RESEARCH PAPER | Zoology | Volume : 4 | Issue : 12 | Dec 2014 | ISSN - 2249-555X

Determination of Minimum Effective Concentration of Honey for Optimal Growth, Metabolism and Silk Production in The Silkworm, *Bombyx Mori*

KEYWORDS: *Bombyx mori*, Honey, Growth, Minimum effective concentration, Proteins

*** S. Sivaprasad**
Department of Zoology, Smt. N.P. S. Government College for Women, Chittoor (517 002). A.P. INDIA. * Corresponding Author

N. Thulasi
Department of Zoology, Smt. N.P. S. Government College for Women, Chittoor (517 002). A.P.

ABSTRACT *The sericultural productivity could be effectively modulated by enriching the silkworm diet with exogenous nutrients like honey. The minimum effective concentration (MEC) that ensures optimal larval growth, active metabolism and higher silk production has been determined in Bombyx mori, by a step- down process using four different concentrations (5%, 3%, 2% and 1%) of honey. While the MEC for larval growth was determined by analyzing changes in the body weight and that of silk production and metabolism by assaying protein levels of the silk gland, fat body and haemolymph. The MEC of honey differs from tissue to tissue. While 2% honey evoked optimal response in body growth and silk protein synthesis, 1% honey triggered optimal protein synthesis in the fat body. Hence, 2% honey is recommended as MEC for modulating growth and silk production and 1 % honey for modulating metabolism in the fat body of silkworm.*

Introduction

It has become customary to investigate the impact analysis of exogenous modulators on the growth and development of silkworm and to make correlational studies with regard to quantum of such modulators applied and the productivity of sericulture. The impact of feeding the silkworm larvae with the nutrient-enriched mulberry diet has been widely practiced in sericulture. A variety of exogenous modulators such as hormones, minerals and vitamins were successfully tried on Bombyx mori (Eg. Javed and Gondal, 2002; Islam et al., 2004; Bhattacharya and Kaliwal, 2005a, 2005b; Kochi and Kaliwal, 2006; Rahmathulla et al., 2007; Raman et al., 2007; Ramakrishna and Bhaskar, 2009; Vitthalrao and Sarwade, 2009; Thulasi and Sivaprasad, 2013, 2014). The determination of minimum effective concentration /dose (MEC / MED) of such exogenous modulators that could elicit response greater than the zero- dose control assumes greater significance in all such treatment studies. Quite often, the dose - response studies are carried out in experimental biology with a view to determine the MEC / MED of such modulators and to derive gainful benefits in terms of increased growth, metabolism, reproductive output and productivity (Ruberg, 1989). One of the exogenous modulator that attracted the attention of investigators is the honey, which is a natural sweetener and multi- factorial nutrient produced by the honey bees (Council of European Union, 2002).

The present investigation was taken up with a view to suggest the minimum effective concentration of honey that could effectively modulate the growth, metabolism and silk production in Bombyx mori and the safe dosage that could be allowed to accumulate its tissues (silk gland, haemolymph, fat body, and muscle) without causing any adverse effect on its growth and metabolism.

Material and Methods

The present investigation was carried out on PM x CSR_2 hybrid variety of the silkworm, ***Bombyx mori***, reared under standard environmental conditions of 28^{0}C, 85 % RH as per Krishna swami, 1986. After hatching, the worms were reared on M_5 variety of mulberry leaves with 5 feeds per day at 6 AM, 10 AM, 2 PM, 6 PM and 10 PM, under normal 12 hr light and 12 hr dark conditions. The minimum effective concentration (MEC), of honey was determined by a step- down process starting from a higher concentration (5%) to a lower concentration (1%), with a Zero- dose control as given by Williams, 1971 and modified by Li Jan, 2005; Kavitha et al., 2011; Thulasi and Siva Prasad , 2014). After the third moult, the fourth instars larvae were divided into five batches; one control and four experimental, each comprising 100 worms. The control batch was fed with normal feedings 5 times a day as stated earlier and the experimental batches were fed with mulberry leaves soaked in four different concentrations (viz., 5%, 3%, 2% and 1%) of honey prepared in distilled water. While the honey soaked leaves were fed to the silkworms, at 6 PM, normal feeding pattern was continued at other timings during the day. Before feeding, the mulberry leaves were soaked in diluted honey solutions and dried under cool weather conditions. Each of the control and experimental batches were examined with reference to silkworm growth and tissue-based protein levels as detailed below.

Silkworm growth: The MEC of honey for the silkworm growth was determined by analyzing the changes in the body weight of the silkworm larvae after feeding them with the mulberry leaves enriched with different concentrations of honey. The larval growth rates were determined by recording the body weights on the first and last day of both fourth (4 days) and fifth (7 days) instars. Since, the body weight is an index of animal growth, the mean body weight of 25 randomly selected larvae were measured in an electronic balance (ELICO; Model BL- 22 OH) and the same was expressed in grams.

Assay of tissue proteins: The MEC of honey for metabolism was determined by analyzing the total protein profiles of silk gland (SG), fat body (FB) and haemolymph (HL) on the first (day-1), and last day (day-7) during fifth instar larval development. While the SG and FB were isolated by mid- dorsal dissection of larval body in the Silkworm Ringer (Yamaoka et al., 1971), the HL was extracted by cutting the telson and prolegs. The total protein content was estimated in 1% homogenates of SG and FB and 1:9 diluted HL (1:9 haemolymph and distilled water) by the method of Lowry et al., 1951 and the

Volume : 4 | Issue : 12 | Dec 2014 | ISSN - 2249-555X

same was expressed in mg protein / gram wet weight of tissue (or) mg/ml of haemolymph.

Statistical analysis: The experimental data were statistically analyzed by mean, standard deviation (SD), percent change and test of significance. While the mean and SD were computed using M.S. Excel, the test of Significance and percent -changes was calculated online using the Graph pad and percent change flatforms (www. Graph pad. Com/quick calcs/index cfm/and www. Percent-change com/ index php). Further, in order to ensure uniformity in data analysis and interpretation and to draw meaningful conclusions thereof, the growth trends in the larval body weight and those of proteins were interpreted in terms of innovative statistical parameter called compound periodical growth rate (CPGR) as given by Sivaprasad, 2012.

RESULTS

The findings of the present investigation on the growth of larval body and tissue proteins in control and experimental batches are presented in Table 1 and Figure 1.

Larval Body Growth

Fourth Instar Larvae: During the fourth instar development, the larval body weight of the control batch showed an overall growth rate (OGR) of ~247% and a compound periodical growth rate (CPGR) of 51.35% (Table.1A). When the silkworm larvae were fed with 5% honey-enriched mulberry diet, the OGR was elevated by 260% with a CPGR of 53.26%, representing an overall increase of ~3.8% from the zero-dose control (ZDC). At 3% and 2 % levels of honey, the body weight recorded an OGR of ~293% and a CPGR of 57.85%, representing a 13% net increase over the ZDC at both the concentrations. At 1% concentration of honey, the larval weight showed an OGR of ~280% and a CPGR of 57.85%, representing ~13% net increase over the ZDC (Table 1A and Fig. 1A).

Fifth Instar Larvae: During fifth instar development, the larval body weight of the control batch showed an OGR of ~287% and a CPGR of 25.28% (Table 1B). When the silkworm larvae were fed with 5% honey-enriched mulberry diet, the OGR was elevated by ~333% with a CPGR of 27.68%, representing an overall increase of 12.06% over ZDC. At 3% level, the body weight recorded an OGR of ~367% and a CPGR of 29.27%, representing an overall increase of 20.68% over ZDC. At 2 % level, the body weight recorded an OGR of ~383% and a CPGR of 30.03%, representing a 25% net increase over the ZDC. At 1% concentration of honey the larval weight showed an OGR of ~375 % and a CPGR of 29.65%, representing ~23 % net increase over the ZDC (Table 1B and Fig.1A).

Table. 1: Effect of different concentrations (5%, 3%, 2%, 1%) of honey on the growth of body weight during fourth (A) and fifth instar (B) larval stages and total protein levels of silk gland (C), fat body (D) and haemolymph (E) in Bombyx mori **during fifth instar larval stage.**

Day	Statistical tools	Control	Experimental (Concentration of Honey)			
			5%	3%	2%	1%
(A) Growth in Fourth Instar Larval Body Weight (mg/g)						
First (1)	Mean	0.15	0.15	0.15	0.15	0.15
	S.D	±0.009	±0.009	±0.009	±0.009	±0.009
Last (4)	Mean	0.52	0.54	0.59	0.59	0.57
	S.D	±0.003*	±0.01*	±0.01*	±0.01*	±0.01*
OGR (%)		(247)	(260)	(293)	(293)	(280)
CPGR (%)		51.35	53.26	57.85	57.85	56.0
(B) Growth in Fifth Instar Larval Body Weight (mg/g)						
First (1)	Mean	0.60	0.60	0.60	0.60	0.60
	S.D	±0.009	±0.009	±0.009	±0.009	±0.009
Last (7)	Mean	2.32	2.60	2.80	2.90	2.85
	S.D	±0.005*	±0.008*	±0.009*	±0.005*	±0.008*
OGR (%)		(287)	(333)	(367)	(383)	(375)
CPGR (%)		25.28	27.68	29.27	30.03	29.65
(C) Growth in Silk Gland Proteins (mg/g)						
First (1)	Mean	19.18	19.18	19.18	19.18	19.18
	S.D	±0.06	±0.06	±0.06	±0.06	±0.06
Last (7)	Mean	86.02	76.67	102.01	115.0	112.0
	S.D	±0.03*	±0.01*	0.08*	±0.05*	±0.08
OGR (%)		(34.8.5)	(299.7)	(431.9)	(499.6)	(483.6)
CPGR (%)		111.78	99.93	130.62	144.86	141.65
(D) Growth in Fat Body Proteins (mg/g)						
First (1)	Mean	29.04	29.04	29.04	29.04	29.04
	SD	±0.35	±0.35	±0.35	±0.35	±0.35
Last (7)	Mean	41.73	43.13	45.37	46.55	49.58
	SD	±1.64*	±1.44**	±1.26*	±1.52*	±0.03*
OGR%		(43.7)	(48.5)	(56.2)	(60.3)	(70.7)
CPGR (%)		19.87	21.87	24.99	26.61	30.66
(E). Growth in Haemolymph Proteins (mg/ml)						
First (1)	Mean	6.71	6.71	6.71	6.71	6.71
	SD	±0.02	±0.02	±0.02	±0.02	±0.02
Last (7)	Mean	8.81	8.60	9.14	9.52	9.20
	SD	±0.008*	±0.01	±0.009*	±0.008*	±0.01*
OGR%		(31.3)	(28.2)	(36.2)	(41.9)	(37.0)

*** Statistically significant; **statistically not significant**

Protein Levels

Silk Gland Proteins (SGP): In general, the SGP levels recorded elevatory trends during fifth instar development. In the control batch, their profiles recorded ~349% OGR and ~111.78% CPGR on day-7. When the larvae were fed with honey-fortified mulberry leaves the SGP levels showed ~300% OGR and 99.93% CPGR at 5% level, ~432% OGR and 130.62% CPGR at 3% level, ~500% OGR and 144.86% CPGR at 2% level and ~484% OGR and 141.65% CPGR at 1% concentration level of honey (Table 1C). When the growth trends were analyzed in terms of deviations from zero dose control (ZDC), the protein levels were deviated by -10.9%, +18.6%, +33.7% and +30.2% at the honey concentrations of 5%, 3%, 2% and 1% respectively (Fig.1B).

Fat Body Proteins (FBP): The impact of honey on FBP was more significant at lower concentrations than those at higher concentrations. In the control batch, the growth rates in FBP levels reflected 43.7% OGR and 19.87% CPGR during fifth instar development. When the larvae were fed with honey-enriched mulberry leaves, the corresponding OGRs and CPGRs were 48.5 and 21.87% at 5% level, 56.2 and 24.99% at 3% level, 60.3 and 26.61% at 2% level and 70.7 and 30.66% at 1% level of honey respectively (Table 1C). When compared with ZDC, the protein levels were enhanced by 3.4% at 5% level, 8.7% at 3% level, 11. 6% at 2% level and by 18.8% at 1% level of honey (Fig.1C).

Volume : 4 | Issue : 12 | Dec 2014 | ISSN - 2249-555X

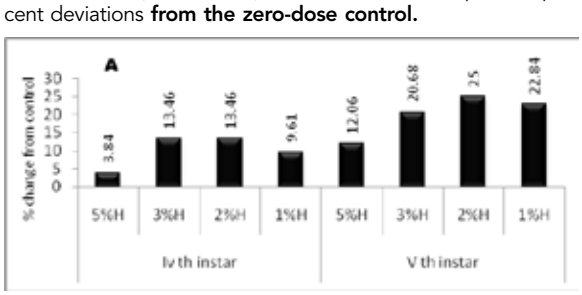
Haemolymph Proteins (HLP): The total proteins levels in the circulating medium of haemolymph were elevated by ~31.3% during fifth instar development and recorded a CPGR of 14.58% during the same period. Under the influence of honey, the HLP levels recorded 28.2% OGR and 13.21% CPGR at 5% level, 36.2% OGR and 16.71% CPGR at 3% level, 41.9% OGR and 19.11% CPGR at 2% level and 37% OGR and 17.09% CPGR at 1% level (Table 1E). When compared with ZDC, the HLP levels of experimental batches showed a negative trend (-2.38%) at 5% level, but positive growth trends (3.74 to 8.05%) at other three concentrations of honey (Fig. 1D).

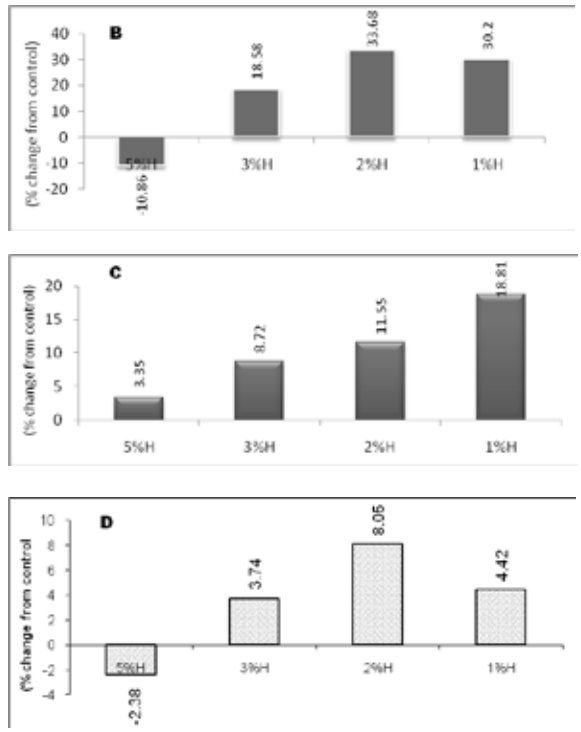
DISCUSSION

Honey is the richest and natural nutrient comprising carbohydrates (82%), proteins, enzymes (e.g. Diastase, invertase, glucose oxidase, catalase, etc.), free amino acids, trace amounts of B-vitamins and vitamin C, and metals such as Cr, Co, Cu, Fe, Mn and Zn (Falco et al., 2003; Garcia et al., 2005; David Ball, 2007). It plays vital role in the growth and metabolism of ***B. mori***. Its impact seems to be concentration- dependent and tissue- specific. The minimum effective concentration (MEC), the lowest concentration level with a response greater than that of the zero-dose control, is powerful tool for detecting the concentration-response relationship in growth related studies (Stewart and Ruberg, 2000; Li Jan, 2005; Amalarani et al., 2011; Kavitha et al., 2011; Thulasi and Sivaprasad, 2014). The present investigation, involving a step-down process with four different concentrations (viz., 5%, 3%, 2% and 1%) and a zero-dose control has revealed that the impact of honey was more pronounced at a concentration of 2% in distilled water. This was clearly reflected through its influence on larval growth and tissue proteins. The analysis of experimental data in terms of CPGR and deviations from ZDC helps ascertain the MEC values of honey.

The CPGRs in the larval body weight under treatment conditions ranged from ~53% to 58% during fourth instar and ~28% to 30% during fifth instar and seems to vary as a function of concentration of honey. Significantly, 2% honey caused greater elevations in the larval growth during fourth (58%) and fifth (~30%) instar developmental stages. The corresponding ZDC figures were +13% and +25% for fourth and fifth instars respectively (Table. 1A, 1B and Fig. 1A). Though, the honey showed positive impact at other concentrations (5%, 3% and 1%) as well, it is not as effective as that of 2% of honey.

Fig.1: Effect of different concentrations (5%, 3%, 2%, 1%) of honey on the growth of body weight during fourth and fifth instars larval stages (A) and total proteins of silk gland (B), fat body (C) and haemolymph (D) during fifth instar larval stage in Bombyx mori. The values represent percent deviations **from the zero-dose control.**





Source: Table 1.

The alterations in the levels of tissue protein profiles under treatment conditions is used as an index of metabolic rate in ***B. mori*** (Kavitha et al., 2011, 2012). Obviously, the MEC of honey for larval metabolism in the silkworm could be determined by analyzing the total protein levels of SG, FB and HL. The day-to-day growth trends in their levels have revealed that the honey caused a decline of 11.85 percentile points (111.78–99.93) in the CPGR of SGP at 5% level, an elevation of 2 percentile points (21.87–19.87%) in the CPGR of FBP and a decline of 1.36 percentile points (13.21–14.58%) in the CPGR of HLP. This culminated in an overall reduction of protein levels by 11% in SG, an elevation of ~3.35% in FB and ~2% reduction in HL. At 3% level, the honey caused an increase of 18.84 percentile points (130.62–111.78%) in the CPGR of SGP, 5.12 percentile points (24.99–19.87%) in the CPGR of FBP and 2.13 percentile points (16.71–14.58%) in the CPGR of HLP. It resulted in an enhancement protein production by ~19% in SG, ~9% in FB and just by ~4% in HL. Significantly, at 2% level, the honey caused maximal elevation of 33.08 percentile points (144.86–111.78%) in the CPGR of SGP, 6.74 percentile points (26.61–19.87%) in the CPGR of FBP and 4.53 percentile points (19.11–4.58%) in the CPGR of HLP. Consequently, the protein profiles were elevated by 34% in SG, ~12% in FB and by ~8% in HL. At 1% level, the nutrient caused an elevation of 29.87 percentile points (141.65-111.78%) in the CPGR of SGP, 10.79 percentile points (30.66-19.87%) in the CPGR of FBP and 2.51 percentile points (17.09-14.58%) in the CPGR of HLP. As a result, the protein repositories were raised by ~30% in SG, 19% in FB and by ~4% in HL.

Needless to say, the MEC of honey differs from tissue to tissue. While 2% honey caused greater response in SGP and HLP levels, the same effect was achieved at 1% concentration level in FBP. Essentially, the FB responds to lower concentration of honey, while SG and HL do so at higher concentrations. Hence, 2% honey is recommended

Volume : 4 | Issue : 12 | Dec 2014 | ISSN - 2249-555X

as MEC for modulating metabolism in respect of SG and HL and 1% for FB. Though, the present study suffers from the paucity of relevant literature, the one involving the zinc salts and their effect on silkworm metabolism (Kavitha et al., 2011) substantiates that SG and FB of silkworm respond positively to lower and higher concentrations / doses of exogenous factors provided through the mulberry diet. The opposite is true with regard to honey wherein SG and HL showed maximal response to 2% honey and FB at 1% honey. Thus, the study demonstrates that the silk output in ***Bombyx mori*** could be enhanced at 2% of honey, while the metabolic rate in the fat body could be stimulated at 1% level. The same proportion of honey could be conveniently accumulated in the tissues of silkworm, without adversely affecting its metabolism.

Each mean under body weight represents the average weight of 25 worms, expressed in grams. Each mean under proteins 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 calculated taking the control as the base value and the compound periodical growth rates (CPGR) were computed on the basis of first and last day values as per Sivaprasad, 2012.

REFERENCE

Amala Rani, G., Padmalatha, C., Sorna Raj & Ranjith Singh, A.J.A. (2011). 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. | Bhattacharya, A., & Kaliwal, B.B. (2005a). Synergetic effects of potassium and magnesium chloride on biochemical contents of the silkworm, Bombyx mori L. Caspian. J. Env. Sci., 3 (1): 1-7. | | Bhattacharya, A., & Kaliwal, B.B. (2005b). Fortification of mulberry leaves with mineral Magnesium chloride (Mgcl2) on Biochemical contents of the silkworm, Bombyx mori L. The Philippine Agricultural Scientist, 38(3): 337-340. | | Council of European Union (2002). Council Directive 2001/110/Ec of 20 December 2001 relating to honey. Offic. J. the. Europ. Comm., LIO.,47-52. | David Ball, W. (2007).The Chemical composition of honey.Chem. Educ., 84 (10): p. 1643. | Falco, G., Gomez- Catalan, C., Llobet, J.M., & Domingo, J.L. (2003). Contribution of medicinal plants to the dietary intake of various toxic elements in Catalonia, Spain. Trace Elem. Electrolyte.,20: 120-124. | Garcia, J.C.R., Garcia, J.B., Latorre, C.H., Martin, S.G., & Crecent, R.M.P. (2005). Comparison of Palladium- magnesium nitrate and ammonium di hydrogen phosphate modifiers for lead determination in honey by electro thermal atomic absorption modifiers spectrometry. Food Chem. 91: 435-439. | Islam, R., Md-Ali, A. O., Md-Paul, D. K., Sultana, S., Akhter Banu,N. A., & Islam, R. (2004). Effect of salt, Nickel chloride supplementation on the growth of silkworm, Bombyx mori L. (Lepidoptera: Bombycidae). J. Biol. Sci. 4: 170- 172. | | Javed, H., & Gondal, M. H. 2002. Effect of food supplementation by N and Ascorbic acid on larval mortality of silkworm (Bombyx mori L). Asian J. Plant Sci. 1: 556-557. | | Kavitha, S., Siva prasad, S., & Yellamma, K. (2011). 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. | | Kavitha, S., Sivaprasad, S., Bano Saidulla & Yellamma, K. (2012). Effect of Zinc Chloride and Zinc Sulphate on the silk worm, Bombyx mori Growth, tissue proteins and economic parameters of sericulture. The Bioscan. 7(2):189-195. | | Kochi, S. C., & Kaliwal, B. B. (2006). The effects of potassium bromide on biochemical contents of the fat body and haemolymph of cross breed races of the silkworm Bombyx mori L. Caspian J. Env. Sci., 4: 17-24. | | Krishnaswami, S (1986). New Technology of silkworm rearing. Central Sericultural research and Training Institute, Mysore, India. | Lowry, O.H., Rosenbrough, N.J., Farra, L., & Randall, R.J. (1951). Protein measurement with Folin phenol reagent. J. Biol. Chem.33:19-27. | | Li Jan, S. (2005). Step contrasts for identifying the minimum effective dose. Communications in Statistics – Theory and Methods. 34:45-47. | | Rahmathulla, V.K., Priyabrata Das, Ramesh, M., & Rajan, R.K. (2007). Growth rate pattern and economic traits of silkworm, Bombyx mori, L. under the influence of folic acid administration. J. Appl. Sci.Environ.Manage.,11: 81-84. | Ramakrishna, S., & Bhaskar, M. (2009). Improvement in cocoon parameters of silkworm larvae, Bombyx mori (L) on induction of thyroxine hormone. The Bioscan. 4: 175-178. | | Raman, C. Suganthi L. M., Xavier, N., & Krishnan, M. K. (2007). Expression of silk gene in response to P. soyatose (hydrolysed soyabean protein) supplementation in the fifth instar male larvae of Bombyx mori. L., J. Cell and Mol. Biol., 6: 163- 174. | | Ruberg, S. J. (1989). Contrasts for identifying the minimum effective dose. J. American Statistical Association. 84: 816-822. | | Sivaprasad, S. (2012). Simple method for calculation periodical growth rates in animals and plants. J. Bio. Innov., (5):114-119. | | Stewart, W.H., & Ruberg, S.J. (2000). Detecting dose response with contrasts. Statistics in Medicine.19:913-921. | | Thulasi, N., & Sivaprasad, S. (2013). 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. | | Thulasi, N., & Sivaprasad, S. (2014). Impact of feeding of lemon juice- enriched mulberry leaves on the larval growth, protein profiles and economic traits in the silk worm, Bombyx mori. Indian Journal of Applied Research.4 (2):36-44. | | Vitthalrao, K. B., & Sarwade, J. P.(2009). Protein profiles in the fifth instar larvae of silkworm, Bombyx mori (L) (PM x CSR) fed with Digoxin treated mulberry leaves. The Bioscan. 4: 41-44. | | Yamaoka, K., Hoshino, M., & Hirai, T. (1971). Role of sensory hairs on the anal papillae in oviposition behaviour of Bombyx mori. L. J. Insect Physiol., 47: 2327-2336. | | Williams. 1971). Minimum effective dose. Biometrics. 27:103-117. |