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Effect of Different Maturity Mulberry Leaves on Biology of Bivoltine Silkworm (*Bombyx mori* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aimed to investigate the effects of feeding mulberry leaves of different maturity stages-tender, medium, and coarse on the biology of bivoltine silkworms (*Bombyx mori* L.), with the goal of enhancing sericulture practices by identifying the most beneficial leaf types for feeding.
Study Design: A randomized block design was employed to evaluate the impact of different mulberry leaf maturity levels on silkworm development.
Place and Duration of Study: The study was conducted during monsoon 2020-21 at the Department of Agricultural Entomology, College of Agriculture, Latur, under Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra.
Methodology: Disease-free layings of FC2 X FC1 bivoltine double hybrid silkworms were reared with seven treatments involving different combinations of mulberry leaves categorized as tender,

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medium, and coarse. Each treatment included three replications, with 100 larvae per replication. Key parameters measured included larval duration, pupal duration, moth emergence, fecundity, and hatching percentage.

Results: Larval duration was shortest for larvae fed tender leaves (23.82 days), significantly shorter compared to those fed coarse leaves (25.38 days). Pupal duration was also shortest for tender leaves (10.67 days) and longest for coarse leaves (11.50 days). Moth emergence was highest in larvae fed tender leaves (97.67%) and lowest in coarse leaves (84.67%). Fecundity ranged from 442.33 to 566.67 eggs per female moth, with the highest observed in larvae fed tender leaves (566.67 eggs). Hatching percentage was highest for tender leaves (94.67%) and lowest for coarse leaves (85.33%).

Conclusion: The study confirms that tender mulberry leaves significantly enhance silkworm development and productivity, resulting in shorter larval and pupal durations, higher moth emergence rates, increased fecundity, and improved hatching percentages. These findings highlight the importance of tender leaves in optimizing sericulture practices and improving silk production efficiency.

Keywords: Mulberry leaves; silkworm; tender leaves; biological traits; sericulture; silk production; bivoltine silkworms.

1. INTRODUCTION

The silkworm, *Bombyx mori*, relies entirely on mulberry leaves (*Morus spp.*) for its nutrition. The growth, development, and silk production of silkworms are significantly influenced by the quality of these leaves. Since mulberry plants are highly variable and vegetatively propagated, it is crucial not only to increase leaf production but also to ensure the leaves are of high quality to maximize silkworm development and cocoon production. Silkworms prefer mulberry leaves at different maturity stages—tender, medium, or mature—depending on their developmental stage [1]. Tender leaves are known to be nutritionally superior, offering higher moisture content and essential nutrients that benefit silkworms [2-5]. Silkworms prefer leaves with high moisture and low dry matter [6]. Tender leaves are not only richer in nutrients but also have less pubescence and a blunt tip, making them more suitable for silkworm consumption [7-10].

Leaves with high water content and essential nutrients are particularly beneficial for young silkworms, which require more moisture for proper digestion. In contrast, older silkworms can handle leaves with less moisture [11,12]. Matsumara et al. [13]. found that the quality of mulberry leaves plays a crucial role in successful cocoon production, alongside other factors such as climate and rearing techniques. Nearly 70% of the silk protein produced by silkworms comes from the protein in mulberry leaves [14]. Therefore, selecting the right mulberry leaves with the right nutrients and features is key to improving sericulture practices [15].

This study aims to explore how different maturity levels of mulberry leaves affect the biology of bivoltine silkworms (*Bombyx mori* L.), with the goal of enhancing practices in sericulture by identifying the optimal leaves for feeding.

2. MATERIALS AND METHODS

2.1 Study Location and Experimental Design

The experiment was conducted during the monsoon season of 2020-21 at the Department of Agricultural Entomology, College of Agriculture, Latur, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra. The aim was to study the effect of different maturity mulberry leaves (Variety V-1) on the biology of bivoltine silkworm (*Bombyx mori* L.). Mulberry variety V-1 is identified as one of the best mulberry varieties in this area. A randomized block design with seven treatments and three replications was employed, using disease-free layings of FC2 X FC1 bivoltine double hybrid silkworm. One hundred silkworm larvae were reared in each replication. The improved technology of silkworm rearing described by Krishnaswami [16] was followed in the present investigation. For defining different maturity stages of leaves, mulberry branches were divided into three regions, namely top tender (high moisture 75-80%), middle medium

(moisture content 65-75%), and bottom coarse leaves (low moisture 60-65%).

2.2 Treatments Details

T_1: Tender leaves
T_2: Medium leaves
T_3: Coarse leaves
T_4: Tender + Medium leaves
T_5: Medium + Coarse leaves
T_6: Tender + Coarse leaves
T_7: Tender + Medium + Coarse leaves

2.3 Equipments

Rearing Trays: Plastic trays (36" x 24" x 3") for housing larvae.

Rearing Stand: Iron frame for supporting trays.

Chopping Board: Softwood board (36.6" x 36.6" x 3.0") for chopping leaves.

Chopping Knife: Iron knife (6") with wooden handle for leaf chopping.

Bamboo Sticks: 8" long, thin sticks for larvae spacing and handling.

Feather: Clean white bird feathers for brushing and cleaning.

Cleaning Nets: Cotton and nylon nets for bed cleaning.

Collapsible Plastic Mountages: For cocoon spinning (2 x 0.95 sq m).

Paraffin Papers: To cover beds and maintain humidity.

Foam Pads: To maintain optimum humidity in rearing beds.

2.4 Rearing Methods

Disease-free layings of FC2 X FC1 were hatched in the laboratory. Upon hatching, chawki worms were placed into rearing trays according to the treatments. Chopped mulberry leaves were provided three times daily (8:00 am, 2:00 pm, and 6:00 pm), with leaf size adjusted to the larval instar. The rearing beds were uniformly prepared, and disinfectants including formalin (2%), bleaching powder (0.3%), lime powder, and vijetha powder @4kg/100 DFL were used. During moulting, larvae were not fed and were undisturbed. Post-moult, beds were cleaned, and vijetha was applied to prevent diseases. Fresh feed was provided after each moult, and the quantity of food was adjusted according to larval growth. Fully developed larvae were transferred to mountages for cocoon spinning. Cocoon harvesting occurred on the fifth day after placement on mountages.

2.5 Data Collection and Observations

The total larval period was recorded from the date of hatching to the onset of spinning, while the total pupal period was noted from spinning to moth emergence. Moth emergence percentage was calculated as (Number of moths emerged / Total number of cocoons) x 100. Fecundity was determined by counting the number of eggs laid by each female moth after mating. Hatching percentage was assessed by counting empty egg shells immediately after brushing, noting late-born larvae, unhatched, and unfertilized eggs.

3. RESULTS AND DISCUSSION

3.1 Larval Duration

The study demonstrated that the larval duration of *Bombyx mori* L. is significantly influenced by the maturity of the mulberry leaves provided, with larvae fed tender leaves (T1) exhibiting the shortest duration of 23.82 days. This duration was statistically comparable to those observed in larvae fed medium leaves (T2) at 24.78 days, tender + medium leaves (T4) at 24.30 days, tender + coarse leaves (T6) at 24.61 days, and a combination of tender + medium + coarse leaves (T7) at 24.83 days. Conversely, larvae fed coarse leaves (T3) had the longest larval duration of 25.38 days, which was at par with those fed medium + coarse leaves (T5) at 25.10 days. These results align with previous studies: Rahmathulla et al. [17] found fifth instar larvae fed tender leaves had a shorter duration of 174 hours, versus 198 hours for mixed feeding, 200 hours for medium leaves, and 211 hours for coarse leaves. Rahmathulla et al. [18] also reported that fourth instar larvae fed tender leaves had the shortest duration of 4.04 days compared to 4.33 days for medium leaves, 4.40 days for coarse leaves, and 4.20 days for mixed feeding. Furthermore, Kale et al. [19] corroborated these findings with a larval duration of 23.21 days for tender leaves, and Sarkar et al. [20,1] observed that tender leaves reduced the fifth instar larval duration to 134.4 hours and 130.4 hours, respectively, compared

to 152 hours and 144.4 hours for over-mature and mature leaves. Collectively, these results underscore that tender leaves are more effective in shortening the larval duration of *Bombyx mori* L. compared to medium and coarse leaves, highlighting the benefits of incorporating tender leaves into feeding regimens to enhance rearing efficiency and optimize sericulture practices.

3.2 Pupal Duration

The study revealed that the pupal duration of *Bombyx mori* L. varied between 10.67 to 11.50 days depending on the maturity of the mulberry leaves provided. Larvae fed tender leaves (T1) had the shortest pupal duration of 10.67 days, which was at par with those fed a combination of tender and medium leaves (T4) with a duration of 10.93 days. In contrast, larvae fed coarse leaves (T3) experienced the longest pupal duration of 11.50 days, comparable to those fed medium + coarse leaves (T5) at 11.30 days. These findings are consistent with previous research indicating a link between higher protein content in mulberry leaves and shorter pupal duration. Syed et al. [21] highlighted that increased protein content is associated with a shorter pupal duration, which supports the current study's observation that tender leaves, being richer in protein, lead to a more efficient transition from larval to pupal stages. Kale et al. [19] further supported this with their observation of a shortest pupal duration of 10.18 days for tender leaves and the longest of 11.50 days for coarse leaves. The consistent results across these studies highlight the importance of tender leaves in reducing pupal duration, attributable to their higher nutritional quality, which facilitates faster development and transition through the pupal stage, thus enhancing the efficiency of silk production and improving sericulture practices.

3.3 Moth Emergence

The study assessed the effect of different maturity mulberry leaves on moth emergence in bivoltine silkworms (*Bombyx mori* L.), revealing emergence rates ranging from 84.67% to 97.67%. The highest emergence rate of 97.67% was observed in larvae fed exclusively on tender leaves (T1), while the lowest rate of 84.67% was recorded in larvae fed coarse leaves (T3). These results are consistent with previous research indicating that tender leaves significantly enhance moth emergence. Basu et al. [22] reported better moth emergence rates with tender leaves compared to medium and mature leaves. Similarly, Krishnaprasad et al. [23] found that larvae fed tender leaves had a higher emergence rate of 78.17%, compared to 75.67% for medium leaves and 75.58% for mature leaves. Kale et al. [19] further corroborated these findings with a highest moth emergence rate of 97.33% for tender leaves and the lowest of 84.00% for coarse leaves. The current study's results support these observations, emphasizing that tender leaves provide superior nutritional benefits that enhance larval health and development, leading to improved moth emergence rates. This highlights the importance of using tender leaves in sericulture to optimize silk production outcomes.

3.4 Fecundity

The impact of feeding different maturity mulberry leaves on the fecundity of bivoltine silkworms (*Bombyx mori* L.) revealed that fecundity ranged from 442.33 to 566.67 eggs per female moth. The highest fecundity of 566.67 eggs was recorded in treatment T1, where larvae were fed exclusively on tender leaves, followed by T4 (tender + medium leaves) with 533 eggs. In contrast, the lowest fecundity was observed in T3 (coarse leaves) with 442.33 eggs. These results are supported by Basu et al. [22] who found that moths developing from larvae fed tender leaves laid significantly more eggs than those fed medium leaves. Singh et al. [24] also confirmed that tender leaves resulted in the highest fecundity, with lower fecundity associated with mature and mixed leaves. Krishnaprasad et al. [23] reported that fecundity was highest at 541.42 eggs per laying for silkworms fed tender leaves, over mature leaves (534 eggs), control (518.83 eggs), water-dipped leaves (486.16 eggs), medium leaves (477.25 eggs), and over-matured leaves (387.08 eggs). Krishnaprasad et al. [25] observed that tender leaves during late-age stages resulted in the highest fecundity (510 eggs per laying), whereas soiled leaves led to significantly lower fecundity (280 eggs per laying). Similarly, Adeduntan [26] found that silkworms fed top leaves produced the highest number of eggs (428), compared to base (288), middle leaves (388). Kale et al. [19] corroborated these findings with a maximum fecundity of 565.33 eggs for larvae fed tender leaves, highlighting that tender leaves are crucial for optimizing fecundity. This underscores the importance of leaf quality in enhancing reproductive performance and silk production efficiency.

Table 1. Effect of feeding different maturity leaves of mulberry on the biology of bivoltine silkworm (*Bombyx mori* L.)

Treatment. No.	Treatment details	Larval duration (days)	Pupal duration (days)	Moth emergence (%)	Fecundity	Hatching (%)
T_1	Feeding with tender leaves	23.82	10.67	97.67 (81.40)*	566.67	94.67 (76.62)*
T_2	Feeding with medium leaves	24.78	11.00	93.33 (75.07)	478.33	89.33 (70.91)
T_3	Feeding with Coarse leaves	25.38	11.50	84.67 (66.98)	442.33	85.33 (67.47)
T_4	Feeding with tender+medium leaves	24.30	10.93	95.33 (77.61)	533.00	92.33 (73.42)
T_5	Feeding with medium+coarse leaves	25.10	11.30	92.00 (74.45)	462.67	89.67 (71.28)
T_6	Feeding with tender+coarse leaves	24.61	11.17	94.00 (75.92)	471.67	91.33 (72.89)
T_7	Feeding with tender+medium + coarse leaves	24.83	11.07	94.33 (76.66)	517.33	90.67 (72.78)
	S.E. ±	0.353	0.091	1.130	11.210	1.140
	C.D. at 5%	1.087	0.281	3.520	34.538	3.553
	C.V. (%)	2.48	1.43	2.594	3.91	2.733

**figures in parentheses are angular transformed values*

3.5 Hatching Percentage

The impact of feeding different maturity mulberry leaves on the hatching percentage of bivoltine silkworms (*Bombyx mori* L.) revealed that hatching percentages ranged from 85.33% to 94.67%. The highest hatching percentage of 94.67% was observed in treatment T1, where larvae were fed exclusively on tender leaves, and this result was statistically comparable to T4, which involved a diet of tender and medium leaves yielding a hatching percentage of 92.33%. In contrast, the lowest hatching percentage was recorded in T3 (coarse leaves only) at 85.33%, which was at par with T2 (medium leaves) at 89.33%. These findings are consistent with previous research, such as Basu et al. (1995), who reported that tender leaves led to higher hatching percentages due to their superior nutritional quality, which supports optimal embryonic development. Krishnaprasad et al. (2002a) also found the highest hatchability of 93.43% with tender leaves, outperforming medium leaves (91.84%), mature leaves (91.15%), control (90.92%), water-dipped leaves (89.40%), and over-matured leaves, with soiled leaves resulting in the lowest hatchability of 86.56%. Additionally, Krishnaprasad et al. (2002b) observed a hatchability of 94.41% with tender leaves during winter, while soiled leaves during summer resulted in a lower hatchability of 87.52%. Kale et al. (2017) further supported these results, noting a hatching percentage of 94.33% with tender leaves. The current study's results affirm that tender leaves consistently lead to the highest hatching percentages, underscoring their critical role in optimizing reproductive success and enhancing silk production efficiency**.**

4. CONCLUSION

The study concluded that feeding tender mulberry leaves of variety V-1 to bivoltine double hybrid silkworms (FC2 X FC1) significantly enhanced various aspects of silkworm development and productivity. Silkworms reared on tender leaves exhibited faster growth, with notably shorter larval and pupal durations compared to those fed coarse leaves. Additionally, these silkworms showed higher moth emergence rates, increased fecundity, and improved hatching percentages, highlighting the superior nutritional benefits of tender leaves. To

maximize sericulture efficiency and silk production, it is recommended to consistently supply tender and succulent mulberry leaves by ensuring frequent irrigation of the mulberry garden, thereby improving overall rearing outcomes and silkworm health.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Chat GPT have been used during writing or editing of this manuscript. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

Details of the AI usage are given below:

1. The AI tool Chat GPT was used in this manuscript to rephrase the sentence only and no artificial data was generated using the AI tool.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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