Influence of Natural Dyes on the qualitative and quantitative parameters of Bivoltine Silkworm Breeds under Sub-tropical Conditions of Poonch District, Jammu and Kashmir

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 $CSR_{16}$and $CSR_{27}$with two concentrations (50% and 100%) of natural dyes: madder*(Rubiacardifolia)*and indigo blue (*Indigoferatinctoria*). 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, $CSR_{16}$and CSR_{27}exhibited the highest values for larval weight, cocoon weight, pupation rateand 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 traits, 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 (Kumar *et al.*, 2020). 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 (Chandrashekhar and Chandrakanth, 2024) 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 (Brahma *et al.*, 2015). 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 (Lakshmi *et al*., 2011).

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 (Ademorotti, 1992; Navarro, 2001 and Sheng & Chi, 2003). Dyes that chemically bond with substrates create vibrant, colourfast fabrics but also pose significant environmental challenges (Yang & Al-Duri, 2001). 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 (Anitha & Prasad, 2007 and Subhashini *et al*., 2009).

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 (Hill, 1997; Han & Yang, 2005& Issa *et al*., 2021). 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 (Adeel *et al*., 2009 and Cristea & Vilarem, 2006). Dyes derived from tannins and flavonoids are effective in binding to various fabrics, making them ideal for textiles including wool, cotton, silk and leather (Mongkholrattanasit & Punrattanasin, 2012). While some natural dyes may demonstrate lower light fastness, others, such as eucalyptus leaf extract, exhibit high fastness even without mordants (Janani & Winifred, 2013).

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 (Bhuyan & Saikia, 2005; Kulkarni *et al.*, 2011& Nisal *et al.*, 2014).

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 $CSR_{16}$and CSR_{27}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 5^{th} 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 8^{th} 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 5^{th} 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 Rangaswami (2010).

3. RESULTS

The study examined the impact of feeding silkworm larvae (CSR$_{16}$and CSR$_{27}$breeds) with mulberry leaves treated with madder and indigo dye solutions on cocoon color, as well as various qualitative and quantitative traits. 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 color. However, at 100% concentration, the cocoons turned blue with indigo and red with madder, as detailed in Tables 1 and 2 and shown in Figure's 1 and2.

3.1.Qualitative Parameters of Bivoltine Silkworm Breeds

3.1.1. Egg Stage observations

The observations are presented in the Figure 1,both CSR_{16} and CSR_{27} breeds produced green, ellipsoidal eggs. CSR_{16} exhibited white serosa and creamish-white chorion, while CSR_{27} had yellow serosa and pale-yellow chorion.

3.1.2. Larval Stage observations

Distinct variations were noted in the larval stage. CSR_{16} larvae had a creamish-white body with markings, whereas CSR_{27} larvae exhibited a pure white and plain body. The haemolymph in both breeds was transparentand 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. CSR_{16} cocoons were dumbbell-shaped, while CSR_{27} cocoons were oval-shaped, with medium-sized grains. Under 100% dye concentrations, indigo-treated larvae produced blue cocoonsand madder-treated larvae produced red cocoons.

3.1.4. Pupal Stage observations

In the pupal stage, CSR_{16} pupae were yellow, while CSR_{27} 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 $CSR_{16}$and CSR_{27}breeds. For CSR_{16}, 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 CSR_{27}, 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 CSR_{16}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 CSR_{27}, 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 CSR_{16}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 CSR_{27}, the highest number of unfertilized eggs was recorded in T0 (controlled) at 13.00 ± 1.080, followed by T1 (50% indigo) at 7.25 ± 0.854and the lowest value in T2 (100% indigo) at 7.00 ± 2.677 (F-cal = 1, P = 0.29810).

The hatching % of CSR_{16}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 CSR_{27}, the hatching % was highest in T2 (100% indigo) at 99.13 ± 0.363, followed by T1 (50% indigo) at 98.39 ± 0.281and 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 numbersand 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 ($CSR_{16}$and CSR_{27}) were examined.

For the CSR_{16}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 CSR_{27}, 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, CSR_{16}showed the longest duration in the T0 (8.32 ± 0.085 days) and the shortest in T1 (8.27 ± 0.048 days). In CSR_{27}, 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 CSR_{16}treated with madder dye was longest in T0 (25.84 ± 0.254 days) and shortest in T2 (24.06 ± 0.019 days). For CSR_{27}, T1 had the longest duration (26.32 ± 0.333 days) and T0 the shortest (25.92 ± 0.250 days). In indigo-treated CSR_{16}, T0 showed the longest duration (26.22 ± 0.217 days), while T1 was the shortest (24.65 ± 0.332 days). For CSR_{27}treated with indigo, T2 had the longest duration (26.27 ± 0.295 days) and T1 the shortest (25.95 ± 0.328 days).

CSR_{16}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 CSR_{27}, 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 $CSR_{16}$was observed in T2 (41.39 ± 0.270 g) and the lowest in T0 (38.87 ± 0.315 g). CSR_{27}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 CSR_{16}, 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 CSR_{27}, T0 showed the longest larvae (6.92 ± 0.193 cm), while T2 had the shortest (6.55 ± 0.299 cm). For indigo-treated CSR_{16}, T1 had the longest larvae (6.92 ± 0.193 cm) and T0 had the shortest (6.25 ± 0.330 cm). CSR_{27}treated with indigo showed the longest larvae in T2 (6.82 ± 0.048 cm) and the shortest in T0 (6.67 ± 0.295 cm).

CSR_{16}treated with madder dye had the highest survival in T0 (98.52 ± 0.675%) and the lowest in T1 (96.74 ± 1.131%). In CSR_{27}, T2 had the highest survival (98.248 ± 0.478%) and T1 the lowest (97.98 ± 0.351%). In CSR_{16}treated with indigo dye, T1 showed the highest survival (98.48 ± 0.558%) and T2 the lowest (97.23 ± 1.567%). In CSR_{27}, the highest survival was in T1 (99.08 ± 0.284%), while T0 had the lowest (95.35 ± 0.928%). CSR_{16}treated with madder dye had the highest mortality in T2 (1.265 ± 0.279%) and the lowest in T0 (1.18 ± 0.048%). For CSR_{27}, T0 had the highest mortality (2.74 ± 0.417%)and T1 the lowest (1.08 ± 0.209%). In CSR_{16}treated with indigo dye, T1 had the highest mortality (2.24 ± 0.417%) and T0 the lowest (1.41 ± 0.672%). CSR_{27}treated with indigo showed the highest mortality in T1 (2.32 ± 0.415%) and the lowest in T2 (1.56 ± 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 Figure 2.

3.2.3. Cocoon and Pupal Stage Observations

The study analyzed cocoon yield and related parameters of $CSR_{16}$and CSR_{27} silkworm breeds treated with varying concentrations of madder and indigo dyes. For CSR_{16}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. CSR_{27} also showed the highest yield in T2 (100% concentration) at 15.19 ± 0.116. In the case of indigo dye treatment, CSR_{16}achieved its highest yield in T1 (50% concentration) at 14.75 ± 0.244and CSR27 recorded the highest yield in the control group (T0) at 14.78 ± 0.252. For dry cocoon yield, CSR_{16}had the highest in T2 (100% madder concentration) at 14.01 ± 0.021, while CSR_{27} recorded the highest in T2 (100% madder concentration) at 13.69 ± 0.314. With indigo dye, CSR_{16}had its highest yield in the control group (T0) at 14.78 ± 0.252, while CSR_{27} had the highest in the control group (T0) at 14.60 ± 0.659. In terms of cocoon yield by number, CSR_{16}treated with madder dye exhibited the highest number of cocoons in T2 (100% concentration) at 9,257.25 ± 5.588, while CSR_{27} showed the highest in T1 (50% concentration) at 9,307.75 ± 25.001. With indigo dye treatment, CSR_{16}highest yield was in T1 (50% concentration) at 9,254.00 ± 10.544and CSR_{27} had the highest in T2 (100% concentration) at 9,635.75 ± 47.068. The dead cocoon % for CSR_{16}treated with madder dye was highest in T1 (50% concentration) at 2.42 ± 0.569, while CSR_{27} recorded the highest in T2 (100% concentration) at 3.96 ± 0.699. With indigo dye treatment, CSR_{16}showed the highest dead cocoon % in the control group (T0) at 3.21 ± 0.608 and CSR_{27} had the highest in T1 (50% concentration) at 4.16 ± 0.295. For defective cocoon %, CSR_{16}treated with madder dye had the highest in the control group (T0) at 4.47 ± 0.604, while CSR_{27} had the highest in T2 (100% concentration) at 4.86 ± 0.271. With indigo dye, CSR_{16}'s highest defective cocoon % was in T2 (100% concentration) at 4.32 ± 0.311 and CSR_{27}'s highest was in T1 (50% concentration) at 3.73 ± 0.356. The cocoon volume per replication showed that CSR_{16}treated with madder dye had the highest volume in T1 (50% concentration) at 107.25 ± 0.854 and CSR_{27} had the highest in T1 (50% concentration) at 105.75 ± 1.493. With indigo dye treatment, CSR_{16}showed the highest cocoon volume in T1 (50% concentration) at 106.25 ± 1.109, while CSR_{27} recorded the highest in T1 (50% concentration) at 107.25 ± 1.109. The pupation % for CSR_{16}treated with madder dye was highest in T2 (100% concentration) at 99.23 ± 0.163, while CSR_{27} recorded the highest in T1 (50% concentration) at 97.39 ± 0.605. With indigo dye treatment, CSR_{16}had the highest pupation % in T2 (100% concentration) at 97.37 ± 0.587and CSR_{27} 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 $CSR_{16}$and CSR_{27} silkworm breeds.

The study on $CSR_{16}$and CSR_{27} breeds treated with natural dyes (madder and indigo) revealed significant variations in cocoon weight, shell weight and shell ratio across different dye concentrations. For CSR_{16}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 CSR_{27}, 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, CSR_{16}exhibited the highest single cocoon weight at 100% concentration (1.69 g) and CSR_{27} 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 CSR_{16}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). CSR_{27} 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 CSR_{16}at 100% concentration (0.556 g) and for CSR_{27} at 50% concentration (0.386 g), both being significantly higher than their respective controls. In terms of shell ratio %, $CSR_{16}$and CSR_{27} showed minor but notable variations under both madder and indigo dye treatments. The highest shell ratio for $CSR_{16}$was observed with 50% madder concentration (22.535%), while for CSR_{27}, the highest ratio was seen with 100% madder concentration (23.138%). Indigo treatments for CSR_{16}resulted in the highest shell ratio at 100% concentration (23.550%), whereas for CSR_{27}, 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 $CSR_{16}$and CSR_{27}, 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 (Asif & Khodadadi, 2013 and Kulkarni *et al*., 2012& Nazeer et al., 2023).

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 coloration 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 (Shankar *et al*., 1994; Murthy *et al*., 2013& Uyen et al., 2023). 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 (Koilpillai, 1995 and Morohoshi, 1969). 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 (Basu *et al*., 1994; Chandrashekar, 1996& Uyen et al., 2024).

In examining larval stages, it was noted that natural dyes like madder and indigo influenced rearing duration, labour inputand 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 coloration without the need for mordants, thereby influencing metabolic processes and growth (Soliman, 1982). These findings align with existing literature, which indicates a correlation between increased larval duration and improved conversion efficiencies (Yamamoto & Gamo, 1976).

The impact of dye treatments on cocoon characteristics was also significant. Experiments involving CSR_{16} and CSR_{27} silkworm breeds demonstrated that madder and indigo dyes affected cocoon yield, weight and pupation rates. Notably, CSR_{16} exhibited the highest cocoon yield at 100% madder concentration, while CSR_{27} 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 CSR_{16} treated with madder, underscoring the importance of dye concentration on rearing efficiency (Minagava & Otsuka, 1975 and Bandopaday, 1990).

Influence of Diet on Silk Production

The diet of silkworms plays a crucial role in their fecundity and overall growth. Breeds such as CSR_{16} and CSR_{27}, when fed with madder and indigo dyes, have shown improvements in hatching rates and overall productivity (Tazima, 1957 and Muthukrishnan & Pandian, 1987). Adequate feeding and optimal environmental conditions during incubation are pivotal for maximizing yield (Rahmathulla, 2012).

Impact of Natural Dyes on Larval and Cocoon Stages

The larval stage is critical for silk production, as food intake significantly influences productivity (Fukuda, 1960). Research shows that CSR_{16} and CSR_{27} silkworms fed with plant dyes like madder or indigo exhibit variations in larval duration, weight and survival rates (Basu *et al*., 1994 and Asif & Khodadadi, 2013). Increased concentrations of dye often lead to higher larval weights but reduced survival, whereas moderate concentrations can optimize both growth and robustness (Kulkarni *et al*., 2012).

In the cocoon stage, dye treatments affect yield, weightand the quality of silk produced. For example, CSR_{16} and CSR_{27} silkworms treated with madder dye achieved the highest cocoon yields at a 100% concentration, while control groups yielded better results with indigo treatments (Basu *etal.*, 1994 and Mustafaev, 1975). The characteristics of cocoons, such as shell weight and volume, were also enhanced with specific dye concentrations.

Pupal and Adult Stages: Effects of Dye Treatments

The weight and size of pupae are significantly influenced by dye treatments. CSR_{16} and CSR_{27} silkworms treated with madder or indigo dyes exhibited varying pupal and moth sizes according to the dye concentration applied (Bhaskar *et al*., 2004). Dietary modifications have been shown to positively affect both cocoon and pupal characteristics (Murugesh & Mahalingam, 2005).

5. CONCLUSION

The study demonstrated that incorporating natural dyes, specifically madder and indigo at a 100% concentration, into the diet of bivoltine silkworm breeds CSR_{16} and CSR_{27} 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 1^{st} 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.

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Table 1: Performance of CSR_{16} and CSR_{27} treated with Madder Dye for Egg, Larval, Cocoon, Pupal and Moth parameters

Treatments	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
T1	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
T2	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} Instarduration(D: Hrs.)				Total larval duration(D: Hrs.)			
	CSR_{16}		CSR_{27}		CSR_{16}		CSR_{27}	
T0	8.35 ± 0.119		8.37 ± 0.048		25.84 ± 0.254		25.92 ± 0.250	
T1	8.52 ± 0.132		8.42 ± 0.111		25.01 ± 0.148		26.32 ± 0.333	
T2	8.32 ± 0.095		8.40 ± 0.091		24.06 ± 0.019		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}
T0	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	2.74 ± 0.417
T1	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
T2	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 %	
			Green Cocoon		Dry Cocoon			
	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}
T0	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
T1	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
T2	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}	**CSR27**	**CSR_{16}**	**CSR_{27}**	**CSR_{16}**	**CSR_{27}**
T0	4.47±0.604	3.79±1.381	103.75±1.377	105.25±1.109	98.29± 0.284	96.47±0.322
T1	3.47±0.618	3.09±0.473	107.25±0.854	105.75±1.493	97.45±0.400	97.39±0.605
T2	2.15±0.532	4.86±0.271	104.75±1.377	104.25±1.377	99.23±0.163	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}**
T0	1.47 ± 0.135	1.19 ± 0.050	0.34 ± 0.007	0.39 ± 0.018	22.07 ± 0.182	21.96 ± 0.577
T1	1.74 ± 0.026	1.47 ± 0.066	0.38 ± 0.001	0.42 ± 0.023	22.53 ± 0.141	22.79 ± 0.157
T2	1.72 ± 0.057	1.62 ± 0.059	0.38 ± 0.001	0.47 ± 0.071	22.46 ± 0.016	23.13 ± 0.380

Pupal and Moth traits								
	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 $CSR_{16}$and CSR_{27}treated with Indigo Dye for Egg, Larval, Cocoon, Pupal and Moth parameters

Egg traits

Treatments	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

Larval traits

	V^{th} Instarduration(D: Hrs.)		Total larval duration(D: Hrs.)	
	CSR_{16}	CSR_{27}	CSR_{16}	CSR_{27}
T_0	8.32 ± 0.085	8.37 ± 0.095	26.22 ± 0.217	26.22 ± 0.217
T_1	8.27 ± 0.048	8.22 ± 0.025	24.65 ± 0.332	25.95 ± 0.328
T_2	8.30 ± 0.071	8.40 ± 0.082	24.90 ± 0.388	26.27 ± 0.295

	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	1.91 ± 0.685
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

Cocoon Parameters								
	ERR (By Number)		ERR (By Weight)				Dead cocoon %	
			Green Cocoon		Dry Cocoon			
	CSR_{16}	**CSR_{27}**	**CSR_{16}**	**CSR_{27}**	**CSR_{16}**	**CSR_{27}**	**CSR_{16}**	**CSR_{27}**
T_0	9,232.00±12.774	9,290.50±49.949	14.64±0.349	14.78±0.252	14.78±0.252	14.60±0.659	3.21±0.608	3.74±0.385
T_1	9,254.00±10.544	9,632.50±36.372	14.75±0.244	14.71±0.331	14.71±0.331	13.06±0.741	2.72±0.363	4.16±0.295
T_2	9,234.00±14.006	9,635.75±47.068	14.56±0.445	14.66±0.201	14.66±0.201	14.36±0.639	2.62±0.587	3.54±0.512
Pupal and Moth traits								
	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
	Single cocoon weight(g)		**Shell ratio %**		**Single shell weight(g)**			
	CSR_{16}	**CSR_{27}**	**CSR_{16}**	**CSR_{27}**	**CSR_{16}**	**CSR_{27}**		
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		
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		
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		
	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	0.39 ± 0.023	0.35 ± 0.029	2.55 ± 0.065	2.30 ± 0.168
T_1	1.04 ± 0.011	1.05 ± 0.007	2.57 ± 0.085	2.55 ± 0.065	0.40 ± 0.009	0.39 ± 0.023	2.27 ± 0.085	2.60 ± 0.108
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

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

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

CSR$_{16}$ marked larvae (left) and CSR$_{27}$ plain larvae (right) for Larval traits

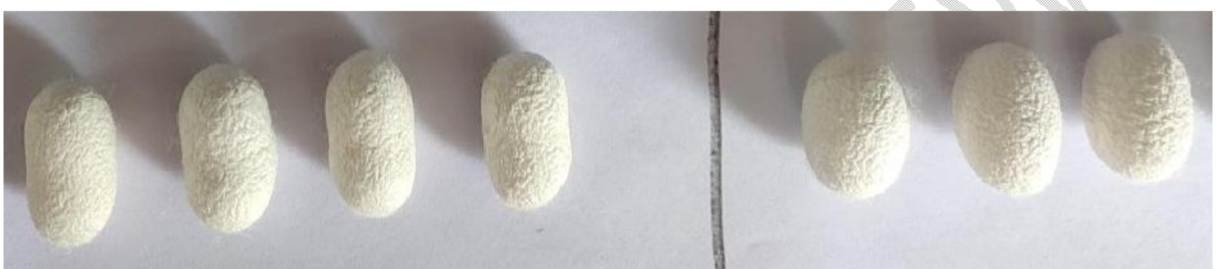
CSR$_{16}$ dumbbell cocoons (left) and CSR$_{27}$ oval cocoons (right) for cocoon traits

CSR_{16} yellow-brown pupae (left) and CSR_{27} yellowish-brown pupae (right) for pupal traits.

Moths of CSR_{16} and CSR_{27} bivoltine silkworm breed for moths' traits

Collection of Haemolymph

Figure 1:Qualitative parameters of CSR_{16} and CSR_{27} bivoltine silkworm breeds for egg, larval, cocoon, pupaland adult stages.



CSR_{16} Madder 50 % conc. on the left CSR_{27} Madder 50 % conc. on the right

CSR_{16} Madder 100 % conc. on the left **CSR_{27} Madder 100 % conc. on the right**

CSR_{16} Indigo 50 % conc. on the left **CSR_{27} Indigo 50 % conc. on the right**

CSR_{16} Indigo 100 % conc. on the left **CSR_{27} Indigo 100 % conc. on the right**

Figure 2: Cocoons of $CSR_{16}$and CSR_{27}bivoltine silkworm breeds treated with 50% and 100% concentrations of madder and indigo dye.