https://doi.org/ 10.33307/entomon.v48i2.891
Entomon 48(2): 219-228 (2023)
Article No. ent. 48206

# Studies on influence of various stages of mulberry leaf in the growth and cocoonic parameters of silkworm *Bombyx mori* (L.)

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**ABSTRACT:** The objective of this work was to determine the effects of different stages of mulberry leaves on growth performance of treated larvae of *Bombyx mori* (L.) (Lepidoptera, Bombycidae) and characters of the resulted cocoons were evaluated. Results showed that the food consumption rate and assimilation rate were greatest for larvae fed on tender and over matured mulberry leaves as compared to larvae fed on mature leaves. Assimilation and conversion efficiencies were very high in the larvae grown on matured mulberry leaves (0.189 and 1.99% respectively). Moreover, the larvae fed on matured mulberry leaves showed higher growth rate, cocoon weight and shell ratio. Among the three different growth stages of leaves, the matured leaves were found to be the best food for final instar larvae of *Bombyx mori* L. which promotes maximum larval growth and it gains to quality cocoon and raw silk production.


**KEY WORDS:** Larval growth, assimilation, shell ratio, cocoon characters

## INTRODUCTION

Sericulture is an industrial trade that involves the cultivation of mulberry plant species, the raising of silkworms, and the manufacture of silk. It is a sustainable, environment friendly and ago-forestry focused trade, because it is integration of mulberry plants and silkworm farming system. It begins with the cultivation of mulberry trees and ends with the raising of silkworms on mulberry leaves to generate cocoons. It is difficult to transport mulberry leaves across vast distances or keep them for lengthy periods of time since they must be fresh. As a result, silkworm raising and mulberry tree farming is now essentially one and the same business (Rama Kant *et al*., 2004).The mulberry silkworm *Bombyx mori* (L.) produces the majority of commercial silk in the world (Yogananda Murthy *et al*., 2013). Apart from environmental issues, the amount and quality of mulberry leaf also have a major impact on silk yield. The nutritional content of the mulberry host leaves, which seems to be a decisive factor of silk quality, has a significant impact on silkworm growth (Chauhan and Tayal, 2017). Silkworm, *B. mori* is a poikilothermic insect that is the primary source of silk manufacturing. Nutritional parameters have a strong influence on silkworm food consumption and growth. Silkworm feeding efficiency iscrucial in the

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conversion of mulberry leaves to silk (Anantha Raman *et al*., 1994).

Several researchers have analysed the quality of mulberry varieties through feeding experiments and reported that MR2 is the best variety in which cocoon weight and silk weight were maximum (Etebari and Matindoost, 2005). The monophagous mulberry silkworm exclusively consumes mulberry leaves, and its growth and development are solely dependent on the nutrients found in those leaves (Shilpi and Praban, 2020).The major goal of silkworm breeding is to enhance the profit for silk producers and other sericultural industries by improving commercially valued economic features including cocoon weight (CW), cocoon shell weight (CSW), cocoon shell percentage (CSP) and shell ratio (SR) (Pouya Zamani *et al*., 2019). Mulberry trees are cultivated in a variety of climates;therefore, leaf quality has a big impact on their growth and development (Rukmangada *et al*., 2019). Mulberry leaves provide silkworms with all of the nutrients and water they require, as a result of massive co-evolution and selective breeding between silkworms and mulberry trees. Proteins, carbohydrates, lipids, inorganic matter, moisture, and vitamin A are the key nutrients in mulberry leaves that silkworms may consume (Fanchi Li *et al*., 2016). Supplemental nutrient enrichment of mulberry leaves is one of the alternate methods of improving larval development and cocoon formation (Thangapandiyan and Dharanipriya, 2019). Masoud *et al*. (2020) found that the leaves of the Kenmochi tree promoted good silkworm growth and development, as evidenced by superior performance qualities. *B. mori* strains reared on Jorhat and TR10 mulberry plant types showed the higher levels of fibrous protein, calcium, potassium, magnesium, and phosphorus than those grown on other mulberry types, resulting in higher silk output and the consumption rate (Lalfelpuii *et al*., 2019). Food conversion efficiency influences the cost effectiveness of silkworm rearing directly or indirectly, and it is regarded as a significant physiological parameter for comparing silkworm breeds (Rahmathulla *et al*., 2005). Slow progress and a longer larval lifespan occurred from restricted feeding period. With the rise in feeding length, absorption, assimilation, conversion, and metabolism, as well as their levels, exhibited a gradual rise (Mathavan *et al*., 1987). In this investigation, an attempt has been made to find out the effect of food quality silk production of the final instar larvae of *B. mori* on the basis of feed, which is focused mainly on different growth stages of mulberry leaves as nutritional intake of growth.

## MATERIALS AND METHODS

The study region was located in Nagercoil, southern region of the state of Tamilnadu, India at 8º 10' N; 77º 25' E, with an average altitude of 157 ft. The summer season is from March to May, followed by the monsoon season from June to mid of September, and the winter season from mid of September to February. The highest temperature recorded in Nagercoil was 34.5ºC, and winter temperatures as low as 23ºC have been recorded. The average annual rainfall received is 985 mm. CSR2 × TN hybrid variety of the silkworm was used in the present investigation, which is a hybridwell-known for its excellent survival, yield, silk ratio, and capacity to produce high-quality bivoltine silk that meets international standards which suits best to the climatic conditions of the study area (Nihal Nila and Stevens Jones, 2021).

The final instar larvae of *B. mori* were obtained from the industrial sericulture training centre at Konam, Nagercoil, India, reared under standard environmental conditions of 28°C, 85 per cent RH (Krishnaswami, 1986) and three types of MR2 variety mulberry leaves were given as food. MR2 is identified as a one of the best mulberry varieties in this area mentioned in the literatures. Therefore, in this analysis, different mulberry leaf stages of MR2 are identified based on the agro-climatic conditions of the study region, to evaluate the cocoon production efficiency and the influence of food, nutritional qualities and silk production of silkworm *B. mori*.

Freshly moulted final instar larvae of *B. mori* were divided into three separate groups containing 50 larvae each. The volume of the terraria (surrounding area of the larvae of caterpillar) as 6.5 × 2.1cm was kept constant for all the groups.

The worms in group I were fed with freshly matured mulberry leaves.

The worms in group II were fed with tender leaves and the

The worms in group III were fed with over matured leaves respectively.

Silkworms were given known quantities of fresh, high-quality mulberry leaves five times, as per the bivoltine rearing package's standard recommendation (Chinnaswamy Ramesha *et al*., 2012). The food given each day was weighed and recorded. Faeces and the unfed leaves were collected every day and oven-dried at 90°C to get the constant weight.

**Determination of food utilization and nutritional indices**

Healthy larvae were measured daily in each replica of each intervention, and uneven, unwell, or dead larvae were removed and replaced from different batches as discovered. After oven drying at a constant temperature of 80°C, the dry weight of residual leaf, excreta, and larvae weight were measured daily. Mounting is the final stage of the rearing process. Mountage is the most significant tool that assists silkworm larvae in spinning their cocoon comfortably. Larvae were replicated and treated individually in plastic, foldable cocoon–making frames .It determines both the quality and quantity of the cocoons. The rectangular- shaped mountage is made from plastic supported by plastic reapers on all sides and a corrugated form with eleven peaks and 0.9m width of plastic mountage is used for this analysis. After the $6^{th}$ day of mounting, the cocoons were collected and assessed. The weights of larva, cocoon, shell, and filament were measured using electronic balance. The following are the formulas for calculating various nutritional metrics (Rahmathulla and Suresh, 2012; Ayandokun and Alamu, 2020).

**Food Utilization:** The scheme of energy budget followed in this present study is the slightly modified IBP formula (Petrusewicz and Macfadyen, 1970) usually represented as -

$$C = P+R+F+U$$

Where, "C" is refereed as consumption, "P" is refereed as production, "R" is referred as energy loss via respiration and "F" is refereed as faeces excretion products and the "U" is referred as nitrogenous excretion products.

The quantity of uric acid in the faeces is negligible in insects (Roy and Kvenberg, 1981). In the present work, the faeces (F) therefore represent the undigested food as well as nitrogenous excretory wastes. "P" was estimated by subtracting the initial dry weight of the larva from the terminal weight at the end of the experiment. Food assimilated was calculated by subtracting the faeces weight from the food consumed. The metabolic loss of energy was found out by subtracting the food converted from the food assimilated. Rate of feeding, assimilation, conversion and metabolism were calculated at the experimentation.

**Feeding rate:** Amount of food consumed per unit body weight of larvae per unit time (g live body wt/ day).

Feeding rate = [Total food consumed (Joules/ Larvae) / Mid body weight (g) × Instar duration (days)] × 1000

**Assimilation rate:** Amount of food assimilated per unit weight of larvae per unit time (g live body wt/ day).

Assimilation rate = [Total food assimilated (Joules/ Larvae) / Mid body weight (g) × Instar duration (days)] × 1000

**Conversion rate:** The food converted per unit weight of larvae per unit time (g live body wt/day).

Conversion rate = [Total food converted (Joules/ Larvae) / Mid body weight (g) × Instar duration (days)] × 1000

**Metabolic rate:** It is difference between the assimilation rate and conversion rate.

Metabolic rate = Assimilation rate × Conversion rate

**Assimilation efficiency:** The percentage of food energy assimilated in relation to the food energy consumed.

Assimilation efficiency = [Total food assimilated (Joules/ Larvae) / Total food consumed (Joules/ Larvae)] × 100

**Conversion efficiency:** Percentage of food converted in relation to food consumed is known as gross conversion efficiency. Net conversion efficiency represents the percentage of food converted in relation to assimilated food.

Gross conversion efficiency = [Total food converted (Joules/ Larvae) / Total food consumed (Joules/ Larvae)] × 100

Net conversion efficiency = Total food converted (Joules/ Larvae) / Total food assimilated (Joules/ Larvae)] × 100

**Shell ratio:** The amount of silk that may be generated from each cocoon is determined by the weight of the shell. As a result, calculating the shell ratio is essential.The cocoon weight includes the weight of shell and weight of pupa inside (Anantha Raman and Magadum, 1994).

Shell ratio = [Weight of cocoon shell / Weight of cocoon] × 100

Using a commercially available statistical software tool (SPSS® for Windows, V. 16.0, Chicago, USA), data were analysed using one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). The results were given as means ± SD, with $P < 0.05$ considered statistically significant (Masoud *et al*., 2020).

## RESULTS AND DISCUSSION

The focus of this research was to determine the effects of various stages of mulberry leaf feed on the growth and characteristics of the final instar larvae of *B. mori*. Several studies have shown that silkworms seem to be more receptive to nutrition supplements during the 4$^{th}$ and 5$^{th}$ stages, which is important for identifying and selecting nutritionally effective silkworm varieties for commercial reasons. As a result, the nutrient usage research was limited to the 5$^{th}$ instar larvae, as this period of silkworm growth saw 80-85 per cent of total leaf consumption (Chinnaswamy *et al*., 2012), which is used in this investigation.

The conversion is greater (0.055± 0.03221g) in mature mulberry fed larvae when compared to the worms fed on tender (0.038± 0.202221 g) and very matured (0.045± 0.03671 g) leaves. Growth is higher in larvae fed with matured leaves of mulberry when compared to other. This shows that the matured leaves are rich in food constituents that promote the growth of the larvae (Table 1). According to Chinnaswamy *et al*. (2012), mulberry leaves are high in protein, carbohydrates, vitamins, sterols, phago-stimulants and minerals all of which aid silkworm larvae in their growth and development. The much-reduced larval growth seen in worms fed solely tender and over matured leaves might be owing to all of the abovementioned nutrients being lower than that in matured leaves, affecting development. When silkworms were fed on mulberry variety on matured leaves or tender or over matured leaves, and when feeding was switched from one mulberry variety to the other, the single pupal weight did not change considerably. This is likely due to the fact that the growth-development effects of protein and vitamins obtained from mulberry leaves, which might impact economic parameters such as pupal weight and cocoon weight, were likely similar in the three mulberry plant kinds studied. There was a favourable association among silkworm larval growth and cocoon production, showing that increasing larval growth will boost silk gland production, which will result in improved cocoon production (Pouya Zamani *et al*., 2019). Mathavan *et al*. (1987) and Hideshi *et al*. (1982) found that leaf ingestion had a direct impact on silkworm body weight and, as a result, silk-producing capacity. Similar findings were observed in the current investigation – silkworm fed on matured leaves had a fast growth rate owing to the influence of high nutritional content.

Insects' growth may be slowed not only by a lack of nutrients in their food, but also by the impact of

Table 1. Growth of fifth Instar *B. mori* fed on various types of mulberry leaves

| Type | Initial dry weight (g) | Final dry weight (g) | Growth (g) |
|---|---|---|---|
| Mature | 0.062± 0.03235$^{a}$ | 0.117± 0.04221$^{b}$ | 0.055± 0.03221$^{ab}$ |
| Tender | 0.063± 0.01046$^{b}$ | 0.101± 0.032231$^{c}$ | 0.038± 0.202221$^{b}$ |
| Over Mature | 0.0625± 0.04215$^{a}$ | 0.106± 0.02321$^{c}$ | 0.045± 0.03671$^{a}$ |

*Values are mean ± SD of ten observations. Values in the same column with different super script letters (a, b, and c) differs significantly at p < 0.05 (DMRT)*

Table 2. Food consumption, assimilation and conversion of fifth Instar *B.mori* fed on various types of mulberry leaves

| Types | Consumption (g) | Assimilation (g) | Conversion (g) |
|---|---|---|---|
| Mature | 2.7586± 0.13425$^{b}$ | 0.5214± 0.12235$^{a}$ | 0.055± 0.13109$^{ab}$ |
| Tender | 6.6462± 0.21235$^{a}$ | 4.0162± 0.16235$^{c}$ | 0.038± 0.19687$^{a}$ |
| Over Mature | 5.6092± 0.16285$^{b}$ | 1.9492± 0.21025$^{a}$ | 0.0435± 0.18615$^{a}$ |

*Values are mean ± SD of ten observations. Values in the same column with different super script letters (a, b, and c) differs significantly at p < 0.05 (DMRT)*

Table 3. Feeding rate, assimilation rate, conversion rate and metabolic rate of fifth Instar *B.mori* fed on different types of mulberry leaves

| Types | Feeding* | Assimilation* | Conversion* | Metabolic* |
|---|---|---|---|---|
| Mature | 3092.6± 0.19316$^{a}$ | 584.52± 0.18460$^{b}$ | 6.16± 0.19225$^{a}$ | 0.4664± 0.18925$^{a}$ |
| Tender | 8144.85± 0.21435$^{c}$ | 4921.81± 0.19421$^{a}$ | 4.65± 0.18315$^{a}$ | 3.978± 0.19425$^{a}$ |
| Over Mature | 6693.55± 0.17425$^{b}$ | 2326± 0.16215$^{b}$ | 5.19± 0.18405$^{c}$ | 1.906± 0.17365$^{b}$ |

**In g live body weight/day; Values are mean ± SD of ten observations. Values in the same column with different super script letters (a, b, and c) differs significantly at p < 0.05 (DMRT)*

Table 4. Assimilation (%) and conversion efficiency (%) of fifth Instar *B. mori* fed on different types of mulberry leaves

| Type | Type | Conversion | |
|---|---|---|---|
| | | Gross | Net |
| Mature | Mature | 1.99± 0.13478$^{ab}$ | 10.54± 0.13969$^{ab}$ |
| Tender | Tender | 0.57± 0.16823$^{b}$ | 0.94± 0.16215$^{b}$ |
| Over Mature | Over Mature | 0.78± 0.14622$^{a}$ | 2.25± 0.15102$^{a}$ |

*Values are mean ± SD of ten observations. Values in the same column with different super script letters (a, b, and c) differs significantly at $p < 0.05$ (DMRT)*

other environmental factors. Growth is also influenced by food consumption and assimilation, which differs across species. Furthermore, in the bivoltine race, a reduction in feeding time at the most active feeding stage (5$^{th}$ instar) resulted in a shorter larval duration, which negatively influenced larval weight and other connected features (Rahmathulla and Suresh, 2012). Consumption and assimilation are separate characteristics that are affected by the type of diet used and the silkworm breeds used. The amount of food digested, is influenced by a number of variables. Higher enzyme production, as seen by increased digestibility, protease, and lipase activity, corresponds to increased food consumption (Muniraju *et al*., 2004).

Consumption and assimilation of larvae fed with different types of mulberry leaves and the experimental values during the calculation of consumption, assimilation and conversion are shown in table 2. Food consumed and food assimilated are higher in larvae fed on over matured leaves (1.9492± 0.21025 g; 5.6092± 0.16285 g) and tender leaves (6.6462± 0.21235 g, 4.0162± 0.16235 g) than that on matured leaves (2.7586± 0.13425 g, 0.5214± 0.12235 g). Thus, food consumption and assimilation are greater when the larvae are fed on tender and over matured leaves. But growth is found to be greater when the larvae are fed with matured leaves. Thus, the type of food which is consumed in small amounts results in high growth. Conversion is greatest in larvae fed on matured leaves and recorded (0.055± 0.03221g) compared to (0.038± 0.202221 g and 0.045± 0.03671 g) for larvae fed on tender and over matured leaves. The food consumption, assimilation effective responses of mulberry silkworm, *B. mori* and its cocoon characteristics enhanced by feeding the last larval instar with mature mulberry leaves. This may be due to the nutritional supplements (Thangapandiyan and Dharanipriya, 2019). Since the matured mulberry leaves were fed to silkworm at the subsequent stage of growth and development, the food consumption and assimilation were found to be lower in the current study. In general, the current findings are consistent with those of previous researchers (Ahmad Nawaz *et al*., 2020; YangYang Li *et al*., 2016; Marilucia Santorum *et al*., 2020; Ming Lei *et al*., 2019).

Protein metabolism is a crucial biochemical process that aids in defining distinct phases of development, and the conversion of host plant nutrients into silk protein occurs mostly during the larval stages. Poor nutrition diets will have a direct impact on insects' basic biochemical and physiological metabolism, altering the detoxification system and increasing illness resistance (Lalfelpuii *et al*., 2019). Similarity rate of feeding and assimilation are high in larvae fed on mature and tender mulberry leaves but rate of conversion is high in mature leaves fed larvae. This corresponds to the high metabolic rate in larvae fed with over matured leaves and tender mulberry leaves (Table 3). Thus, it is strong evident that though the food consumption, assimilation and rate of feeding are high in larvae feed on over matured leaves and tender mulberry leaves, because of high of metabolic rate the conversion and the conversion rate are low in these larvae.

Assimilation and conversion efficiencies are very high in the larvae grown matured mulberry leaves (0.189± 0.12565%, 1.99± 0.13478%) when compared to that of larvae fed on tender mulberry leaves (0.005± 0.14231%, 0.57± 0.16823%) and over matured leaves (0.007± 0.14952%, 0.78± 0.14622%). The findings indicate the food which is assimilated and converted with high efficiency by organisms is proved to be the best food for the larvae (Table 4).

The nutritional efficiency of an insect may be considered a significant factor in a basic entomological situation. Nutritional efficiency, on the other hand, becomes a major concern in sericulture. The potential of various silkworm races and variants to digest, assimilate, and convert mulberry leaves to body substance, and then to the major commercial product, the cocoon, varies significantly. The conversion efficiency is influenced by numerous factors such as rearing procedures, rearing conditions, leaf quality, feeding proportion, and numerous compounds like as food additives, vitamins, antibiotics, and hormones (Rahmathulla and Suresh, 2012).

Silkworm growth, development, and production are all influenced by temperature. Evaluating the effect of temperature variations rather than constant-temperature rearing has been proven to be more effective in commercial silkworm rearing. Food consumption, production, and larval duration are all affected by the temperature combination (Rahmathulla, 2012). Variations in the amount of food consumed in a range of temperature combinations might explain variations in the amount of poor matter generated. During early instars, raising rearing temperature reduced food absorption and increased assimilation efficiency. Increased consumption may increase the pace at which food passes through the multivoltine silkworm larva's stomach, leaving less time for digestion and thereby lowering food assimilation efficiency (Stuart and Stephen, 1985). Despite modest food consumption, the conversion rate was higher at higher rearing temperatures. This finding suggests that larvae reared with little input can collect more nutrients by improving efficiency and feeding durations, allowing them to retain crucial levels of growth-limiting nutrients like nitrogen and water (Muniraju *et al.,* 2004).

Cocoon and shell weights are crucial production indicators, while shell ratio indicates the quantity of raw silk spun from cocoons, which varies depending on silkworm age and strain. It is identified that the shell ratio is high in the larvae fed on matured leaves (16.22± 0.21114%) and low in larvae fed on over matured leaves (11.29± 0.19112%) shown in Table 5. A higher value of Shell ratio was evident on the *B. mori* strains fed with the matured mulberry leaves. Feeding mulberry leaves enriched in potassium, magnesium, and calcium greatly enhanced the shell ratio,which has been revealed in larvae raised on matured mulberry leaves, which could be due to the high levels of amino acids, potassium, magnesium, and calcium in the leaves (Lalfelpuii *et al*., 2019). It is showed that the larvae fed on matured mulberry leaves have spun cocoons with highest shell ratios, which might be attributed

Table 5. Cocoon weight (g), shell weight (g)and shell ratio of fifth Instar *B. mori* fed on different types of mulberry leaves

| Types | Cocoon | Shell without pupa | Shell Ratio |
|---|---|---|---|
| Mature | 11.4± 0.21015$^c$ | 1.85± 0.21116$^c$ | 16.22± 0.21114$^c$ |
| Tender | 8.62± 0.20315$^c$ | 1.32± 0.19273$^c$ | 15.31± 0.19547$^c$ |
| Over Mature | 10.8± 0.19208$^c$ | 1.22± 0.18748$^c$ | 11.29± 0.19112$^c$ |

*Values are mean ± SD of ten observations. Values in the same column with different super script letter (c) differs significantly at $p < 0.05$ (DMRT)* to high amino acid and carbohydrate levels.

Food utilization studies on *Riccinus communis* (Sujatha *et al*., 2014) and *B. mori* (Muniraju *et al*., 2004) support the facts from this present study: it is evident that the matured mulberry leaves is the best food for the final instar larvae of *B. mori* because of its high assimilation and conversion efficiencies. Moreover, the conversion and the rate of conversion are also high. The larvae fed on matured leaves shows high growth rate. Shell ratio and cocoon weight are high in the worms fed in matured mulberry leaves. This might be due to the presence of more amount of nutrients in their leaves. It is also found that amino acid presents in the food will plays an important role in growth of the larvae, cocoon weight and shell ratio.

In this study it is identified that the significant growth was observed on larvae fed on matured mulberry leaves as 0.055± 0.03221 g. Amount of food consumed and assimilated were high in larvae fed on tender (6.6462± 0.21235 g; 4.02± 0.16235 g) and over matured leaves (5.6± 0.16285 g; 1.95± 0.21025 g) of mulberry compared with matured mulberry leaves (2.75± 0.13425 g; 0.52± 0.12235 g), because of the nutritional supplements in proper availability aspect. The same results were reflected in rate of feeding and metabolism, which is higher in tender and over matured leaves of mulberry. It results that the poor nutrition diets will have a direct impact on the insect's basic biochemical and physiological metabolism. Since the matured mulberry leaves are high in nutritional qualities, the shell ratio, assimilation and conversion efficiencies are high in larvae fed on matured mulberry leaves. Thus the results reveal that the matured leaves are best food for final instar larvae of *B. mori* which promotes maximum possible growth and silk production.This information is vital for suitable management of healthy silkworms, improvement of silkworm strains and production of high quality cocoons with good commercial value.

## ACKNOWLEDGEMENTS

The authors are grateful to the authorities of Industrial Sericulture Training Centre at Konam, Nagercoil, India for providing the silkworm DFLS and experimental design methodology during the investigation. The authors are thankful to the authorities of Scott Christian College, Nagercoil, India for providing lab facilities during the experimentation.

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*(Received December 05, 2022; revised ms accepted March 14, 2023; published June 301, 2023)*