Effect of Vitamin Supplements on Economic Traits of Silkworm *Bombyx mori* L.

Ifrah Shafi
(MSS/2020/85)

College of Temperate Sericulture
Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir

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Submitted to

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Dedicated

To

My Beloved Parents

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
College of Temperate Sericulture, Mirgund

Certificate-I

This is to certify that the thesis entitled, **"Effect of Vitamin Supplements on Economic Traits of Silkworm *Bombyx mori* L."** submitted in partial fulfilment of the requirements for the award of **Master of Science in Sericulture,** to the **College of Temperate Sericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir,** is a record of bonafide research work carried out by **Ms. Ifrah Shafi (Regd. No. MSS/2020/85)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that the information received during the course of investigation has duly been acknowledged.

(Dr. Firdose Ahmad Malik)
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Advisory Committee

Endorsed

Prof. & Head,
Division of Sericulture Crop Improvement,
College of Temperate Sericulture, SKUAST-K.

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
College of Temperate Sericulture, Mirgund

Certificate-II

We, the members of the Advisory committee of **Ms. Ifrah Shafi (Regd. No. MSS/2020/85),** a candidate for the degree of **Master of Science in Sericulture,** have gone through the manuscript of the thesis entitled **"Effect of Vitamin Supplements on Economic Traits of Silkworm *Bombyx mori* L."** and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of degree.

Advisory Committee

Chairman

Dr. Firdose Ahmad Malik
Assistant Professor
D.S.C.I, CoTS, Mirgund
SKUAST-Kashmir

Members

Dr. Shabir Ahmad Bhat
Assistant Professor
CTS, Mirgund
SKUAST-Kashmir

Dr. Nageena Nazir
Associate Professor
Division of Agri Statistics
SKUAST-Kashmir

Dr. Saima Masood Wani
Assistant Professor
Division of BS&H
SKUAST-Kashmir

Dr. K.A Sahaf
Professor & Head,
Division of Silk Product Science
CoTS, Mirgund, SKUAST-K
(Dean's Nominee)

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
College of Temperate Sericulture, Mirgund

Certificate-III

This is to certify that the thesis entitled, **"Effect of Vitamin Supplements on Economic Traits of Silkworm *Bombyx mori* L."** submitted by **Ms. Ifrah Shafi (Regd. No. MSS/2020/85),** to **College of Temperate Sericulture Mirgund, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** in partial fulfillment of the requirements for the award of the degree of **Master of Science in Sericulture** was examined and approved by the Advisory Committee and external examiner on ……………………….

Chairman
Advisory Committee

External Examiner

Prof. & Head
Division of Sericulture Crop Improvement
College of Temperate Sericulture, Mirgund
SKUAST-Kashmir

Associate Dean
College of Temperate Sericulture, Mirgund
SKUAST-Kashmir

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
College of Temperate Sericulture, Mirgund

Name of the student	:	**Ifrah Shafi**
Registration no	:	MSS-2020-85
Major subject	:	Sericulture
Minor subject	:	Biochemistry
Major advisor	:	**Dr. Firdose Ahmad Malik** Assistant Professor CoTS, Mirgund SKUAST-Kashmir
Title of the Thesis	:	**"Effect of Vitamin Supplements on Economic Traits of Silkworm, *Bombyx mori* L."**

ABSTRACT

The investigation entitled "Effect of Vitamin Supplements on Economic Traits of Silkworm, *Bombyx mori* L." was carried out at Division of Sericulture Crop Improvement in College of Temperate Sericulture, Mirgund, during spring (May - June) 2022. The study material comprised silkworm Double hybrid (CSR6×CSR26)×(CSR2×CSR27). The mass rearing of silkworms was conducted upto 3rd instar. After 3rd moult, three replications each of 100 worms were maintained for each treatment as well as for control. For the present study eight vitamins *viz.* Thiamine, Riboflavin, Niacin, Pantothenic acid, Pyridoxine, Biotin, Folic acid and Ascorbic acid with two different concentrations *viz.,* 10µg/ml and 20µg/ml were selected to determine their effect on growth, development and commercial cocoon characters of silkworm. These vitamin solutions were sprayed @50ml/200gm mulberry leaf with the help of an atomizer and fed to silkworm larvae on alternate days once as first feed during 4th and 5th instar. One control was also maintained (worms fed with mulberry leaves treated with distilled water for comparison). The shortest 5th instar and total larval duration of 163 and 651 hours, respectively were recorded when mulberry leaves were fortified with Pyridoxine at 20µg/ml concentration and fed to the silkworms. The highest weight of 10 mature larvae of 52.25 grams, Silk gland weight of 1.97 grams, Silk gland somatic index of 36.52 per cent, cocoon yield of 9615 cocoons/10,000 larvae

by number, cocoon yield of 18.17 kg by weight, silk productivity of 7.54 cg/day, single cocoon weight of 1.887 grams, single shell weight 0.430 grams and shell percentage 22.84 per cent were recorded in 20µg/ml Pyridoxine treatment. In case of post cocoon parameters longest filament length of 1184 meters, thinnest filament size of 2.47 and highest raw silk percentage of 17.45 per cent were also recorded in same treatment. The highest fecundity of 685 eggs per layings was recorded with Ascorbic acid at 20µg/ml concentration. Thus results of this study clearly indicated by improving the nutritional value of mulberry leaf by fortification with Pyridoxine followed by Ascorbic acid, Folic acid, Biotin, Riboflavin, Niacin and Pantothenic acid at 20µg/ml concentration resulted better growth and development of the silkworm. As such these vitamins can be utilized as feed supplement to improve productivity at field level especially when mulberry leaf is deficient in nutrients.
Keywords: Vitamins, Silkworm, Egg laying, Silk gland

Signature of Student | Signature of Major Advisor

Dated: ___________ | Dated: ________________

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"No scientific endeavour is a result of an individual efforts and so comes the time to look back on the path traversed during this endeavour and to remember the faces and spirits behind the action with sense of gratitude."

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Place: CoTS, Mirgund **Ifrah Shafi**

Dated:

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INTRODUCTION

Chapter-1

INTRODUCTION

Sericulture is an art of silk production that comprises cultivation of mulberry, silkworm rearing and post cocoon activities leading to production of silk yarn. Nearly 30 million people rely exclusively on sericulture for their livelihood in a number of countries including China, India, Uzbekistan, Brazil, Japan, and Vietnam, with 26 million farmers are engaged in the sericulture and silk processing business in China and India alone (Anonymous, 2022).

India is the second largest producer of silk in the world after China. India is the only country in the world which produces all the four types of silk. Of the 4 kinds of silk produced during 2021-22, Mulberry comprised 74.03% (25,853 MT), of the total raw silk output in 2021-2022, which was 34,923 MT, 3.4% more than the production during 2020-2021. Tasar made up 4.17% (1,456 MT), Eri 21.07% (7,359 MT) and Muga 0.73% (255 MT) of the raw silk production (33,770 MT). The bivoltine raw silk production increased substantially by 17.6% from 6,783 MT during 2020-21 to 7,978 MT during 2021-22. The employment generation was 8.8 million persons during 2020-2021, indicating increase of 1.1% (Anonymous, 2022).

In the Union Territory of Jammu and Kashmir, sericulture is considered as an age-old vocation and serves as subsidiary occupation to a large section of population. In Jammu and Kashmir, about 27,000 families are directly or indirectly associated with silk industry (Anonymous, 2020). In the present scenario, the sericulture industry is distributed across 20 districts of Jammu and Kashmir. The main districts that produce cocoons are Udhampur, Rajouri, Kathua, Anantnag, Bandipora, Baramulla and Kupwara (Anonymous 2021). The Union Territory of J&K registered a cocoon production of 800 MT during 2019-2020 while raw silk production stood at 99 MT during 2020-21 (Anonymous, 2021).

Efforts have been made by the Sericulture Development Department with the collaboration of Central Silk Board and SKUAST-K from time to time for the upliftment of the industry. Silkworm (*Bombyx mori* L.) being a monophagous insect survives solely on mulberry leaf which plays an important role in the nutrition of the silkworm (Etebari, 2002; Tang *et al*., 2005). Therefore, in order to improve quality and quantity of cocoons and silk, the production of quality mulberry leaf is of paramount importance.

Mulberry leaf is the solitary nutriment for the silkworms and its quality plays an important role in determining the larval growth *viz-a-viz* cocoon productivity (Paul *et al*., 1992; Yokoyama, 1963) and success of silk industry (Choudhary, 1991).

The silkworm *Bombyx mori*, benefits from proper nutrition in terms of growth and development. According to Legay, (1958) the nutritional value of mulberry leaves plays a crucial role in developing high-quality cocoons. Silkworm obtains its entire nutritional requirement from mulberry leaves because this insect is monophagous and can complete the life cycle on mulberry leaves exclusively. Feeding silkworms nutrient-rich leaves resulted in enhanced silkworm growth and development as well as improved cocoon quality (Krishnaswami *et al.*1971).

Miyashita (1986) observed that leaf quality alone contributes about 38.2% to success of cocoon crop. The nutritional content and composition of mulberry leaves vary significantly from season to season based on a variety of elements including weather, soil nutrition, insect and disease infestation, and agricultural techniques (Ito, 1978). Nutritive components like moisture, amino acids, carbohydrates, proteins, vitamins, and minerals in feed play a central role in determining the quality of feed. The biochemical substance in the mulberry leaves highly influence the growth and development of silkworm.

Vitamins are organic molecules that are required in the diet and play a major role in various physiological processes in an organism like growth, reproduction and metabolism (Bentea *et al*., 2011). They are needed in very small quantity because they function as coenzymes, they facilitate enzymatic reactions without being either consumed or produced in the reaction. (Douglas, 2017). The Vitamins are of two distinct types, water soluble vitamins and fat soluble

Vitamins.

Water soluble Vitamins

- B1-Thiamine
- B2-Riboflavin
- B3-Niacin
- B5-Pantothenic acid
- B6-Pyridoxine,pyridoxal, pyridoxamine
- B8-Biotin
- B9-Folic Acid
- Vitamin-C

Fat soluble Vitamins

- Vitamin A
- Vitamin D
- Vitamin E
- Vitamin K

Role of water soluble Vitamins:

- **Thiamine (B1)**

Thiamine is also known as Vitamin-B1. Thiamine is the precursor of the cofactor thiamindiphosphate, is required by numerous enzymes participating in the metabolism of carbohydrates and amino acid (Settembre *et al.*, 2003).

- **Riboflavin (B2)**

Riboflavin is also known as Vitamin-B2. Riboflavin is the precursor for the coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The enzymes that require FMN or FAD as cofactors are termed flavoproteins. Several flavoproteins also contain metal ions and are termed metalloflavoproteins. Both classes of enzymes are involved in a wide range of redox reactions, e.g. succinate dehydrogenase and xanthine oxidase. Riboflavin is important in promoting the release of energy from carbohydrates, fats and proteins i.e. in the metabolic pathway for ATP production (Husain, 2017).

- **Niacin (B3)**

Niacin (nicotinic acid and nicotinamide) is also known as Vitamin B3. Niacin is important for the release of energy from carbohydrates and fats, the metabolism of proteins and production of several hormones (National Research Council (U.S), 1987). Horie and Ito, (1965) showed the required level of niacin for silkworm is highly regulated to the most appropriate level of 33 µg/l of dry weight and the increase of niacin reduced the larval weight.

- **Pantothenic acid (B5)**

Pantothenic acid is also known as Vitamin-B5. It is the precursor of coenzyme A that is vital for the metabolism of carbohydrates, the synthesis and degradation of fats, the synthesis of sterols and the resultant steroid hormones, and the synthesis of many other important compounds (Kanafi *et al*., 2007).

- **Pyridoxine, pyridoxal, pyridoxamine (B6)**

Pyridoxal, pyridoxamine and pyridoxine are collectively known as Vitamin-B6. All three compounds are efficiently converted to the biologically active form of Vitamin-B6, pyridoxal phosphate. This conversion is catalyzed by the ATP requiring enzyme, pyridoxal kinase, for numerous enzymatic reactions predominantly in amino acid metabolism.

- **Biotin (B8)**

Biotin is the cofactor for a small group of enzymes that catalyze carboxylation, decarboxylation, and transcarboxylation reactions in carbohydrate and fatty acid metabolism. It has been showed that biotin is one of the essential vitamins for the silkworm *Bombyx mori* L. (Horie *et al*., 1966).

- **Folic Acid (B9)**

Folic acid is a conjugated molecule consisting of a pteridine ring structure linked to para-aminobenzoic acid (PABA) that forms pteroic acid. Folic acid itself is then generated through the conjugation of glutamic acid residues to pteroicacid. Folic acid plays a major role in cellular metabolism including the synthesis of some of the components of DNA and pigment precursor (Chapman, 1998). Yosuhiro and Sholchi, (1971) noted that the silkworm growth decreased when folic acid was eliminated from artificial diet. Nirwani and Kaliwal, (1996) determined that folic acid has phagostimulatory effects with significant increase in female and male cocoon and shell weight.

- **Vitamin C**

Ascorbic acid is more commonly known as Vitamin-C. Ascorbic acid is derived from glucose via the uronic acid pathway. The enzyme L-gulonolactone oxidase responsible for the conversion of gulonolactone to ascorbic acid is absent in primates making ascorbic acid required in the diet. The active form of Vitamin-C is ascorbate acid itself. The main function of ascorbate is as a reducing agent in a number of different reactions. The silkworm, *B. mori* has been classified among the insects which are unable to synthesize Vitamin-C in their body and depend on exogenous supply to fulfill the requirement (Ito and Arai, 1967).

Keeping in view the importance of Vitamins in silkworm nutrition, the present study was undertaken to assess the supplementation of mulberry leaves by Vitamins on the growth and economic traits of silkworms, *Bombyx mori* L.

OBJECTIVES:

1. To study the influence of vitamin supplements on silkworm larval growth and development.
2. To study the influence of vitamin supplements on economic traits of silkworm, *Bombyx mori* L.

Chapter – 2

REVIEW OF LITERATURE

Chapter-2

REVIEW OF LITERATURE

Keeping in view the objectives of the present study, the available literature on the subject has been reviewed and presented as under:

Ito (1961) recorded relationship of ascorbic acid supplementation and growth of silkworm and revealed that absence of ascorbic acid in the diet of first and second instar larvae adversely affected growth and development of silkworm.

Sengupta *et al.* (1972) showed that *Bombyx mori* L. requires specific essential sugars, amino acids, proteins and vitamins for its normal growth, survival and also for the silk gland growth and development.

Karaksy and Idriss (1990) reported that ascorbic acid at different concentrations (0.25, 0.5, 1 and 2%) increased significantly the weight of both larvae and pupae. Further, fifth instar larvae of *B.mori* fed on mulberry leaves treated with ascorbic produced better cocoons. Statistical analysis of data showed that 2% ascorbic acid produced better quality cocoons and silkworms fed with treated leaves enhanced 29.85% silk production against control. In addition ascorbic acid proved to exhibit a significant effect on the increasing of fecundity of emerged females.

Babu *et al*. (1992) observed that 1.5 % ascorbic acid enriched mulberry leaves resulted in higher silk filament length, silk weight and denier values.

Chauhan and Singh (1992) showed that 1% concentration of ascorbic acid supplemented leaves increased the number of eggs in the silkmoths.

Suprakash and Madhuri (1992) studied the effect of ascorbic acid on the growth and development of two races of mulberry silkworm *Bombyx mori* L. under laboratory conditions. It was found that larval weight, cocoon and shell weight and shell ratio were increased significantly due to leaf supplementation with vitamin C. Supplementation with 1% ascorbic acid produces the best results but ascorbic acid at higher concentration was found have negative impact. The larval and pupal duration were reduced significantly due to leaf fortification with ascorbic acid. Fecundity of female moths and hatching percentage of eggs also increased significantly due to treatments with ascorbic acid.

Sarker *et al*. (1995) showed that the dietary supplementation with Vitamin -B complex to silkworm larvae resulted in a significant increase of female fecundity and improved cocoon yield and filament length of the silk.

Nirwani and Kaliwal (1996) observed that dietary supplementation of folic acid to silkworm larvae in different concentration (100, 200 and 300 µg/ml) to the 4th and the 5th instar resulted in significant increase in economic parameters, such as female and male cocoon weights, shell weights, egg productivity and egg hatching percentage.

Faruki (1998) reported that the thiamine derivative thianomin enhanced the growth of silkworm larvae, pupae and adults in all concentrations used (50, 100, 500 and 1000 ppm) of thiamine supplemented leaves.

Etebari (2002) showed that the high concentrations of nicotinamide (10, 20 and 30 g/l) could cause intensive mortality in the larval stage. From the first instar, the larvae in this group were treated with high doses. Moulting was disrupted in each stage and many larvae died. Many of larvae were unable to continue moulting and spent the rest of their lives in the same stage. Only 1.2% of larvae could reach to fifth instar. None of the larvae from the 20 and 30 g/l concentrations entered the 5^{th} instar.

Talebi *et al*. (2002) reported that supplementation of 0.2g /litre of vitamin C solution to the fourth anf fifth instar silkworm resulted significantly increase in the cocoon, shell, pupal weight, and shell ratio.

Humayun and Mubashir (2002) carried out study to determine the effect of 0.2 % N in combination with different doses of ascorbic acid (0.025, 0.075, 0.100, 0.125 and 0.150%) on larval growth and silk production of silkworm larvae (*Bombyx mori* L.) the larvae which were offered mulberry leaves treated with 0.2% N + 0.150% ascorbic acid showed higher mean values of body weight, body length, food consumption, coefficient of utilization and cocoon shell ratio.

Etebari and Matindoost (2004) while studying the effects of hypervitaminosis of Vitamins B3 on silkworm biology reported that a high dose of Vitamin B3 in silkworm diet interrupts larval feeding and normal growth. Also, larvae exhibit Vitamin B3 hypervitaminosis symptoms like immobility, dyspepsia, darkening of skin, excreting brownish fluid and swelling of rectal muscles.

Etebari *et al.* (2004) demonstrated that feeding on mulberry leaves enriched with ascorbic acid at 3% concentration decreased larval weight due to hypervitaminosis.

Kayvan *et al*. (2004) studied the effects of dietary supplements of ascorbic acid 1, 2, and 3% to silkworm larvae through 1st to 5th instar.These treatments resulted in a significant increase of biological parameters such as larval weight,the rate of food consumption and the approximate digestibility of the food. But, the economical parameters such as cocoon weight and cocoon shell weight didn't show considerable difference compared to control.

Prasad (2004) reported that supplementation of 2% ascorbic acid significantly increased the cocoon parameters in all three races i.e. multivoltine (pure mysore), bivoltine (NB4D2) and cross breed (PM×NB4D2).The highest single shell weight of 0.167, 0.394 and 0.355g was recorded in pure mysore, NB4D2, PM×NB4D2 races respectively, with 3% ascorbic acid supplementation.

Etebari and Matindoost (2005) reported that feeding of silkworm on mulberry leaves enriched with multi-Vitamins from 4th instar increased female cocoon shell weight in 2.5% concentration, while female pupal weight increased in 1% concentration.

Luciano *et al.* (2005) studied that an artificial diet containing 2% L-ascorbic acid was given to silkworm (*Bombyx mori* L.) larvae throughout larval life, or only in some larval instars in order to make a comparison of larvae fed on a diet without L-ascorbic acid throughout larval life. Obtained results show that, when complete L-ascorbic acid deprivation is done during the larval cycle, it affects larval growth and cocoon production. Furthermore, L-ascorbic acid absence from larval food, particularly during the first and last instars, generates beneficial effects to cocoon production without affecting the survival rate or delaying the larval cycle.

Rajabi *et al*. (2006) while analyzing the enrichment of mulberry leaves with riboflavin at 77 ppm enhanced economic characters of silkworm, and improved silk production in north climatic conditions of Iran. Male cocoon weight (1.195 g) was higher at 77 ppm while female cocoon weight (1.622 g) was higher at 127 ppm. Maximum male pupal weight was recorded at 37 ppm (0.895 g) compared to 127 ppm for the female (1.169 g). Male and female shell weight (0.311 and 0.318 g) had significant increase at 77 ppm compared to control (0.276 and 0.277 g). Male and female cocoon shell percentage reached their maximum at 77 ppm treatment, which were 26.06 % and 21.46 % respectively.

Rahmathulla *et al*. (2007) studied the influence of oral administration of folic acid to 5th instar silkworm larvae of a popular Indian bivoltine hybrid (CSR2 x CSR4). In this experiment, folic acid solution fortified on mulberry leaf significantly improved larval weight, silk gland weight and growth rate. Higher larval and silk gland weight subsequently improved the economic parameters like cocoon weight, shell weight and shell ratio of folic acid treated batches.

Munghate *et al*. (2009) studied the effect of supplementation of ascorbic acid at different concentration on silkworm *Bombyx mori* L. CSR2×CSR4 hybrid. The result showed highest larval weight (31.22g/ 10 larvae), lowest larval period (699hours), maximum pupal weight (14.13/10 pupae), highest silk percentage (24.24%) highest filament length (803.33m/50 cocoon) and low mortality (5.58%) at 2% ascorbic acid fortification.

Tantray *et al*. (2011) reported that treatment of Vitamin- C doses, (0.50% to 3.00%), improved all the economic traits significantly and higher doses of Vitamin-C, (5.00 to 15.0%), brought out significant reduction in economic traits (3.00-13.79%) as compared to the control.

Rahamthulla *et al.* (2011) studied the influence of folic acid on nutritional indices and nutritional efficiency parameters of the silkworm *Bombyx mori* L. The result indicated that nutritional indices such as ingesta, digesta, reference ratio, approximate digestibility and consumption index were significantly higher in the folic acid-treated silkworm batches. Similarly, nutritional efficiency parameters such as efficiency of ingested and digested food into larva, cocoon and shell weight were significantly higher in the folic acid treated batches.

Singh and Bandey (2012) studied that the supplementation of Vitamin-C in the last larval stages in the bivoltine hybrid NB4D2×SH6 of silkworm and recorded significant improvement in the parameters such as larval weight, cocoon weight, shell weight, shell ratio in vitamin C fortified leaf over the control.

Ahsan *et al*. (2013) studied the effect of Vitamin B and Vitamin C on the growth and development of *Bombyx mori* L. Oral supplementation of different concentration of Vitamin B and C to the 3rd, 4th and 5th instar larvae of multivoltine silkworm race, (BSR-95/14) resulted in significant increase in larval and pupal characters. It was observed that the development of larvae and pupae took place up to a particular dose. There was also a significant increase in the various growth indices by Vitamin supplementation.

Balasundaram and Ganesh (2013) observed that the feed efficacy and growth rate of silkworm larvae (V instar), enhanced by 0.2% Vitamin C treated group than control and other Vitamin C treated groups (0.1%, 0.4% and 0.8%). This study has indicated that the Vitamin C exhibits the presence of certain growth stimulant activity and can be used to increase the feed efficacy in commercial silkworm rearing with reference to sericulture.

Tantray *et al*. (2015) studied that seven different doses of vitamin C were orally fed to 5th instar larvae of Bombyx mori hybrid (PM × CSR2) at seven varied application times. The supplement had a concentration and application time-dependent effect on reeling traits. Maximum improvement in filament length (14.97-17.20%), non-breakable filament length (13.91-17.00%), reelability (12.38-16.71%) and renditta (9.52-10.23%) was recorded in 3.00% dose when fed at 0 hour of 5th instar once and daily once from 0 hour to the onset of spinning over the control. No significant difference was observed between the effects exhibited at the two application times and therefore, application at 0 hour of 5th instar was concluded as economically viable and recommendable.

Ravi and Anil (2016) reported that the effect of mulberry leaves supplemented with folic acid at different concentrations *viz.*, 02, 04, and 06% on the economic traits in multivoltine silkworm breeds namely MU-1 and Hosa Mysore and the result of the study revealed that, the expression for economic traits *viz.*, mature larval weight, cocoon weight, shell weight, shell ratio, filament length, denier, and fecundity were maximum in both the breeds at 6% concentration over control batch.

Meeramaideen *et al.* (2017) studied the feed efficacy, growth rate and economic parameters of silkworm *Bombyx mori* L. (V instar larvae) fed by KANVA-2 mulberry (*Morus alba*) leaves and B-complex Vitamins (Riboflavin (0.5%), Pantothenic acid (0.5%), Pyridoxal phosphate (0.5%) and Biotin (0.5%)) treated KANVA-2 mulberry leaves in relation to feed efficacy parameters like food consumption, food utilization, approximate digestibility, consumption index and coefficient of food utilization. It has been observed that the feed efficacy, growth and economic parameters of *Bombyx mori* L. enhanced by 0.5% of Riboflavin treated group followed by Pantothenic acid (0.5%, Pyridoxal phosphate (0.5%) and Biotin (0.5%). It indicated that among the Vitamin treatments Riboflavin exhibits the presence of more growth stimulant activity and can be used to increase the silk yield in commercial silkworm rearing with reference to sericulture.

Kamala and Karthikeyan (2019) studied the effect of nutritional fortification of nanoparticles of riboflavin on the growth and development of mulberry silkworm, *Bombyx mori* L. Supplementation of nanoparticles of Vitamin B2 showed a positive impact and significantly enhanced the growth of the larvae (28.98%), silk gland weight (111.39%) and silk yield (194.44%) when compared to control.

Kamala and Kathikeyan (2020) reported that the fifth instar larvae of mulberry silkworm *B.mori* L. were fed with enriched mulberry leaves thrice a day.

Economic characters and fecundity were studied and recorded. Synergetic supplementation of nanoparticles showed a positive impact and significantly enhanced the cocoon weight by 94.366%, pupal weight by 64.285%, silk yield by 89.44% and fecundity by 48.44% when compared to control.

Rania and Dina (2021) investigated the effectiveness of Vitamin C on different characteristics of silkworm, *Bombyx mori* L. Silkworm larvae through 4th to 5th instar were treated with dietary supplements of ascorbic acid and were fed four times per day. Results revealed that treated batches of Vitamin C is more effective in growth improvement than concentration. It had a significant increase in biological criteria like larval weight and larval period and economic criteria like cocoon weight, cocoon shell weight and pupal weight of silkworm, *Bombyx mori* L.

Aneesha and Kumar (2022) found that the dietary supplementation of ascorbic acid was effective in modifying the composition of essential biomolecules. A relative investigation on effects of exogenous dietary supplementation of 0.2% ascorbic acid on the fifth instar larvae of silkworm, *Bombyx mori* L. exposed to a high thermal stress of range 40 ± 2 °C. The results reveals that foliar supplementation of ascorbic acid has been effective in positively-modulating the biochemical performance in larvae exposed to thermal stress. Moreover, the study also revealed the possibilities of ascorbic acid as a potential treatment, capable of facilitating the production of good quality cocoons, from larvae exposed to thermal stress.

Chapter – 3

MATERIALS AND METHODS

Chapter-3

MATERIALS AND METHODS

The present study was conducted at Division of Sericulture Crop Improvement, College of Temperate Sericulture, Mirgund during spring (May-June) in the year 2022.

3.1 Mulberry varieties

Mulberry varieties namely Ichinose (for chawki rearing) and Goshoerami (for late age rearing), available at the mulberry farm of College of Temperate Sericulture, Mirgund were utilized for the present study.

3.2 Silkworm rearing

The disease free layings of commercial silkworm bivoltine double hybrid (CSR6×CSR26)×(CSR2×CSR27), were incubated, brushed and reared by following the recommended package of practices (Anonymous, 2003). Mass rearing was done up to 3rd instar by feeding Ichinose mulberry leaf without any treatment. Just after 3rd moult, three replications each of 100 worms were maintained for each treatment as well as for control batches.

3.3 Preparation of different vitamins

The 10 µg/ml and 20µg/ml concentrations of each vitamin was prepared by dissolving 1gram and 2 grams of Thiamine, Riboflavin, Niacin, Pantothenic acid, Pyridoxine, Biotin, Folic acid and Ascorbic acid in 100ml of distilled water.

3.4 Fortification of mulberry leaf

For the present study eight vitamins *viz*. Thiamine, Riboflavin, Niacin, Pantothenic acid, Pyridoxine, Biotin, Folic acid and Ascorbic acid with two different concentrations i.e., 10µg/ml and 20µg/ml were selected. After 3rd moult, vitamin solutions were prepared and were sprayed on the mulberry leaf @50ml/200gm of mulberry leaf uniformly with the help of atomizer and the treated leaves were air dried for 10-20 minutes before feeding them to silkworm, *B. mori* L. on alternate days as first feed during 4th and 5th instar.

3.5 TECHNICAL PROGRAMME:

Target crop	:	Silkworm
Silkworm breed	:	Double-hybrid (CSR6×CSR26)×(CSR2×CSR27)
Factor 1	:	Vitamin
Levels of factor 1	:	08 V1: Thiamine V2: Riboflavin V3: Niacin V4: Pantothenic acid V5: Pyridoxine V6: Biotin V7: Folic Acid V8: Ascorbic Acid
Factor 2	:	Concentration of Vitamins: 03
Levels of factor 2	:	• 0 µg/ml(Control) • 10µg/ml • 20 µg/ml
Total no. of treatment	:	24
No. of replications per treatment	:	03
No. of silkworm per replication	:	100
Stage of treatment	:	4^{th} and 5^{th} instar
Design of experiment	:	Completely Randomized Design (factorial).

3.5.1 TREATMENT COMBINATION DETAILS:

Code	Vitamins	Dose
T1	Control	0 µg/ml
T2	Thiamine	10 µg/ml
T3	Thiamine	20 µg/ml
T4	Control	0 µg/ml
T5	Riboflavin	10 µg/ml
T6	Riboflavin	20 µg/ml
T7	Control	0 µg/ml
T8	Niacin	10 µg/ml
T9	Niacin	20 µg/ml
T10	Control	0 µg/ml
T11	Pantothenic acid	10 µg/ml
T12	Pantothenic acid	20 µg/ml
T13	Control	0 µg/ml
T14	Pyridoxine	10 µg/ml
T15	Pyridoxine	20 µg/ml
T16	Control	0 µg/ml
T17	Biotin	10 µg/ml
T18	Biotin	20µg/ml
T19	Control	0µg/ml
T20	Folic acid	10µg/ml
T21	Folic acid	20µg/ml
T22	Control	0µg/ml
T23	Ascorbic acid	10µg/ml
T24	Ascorbic acid	20µg/ml

3.6 PARAMETERS RECORDED

The various larval and cocoon parameters which were recorded during this study are as under:

1. Fifth age larval duration (hours):

The 5th age larval duration was calculated by recording the time in terms of total number of hours taken from the first day of 5th instar to date of seriposition.

2. Total larval duration (hours):

The total larval duration was calculated by recording the total number of hours taken from date of brushing to seriposition.

3. Weight of 10 mature larvae (g):

Ten mature larvae were randomly selected on 5th /6th day of last instar from each replication and weighed on digital balance to determine the average larval weight.

4. Silk gland weight (g):

10 larvae were randomly collected during 5th instar when the larvae attained the maximum weight (preferably on 5th or 6th day) from each replication and were dissected in order to take silk gland weight by using the formula:

$$\text{Silk gland weight} = \frac{\text{Total weight of silk gland}}{\text{No. of larvae taken for dissection}}$$

5. Silk gland somatic index:

Silk gland somatic index is the ratio between the weight of silk gland and larval body weight in grams. Silk gland somatic index was calculated as:

$$\text{Silk gland somatic index} = \frac{\text{Silk gland weight (g)}}{\text{Mature larval weight (g)}} \times 100$$

6. Cocoon yield/10,000 larvae (by number:)

The number of larvae retained and the resultant number of cocoons harvested from a standard unit of 10,000 larvae was calculated as:

$$\text{Cocoon yield by number} = \frac{\text{Number of cocoons harvested}}{\text{No. of worms retained after } 3^{rd} \text{ moult - No.of larvae taken for dissection}} \times 10000$$

7. **Cocoon yield/10,000 larvae (by weight kg:)**

The quantum of viable cocoons as expressed in kilograms procured from a standard unit of 10,000 larvae was calculated as:

$$\text{Cocoon yield by weight} = \frac{\text{Weight of cocoons harvested}}{\text{No. of worms retained after } 3^{rd} \text{ moult-No. of larvae taken for dissection}} \times 10000$$

8. **Silk productivity (cg/day):**

The silk productivity (cg/day) was calculated as:

$$\text{Silk productivity} = \frac{\text{Shell weight}}{5^{th} \text{ instar larval duration}}$$

9. **Single cocoon weight (g):**

In order to calculate single cocoon weight, 10 cocoons were randomly selected from each replication of each treatment and weighed on digital balance to determine average cocoon weight.

10. **Single shell weight (g):**

10 male and 10 female cocoons were taken randomly from each replication and cut open to take their shells. These 20 cocoon shells was weighed on a digital balance in order to calculate average single cocoon shell weight.

11. **Shell ratio:**

Shell percentage was calculated by using the formula:

$$\text{Shell percentage} = \frac{\text{Single cocoon shell weight (g)}}{\text{Single cocoon weight (g)}} \times 100$$

12. Filament length:

Average filament length was calculated by selecting 10 cocoons from each replication and reeled on an eupprouvette to get total length of raw silk spun by the larvae.

$$\text{Average filament length (m)} = \frac{\text{Total filament length (m)}}{\text{Total no. of cocoons reeled}}$$

13. Filament size:

It was calculated by using the following formula:

$$\text{Filament size} = \frac{\text{Conditioned weight of raw silk (g)}}{\text{Total length of raw silk (m)}} \times 9{,}000$$

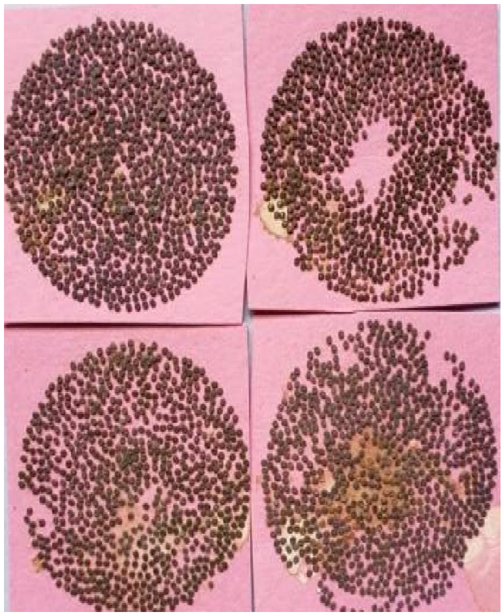
14. Raw silk percentage:

Raw silk percentage was calculated for the same cocoons used in filament length by using the following formula:

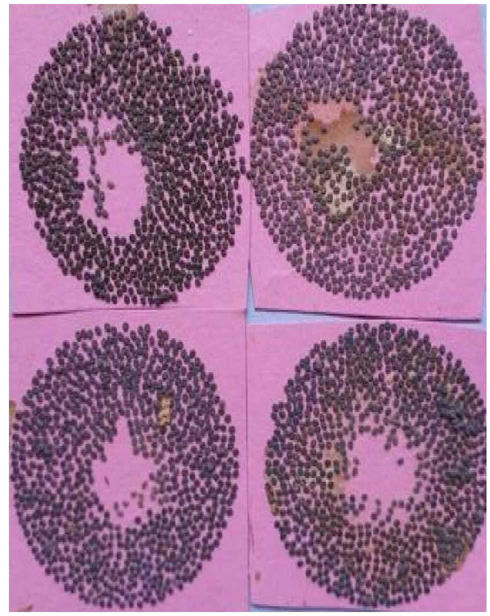
$$\text{Raw silk percentage} = \frac{\text{Weight of silk reeled (g)}}{\text{Weight of green cocoons (g)}} \times 100$$

15. Fecundity:

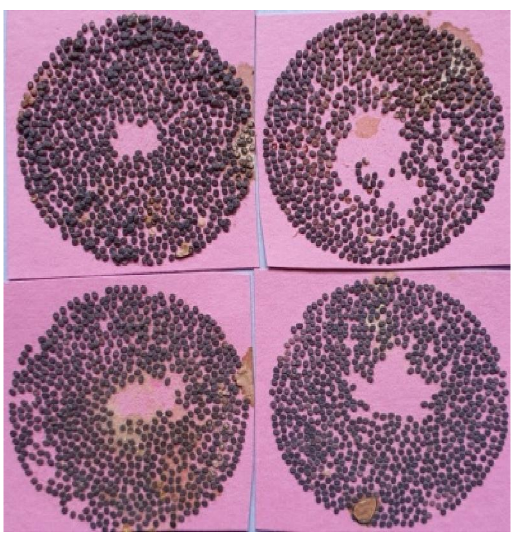
4 fertilized silkworm moths selected randomly from each replication of each treatment were allowed to lay eggs in plastic cellules. The eggs were counted and average fecundity worked out.



Eggs laid by silkworm batches treated with Ascorbic Acid



Eggs laid by silkworm batches treated by Riboflavin



Eggs laid by silkworm batches treated with Folic Acid

Plate 1: Egg laying of moths supplemented by different vitamin treatments

Spraying of Vitamins on mulberry leaves

Feeding of vitamin supplemented mulberry leaves

Plate 2: Spraying and feeding of Vitamins fortified mulberry leaves to silkworm

Dissecting silkworm larva

Dissected out silk gland

Plate 3: Dissection of silkworm to obtain silk gland from 5th instar larva

Chapter – 4

EXPERIMENTAL FINDINDS

Chapter-4

EXPERIMENTAL FINDINGS

The present chapter describes the experimental findings of the study entitled "Effect of vitamin supplements on the economic traits of silkworm *Bombyx mori* L." The observations recorded during the study are presented below:

4.1 5th instar larval duration (hours):

Significant difference *($p \leq 0.05$)* was observed in 5th instar larval duration among different vitamins and concentrations of vitamins (Table.1) Shortest 5th instar larval duration (168 hours) was recorded at 20ug/ml concentration than by concentration, 10ug/ml (171 hours) which was again significantly shorter than that of average control (186 hours). However, among the vitamins, there was a significant decrease in 5th instar and larval duration in Pyridoxine treated batches (173 hours) irrespective of concentrations followed by Ascorbic acid (175 hours), Folic acid (175 hours), Biotin (176 hours), Riboflavin (176 hours), Niacin (177 hours), Pantothenic acid (176 hours) and Thiamine (177 hours) respectively.

Among the treatment combinations, the interaction effect of vitamins supplemented mulberry leaf with concentration showed a significantly shortest 5th instar larval duration of 163 hours in silkworm batches fed with Pyridoxine at 20µg/ml concentration which was followed by Ascorbic acid, (167 hours) and Folic acid (168 hours) and Pyridoxine at 10µg/ml with a larval duration of 169 hours occupied fourth place in the overall performance of all treatment combinations and longest 5th instar larval duration of 174 hours was recorded in Thiamine at 10µg/ml concentration. However, the overall performance of the vitamin treatments observed was better at 20µg/ml concentration as compared to 10µg/ml.

4.2 Total larval duration (hours):

Significant difference *($p \leq 0.05$)* was observed in total larval duration among different vitamins and concentrations of vitamins. Shortest larval duration

(654 hours) was recorded at 20ug/ml concentration than (660 hours) at 10ug/ml concentration which was again significantly shorter than that of control (670 hours). However, among the vitamin treatments, there was a significant decrease in total larval duration in Pyridoxine and Ascorbic acid treated batches (659 hours) irrespective of concentrations followed by Ascorbic acid (659 hours), Folic acid (661 hours), Biotin (661 hours), Riboflavin (661 hours), Niacin (663 hours), Pantothenic acid (663 hours) and Thiamine (663 hours) respectively.

However, the interaction effect of vitamins supplemented mulberry leaf with concentrations showed a significantly shortest total larval duration of 650 hours in silkworm batches fed with Ascorbic acid at 20µg/ml concentration which was followed by 651 hours in Pyridoxine, 652 hours in Folic acid, 654 hours in Biotin, 655 hours in Riboflavin, 656 hours in Niacin, 658 hours in Pantothenic acid respectively at the same concentration and longest larval duration of 662 hours was recorded in Thiamine at 10µg/ml concentration.

Table 1: Effect of vitamins supplemented mulberry leaf on 5th instar larval duration (hours) of silkworm, *Bombyx mori* L.

Concentration / Vitamin	C0: 0µg/ml (Control)	C1:10µg/ml	C2: 20µg/ml	Mean
Thiamine	187	174	170	**177**
Riboflavin	186	173	170	**176**
Niacin	188	172	171	**177**
Pantothenic acid	186	172	170	**176**
Pyridoxine	188	169	163	**173**
Biotin	185	172	170	**176**
Folic acid	185	171	168	**175**
Ascorbic acid	188	171	167	**175**
Mean	**186**	**171**	**168**	

C.D ($p \leq 0.05$)

Vitamins (V) : 1.207
Concentration (C) : 0.904
V×C : 2.111

Table 2: Effect of vitamins supplemented mulberry leaf on total larval duration (hours) of silkworm, *Bombyx mori* L.

Vitamin \ Concentration	C0:10µg/ml (Control)	C1:10µg/ml	C2: 20µg/ml	Mean
Thiamine	669	661	659	**663**
Riboflavin	670	659	655	**661**
Niacin	671	662	656	**663**
Pantothenic acid	670	662	658	**663**
Pyridoxine	671	657	651	**659**
Biotin	669	660	654	**661**
Folic acid	670	661	652	**661**
Ascorbic acid	669	658	650	**659**
Mean	**670**	**660**	**654**	

C.D ($p \leq 0.05$)

Vitamins (V) : 1.71

Concentration (C) : 1.047

V×C : 2.962

4.3 Larval weight (g):

The larval weight showed significant difference ($p \leq 0.05$) among different vitamins and concentrations of vitamins, 20ug/ml concentration showed significantly higher larval weight (48.23 g) than by the concentration, 10ug/ml (45.94 g) which was again significantly superior than that of control (40.64 g.) However, there was a significant increase in larval weight in Pyridoxine treated batches (46.78) irrespective of concentrations followed by Ascorbic acid (46.29), Folic acid (46.22), Biotin (45.58), Riboflavin (44.76), Niacin (43.66), Pantothenic acid (43.25), Thiamine (42.94.)

Among the treatment combinations, the interaction effect of vitamins supplemented mulberry leaf with respect to control treatments showed a significantly highest larval weight of 52.25 g in silkworm batches fed with Pyridoxine at 20µg/ml concentration, which was followed by Ascorbic acid, (51 g) Folic acid (50.74), Biotin (50.24), Riboflavin (46.99), Niacin (45.56), Pantothenic acid (44.99) respectively at 20µg/ml and the lowest larval weight of 43.89 g was recorded in Thiamine at 10µg/ml concentration.

4.4 Silk gland weight (g):

The silk gland weight showed significant difference *($p \leq 0.05$)* among different treatments and concentrations of vitamins showed significantly higher silk gland weight of 1.86 grams was recorded at 20ug/ml concentration than by concentration of 10ug/ml (1.74 grams) which was again significantly superior than that of control (1.42 grams). However, there was a significant increase in silk gland weight of Pyridoxine 1.74 treated batches irrespective of concentrations followed by Ascorbic acid 1.72, Folic acid 1.71, Biotin 1.69, Riboflavin 1.67, Niacin 1.65, Pantothenic acid 1.62, Thiamine 1.59.

The statistical analysis of the data revealed that among the 16 treatment combinations, the interaction effect of vitamins supplemented mulberry leaf with concentrations showed a significantly higher silk gland weight of 1.97 grams in silkworm batches fed with Pyridoxine at 20µg/ml concentration, which was followed silkworm batches fed with Ascorbic acid (1.94 grams), Folic acid (1.92

grams), Biotin (1.90 grams), Riboflavin (1.86 grams), Niacin (1.81 grams), Pantothenic acid (1.78 grams) respectively at 20µg/ml and lowest silk gland weight of 1.63 grams was recorded in Thiamine at 10µg/ml concentration.

4.5 Silk gland somatic index:

Significant difference *($p \leq 0.05$)* was observed in silk gland somatic index among different treatments and concentrations of vitamins showed significantly higher silk somatic index of 34.17 per cent at 20µg/ml concentration than by concentration of 10ug/ml 32.65 per cent which was again significantly superior than that of control (29.58 per cent). However, there was a significant increase in silk gland somatic index in Pyridoxine 33.77 treated batches irrespective of concentrations followed by Ascorbic acid 32.86, Folic acid 32.29, Biotin 32.43, Riboflavin 31.75, Niacin 31.43, Pantothenic acid 31.35, Thiamine 31.20.

The statistical analysis of the data revealed that among the 16 treatment combinations, the interaction effect of vitamins supplemented mulberry leaf with concentrations showed a significantly higher silk gland somatic index of 36.52 per cent in silkworm batches fed with Pyridoxine at 20µg/ml concentration, which was followed by 35.64 per cent in Ascorbic acid, Pyridoxine at 10µg/ml with a silk gland somatic index of 35.22% occupied 3rd rank in the overall performance of 16 treatment combinations under study followed by Folic acid (34.74%) and Biotin 34.45%, as some of the other outstanding combinations. However, lowest silk gland somatic index of 31.33 per cent was recorded in Thiamine at 10µg/ml concentration.

Table 3: Effect of vitamins supplemented mulberry leaf on weight of 10 mature larvae (g) silkworm, *Bombyx mori* L.

Concentration / Vitamin	C0:0µg/ml (Control)	C1:10µg/ml	C2:20µg/ml	Mean
Thiamine	40.89	43.89	44.03	**42.94**
Riboflavin	40.40	46.90	46.99	**44.76**
Niacin	40.79	44.63	45.56	**43.66**
Pantothenic acid	40.66	44.10	44.99	**43.25**
Pyridoxine	40.60	47.49	52.25	**46.78**
Biotin	40.00	46.52	50.24	**45.58**
Folic acid	41.09	46.82	50.74	**46.22**
Ascorbic acid	40.69	47.20	51.00	**46.29**
Mean	**40.64**	**45.94**	**48.23**	

C.D ($p \leq 0.05$)

Vitamins (V) : 0.31
Concentration (C) : 0.19
V×C : 0.53

Table 4: Effect of vitamins supplemented mulberry leaf on silk gland weight (g) of silkworm, *Bombyx mori* L.

Concentration / Vitamin	C0:0µg/ml (Control)	C1:10µg/ml	C2: 20µg/ml	Mean
Thiamine	1.40	1.63	1.74	**1.59**
Riboflavin	1.42	1.74	1.86	**1.67**
Niacin	1.43	1.71	1.81	**1.65**
Pantothenic acid	1.41	1.66	1.78	**1.62**
Pyridoxine	1.42	1.83	1.97	**1.74**
Biotin	1.42	1.74	1.90	**1.69**
Folic acid	1.43	1.78	1.92	**1.71**
Ascorbic acid	1.42	1.81	1.94	**1.72**
Mean	**1.42**	**1.74**	**1.86**	

C.D ($p \leq 0.05$)

Vitamins (V) : 0.022

Concentration (C) : 0.011

V×C : 0.033

Table 5: Effect of vitamins supplemented mulberry leaf on silk gland somatic index (%) of silkworm, *Bombyx mori* L.

Concentration / Vitamin	C0:0µg/ml (Control)	C1:10µg/ml	C2: 20µg/ml	Mean
Thiamine	29.52	31.33	32.76	**31.20**
Riboflavin	29.59	32.24	33.42	**31.75**
Niacin	29.53	31.78	32.97	**31.43**
Pantothenic acid	29.61	31.59	32.86	**31.35**
Pyridoxine	29.56	35.22	36.52	**33.77**
Biotin	29.61	33.24	34.45	**32.43**
Folic acid	29.65	32.48	34.74	**32.29**
Ascorbic acid	29.61	33.34	35.64	**32.86**
Mean	**29.58**	**32.65**	**34.17**	

C.D ($p \leq 0.05$)

Vitamins (V) : 0.31

Concentration (C) : 0.19

V×C : 0.53

4.6 Cocoon yield/10,000 larvae (by number):

Significant difference ($p \leq 0.05$) was observed in Cocoon yield/10,000 larvae by number among different treatments and concentrations of vitamins 20ug/ml concentration showed significantly higher cocoon yield by number (8927) than by concentration of 10ug/ml (8413) which was again significantly superior than that of control (7717). However, there was a significant increase in cocoon yield by number (8782) in Pyridoxine treated batches irrespective of concentrations followed by Ascorbic acid (8682), Folic acid (8590), Biotin (8504), Riboflavin (8371), Niacin (8078), Pantothenic acid (7921), and Thiamine (7892.)

The statistical analysis of the data revealed that among the 16 treatment combinations, maximum cocoon yield by number (9615) was recorded in silkworm batches fed with pyridoxine at 20ug/ml concentration. This treatment combination was significantly superior to all the treatments followed by Ascorbic acid (9410), Folic acid (9323), Biotin (9284) and Riboflavin (9117) at the same concentration. Pyridoxine, again at 10ug/ml occupied sixth rank with a yield of 8964 followed by Ascorbic acid (8874) and Folic acid (8680) at some of the other significant treatment combinations. Lowest yield (7990) was recorded with Thiamine at 10ug/ml concentration.

4.7 Cocoon yield/10,000 larvae (by weight kg):

Among the different treatments and concentrations there was a significant difference ($p \leq 0.05$) in cocoon yield/10,000 larvae by weight. The different vitamins of 20ug/ml concentration (16.25 kg) showed significantly higher cocoon yield by weight than by concentration 10ug/ml (15.17 kg) which was again significantly superior than that of control (13.14 kg). However, there was a significant increase in cocoon yield by weight in Pyridoxine 15.95 treated batches irrespective of concentrations followed by Ascorbic acid 15.71, Folic acid 15.31, Biotin 15.00, Riboflavin 14.68, Niacin 14.35, Pantothenic acid 13.92, Thiamine 13.91.

The statistical analysis of the data revealed that among the 16 treatment combinations, the interaction effect of vitamins supplemented mulberry leaf with respect to control treatments showed a significantly highest cocoon yield weight of 18.17 kg in silkworm batches fed with Pyridoxine at 20µg/ml concentration, which was followed by 17.59 kg in Ascorbic acid, 17.16 kg in Folic acid, 16.69 kg in Biotin, 16.20 kg in Riboflavin, 15.49 kg in Niacin 14.38 kg in Pantothenic acid respectively at concentration 20ug/ml and lowest cocoon yield by weight of 14.19 kg was recorded in Thiamine at 10ug/ml concentration.

4.8 Silk productivity (cg/day):

Significant difference *($p \leq 0.05$)* was observed in silk productivity among different treatments and concentrations of vitamins 20ug/ml concentration (6.87cg) showed significantly higher silk productivity than by concentration 10ug/ml (5.63cg) which was again significantly superior than that of control (4.26 cg). However, there was a significant increase in silk productivity of Pyridoxine 6.04 treated batches irrespective of concentrations followed by Ascorbic acid 5.88, Folic acid 5.79, Biotin 5.71, Riboflavin 5.35, Niacin 5.37, Pantothenic acid 5.37, Thiamine 5.18.

The statistical analysis of the data represented that among the 16 treatment combinations, the interaction effect of vitamins supplemented mulberry leaf with respect to control treatments showed a significantly higher silk productivity of 7.54 cg in silkworm batches fed with Pyridoxine at 20µg/ml concentration, which was followed by 7.41 cg in Ascorbic acid, 7.33 cg in Folic acid, 7.21 cg in Biotin. 6.54 cg in Riboflavin, 6.41 cg in Niacin, 6.32 cg in Pantothenic acid respectively at 20µg/ml concentration and lowest silk productivity of 5.12 cg was recorded in Thiamine at 10ug/ml concentration.

Table 6: Effect of vitamins supplemented mulberry leaf on cocoon yield/10,000 larvae by number of silkworm, *Bombyx mori* L.

Concentration / Vitamin	C0:0µg/ml (Control)	C1:10µg/ml	C2: 20µg/ml	Mean
Thiamine	7664	7990	8023	**7892**
Riboflavin	7665	8332	9117	**8371**
Niacin	7672	8011	8550	**8078**
Pantothenic acid	7677	7991	8095	**7921**
Pyridoxine	7767	8964	9615	**8782**
Biotin	7762	8467	9284	**8504**
Folic acid	7767	8680	9323	**8590**
Ascorbic acid	7761	8874	9410	**8682**
Mean	**7717**	**8413**	**8927**	

C.D ($p \leq 0.05$)
Vitamins (V) : 52.275
Concentration (C) : 37.517
V×C : 89.792

Table 7: Effect of vitamins supplemented mulberry leaf on cocoon yield/10,000 larvae by weight (kg) of silkworm, *Bombyx mori* L.

Concentration / Vitamin	C0:0µg/ml (Control)	C1:10µg/ml	C2: 20µg/ml	Mean
Thiamine	13.20	14.19	14.37	**13.91**
Riboflavin	13.10	14.75	16.20	**14.68**
Niacin	13.16	14.40	15.49	**14.35**
Pantothenic acid	13.15	14.22	14.38	**13.92**
Pyridoxine	13.10	16.59	18.17	**15.95**
Biotin	13.17	15.15	16.69	**15.00**
Folic acid	13.13	15.64	17.16	**15.31**
Ascorbic acid	13.14	16.41	17.59	**15.71**
Mean	**13.14**	**15.17**	**16.25**	

C.D ($p \leq 0.05$)

Vitamins (V) : 0.43
Concentration (C) : 0.18
V×C : 0.61

Table 8: Effect of vitamins supplemented mulberry leaf on silk productivity (cg/day) of silkworm, *Bombyx mori* L.

Concentration / Vitamin	C0:0µg/ml (Control)	C1:10µg/ml	C2: 20µg/ml	Mean
Thiamine	4.23	5.12	6.19	**5.18**
Riboflavin	4.26	5.26	6.54	**5.35**
Niacin	4.24	5.47	6.41	**5.37**
Pantothenic acid	4.24	5.55	6.32	**5.37**
Pyridoxine	4.31	6.27	7.54	**6.04**
Biotin	4.27	5.65	7.21	**5.71**
Folic acid	4.27	5.77	7.33	**5.79**
Ascorbic acid	4.29	5.95	7.41	**5.88**
Mean	**4.26**	**5.63**	**6.87**	

C.D ($p \leq 0.05$)

Vitamins (V) : 0.25

Concentration (C) : 0.16

V×C : 0.44

4.9 Single cocoon weight (g):

Significant difference *(p≤0.05)* was observed in single cocoon weight among different treatments and concentrations, of vitamins 20ug/ml concentration (1.809 grams) showed significantly higher single cocoon weight than by concentration of 10µg/ml (1.772 grams) which was again significantly superior than that of control (1.689 grams). However, there was a significant increase in single cocoon weight of Pyridoxine 1.808 treated batches irrespective of concentrations followed by Ascorbic acid 1.788, Folic acid 1.774, Biotin 1.748, Riboflavin 1.748, Niacin 1.738, Pantothenic acid 1.734, Thiamine 1.717.

Among the 16 treatment combinations, the interaction effect of vitamins supplemented mulberry leaf showed a significantly higher single cocoon weight of 1.887 grams in silkworm batches fed with Pyridoxine at 20µg/ml concentration, which was followed by 1.867 grams in Ascorbic acid and 1.860 grams with Pyridoxine at 10µg/ml Folic acid (1.840 grams), Biotin (1.797), Riboflavin (1.790 grams), Niacin (1.780 grams), Pantothenic acid (1.767 grams) respectively at 20µg/ml were some of the other promising treatment combinations. Lowest single cocoon weight of 1.720 grams was recorded in Thiamine at 10µg/ml concentration.

4.10 Single shell weight (g):

A significant difference *(p≤0.05)* was observed in single shell weight among different treatments and concentrations, of vitamins the significantly higher single shell weight was recorded at 20ug/ml concentration (0.403g) than by the concentration of 10ug/ml (0.391g) which was again significantly superior than that of control (0.331 g).

Among the 16 treatment combinations, the interaction effect of vitamins supplemented mulberry leaf with concentration showed a significantly higher single shell weight of 0.430 g in silkworm batches fed with Pyridoxine at 20µg/ml concentration, which was followed by 0.425 g in Ascorbic acid, 0.415 g in Folic acid, 0.399 g in Biotin, 0.395 g in Riboflavin, 0.392 g in Niacin, 0.384 g in Pantothenic acid respectively at 20µg/ml concentration and lowest single shell weight of 0.372 g was recorded in Thiamine at 10µg/ml concentration.

4.11 Shell ratio

Significant difference *($p \leq 0.05$)* was observed in shell ratio among different treatments and concentrations, of vitamins the significantly higher shell ratio was recorded at 20ug/ml concentration (22.26 per cent) than by the concentration of 10ug/ml (22.02 per cent) which was again significantly superior than that of control (19.59 per cent). However, there was a significant increase in shell ratio of Pyridoxine 21.76 treated batches irrespective of concentrations followed by Ascorbic acid 21.77, Folic acid , Biotin 0.371, Riboflavin 0.369, Niacin 0.367, Pantothenic acid 0.363, Thiamine 0.360.

Among the treatment combinations, the interaction effect of vitamins supplemented mulberry leaf with concentrations showed a significantly higher shell ratio of 22.84 per cent in silkworm batches fed with Pyridoxine at 20µg/ml concentration, which was followed by 22.82 per cent in Ascorbic acid, 22.60 per cent in Folic acid. Both Pyridoxine and Ascorbic acid at 10µg/ml registered shell ratio of 22.25 per cent. However, Lowest shell ratio of 21.62 was recorded in Thiamine at 10µg/ml concentration.

4.12 Filament length (m):

Significant difference *($p \leq 0.05$)* was observed in filament length among different treatments and concentrations, of vitamins 20ug/ml concentration showed significantly higher filament length of 1145 meters which was followed by 10ug/ml (1090 meters) which was again significantly superior than that of control (960 meters). However, there was a significant increase in filament length of Pyridoxine 1096 treated batches irrespective of concentrations followed by Ascorbic acid 1082, Folic acid 1075, Biotin 1071, Riboflavin 1057, Niacin 1053, Pantothenic acid 1047, Thiamine 1039.

The statistical analysis of the data revealed that among the 16 treatment combinations, the interaction effect of vitamins supplemented mulberry leaf with concentration a significantly higher filament length of 1184 meters in silkworm batches fed with Pyridoxine at 20µg/ml concentration, which was followed by Ascorbic acid 1174 meters, 1167 meters in Folic acid, 1154 meters in Biotin, Pyridoxine at 10µg/ml with a filament length of 1145 meters occupied 5th place in the overall perform of 16 treatment combinations. However, lowest filament length of 1057 meters was recorded in Thiamine at 10µg/ml concentration.

Table 9: Effect of vitamins supplemented mulberry leaf on single cocoon weight (g) of silkworm, *Bombyx mori* L.

Concentration / Vitamin	C0:0µg/ml (Control)	C1:10µg/ml	C2: 20µg/ml	Mean
Thiamine	1.683	1.720	1.747	**1.717**
Riboflavin	1.703	1.750	1.790	**1.748**
Niacin	1.690	1.743	1.780	**1.738**
Pantothenic acid	1.707	1.730	1.767	**1.734**
Pyridoxine	1.677	1.860	1.887	**1.808**
Biotin	1.690	1.757	1.797	**1.748**
Folic acid	1.687	1.797	1.840	**1.774**
Ascorbic acid	1.677	1.820	1.867	**1.788**
Mean	**1.689**	**1.772**	**1.809**	

C.D ($p \leq 0.05$)

Vitamins (V) : 0.014
Concentration (C) : 0.009
V×C : 0.024

Table 10: Effect of vitamins supplemented mulberry leaf on Single shell-weight of silkworm, *Bombyx mori* L.

Vitamin \ Concentration	C0:0µg/ml (Control)	C1:10µg/ml	C2: 20µg/ml	Mean
Thiamine	0.328	**0.372**	0.381	**0.360**
Riboflavin	0.331	0.380	0.395	**0.369**
Niacin	0.330	0.379	0.392	**0.367**
Pantothenic acid	0.329	0.377	0.384	**0.363**
Pyridoxine	0.336	0.421	**0.430**	**0.396**
Biotin	0.330	0.386	0.399	**0.371**
Folic acid	0.331	0.400	0.415	**0.382**
Ascorbic acid	0.334	0.412	0.425	**0.390**
Mean	**0.331**	**0.391**	**0.403**	

C.D ($p \leq 0.05$)

Vitamins (V) : 0.002

Concentration(C) : 0.001

V×C : 0.004

Table 11: Effect of vitamins supplemented mulberry leaf on shell ratio of silkworm, *Bombyx mori* L.

Concentration / Vitamin	C0:0µg/ml (Control)	C1:10µg/ml	C2: 20µg/ml	Mean
Thiamine	19.36	21.62	21.69	**20.89**
Riboflavin	19.54	22.00	22.07	**21.20**
Niacin	19.58	21.89	21.95	**21.14**
Pantothenic acid	19.39	21.79	21.84	**21.00**
Pyridoxine	19.98	22.52	22.84	**21.77**
Biotin	19.40	21.53	22.29	**21.07**
Folic acid	19.56	22.31	22.60	**21.49**
Ascorbic acid	19.92	22.52	22.82	**21.76**
Mean	**19.59**	**22.02**	**22.26**	

C.D ($p \leq 0.05$)
Vitamins (V) : 0.25
Concentration (C) : 0.15
V×C : 0.43

Table 12: Effect of vitamins supplemented mulberry leaf on average filament length (m) of silkworm, *Bombyx mori* L.

Concentration / Vitamin	C0:0µg/ml (Control)	C1:10µg/ml	C2: 20µg/ml	Mean
Thiamine	952	1057	1108	**1039**
Riboflavin	956	1083	1132	**1057**
Niacin	959	1075	1127	**1053**
Pantothenic acid	963	1061	1116	**1047**
Pyridoxine	961	1145	1184	**1096**
Biotin	966	1095	1154	**1071**
Folic acid	958	1101	1167	**1075**
Ascorbic acid	963	1109	1174	**1082**
Mean	**960**	**1090**	**1145**	

C.D ($p \leq 0.05$)

Vitamins (V) : 3.777
Concentration (C) : 1.926
V×C : 8.275

4.13 Filament size:

Significant difference *($p \leq 0.05$)* was observed in filament size among different treatments and concentrations, of vitamins 20ug/ml concentration showed thinnest filament size of 2.62 which was followed by 10ug/ml (2.69) as against control (2.90) which showed the highest filament size. However, there was a significant decrease in filament length of Pyridoxine 2.66 treated batches irrespective of concentrations followed by Ascorbic acid 2.69, Folic acid 2.71, Biotin 2.71, Riboflavin 2.77, Niacin 2.77, Pantothenic acid 2.79, Thiamine 2.78.

Among the treatment combinations, the interaction effect of vitamins supplemented mulberry leaf with respect to control treatments showed the lowest filament size of 2.47 in silkworm batches fed with Pyridoxine at 20µg/ml concentration, which was followed by Ascorbic acid 2.52, 2.55 in Folic acid, 2.59 in Biotin, 2.67 in Riboflavin, 2.69 in Niacin, 2.72 in Pantothenic acid respectively at 20µg/ml concentration and highest filament size of 2.78 was recorded in Thiamine at 10µg/ml concentration.

4.14 Raw silk percentage:

Among the treatments and concentrations a significant difference *($p \leq 0.05$)* was observed in raw silk percentage of vitamins 20ug/ml concentration showed higher raw silk percentage of (16.85 per cent) followed by concentration 10ug/ml (16.35 per cent) which was again significantly superior than that of control (14.56 per cent). However, there was a significant increase in raw silk percentage of Pyridoxine 16.28 treated batches irrespective of concentrations followed by Ascorbic acid 16.19, Folic acid 16.01, Biotin 15.95, Riboflavin 15.88, Niacin 15.84, Pantothenic acid 15.63, Thiamine 15.57.

The statistical analysis of the data represented that among the treatment combinations, the interaction effect of vitamins supplemented mulberry leaf with respect to control treatments showed a significantly higher raw silk percentage of 17.45 per cent in silkworm batches fed with Pyridoxine at 20µg/ml concentration, which was followed by 17.23 per cent in Ascorbic acid, 16.94 per cent in Folic acid, 16.82 per cent in Biotin, 16.74 per cent in Riboflavin, 16.61 in Niacin, 16.57

in Pantothenic acid respectively at 20µg/ml concentration and lowest raw silk per cent of 15.74 per cent was recorded in Thiamine at 10ug/ml concentration.

4.15 Fecundity:

Significant difference *($p \leq 0.05$)* was observed in fecundity among different treatments and concentrations, of vitamins 20ug/ml concentration showed significantly higher fecundity of 626 eggs per layings which was followed by concentration of 10ug/ml (617) which was again significantly superior than that of control (523). However, there was a significant increase in fecundity of Ascorbic acid 619 treated batches irrespective of concentrations followed by Riboflavin 611, 608 in Folic acid, 603 in Biotin, 601 in Thiamine, 598 in Niacin, 595 in Pantothenic acid, 476 in Pyridoxine.

The statistical analysis of the data represented that among the treatment combinations, the interaction effect of vitamins supplemented mulberry leaf with respect to control treatments showed a significantly higher fecundity 685 eggs in silkworm batches fed with Ascorbic acid at 20µg/ml concentration, which was followed by 667 eggs per layings in Riboflavin, 662 in Folic acid, 656 in Biotin, 653 in Thiamine, 648 in Niacin, 640 in Pantothenic acid respectively at 20µg/ml concentration and lowest fecundity of 401 was recorded in Pyridoxine at 20µg/ml concentration.

Table 13: **Effect of vitamins supplemented mulberry leaf on filament size (denier) of silkworm, *Bombyx mori* L.**

Concentration / Vitamin	C0:0µg/ml (Control)	C1:10µg/ml	C2: 20µg/ml	Mean
Thiamine	2.83	2.78	2.74	**2.78**
Riboflavin	2.90	2.74	2.67	**2.77**
Niacin	2.87	2.76	2.69	**2.77**
Pantothenic acid	2.91	2.75	2.72	**2.79**
Pyridoxine	2.85	2.65	2.47	**2.66**
Biotin	2.92	2.62	2.59	**2.71**
Folic acid	2.94	2.65	2.55	**2.71**
Ascorbic acid	2.97	2.59	2.52	**2.69**
Mean	**2.90**	**2.69**	**2.62**	

C.D ($p \leq 0.05$)
Vitamins (V) : 0.063
Concentration (C) : 0.039
V×C : 0.109

Table 14: Effect of vitamins supplemented mulberry leaf on raw silk percentage of silkworm, *Bombyx mori* L.

Concentration / Vitamin	C0:0µg/ml (Control)	C1:10µg/ml	C2: 20µg/ml	Mean
Thiamine	14.55	15.74	16.44	**15.57**
Riboflavin	14.53	16.37	16.74	**15.88**
Niacin	14.60	16.31	16.61	**15.84**
Pantothenic acid	14.50	15.83	16.57	**15.63**
Pyridoxine	14.54	16.85	17.45	**16.28**
Biotin	14.59	16.44	16.82	**15.95**
Folic acid	14.54	16.56	16.94	**16.01**
Ascorbic acid	14.63	16.72	17.23	**16.19**
Mean	**14.56**	**16.35**	**16.85**	

C.D ($p \leq 0.05$)

Vitamins (V)	:	0.24
Concentration (C)	:	0.15
V×C	:	0.41

Table 15: Effect of vitamins supplemented mulberry leaf on fecundity of silkworm, *Bombyx mori* L.

Concentration / Vitamin	C0:0µg/ml (Control)	C1:10µg/ml	C2: 20µg/ml	Mean
Thiamine	520	630	653	**601**
Riboflavin	525	641	667	**611**
Niacin	523	625	648	**598**
Pantothenic acid	527	620	640	**595**
Pyridoxine	526	503	**401**	**476**
Biotin	519	635	656	**603**
Folic acid	523	639	662	**608**
Ascorbic acid	522	650	**685**	**619**
Mean	**523**	**617**	**626**	

C.D ($p \leq 0.05$)
Vitamins (V) : 2.703
Concentration (C) : 1.655
V×C : 4.682

Chapter – 5

DISCUSSION

Chapter-5

DISCUSSION

An insect receives energy from food to support its demands for growth, development, reproduction, and a variety of other functions (Chapman, 1998). Because their body's primary chemical constituents and metabolic pathways are similar throughout, so are their dietary requirements. Although insects are capable of producing some of the nutrients on their own, others must be obtained through food or symbiotic organisms that they harbour (Bursell, 1970; Chapman, 1998). Despite having incredibly diverse nutritional requirements, silkworms get the majority of their nutrition from feeding mulberry leaves. Although the primary food supply for silkworms is mulberry leaves, it is still possible for some deficiencies to occur for a variety of reasons. The silkworm, *B.mori* L. like other organisms, benefits from proper nutrition in terms of growth and development. According to Legay (1958), the nutritional value of mulberry leaves has a significant impact on the ability to produce high-quality cocoons. When silkworm larvae were fed on nutritionally enhanced leaves, better growth and development as well as high-quality cocoons are produced (Seki and Oshikane 1959).

Insects and other animals need the water-soluble organic micronutrients known as the B- vitamins for uniform growth and survival. They are only needed in relatively small quantities since they function as coenzymes and support enzymatic reactions without being consumed or produced during the reaction. Although at least 20 compounds have historically been referred to be B vitamins, considerable nutritional study on humans and other mammals has only identified eight B vitamins that are required for diets and function as coenzymes (Douglas, 2017) and therefore, recent research on the B vitamin nutrition of insects has mostly focused on these eight compounds.

Surprisingly, we know very little about the role of the B-vitamin family: Thiamine (B1), Riboflavin (B2), Niacin (B3), Pantothenic acid (B5), Pyridoxine B6, Biotin (B8), Folic acid (B9) in silkworm nutrition. The amount of vitamins present in mulberry leaves varies depending on environmental factors, the use of fertilizers in the field, mulberry variety, and other field practices, according to research by Ito (1978). Nutrient supplements leads to a higher yield since the development of silk in both quality and quantity depends on the health and nutrition of the larva, both of which are influenced by the nutritional value of mulberry leaves (Ito, 1978). Keeping in view the importance of Vitamins in silkworm nutrition, the present study was carried out to determine enrichment efficacy of mulberry leaves by vitamins on the growth and economic traits of silkworms, *B. mori* L. During the present study, eight water-soluble vitamins, including Thiamine (B1), Riboflavin (B2), Niacin (B3), Pantothenic acid (B5), Pyridoxine (B6), Biotin (B8), Folic acid (B9), and Vitamin C (Ascorbic acid), were used to fortify mulberry leaves and determine their effects on the growth and economic traits of silkworm, *B. mori* L.

5.1 Impact of supplemented Vitamins on commercial traits of silkworms, *Bombyx mori* L.

The present results revealed that there was a significant decrease in 5th instar and total larval duration with Pyridoxine supplemented mulberry leaves (163, 651 hours) followed by Ascorbic acid (167, 650 hours) and Folic acid (168, 652 hours) at 20µg/ml concentration as against control (186, 670 hours). The current results are in conformity with the findings of Ahsan *et al.* (2013) who found that larval and pupal periods were significantly reduced at lower concentration by mixing of 5.8% Vitamin B+25% Vitamin C supplemented leaves as compared to control. When the amount of meals was lowered from 8 to 2, Haniffa *et al*. (1994) noticed longer larval duration in each instar. The nutritional value of the vitamin-treated mulberry leaves may be the cause of the variation in the larval duration between control and vitamin-supplemented leaves. Different larval lengths in the same race throughout various seasons and with various mulberry types have been documented by a number of researchers (Muthukrishnan and Delvi, 1974 in Grasshoper) Mathawan and Muthukrishnan, 1976 in Danaus Chrysippus) Haniffa and Periasamy, 1981 in Acrotylus insubricus).

In the present study, It has also been found that among the vitamin treatments, significantly highest larval weight was recorded in Pyridoxine supplemented batches at both 10 & 20 µg/ml concentrations (47.49, 52.25 g) followed by Ascorbic acid (47.20, 51.00 g), Folic acid (46.82, 50.74 g) and the lowest mean larval weight of 40.64 g was recorded in Control. Among the vitamin concentrations, highest larval weight irrespective of vitamins was recorded at 20 µg/ml concentration (48.23 g) than by 10 µg/ml concentration (45.94 g), respectively. El-Karkasy and Idriss (1990) also reported that supplementation of mulberry leaves with 0.25-2.0% ascorbic acid increases the larval weight of silkworm, *Bombyx mori* L. Dietary supplementation of 2% ascorbic acid increased larval weight by 7.8% (Etebari *et al*., 2004).

Observations on the growth of silk gland revealed significantly higher silk gland weight of 1.97 g in silkworm batches fed with Pyridoxine at 20µg/ml concentration supplemented mulberry leaves, followed by ascorbic acid (1.94 g) and folic acid (1.90 g) as against average control (1.42 g). Saha and Khan (1995) also found that addition of 2.0% Ascorbic acid resulted in an improvement in larval weight, silk gland weight, cocoon and shell weight. The addition of vitamin B2 nanoparticles had a favourable effect and considerably increased the larval growth (28.985%), the weight of silk gland (111.392%), and silk yield (194.44%) when compared to the control.

The silk gland somatic index (SSI) represents the biomass of the silk gland in relation to total body weight. In the present study, the values among all the treatments are relatively higher in Pyridoxine, Ascorbic acid, Folic acid and Biotin than other vitamins (Riboflavin, Niacin, Pantothenic acid and Thiamine). The present result revealed maximum silk gland somatic index at 20µg/ml vitamin concentration resulted in higher growth rates for silk gland. The difference in silk gland somatic index could be traced to the high silk gland somatic index values in all the two amino acid concentration of Pyridoxine and Ascorbic acid. The results of the silk gland somatic index which is the weight of the silk gland relative to body mass showed that the growth of the silk gland is increased by the stimulatory influence of Pyridoxine to protein synthesis. Pyridoxine is a rate-limiting cofactor in the synthesis of neurotransmitters like dopamine, serotonin, aminobutyric acid (GABA), nor adrenaline, and the hormone melatonin because of its role in amino acid metabolism (Kenedy, 2016). Singh and Kumar (1996) noted a 27.9% somatic index for silk glands in silkworm breed NB18. The accumulation of organic substances, namely proteins, is indicative of the formation of the silk gland (Ito and Arai, 1967; Tazima, 1978).

Cocoon yield/10,000 larvae by number and weight are correlated traits and in present study they were found higher among all the treatments irrespective of concentrations with significantly higher yield (8782, 15.95 kg) in silkworm batches fed with Pyridoxine followed by Ascorbic acid (8682, 15.71 kg), Folic acid (8590, 15.31 kg) and Biotin (8504, 15.00 kg) as compared to control (7717, 13.14 kg). The cocoon yield was significantly increased in the batches fed with Pyridoxine supplemented mulberry leaves at 10µg/ml and 20µg/ml vitamin concentrations as against control batches, thus showing the influence of vitamins on the survivability and cocoon production.

Silk productivity (cg/day) again better in Pyridoxine fed worms (7.54) followed by Ascorbic acid (7.41), Folic acid (7.33), and Biotin (7.21) than the average of control batches (4.26). According to Horie and Inokuchi (1978), distinct races of the mulberry silkworm, *B.mori*, use amino acids that have undergone deamination for the production of fatty acids.

The observations on cocoon weight and shell weight revealed that Pyridoxine fed worms expressed significantly higher cocoon weight and shell weight followed by Ascorbic acid, Folic acid, and Biotin. According to Devi and Yellama (2013), the increase in cocoon weight and shell weight might be due to the increase in absorption of supplemented vitamins. In the posterior silk gland, zinc + vitamin B6 + hormone caused active turnover of all protein profiles and metabolic activities, generating favourable metabolic circumstances for growth and silk production in silkworm larvae fed with mixed dosage. According to Nirwani and Kaliwal (1996), adding folic acid to the diet significantly enhanced the silkworm's economic traits by 20–30%. The co-factor folic acid is crucial for the process that turns phenyl alanine into tyrosine. Additionally, it was discovered that providing folic acid supplements to silkworm did not significantly enhance the glycogen content of the fat body, but did considerably raise the haemolymph trehalose content in all treatment groups. This might be because the fat body releases trehalose into the haemolymph after converting glycogen into trehalose (Thorsteinson, 1958). According to reports on vitamin C in *B. mori*, the phagostimulatory impact of the vitamin may be the cause of the rise in trehalose and glycogen levels (Ito, 1961).

In silkworm, Shell weight and filament length are positively correlated. In the present study, the length of the filament was significantly influenced by the vitamins added to the mulberry leaf that the silkworms consumed in a variety of doses. In comparison to the average filament length of control batches (960 meters), the leaf that had been supplemented with pyridoxine, ascorbic acid, folic acid, and biotin showed noticeably longer filament lengths of 1184, 1174, 1167, and 1154 meters respectively. A faster rate of silk protein synthesis brought on by extra vitamin intake may be the cause of the increased filament length. According to Balasundaram *et al*. (2013), adding vitamin C to feed at a concentration of 0.2% may improve feed efficacy, enhance silk production, and grow silkworm larval, pupal, and cocoon length, width, and weight. According to Meeramaideen *et al.* (2017), silkworms fed with leaves treated with 0.5% Riboflavin have better growth-stimulating activity, feed efficacy, and economic features than silkworms fed with control leaves.

The average filament size is significantly lower in Pyridoxine (2.47) followed by Ascorbic acid (2.52), Folic acid (2.55), and Biotin (2.59) as against average control (2.90). Raj *et al*. (2001) found that feeding Pure Mysore race with mulberry leaves supplemented with soyabean flour resulted in fine denier (1.82) compared to control (2.14). Additionally, Kumar and Kumar (2018) showed decreased deniers of 1.650 in MU11 and 1.70 in MU303 silkworm breeds on methionine-supplemented mulberry leaves.

There was an improvement of 17.45 %, 17.23 %, 16.94% and 16.82 % in raw silk percentage when the larvae were fed on mulberry leaves supplemented with Pyridoxine, Ascorbic acid Folic acid and Biotin at concentration of 20ug/ml as against in control (14.56%).

The results of present study showed that among the treatments there was a significant increase in fecundity with Ascorbic acid supplemented mulberry leaves (685) followed by Riboflavin (667), Folic acid (662) and Biotin (656) at 20µg/ml as against average control (523). These results are in conformity with finding of Chauhan and Singh (1992) who reported that 1% concentration of ascorbic acid could increase the number of eggs in the silkworm. Nirwani and Kaliwal (1998) have also reported that oral supplementation with thiamine with different concentrations to silkworms larvae resulted in a significant increase in the fecundity in the silkworm, *B. mori*. Rahman *et al.,* (1990) recorded the significant improvement in fecundity by daily supplementation of 5% VC with regular feeding schedule from 3^{rd}-5^{th} instar and with 3 % VC in 5^{th} instar. In present study, a significant decrease in fecundity was noticed in Pyridoxine supplemented batches and relatively decrease at higher concentration of Pyridoxine treated leaves. The current results are in agreement with the findings of Faruki (2005) who reported significant reduction in fecundity upon Pyridoxine supplemented feed in comparison to control. Faruki *et al.* (1992) who observed that para-amino benzoic acid (PABA) significantly reduced the fecundity and egg-viability of *B.mori* females. Pai *et al.* (1988) recorded a reduced reproductive potentials in the females of *B.mori* resulting from PABA treatment.

Chapter – 6

SUMMARY AND CONCLUSION

Chapter-6

SUMMARY AND CONCLUSION

The study entitled "Effect of Vitamin Supplements on Economic Traits of Silkworm" was carried out in the Division of Sericulture Crop Improvement at College of Temperate Sericulture, Mirgund.

- Disease free layings of silkworm breed i.e., Double hybrid were incubated, brushed and reared on Ichinose mulberry leaves upto 3rd instar by following standard rearing procedure.
- Just after 3rd moult, three replications containing 100 worms were maintained for each treatment as well as for control batches. After 3rd instar till spinning, the worms were reared on Goshoerami leaves on which the selected vitamin solutions were sprayed on alternate days once as first feed during 4th and 5th instar with the help of an atomizer and the treated leaves were shade dried for 10-15 minutes before feeding the silkworms in order to study the influence of mulberry leaf supplemented with vitamins on economic traits of silkworm *Bombyx mori* L.
- Eight vitamins *viz.,* Thiamine, Riboflavin, Niacin, Pantothenic acid, Pyridoxine, Biotin, Folic acid, Ascorbic acid and two different concentrations *viz.,* 10µg/ml and 20µg/ml were prepared for the present study.
- Result revealed that all vitamins fortifications significantly reduced 5th instar and total larval duration in the silkworm batches fed with 20µg/ml concentration of Pyridoxine as compared to that of control batch.
- The findings of the current investigation also showed that vitamin fortification significantly improved the weight of 10 mature larvae, silk gland weight, silk gland somatic index, cocoon yield per 10,000 larvae (by number and weight), silk productivity, single cocoon weight, single shell weight, shell percentage, filament length, raw silk percentage and denier in

the silkworm batches fed with 20µg/ml concentration of Pyridoxine as compared to that of control.

- However, the reproductive trait i.e., fecundity was found significantly superior in silkworm batches fed with 20µg/ml concentration of Ascorbic acid.

The study has shown significant improvement in all the recorded parameters as compared to the control batches wherein no vitamin supplementation was applied to the feed. Pyridoxine, Ascorbic acid, Folic acid at 20µg/ml showed maximum improvement in larval and cocoon characteristics as such these vitamins can be utilized as feed supplement to improve productivity.

LITERATURE CITED

LITERATURE CITED

Ahsan, M. K., Khan, A. R., and Ferdous, T. 2013. Growth and development of the mulberry silkworm, *Bombyx mori* L. on vitamin B and C supplemented diet. *Bangladesh Journal of Zoology*, **41**(2), 199-206.

Aneesha, U., and Kumar, C. S. 2022. Foliar supplementation of ascorbic acid modulates biochemical performance of silkworm, *Bombyx mori* under thermal stress. *Journal of Thermal Biology*, *104*, 103-184.

Anonymous, 2003. Package of practices for silkworm rearing and mulberry cultivation in Kashmir pp.13-20.

Anonymous, 2021. Note on the functioning of Central Silk Board and performance of Indian silk industry, pp: 21-23.

Anonymous. 2022. Functioning of Central Silk Board and performance of Indian Silk Industry.*Technical document.* Central Silk Board-Ministry of Textiles, Govt. of India, Bangalore, pp.22.

Anonymous.2020. Jammu and Kashmir draft policy for sericulture development for production of bivoltine silk. *A report by sdd*, p:6.

Babu M, Swamy MT, Rao PK, Rao MS. 1992. Effect of ascorbic acid-enriched mulberry leaves on rearing of *Bombyx mori* L. Indian J. Seric. **31**: 11-114.

Bentea, M.; L. A. Marghitas and A. Sara 2011. The effect of some additives on bioproductive performances of silkworm, *Bombyx mori* L., Animal Science and Biotechnologies, **44** (1): 9-12.

Balasundaram, D., P. P. Ganesh, Mathivanan V. Selvisabhanayakam, and V. Ramesh 2013. "Studies on the nutritional supplementation of vitamin C treated MR2 mulberry leaves fed by V instar larvae of silkworm, *Bombyx mori* (L.) (Lepidoptera: Bombycidae) in relation to feed efficacy and growth rate." *International Journal of Research in Biotechnology and Biochemistry* **3**(1), 11-18.

Balasundaram, D., Prabu, P. G., Selvisabhanayakam, V. M., & Ramesh, V. 2013. Biotechnological Applications and Nutritional Supplementation of

Ascorbic Acid (Vitamin C) Treated Morus alba (L.) Leaves Fed by Silkworm, *B. mori* (L.) (Lepidoptera: Bombycidae) in Relation to Silk Production. *International Journal of Research in Biomedicine and Biotechnology*, **3**(1), 11-16.

Bursell, E. 1970. An introduction to insect physiology. *An introduction to insect physiology*.

Chapman, R.F.1998. The insects structure and function, 4^{th} edition, Cambridge University Press Cambridge.

Chauhan T, Singh K. 1992. Studies on the effect of ascorbic acid on the fecundity in the mulberry silkworm. Sericologia **32:** 567-574.

Choudhury.1991. Varietal influence of mulberry on silkworm *Bombyx mori*.L growth and development , *International journal of Advanced Research* **2**:921-927.

Cappellozza, L., Cappellozza, S., & Saviane, A., & Sbrenna, G. 2005. Artificial diet rearing system for the silkworm *Bombyx mori* L. Effect of vitamin C deprivation on larval growth and cocoon production. *Applied Entomology and Zoology*, **40** (3), 405-412.

Devi, K. L., and Yellamma, K. 2013. Cocoon parameters in the silkworm, *Bombyx mori* on exposure to trace element and nutrients. *J Bio. Innov*, **2**(5), 260-284.

Douglas, A. E. 2017. The B vitamin nutrition of insects: the contributions of diet, microbiome and horizontally acquired genes. *Current opinion in insect science*, **23,** 65-69.

El-Karaksy,I.A., and Idriss, M.1990. Ascorbic acid enhances the silk yield of the mulberry silkworm *Bombyx mori* L. *Journal of applied entomology*, **109** (1-5),81-86.

Etebari K, Edabi R. and Matindoost .2004.Effect of feeding mulberry's enriched leaves with ascorbic acid on some biological, biochemical and economical characteristics of silkworm *Bombyx mori* L; *Int.J.Indust.Entamol*.**8** 81-87.

Etebari, K and Matindoost, L.2005. Application of multivitamins as nutrients on biological and economical characteristics of silkworm *Bombyx mori* L. *J. Asia-Pacefic Entomol*. **8**: 1- 6.

Etebari, K and Matindoost,L. 2004. Effects of hypervitaminosis of vitamin B3 on silkworm biology. *J. Biosci*. **29**: 417–422.

Etebari, K. 2002. Effect of enrichment of mulberry leaves *(Morus alba)* with some vitamins and nitrogenous compounds on some economic traits and physiological characters of silkworm *Bombyx mori* (Lep.), Bombycidae, Isfahan University of Technology, Iran.

Etebari, K., Ebadi, R., and Matindoost, L.2004. The Effects of Vitamin C on Biological, Biochemical and Economical Characteristics of the Silkworm, *Bombyx mori* L. *International Journal of Industrial Entomology*, **8**(1), 81-87.

Faruki, S. I., Khan, A. R., & Mannan, A. 1992. Fecundity and fertility of the silkworm, *Bombyx mori* L. fed on mulberry leaves supplemented with paraamino benzoic acid. *Bangladesh J. Zool*, **20**, 351-353.

Faruki, S. I. 1998. Nutritive effects of thianomin enriched mulberry leaves on the silkworm, *Bombyx mori* L. Univ. J. Zool. Rajshahi Univ. **17**: 39-44.

Faruki, S. I. 2005. Effect of pyridoxine on the reproduction of the mulberry silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). *Invertebrate Survival Journal*, **2(**1), 28-31.

Gad, R., & Fathy, D. M. 2021. Effectiveness Vitamin C on different Characteristics of Silkworm, *Bombyx mori* L. *Journal of Plant Protection and Pathology*, **12**(9), 651-654.

Haniffa, M. A. and Periasamy, K. 1981. Effect of ration level on nymphal development and food utilization in *Acrotylus insubricus* (Scopali) (Orthoptera: Acrididae). *Acrida* **10**: 91-102.

Horie,Y and Ito,T. 1965. Nutrition of the silkworm, *Bombyx mori*-X.Vitamin B requirments and the effects of several analogues.*J. Insect Physiol.* **11**:1585–1593.

Horie,Y., Watanabe, K and Ito,T.1966. Nutrition of silkworm, *Bombyx mori* L. XIV.Further studies on then requirements for B vitamins.*Bull. Seric.Exp.Stn Japan*, **20**:393-409.

Horie Y Inokuchi T and Watanabe K 1978. Quantitative studies of food utilization by the silkworm (*Bombyx mori* L) through the life cycle II Economy of nitrogen and amino acids. Bull Seri Expt Station, Japan, **27**:531-578.

Haniffa, M.A., Thomas-Punitham, M. and Arunachalam, S. 1994. Effect of larval nutrition on survival, growth and reproduction in the silkworm *Bombyx mori* L. *Sericologia* **28**: 563-575.

Husain, A. 2017. Vitamins and related compounds (Medicinal Chemistry). *Pharmacy e-Book*: p. 1-43.

Hussain, Mubashir and Javed, Humayun. 2002. Effect of 0.2 per cent N with various combinations of Ascorbic acid on growth and silk production of silkworm (*Bombyx mori* L.). *Asian J. Pl. Sci.,* **1**: 650-651.

Ito T. Silkworm Nutrition; 1978. In the Silkworm an Important Laboratory Tool. Tazima, Y. (Ed), pp.121-157*, Kodansha Ltd, Tokyo.*

Ito, T. 1961. Nutrition on the silkworm, *Bombyx mori* L. IV.Effects of ascorbic acid (English*).Bull.Seric. Expt. Statn*., **17**: 134-137.

Ito, T., and Arai, N. 1967. Nutritive effects of alanine, cystine, glycine, serine, and tyrosine on the silkworm, Bombyx mori. *Journal of Insect Physiology*, **13**(12), 1813-1824.

Javed, Humayun, and Mubashir Hussain Gondal 2002. "Effect of food supplementation by N and ascorbic acid on larval mortality of silkworm (*Bombyx mori* L.)." *Asian Journal of Plant Sciences*.

Kamala, M., and Karthikeyan, A.2020. Synergetic effect of the nutritional fortification of nanoparticles of alanine and riboflavinon the economic parameters and fecundity of mulberry silkworm, *bombyx mori* l. *Journal of Advanced Scientific Research*, **11**(03), 127-133.

Kamala, M., and Karthikeyan, A.2019. Effect of Nutritional Fortification of Nanoparticles of Riboflavin on the Growth and Development of Mulberry Silkworm, *Bombyx mori* L. *Research Biotica*, **1**(1), 23-30.

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

Karasky,I.A. and Idriss, M., 1990, Ascorbic acid enhances the silk yield of mulberry silkworm *Bombyx mori* L. *J.Appl.Entomol*., **109**(1-5): 81-86.

Kennedy, D. O. 2016. B vitamins and the brain: mechanisms, dose and efficacy, a review. *Nutrients*, **8**(2), 68.

Krishnaswami, S. Kurnararaj, S.S.,Ijayaragliavan.K. and Kastviswanathan ,K.1971.Silkworm feeding traits for evaluating the quality of mulberry as influenced by variety, spacing and nitrogen fertilization. *Indian J.Seric*.**10** (1): 79-90.

Kumar, A. M. N. and Kumar, S.B. 2018. Influence of fortified mulberry leaf with Methionine on the economic traits and aminotransferase activity in *Bombyx mori* (Lepidoptera; Bombycidae). *International Journal of Current Advanced Research* **7** (1): 9258-9262.

Kumara,R.R., & Kumar, M.A.2016. Folic acid as a nutrient supplement with mulberry leaf and its impact on the economic traits of silkworm (*Bombyx mori* L.) *International journal of Advanced Research*, **4** (12), 1159-1165.

Laz, R., Gani, A. and Reza, S. A. M. 2006. Effect of methionine and Tryptophan on some economic characters in the mulberry silkworm, *Bombyx mori* L. *J. Zool. Rajshahi Univ*. **25**: 57-62.

Legay, J. M. 1958, Recent advances in silkworm nutrition. *A review Entomology* **3**: 75-86.

Malik, F.A., & Reddy, Y. S. 2009. Role of mulberry nutrition in the manifestation of economic traits of silkworm races under temperature stress conditions. *Revue des Vers a Soie Journal of Silkworms*, 461.

Mathawan, S. and Muthukrishnan, J. 1976. Effect of ration levels and restriction of feeding duration on food utilization in *Danaus chrysippus* (Lepidoptera: Danidae). *Ent. Exp. Appl*. **19**: 155-162.

Meeramaideen, M., Rajasekar, P., Balamurugan, M., & Prabu, P. G. 2017. Studies on the feed efficacy, growth rate and economic traits of silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae) fed with riboflavin treated kanva-2 mulberry leaves. *International Journal of Modern Research and Reviews*, **5**(1), 1460-1467.

Miyashita,Y. 1986. A report on mulberry cultivation and training methods suitable to bivoltine rearing in Karnataka.Pp.1-7.

Munghate, R.S., Wankhede, S.M., Mane, P.N., Somkuwar, M. R. and Dhurve, S.B., 2009, Effect of fortified mulberry leaves with ascorbic acid on growth and development of *Bombyx mori* L. *J.Soil. Crops*, **19** (1):153-157.

Muthukrishnan, J. And Delvi, M.R. 1974. Effect of ration level on food utilization in the grasshopper *Poecilocerus pictue. Oecologia*. **16**:227-236.

National Research Council. 1987. *Vitamin tolerance of animals*. National Academies Press. pp 60.

Nirwani ,R. B., and Kaliwal, B.B. 1996. Effect of Folic Acid on Economic Traits and the Change of Some Metabolic Substances of the Silkworm, *Bombyx mori* L. *Journal of Sericultural and Entomological Science*, **38**(2), 118-123.

Nirwani, R. B., and Kaliwal, B. B. 1998. Effect of thiamine on commercial traits and biochemical contents of the fat body and haemolymph in the silkworm. *Bombyx mori* L.*Sericologia.* **38** 639-646.ss

Pai, I. K., Hedge, S. N., & Krishnamurthy, N. B. 1988. PABA induced genotoxic effects in silkworm, *Bombyx mori*. In *Proc. Int. Sym. On nature of genetic variation in man (XII Ann. Conf. of EMSI), Hydarabad* (p. 54).

Paul, D.C., G.S. Rao and D.C. Deb. 1992. Impact of dietary moisture on nutritional indices and growth of *Bombyx mori* L. and concomitant larval duration J. Insect Physiol. **38:** 229-235.

Prasad, P.R., 2004. Effect of fortification of ascorbic acid through mulberry leaf on cocoon traits of pure mysore of silkworm *Bombyx mori* L. *Indian J .Entomol*.,**66** (1):37-39.

Rahman, S. M., Khanam, L. A. M. and Ferdoud, J. 1990.Effect of various concentrations of ascorbic acid on the oviposition and hatchability of silkworm, *Bombyx mori* L. Bull. Sericult. Res., **1**: 47-50.

Radjabi, R. 2010. Effect of mulberry leaves enrichment with amino acids supplementary nutrient on silkworm, *Bombyx mori* L. *Academic Journal of Entomology* **3**(1): 45-51.

Rahmathulla, V. K., Priyabrata Das, and M. Ramesh 2011. "Effect of folic acid supplementation on nutritional and feed conversion efficiency of mulberry silkworm (*Bombyx mori* Linnaeus 1758)." *Tropical Zoology* 24, no. 2.

Rahmathulla,V.K., DAS, P., Ramesh ,M. and Rajan ,R.K.2007. Growth rate pattern and economic traits of silkworm, *Bombyx mori*.L.under the influence of folic acid administration.*Sci.Environ.Man. J.Applage*.**11**(4): 81-84

Raj, S. S., Nangia, N., Chinnaswany, K. P. and Sannappa, B. 2001. Enrichment of rainfed mulberry leaves with protein and its influence on rearing performance of *Bombyx mori* L. *Res. On Crops*. **2** (2): 179-184.

Rajabi R., Ebadi R, Fazilati M., Mirhoseini.SZ 2006. Nutritive effects of mulberry leaves enrichment with riboflavin vitamin on bio-economic characters of silkworm, *Bombyx mori* L. 9^{th} Arab Congress of Plant Protection.19-23 November, Damascus, Syria.

Ravi Kumara, R., & Kumar, A. 2016. Folic acid as a nutrient supplement with mulberry leaf and its impact on the economic traits of silkworm (*Bombyx mori* L.). *International Journal of Advanced Research (IJAR)*, **4**(12), 1159-1165.

Seki K and Oshikane K. 1959. Res. Reports. Fac. Textile and Sericulture, Shinshu University.

Saha B.N. and Khan ,A.R. 1997.The nutritive effects of Sinafort-B on *Bombyx mori* L. *Entomon* **22**(1): 29-34.

Saha, A. K., & Khan, Z. H. 1995. Chemical beneficiation of low grade ferroginous bauxite. *NML Technical Journal*, **37**(3), 83-87.

Sarker, A., Haque, M., Rab, M. & Absar, N.1995. Effect of feeding Mulberry (Morus sp.) leaves supplemented with different nutrients to silkworm (*Bombyx mori* L.). Current Science **69**(2): 185-188.

Sengupta K,. Singh BD, Mustafi JC. 1972. Nutrition of silkworm, *Bombyx mori* L. I. Studies on the enrichment of leaf with various sugars, proteins, amino acids and vitamins for vigorous growth of the worm and increased cocoon crop protection.*Indian J. Seric*.**11***:* 11-27.

Settembre E, Begley T.P, Ealick S.E. 2003. Structural biology of enzymes of the thiamin biosynthesis pathway. Curr Opin Struct Biol.**13**: 739-47.

Singh, A., and Bandey, S. A. 2012.Supplementation of Synthetic Vitamin C in The Fifth Instars Bivoltine Hybrid Larvae of Nb4d2× Sh6 of Silkworm, *Bombyx mori* L. *Int. J. Agri. Vet. Sci*, **2**(1), 54-57.

Singh, G. P., & Kumar, V. 1996. Correlation Study on Larval Silk Gland and Shell Weight in the Silkworm, *Bombyx mori* L. *ENTOMON-TRIVANDRUM-*, **21**, 277-280.

Srikanth, S. Guru 2020. Seven month market silence hits Anantapur silk farmers hard. New Indian Express. *https://www.newindianexpress.com/states/Andhrapradesh/2020/oct/18/7-month-market-silence-hits-anantapur-silkfarmers–hard-2211822.html.*

Suprakash,P. and Madhuri, D., 1992, Effect of vitamin C or ascorbic acid supplementation on the growth and development of mulberry silkworm, *Bombyx mori* L.*Environ .Health*, **3** (5):297-299.

Thorsteinson, A. *Entomol . Exp. Appl*. 1958, **1**, 23-27.

Talebi, E.M., Bahreyni, R. and Tajabadi ,N.,2002. Effect of vitamin C on some trait of the silkworm, *Bombyx mori* L. *Pajouhesh.Va. Sazandegi.*,**15** (2): 92-94.

Tang, M. A. K., Salam, M. A. and Absar, N. 2005. Nutritional changes of four varieties of mulberry leaves infected with fungus (*cercospora moricola*). *Pakistan journal of Biological Sciences* **8**: 127-131.

Tantray, A. K., Shahnawaz, A., Bhat, S. A., Ifat, B., & Kanika, T. 2015. Studies on the effect of fortification with synthetic vitamin C on reeling traits of the silkworm, *Bombyx mori* L. *Journal of Experimental Zoology, India*, **18** (2), 1005-1009.

Tantray, A. K., Trivedy, K., and Nirmal Kumar, S. 2011. Studies on hypervitaminosis and early larval maturation in the silkworm. *Bombyx mori* L. Induced by vitamin-C treatment. *Revue des Vers a Soie Journal of Silkworms*, 471.

Tazima, Y. 1978. The silkworm: an important laboratory tool.

Yokoyama,T. 1963. Sericulture. *Annual Review of Entomology* **8**:287-298.

Yosuhiro and Sholchi. 1971. noted that the silkworm growth decreased when folic acid was eliminated from artificial diet.

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
College of Temperate Sericulture, Mirgund

CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner **Dr. Gh. Nabi Malik,** Ex. Professor College of Temperate Sericulture, Mirgund, SKUAST- K during Viva-Voce examination held on **03-04-2023** have been incorporated in the manuscript entitled **"Effect of Vitamin Supplements on Economic Traits of Silkworm *Bombyx mori* L."** submitted by **Ms. Ifrah Shafi (Regd. No. MSS/2020/85).**

(Dr. Firdose Ahmad Malik)
Chairman
Advisory Committee