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Application of Amla Juice as Supplementary Nutrient on Biomolecules and Commercial 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. 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 amla juice at varied concentrations viz., 0.2, 0.4, and 0.6% on protein and carbohydrate contents as well as commercial parameters in the FC_1 silkworm hybrid. The larvae reared on amla at 0.2% concentration registered higher protein and carbohydrate contents in the fat body and midgut tissue over absolute and distilled water control. However, carbohydrate and protein contents were relatively higher in the fat body when compared to midgut tissue in all the treatments. A similar trend was also observed at 0.2% concentration for commercial parameters such as larval weight, cocoon weight, shell weight, pupal weight, shell ratio, filament length, filament weight, denier, renditta and raw silk percentage.

Key words: Bivoltine hybrids, Amla juice, Supplementation, Biomolecules, Commercial parameters

The silkworm, *Bombyx mori* is a monophagous lepidopteran insect and feed exclusively on the mulberry leaf (*Morus* sps) as a source of food. Man has immensely benefited from the silk produced by the silkworm, and major quality of silk is utilized in textile industry [1]. Moreover, *B. mori* complete its life cycle by the assistance of manmade rearing practices [2]. Needless to say, silkworm has to derive its nutrients from mulberry leaves for metamorphosis and successful completion of life cycle. The nutrients in the mulberry leaf are chiefly governed by agronomical and environmental factors. It is well known fact that nutrients solidify sharply among mulberry varieties [3-4]. Nearly about 60% of silk protein are synthesized by dietary protein and amino acids [5]. Thus, dietary nutrients regulate growth, development and reproduction of silkworm in turn reflects on cocoon quality, and yield [6]. Nevertheless, the quality and quantity of leaf fed to the silkworm larvae has greater importance for accumulating biomass that should culminate in the improvement of raw silk production. Whenever the leaf quality is inferior, there arises a need of fortifying the leaf with suitable supplementary nutrients.

Fortification of mulberry leaves with different nutrients is a recent technique which enhance economic parameters of silkworm [7]. Quite a good number of reports are available on synthetic fortifying agents such as tryptophan [8], folic acid [9], zinc salts [10], juvenile hormone [11], antibiotics [12], amway protein [13], pectin [14] and steric and linoleic acid [15] enhances commercial parameters of silkworm *viz.*, larval weight, cocoon weight, shell weight, shell percentage, filament length, renditta, diner and fecundity. In recent year many attempts have been made by researches to enhance commercial parameters of silkworm by supplementing botanical extraction with (or) without combination at varied concentrations. For instance, increase in larval weight is due to supplementation solanaceous leaf extracts [16], soya bean flour [17-18], wheat bran [19], *Phyllanthus niruri* [20] and soya bean and mushroom [21].

Similarly, increase in cocoon and post cocoon parameters such as cocoon weight, shell weight, shell percentage, filament length and renditta were reported by supplementing *Amaranthus hybridus* [22] *Xanthium indicum* [23] and *Lantana camara* [24]. It is well documented that carbohydrates, proteins and lipids are crucial biomolecules of the diet which regulate growth, development, reproduction and protein synthesis in the silkworm. The quantity of glycogen and trehalose (blood sugar) are existing in fat body and haemolymph of silkworm is determined by carbohydrate contents in the diet [25]. It is the dietary protein regulate nitrogen metabolism and synthesis of sericin and fibroin silk proteins [26]. Likewise, levels of fatty acids in the silkworm are determined by the quantity of fatty acids and carbohydrates contents in the diets [27].

The mulberry leaf supplemented with both synthetic and natural fortifying agents play a pivotal role on metabolism of

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silkworm. For instance, silkworm reared on mulberry leaf fortified with *Dolichos lablab*, *Vigna unguiculata* and soya bean flour enhances alanine and aspartate aminotranferase enzyme activities [28-30]. Similarly, supplementation of honey increases protein content in the silk gland [31]. Furthermore, supplementation of *Tribulus terrestris* and *Phyllanthus niruri* extracts increased with mulberry excerted protein content in the silk gland [32]. However, *Phyllanthus emblica* (amla) is a member phyllanthaceae family and in rich vitamin c, iron, calcium, gallic acid and phenolic compounds and utilized as fortifying agent to enhance larval and cocoon characters [33] and few reports are available on biomolecules. A good number reports are available on exogenous supplementation of various botanical extracts which enhances commercial parameters of the silkworm. However, information available on supplementation of amla juice on bimolecules and commercial parameters is meager. Keep this in view, current investigation is initiated.

MATERIALS AND METHODS

The bivoltine hybrid FC_1 was selected after brushing of the hatched eggs, young and late-age silkworm rearing was carried out as per the methods of [34]. The larvae were fed with the V1 mulberry variety during rearing.

Supplementation of amla juice

The commercially available amla juice tonic a product of Dabur, procured from charaka ayurvedic medicines and general stores, Brindavan Extension, Mysuru-20. The amla tonic stock solution was diluted at the rate of 0.2, 0.4 and 0.6 per cent by using distilled water. The prepared solutions were preserved in refrigerator at 4^oC until further use. For the experimentation, the larvae is divided into five batches viz., batch I (T_1), batch II (T_2) and batch III (T_3) were reared with mulberry leaf fortified with amla juice at 0.2, 0.4 and 0.6%, respectively. Whereas batch IV (T_4) larvae were reared on mulberry leaf sprayed with distilled water (control) and batch V (T_5) larvae were reared on mulberry leaf alone (absolute control). For each treatment, three replications were maintained and each with 100 larvae along with controls. A minimum of twenty larvae were used from each treatment to record protein and carbohydrate contents as well as larval weight, cocoon weight, shell weight, pupal weight and filament weight. The parameters such as shell ratio, filament length, raw silk percentage, denier and renditta were calculated by using following formulae:

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

Filament Length (L) = R×1.125

R = Number of revolutions recorded by an epprouvette.

1.125 = Circumference of epprouvette in meter.

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

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

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

Preparation of samples

The midgut and fat body tissues were collected by dissecting 5^{th} instar 1^{st} day to 6^{th} day old larvae in respective treatments and control batches. The tissue homogenate (1%) was prepared by using distilled water. The contents were centrifuged at 3000 rpm for 10 minutes, and the supernatant was used for the estimation of protein and carbohydrate contents in the midgut and fat body tissue.

Estimation of protein

The protein content in the fat body and midgut tissue was estimated by the method of [35]. *One ml of* tissue supernatant was taken with 5 ml of protein reagent, then the reaction mixture was incubated at room temperature for 10 minutes and 0.5 ml of Folin's reagent was added. The contents were mixed thoroughly and kept for 30 minutes until the blue color developed. One ml of bovine serum albumin (BSA) was used as a standard. A blank was prepared using 1 ml of distilled water. The optical density (OD) was measured using a spectrophotometer at 660nm against a blank. The results were expressed in mg/g of tissue.

$$\text{Amount of protein (mg/g)} = \frac{\text{OD of the sample} \times \text{mg of BSA in standard}}{\text{OD of the standard} \times \text{mg of tissue taken}} \times 1000$$

Estimation of carbohydrate

Total carbohydrate was determined by the Anthrone method [36] using glucose as a standard. To the 1 ml sample, 4 ml of anthrone reagent was added and the content was boiled for 8 minutes in a water bath, then cooled with running water. The absorbency was measured using a spectrophotometer at 630 nm against a blank and the carbohydrate content was expressed in mg/g of tissue. The obtained data were statistically analyzed by adopting the standard deviation method and mean values were expressed.

$$\text{Amount of carbohydrate (mg/g)} = \frac{\text{OD of the sample x mg of glucose in standard}}{\text{OD of the standard} \times \text{mg of tissue taken}} \times 1000$$

RESULTS AND DISCUSSION

Influence of mulberry leaf supplemented with amla juice on protein content

The protein plays pivotal role in growth, development and metamorphosis and silk production. It is the dietary proteins and amino acids regulate the synthesis of storage (heamolymph) proteins [26] and silk protein in the silk gland [37]. The fat body is the main site of intermediary metabolism in which many biomolecules are synthesized including protein [38]. It is well documented that protein content progressively increased during embryogenesis and reached maximum in 5^{th} instar 6^{th} day old larva [39-40]. It is evident from the present study that silkworm larvae nourished with amla juice registered highest protein content in the fat body at 0.2% concentration (118.15mg of protein/g of tissue) when compared to midgut (75.28mg) during 5^{th} instar 6^{th} day over respective control batches (106.32 and 70.83mg) (Fig 1-2). Increase in protein content in the fat body and midgut tissue might be due to utilization of nutrients that are existed in the amla juice. Obviously, these nutrients might have enhanced both soluble and structural protein synthesis at cellular level besides to sericin and fibroin proteins. The results of present investigation are in concurrence with the finding of [32] wherein NB_4D_2 silkworm larvae reared on mulberry leaves extrafoliated with *Tribulus terresteris*, *Boerhavia diffusa* and *Phyllanthus niruri* at 100ppm concentration recorded increase in protein content in the silk gland (136, 130 and 125mg), respectively against control (98mg). Similarly, [41] have observed when silkworm larvae supplemented with honey, lime and combination of honey and lime registered highest total proteins content in the midgut (20.67, 17.76 and 26.00 mg), respectively over control and trend was similar for fat body protein with honey and lime mixture supplementation [41]. Furthermore, supplementation of *Curcuma longa* at different concentration *viz.*, 1, 10 and 100% enhance protein content in the haemolymph, silk gland, muscle and midgut tissues as against control [42] and with honey (2%) exerted elevated levels of protein content in the silk gland [43].

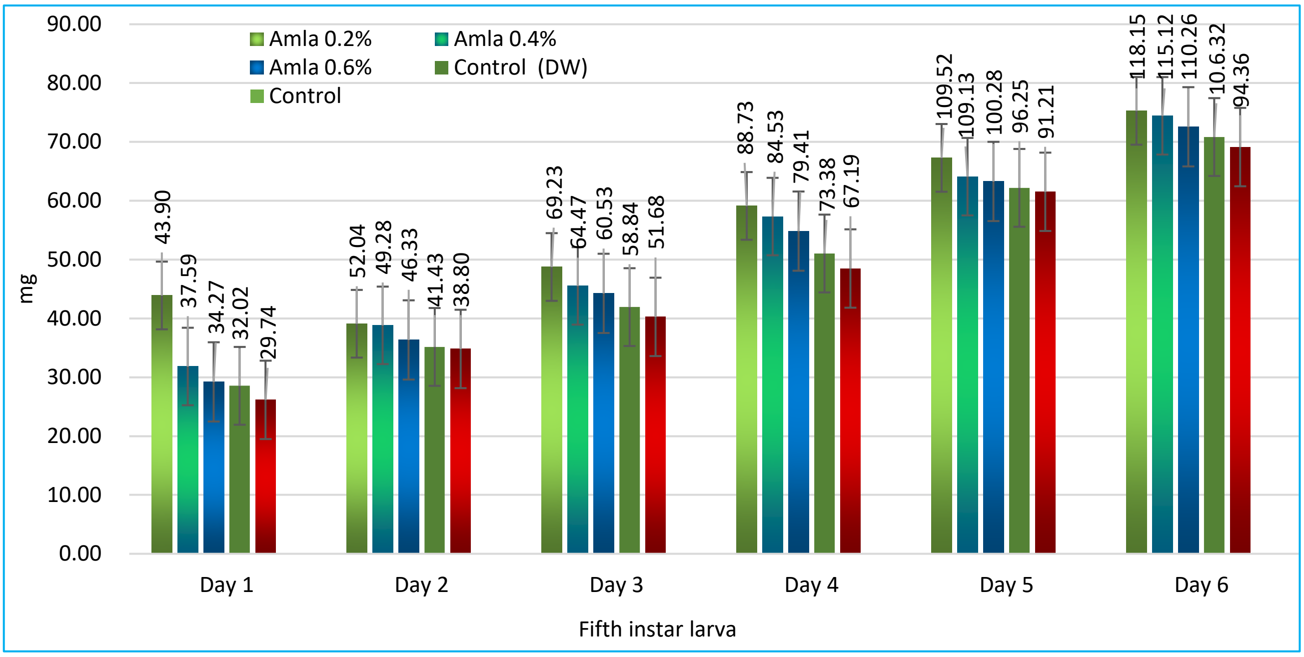

Fig 1 Effect of fortified mulberry leaf with amla on protein content in the fat body of FC_1 silkworm

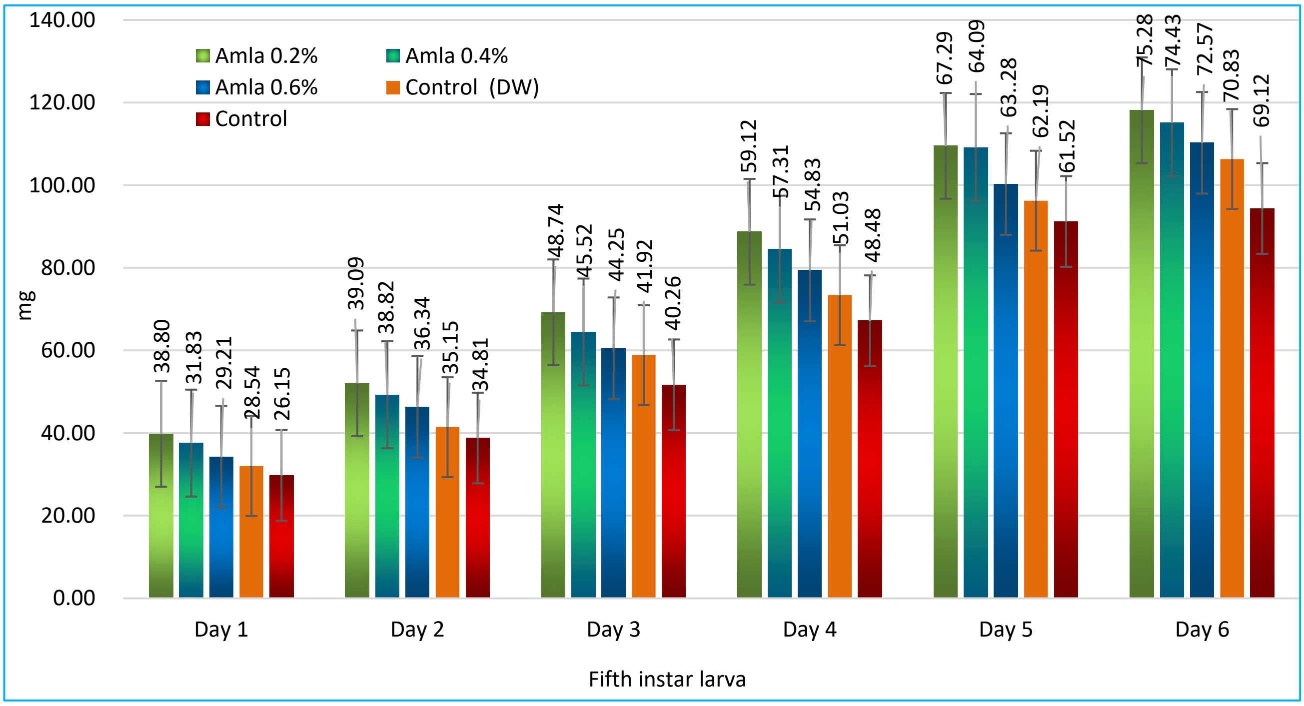

Fig 2 Effect of fortified mulberry leaf with amla on protein content in midgut of FC1 silkworm

Influence of mulberry leaf supplemented with amla juice on carbohydrate content

The carbohydrates are chief energy source for metabolic process and they stored in the form of glucose and glycogen in the haemolymph and fat body, respectively. Interestingly, carbohydrate content decrease during embryogenesis [39] and increase during larval stage indicating energy resource for the cocoon spinning and synthesis of pupal and moth cuticle [44-45]. During the present study, the mulberry fortified with amla juice on total carbohydrate content in silkworm revealed varied results. The FC_1 larvae fed on mulberry leaves enriched with amla juice at 2% recorded maximum carbohydrate content in the fat body (34.61mg) and midgut (29.84mg) during 5th instar 6th day larvae as compared to their respective controls (28.49 and 25.85mg) (Fig 3-4). In contrast, it was lowest in the midgut during 5th instar 1st day larvae. Increased carbohydrate content in the midgut and fat body tissue might be due to utilization amla nutrients. The present findings are in harmony with [46] where in $FC_1 \times FC_2$ silkworm larvae reared on the mulberry leaves enriched with honey, lime and their combination exerted highest total carbohydrate content in the midgut (14.00, 13.25 and 14.66mg), respectively as compared to control batch (12.52mg). Similar results were also observed in fat body [41]. According to [33], the larvae supplemented with spirulina (BGA) at 1per cent concentration recorded maximum carbohydrate content when compared to control batch. Similarly, worms nourished on mulberry leaves extra foliated spirulina at 3% concentration scored highest carbohydrate content in the silk gland (21.34mg/g) and fat body (16.34mg/g) and at 4% in heamolymph (17.075mg/ml) and muscle (10.82mg/g) against their respective controls (15.92, 12.74, 12.07 and 8.18mg/g) [47]. Furthermore, the larva supplemented with honey, lime and their mixture registered maximum carbohydrate content of 22.80, 22.28, 24.53 mg/g in the silk gland of V instar 7th day larva, respectively against control 22.05mg/g [48].

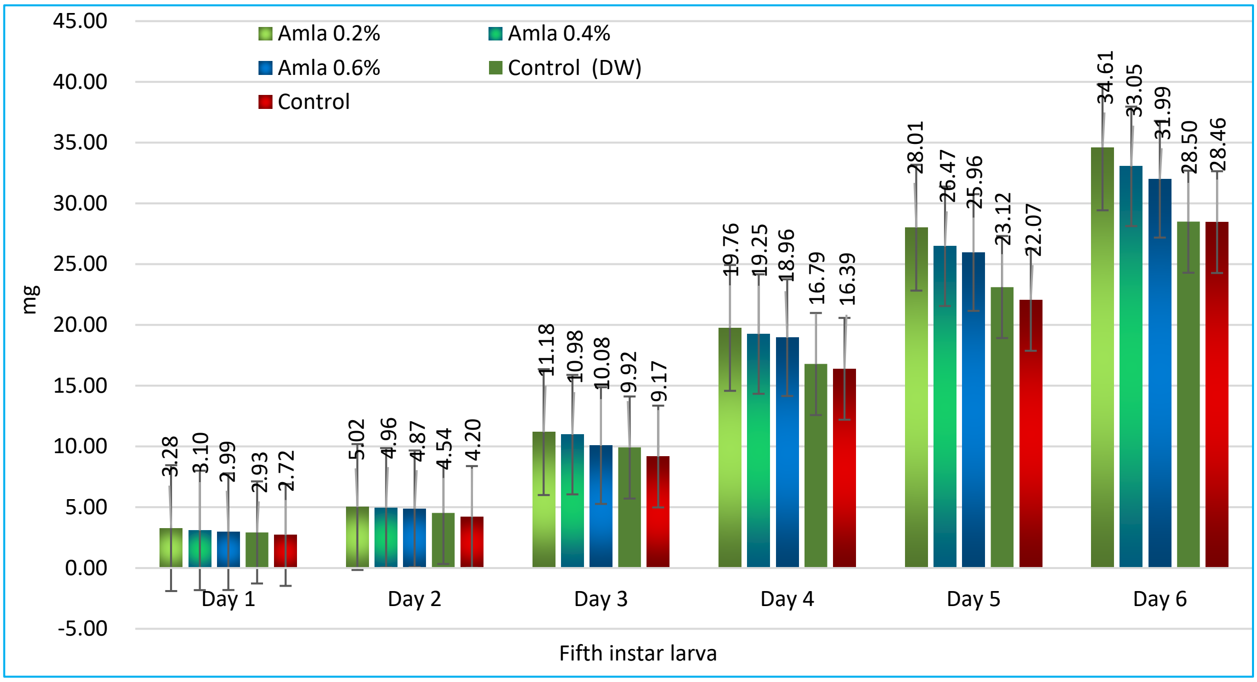

Fig 3 Effect of fortified mulberry leaf with amla on carbohydrate content in fat body of FC1 silkworm

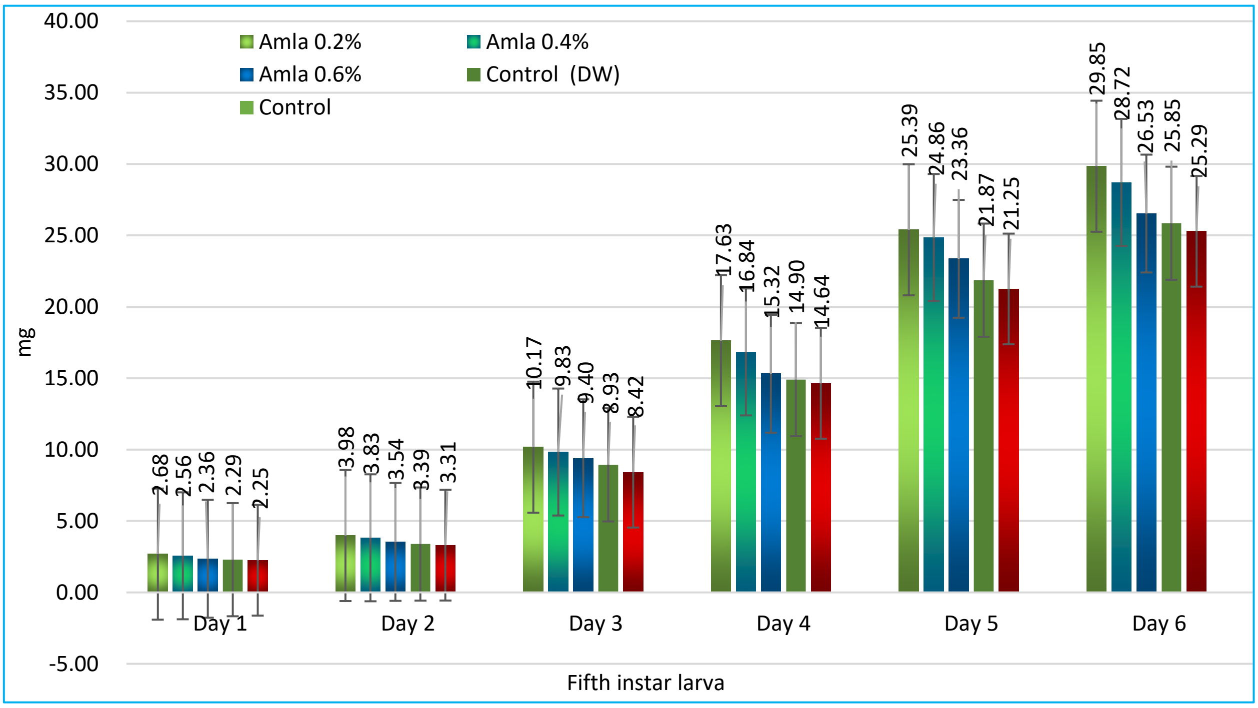

Fig 4 Effect of fortified mulberry leaf with amla on carbohydrate content in midgut of FC1 silkworm

Influence of mulberry leaf supplemented with amla juice on commercial parameters.

Larval weight and cocoon weight

The larva and cocoon weight are strongly influenced by quantity and quality of diet provided to silkworm. The 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 (4.366 and 1.914g) (Table 1). The increase in larval and cocoon weight might be due to additional and supplement of nutrients that are found in amla juice. It is evident that amla juice is a richest source of ascorbic acid and other nutrients with good antioxidant property and supplementation of amla 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 cocoon weight [49]. These results are on line with earlier observations of [50] who have reported that silkworm larvae supplemented with *Achyranthes aspera* (40.41g), *Achyranthes recimosus* (40.00g), *Parthenium hysterophorus* (39.20g), *Tribulus terrestris* (40.23g) and *Withania somnifera* (40.21g) at 5% registered highest larval weight over remaining concentrations (3 and 8%) as well as control batchs (38.61g). [51] 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, [30] have noticed that, larvae supplemented with soya bean flour at 2, 4 and 6% exerted higher larval and cocoon weight over control. As per [28-29], 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.

Table 1 Influence of mulberry leaves supplemented with amla juice at different concentrations on commercial parameters of FC1 silkworm

Concentration (%)	Larval weight (g)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Pupal weight (g)	Filament length (m)	Filament weight (g)	Raw silk (%)	Denier	Renditta
0.2	4.665 ± 0.015	2.032 ± 0.016	0.469 ±0.192	23.08 ± 0.436	1.563 ± 0.213	1204 ±106.532	0.401 ± 0.196	19.73 ±0.699	2.998 ± 0.193	5.067 ±0.058
0.4	4.492 ±0.009	2.004 ±0.006	0.455 ±0.218	22.70 ±0.516	1.549 ±0.215	1174 ±220.837	0.387 ±0.182	19.31 ±0.417	2.967 ±0.082	5.178 ±0.083
0.6	4.453 ±0.199	1.964 ±0.080	0.438 ±0.134	22.30 ± 0.756	1.526 ±0.082	1125 ±222.178	0.370 ±0.101	18.84 ±0.160	2.960 ±0.081	5.308 ±0.123
Control (Distilled water)	4.366 ±0.004	1.914 ±0.078	0.409 ±0.126	21.37 ±0.290	1.505 ±0.204	1066 ±62.865	0.341 ±0.074	17.82 ±0.524	2.879 ±0.406	5.613 ±0.098
Absolute control	4.287 ±0.002	1.861 ±0.070	0.391 ±0.121	21.01 ±0.940	1.470 ±0.249	1029 ±80.037	0.323 ±0.093	17.36 ±0.346	2.825 ±0.258	5.762 ±0.245

Shell weight and pupal weight

Silkworms nourished with mulberry leaves enriched with amla juice at varied concentrations registered variation with respect to shell and pupal weight. The worms supplemented with amla at 2.0% concentration recorded higher shell and pupal weight of 0.469 and 1.563g, respectively. However, control batch scored (0.409 and 1.505g) for this parameter (Table 1). The significant increase in shell and pupal weight might be due to utilization of amla nutrients for the synthesis of silk protein and also enhances body mass. The present findings are in harmony with observation of [52] who have opined that oral administration of *Ocimum sanctum* (steroids, alkaloids and flevonoids) extract at 3% concentration to 5th instar silkworm larva had the positive impact on shell and pupal weight (1.316 and 0.258g) against respective controls (1.287 and 0.195g). According [53] silkworm larvae CSR_2 and PM X CSR_2 fed on mulberry leaves extra foliated with *Asparagus officinalis* at 6% enhances shell weight (3.743 and 2.480g/10 cocoon shells) and pupal weight (14.32 and 11.15g/10 pupae), respectively. Similar results were also noticed with supplementing drumstick extract at 4% [54], *Alternanthera sessilis* at 2% [55] and *Tinospora cortifolia* at 8% concentrations [56].

Shell ratio and filament length

The FC_1 larvae fed on mulberry leaves fortified with amla juice at different concentrations exhibited marked variation in respect of shell ratio and filament length. The worms supplemented with amla at 0.2% concentration expressed higher shell percentage of 23.08% and filament length 1204m as against respective controls (21.37% and 1066m) (Table 1). The shell ratio and filament length are the most important economic parameters [57] and supplementation amla 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 [58] 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. [59] have reported increased shell ratio and filament length with amla extract. Similar results were also observed with the supplementation of tender coconut [60] and with Aloe vera [61]. According to [62] larvae reared on soya bean flour registered more shell ratio and filament length. Next best was defatted soya bean, wheat, ragi, rice and mushroom.

Filament weight and denier

Silkworm nourished with mulberry leaves enriched with amla at different concentrations exhibited notable influence on filament weight and denier. The larvae supplemented with amla at 0.2% concentration exerted higher filament weight of 0.401g over control (0.341g) (Table 1). However, control batch recorded lowest denier of 2.82 against larvae treated at 0.2% (2.99). The denier important quality trait which donates thickness of the silk filament. The increase in filament weight and denier may be due to higher utilization of amla juice for the synthesis silk proteins. The results corroborate the earlier finding of [22], [63] who have noticed that worms 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 [64], 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, [65] have noticed increased filament 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% [23].

Renditta and raw silk percentage

The renditta and raw silk percentage are imported parameters which depicts silk available from the cocoon and silk obtained from reeled cocoons, respectively. Marked variation was observed with respect to renditta and raw silk percentage when the larvae reared on extra foliated mulberry leaf with amla juice. The worms supplemented with amla at 0.2% concentration registered lowest renditta (5.067) and highest raw silk percentage (19.73%) over respective control (5.613 and 17.82%). It is presumed that the silkworm larvae absorbed and utilized the nutrients of amla juice might have enhance these parameters. These results are in agreement with the earlier observation of [30] who have opined that silkworm larvae CSR_2 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. Likewise, worms supplemented with drumstick (4%) and coriander (2%) extracts expressed lower renditta (6.76 and 7.34) and higher raw silk percentage (19.36 and 19.12%), respectively [54]. Similar trend was observed with supplementation *Ocimum sanctum* at 2% and phytochemicals such as resepine and picrotoxin [52], [66] respectively.

CONCLUSION

The silkworm larvae nourished by mulberry larvae fortified with amla juice reveled promising results. The larvae fed mulberry leaves supplemented with 2% amla juice, enhancing all the commercial parameters. Hence, farmers can use amla juice (2%) for commercial rearing. Mulberry leaves are a traditional food for silkworms, but adding amla juice to their diet seems to have improved their growth and other commercial parameters. Amla, also known as Indian gooseberry, is rich in vitamin C and other nutrients, which could be contributing to the positive effects observed in the silkworms. By supplementing mulberry leaves with 2% amla juice, 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.

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