



Influence of Natural Dyes on the Qualitative and Quantitative Parameters of Bivoltine Silkworm Breeds under Sub-Tropical Conditions of Poonch District, Jammu and Kashmir

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Authors' contributions

This work was carried out in collaboration among all authors. Author RB designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MA and AS managed the analyses of the study. Author AG managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: <https://doi.org/10.9734/jabb/2024/v27i101575>

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/125246>

Original Research Article

Received: 17/08/2024
Accepted: 19/10/2024
Published: 26/10/2024

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ABSTRACT

The study on Influence of Natural Dyes on the qualitative and quantitative parameters of Bivoltine Silkworm Breeds under Sub-tropical Conditions of Poonch District, UT Jammu and Kashmir,” was conducted during the spring of 2022 at the PG, Department of Sericulture, Poonch Campus, University of Jammu. The study examined the effects of feeding bivoltine silkworm breeds CSR₁₆ and CSR₂₇ with two concentrations (50% and 100%) of natural dyes: madder (*Rubia cardifolia*) and indigo blue (*Indigofera tinctoria*). A comprehensive evaluation of both qualitative and quantitative parameters was undertaken, including fecundity, hatching %, larval duration, weight of 10 mature larvae, survival rate, mortality %, pupation % and cocoon quality parameters. The results indicated significant enhancements in fecundity, hatching % and cocoon yield for silkworms treated with both dyes at 100% concentration (T2). Specifically, CSR₁₆ and CSR₂₇ exhibited the highest values for larval weight, cocoon weight, pupation rate and shell ratio % in T2 treatments. The findings suggest that feeding silkworms natural dyes, particularly at higher concentrations, significantly improved the economic parameters of both breeds. While the cocoons produced were intrinsically coloured without compromising commercial parameters, the colour intensity was lighter than anticipated. This implies that introducing dyed mulberry leaves during the 1st instar stage may yield more favourable results. Overall, the study demonstrates that madder and indigo dyes are suitable for producing coloured silk, offering potential for large-scale applications in sustainable silk production.

Keywords: Silkworm; madder dye; indigo dye; colour silk; economic parameters.

1. INTRODUCTION

Sericulture, the art and science of breeding silkworms for silk production, has long been a crucial agro-based sector that significantly contributes to rural economies through revenue generation and job creation. India stands as the second-largest producer of silk globally, following China, with a diverse range of agroclimatic conditions conducive to sericulture, including plains, hills and forests [1]. Notably, India is the only country capable of producing all four commercial varieties of natural silk; mulberry, tasar, eri and muga. However, approximately 68% of mulberry silk is crossbreed [2] which are of lower quality compared to bivoltine silk, known for its superior quality and productivity. The agricultural significance of sericulture is evident, as it provides year-round employment and enhances income for rural farmers. Despite India's status as a leading raw silk producer, the quality of yarn is compromised due to the prevalence of multivoltine races, particularly in tropical climates where the potential for high-quality bivoltine silk is underexploited [3]. The need for improved silkworm breeding strategies, particularly the introduction of compatible bivoltine hybrids in tropical areas, has become increasingly apparent to enhance silk production [4].

The textile industry, a critical component of both developed and developing economies, relies heavily on sericulture. However, the production

process generates vast amounts of polluted wastewater due to the use of various chemicals, including synthetic dyes [5-7]. Dyes that chemically bond with substrates create vibrant, colourfast fabrics but also pose significant environmental challenges [8]. The growing global awareness of environmental sustainability has spurred interest in the use of natural dyes, which are biodegradable and less toxic compared to their synthetic counterparts [9] and [10].

The silk dyeing industry is increasingly embracing sustainable practices, particularly through the use of naturally coloured silk cocoons dyed with eco-friendly plant and animal-based dyes. These natural dyes, being biodegradable and less toxic, present viable alternatives to synthetic options. Recent studies emphasize the necessity for continued research on dye retention in textiles, noting that silkworms fed with natural dyes maintain normal growth and feeding behaviours [11-13]. Additionally, natural dyes like turmeric have been shown to possess medicinal properties, including antibacterial effects.

Natural dyes are capable of producing vibrant colours that can rival those of synthetic dyes while being environmentally safer. Pigments such as chlorophyll and quercetin exhibit excellent colourfastness and ecological benefits [14] and [15]. Dyes derived from tannins and flavonoids are effective in binding to various fabrics, making them ideal for textiles including

wool, cotton, silk and leather [16]. While some natural dyes may demonstrate lower light fastness, others, such as eucalyptus leaf extract, exhibit high fastness even without mordants [17].

In light of these considerations, the present study aims to investigate the influence of natural dyes on the qualitative and quantitative parameters of bivoltine silkworm breeds in the sub-tropical conditions of Poonch District, Jammu and Kashmir. This investigation is particularly relevant given the region's potential for sericulture and the opportunity to enhance silk quality through environmentally sustainable practices. The exploration of natural dyes could not only improve the economic viability of sericulture in the area but also contribute to the reduction of chemical pollution in the textile industry, aligning with global sustainability trends [18-20].

2. MATERIALS AND METHODS

2.1 Study Location and Duration

The study was conducted in the Department of Sericulture at Poonch Campus, University of Jammu, during Spring 2022. It aimed to assess the impact of natural dye-feeding on the qualitative and quantitative parameters of bivoltine silkworm breeds CSR₁₆ and CSR₂₇ under the sub-tropical conditions of Poonch District, Jammu and Kashmir.

2.2 Procurement of Silkworm Seed

Disease-free layings (DFLs) of CSR₁₆ and CSR₂₇ were sourced from the Central Sericultural Research Training and Institute (CSR&TI), Mysore.

2.3 Silkworm Rearing and Environmental Conditions

The experimental design followed the standard sericulture practices, both natural and modified diets, as specified in Table 1, were used in the study. The rearing room and equipment were disinfected using a 2% bleaching powder solution followed by a 2% formalin solution, sealed for 24 hours, and ventilated for another 24 hours to remove residual formalin. DFLs were incubated at 25±1°C and 75±5% relative humidity until reaching the head pigmentation stage. Black boxing was employed to synchronize hatching prior to light exposure. Hatched larvae were

placed in trays lined with paraffin paper to retain humidity and were fed chopped tender mulberry leaves as the primary food source. Temperature and humidity were strictly managed during sensitive larval stages, particularly during moulting. Paraffin paper and foam strips were used to regulate humidity

2.4 Dye Treatments and Experimental Design

Natural dyes (madder and indigo) were sourced from SKYMORN Herbs and Dye Export, India. Two dye concentrations (50% and 100%) were prepared, and larvae were divided into control (T0) and treated groups (T1 for 50%, T2 for 100%), receiving mulberry leaves sprayed with dye solutions during the 5th instar. Silkworms were fed fresh mulberry leaves four times daily, with regular cleaning of beds to maintain hygiene by removing waste and unhealthy larvae. Adequate spacing between trays ensured proper ventilation and feeding was paused during moulting to reduce stress. When the larvae reached maturity, they were transferred to spinning mountages, where they spun cocoons over a three-day period under controlled conditions of 25°C and 60-70% relative humidity. The cocoons were harvested on the 8th day after mounting for analysis of cocoon parameters, such as shell weight, shell ratio % and other parameters.

2.5 Data Collection

Qualitative and quantitative parameters were recorded at various silkworm development stages, including:

2.5.1 Qualitative Parameters

- Egg Stage: Serosa colour, egg shape and chorion colour.
- Larval Stage: Body colour, larval markings, body build and haemolymph colour.
- Cocoon Stage: Cocoon colour, shape and texture.
- Pupal Stage: Pupal colour and shape.
- Adult Stage: Body colour of moths.

2.5.2 Quantitative Parameters

- Egg Stage: Fecundity, hatching percentage and brushing percentage.
- Larval Stage: Duration of the 5th instar, total larval duration, weight of single and ten mature larvae, length of mature larvae, survival rate and mortality percentage.

- Cocoon and Pupal Stage: Cocoon yield, pupation percentage, cocoon and shell weights and shell ratio.

2.6 Statistical Analysis

For statistical analysis, a Completely Randomized Design (CRD) was employed, with square root transformations applied where necessary. Treatment means were compared using the Least Significant Difference (LSD) test, following [21].

3. RESULTS

The study examined the impact of feeding silkworm larvae (CSR₁₆ and CSR₂₇ breeds) with mulberry leaves treated with madder and indigo dye solutions on cocoon colour, as well as various qualitative and quantitative parameters. Both breeds were fed the dyed leaves from the first day of the 5th instar until cocoon spinning. Feeding with 50% concentrations of both dyes showed no visible changes in cocoon colour. However, at 100% concentration, the cocoons turned blue with indigo and red with madder, as detailed in Tables 1 and 2 and shown in Figs. 1 and 2.

3.1 Qualitative Parameters of Bivoltine Silkworm Breeds

3.1.1 Egg Stage observations

The observations are presented in the Fig. 1, both CSR₁₆ and CSR₂₇ breeds produced green, ellipsoidal eggs. CSR₁₆ exhibited white serosa and creamish-white chorion, while CSR₂₇ had yellow serosa and pale-yellow chorion.

3.1.2 Larval Stage observations

Distinct variations were noted in the larval stage. CSR₁₆ larvae had a creamish-white body with markings, whereas CSR₂₇ larvae exhibited a pure white and plain body. The haemolymph in both breeds was transparent and the larvae possessed a cylindrical and stout body structure.

3.1.3 Cocoon Stage observations

At the cocoon stage, both breeds produced bright white cocoons. CSR₁₆ cocoons were dumbbell-shaped, while CSR₂₇ cocoons were oval-shaped, with medium-sized grains. Under 100% dye concentrations, indigo-treated larvae produced blue cocoons and madder-treated larvae produced red cocoons.



Egg laying of CSR16 and CSR27 bivoltine silkworm breed for Egg Parameters



Newly hatched larvae of CSR16 and CSR27 bivoltine silkworm breed for larval Parameters



CSR16 marked larvae (left) and CSR27 plain larvae (right) for Larval Parameters



CSR16 dumbbell cocoons (left) and CSR27 oval cocoons (right) for cocoon Parameters



CSR16 yellow-brown pupae (left) and CSR27 yellowish-brown pupae (right) for pupal Parameters



Moths of CSR16 and CSR27 bivoltine silkworm breed for adult Parameters



Collection of Haemolymph

Fig. 1. Qualitative parameters of CSR₁₆ and CSR₂₇ bivoltine silkworm breeds for egg, larval, cocoon, pupal and adult stages

Table 1. Performance of CSR₁₆ and CSR₂₇ treated with Madder Dye for Egg, Larval, Cocoon, Pupal and adult parameters

Treatments	Egg Parameters							
	Fecundity (no.)		Number of Fertilised eggs		Number of unfertilised eggs		Hatching %	
	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇
T ₀	515.50±10.720	508.75 ± 11.146	493.25 ± 4.535	502.50 ±11.637	22.25 ± 8.107	6.25 ± 0.946	94.55 ± 0.401	98.76 ± 0.208
T ₁	526.75 ± 7.375	525.00 ± 7.906	498.75 ± 1.493	518.25 ± 7.663	28.00 ± 6.519	6.75 ± 0.629	90.56 ± 1.240	98.71 ± 0.113
T ₂	537.50 ± 5.204	531.25 ± 8.985	515.50 ± 4.406	524.00 ± 8.573	22.00 ± 3.136	7.25 ± 1.315	95.01 ± 0.966	98.63 ± 0.238
	Larval parameters							
	V th Instar duration (D: Hrs.)				Total larval duration (D: Hrs.)			
	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇
T ₀	8.35 ± 0.119	8.37 ± 0.048	8.37 ± 0.048	8.37 ± 0.048	25.84 ± 0.254	25.84 ± 0.254	25.92 ± 0.250	25.92 ± 0.250
T ₁	8.52 ± 0.132	8.42 ± 0.111	8.42 ± 0.111	8.42 ± 0.111	25.01 ± 0.148	25.01 ± 0.148	26.32 ± 0.333	26.32 ± 0.333
T ₂	8.32 ± 0.095	8.40 ± 0.091	8.40 ± 0.091	8.40 ± 0.091	24.06 ± 0.019	24.06 ± 0.019	26.12 ± 0.295	26.12 ± 0.295
	Weight of 10 Mature larvae (g)		Length of single mature larvae (cm)		Survival (%)		Larval Mortality (%)	
	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇
	T ₀	40.06 ± 0.480	40.45 ± 0.151	6.65 ± 0.312	6.92 ± 0.193	98.52 ± 0.675	98.08 ± 0.686	1.18 ± 0.048
T ₁	40.83 ± 0.010	41.82 ± 0.016	6.87 ± 0.025	6.67 ± 0.295	96.74 ± 1.131	97.98 ± 0.351	1.83 ± 0.645	1.08 ± 0.209
T ₂	40.82 ± 0.015	42.67 ± 0.085	7.37 ± 0.180	6.55 ± 0.299	98.07 ± 0.829	98.24 ± 0.478	1.265 ± 0.279	2.16 ± 0.347
	Cocoon Parameters							
	ERR by No.		ERR By Weight.				Dead cocoon %	
	CSR ₁₆	CSR ₂₇	Green Cocoon		Dry Cocoon		CSR ₁₆	CSR ₂₇
T ₀	9,225.25 ± 8.635	9,288.25±22.410	14.74 ± 0.310	13.95 ± 0.425	13.71±0.239	13.47±0.332	1.69±0.284	3.74±0.315
T ₁	9,253.00 ± 4.813	9,307.75±25.001	14.66 ± 0.537	14.75 ± 0.619	13.08±0.422	13.64±0.362	2.42±0.569	2.68±0.644
T ₂	9,257.25 ± 5.588	9,243.25±4.535	15.63 ± 0.217	15.19 ± 0.116	14.01±0.021	13.69±0.314	1.61±0.950	3.96±0.699
	Defective cocoon %		Volume of cocoon per litre (no.)		Pupation %			
	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇
	T ₀	4.47±0.604	3.79±1.381	103.75±1.377	105.25±1.109	98.29± 0.284	98.29± 0.284	96.47±0.322
T ₁	3.47±0.618	3.09±0.473	107.25±0.854	105.75±1.493	97.45±0.400	97.45±0.400	97.39±0.605	97.39±0.605
T ₂	2.15±0.532	4.86±0.271	104.75±1.377	104.25±1.377	99.23±0.163	99.23±0.163	96.16 ± 0.645	96.16 ± 0.645
	Single cocoon weight (g)		Shell ratio %		Single shell weight (g)			
	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇
	T ₀	1.47 ± 0.135	1.19 ± 0.050	0.34 ± 0.007	0.39 ± 0.018	22.07 ± 0.182	22.07 ± 0.182	21.96 ± 0.577
T ₁	1.74 ± 0.026	1.47 ± 0.066	0.38 ± 0.001	0.42 ± 0.023	22.53 ± 0.141	22.53 ± 0.141	22.79 ± 0.157	22.79 ± 0.157
T ₂	1.72 ± 0.057	1.62 ± 0.059	0.38 ± 0.001	0.47 ± 0.071	22.46 ± 0.016	22.46 ± 0.016	23.13 ± 0.380	23.13 ± 0.380
	Pupal and adult Parameters							
	Weight of single pupa (g)		Size of single pupa (cm)		Weight of single moth (g)		Size of single moth (cm)	
	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇
T ₀	1.05 ± 0.005	1.06 ± 0.001	2.10 ± 0.041	2.30 ± 0.168	0.45 ± 0.030	0.41 ± 0.009	2.32 ± 0.019	2.30 ± 0.168
T ₁	1.07 ± 0.001	1.06 ± 0.001	2.40 ± 0.071	2.57 ± 0.111	0.39 ± 0.023	0.39 ± 0.023	2.21 ± 0.075	2.57 ± 0.111
T ₂	1.06 ± 0.001	1.08 ± 0.002	2.17 ± 0.048	2.47 ± 0.165	0.40 ± 0.009	0.40 ± 0.009	2.33 ± 0.128	2.47 ± 0.165

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

Table 2. Performance of CSR₁₆ and CSR₂₇ treated with Indigo Dye for Egg, Larval, Cocoon, Pupal and Moth parameters

Treatments	Egg Parameters							
	Fecundity (no.)		Number of Fertilised eggs		Number of unfertilised eggs		Hatching %	
	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇
T ₀	495.0 ± 6.608	511.00 ± 9.626	536.00 ± 1.633	498.00 ± 8.612	3.00 ± 0.408	13.00 ± 1.080	96.45 ± 0.897	97.46 ± 0.170
T ₁	505.0 ± 5.066	536.25 ± 4.270	534.75 ± 1.702	529.00 ± 4.916	4.00 ± 0.913	7.25 ± 0.854	93.27 ± 0.873	98.39 ± 0.281
T ₂	506.7 ± 8.045	526.25 ± 8.985	524.25 ± 2.462	521.75 ± 10.061	3.00 ± 0.707	7.00 ± 2.677	94.22 ± 1.651	99.13 ± 0.363
Treatments	Larval Parameters				Cocoon Parameters			
	V th Instar duration (D: Hrs.)		Total larval duration (D: Hrs.)		ERR (By Number)		ERR (By Weight)	
	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	Green Cocoon	Dry Cocoon	Dead cocoon %	
T ₀	8.32 ± 0.085	8.37 ± 0.095	26.22 ± 0.217	26.22 ± 0.217	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇
T ₁	8.27 ± 0.048	8.22 ± 0.025	24.65 ± 0.332	24.65 ± 0.332	9,232.00±12.774	9,290.50±49.949	14.64±0.349	14.78±0.252
T ₂	8.30 ± 0.071	8.40 ± 0.082	24.90 ± 0.388	24.90 ± 0.388	9,254.00±10.544	9,632.50±36.372	14.75±0.244	14.71±0.331
Treatments	Weight of Ten Mature larvae (g)		Length of single mature larvae (cm)		Survival (%)		Larval Mortality (%)	
	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇
	T ₀	38.87 ± 0.315	39.72 ± 0.408	6.25 ± 0.330	6.67 ± 0.295	97.99 ± 0.528	95.35 ± 0.928	1.41 ± 0.672
T ₁	40.99 ± 0.050	40.84 ± 0.011	6.92 ± 0.193	6.80 ± 0.208	98.48 ± 0.558	99.08 ± 0.284	2.24 ± 0.417	2.32 ± 0.415
T ₂	41.39 ± 0.270	40.84 ± 0.018	6.67 ± 0.295	6.82 ± 0.048	97.23 ± 1.567	97.83 ± 0.346	1.99 ± 0.793	1.56 ± 0.516
Treatments	Pupal and adult Parameters				Moth Parameters			
	Good cocoon %		Defective cocoon %		Volume of cocoon per litre (no.)		Pupation %	
	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇
T ₀	96.33±0.432	96.86±0.587	3.73±0.464	3.08±0.497	105.75±1.031	104.00±0.408	96.77±0.610	96.25±0.385
T ₁	95.97±0.451	96.77±0.515	4.02±0.451	3.73±0.356	106.25±1.109	107.25±1.109	96.84±0.167	95.82±0.295
T ₂	95.53±0.313	97.18±0.520	4.32±0.311	3.16±0.397	105.00±1.291	104.25±1.031	97.37±0.587	96.44±0.511
Treatments	Single cocoon weight (g)		Shell ratio %		Single shell weight (g)		Moth Parameters	
	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	Weight of single moth (g)	Size of single moth (cm)
	T ₀	1.26 ± 0.085	1.60 ± 0.001	21.46 ± 0.294	22.08 ± 0.178	0.34 ± 0.007	0.39 ± 0.018	0.39 ± 0.023
T ₁	1.51 ± 0.084	1.72 ± 0.001	22.59 ± 0.256	22.54 ± 0.145	0.38 ± 0.001	0.42 ± 0.023	0.39 ± 0.023	2.30 ± 0.168
T ₂	1.69 ± 0.031	1.73 ± 0.011	23.55 ± 0.357	22.45 ± 0.014	0.38 ± 0.001	0.47 ± 0.071	0.40 ± 0.009	2.27 ± 0.085
Treatments	Weight of single pupa (g)		Size of single pupa (cm)		Weight of single moth (g)		Size of single moth (cm)	
	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇	CSR ₁₆	CSR ₂₇
	T ₀	1.06 ± 0.016	1.08 ± 0.009	2.15 ± 0.029	2.27 ± 0.075	0.43 ± 0.018	0.40 ± 0.009	2.62 ± 0.138
T ₁	1.04 ± 0.011	1.05 ± 0.007	2.57 ± 0.085	2.55 ± 0.065	0.43 ± 0.018	0.40 ± 0.009	2.62 ± 0.138	2.50 ± 0.178
T ₂	1.06 ± 0.004	1.08 ± 0.006	2.37 ± 0.138	2.42 ± 0.165	0.43 ± 0.018	0.40 ± 0.009	2.62 ± 0.138	2.50 ± 0.178

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

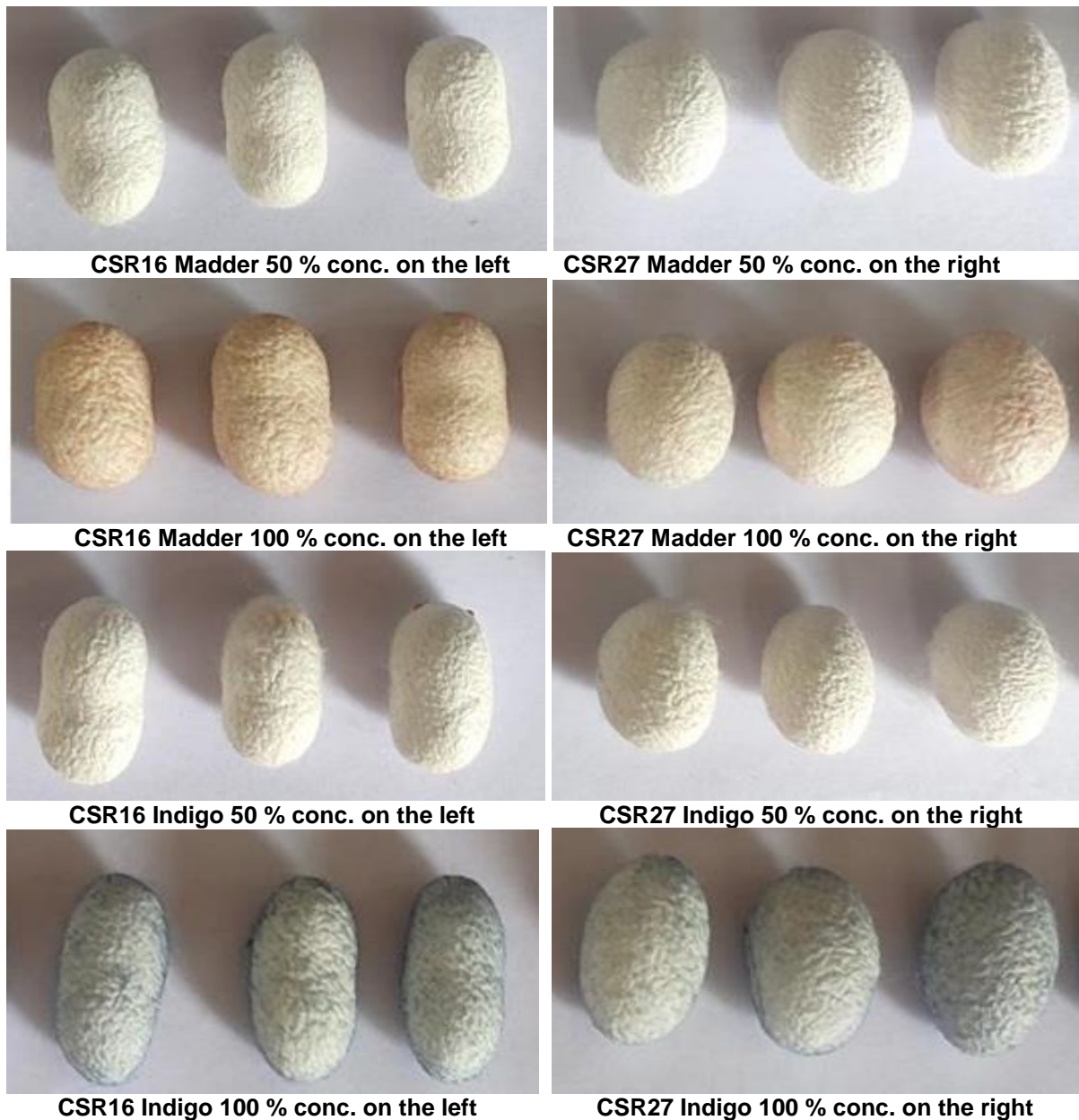


Fig. 2. Cocoons of CSR₁₆ and CSR₂₇ bivoltine silkworm breeds treated with 50% and 100% concentrations of madder and indigo dye

3.1.4 Pupal Stage observations

In the pupal stage, CSR₁₆ pupae were yellow, while CSR₂₇ pupae displayed a yellowish-brown hue. Both breeds exhibited an elliptical pupal shape.

3.1.5 Adult Stage observations

Post-cocoon emergence, male moths of both breeds were creamish-white, while female moths exhibited a dirty colouration.

3.2 Quantitative Parameters of Bivoltine Silkworm Breeds

3.2.1 Egg Stage observations

The fecundity of silkworms treated with indigo dye revealed significant variations among different treatments for both CSR₁₆ and CSR₂₇ breeds. For CSR₁₆, the highest fecundity was observed in T2 (100% indigo concentration), with a recorded value of 506.7 ± 8.045 , followed by T1 (50% indigo concentration) at 505.0 ± 5.066 .

The lowest fecundity was seen in the control group (T0) at 495.0 ± 6.608 (F-cal = 0.883, P = 0.92117). For CSR₂₇, the highest fecundity was recorded in T1 (50% indigo concentration) at 536.25 ± 4.270 , followed by T2 (100% indigo concentration) at 526.25 ± 8.985 , with the lowest value in T0 (controlled) at 511.00 ± 9.626 (F-cal = 1.513, P = 0.27134).

The number of fertilized eggs for CSR₁₆ treated with indigo dye showed the highest value in T0 (controlled) at 536.00 ± 1.633 , followed by T1 (50% indigo) at 534.75 ± 1.702 , with the lowest value in T2 (100% indigo) at 524.25 ± 2.462 (F-cal = 1.513, P = 0.27134). In CSR₂₇, the highest number of fertilized eggs was recorded in T1 (50% indigo) at 529.00 ± 4.916 , followed by T2 (100% indigo) at 521.75 ± 10.061 and the lowest value in T0 (controlled) at 498.00 ± 8.612 (F-cal = 1.389, P = 0.29810).

For unfertilized eggs, the CSR₁₆ breed treated with indigo dye showed the highest value in T1 (50% indigo) at 4.00 ± 0.913 , followed by T2 (100% indigo) at 3.00 ± 0.707 and the lowest value in T0 (controlled) at 3.00 ± 0.408 (F-cal = 1.513, P = 0.27134). In CSR₂₇, the highest number of unfertilized eggs was recorded in T0 (controlled) at 13.00 ± 1.080 , followed by T1 (50% indigo) at 7.25 ± 0.854 and the lowest value in T2 (100% indigo) at 7.00 ± 2.677 (F-cal = 1, P = 0.29810).

The hatching % of CSR₁₆ treated with indigo dye showed significant variations, with the highest value recorded in T0 (controlled) at 96.45 ± 0.897 , followed by T2 (100% indigo) at 94.22 ± 1.651 and the lowest in T1 (50% indigo) at 93.27 ± 0.873 . In CSR₂₇, the hatching % was highest in T2 (100% indigo) at 99.13 ± 0.363 , followed by T1 (50% indigo) at 98.39 ± 0.281 and the lowest in T0 (controlled) at 97.46 ± 0.170 (F-cal = 0.069, P = 0.93350).

Overall, the results from the egg stage indicate that both breeds exhibited enhanced fecundity, fertilized egg numbers and hatching % under indigo dye treatment, particularly at higher concentrations. However, the control group also showed competitive values for certain parameters, indicating variability based on the treatment applied.

3.2.2 Larval Stage observations

In this study, the effects of madder and indigo dyes on the 5th instar duration, total larval

duration, larval weight, length, survival and mortality % in two breeds of silkworms (CSR₁₆ and CSR₂₇) were examined.

For the CSR₁₆ breed treated with madder dye, the highest 5th instar duration was observed in the T1 (50% concentration) group (8.52 ± 0.132 days), while the shortest was in the T2 (100% concentration) group (8.32 ± 0.095 days). In CSR₂₇, T1 also showed the highest duration (8.42 ± 0.111 days) and T0 (control) showed the shortest (8.37 ± 0.048 days). For indigo dye treatments, CSR₁₆ showed the longest duration in the T0 (8.32 ± 0.085 days) and the shortest in T1 (8.27 ± 0.048 days). In CSR₂₇, the longest duration was in T2 (8.40 ± 0.082 days) and the shortest was in T1 (8.22 ± 0.025 days).

The total larval duration of CSR₁₆ treated with madder dye was longest in T0 (25.84 ± 0.254 days) and shortest in T2 (24.06 ± 0.019 days). For CSR₂₇, T1 had the longest duration (26.32 ± 0.333 days) and T0 the shortest (25.92 ± 0.250 days). In indigo-treated CSR₁₆, T0 showed the longest duration (26.22 ± 0.217 days), while T1 was the shortest (24.65 ± 0.332 days). For CSR₂₇ treated with indigo, T2 had the longest duration (26.27 ± 0.295 days) and T1 the shortest (25.95 ± 0.328 days).

CSR₁₆ treated with madder dye showed the highest weight in T2 (40.82 ± 0.015 g) and the lowest in T0 (40.06 ± 0.480 g). In CSR₂₇, T2 recorded the highest weight (42.67 ± 0.085 g) and T0 had the lowest (40.45 ± 0.151 g). For indigo treatments, the highest weight in CSR₁₆ was observed in T2 (41.39 ± 0.270 g) and the lowest in T0 (38.87 ± 0.315 g). CSR₂₇ treated with indigo dye showed the highest weight in T2 (40.84 ± 0.018 g) and the lowest in T0 (39.72 ± 0.408 g).

In CSR₁₆, the longest larvae treated with madder dye were recorded in T2 (7.37 ± 0.180 cm) and the shortest in T0 (6.65 ± 0.312 cm). In CSR₂₇, T0 showed the longest larvae (6.92 ± 0.193 cm), while T2 had the shortest (6.55 ± 0.299 cm). For indigo-treated CSR₁₆, T1 had the longest larvae (6.92 ± 0.193 cm) and T0 had the shortest (6.25 ± 0.330 cm). CSR₂₇ treated with indigo showed the longest larvae in T2 (6.82 ± 0.048 cm) and the shortest in T0 (6.67 ± 0.295 cm).

CSR₁₆ treated with madder dye had the highest survival in T0 ($98.52 \pm 0.675\%$) and the lowest in T1 ($96.74 \pm 1.131\%$). In CSR₂₇, T2 had the highest survival ($98.248 \pm 0.478\%$) and T1 the

lowest ($97.98 \pm 0.351\%$). In CSR₁₆ treated with indigo dye, T1 showed the highest survival ($98.48 \pm 0.558\%$) and T2 the lowest ($97.23 \pm 1.567\%$). In CSR₂₇, the highest survival was in T1 ($99.08 \pm 0.284\%$), while T0 had the lowest ($95.35 \pm 0.928\%$). CSR₁₆ treated with madder dye had the highest mortality in T2 ($1.265 \pm 0.279\%$) and the lowest in T0 ($1.18 \pm 0.048\%$). For CSR₂₇, T0 had the highest mortality ($2.74 \pm 0.417\%$) and T1 the lowest ($1.08 \pm 0.209\%$). In CSR₁₆ treated with indigo dye, T1 had the highest mortality ($2.24 \pm 0.417\%$) and T0 the lowest ($1.41 \pm 0.672\%$). CSR₂₇ treated with indigo showed the highest mortality in T1 ($2.32 \pm 0.415\%$) and the lowest in T2 ($1.56 \pm 0.516\%$). These findings suggest that different concentrations of natural dyes influence various parameters of larval development and survival, with distinct breed-specific responses to madder and indigo treatments. The data is presented in Tables 1 and 2 and Fig. 2.

3.2.3 Cocoon and Pupal Stage Observations

The study analyzed cocoon yield and related parameters of CSR₁₆ and CSR₂₇ silkworm breeds treated with varying concentrations of madder and indigo dyes. For CSR₁₆ treated with madder dye, the highest yield by weight (green cocoon) was observed in T2 (100% concentration) with 15.63 ± 0.217 , while the lowest yield was in T1 (50% concentration) at 14.66 ± 0.537 . CSR₂₇ also showed the highest yield in T2 (100% concentration) at 15.19 ± 0.116 . In the case of indigo dye treatment, CSR₁₆ achieved its highest yield in T1 (50% concentration) at 14.75 ± 0.244 and CSR₂₇ recorded the highest yield in the control group (T0) at 14.78 ± 0.252 . For dry cocoon yield, CSR₁₆ had the highest in T2 (100% madder concentration) at 14.01 ± 0.021 , while CSR₂₇ recorded the highest in T2 (100% madder concentration) at 13.69 ± 0.314 . With indigo dye, CSR₁₆ had its highest yield in the control group (T0) at 14.78 ± 0.252 , while CSR₂₇ had the highest in the control group (T0) at 14.60 ± 0.659 . In terms of cocoon yield by number, CSR₁₆ treated with madder dye exhibited the highest number of cocoons in T2 (100% concentration) at $9,257.25 \pm 5.588$, while CSR₂₇ showed the highest in T1 (50% concentration) at $9,307.75 \pm 25.001$. With indigo dye treatment, CSR₁₆ highest yield was in T1 (50% concentration) at $9,254.00 \pm 10.544$ and CSR₂₇ had the highest in T2 (100% concentration) at $9,635.75 \pm 47.068$. The dead cocoon % for CSR₁₆ treated with madder dye was highest in T1 (50% concentration) at 2.42 ± 0.569 , while

CSR₂₇ recorded the highest in T2 (100% concentration) at 3.96 ± 0.699 . With indigo dye treatment, CSR₁₆ showed the highest dead cocoon % in the control group (T0) at 3.21 ± 0.608 and CSR₂₇ had the highest in T1 (50% concentration) at 4.16 ± 0.295 . For defective cocoon %, CSR₁₆ treated with madder dye had the highest in the control group (T0) at 4.47 ± 0.604 , while CSR₂₇ had the highest in T2 (100% concentration) at 4.86 ± 0.271 . With indigo dye, CSR₁₆'s highest defective cocoon % was in T2 (100% concentration) at 4.32 ± 0.311 and CSR₂₇'s highest was in T1 (50% concentration) at 3.73 ± 0.356 . The cocoon volume (in no.) per replication showed that CSR₁₆ treated with madder dye had the highest volume in T1 (50% concentration) at 107.25 ± 0.854 and CSR₂₇ had the highest in T1 (50% concentration) at 105.75 ± 1.493 . With indigo dye treatment, CSR₁₆ showed the highest cocoon volume in T1 (50% concentration) at 106.25 ± 1.109 , while CSR₂₇ recorded the highest in T1 (50% concentration) at 107.25 ± 1.109 . The pupation % for CSR₁₆ treated with madder dye was highest in T2 (100% concentration) at 99.23 ± 0.163 , while CSR₂₇ recorded the highest in T1 (50% concentration) at 97.39 ± 0.605 . With indigo dye treatment, CSR₁₆ had the highest pupation % in T2 (100% concentration) at 97.37 ± 0.587 and CSR₂₇ had the highest in T2 (100% concentration) at 96.44 ± 0.511 . These results highlight the significant influence of natural dyes (madder and indigo) on cocoon yield, dead cocoon %, defective cocoon % and pupation % in CSR₁₆ and CSR₂₇ silkworm breeds.

The study on CSR₁₆ and CSR₂₇ breeds treated with natural dyes (madder and indigo) revealed significant variations in cocoon weight, shell weight and shell ratio across different dye concentrations. For CSR₁₆ treated with madder, the highest single cocoon weight was observed in the 50% concentration (1.748 g), followed closely by the 100% concentration (1.721 g), while the control group recorded the lowest (1.470 g). Similar trends were seen in CSR₂₇, where the highest cocoon weight was in the 100% madder concentration (1.624 g), with the lowest in the control (1.195 g). In the indigo-treated groups, CSR₁₆ exhibited the highest single cocoon weight at 100% concentration (1.69 g) and CSR₂₇ showed its maximum at 100% concentration as well (1.73 g). Both breeds demonstrated that the indigo dye concentrations yielded significantly higher cocoon weights compared to controls. The single shell weight also showed substantial variations. For CSR₁₆

treated with madder, the 50% concentration recorded the highest shell weight (0.386 g), followed by the 100% concentration (0.384 g), while the control was the lowest (0.345 g). CSR₂₇ showed a similar pattern with the highest shell weight at 100% madder concentration (0.471 g). The indigo dye treatments resulted in the highest shell weight for CSR₁₆ at 100% concentration (0.556 g) and for CSR₂₇ at 50% concentration (0.386 g), both being significantly higher than their respective controls. In terms of shell ratio %, CSR₁₆ and CSR₂₇ showed minor but notable variations under both madder and indigo dye treatments. The highest shell ratio for CSR₁₆ was observed with 50% madder concentration (22.535%), while for CSR₂₇, the highest ratio was seen with 100% madder concentration (23.138%). Indigo treatments for CSR₁₆ resulted in the highest shell ratio at 100% concentration (23.550%), whereas for CSR₂₇, the highest was recorded at 50% indigo concentration (22.543%).

Overall, the use of natural dyes, particularly at higher concentrations, significantly improved cocoon weight, shell weight and shell ratio % for both CSR₁₆ and CSR₂₇, indicating a potential enhancement in silk yield under these treatments.

4. DISCUSSION

This study emphasizes the promising potential of plant-based natural dyes in sericulture, particularly in the production of silk. The ability to colour silk cocoons with eco-friendly dyes without adversely affecting silkworm growth or feeding patterns is encouraging. However, challenges remain, particularly regarding colour retention after processing. Research indicates that dyes with a positive partition coefficient may struggle to retain colour effectively post-degumming, highlighting the need for further investigation to identify dyes that ensure vibrant colouration alongside durability [22-24].

Recent investigations have focused on supplementing mulberry leaves with natural dyes such as madder and indigo, which have yielded mixed results in terms of silk colouration and the overall biology of silkworms. Some dietary supplements have shown potential to enhance traits like fecundity and growth; however, the biological impact of these supplements remains inconsistent, suggesting a need for optimized dietary formulations [25-27]. The increasing preference for natural dyes can be attributed to their biodegradability, reduced toxicity and

cultural significance, as seen in the calming effects associated with indigo in Japan.

Historically, natural dyes have played a significant role in textiles, yet modern challenges such as colourfastness persist. The use of tannins and flavonoids has been shown to improve dye retention, offering a sustainable alternative that does not rely on synthetic mordants [28] and [29]. This study further highlights the potential for natural dyes to enhance reproductive outcomes, fecundity, hatching and larval development in silkworms, particularly with madder dye, which may contribute to improved breeding success [30-32].

In examining larval stages, it was noted that natural dyes like madder and indigo influenced rearing duration, labour input and growth rates. Lower concentrations of madder (50%) extended the duration of the 5th instar, whereas higher concentrations (100%) resulted in a reduction. Phytochemicals such as tannins and quercetin appear to enhance silk colouration without the need for mordants, thereby influencing metabolic processes and growth [33]. These findings align with existing literature, which indicates a correlation between increased larval duration and improved conversion efficiencies [34].

The impact of dye treatments on cocoon characteristics was also significant. Experiments involving CSR₁₆ and CSR₂₇ silkworm breeds demonstrated that madder and indigo dyes affected cocoon yield, weight and pupation rates. Notably, CSR₁₆ exhibited the highest cocoon yield at 100% madder concentration, while CSR₂₇ performed optimally at 50% madder concentration. Variability in results was also observed in indigo-treated groups, with control samples showing higher dry cocoon weights. Pupation rates peaked in CSR₁₆ treated with madder, underscoring the importance of dye concentration on rearing efficiency [35] and [36].

4.1 Influence of Diet on Silk Production

The diet of silkworms plays a crucial role in their fecundity and overall growth. Breeds such as CSR₁₆ and CSR₂₇, when fed with madder and indigo dyes, have shown improvements in hatching rates and overall productivity [37] and [38]. Adequate feeding and optimal environmental conditions during incubation are pivotal for maximizing yield [39].

4.2 Impact of Natural Dyes on Larval and Cocoon Stages

The larval stage is critical for silk production, as food intake significantly influences productivity [40]. Research shows that CSR₁₆ and CSR₂₇ silkworms fed with plant dyes like madder or indigo exhibit variations in larval duration, weight and survival rates [30] and [22]. Increased concentrations of dye often lead to higher larval weights but reduced survival, whereas moderate concentrations can optimize both growth and robustness [23].

In the cocoon stage, dye treatments affect yield, weight and the quality of silk produced. For example, CSR₁₆ and CSR₂₇ silkworms treated with madder dye achieved the highest cocoon yields at a 100% concentration, while control groups yielded better results with indigo treatments [30] and [41]. The characteristics of cocoons, such as shell weight and volume, were also enhanced with specific dye concentrations.

4.3 Pupal and Adult Stages: Effects of Dye Treatments

The weight and size of pupae are significantly influenced by dye treatments. CSR₁₆ and CSR₂₇ silkworms treated with madder or indigo dyes exhibited varying pupal and moth sizes according to the dye concentration applied [42]. Dietary modifications have been shown to positively affect both cocoon and pupal characteristics [43].

5. CONCLUSION

The study demonstrated that incorporating natural dyes, specifically madder and indigo at a 100% concentration, into the diet of bivoltine silkworm breeds CSR₁₆ and CSR₂₇ significantly enhanced key economic and biological parameters. Improvements were noted in fecundity, hatching %, larval and cocoon weight, pupation rate and shell ratio % for both breeds when fed mulberry leaves treated with dye. Although the cocoons produced exhibited intrinsic colouration, the colour intensity was lighter than expected. These findings indicate that feeding dyed mulberry leaves from the 1st instar stage could yield deeper hues. These findings suggest that madder and indigo dyes could serve as effective dietary additives to optimize sericulture outcomes in sub-tropical conditions. Future research should focus on refining dye concentrations for maximum benefit

and exploring other natural dyes for similar effects, thereby contributing to more profitable and sustainable silk production in the region.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kumar S, et al. Global silk market trends and challenges: A review. *J Textile Sci Technol.* 2020;6(3):201–15.
2. Chandrashekhar KB, Chandrakanth N. Silkworm breeds and their characteristics. In: *A journey of 75 years: technological advancements in tropical mulberry silk production.* Mysore: CS-CSR&TI; 2024. p. 131–44.
3. Brahma S, Das K, Sinha A. Hybridization in silkworm breeding for increased productivity. *J Entomol Res.* 2015;59(1): 75–80.
4. Lakshmi R, Srinivas D, Rao M. Bivoltine silkworm breeds and their role in enhancing silk quality in India. *J Indian Seric.* 2011;45(1):35–42.
5. Ademorotti G. Environmental impacts of traditional silk dyeing methods. *J Textile Sustain.* 1992;12(3):45–50.
6. Navarro DM. Toxicity and environmental impact of synthetic dyes in textile industry. *Environ Pollut.* 2001;112(2):345–50.
7. Sheng Q, Chi M. Wet processing in textiles: Issues and solutions. *J Clean Prod.* 2003;11(4):321–9.
8. Yang Z, Al-Duri B. Reactive dyes in the textile industry: An overview. *J Chem Technol Biotechnol.* 2001;76(1):5–12.
9. Anitha K, Prasad MNV. Natural dyes: A potential alternative to synthetic dyes in the textile industry. *Textile Res J.* 2007;77(8):569–75.
10. Subhashini V, Kumar P, Reddy K. Natural dyes as sustainable alternatives in silk production. *Int J Environ Sci.* 2009; 3(2):95–100.

11. Hill P. Dye retention in natural silk fibers: Challenges and solutions. *Textile Sci J*. 1997;29(2):110–5.
12. Han J, Yang L. Antimicrobial properties of natural dyes in silk production. *J Nat Prod*. 2005;68(3):400–6.
13. Issa KT, Khalaf AAA, Saad MSI, Ahmed HHR. Assessment of use of some dyes for production of coloured silk from silkworm, *Bombyx mori* L. in Egypt. *Ann Biol*. 2021;37(2):201–8.
14. Adeel M, Khan S, Ali M. Chlorophyll and quercetin as natural dyes: Colourfastness and ecological benefits. *Nat Dyes Colourants*. 2009;7(2):123–30.
15. Cristea A, Vilarem J. Tannins and flavonoids in natural dye binding to textiles. *Textile Chem Technol*. 2006;38(4):220–5.
16. Mongkholrattanasit J, Punrattanasin T. Binding effectiveness of tannins and flavonoids in natural dyes for silk. *J Nat Dyes*. 2012;25(2):140–8.
17. Janani M, Winifred S. Eucalyptus leaf extract as a high fastness natural dye for silk textiles. *J Textile Fiber Res*. 2013;64(3):225–30.
18. Bhuyan K, Saikia B. Eco-friendly dyes: A review. *Dyes Pigments*. 2005;67(1):65–80.
19. Kulkarni AR, et al. Biodegradable natural dyes: A review. *J Environ Sci Technol*. 2011;4(3):165–70.
20. Nisal A, Trivedy K, Mohammad H, et al. Uptake of azo dyes into silk glands for production of colored silk cocoons using a green feeding approach. *ACS Sustain Chem Eng*. 2014;2(2):312–7.
21. Rangaswami R. A textbook of agricultural statistics. 2nd ed. New Delhi: New Age International Publishers; 2010.
22. Asif M, Khodadadi N. Colour retention challenges of natural dyes in silk post-degumming. *J Textile Chem*. 2013;58(3):210–5.
23. Kulkarni P, Sharma R, Deshpande M. Optimizing natural dye concentrations for enhanced silk quality. *Int J Seric Res*. 2012;19(2):145–50.
24. Nazeer MB, Nawas MA, Kebaraj R, Balasundaram A. Effect of vital dye supplementation on nutritional indices of mulberry silkworm, *Bombyx mori* L. *Int J Entomol Res*. 2023;8(8):24–8.
25. Shankar R, Suresh HM, Devaiah MC, Gnanesh BN. Supplementation of mulberry leaves with natural dyes: Effects on silk coloration and silkworm biology. *J Seric Res*. 1994;28(4):200–5.
26. Murthy VVN, Ravikumar C, Ramesh KM. Impact of temperature and humidity on the performance of silkworm *Bombyx mori* L. during different seasons. *J Glob Biosci*. 2013;2(4):115–21.
27. Uyen NTT, Hung NP, Thao TH, Nu THL, Huong MB. Characterization of self-dyed silk yarn with Rhodamine B dye for fashion applications. *Int J Clothing Sci Technol*; 2023. DOI:10.1108/IJCST-10-2022-0147.
28. Koilpillai P. Role of tannins and flavonoids in natural dye retention in silk. *Indian J Seric*. 1995;32(2):120–5.
29. Cheng Y, Fan X, Liu Z. Sustainable silk production through natural dye feeding methods. *J Sustain Text*. 2019;22(3):150–60.
30. Basu S, Roy P, Das D. Influence of natural dyes on reproductive outcomes in silkworms. *Sericulture J*. 1994;40(4):200–5.
31. Chandrashekar K. Impact of madder dye on silkworm fecundity and larval development. *J Appl Seric*. 1996;44(2):98–104.
32. Uyen TNT, Vy DK, Phuc NH, Thanh H. Self-dyed silk by feeding silkworms with coloured mulberry leaves—approaching sustainable silk treatment technology. *J Sustain Sci Manag*. 2024; 19(1):63–73.
33. Soliman A. Phytochemical effects on silk coloration and silkworm metabolism. *J Appl Phytochem*. 1982;15(1): 70–5.
34. Yamamoto Y, Gamo M. Correlation between larval duration and conversion efficiencies in *Bombyx mori*. *Entomol Stud*. 1976;25(2):120–5.
35. Minagava K, Otsuka T. Impact of madder dye concentration on cocoon yield in *Bombyx mori*. *Jpn J Seric*. 1975;15(1):50–5.
36. Bandopaday S. Effects of indigo dye on cocoon yield and quality in *Bombyx mori*. *Indian J Seric*. 1990;29(2):30–5.
37. Tazima M. Standard sericulture practices for optimal silk production. *J Seric*. 1957;2(1):10–5.
38. Muthukrishnan K, Pandian R. Silkworm feeding practices for enhanced silk production. *J Appl Entomol*. 1987;24(3): 150–5.
39. Rahmathulla VK. Management of climatic factors for successful silkworm (*Bombyx mori* L.) crop and higher silk production: a

- review. Psyche: J Entomol. 2012;2012(1): 121234.
40. Fukuda T. Nutrition and productivity in silkworms. J Seric. 1960;8(1): 50–5.
41. Mustafaev AA. Effects of indigo dye on cocoon weight and silk quality. Sov J Seric. 1975;20(1):90–5.
42. Fan X, Cheng Y, Li H. Environmental benefits of dye-fed sericulture practices. Int J Textile Res. 2019;45(2):134-42.
43. Muruges T, Mahalingam M. Dietary modifications and their effects on pupal characteristics in silkworms. J Entomol Stud. 2005;48(3):180–5.

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