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IMPACT OF FEEDING SILKWORM *BOMBYX MORI* L. (LEPIDOPTERA-BOMBYCIDEA) WITH MULBERRY LEAVES BIOFORTIFIED WITH DYES ON THE SILK FIBER PARAMETERS

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ABSTRACT

The silkworm (*Bombyx mori* L.) is a significant monophagous insect that primarily feeds on mulberry leaves (*Morus* spp.), with the quality of silk being highly dependent on the quality of the mulberry foliage. Natural dyes have long played an important role in the textile industry, but modern challenges, such as colourfastness, persist. Developing an environmentally friendly dyeing process for silk hinges on the dyeing method used. In this study, two bivoltine silkworm breeds were investigated by feeding them mulberry leaves biofortified with natural dyes at two concentrations (50% and 100%). A control group (T0) was established, where silkworms were not fed with any type of dye. The results demonstrated that silkworms fed mulberry leaves biofortified with indigo dye achieved the highest filament length (FL) of 979.25 m in the CSR16 breed, compared to 958.00 m in the control group. Similarly, for the CSR27 breed treated with madder dye, a filament length of 977.50 m was observed at the T2 treatment level. Notably, significant improvements in filament length were observed in the CSR16 breed when fed biofortified leaves with indigo dye at 100% concentration (T2). Further, reelability (84.50%) and raw silk percentage (18.50%) were enhanced in both CSR_{16} and CSR_{27} breeds when the silkworms were fed mulberry leaves treated with 100% madder dye. Similar improvements in reelability and raw silk percentage were recorded for both breeds when indigo-dyed mulberry leaves were used. This study highlights that biofortifying mulberry leaves with natural dyes, particularly at 100% concentration, can improve silk production parameters in bivoltine silkworm breeds. The findings contribute to advancing sustainable practices in the textile industry, promoting eco-friendly silk production with enhanced quality.
Keywords : Silkworm, Biofortified mulberry leaves, Filament length, Renditta, Denier

Introduction

Mulberry silk, produced by silkworms that feed on mulberry leaves, is renowned for being one of the highest quality fibers, characterized by outstanding properties. The qualitative and quantitative production of silk is influenced by various factors, including the silkworm breed, the quality of mulberry leaves, rearing practices and environmental conditions during the rearing process. To produce superior quality cocoons, the silkworm *Bombyx mori L.* must be nourished with healthy and nutritious mulberry leaves, alongside these other factors Sarker, 2018 and Datta, 2019.

Natural dyes have historically played a crucial role in textile dyeing; however, modern challenges, particularly regarding colourfastness, persist (Yadav & Sharma, 2021). Research indicates that the incorporation of tannins and flavonoids can enhance dye retention, providing a sustainable alternative that does not depend on synthetic mordants (Angelini *et al.*, 2003 and Bechtold & Mussak, 2009). Although traditional silk dyeing methods are widely used, they pose significant environmental concerns due to the release of toxic chemicals and pollutants (Melba & Queensly, 2023). Synthetic dyes, especially azo dyes, dominate the textile industry but are detrimental to both human health and the environment (Melgoza *et al.*, 2004).

The silk dyeing industry is increasingly adopting sustainable practices, particularly through the use of naturally coloured silk cocoons dyed with eco-friendly plant and animal-based dyes (Baburaj & Das (2022). These natural dyes are biodegradable and less toxic, representing viable alternatives to synthetic options. Recent studies underscore the need for ongoing research into dye retention in textiles, with findings suggesting that silkworms fed natural dyes can maintain normal growth and feeding behaviours (Khan & Hussain, 2024). Furthermore, natural dyes such as turmeric have been noted for their medicinal properties, including antibacterial effects.

Current trends emphasize the adoption of eco-friendly, natural dyes to mitigate environmental impacts. Derived from plants and minerals, these natural dyes are non-toxic, biodegradable and present an environmentally sustainable alternative to synthetic dyes (Subhashini *et al.*, 2009). Moreover, natural dyes have been shown to possess antimicrobial properties, further enhancing their appeal (Sinha & Prasad, 2023).

An innovative approach to silk dyeing involves feeding silkworms dye-laced diets, enabling them to produce coloured cocoons (Trivedy *et al.*, 2016). Studies have demonstrated that silkworms fed dye-modified diets can generate naturally coloured cocoons without negatively impacting their growth or silk quality (Bukhari *et al.*, 2024). This method not only reduces the environmental impact of traditional dyeing processes but also provides a sustainable means of producing coloured silk (Fan *et al.*, 2019 and Cheng *et al.*, 2019).

The textile industry faces a critical sustainability challenge, particularly in the context of dyeing processes. This manuscript addresses this issue by investigating the use of biofortified mulberry leaves as a natural dye source for silkworms. This innovative approach not only provides an eco-friendly alternative to harmful synthetic dyes but also seeks to enhance key silk fiber parameters, with a particular focus on increasing silk fiber length. By feeding silkworms mulberry leaves fortified with natural dyes, the research aims to create a more sustainable dyeing ecosystem, promoting environmentally friendly practices in both sericulture and textile production. The integration of natural dyes into the silk production process represents a significant step towards reducing the environmental footprint of traditional dyeing methods. This practice supports the growing global emphasis on green technologies and presents a viable solution for improving silk quality while maintaining ecological balance (Smith *et al.*, 2018 and Gupta and Kumar, 2020).

Thus, this study, "Impact of feeding Silkworm *Bombyx Mori* L. (Lepidoptera-Bombycidea) with mulberry leaves biofortified with Dyes on the Silk fiber parameters," is undertaken to explore the potential of natural dye feeding in bivoltine silkworm breeds concerning various silk parameters. The findings will offer insights into sustainable silk production practices that align with global environmental trends, providing valuable contributions to the development of eco-friendly methodologies in the textile industry.

Materials and Methods

Silkworm Breeds

Bivoltine silkworm seeds of CSR_{16} and CSR_{27} were sourced from the Silkworm Breeding and Genetics Section at CSB-CSR&TI, Mysore. The silkworms were reared in the laboratory using standard rearing techniques as outlined by Dandin & Kumari (2021). The rearing conditions were maintained at a temperature of 25°C, with a relative humidity of 75 ± 5% and a photoperiod of 16 hours light and 8 hours dark. This study was conducted under the sub-tropical conditions of Poonch District, Jammu and Kashmir, during the spring of 2022, at the Department of Sericulture, Poonch Campus, University of Jammu.

Biofortification of mulberry leaves

The mulberry leaves utilized for rearing were of a local variety, collected daily from nearby mulberry gardens. The natural dyes, madder and indigo, used in this study were procured from SKYMORN Herbs and Dye Export, India. Two dye concentrations were prepared: 50% and 100%. The larvae were divided into three groups: a control group (T0) and two treated groups (T1 for 50% dye concentration and T2 for 100% dye concentration). The biofortification was administered during the 5^{th} instar larval stage of the silkworm breeds.

Data recording

Upon reaching full growth, the matured worms ready for spinning were gently collected and mounted on plastic mountages. The harvesting of cocoons was carried out on the 8^{th} day following the commencement of spinning. Data regarding various silk parameters, including total filament length, number of broken filament length (NBFL), denier, renditta, reelability and silk waste percentages, were recorded as per the equations 1-5.

$$L = R \times 1.125\,m \quad (1)$$

Where, L-Length of the filament (m); R-Number of revolutions; 1.125 m-Circumference of the eprouvette reel

$$NBFL = \frac{\text{Total Filament length}}{1 + \text{number of breaks}} \quad (2)$$

$$Denier = \frac{\text{Weight of the filament}}{\text{Length of the filament}} \times 9000 \quad (3)$$

$$Renditta = \frac{\text{Weight of cocoon reeled}}{\text{Weight of raw silk obtained}} \quad (4)$$

$$\text{Raw Silk \%} = \frac{\text{Weight of raw silk obtained}}{\text{Weight of cocoon reeled}} \quad (5)$$

Statistical Analysis

The data were analyzed using a Completely Randomized Design (CRD), applying square root transformations as necessary. Treatment means were compared using the Least Significant Difference (LSD) test, as described by Rangaswami (2010). The statistical analyses were performed using IBM SPSS Statistics software.

Results and Discussions

The study investigated the effects of feeding silkworm larvae (CSR_{16} and CSR_{27} breeds) mulberry leaves treated with madder and indigo dye solutions on various silk parameters. In assessing filament length, CSR_{16} demonstrated significant variations across different dye treatments. The Madder Dye revealed that the control group (T0) exhibited the longest filament length at 968.00 ± 1.291 m, followed closely by T1 (967.50 ± 0.645 m) and T2 (965.50 ± 1.041 m). In contrast, when treated with Indigo Dye, the maximum filament length was recorded at T2 (100% concentration) at 979.25 ± 0.629 m, followed by T1 at 977.00 ± 0.408 m and T0 at 958.00 ± 1.181 m. For CSR_{27}, the Madder Dye treatment showed the highest filament length in T2 at 977.50 ± 1.323 m, with T1 and T0 at 968.00 ± 0.408 m and 962.50 ± 1.848 m, respectively. Conversely, under the Indigo Dye treatment, T0 achieved the longest filament length at 969.25 ± 2.016 m, while T2 and T1 recorded lengths of 967.50 ± 1.323 m and 967.50 ± 0.645 m respectively.

Filament length, a crucial indicator of silk quality, was affected differently by dye treatments across breeds. For CSR_{16}, filament length decreased with higher concentrations of madder, suggesting potential negative impacts on filament elongation. Conversely, CSR_{27} exhibited an increase in filament length at 100% madder concentration, indicating a breed-specific response (Han and Yang, 2005). The Indigo Dye at 100% concentration enhanced filament length in CSR_{16}, likely due to increased larval weight (Satenahalli *et al.*, 1990 and Adeel *et al.*, 2009), whereas CSR_{27} showed a reduction in filament length, potentially due to a higher fecundity negatively correlating with filament length (Ram, 1994).

The results for non-breakable filament length (NBFL) indicated that CSR_{16} treated with Madder Dye at T2 yielded the maximum length of 843.00 ± 1.291 m, with T1 and T0 at 842.25 ± 0.629 m and 840.75 ± 0.479 m, respectively. For Indigo Dye, the longest NBFL was again recorded in T2 at 842.50 ± 0.957 m. CSR_{27} achieved the highest NBFL in T1 (Madder Dye) at 845.25 ± 3.683 m, followed by T2 and T0 at 844.75 ± 1.887 m and 841.25 ± 0.750 m, respectively. Under Indigo Dye, the maximum NBFL was recorded in T1 at 844.25 ± 2.016 m. The NBFL, crucial for reelability, was found to be longer with higher dye concentrations, thereby improving filament integrity and minimizing breakage during reeling (Asif & Khodadadi (2013); Janani & Winifred (2013); Ji *et al.*, 2014; Bahl & Kaur (2020) and Sharma & Gupta (2022).

Enhanced reelability and neatness percentages in dye-treated groups emphasize the optimization of silk fibers for industrial applications, supporting the assertions made by Anumol Anto *et al.*, 2017; Ram *et al.* (2003) and Mongkholrattanasit *et al.* (2012) regarding the necessity of optimizing nutrigenetic traits and silk-contributing parameters for superior silkworm hybrids.

The filament size (denier) for CSR_{16} indicated that T2 (Madder Dye) had the largest size at 2.89 ± 0.010, followed by T1 at 2.87 ± 0.010 and T0 at 2.85 ± 0.009. For Indigo Dye, T2 similarly had the highest denier size at 2.86 ± 0.005. In CSR_{27}, T2 (Madder Dye) showed the greatest filament size at 2.93 ± 0.017, with T1 at 2.91 ± 0.015 and T0 at 2.88 ± 0.006. The Indigo Dye also had the highest value in T2 at 2.86 ± 0.006. This increase in filament size (denier) with higher dye concentrations in both breeds suggests that dye treatments effectively enhance filament thickness, corroborating findings by Murugesh *et al.* (2005); Nagasawa *et al.* (2006); Lakshmi *et al.* (2011).

The renditta was highest in CSR_{16} with mulberry leaves treated with Madder Dye at T1, recording a value of 6.87 ± 0.642, followed by T0 at 6.75 ± 0.511

and T2 at 6.37 ± 0.368. In CSR_{27}, the highest renditta was recorded in T1 at 7.10 ± 0.507. For Indigo Dye, CSR_{16} achieved a maximum renditta of 6.72 ± 0.578 in T1, while CSR_{27} recorded a peak of 7.19 ± 0.420 under the same treatment. The reelability percentage for CSR_{16} under Madder Dye was highest in T2 at 84.50 ± 1.708%, with T1 and T0 at 83.25 ± 1.493% and 82.00 ± 1.809%, respectively. Under Indigo Dye, T2 again recorded the highest at 83.50 ± 0.957%. For CSR_{27}, T1 and T2 both demonstrated the highest reelability percentage of 85.00 ± 1.780%, followed by T0 at 82.50 ± 1.500%. These results are corroborated with the earlier researchers (Anumol Anto *et al* 2018; Anumol *et al*., 2017)

The weight of cocoons for CSR_{16} treated with Indigo Dye was highest in T1 (50% concentration) at 0.28 ± 0.025 kg, closely followed by T2 (100% concentration) at 0.28 ± 0.013 kg, while the control group (T0) yielded the lowest at 0.25 ± 0.013 kg. For CSR_{27}, T2 (control) showed the highest weight at 0.27 ± 0.019 kg, with T0 at 0.25 ± 0.014 kg and the lowest weight noted in T1 at 0.24 ± 0.013 kg. Under Madder Dye, the highest weight of reelable cocoons for CSR_{16} was found in T0 at 0.26 ± 0.021 kg, while CSR_{27} exhibited maximum reelable cocoon weight in T0 at 0.24 ± 0.027 kg.

Statistical analyses indicated significant variations in cocoon weights across treatments. In terms of silk weight, CSR_{16} demonstrated the highest under Madder Dye at T2 with 52.22 ± 2.254 g, closely followed by T0 at 51.42 ± 1.067 g and T1 at 49.67 ± 0.965 g. With Indigo Dye, T0 had the highest silk weight at 52.60 ± 1.037 g. CSR_{27} revealed the highest silk weight with Madder Dye in T2 at 51.70 ± 1.907 g, while T1 with Indigo Dye recorded the highest at 52.67 ± 0.997 g. Silk waste analysis indicated that CSR_{16} with Madder Dye had the maximum silk waste at 7.10 ± 0.576 g in T0, with T2 at 6.25 ± 0.366 g and T1 at 6.15 ± 1.05 g. CSR_{27}, when treated with Madder Dye, recorded the highest waste value at 7.18 ± 0.158 g in T1.

Cocoon and silk weight are key indicators of silk yield and economic viability, with CSR_{16} showing increased weights at 100% madder concentration, while CSR_{27} achieved the highest silk weight at 50% indigo concentration, indicating optimal physiological enhancements from specific dye treatments. The reduction in silk waste and improved renditta values with higher dye concentrations indicate enhanced silk quality and minimized production losses, consistent with Kulkarni *et al*. (2012) and Ibrahim *et al*. (2017).

The results indicated that the performance of CSR16 and CSR27 silkworm bivoltine breeds varied significantly when treated with madder dye. For CSR16, the weight of waste recorded was 6.27 ± 0.366 g in T0, increasing to 7.92 ± 0.272 g in T2, reflecting a consistent increase in waste across treatments. Conversely, CSR27 exhibited a similar trend, with waste weight starting at 6.57 ± 0.180 g in T0 and reaching 7.80 ± 0.389 g in T2. In the case of indigo dye, CSR16 silkworms produced waste weights of 6.15 ± 0.612 g in T0, increasing to 7.87 ± 0.610 g in T2, while CSR27 showed a slightly lower range, with waste weights from 6.12 ± 0.551 g in T0 to 7.77 ± 0.567 g in T2.

The silk waste percentage revealed that CSR_{16} with Madder Dye had a maximum waste percentage of 7.10 ± 0.576 in the control group (T0), while CSR_{27} with the same dye showed the highest percentage in T1 at 7.18 ± 0.158. For raw silk percentage, CSR_{16} indicated the highest value in T0 at 18.32 ± 0.193, while CSR_{27} recorded the highest in T1 at 18.50 ± 0.269. The neatness percentage for CSR_{16} treated with Madder Dye peaked in T2 at 94.25 ± 1.109%, while CSR_{27} also displayed a high neatness percentage in T2 at 94.75 ± 1.652%. Floss percentage details were not provided in the initial results, necessitating further analysis to obtain this parameter for CSR_{16} and CSR_{27}. The raw silk percentage reflects silk purity, with CSR_{16} achieving the highest in the control group and CSR_{27} optimal at 50% madder concentration, indicating that moderate dye concentrations may enhance silk purity without compromising quality. Indigo treatments maintained or slightly improved raw silk percentages, particularly at 50% concentration in CSR_{27}, aligning with Rajalakshmi *et al*. (1998) and Hosseini *et al*. (2014), who emphasized minimizing filament breaks during reeling.

Conclusion

This study demonstrates the significant impact of dye treatments on silk quality parameters, reinforcing findings from previous research. The differential responses of CSR_{16} and CSR_{27} emphasize the need for breed-specific optimization in sericulture. Tailoring dye concentrations to meet the requirements of each silkworm breed enhances key silk attributes and improves both reeler performance and industrial outcomes.

Feeding mulberry leaves treated with natural dyes to the bivoltine breeds CSR_{16} and CSR_{27} led to notable improvements in several critical silk parameters. This innovative dyeing method lays the groundwork for environmentally friendly processes in silk production, potentially reducing water usage and wastewater generation compared to traditional methods.

Future research should explore the biochemical mechanisms behind dye-silk interactions and expand the investigation to other silkworm breeds and dye types. These efforts are essential for optimizing silk production technologies further.

Table 1: Performance of CSR_{16} and CSR_{27} Silkworm Bivoltine Breeds treated with Madder Dye for Silk Parameters

Treatments	Average Filament Length (mtrs.)		Average Non-Breakable Filament Length (mtrs.)		Raw Silk %		Renditta		Filament size (Denier)		Reelability %	
	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}
T_0	968.00 ± 1.291	962.50 ± 1.848	840.75 ± 0.479	841.25 ± 0.750	18.32 ± 0.193	18.22± 0.183	6.75 ± 0.511	6.55 ± 0.290	2.85± 0.009	2.88± 0.006	82.00 ± 1.080	82.50 ± 1.500
T_1	967.50 ± 0.645	968.00 ± 0.408	842.25 ± 0.629	845.25 ± 3.683	18.22± 0.020	18.50 ±0.269	6.87 ± 0.642	7.10 ± 0.507	2.87± 0.010	2.91± 0.015	83.25 ± 1.493	85.00 ± 1.780
T_2	965.50 ± 1.041	977.50 ± 1.323	843.00 ± 1.291	844.75 ± 1.887	18.23± 0.019	18.25 ±0.017	6.37 ± 0.368	6.22 ± 0.170	2.89± 0.010	2.93± 0.017	84.50 ± 1.708	85.00 ± 1.414
	Weight of silk (g)		Weight of Waste (g)		Weight of Cocoons (kgs)		Weight of reelable cocoons (kgs)		Floss percentage		Neatness	
	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}
T_0	51.42 ± 1.067	50.43 ± 1.017	6.27 ± 0.366	6.57 ± 0.180	0.24 ± 0.017	0.25 ± 0.014	0.26 ± 0.021	0.24 ± 0.027	Negligible	Negligible	91.75 ± 0.750	92.00 ± 0.577
T_1	49.67 ± 0.965	49.61 ± 0.967	7.92 ± 0.272	7.80 ± 0.389	0.26 ± 0.027	0.23 ± 0.014	0.19 ± 0.016	0.23 ± 0.022	Negligible	Negligible	93.25 ± 0.854	93.25 ± 1.377
T_2	52.22 ± 2.254	51.70 ± 1.907	6.45 ± 0.218	7.12 ± 0.229	0.27 ± 0.013	0.25 ± 0.016	0.19 ± 0.022	0.18 ± 0.023	Negligible	Negligible	94.25 ± 1.109	94.75 ± 1.652

Values are Means ± SE
Means within a column followed by different letters are significantly different $P<0.05$

Table 2: Performance of CSR_{16} and CSR_{27} Silkworm Bivoltine Breeds treated with Indigo Dye for Silk Parameters

Treatments	Average Filament Length (mtrs.)		Average Non-Breakable Filament Length (mtrs.)		Raw Silk %		Renditta		Filament size (Denier)		Reelability %	
	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}
T_0	958.00 ±1.181	969.25 ± 2.016	841.25 ± 0.479	840.75 ± 0.479	18.12 ± 0.004	18.32 ± 0.193	6.69 ± 0.489	6.48 ± 0.305	2.82 ± 0.009	2.84 ± 0.007	81.00 ± 0.408	82.00 ± 1.414
T_1	977.00 ± 0.408	967.50 ± 0.645	841.75 ± 0.479	844.25 ± 2.016	18.20 ± 0.021	18.49 ± 0.272	6.72 ± 0.578	7.19 ± 0.420	2.83 ± 0.004	2.84 ± 0.009	82.00 ± 0.816	84.75 ± 1.797
T_2	979.25 ± 0.629	967.50 ± 1.323	842.50 ± 0.957	843.00 ± 1.291	18.21 ± 0.004	18.24 ± 0.017	6.45 ± 0.298	6.36 ± 0.180	2.86 ± 0.005	2.86 ± 0.006	83.50 ± 0.957	85.55 ± 1.808
	Weight of silk (g)		Weight of Waste (g)		Weight of Cocoons (kgs)		Weight of reelable cocoons (kgs)		Floss percentage		Neatness	
	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}
T_0	52.60 ± 1.037	52.33 ± 1.006	6.15 ± 0.612	6.12 ± 0.551	0.25 ± 0.013	0.25 ± 0.014	0.24 ± 0.017	0.23 ± 0.016	Negligible	Negligible	91.25 ± 0.629	91.75 ± 0.750
T_1	51.37 ± 0.985	52.67 ± 0.997	6.42 ± 0.519	6.28 ± 0.366	0.28 ± 0.025	0.24 ± 0.013	0.22 ± 0.017	0.26 ± 0.024	Negligible	Negligible	93.25 ± 0.479	93.00 ± 1.080
T_2	49.19 ± 2.246	52.60 ± 1.037	7.87 ± 0.610	7.77 ± 0.567	0.28 ± 0.013	0.27 ± 0.019	0.27 ± 0.032	0.29 ± 0.026	Negligible	Negligible	95.00 ± 0.816	93.75 ± 1.750

Values are Means ± SE
Means within a column followed by different letters are significantly different $P<0.05$

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Conflicts of Interest

Declare conflicts of interest or state "The authors declare no conflict of interest."

Disclaimer (Artificial intelligence)

We hereby declare that NO generative AI technologies such as (ChatGPT, COPILOT, etc.) or text-to-image generators have been used during the writing or editing of this manuscript.

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