41±0.18	6.41±0.18
Day-7	Mean SD	8.22±0.02*	8.46±0.03*	8.84±0.08*	8.91±0.03*	8.83±0.02*
OGR (%)		28.2	32.0	37.9	39.0	37.8
CPGR (%)		13.24%	14.88%	17.43%	17.90%	17.37%

* Statistically significant; **statistically not significant

Each mean represent the mean, ± standard deviation of four individual observations, expressed in mg/g or mg/ml (P value < 0.001). The overall growth rates (OGRs) were computed taking the zero dose control as the base value and the compound periodical growth rates (CPGR) on the basis of first and last day values as per Sivaprasad[17].

Fig.1: Effect of different doses (5%, 3%, 2%, 1%) of nutrilite on the larval growth of *Bombyx mori* during fourth and fifth instar larval stages. The growth trends represent changes in compound periodical growth rates (CPGRs)

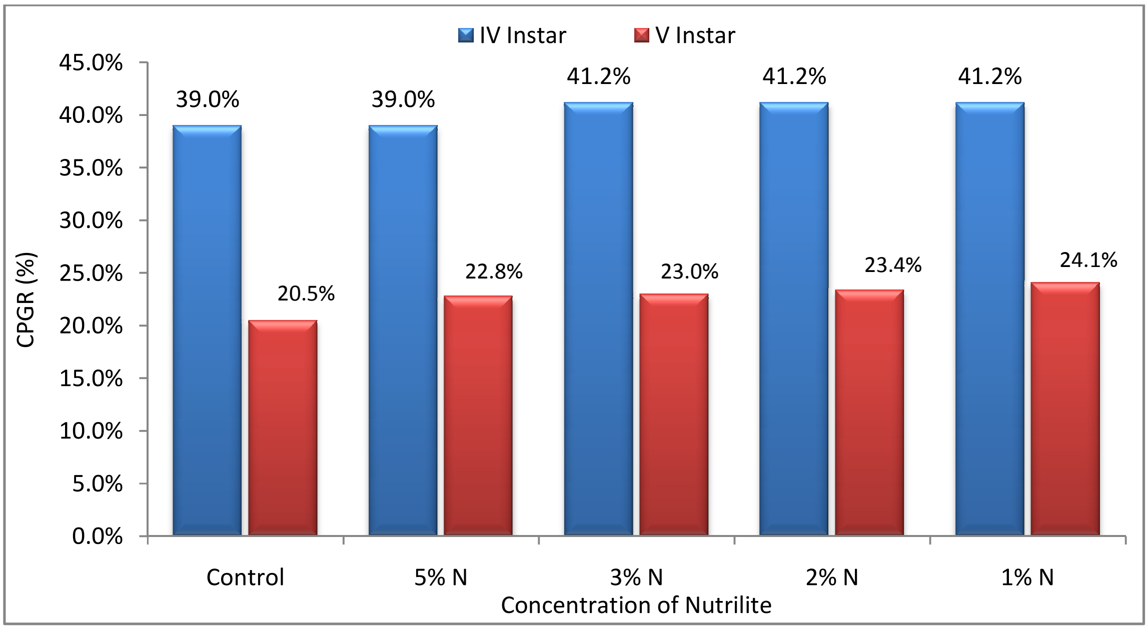
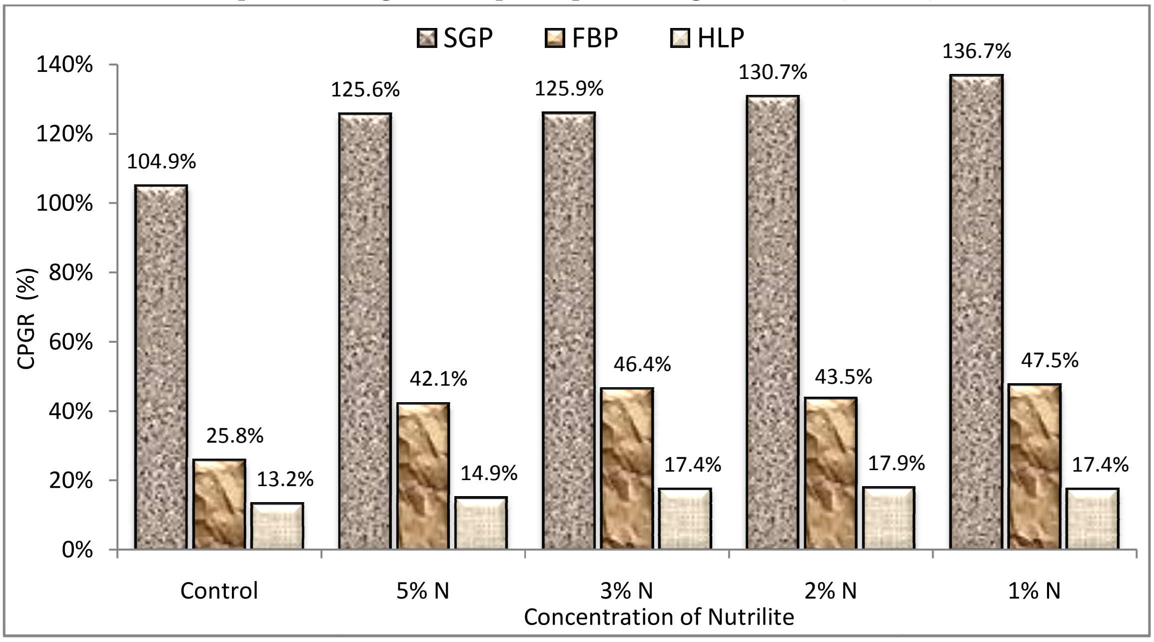


Fig.2: Effect of different doses (5%, 3%, 2%, 1%) of nutrilite on protein levels in silk gland (SGP), fat body (FBP) and haemolymph (HLP) during fifth instar larval development in *Bombyx mori*. The growth trends represent changes in compound periodical growth rates (CPGRs)



CONCLUSION

The commercially available protein- rich nutrilite, an Amway Product, has potential to stimulate growth, protein synthesis and metabolism in the silkworm *Bombyx mori*. Its potential can be realized at a minimum effective dose (MED) of 1% in distilled water. It selectively accelerates the growth rate in the silkworm larvae, at a time when it is naturally slower and stimulates protein synthesis, even in the inert regions of the silk gland. As discussed earlier, the nutrilite enhances the growth rate during fifth instar larval life, more effectively than that of fourth instar. Similarly, it stimulates silk protein synthesis in the silk gland more than it does so in the fat body and haemolymph. By virtue of its accelerating role on growth and silk protein synthesis, the nutrilite has potential to improve the productivity and quality of silk produced by *B. mori*. The possibility of its application in sericulture in the form of enriched mulberry diet can be explored after conducting relevant field trials.

Acknowledgements

Authors thank the University Grants Commission, New Delhi for providing financial assistance in the form of Rajiv Gandhi National Fellowship for this research work.

REFERENCES

1. Laskar, N. Datta, M. Effect of alfalfa tonic and its organic ingredients on growth and development of silkworm, *Bombyx mori* L. race instar. *Environ. Ecol.,* **18**: 591-596 (2000)
2. Kanafi, R.R. Ebadi, R. Mirhosseini, S.Z. Seindavi, A.R. Zolfaghari, M. Eteban, K. A review on nutritive effect of mulberry leaves enrichment with vitamins on economic parameters traits and biological parameters of silkworm, *Bombyx mori L. Indian Sericultture Journal.* **4**: 86-91 (2007)
3. Sanappa, B. Ramaiah, M.J. Chandrappa, D. Influence of castor genotype on consumption indices of eri silkworm, *Samia cynthia ricini*. *Bioduval. Environ. Ecol.,* **20**: 960-964 (2002)
4. Etebari, K. Matindoost, L. Effect of hypervitaminosis of B_3 on silkworm biology. *J. Biosci.,* **29**: 417-422 (2004)
5. Bhattacharya, A. Kaliwal, B.B. Synergetic effects of potassium and magnesium chloride on biochemical contents of the silkworm, *Bombyx mori* L. *Caspian. J. Env. Sci.,* **3(1)**: 1-7 (2005 b)

6. Raman, C. Suganthi, L.M. Xavier, N. and Krishnan, M.K. Expression of silk gene in response to P. Soyatose (hydrolysed soya bean protein) supplementation in the fifth instar male larvae of *Bombyx mori L. J. Cell and mol. Biol.*, **6**: 163-174 (2007)
7. Sheeba, Quraiza Fatima, Thilsath, M., Das, Manohar, Sam, S. and Bai, Ramani, M. Effect of prophylactic antibiotic treatment on growth and cocoon characteristics of *Bombyx mori* L., *Journal of Basic and Applied Biology.* **2(1)**: 19-22 (2008)
8. Ramakrishna, S. and Bhaskar, M. Improvement in cocoon parameters of silkworm larvae, *Bombyx mori (L)* on induction of thyroxine hormone. *The Bioscan.* **4**: 175-178 (2009)
9. Amala Rani, G. Padmalatha, C. Sorna Raj and Ranjith Singh ,A.J.A. Impact of supplementation of Amway protein on the economic characters and energy budget of silkworm *Bombyx mori L. Asian Journal of Animal Sciences*. **5(3)**: 190-195 (2011)
10. Chakrabarty, S, Kaliwal, B.B. Supplementation with potassium carbonate, magnesium carbonate and their synergetic effects on the economic traits of the silkworm, *Bombyx mori* L. *World Journal of Science and Technology*, **1(5)**: 10-23 (2011)
11. Ganesh Prabhu, P. Balasundaram, D. Selvi, S. Mathivanan,V. and Ramesh, V. Biotechnological applications and nutritional supplementation of ascorbic acid (Vitamin C) treated *Morus alba* (L.) leaves fed by silkworm, *Bombyx mori*(L.) (Lepidoptera: *Bombycidae*) in relation to silk production. *Int. J. Res. in Biomed.& Biotech.*, **3(1)**: 11-16 (2013)
12. Krishnaswami,S. New Technology of silkworm rearing. *Central Sericultural Research and Training Institute, Mysore, India* (1986)
13. Williams. Minimum effective dose. *Biometrics*. **27**: 103-117 (1971)
14. Li Jan, S. Step contrasts for identifying the minimum effective dose. *Communications in Statistics – Theory and Methods*. **34**: 45-47 (2005)
15. Kavitha, S. Siva prasad, S. and Yellamma, K. Determination of minimum effective dose of Zinc chloride for modulation of metabolism and silk production in the silkworm, *Bombyx mori*. *The Bioscan*. **6(1)**: 147-152. (2011)
16. Lowry, O.H. Rosenbrough, N.J. Farra, L. Randall, R.J. Protein measurement with folin phenol reagent. *J. Biol. Chem.,* **33**: 19-27 (1951)
17. Sivaprasad, S. Simple method for calculation periodical growth rates in animals and plants. *J. Bio. Innov* **(5)**: 114-119 (2012)
18. Stewart, W.H. and Ruberg, S.J. Detecting dose response with contrasts. *Statistics in Medicine*.**19**: 913-921(2000)
19. Thulasi, N. Sivaprasad, S. Determination of minimum effective concentration of alfalfa for optimal growth, metabolism and silk production in the silkworm, *Bombyx mori*. *J. Bio. Innov*., (2014a)
20. Sarker A, Haque M, Rab M, Absar N. Effect of feeding Mulberry (*Morus* sp.) leaves supplemented with different nutrients to silkworm (*Bombyx mori* L.). *Current Science.* **69(2)**: 185-188 (1995)
21. Nirmala, M.K. Vanisree, V. Zurovec, M. Sehnal, F. Identification of four small molecular mass proteins in the silk of *Bombyx mori*. *Insect Mol.*, **10***:* 437-445 (2001)
22. Jin, Y.X. Chen, Y.Y. Xu, M.K. Jiang, Y.H. Studies on middle silk gland portions of cocoon colour sex-limited silkworm (*Bombyx mori*) using two dimensional Polyacrylamide gel electrophoresis. *J. Biosci.*, **29**: 45-49 (2004)
23. Takasu, Y. Yamada, H. Saito, H. Tsubouchi, K. Characterization of *Bombyx mori* sericins by the partial amino acid sequences. *J. Insect Biotech. & Sericol.*, **74**: 103-109 (2005)
24. Kyung, H.S. Su, J.J. Yong, R.S. Seok, W.K. Sung, SH. Identification of up-regulated proteins in the haemolymph of immunized *Bombyx mori* larvae. *Comp. Biochem. Physiol.*, **Dl**: 260-266 (2006)
25. Zhang, PB. Aso, YK. Yamamoto, BY. Wang, YQ. Tsuchida, K.Y. Kawagnchi, H. Fujii. Proteome analysis of silk gland proteins from the silkworm, *Bombyx mori*. *Proteomics.* **6**: 2586-2599 (2006)
26. Hou, Y. Zhao, P. Liu, H.L. Zou, Y. Guan, J. and Xia, Q.Y. Proteomics analysis of fat body form silkworm (*Bombyx mori*). *Sheng. Wu gong. Cheng. XueBao.* **23**: 867-872 (2007 b)

27. Etebari, K, Matindoost, L. Application of multi- vitamins as nutrients on biological and economical characteristics of silkworm *Bombyx mori* L. *J. Asia- Pacific Entomol*., **8**: 1-6 (2005)
28. Thulasi, N. Sivaprasad, S. Synergetic effect of ascorbic acid and lemon juice on the growth and protein synthesis in the silkworm, *Bombyx mori* and its influence on economic traits of sericulture. *J.Bio. Innov*., **2(4)**: 168-183 (2013)
29. Thulasi, N. Sivaprasad, S. Impact of feeding of lemon juice- enriched mulberry leaves on the larval growth, protein profiles and economic traits in the silk worm, *Bombyx mori. Ind. J. Appl. Res*4 **(2)**: 36-44 (2014 b)
30. Scott, R.C. Schuldiner, O. and Neufeld, T.P. Role and regulation of starvation – induced autophagy in the Drosophila fat body. *Dev. Cell*. **7**: 167-178 (2004)
31. Hak Ryul Kim, Soo J. Seo, Dr. Richard T. Mayer. *Insect Biochemistry and Physiology*.**10**: 215-228 (2005)
32. Hou, Y. Xia, Q. Zhao, P. Zou, Y. Liu, H. Guan, J. Gong, J. and Xiang, Z. Studies on middle and posterior silk glands of silkworm (*Bombyx mori*) using two- dimensional electrophoresis and mass spectrometry. *Insect Biochem. Mol. Biol.* **37**: 486-496 (2007 a)
33. Lix, H. Wu, X.F. Liu, J.M. Li, G.L. and Miao, Y.G. Proteomic analysis of the silkworm (*Bombyx mori* L,) Haemolymph during developmental stages. *J. Proteome. Res.* **5**: 2809-2814 (2006)
34. Chai, S.S. Rhee, W.J. and Park, T.H. Inhibition of stem cell apoptosis by silkworm haemolymph, *Biotechnology progress*. **18**: 874-878 (2008)
35. Satake, S. Kawabe Y. and Mizoguchi, A. Carbohydrate metabolism during starvation in the silkworm *Bombyxmori L. Arch. Insect Biochem.Physiol.,* **44**: 90-98 (2000)
36. Nijhout, H.F. and Grunert L.W. Bombyxin is a growth factor for wing imaginal disks in Lepidoptera. *Proceedings of the National Academy of Sciences USA.* **99(24)**: 15446-15450 (2002)
37. Kiran Kumar, N. Ismail, S.M. and Dutta, A. Differential uptake of storage protein by the fat body of Rice moth, *Corcyra cephalonica* during the larval pupal development. *Entomol.*, **23**: 83-90 (1998)