Influence of herbal tonic Alloe on the overall performance of the mulberry silkworm, *Bombyx mori* L.

M. Manimuthu and L. Isaiarasu*

ABSTRACT

The influence of the dietary supplementation of the herbal tonic "Alloe" containing principally the extract of *Aloe vera* Linne. on the growth and the cocoon characteristics of the mulberry silkworm *Bombyx mori* L. commercial cross breed race L x CSR2 during its fifth instar was experimented. This, in general, elicited better response of the growth and the cocoon characteristics of this silkworm. Specifically, the A*loe vera* tonic at 2.0 per cent concentration resulted higher larval growth and increased the weight of cocoon. The mean larval weight, relative growth rate, effective rearing rate, larval consumption index of the final instar larvae of *B. mori* increased with this supplementation of *Aloe vera* tonic. The average pupal weight and mother moth weight also increased as a result of this supplementation. Maximum shell weight and shell ratio were noted in the experimental sets treated with the *A. vera* tonic. In addition, the fibroin content of the cocoon shell produced by this silkworm in response to the dietary supplementation of *Aloe vera* tonic treatment also showed significant increase over the control The overall performance of *Bombyx mori* in response to this *A. vera* tonic treatment observed in the present study and the evaluation index values worked out showed that the growth and the cocoon parameters could be improved with the supplementation of *A. vera* commercial preparations.

Key words : Herbal tonic, *Aloe vera* Linnae, Performance of *Bombyx mori* L.

INTRODUCTION

The silkworm, *Bombyx mori* L. is a typical monophagous insect and mulberry (*Morus* spp.) leaf is its sole food. Man has immensely benefited from the silk produced by silkworms and subsequently researchers have always been trying to unveil the factors that can be manipulated to the benefit of the silkworm rearers (Nair and Kumar, 2004). Sericulture is an age-old land-based practice in India with high employment potential and economic benefits to agrarian families. No doubt, India is the second largest producer of mulberry silk next only to China (Vijayaprakash and Dandin, 2005). Plants are the richest source of organic chemicals on earth and phytochemicals have been reported to influence the life and behaviour of different insects (Rajasekaragouda *et al*., 1997). Various extracts of medicinal plants have been tested by supplementation in the silkworm *Bombyx mori* and were seen to influence the body weight, silk gland weight and the silk thread length in *Bombyx mori* (Murugan *et al*., 1998). Dietary supplementation of the leaf, flower and pod extracts of *Moringa oleifera* (Rajeswari and Isaiarasu, 2004) and chitosan solution (Bin Li *et al*., 2010) elicited varied responses in the final instar larvae of *Bombyx mori*. A commercial herbal tonic 'Iogen" having the extracts of some selected medicinal plants elicited better response from the larvae of *Bombyx mori* (Balamurugan and Isaiarasu, 2007)

Numerous scientific studies on *Aloe vera* had demonstrated its analgesic, anti - inflammatory, wound healing, immune modulating and anti-tumor activities as well as antiviral, antibacterial and antifungal properties (Reynolds and Deweck, 1999). Besides, *Aloe vera* products are also used in pharmaceutical and food industries (Eshun and He, 2004). *Aloe vera* contains over 75 nutrients and 200 active compounds, including vitamins, enzymes, minerals, sugars, lignin, anthraquinones, saponins, salicilic acid and amino acids (Park and Jo, 2006). Preparations of *Aloe* are, therefore, used both topically and as dietary supplement (Buenz, 2008). There has been no attempt so far to study the effect of the *Aloe vera* preparation on the silkworm *B. mori*. The present study is an attempt to assess the influence of a commercial health drink "Alloe" Health drink, an ayurvedic preparation having the *Aloe vera* plant juice on the growth and the cocoon parameters of mulberry silkworm.

MATERIALS AND METHODS

Final instar larvae of the mulberry silkworm (LX. CSR_2) were used in this study. Disease free layings of the silkworm were obtained and raised on fresh mulberry leaves as per the new technology for silkworm rearing (Dandin *et al*., 2000). After third moult, the larvae were acclimatized to the laboratory conditions by rearing them during the fourth instar in plastic trays of size 26 x 20 x 6 cm. During this period, they were fed four times a day. Sufficient ventilation was ensured to the larvae by placing the trays one above the other crosswise. Coolant gel bags were used to bring down the temperature and wet synthetic foam pads were used to enhance the relative humidity near the larval bed within the optimum level. A Thermo-Hygrometer was used to record the temperature and relative humidity near the larval bed.

Fresh and healthy leaves of MR_2 variety of mulberry were used in the present study. The leaves were harvested daily from the mulberry garden during the early hours of the day and stored cool to maintain its freshness until use using wet gunny cloth in a wooden chamber. Disinfection was carried out prior to the commencement of silkworm rearing as a precautionary measure against pathogens, which may remain in the rearing room and are likely to infect the silkworm. For this, the rearing room was disinfected by spraying 2% formalin solution 3 days prior to the commencement of rearing. The rearing materials such as trays and mountages were washed with Chloralk solution. Dettol solution was used to wash the hands before and after handling the worms during the time of rearing. A bed disinfectant powder prepared by grinding Lime Powder, Paraformaldehyde and Benzoic acid in 97:2:1 ratio was dusted mildly on the worms daily after bed cleaning. Dead larvae if any, during the course of rearing were immediately removed and discarded properly. The herbal tonic *"Alloe"* Manufactured in India by C.P. Plantations, Kottampatty, Madurai (Dist) was used in this experiment as the study material. Required quantities of this tonic were taken and diluted with distilled water to get required conditions. The larvae were taken in equal number of 20 each is 15 trays to form five triplicate sets for the control (A_0, B_0 and C_0) and treatment with 0.5 per cent (A_1, B_1, C_1), 1 per cent (A_2, B_2, C_2), 1.5 per cent (A_3, B_3, C_3) and 2 per cent (A_4, B_4, C_4) solutions of the herbal tonic respectively. Accordingly, the herbal tonic was diluted separately in 100ml distilled water. The mulberry leaves were kept soaked in these solutions for 10 minutes before feeding them to the larvae in the respective experimental trays. The larvae in the control trays were fed with untreated mulberry leaves. Thus, the larvae in both the control and experimental trays were reared with equal quantities of leaves. The temperature and relative humidity were maintained at about 26 ± 2°C and around 70 ± 10 per cent respectively.

Several parameters were studied to assess the growth and the cocoon characteristics of *B. mori*. The daily increment in the weight of the larvae in each tray was monitored by weighing them daily at the time of bed cleaning. Sample larvae both at the beginning and end of the feeding were collected, weighed and dried to weight constancy in hot air oven. The initial and final dry weights of the larvae were used to calculate the production or absolute growth on dry weight basis. The mature larvae from both control and experimental sets were isolated and mounted on separate plastic mountage (Netrika). They were left undisturbed for four days to spin the cocoon. The cocoon were harvested and the commercial characteristics were assessed. The overall performance of the silkworm in the present study was assessed by taking into account six growth and six commercial parameters and subjecting them to evaluation index analysis (Mano *et al*., 1993).

RESULTS AND DISCUSSION

Nutrition plays an important role in improving the growth and development of *B. mori* (Kanafi *et al*., 2007). Alagumalai *et al.* (1991) observed fortification of mulberry leaves with the flour of black gram and red gram to improve the larval growth and cocoon characteristics in *B. mori*. Similarly, the growth of silkworm larvae improved significantly upon feeding them with mulberry leaves supplemented with different nutrients (Sarker, 1993). In the present study, the mean larval weight of the final instar larvae increased in the case of the larvae that received "Alloe" tonic as the dietary supplement. Rajasekaragouda *et al.* (1997) noticed the growth promoting effect of the water and ether extracts of the plants such as *Tribulus terrestis* and *Psoralea corylifolia*. Subburathinam and Krishnan, (1998) noticed Soya bean meal to accelerate the larval growth significantly at certain level when supplemented along with food to the silkworm *B. mori*. Sundarraj *et al.* (2000) observed silkworm reared on the leaf supplemented with soyabean flour to record significantly higher larval weight on the account of the additional protein supplemented. It was estimated that the gel of *Aloe vera* contains nearly 1.7 per cent protein (Luta *et al*., 2009). The observation of increase in the live weight of the final instar larvae of *B. mori* upon receiving the dietary supplementation of "Alloe" tonic in the present

study may, therefore, be attributed to the nutritive nature of this plant extract.

The average consumption by the larvae in the control ranged from 294 ± 27.7 mg dry weight / larvae in the first day to the maximum of 960 ± 69 mg dry weight / larvae on the third day with the total consumption being 3764 ± 130 mg dry weight / larvae. At the same time, the larvae in the experimental sets showed an increase in the total consumption over the control. Accordingly, the larval consumption indices worked out also showed considerable increase as a result of "Alloe" tonic treatment. The duration of the instar was about six days with the total number of feedings around 27 in all sets. However, the larvae in the experimental sets began to spin earlier than those in the control. Some larval mortality was seen in the control and in the experimental sets with "Alloe" tonic treatment. Accordingly the effective rearing rate was calculated and the relative growth rates of the larvae were increased with "Alloe" tonic supplementation. The weight of pupae and the weight of the mother moth in the present study were also increased as a result of "Alloe" tonic treatment. The quantity and the quality of dietary protein has long been considered to be important in the growth of the silkworm. Higher growth rate as well as weight gain can be observed in higher protein utilized group and the relative growth rate varied among the different breeds of the silkworm (Magadum *et al*., 1996) and were influenced by the season (Isaiarasu and Suriabraman., 1999). The difference in the relative growth rate of *Aloe vera* tonic supplemented larvae from the control observed in the present study indicates that the *Aloe vera* supplementation results in higher protein utilization. Sundaramahalingam *et al.* (1998) noticed that the growth rate and protein utilization of silkworm are high as a result of the supplementation of Miraculan, a plant growth regulator. Murugan *et al.* (1998) noticed a strong correlation between the growth of silkworm and the silk production in the silkworm after the treatment with plant extracts and attributed the growth promoting effect of the plant extracts to the stimulation of biochemical processes leading to protein synthesis.

The economic characters of the silk cocoon were reported to improve by feeding the silkworm with mulberry leaves treated with amino acids (Sridhar and Radha, 1986). Subburathinam *et al*. (1990) observed the enrichment of mulberry leaves with calcium chloride to increase the cocoon characters like cocoon weight, shell weight cocoon /shell ratio and silk proteins. The cocoon weight increased when the silkworm larvae were fed with blood meal fortified mulberry leaves (Matsura, 1994).

Table 1. Overall performance of the silkworm *B. mori* in response to the influence of *Aloe vera* tonic during final instar

Parameter	Per cent mortality				
	Control	0.5%	1.0 %	1.5 %	2.0 %
Mean larvalweight (mg live weight/larva)	1985 ± 25	2016 ± 46*	2019 ± 13*	2035 ± 35*	2180 ± 52*
Relative Growth rate	0.123 ± 0.000	0.124 ± 0.000*	0.124 ± 0.000*	0.124 ± 0.001*	0.218 ± 0.000*
Effective rate of rearing (%)	95 ± 7.07	95 ± 0	96.6 ± 4.71	98.33 ± 2.35	98.33 ± 2.35
Larval consumption index	0.290 ± 0.005	0.296 ± 0.015*	0.331 ± 0.03*	0.334 ± 0.01*	0.336 ± 0.004*
Average pupal weight	318 ± 13	322 ± 7	327 ± 17	330 ± 14	351 ± 18*
Mother mothweight (mg live weight/moth)	335 ± 11	345 ± 11	361 ± 24*	383 ± 6*	407 ± 12*
Average cocoon weight (mg wet weight/cocoon)	1690 ± 16	1743 ± 17*	1771 ± 14*	1792 ± 10*	1815 ± 19*
Average cocoonVolume (cm^2)	3.47 ± 0.66	3.54 ± 0.80	4.37 ± 1.34	4.37 ± 0.67	4.61 ± 1.16
Average shellweight (mg dry weight/shell)	208 ± 8	228 ± 53	233 ± 32	243 ± 33	251 ± 20*
Cocoon shellRatio (%)	7.8 ± 0.50	8.42 ± 3.99	9.14 ± 0.78	10.0 ± 1.41	10.0 ± 1.84
Silk fibroincontent (mg dry weight/shell)	114 ± 5	122 ± 6*	128 ± 8*	153 ± 11*	153 ± 6*
Silk filament length (M)	798 ± 58	860 ± 8	945 ± 18*	977 ± 20*	1003 ± 12*

* Student't' test value for the difference from control significant at 5%.

Table 2. Evaluation Index Values (%) calculated for influence of *Aloe vera* tonic during final instar on the growth and commercial parameters of *B. mori*

Parameter	Experimental Rearing (%)				
	Control	0.5%	1.0 %	1.5 %	2.0 %
Mean larval weight	40.94	45.47	45.90	48.24	69.43
Relative Growth rate	49.99	49.99	49.99	49.99	50.01
Effective rate of rear`ing	38.85	38.85	49.66	61.35	61.35
Larval consumption index	36.5	39.5	57.0	58.5	59.5
Average pupal weight	40.0	44.0	48.0	49.9	68.17
Mother moth weight	37.7	41.66	47.83	56.44	65.44
Average cocoon weight	33.33	45.60	52.08	56.94	62.28
Average cocoon volume	37.2	38.74	56.36	56.36	61.46
Average shell weight	36.14	42.67	50.52	55.75	64.90
Cocoon shell ratio	35.20	42.41	50.79	60.79	60.79
Silk fibroin content	37.10	42.13	46.35	61.44	61.44
Silk filament length	34.47	42.59	53.70	57.88	61.27
Average	38.11	42.80	50.68	56.13	62.17

Chamudeswari and Radhakrishnaiah (1994) reported the increased of cocoon weight, when the silkworm larvae were fed with zinc and nickel fortified mulberry leaves. Majumdar and Medda (1995) reported the supplementation of tyrosine to enhance the cocoon weight due to the increased synthesis of DNA, RNA and proteins in silk gland. The weight and the size of coccon shell ratio and fibroin content of the shell increased with the supplementation of the amino acid, glycine (Isaiarasu and Ganga, 2000) reported that administration of JH analogue, Methaprene, to fifth instar larvae of *B. mori* through hypodermic injection increased the shell weight by 16 per cent over the control. Improvement in economic characters of silkworm was also noticed with folic acid administration. Sevarkodiyone an reported a greater stimulatory effect resulting in an increase in shell weight by 30.7 per cent over the control with the supplementation of aqueous leaf extracts of some plants along with mulberry leaves. In the present study, the weight of the cocoon were found increased significantly over the control in the experimental sets that had received the supplementation of 0.5 per cent, 1.0 per cent, 1.5 per cent, and 2.0 per cent "Alloe" tonic. This indicates that Aloe *vera* is also capable of influencing the cocoon parameters.

The plant extracts could benefit sericulture by improving the silk yield of *B. mori* and commercial silk production (Rajaeshekaragouda *et at.*, 1997). The silkworm larvae fed on mulberry leaves treated with *Coffea arabica* leaf extracts at 1:25 concentration recorded significantly higher shell weight (0.296g) than control (Jeyapaul *et al*., 2003). Eswaran and Sevarkodiyone (2004) reported that the 3 per cent suspension of wheat and Tapioca flours along with mulberry leaves showed greater shell weight respectively by 28 per cent and 15 per cent. The fibroin content of the cocoon shell produced by the silkworm *B. mori* in response to the dietary supplementation of "Alloe" tonic in the present study also showed a significant increase in the fibroin content and indicate that dietary supplementation of *Aloe vera* would lead to an increased silk productivity.

The overall performance of *B. mori* with response the to the influence of "Alloe" tonic treatment observed in the present study (Table 1) show that most of the parameters increase in the set that received of *Aloe vera* tonic treatments. The evaluation index values worked out for the performance of the silkworm larvae in this experimental rearing are given in the (Table 2). They averaged 38.11, 42.80, 50.68, 56.13 and 62.17 for the control larvae and the larvae that experimental supplementation respectively. The improvement noticed in the overall rearing performance of the final instar larvae of *B. mori* in response to the dietary supplementation of *Aloe vera* tonic in the present study indicate the possibility of using it to realize the ultimate applied objective of qualitative and quantitative improvements of silk yield in sericulture research (Mathavan *et al*., 1984). Numerous medicinal plants have become key ingredients for dietary supplements and related industries. The controversy over the identity of

the active substances in *Aloe vera* has not been settled and various mechanisms have been proposed for the alleged medicinal properties of this plant. It is commonly suggested that there may be some synergistic action (Lawless and Allan, 2000). The present study was only an attempt to assess the influence of *Aloe vera*, which is used extensively as a food additive by virtue of its nutritive and medicinal properties. Screening of such growth promoting tonics that are readily available in the market may prove to be useful in augmenting commercial silk production.

ACKNOWLEDGEMENT

The provision of facilities in our college for the rearing of silkworm and help rendered by the Sericulture Department staff during this study are gratefully acknowledged.

REFERENCES

Alagumalai, K., Hepshyba, C.S.S. and Ramaraj, P. 1991. Bran of pulses as extra nutrient to silkworm. *India Silk,* **30** (6) : 10 - 11

Balamurugan, R. and Isaiarasu, L. 2007. Effect of the herbal tonic "Iogen" on the growth and cocoon parameters of mulberry silkworm, *Bombyx mori* L. Proceedings of the National Seminar on Appliec Zoology ANJA College, Sivakasi.

Bin Li, Ting Su, Xiaoling Chen, Baoping Liu, Bo Zhu, Yuan Fang, Wen Qiu and Guanlin Xie. 2010. Effect of chitosan solution on the bacterial septicemia disease of *Bombyx mori* (Lepidoptera: Bombycidae) caused by *Serratia marcescens.*" *Applied Entomology and Zoology,* **45** : 145 - 152 .

Buenz, E.J. 2008. Aloin induces apoptosis in Jurkat cells. *Toxicology in vitro,* **22** (2008) : 422 - 429.

Chamudeswari, P. and Radhakrishnaiah, K. 1994. Effect of Zinc and Nickel on the silkworm, *Bombyx mori* L. *Sericologia,* **34** (2) : 327 - 332.

Dandin, S.B., Jayaswal, J. and Giridhar, K. 2000. Hand book of Sericulture Technologies, Central Silk Board, Bangalore. pp. 259.

Eshun, K. and He, Q. 2004. *Aloe vera* : A valuable ingredient for the food, pharmaceutical and cosmetic industries: A review. *Critical Review of Food Science and Nutrition,* **44** (2) : 91 - 96.

Eswaran, R. and Sevarkodiyone, SP. 2004. Comparative effect of tapioca flour and amaranths green leaves on feeding characters of mulberry silkworm, *Bombyx mori. Bionotes,* **6** (3): 88 - 89.

Isaiarasu, L. and Suriabraman, S. 1999. Seasonal differences in the biochemical composition of M_5 and MR_2 varieties grown in Sivakasi and their influence on the growth of the late age larvae of *Bombyx mori*. *Journal of Ecobiology,* **11** (3) : 229 - 231.

Isaiarasu, L. and Ganga, G. 2000. Influence of dietary glycine supplementation on the mulberry silkworm, *Bombyx mori. ANJAC Journal,* **17** : 47 - 53.

Jeyapaul. C., Padmalatha, C., Ranjitsingh, A.J.A., Murugesan, A.G. and Dhasarathan, P. 2003. Effect of plant extracts on nutritional efficiency in mulberry silkworm *Bombyx mori. Indian Journal of Sericulture,* **42** (3) : 128 - 131.

Kanafi, R.R., Ebadi, R., Mirhosseini, SZ., Seidavi, AR., Zolfaghari, M. and Eteban, K. 2007. A review on nutritive effect of mulberry leaves enrichment with vitamins on economic traits and biological parameters of silkworm, *Bombyx mori* L. *Indian Sericulture Journal,* **4** : 86 - 91.

Lawless, J. and Allen, J. 2000. Aloe vera - Natural Wonder Cure. Harper Collins Publishers, London.

Luta, G., Duncan, and Sinnott, R. 2009. Chemical Characterization of polysaccharide rich ingredients from *Aloe vera,* Larix taricina, Larix occidentalis, and *Undaria pinnatifida*. Poster presentation scripts, Center for Integrative Medicine. Annual Natural Supplements Conference, San Diego, California, *22 - 25.*

Magadum, S.B., Hooli, M.A. and Magadum, V.B. 1996. Effect of the application of Juvenile hormone analogue in V instar followed by thyroxin in the Pure Mysore Breed of *Bombyx mori*. L. *Sericologia,* **32** (3): 385 - 390.

Majumdar A.C. and Medda, A.K. 1995. Studies on the thyroxin and vitamin B_2 induced changes in the cycle of Silkworm *Bombyx mori. Indian Journal of Physiology and Applied Science,* **29** : 1 - 13.

Mano, Y., Nirmalkumar, S, Basavaraju, H.K., Malreddy, N. and Datta, R.K. 1993. A new method to select promising silkworm breeds / combinations. *Indian Silk,* **31** : 53 - 54.

Mathavan, S., Baskaran. R., Sironmani, A. and Pandian, T.J. 1984. Studies on the utilization of single cell protein by the Silkworm, *Bombyx mori. Entomology Experiments and Application,* **36** : 61- 68.

Matsura,Y. 1994. Utilization of blood meal as the source of dietary protein for the silkworm, *Bombyx mori*. L. *Japan Agriculture Research Quarterly,* **28** (2): 133 - 137.

Murugan, K., Jeyabalan, D., Senthikumar, N., Senthilnathan, S. and Sivaprakasam, N. 1998. Growth Promoting effect of plant products on silkworm. A

Biotechnological Approach. *Journal of Scientific and Industrial Research,* **57** : 740 - 745.

Nair, J.S. and Kumar, S.N. 2004. Artificial diet for the silkworm, *Bombyx mori,* L., A retrospection through the decades. *Indian Journal of Sericulture*, **43** (1) : 1 - 17.

Park, Y.I. and Jo, T.H. 2006. Perspective of Industrial Application of Aloe vera. In : New perspective on Aloe (Eds.) Park, Y.I. and S.K. Lee. Springer Verlag, New York. 109 - 200 **PP**.

Rajasekaragouda, R., Gopalan. M., Jeyaraj and N. Natarajan. 1997. Field performance of plant extracts on mulberry silkworm, *Bombyx mori* L. *Entomon,* **22** (3 & 4) : 235 - 238.

Rajeswari, K. and Isaiarasu, L. 2004. Influence of the leaf, flower and pod extracts of *Moringa oleifera* on the growth and reproductive parameters of *Bombyx mori* L. *Entomon,* **29** (4) : 331- 338.

Reynolds, T. and Dweck, A.C. 1999. *Aloe vera* leaf gel: a review update. *Ethnopharmocology,* **68** (1- 3): 3-37.

Sarker. A. 1993. Effects of feeding different races of silkworm (*Bombyx mori* .L) with leaves varying in ploidy level. *Seriologia,* **33** (1) : 25 - 34

Sridhar, P. and Radha, N.V. 1986. Effect of supplementing glycine to the feed silkworm, Proceedings of the National Seminar on Prospects and Problems of Sericulture in India, TNAU, Coimbatore. 8 - 11.

Subburathinam, K. M. and Krishnan, M. 1998. Role of soyabean protein in silkworm nutrition, *Indian Silk*, **37** : 9 - 10.

Subburathinam, K.M., Kabila, V. and J. Sulochanachetty, 1990. Mineral spray can increase cocoon quality. *Indian Silk*, **28** (12) : 35 - 36.

Sundarraj, S., Nangia, N., Chinnaswamy, K.P. and Sannappa, B. 2000. Influence of protein supplement on performance of $PMxNB_4D_2$ silkworm breed. *Mysore Journal of Agricultural Science*, **34** : 302 - 307.

Sundaramahalingam, A., Muthuchelian, K. and Haridasan, T. M.1998. Influence of miraculan in the food and protein utilization of different larval stages of silkworm, *Bombyx mori* L. *UP Journal. Zoology,* **18** (2) : 95 - 98.

Vijayaprakash, N.B. and Dandin, S.B. 2005. Yield gaps and constraints in bivoltine cocoon production in Mandya district of Karnataka - An economic analysis. *Indian Journal of Sericulture*, **44** (1) : 50 - 54.

M. Manimuthu and L . Isaiarasu*

Department of Zoology, ANJA College (Autonomous), Sivakasi - 626 124, Tamil Nadu, India, Phone: 04562-254100 Fax: 04562-254970; *E - mail: isaiarasuin @ yahoo.co.in

Received: April 10, 2010 Revised: July 13, 2010 Accepted: August 13, 2010