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Chavan KK
M.Sc. Agriculture, Department of Entomology, RCSM College of Agriculture Kolhapur, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Galande SM
Associate Professor, Department of Entomology, RCSM College of Agriculture, Kolhapur, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Kharbade SB
Head of Division, Department of Entomology, RCSM College of Agriculture, Kolhapur, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Hole UB
Professor, Department of Entomology, College of Agriculture, Nandurbar, Maharashtra, India

Badge AS
Assistant Professor of Entomology, RCSM College of Agriculture, Kolhapur, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Kuruva Veeranjaneyulu
M.Sc. Agriculture, Department of Entomology, RCSM College of Agriculture Kolhapur, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Corresponding Author:
Chavan KK
M.Sc. Agriculture, Department of Entomology, RCSM College of Agriculture Kolhapur, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Effect of mulberry leaves enrichment with nutritional supplements on growth parameters of FC_1 x FC_2 Silkworm, *Bombyx mori* L

Chavan KK, Galande SM, Kharbade SB, Hole UB, Badge AS and Kuruva Veeranjaneyulu

Abstract
The nutritive value of mulberry leaves has a direct influence on various growth parameters of the silkworm. The mulberry leaves enriched with additional nutritional supplements have often proved to be beneficial for larval and pupal parameters. The present investigation was undertaken to know the effect of mulberry leaves enrichment with combinations of various nutritional supplements *viz.*, vitamin C, vitamin B-complex, soya flour, methionine and L-serine on growth characters in FC_1X FC_2 silkworm hybrid. The result of the study revealed that marked improvement in the growth parameters *viz.* larval length (cm), larval breadth (mm), larval weight (g), larval duration (days), pupal length (cm), pupal breadth (mm), pupal weight (g) and pupal duration (days) when the FC_1 X FC_2 silkworm larvae reared on vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5% over control batches.

Keywords: Silkworm, *Bombyx mori* L., nutritional supplements, growth parameters

1. Introduction
Sericulture is the practice of rearing silkworm for the production of silk. Silk is the most elegant textile in the world with unparalleled grandeur, natural sheen, inherent affinity for dyes, light weight, high absorbance, soft touch and high in longevity. Due to these unique features silk is known as the "Queen of Textiles" the world over (CSB; 2022). Silkworm, *Bombyx mori* L. is monophagus and feeds entirely on mulberry leaves only due to the presence of a chemical morin (Masthan *et al*., 2017) [15].
The life cycle of *Bombyx mori* L. includes four stages *viz.*, embryo, larva, pupa and adult moth and considered one of the most advanced forms of metamorphosis. The silk worm is the larval stage of the silk moth's life cycle. Moths lay eggs which develop into larvae (Caterpillars) commonly called Silkworms. They eat for 20-30 days, consuming large amounts of mulberry leaves and undergo moulting through five changes of skin or instars. (Soumya *et al*., 2017) [26]. Two kinds of silk proteins, first being real silk fiber namely fibroin and second being adhesive, sticky substance i.e. sericin noted as major component of silk cocoon (Selin, *et al*., 2018) [25].
Nutrition plays an important role in improving the growth and development of silkworm *Bombyx mori* L. Silk production is largely dependent on larval growth and nutritive value of mulberry leaves (Masthan *et al*., 2017) [15]. As the quality of mulberry leaf has an intimate relation to the healthy growth of the larvae and quality of their cocoon, the chemical composition of the leaves has a great scope in determining the food value (Anil Kumar and Sunil Kumar 2018) [2]. Fortification of mulberry leaves by using extra nutrients and feeding to the silkworms is a useful modern technique to increase the economic value of cocoon. (Rahmathulla *et al*., 2007) [22]. Several dietary compounds such as sugars proteins, amino acids, minerals, vitamins, salts, etc. whose supplementation can considerably enhance the growth and development of silkworm and hence improve the quality of cocoons (Anil Kumar and Jayaraju 2021) [3].
Vitamins are a group of unrelated organic compounds needed only in little quantities in the diet that are essential for specific metabolic reactions within the cell and necessary for normal

growth and maintenance of health. It prevents associated deficiency diseases. Dosages of vitamins are very definitive for normal growth of silkworm (Ahsan *et al*., 2013) [1]. In silkworms, silk fibroin is mainly obtained from four amino acids *viz*., alanine, serine, glycine and tyrosine. Silkworm derive 72-86% of their amino acids from mulberry leaves and more than 60% of the absorbed amino acids are used for silk production (Radjabi, 2010) [20]. It is known that the rich sources of food proteins improve the economic features and promote the growth of silkworm such as soy protein (Rahman, 2018) [21].

2. Materials and Methods

Present experimental analysis was conducted in laboratory Silkworm Seed Production Center, Gadhinglaj, Kolhapur, Maharashtra, to study the effect of mulberry leaves enrichment with nutritional supplements on economic parameters of silkworm *Bombyx mori* L. In the present experiment nine treatments and three replications were used. The experiment was carried out by using Completely Randomized Design. The silkworm race FC_1XFC_2 Bivoltine Double Hybrid, was obtained from the Silkworm Seed Production Center, Gadhinglaj, Kolhapur district, Maharashtra State. Two per cent formalin solution used as disinfectant, 0.3 per cent bleaching powder, Vijetha powder and lime powder was also used. The nutritional supplements used were vitamin C, vitamin B-complex, soya flour, L-serine and methionine. The fresh mulberry leaves of variety V_1 obtained from previous year established mulberry garden at Silkworm Seed Production Centre, Gadhinglaj, Kolhapur district (Maharashtra State). Rearing method of silkworm breeds: The improved method of silkworm rearing used in this experiment. The disease free layings (Dfl's) of mulberry silkworm races were procured from Silkworm Seed Production Center, Gadhinglaj, Kolhapur, Maharashtra. The Dfl's were incubated at 25 ºC and 75 per cent relative humidity. In the rearing process, egg sheets were spread out in trays with single layer. Trays were covered by using paraffin paper to maintain the necessary humidity for incubation. When obtained the blue egg stage, the egg sheets were put inside black box and covered with black piece of fabric and left undisturbed for 48 hours to promote homogeneous embryo growth referred as "Black boxing". The eggs were exposed to bright day light for few minutes in the morning hour to ensure evenly hatching. Newly hatched silkworm larvae were brushed with the help of sterilized feather of bird and immediately fed with chopped pieces (0.5-1 sq. cm) of mulberry leaves of variety V_1. After passing each moult bed was disinfected with vijetha powder for control of infection caused by fungi, bacteria, protozoa and viruses. After reaching their full growth, the matured silkworms were released on chandrika. Silkworm spin the cocoon in between 48-72 hr. Fifth day after the discharge of the silkworm for mounting on chandrika, the cocoons were harvested. (Krishnaswami, 1979) [12].

Treatment Details

The details of treatment used during the present investigation are given in Table 3.1 Fresh mulberry leaves were soaked in each concentration for 15 min and then were dried in air for 20 min. The supplementary leaves were fed to the mulberry silkworm larvae till pupation. The procedure outlined by Etebari *et al*. (2007) [8] was used for preparation of test solution.

Table 1: Treatment details

Tr. No	Treatment details
T_1	Vitamin C 0.25% + soya flour 0.25%
T_2	Vitamin B-complex 0.25%+ soya flour 0.25%
T_3	Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5%
T_4	Vitamin B-complex 0.25%+ soya flour 0.25% + vitamin C 0.5%
T_5	Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%
T_6	Vitamin B-complex 0.25%+ soya flour 0.25% + vitamin C 0.5% + methionine 0.5%
T_7	Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%+ L-serine 0.5%
T_8	Vitamin B-complex 0.25%+ soya flour 0.25% + vitamin C 0.5% + methionine 0.5%+ L-serine 0.5%
T_9	Untreated control

2.1 Method of recording observation

Economic parameters such as single cocoon weight, single shell weight, cocoon shell ratio, ERR by number, ERR by weight, filament length, filament weight and denier were recorded (Formulae given).

Larval length (cm)

At the end of each instar, the length of silkworm larvae were measured in cm using a rural scale.

Larval breadth (mm)

At the end of each instar, the breadth of silkworm larvae were recorded in mm by using vernier caliper.

Larval weight (g): The weight of 10 silkworm larvae at the end of each instar were measured by using following formula and which was expressed in grams.

$$\text{Weight of single larva (g)} = \frac{\text{Weight of 10 larvae (g)}}{\text{Total no. of larvae taken}}$$

Larval duration (days)

The entire larval period was measured from the first day that newborn larvae were alive until they began to spin their cocoons.

Pupal length (cm)

Length of silkworm pupa was measured in cm by using a ruler scale.

Pupal breadth (mm)

Breadth of silkworm pupa was recorded by using a vernier caliper.

Pupal weight (g): The maximum weight of 10 pupae were measured and expressed in grams. The weight of single pupa which was measured by using following formula.

$$\text{Weight of single pupa (g)} = \frac{\text{Weight of 10 pupae (g)}}{\text{Total no. of pupae taken}}$$

Pupal duration (Days)
The entire pupal period was calculated by noting the time from spinning to the day of moth emergence.

Statistical analysis
The experimental data collected on various growth traits were statistically analyzed by standard 'analysis of variance' as per method and the critical difference (CD) was calculated wherever the 'F' test was found significant. The data are presented with the level of significance at 5 per cent (Gomez and Gomez, 1984) [27].

3. Results and Discussions

3.1 Larval length (cm)

The mean larval length observations for different dietary supplements are presented in Table 2.

The data indicated that the larval length of silkworm ranged from 0.27-8.27 cm. The impact of treatment T_5 comprising of vitamin C 0.25% + soya flour 0.25%+ vitamin B-complex 0.5% + methionine 0.5% on the mean larval length of first to fifth instar silkworm larvae was significantly superior compared to other treatments. The recorded lengths for the first to fifth instar larvae were 0.45, 2.07, 2.69, 6.17, and 8.27 cm, respectively, and these values were notably better than those of other treatments for each corresponding instar.

In the first instar, the highest larval length (0.45 cm) was observed in T_5 the treatment of mulberry leaves enriched with vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%. This was followed by two treatments: T_3 *viz.* vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% with a length of 0.44 cm, and T_1 *viz.* vitamin C 0.25% + soya flour 0.25% with a length of 0.43 cm, which were at with each other. As against, the lowest larval length (0.27 cm) was recorded in the control, which was fed with untreated mulberry leaves.

During the second instar, the treatment involving mulberry leaves enriched with 0.25% vitamin C, 0.25% soya flour, 0.5% vitamin B-complex, and 0.5% methionine (T_5) exhibited the highest larval length at 2.07 cm. Following closely were two other treatments: T_3, which involved feeding with 0.25% vitamin C, 0.25% soya flour, and 0.5% vitamin B-complex, resulting in a length of 2.02 cm, and T_1, fed with 0.25% vitamin C and 0.25% soya flour, yielding a length of 2.00 cm. Both T_3 and T_1 were at par with each other. In contrast, the control group, which was fed untreated mulberry leaves, recorded the lowest larval length at 0.68 cm.

In case of third instar, the highest larval length (2.69 cm) was observed in T_5 the treatment of mulberry leaves enriched with vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%, which was significantly superior rest of the treatments except: T_3 which involved vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% with a length of 2.64 cm, and another T_1 *viz.* vitamin C 0.25% + soya flour 0.25% with a length of 2.61 cm, which was at par with each other. However the lowest larval length (1.86 cm) was recorded in the control, fed with untreated mulberry leaves.

During fourth instar of silkworm larvae, the highest larval length (6.17 cm) was observed in T_5 the treatment of mulberry leaves enriched with vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%. This was followed by two groups: T_3 which involved vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% with a length of 6.05 cm, and T_1 *viz.* vitamin C 0.25% + soya flour 0.25% with a length of 2.00 cm, which were at par with each other. In contrast, the lowest larval length (6.02 cm) was recorded in the control group, which was fed untreated mulberry leaves.

In the fifth instar of silkworm larvae, the treatment (T_5) involving mulberry leaves enriched with 0.25% vitamin C, 0.25% soya flour, 0.5% vitamin B-complex, and 0.5% methionine exhibited the highest larval length at 8.27 cm. Following closely were two treatments: T_3 *viz.* 0.25% vitamin C, 0.25% soya flour, and 0.5% vitamin B-complex, resulting in a length of 8.17 cm, and T_1 involving 0.25% vitamin C and 0.25% soya flour, yielding a length of 8.16 cm, which were at par with each other. In contrast, the control, which was fed untreated mulberry leaves, recorded the lowest larval length at 0.68 cm.

These results show similarity with findings of Yadav and Bagdi (2015) who observed that caster leaves supplemented with a concentration of 300 ppm of Spirulina thrice per day resulted in a significant increase in the larval length of eri silkworm, reaching 6.70 cm. This notable effect could be attributed to the growth-promoting properties of water-soluble proteins and vitamins such as B2, B6, and C present in spirulina. According to Balasundram *et al*. (2013) [5], feeding vitamin C-treated MR_2 mulberry leaves to *B. mori* larvae in their last instar resulted increase in larval length of group fed with 0.2% vitamin C, with the silkworm larvae (V instar) being longer than those in the control and other vitamin C-treated groups (0.1, 0.4, and 0.8 percent). Rani *et al*. (2011) [23] proved that larvae of *B. mori* fed with mulberry leaves enriched with amway nutrilite protein (10 per cent) revealed significant enhancement in larval characters. Borgohain (2015) [6] studied the nutritional supplement and its effect on *B. mori* and concluded that the fortification of mulberry leaves with nutrient supplements *viz*., royal jelly, dietary proteins, amino acids, vitamin B3 and vitamin B6 had affirmative effects on the growth and development of silkworm larvae.

3.2 Larval breadth (mm)

The mean larval breadth observations for different dietary supplements are given in Table 3. It is seen from data that the larval breadth of silkworm ranged from 0.50-9.41 mm. The impact of treatment T_5 comprising of vitamin C 0.25% + soya flour 0.25%+ vitamin B-complex 0.5% + methionine 0.5% on the mean larval breadth of first to fifth instar silkworm larvae was significantly superior compared to other treatments. The recorded breadths for the first to fifth instar larvae were 0.83, 1.43, 3.35, 6.67, and 9.41 mm, respectively, and these values were notably better than those of other treatments for each corresponding instar.

During first instar, the highest larval breadth (0.83 mm) was observed in T_5 the treatment of mulberry leaves enriched with vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%, which was significantly superior rest of the treatments except, T_3 involving vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% with a breadth of 0.81 mm, and T_1 *viz.* vitamin C 0.25% + soya flour 0.25% with a breadth of 0.78 mm, both of which were statistically similar and at par with each other. In contrast, the lowest larval breadth (0.50 mm) was recorded in the control, which was fed untreated mulberry leaves.

In the second instar, the highest larval breadth (1.43 mm) was observed in T_5 the treatment of mulberry leaves enriched with vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%, which was significantly superior rest of the treatments except, T_3 which involve vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% with a

breadth of 1.42 mm and T_1 comprising of vitamin C 0.25% + soya flour (0.25% with a breadth of 1.39 mm, both of which were statistically similar. In contrast, the lowest larval breadth (1.27 mm) was recorded in the control, which was fed untreated mulberry leaves.

During third instar, the highest larval breadth (3.35 mm) was observed in T_5 the treatment of mulberry leaves enriched with vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%, This was followed by T_3 comprising of vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% with a breadth of 3.32 mm, and T_1 involving vitamin C 0.25% + soya flour 0.25% with a breadth of 3.31 mm, both of which were statistically similar. In contrast, the lowest larval breadth (2.33 mm) was recorded in the control (T_9), which was fed untreated mulberry leaves.

In the fourth instar of silkworm larvae, the highest larval breadth (6.67 mm) was observed in T_5 the treatment of mulberry leaves enriched with vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%. This was followed by T_3 *viz.* vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% with a breadth of 6.59 mm, and T_1 which involved vitamin C 0.25% + soya flour 0.25% with a breadth of 6.53 mm, both of which were statistically similar. In contrast, the lowest larval breadth (6.02 mm) was recorded in the control (T_9), which was fed untreated mulberry leaves.

In the fifth instar, the highest larval breadth (9.41 mm) was observed in T_5 the treatment of mulberry leaves enriched with vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%. This was followed by T_3 comprising of vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% with a breadth of 9.27 mm, and T_1 *viz.* vitamin C 0.25% + soya flour 0.25% with a breadth of 9.20 mm, both of which were statistically similar. In contrast, the lowest larval breadth (7.83 mm) was recorded in the control (T_9) fed untreated mulberry leaves.

These results are similar to findings of Balasundram *et al.* (2013) [5] who observed, feeding vitamin C-treated MR_2 mulberry leaves to *B. mori* larvae in their last instar resulted increase in larval width of group fed with 0.2% vitamin C, with the silkworm larvae (V instar) being longer than those in the control and other vitamin C-treated groups (0.1, 0.4, and 0.8 percent). Pal (2003) [7] found that there is positive effect of 0.5% vitamin B-complex on growth and development of silkworm, *Bombyx mori* L. Proteins play a crucial role in the growth and development of silkworm larvae. These findings align with the research conducted by Rani *et al.* (2011) [23], demonstrating that the larvae of *B. mori*, when fed with mulberry leaves enriched with amway nutrilite protein (10%), exhibited noteworthy improvements in larval characteristics. Borgohain (2015) [6] studied the nutritional supplement and its effect on *B. mori* and concluded that the fortification of mulberry leaves with nutrient supplements *viz*., royal jelly, dietary proteins, amino acids, vitamin B3 and vitamin B6 had affirmative effects on the growth and development of silkworm larvae.

3.3 Larval weight (g)

The mean larval weight observations for different dietary supplements are shown in Table 4.

The data indicated that the larval weight of silkworm ranged from 0.18-40.13 g. The effect of treatment T_5 comprising of vitamin C 0.25% + soya flour 0.25%+ vitamin B-complex 0.5% + methionine 0.5% on the mean larval weight of first to fifth instar silkworm larvae was significantly superior compared to other treatments. The recorded weights for the first to fifth instar larvae were 0.18, 0.81, 3.02, 10.50, and 40.13 g, respectively, and these values were notably better than those of other treatments for each corresponding instar.

In the first instar of silkworm larvae, the highest larval weight (0.19 g) was observed in T_5 the treatment of mulberry leaves enriched with vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%. This was followed by treatment T_3 involving vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% with a weight of 0.18 g which was statistically significant and at par. In contrast, the lowest larval weight (0.06 g) was recorded in the control, which was fed untreated mulberry leaves.

In the second instar of silkworm larvae, the highest larval weight (0.81 g) was observed in T_5 the treatment of mulberry leaves enriched with vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%. This was followed by T_3 *viz.* vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% with a weight of 0.79 g, T_1 comprising of vitamin C 0.25% + soya flour 0.25% with a weight of 0.78 g, which were at par with each other. In contrast, the lowest larval weight (0.42 g) was recorded in the control group, which was fed untreated mulberry leaves.

In the third instar of silkworm larvae, the highest larval weight (3.02 g) was observed in T_5 the treatment of mulberry leaves enriched with vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%. This was followed by T_3 *viz.* vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% with a weight of 2.98 g, and T_1 involving vitamin C 0.25% + soya flour 0.25% with a weight of 2.87 g, which were at par with each other. In contrast, the lowest larval weight (2.03 g) was recorded in the control, which was fed untreated mulberry leaves.

In the fourth instar of silkworm larvae, the highest larval weight (10.50 g) was observed in T_5 the treatment of mulberry leaves enriched with vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%. This was followed by T_3 which involved vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% with a weight of 10.30 g, and T_1 *viz.* vitamin C 0.25% + soya flour 0.25% with a weight of 10.27 g, which were at par with each other. In contrast, the lowest larval weight (7.90 g) was recorded in the control, which was fed untreated mulberry leaves.

In the fifth instar of silkworm larvae, the highest larval weight (40.13 g) was observed in T_5 the treatment of mulberry leaves enriched with vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%. This was followed by T_3 comprising of vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% with a weight of 39.87 g, and T_1 involved vitamin C 0.25% + soya flour 0.25% with a weight of 39.50 g, which were at par with each other. In contrast, the lowest larval weight (25.60 g) was recorded in the control, which was fed untreated mulberry leaves.

These results are similar to findings of Balasundram *et al.* (2013) [5] who examined, feeding vitamin C-treated MR_2 mulberry leaves to *B. mori* larvae in their last instar resulted increase in larval weight of group fed with 0.2% vitamin C, with the silkworm larvae (V instar) being more than those in the control and other vitamin C-treated groups (0.1, 0.4, and 0.8 percent). According to Sawant *et al.* (2016) [24], supplementing silkworms (*Bombyx mori* L.) with 0.2% ascorbic acid improved their economic metrics, such as larval weight. Pal (2003) [7] found that there is positive effect of 0.5% vitamin B-complex on weight of 5th instar larvae of silkworm, *Bombyx mori* L. Krishanan *et al.* (1995) [11] validated that the highest larval weight was observed with a

2.00% concentration of hydrolyzed protein (P-soyatose). Anil Kumar and Sunil Kumar (2018) [2] found that mulberry leaves enriched with 0.5% methionine enhanced larval weight of silkworm, *Bombyx mori* L.

3.4 Total larval duration (days)

The mean larval duration observations for different dietary supplements are given in Table 5.

The treatment T_5 involving vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5% exhibited significantly superior performance, with the lowest mean larval duration recorded at 20.00 days, surpassing other treatments. This was followed by T_3 involving vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% at 20.33 days and T_1 consisting vitamin C 0.25% + soya flour 0.25% at 20.33 days, both statistically similar and at par with each other. The control group, fed with untreated mulberry leaves, showed the highest larval duration at 27.00 days. The impact of nutritional supplements on mean larval duration ranged from 20.00 to 27.00 days.

Results on larval duration are in accordance with Pal and Datta (2003) [8] found that ascorbic acid treatment of leaves resulted in a considerable reduction in the duration of the larval and pupal stages. According to Deepa *et al*. (2020) [7], silkworms (PM x CSR) fed V_1 mulberry supplemented with amino acids in their late stages of development showed noticeably reduced durations for both the fifth and final instars (169.00 and 608.00 hours), respectively. According to Kamaraj *et al*. (2017) [10], tasar silkworm larvae treated with soy solution spray were observed two days earlier than those treated with soy powder dust and control treatments. Borgohain (2015) [6] came to the conclusion that adding nutritional supplements, such as royal jelly, dietary proteins, amino acids, vitamin B3, and vitamin B6, to mulberry leaves had a positive impact on the growth and development of *B. mori* silkworm larvae.

3.5 Pupal length (cm)

The mean pupal length observations for different nutritional supplements are given in Table 5.

The treatment T_5 comprising a combination of vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5% demonstrated notably superior results, with the highest average pupal length reaching 3.47 cm, outperforming other treatments. Following closely were the treatments T_3 involving vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5%, with a recorded length of 3.40 cm, and T_1 consisting vitamin C 0.25% + soya flour 0.25%, with a length of 3.37 cm. Both of these treatments showed statistical significance and were at par with each other. The control group, fed with untreated mulberry leaves, showed the lowest pupal length 2.90 cm. The nutritional supplements showed significant effect on mean pupal length varied from 2.90 to 3.47 cm.

These results are similar to findings of Balasundram *et al.* (2013) [5] who examined, feeding vitamin C-treated MR_2 mulberry leaves to *B. mori* larvae in their last instar resulted increase in pupal length of group fed with 0.2% vitamin C, with the silkworm larvae (V instar) being longer than those in the control and other vitamin C-treated groups (0.1, 0.4, and 0.8 percent). Quraiza *et al.* (2016) [19] demonstrated fortification of the mulberry leaves by nutrient supplementation, it resulted that 7% plant protein (Soya flour) showed significant increase in parameters such as cocoon length over control. Laz *et al*. (2006) [13] carried out an experiment and stated that when mulberry silkworm fed with mulberry leaves treated with methionine and tryptophan, there is enhancement in pupal characters of silkworm. Meeramaideen *et al*. (2017) [16] found affirmative results of feeding mulberry leaves treated with vitamin B-complex on growth parameters of silkworm.

3.6 Pupal breadth (mm)

The mean pupal breadth observations for different dietary supplements are presented in Table 5.

The treatment T_5 involving vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5% exhibited significantly superior performance, with the highest mean pupal breadth recorded 13.97 mm, surpassing other treatments. This was followed by T_3 comprising of vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% recorded 13.50mm and T_1 involving vitamin C 0.25% + soya flour 0.25% recorded 13.40 mm, which were at par with each other. The control, fed with untreated mulberry leaves, showed the lowest pupal breadth 9.18 mm. The impact of nutritional supplements on mean pupal breadth ranged from 9.18 to 13.97 mm.

These results are similar to findings like Balasundram *et al.* (2013) [5] examined, feeding vitamin C-treated MR_2 mulberry leaves to *B. mori* larvae in their last instar resulted increase in pupal width of group fed with 0.2% vitamin C, with the silkworm larvae (V instar) being more than those in the control and other vitamin C-treated groups (0.1, 0.4, and 0.8 percent). Quraiza *et al.* (2016) [19] demonstrated fortification of the mulberry leaves by nutrient supplementation, it resulted that 7% plant protein (Soya flour) showed significant increase in parameters such as cocoon width over control. Laz *et al*. (2006) [13] carried out an experiment and stated that when mulberry silkworm fed with mulberry leaves treated with methionine and tryptophan, there is enhancement in pupal characters of silkworm. Meeramaideen *et al*. (2017) [16] found affirmative results of feeding mulberry leaves treated with vitamin B-complex on growth parameters of silkworm.

3.7 Pupal weight (g)

The observation recorded of mean pupal weight of silkworm are given in Table 5.

The results indicated that T_5, vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%, exhibited the highest mean pupal weight at 18.13 g, followed closely by T_3, vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5%, with a recorded weight of 17.67g. Additionally, T_1, vitamin C 0.25% + soya flour 0.25%, demonstrated a comparable mean pupal weight of 17.50 g, these three treatments were at par with each other. On the other hand, the lowest mean pupal breadth was observed in T_9, control, at 12.43 g. The range of mean pupal weights across mulberry leaves enriched with nutritional supplements varied from 12.43 g to 18.13 g.

These results are similar to findings like Balasundram *et al*. (2013) [5] examined, feeding vitamin C-treated MR_2 mulberry leaves to *B. mori* larvae in their last instar resulted increase in pupal weight of group fed with 0.2% vitamin C, with the silkworm larvae (V instar) being more than those in the control and other vitamin C-treated groups (0.1, 0.4, and 0.8 percent). Etebari *et al.* (2005) [9] reported as fed once daily to 4th and 5th instar silkworm larvae, mulberry leaves enhanced with multi-vitamins (1, 2.5, and 5%) significantly boosted biological and economic metrics such pupal weight as compared to the normal control. Quraiza *et al.* (2016) [19]

demonstrated fortification of the mulberry leaves by nutrient supplementation, it resulted that 7% plant protein (soya flour) showed significant increase in parameters such as pupal weight over control. Deepa *et al*. (2020) [7] observed that when silkworm larvae fed with mulberry leaves supplemented with amino acids during late instars resulted in maximum single pupal weight.

3.8 Total pupal duration (days)

The observation recorded on mean pupal duration of silkworm are given in Table 5. The T_5, vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5% significantly superior with lowest pupal duration (4.00 days) except the T_3, vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% (4.33 days) was similar with T_1, vitamin C 0.25% + soya flour 0.25% (4.33 days). Whereas, the highest pupal duration was recorded in T_9 control (7.33 days). The results show that the mean pupal duration of silkworm ranged from 4.00 to 7.33 days.

Results on pupal duration are in accordance with Pal and Datta (2003) [8] who found that ascorbic acid treatment of leaves resulted in a considerable reduction in the duration of the larval and pupal stages. Meeramaideen *et al*. (2017) [16] found affirmative results of feeding mulberry leaves treated with vitamin B-complex on growth parameters of silkworm. Ahsan *et al.* (2013) [1] observed decrease in larval and pupal duration of mulberry silkworm fed with vitamin B and C. Mahmoud *et al*. (2013) [14] reported that various protein sources has impact on larval and pupal durations.

Table 2: Effect of mulberry leaves enrichment with various nutritional supplements on mean larval length of silkworm (cm/10 larvae)

Sr. No.	Treatments	Mean larval length (cm/10 larvae)					Treatment mean
			Initial instars		Late instars		
		First instar	Second instar	Third instar	Fourth instar	Fifth instar	
T_1	Vitamin C 0.25% + soya flour 0.25%	0.43	2.00	2.61	6.02	8.16	3.84
T_2	Vitamin B-complex 0.25%+ soya flour 0.25%	0.29	0.78	1.95	4.20	6.30	2.70
T_3	Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5%	0.44	2.02	2.64	6.05	8.17	3.86
T_4	Vitamin B-complex 0.25%+ soya flour 0.25% + vitamin C 0.5%	0.30	0.83	1.98	4.52	6.60	2.84
T_5	Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%	0.45	2.07	2.69	6.17	8.27	3.93
T_6	Vitamin B-complex 0.25%+ soya flour 0.25% + vitamin C 0.5% + methionine 0.5%	0.38	1.70	2.45	5.72	7.57	3.56
T_7	Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5% + L-serine 0.5%	0.37	1.31	2.29	5.22	7.20	3.27
T_8	Vitamin B-Complex 0.25%+ soya flour 0.25% + vitamin C 0.5% + methionine 0.5%+ L-serine 0.5%	0.32	1.20	2.03	4.90	6.68	3.02
T_9	Untreated control	0.27	0.68	1.86	4.01	5.70	2.50
S.Em ±		0.007	0.032	0.036	0.06	0.131	0.053
C.D at 5%		0.02	0.10	0.11	0.18	0.39	0.27
C.V. (%)		3.72	3.98	3.05	2.02	3.17	3.18

Table 3: Effect of mulberry leaves enrichment with various nutritional supplements on mean larval breadth of ten larvae (mm)

Sr. No.	Treatments	Mean larval breadth (mm/10 larvae)					Treatment mean
			Initial instars		Late instars		
		First instar	Second instar	Third instar	Fourth instar	Fifth instar	
T_1	Vitamin C 0.25% + soya flour 0.25%	0.78	1.39	3.31	6.53	9.20	4.24
T_2	Vitamin B-complex 0.25%+ soya flour 0.25%	0.51	1.28	2.65	5.17	8.37	3.59
T_3	Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5%	0.81	1.42	3.32	6.59	9.27	4.28
T_4	Vitamin B-complex 0.25%+ soya flour 0.25% + vitamin C 0.5%	0.54	1.30	2.67	5.55	8.48	3.70
T_5	Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%	0.83	1.43	3.35	6.67	9.41	4.33
T_6	Vitamin B-complex 0.25%+ soya flour 0.25% + vitamin C 0.5% + methionine 0.5%	0.75	1.35	2.91	6.30	8.80	4.02
T_7	Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5% + L-serine 0.5%	0.60	1.33	2.82	6.13	8.81	3.93
T_8	Vitamin B-complex 0.25%+ soya flour 0.25% + vitamin C 0.5% + methionine 0.5% + L-serine 0.5%	0.58	1.32	2.69	5.60	8.51	3.74
T_9	Untreated control	0.50	1.27	2.33	4.93	7.83	3.37
S.Em +		0.016	0.019	0.073	0.123	0.195	0.085
C.D at 5%		0.05	0.06	0.22	0.37	0.58	0.25
C.V. (%)		4.28	2.53	4.40	3.60	3.87	3.73

Table 4: Effect of mulberry leaves enrichment with various nutritional supplements on mean larval weight of ten larvae (g)

Sr. No.	Treatments	Mean larval weight (g/10 larvae)					Treatment mean
			Initial instars		Late instars		
		First instar	Second instar	Third instar	Fourth instar	Fifth instar	
T_1	Vitamin C 0.25% + soya flour 0.25%	0.17	0.78	2.87	10.27	39.50	10.72
T_2	Vitamin B-complex 0.25%+ soya flour 0.25%	0.07	0.47	2.66	8.33	27.05	7.71
T_3	Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5%	0.18	0.79	2.98	10.30	39.87	10.82
T_4	Vitamin B-complex 0.25%+ soya flour 0.25% + vitamin C 0.5%	0.08	0.52	2.68	8.47	29.28	8.20
T_5	Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%	0.19	0.81	3.02	10.50	40.13	10.85
T_6	Vitamin B-complex 0.25%+ soya flour 0.25% + vitamin C 0.5% + methionine 0.5%	0.10	0.68	2.81	9.82	34.80	9.64
T_7	Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5% + L-serine 0.5%	0.10	0.62	2.71	9.37	32.07	8.97
T_8	Vitamin B-complex 0.25%+ soya flour 0.25% + vitamin C 0.5% + methionine 0.5% + L-serine 0.5%	0.09	0.57	2.70	8.75	30.23	8.47
T_9	Untreated control	0.06	0.42	2.03	7.90	25.60	7.20
S.Em ±		0.002	0.015	0.055	0.175	0.651	0.179
C.D at 5%		0.01	0.05	0.16	0.52	1.94	0.53
CV at 5%		3.50	4.18	3.49	3.25	3.40	3.56

Table 5: Effect of mulberry leaves enrichement with various nutritional supplements on growth parameters of mulberry silkworm

Sr. No	Treatments	Mean larval duration (Days)	Mean pupal length (cm)	Mean pupal breadth (mm)	Mean pupal weight (g)	Mean pupal duration (Days)
T_1	Vitamin C 0.25% + soya flour 0.25%	20.33 (4.56)	3.37	13.40	17.50	4.33 (2.20)
T_2	Vitamin B-complex 0.25%+ soya flour 0.25%	25.00 (5.05)	3.13	10.13	13.00	7.00 (2.74)
T_3	Vitamin C 0.25% + soya flour 0.25% + Vitamin B-complex 0.5%	20.33 (4.56)	3.40	13.50	17.67	4.3 (2.20)
T_4	Vitamin B-complex 0.25%+ soya flour 0.25% + Vitamin C 0.5%	24.00 (4.94)	3.10	10.29	13.73	6.33 (2.61)
T_5	Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%	20.00 (4.53)	3.47	13.97	18.13	4.00 (2.12)
T_6	Vitamin B-complex 0.25%+ soya flour 0.25% + vitamin C 0.5% + methionine 0.5%	22.00 (4.74)	3.23	11.80	17.17	5.00 (2.35)
T_7	Vitamin C 0.25% + soya flour 0.25% + Vitamin B-complex 0.5% + methionine 0.5%+ L-serine 0.5%	23.00 (4.85)	3.17	11.53	16.27	5.33 (2.41)
T_8	Vitamin B-Complex 0.25%+ soya flour 0.25% + vitamin C 0.5% + methionine 0.5%+ L-serine 0.5%	23.67 (4.92)	3.07	10.53	14.63	5.67 (2.48)
T_9	Untreated control	27.00 (5.24)	2.90	9.18	12.43	7.33 (2.80)
S.Em ±		0.119	0.063	0.203	0.217	0.055
C.D at 5%		0.35	0.19	0.61	0.65	0.16
C.V. (%)		4.28	3.42	3.04	2.41	3.90

Conclusion

The present investigation of research topic concluded that all growth parameters based on overall performance it was noticed that vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5% and vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% performed best among all combinations of nutritional supplements for larval length (cm), larval breadth (mm), larval weight (g), larval duration (days), pupal length (cm), pupal breadth (mm), pupal weight (g) and pupal duration (days).

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