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Impact of Aloe vera Juice as Supplementary Nutrient on Larval, Cocoon and Silk Parameters in *Bombyx mori* L.

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ABSTRACT: The silkworm *Bombyx mori* L. is a lepidopteran insect used as laboratory tool for various experiments. Being a domesticated insect it has been reared mainly for the production of silk. The successful production of the silk not only governed by dynamic environmental factors but also affected by quality of mulberry leaf provided to silkworm. The feeding of nutritionally enriched leaves provide better growth and development of silkworm larvae, as well as directly influence the quality and quantity of silk production. Hence, dietary nutrients are the most important factor which determine quality and quantity of silk production. An experiment was carried out to know the impact of mulberry leaf fortified with aloe vera at varied concentrations *viz.*, 0.2, 0.4, and 0.6% on larval, cocoon and silk parameters in the FC2 silkworm bivoltine hybrid. The study reveals that larvae feed on mulberry fortified with aloe vera at 0.6% treatment yielded the most pronounced improvements in economic parameters. Specifically, larval weight (4.96 g), cocoon weight (2.13 g) shell weight (0.50 g), pupal weight (1.62 g), shell ratio (23.64 %), filament length (1288m), filament weight (0.43 g), renditta (0.43), denier (3.04) and raw silk percentage (20.45 %) were all significantly higher compared to both control and lower-concentration treatments. The outcome of the study indicated that, there is profound increase due to aloe vera treatment in larval growth and cocoon characters than the control with enhanced quantity and quality silk production.

Keywords: Aloe vera, fortification, mulberry, silkworm, cocoon and silk parameter.

INTRODUCTION

Silkworm Bombyx mori L. is a well-known lepidopteran (Family: Bombycidae), the larval instars of which feed on the leaves of mulberry (Morus sp). The growth, development of larva and subsequent cocoon production are greatly influenced by nutritional quality of mulberry leaves. Needless to say, it has to derive its nutritional requirements from mulberry leaves for its growth and development. Any variation in the nutritional components of mulberry leaves may have some influence on the growth and development of silkworm. Though the availability of silkworm nutrients in mulberry leaf is ensured on most occasions, some time they may not be available in adequate quantities for the larval growth. The quality of the leaf can have profound influence on the superiority of silk produced by B. mori. Therefore, the production of good cocoon crop is largely dependent on the quality of leaves. In other words, leaves of superior quality enhance the chances of realizing good cocoon crop (Ravikumar, 1988). It has also been demonstrated that the dietary nutrition management has a direct influence on quality and quantity of silk production in B. mori. The nutritional parameters of mulberry leaves and silk production are related to each other. The nutritional composition of mulberry leaves varies depending on the nature of the genotype, soil and environmental conditions, precipitation (rainfall/irrigation), agronomical practices employed, plant protection measures adopted, etc. (Murugan *et al.*, 1998).

Cocoon production by B. mori depends on its rearing on mulberry leaves. For this reason, the quality of its food (mulberry leaf) needs to be high for realizing the full potential of the silkworm in terms of cocoon production. Nevertheless, the quantity of leaf fed by the larva also assumes great significance for accumulating considerable biomass that should culminate in the improvement of cocoon yield. Understandably, whenever the leaf quality if inferior, there arises a need for fortifying the leaf using supplementary nutrients such as vitamins, proteins, amino acids, carbohydrates, minerals, etc. so that the nutritional status of the leaf would be ultimately matching with the nutritional requirement of the silkworm. In addition, there is also a necessity to enhance the appetite in the silkworm so that it would grow better and to improve the disease

tolerance that should facilitate higher rate of survival of the larvae (Legay, 1958).

Like in other organisms, in the silkworm, B. mori too, nutrition plays an important role in improving the growth and development. The intake of nutrient by the silkworm larvae is influenced by the availability of feed. Therefore, silkworm nutrition is considered as a major area of research in sericulture (Legay, 1958). Nutrition study in silkworm is an essential pre requisite for its proper commercial exploitation. B. mori requires specific essential sugars, amino acids, proteins and vitamins for its normal growth, survival and also for the silk gland growth. Akhtar and Asghar (1972) found that vitamins and mineral salts played an important role in the nutrition of silkworm. Karaksy (1990) showed that silkworms fed on mulberry leaves enriched with ascorbic acid showed better growth and produced heavier cocoons. Similar observations were made by Madhu Babu (1992).

The feeding of nutritionally enriched leaves showed better growth and development of silkworm larvae and, in turn, influenced the quality and quantity of silk produced. Nearly 70% of the silk proteins produced by silkworm are directly derived from the protein of the mulberry leaves. In recent years a number of botanicals have been identified with insect growth regulation (IGR), juvenile hormone (JH) secretion, Experimental evidence consistently shows fortifying mulberry leaf with vitamin C, B-complex vitamins or minerals viz., Zn significantly improves larval growth, silk gland development and cocoon productivity in silkworm, Bombyx mori (Yashaswini et al., 2025). Fortifying mulberry leaf with amino acids (L-Serine and glycine) or protein rich supplements (drone brood, protinex) significantly improves larval silk gland development and cocoon growth, productivity in silkworm, Bombyx mori. Optimal gains are observed at moderate supplementation levels during the fifth instar (Yashaswini et al., 2025). However, silkworm, B. mori supplemented with plant extracts namely Tribulus terrestris, Pssoralea coryleifolia, Phyllanthus niruri, Polypodium Sp., Parthenium hysterophorus and Tridax procumbens increases larval growth as well as cocoon parameters (Rajashekargouda, 1991; Murugan et al., 1998). According to Mamadapur (1994); Santosh Kumar (1997), application of Lantana camara and Clerodendroninermae at 5% once on 48h old fifth instar larvae increased all the economic traits. A good number of reports are available on fortifying agents, feed additives and botanical extractions which enhances economics parameters of the silk worm. However, study on impact of botanical extracts on larval, cocoon and silk parameters of silkworm is meager. In this context, the present study was under taken.

MATERIAL AND METHODS

The experiment was carried out in the Department of studies in sericulture science, Manasagangaotri, University of Mysuru, which is which is located at 12° 18′ 26″ North Latitude and 76° 38′ 59″ East Longitude at an altitude of 770 m (2,530 ft) above mean sea level.

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Maintenance of mulberry garden: The experiment was conducted using mulberry garden (V-1 variety) maintained at Manasagangothri campus, Mysuru. The tender and medium mulberry leaves were used for young-age silkworm rearing. While, coarse leaves were used for late-age rearing. To maintain good quality leaf for feeding, proper methods were followed during harvesting and storage. Fresh leaves were collected during morning and evening hours of the day and stored in leaf preservation chamber covered with wet gunny cloth to reduce loss of moisture and degradation of nutrients (Sekharappa *et al.*, 1991).

Disinfection of rearing house and appliances: To ensure the rearing house and appliances pathogen free, they were thoroughly cleaned with water and 2% bleaching powder solution followed by 2.5% Sanitech solution @1.5 liter per square meter to achieve disinfection (Dandin *et al.*, 2003).

Procurement of disease free layings (DFLs): The disease free layings (eggs) of popular bivoltine silkworm hybrids namely FC2 (CSR6 × CSR26) was procured from the National Silkworm Seed Organization (NSSO), Central Silk Board (CSB), Mysuru. The egg cards were surface sterilized by dipping them in 2% formalin for 8-10 minutes and dried in shade before use.

Incubation of DFLS: The eggs of FC2 were incubated at room temperature (25°C) and 70-80% relative humidity by adopting standard incubation method and were subjected to black boxing for about 48 h at pin head stage (Benchamin and Nagaraj 1987).

Rearing of FC2 silkworm: After brushing the eggs, newly hatched silkworms were carefully shifted on to the rearing bed and fed fresh tender leaves and reared by employing stranded rearing techniques advocated by Dandin and Giridhar (2010).

Selection of plant extract Aloe vera Classification Kingdom-Plantae Order - Asparagales Family - Asphodelaceae Sub family- Asphodeloideae Genus - Aloe

Species - vera

Aloe vera is a stem less or very short-stemmed plant growing to 60-100cm tall, spreading by offsets. The leaves are thick and fleshy, green to grey-green with some varieties showing white flecks on their upper and lower stem surfaces. The margin of leaf is serrated and has small white teeth. The flowers are produced in summer on a spike up to 90cm tall. Aloe vera forms arbuscular mycorrhiza, a symbiosis that allows the plant better access to mineral nutrients in soil (Damodhara *et al.*, 2024).

Preparation of plant extract. The aloe vera (Dabur) procured from Charaka Ayurvedic Medicines and General Stores, Brindavan Extension Mysuru-20. The stock solution was diluted at the rate of 0.2, 0.4 and 0.6% by using distilled water. The prepared solution was preserved in refrigerator at 4°C until further use. For the experimentation, the larvae is divided into five batches *viz.*, batch I (T1), batch II (T2) and batch III

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(T3) were reared with mulberry leaf fortified with amla juice at 0.2, 0.4 and 0.6%, respectively. Whereas batch IV (T4) larvae were reared on mulberry leaf sprayed with distilled water (control) and batch V (T5) larvae were reared on mulberry leaf alone (absolute control). For each treatment, three replications were maintained and each with 100 larvae along with controls.

Silkworm larval and economic parameters: The silkworms were carefully monitored every day and from the first day of V instar moulting, the larval weight was determined everyday till the worms started to spin. Then the cocoon weight, pupal weight, shell weight, shell ratio, silk productivity, filament length, filament weight and denier were recorded.

Larval weight (g): For recording mature larval weight (g), ten larvae were randomly selected from each treatment, replication-wise during fifth day of fifth instar.

Cocoon weight (g): Cocoon weight (g) was calculated by taking ten randomly selected cocoons from all groups and weighed using an electronic balance. Weight of each cocoon from each group was recorded separately.

Pupal weight (g): Pupal weight (g) was weighed using an electronic balance, after removing the floss. The cocoons were cut open and the pupae were taken out without causing any damage to them.

Shell weight (g): Shell weight (g) was calculated by taking randomly selected 10 cocoons and cut open with

the help of a blade and the shell weight was taken accurately.

The parameters such as shell ratio, silk filament length, denier, renditta and raw silk percentage were calculated by using following formulae:

Shell ratio (%) =
$$\frac{\text{Shell weight } (g)}{\text{Cocoon weight } (g)} \times 100$$

Filament length (L)= $R \times 1.125$

Where, L = total length of the silk filament

R = number of revolutions recorded

1.125 = circumference of epprouvette in meter

Denier =
$$\frac{Total\ weight\ of\ reeled\ silk\ (g)}{Total\ length\ of\ reeled\ silk\ (m)} \times 9000$$

Renditta =
$$\frac{\text{Weight of cocoon reeled (g)}}{\text{Weight of raw silk (g)}} \times 9000$$

Raw silk percentage =
$$\frac{Silk \ weight \ (g)}{Coocon \ weight \ (g)} \times 100$$

RESULT AND DISCUSSIONS

Larval and Cocoon weight: Silkworm larvae fed on mulberry leaves fortified with aloe vera juice expressed variation in respect of larval weight with maximum being at 0.6% (4.965 g) followed by 0.4 % (4.692) and 0.2% (4.523g) respectively. While, it was minimum in absolute (4.487g) and distilled water controls (4.564g). The highest cocoon weight of 2.132g 0.6% followed by 0.4% (2.090g) and 0.2% (2.010g). As against to this, it was lowest in absolute (1.901g) and distilled water (1.950g) control batches (Table 1).

Table 1: Effect of mulberry leaves fortified with aloe vera juice at different concentrations on larval and cocoon parameters of FC2 silkworm hybrid.

Concentration (%)	Larval Weight	Cocoon	Pupal	Shell Weight	Shell ratio (%)
	(g)	Weight (g)	Weight (g)	(g)	
0.2	4.52+0.04	2.01±0.01	1.56±0.12	0.44±0.12	21.94±0.54
0.4	4.69±0.007	2.09±0.002	1.61±0.07	0.47±0.07	22.54±0.25
0.6	4.96±0.05	2.13±0.53	1.62±0.03	0.50±0.13	23.64±0.42
Control(Dil. water)	4.56±0.03	1.95±0.42	1.53±0.09	0.41±0.09	21.38±0.52
Absolute control	4.48±0.02	1.90±0.003	1.49±0.10	0.40±0.10	21.15±0.35

It is evident that aloe vera juice is a richest source of vitamin A, E and other nutrients and supplementation of aloe vera juice obviously boost the immune system of the larva which enhance larval weight in turn reflect on more cocoon weight. Perhaps, many vitamins and biomolecules are found in the diet are phagostimulatory in nature and it could be one of the reasons which enhance food intake by the silkworm resulting in increased larval and cocoon weight (Mane et al., 1997). These results are on line with earlier observations of Pratheesh et al. (2007), who have reported that silkworm larvae supplemented with Achyranthes aspera (4.04g), Achyranthes recimosus (4.00g), Parthenium hysterophorus (3.920g), Tribulus terrestris (4.02g) and Withania somnifera (4.02g) at 5% registered highest larval weight over remaining concentrations (3 and 8%) as well as control batches (3.8g). Further, Rajashekharagouda (1991) opined that administration of Psoralea corylifolia L. and Tribulus terrestris L. plant extracts at 10µg per larvae increased the cocoon weight. Barge and Pardeshi 2018, also noticed that increase in larval weight due to Sida acuta

plant extract at 0.5, 1.0, 1.5 and 2.0%, (2.336, 2.401, 2.419 and 2.559g), respectively against control (2.319g), similarly, Anil Kumar and Prashanth (2018), have noticed that, larvae supplemented with soya bean flour at 2, 4 and 6% exerted higher larval and cocoon weight over control. As per Manjula et al. (2011) larvae fed on mulberry leaf enriched with Dolichos lablab and Vigna unguiculata at 7.5% concentration has resulted increased larval and cocoon weight as against control. Mamadapur (1994) who reported the administration of 4µl of Latana camara and 7µl of Clerodendron inermae leaf extracts increases the larval weight. Kiran Kumara et al. (2024) also found that worms fed on mulberry leaves fortified with amla pulp juice at 0.2% concentration recorded higher larval and cocoon weight of 4.665 and 2.032g, respectively over control. Further, Krishna Prasad et al. (2001) opined that increase in larval weight in the cross breed PMxNBAD, was due to supplementation of mulberry leaves with potato leaf extract. Similar trend was also noticed by Shivakumar et al. (1995); Rajashekaragouda (1991); Takhlique (2012) in other plant extracts.

Pupal weight: The pupa formed by the larvae reared on mulberry leaves treated with aloe vera juice at 0.6% registered maximum pupal weight of (1.628g) followed by 0.4% (1.619g) and 0.2% (1.569g) over distilled water (1.533g) and absolute (1.499g) controls (Table 1). The silkworms fed on mulberry leaves sprayed with aloe vera at 0.6% concentration exerted highest pupal weight (1.628 g). The increase in pupal weight may be due to the increase in feeding efficacy during Vth instar larvae by the additional supplementation of aloe vera juice. These results are also supported by the observations of Sundar Raj et al. (2001) who reported that supplementation of soyabean flour to the silkworm recorded highest pupal weight of 1.23g over control batch (0.85g). Similarly, Kiran Kumara et al. (2024) found that silkworms supplemented with amla at 2.0% concentration recorded higher pupal weight of 1.563g over control.

Shell weight and Shell ratio: The larvae reared on mulberry leaves fortified with aloe vera juice at varied concentrations exhibited notable differences in respect of shell weight with highest being at 0.6% (0.504g) followed by 0.4% (0.471g) and 0.2% (0.441g) respectively. Whereas, it was least in absolute and distilled water controls (0.417 and 0.402g) respectively (Table 1).

The shell ratio and filament length are the most important economic parameters (Chanda et al., 2013) and supplementation aloe vera juice might have accelerated biosynthesis of sericin and fibroin in turn resulting in increased shell ratio and filament length. These results are more or less parallel with finding of Walaa et al. (2018), who have opined that larvae supplemented with ascorbic acid, lemon and sweet orange enhance shell percentage of 23.81, 22.07 and 21.49% over control (21.15%). This type of trend was observed for filament length at 4% concentration. Sisodia et al. (2019) have reported increased shell ratio and filament length with amla extract. Similar results were also observed with the supplementation of tender coconut (Amit et al., 2015) and with Aloe vera (Vitthalrao and Anil 2012). These results are in agreement with the earlier observations Rajashekaragouda (1991) who reported that increased shell weight in petroleum ether extract of P. corylifolia and T. terrestris and same was reported by Mamadupur (1994) with plant extract of C. inermae and L. camara. Further, significant higher shell ratio of 16.37% on potato leaf extract was also observed by Krishna Prasad et al. (2001). Kiran Kumara et al. (2024) also found that silkworms supplemented with amla at 2.0% concentration recorded higher shell weight and shell ratio of 0.469 and 23.08%, respectively over control.

Table 2: Effect of mulberry leaves fortified with aloe vera juice at different concentrations on silk parameters of FC2 silkworm hybrid.

Concentration (%)	Filament Length (m)	Filament Weight(g)	Denier	Renditta	Raw silk %
0.2	1197±18.52	0.37±0.07	2.80±0.10	5.38±0.29	18.56±0.63
0.4	1224±44.23	0.40±0.02	2.96±0.08	5.18±0.10	19.28±0.38
0.6	1288±84.25	0.43±0.08	3.04±0.78	4.89±0.30	20.45±0.72
Control(Dil. water)	1060±119.4	0.34±0.04	2.96±0.09	5.58±1.14	17.9±0.70
Absolute control	1034±62.42	0.33±0.02	2.90±0.12	5.69±0.24	17.57± 1.07

Filament length: It is one of the major contributing quantitative traits in silkworm. The silkworm reared on mulberry leaves supplemented with aloe vera recorded marked differences with regard to filament length with maximum being at 0.6% (1288 m) followed by 0.4% and 0.2% (1224 and 1197m). On the other hand, it was shortest in absolute (1034m) and distilled water controls (1060m) (Table 2).

The silkworms fed on mulberry leaves sprayed with aloe vera at 0.6% concentration exerted longer filament length of 1288m against control batches (1060m). The increase in filament length may be due to higher silk protein synthesis by the additional supplementation of aloe vera juice. These results are also supported by the administration of 2% aloe vera extract to silkworm increases filament length of 1003.0m over control batch (Vitthalrao and Anil 2012). Similar results were also noticed with *Clerodendron inermae* supplementation (Mamadapur, 1994). Further, Kiran Kumara *et al.* (2024) revealed that silkworms supplemented with amla at 0.2% concentration expressed higher filament length 1204m as against respective control.

Filament weight and Denier: The silkworm larvae supplemented with aloe vera at 0.6% exerted highest filament weight of (0.436g) followed by 0.4% (0.403 g) and 0.2% (0.373g) over absolute (0.349g) and distilled water (0.334g) controls. Denier denotes size of filament obtained from cocoons of silkworm breeds. Marginal variation was noticed in respect of denier among breeds provided with mulberry leaves fortified with aloe vera juice at different concentrations. In FC2 silkworm shows lowest denier at 0.2% (2.80) followed by 0.4% (2.96), absolute control (2.90). As opposed to this, highest denier was noticed with 0.6% (3.04) (Table 2). The larvae supplemented with aloe vera at 0.6% concentration recorded highest filament weight of 0.43g and on the other hand, lowest denier of 2.80 recorded at 0.2% concentration. However, not much more variations was registered for denier in distilled water control (2.96) and absolute control (2.90). The increase in filament weight and denier may be due to higher utilization of aloe vera juice for the synthesis silk proteins. The results corroborate the earlier finding of Pardeshi et al. (2014); Pardeshi et al. (2017) who have noticed that silkworms supplemented with Amaranthus

hybridus extract at 2% concentration enhance the filament weight (0.198g) and denier (2.004). Similar results were observed with Alpermanthera sessilis extract at 2per cent. According to Saritha Kumari et al. (2011), lowest denier of 2.31 and 2.36 were recorded by the silkworm supplemted with Phyllanthus niruri and Adathoda vasica extracts against control (2.41). Furthermore, Sridevi (2003), noticed increased filament 535 weight and denier with Withania somnifera, Terminalia arjuna and Terminalia cordifolia plant extracts. Similar result was also observed with supplementation of *Xanthium indicum* extract at 2.0% (Pardeshi et al., 2014). Kiran Kumara et al. (2024) also found that silkworm supplemented with amla at 0.2% concentration exerted higher filament weight and denier of 0.401g and 2.998 respectively, over control.

Renditta and Raw silk percentage: Renditta trait indicates actual silk available from the cocoon. Notable variation was noticed with respect to renditta among breed provided with mulberry leaves fortified with alovera juice at varied concentration. In the FC2 silkworm lowest renditta was recorded at 0.6% (4.489) followed by 0.4% (5.186) and 0.2% (5.389). In contrast highest renditta was seen in absolute and distilled water control batches. The raw silk percentage indicates that the amount of silk obtained or produced from the cocoon. The mulberry leaves fortified with the aloe vera juice shows raise in the raw silk percentage. The 0.2% concentration shows the lowest raw silk percentage of 18.56% followed by 0.4% and 0.6% concentrations (19.28 and 20.45%), respectively. While, it was lowest in absolute and distilled water control (17.57 and 17.9%) (Table 2).

The silkworms supplemented with aloe vera at 0.6% concentration expressed lowest renditta and higher raw silk percentage (4.89 and 20.45%) respectively as against control batch (5.58 and 17.9%). It is presumed that the silkworm larvae absorbed and utilized the nutrients of aloe vera juice might have enhance these parameters. These results are in conformity with those of Sujatha et al. (2015) who reported that mulberry leaves fortified with phytochemicals resepine and picrotoxin enhance raw silk percentage. Similarly, lowest renditta was recorded in drumstick leaf extract over other leaf extracts might be due to utilization of additional nutrients for the cocoon formation (Anil Kumar et al., 2017). These results are in agreement with the earlier observation of Anil Kumar and Prashanth, (2018) who have opined that silkworm larvae CSR2 supplemented with 4% soybean flour registered higher raw silk percentage of 19.22% and lower renditta of 6.850 as against control 15.93% and 7.550, respectively. Similarly, Kiran Kumara et al. (2024) reported that silkworms supplemented with amla at 0.2% concentration registered lowest renditta (5.067) and highest raw silk percentage (19.73%) over respective control.

CONCLUSIONS

The silkworm larvae nourished by mulberry larvae fortified with aloe vera reveled promising results. The larvae fed mulberry leaves supplemented with 0.6% **Biological Forum**

aloe vera, enhancing all the commercial parameters. Hence, farmers can use aloe vera (0.6%) for commercial rearing. Mulberry leaves are a traditional food for silkworms, but adding aloe vera to their diet seems to have improved their growth and other commercial parameters. Aloe vera is rich in vitamin A, C, E and other nutrients, which could be contributing to the positive effects observed in the silkworms. By supplementing mulberry leaves with 0.6% aloe vera, farmers can potentially enhance the quality and yield of silk produced by silkworms. This could be particularly beneficial for commercial silk production, as it may lead to higher yields or better-quality silk, ultimately increasing profitability for farmers. Overall, this finding holds promise for silk farmers looking to improve their yields and product quality of mulberry.

FUTURE SCOPE

Systematic field trials (scaling up the best performing supplements e.g. 0.6 % aloe vera), implementation studies (evaluating cost, ease of use and farmer acceptance under real-world conditions), genotypespecific trials (assessing whether particular silkworm strains respond better to certain supplements) and interdisciplinary collaborations integrating entomology, molecular biology, biotech and sustainable agriculture.

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