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Research Article

Effect of Spirulina supplementation on post-cocoon parameters of Tasar Silkworm (*Antheraea mylitta*)

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ABSTRACT

Antheraea mylitta Drury is a wild sericigenous insect known for producing economically valuable silk. India ranked second in the world for Tasar silk production, following China. Tasar culture serves as an important resource for the socio-economic development of tribal communities. It primarily feeds on *Terminalia arjuna*, *Terminalia tomentosa*, and *Shorea robusta*. The present study investigates the impact of *Spirulina platensis* supplementation on the growth of the Tasar silkworm through foliar application on *T. arjuna* leaves, the primary host plant. Spirulina, a nutrient-dense microalgae, was applied in three different concentrations to assess its influence on cocoon quality and silk yield. They were reared under natural outdoor conditions and fed exclusively on treated leaves in 4th and 5th instars. Among the tested concentrations, the moderate ratio resulted significantly enhanced post-cocoon performance, with notable improvement in cocoon weight, shell weight, and silk filament length. These results suggest that moderate Spirulina supplementation effectively boosts nutritional intake, supporting better silk production outcome. The study highlights the potential of Spirulina as a natural and sustainable bio-supplement to improve silk quality in sericulture, especially in tribal and forest-based Tasar farming systems

1. Introduction

The Tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae), is a wild sericigenous insect of great economic importance in India. Tasar silk, valued for its natural golden luster, strength, and durability, contributes significantly to the rural economy, particularly supporting tribal and forest-dependent communities (Suryanarayana & Srivastava, 2005; Singh et al., 2014). India is the second-largest producer of Tasar silk after China, with major cultivation areas spread across Jharkhand, Chhattisgarh, Odisha, and parts of Telangana. Food plant variety and its nutritional value highly affect cocoon yield and seed quantity of Tasar silk. *A. mylitta* which feeds on *T. tomentosa* (Asan), *T. arjuna* (Arjun) and *S. robusta* (Sal) and secondarily on *Lagerstromia parviflora*, *Zizyphus mauritiana*, *Anogcissus latifolia*, *Syzigium cumini*, *Lareya arborea* and *Hardwickia binata* (Jadhav, A, et al., 2014), (Giri, S., et al., 2023).

Improving the nutritional quality of host plants has been considered an effective strategy to enhance larval performance and silk yield. Nutrient supplementation, either through fortification of host leaves or microbial additives, has been reported to improve cocoon parameters and overall silk productivity (Borah, S. D, et., al. 2020).

Spirulina (*Arthrospira* sp.) is an edible, filamentous, spiral-shaped cyanobacterium, formally classified as a blue-green microalga (Becker 2007; Gupta et al., 2008).

Spirulina (*Spirulina platensis*), is widely recognized as a rich source of proteins, vitamins, minerals, essential amino acids, and antioxidants (Belay et al., 1993). It has been extensively used as a dietary supplement in human nutrition, aquaculture, poultry, and livestock due to its immune-boosting and growth-promoting effects (Khan et al., 2005; Holman & Malau-Aduli, 2013). In sericulture, Spirulina offers a promising bio-supplement for enhancing the nutritive value of mulberry and non-mulberry host plants, thereby improving larval metabolism, cocoon quality, and silk filament length. However, studies on its application in Tasar culture remain limited.

Although the nutritional benefits of *Spirulina* have been well documented in animal and aquaculture systems, its utilization in non-mulberry sericulture, particularly for the Tasar silkworm (*Antheraea mylitta*), remains largely unexplored or limited. The novelty of this work lies in the first-time application of *Spirulina* in Tasar culture to enhance larval growth and cocoon productivity.

Therefore, the present investigation evaluates the effect of Spirulina supplementation through foliar application on *T. arjuna* leaves in *A. mylitta* rearing. The study specifically aims to determine its impact on post-cocoon parameters such as cocoon weight, shell weight, and filament length, thereby exploring Spirulina as a sustainable approach to improve Tasar silk quality.

2. Materials and Methods

2.1 Rearing activity

The Tasar silkworm (*Antheraea mylitta* Drury) is a bivoltine species, producing two crops per year (August and October). In the present preliminary study, one disease-free laying (1 DFL) containing approximately 200 eggs was utilized. The larvae were reared on healthy *Terminalia arjuna* host plants under natural conditions by brushing of larvae was carried out in the early morning using fresh, stage-specific leaves. The rearing process was conducted outdoors under close supervision to ensure protection from pest attack throughout the larval development.

Climatic parameters, including temperature, humidity, and rainfall, were recorded for each instar. Temperature and rainfall data were noted using a mobile-based cloud weather application, while humidity was measured using a hygrometer. Larval parameters such as larval height and larval weight were measured, and instar-wise mortality was calculated by recording the number of dead and diseased larvae, which were subsequently removed.

During moulting and cocooning, adequate foliage was provided while minimizing disturbances. After the third moulting, healthy fourth-instar larvae were randomly selected for the experiment. The larvae were divided into four groups are three treatment groups and one control group, with each treatment group consisting of ten larvae. The remaining larvae were maintained as the control group and reared until cocoon formation for post-cocoon analysis. Cocoons were harvested 6–7 days after the completion of spinning. The success of rearing was primarily attributed to proper site selection, continuous supply of high-quality host leaves, and careful monitoring to prevent pest infestation and unfavourable environmental influences.

2.2 Rearing site

The experiment was conducted in the Tasar plantation at Kakatiya University, Warangal, Telangana, under natural outdoor rearing conditions during the crop season of 2023–2024 (Fig.1).

Fig:1. *Terminalia arjuna* plantation @ Zoology Department, Kakatiya university, Warangal.

Newly hatched larvae of *Antheraea mylitta* were reared on *Terminalia arjuna* plantations maintained under clean conditions, free from weeds and major insect predators, particularly ants. The average temperature ranged between 26–30°C with 70–80% relative humidity, which are considered optimum for Tasar silkworm rearing (Suryanarayana & Srivastava, 2005).

2.3 Preparation of Spirulina solution

Commercially available dried Spirulina (*Spirulina platensis*) powder was obtained from a certified supplier. Aqueous suspensions of Spirulina were prepared in three concentrations: 1:10, 1:25 and 1: 50. In terms of weight/volume solutions were prepared as 0.01%, 0.004%, and 0.001% (w/v) solutions by dissolving 10 mg, 4 mg, and 1 mg of *Spirulina platensis* powder, respectively, in 100 mL of distilled water. Each suspension was uniformly sprayed on both surfaces of *Terminalia arjuna* leaves before feeding.

2.4 Supplementation of Spirulina fortified leaves to Tasar silkworm, *Antheraea mylitta*

Fortifying mulberry leaves with supplementary Spirulina and feeding them to silkworms represents a modern and effective approach to enhance their economic and commercial value. (Kumar, K., et al., 2015). In the present study, healthy fourth-instar larvae of similar size and weight were randomly picked and divided into three groups, each consisting of ten individuals (Fig.2). Ten larvae were used per treatment group in this preliminary experiment. The sample size was determined by the limited availability of *A. mylitta* larvae and rearing constraints. *Spirulina platensis* was sprayed to both surfaces of *Terminalia arjuna* leaves and Treated leaves were provided once during each of the two instar stages. The experiment was replicated during two rearing seasons (August and October 2024) to ensure reproducibility. The Spirulina was administered at three concentrations: 1:10, 1:25, and 1:50, while a separate control group received untreated leaves.

Fig:2. Supplementation of spirulina solution to silkworm, divided into three different groups and covered them with net to prevent from pest and predators.

2.5 Economic Parameters:

All the post cocoon parameters were measured based on the standard procedures, Food and Agriculture Organization Manual, (FAO Manual 1972). Larval weight, cocoon weight and Pupal weight were measured using a standard electronic balance. By the end of fifth instar stage, cocoons were formed in the experimental groups. The cocoons were harvested,

measured post-cocoon parameters, including cocoon weight, shell weight, shell ratio, and reeled silk weight, were recorded using a Citizen electronic balance with measurements expressed in grams. The cocoons were cut open, and the pupal weights were also recorded. Cocoon length, cocoon width, were measured using Vernier callipers, while shell thickness was determined with the help of a screw gauge.

2.6 Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Statistical comparisons between the control and *Spirulina*-treated groups were performed using Student's *t*-test (two-tailed) in Microsoft Excel. Differences were considered statistically significant at $p < 0.05$ and highly significant at $p < 0.001$. Statistically significant differences are indicated by asterisks (*) in the tables.

3. Results and Discussion

3.1 Supplementation of Spirulina fortified leaves to Tasar silkworm, *Antheraea mylitta*

Feeding silkworms with *Terminalia arjuna* leaves, fortified with spirulina showed a beneficial impact on economic traits compared to the untreated group as given below.

3.2 Larval parameters

Fortification of *Terminalia arjuna* leaves with spirulina resulted in significant variations in larval weight ($p < 0.05$). The 1:25 concentration group exhibited the maximum larval weight (33.37± 0.37 g) and larval length (12.4 cm), were highly significant ($p < 0.001$), indicating the most pronounced growth response. Seasonal differences were also evident, with larvae reared during August 2024 (crop-1) showing significantly higher performance compared to those reared in October 2024 (crop-2). The reduced growth in the latter season may be attributed to excessive winter rainfall, which adversely affected larval development (Table-1). These variations are further illustrated in the graphical representation (Fig-4), which clearly depicts the enhanced larval performance in crop-1 and the superior effect of the 1:25 *Spirulina* concentration.

Table-1. The larval parameters of control and treated with spirulina in different ratios of Tasar silkworm, *Antheraea mylitta* Drury. Asterisk (*) indicates values that are significantly different from the control group ($p < 0.05$; highly significant at $p < 0.001$).

Parameters Category		Larval weight (g)	Larval length (cm)
Crop-1			
Control		30.41± 0.35	8.7
Treated	**1:10**	31.38 ± 0.46	11.0
	1:25	*33.37± 0.37	*12.4
	1:50	31.99 ± 0.35	10.2
Crop-2			
Control		29.1 ± 0.55	8.0
Treated	**1:10**	30.58 ± 0.36	10.7
	1:25	*32.04 ± 0.16	*11.6
	1:50	31.52 ± 0.33	9.9

3.3 Post-cocoon parameters

Feeding silkworms with *Terminalia arjuna* leaves supplemented with spirulina significantly improved cocoon parameters. Treated groups demonstrated notable enhancements over the control ($p < 0.05$), with the 1:25 concentration yielding the highest cocoon weight (13.38±0.59 g), shell weight (1.79± 0.14 g), pupal weight (11.36±0.61 g), shell ratio (15.2%), cocoon length (3.9 cm), cocoon width (1.8 cm), filament length (869 m), reelability (8.7%), denier (9.0) (Fig 3). The difference between the control and 1:25 *Spirulina* treatment was highly significant ($p < 0.001$), indicating the strong positive effect of optimal *Spirulina* concentration on silk productivity. Furthermore, cocoon performance was superior in the first season/ crop-1 (August 2024) compared to the second season/ crop-2 (October 2024), which may be attributed to excessive winter rainfall adversely affecting silkworm productivity (Table 2). As shown in the graphical representation (Fig.5), these variations reveal improved cocoon parameters in crop-1 and a stronger influence of the 1:25 *Spirulina* concentration.

Fig 3. Cocoons of the control and *Spirulina*-treated groups (1:10, 1:25, and 1:50) of Tasar silkworm (*Antheraea mylitta* Drury). All treated groups showed significant improvement over the control ($p < 0.05$), with the 1:25 concentration showing a highly significant difference ($p < 0.001$).

The present study demonstrates that foliar supplementation of *Spirulina platensis* to *Terminalia arjuna* leaves significantly enhanced larval and post-cocoon traits of *Antheraea mylitta*. This improvement can be attributed to Spirulina's rich nutritional profile, extremely rich source of nutrients, comprising up to 70% protein by dry weight and providing all essential amino acids. It also contains essential fatty acids, a wide range of vitamins and minerals, and various pigments such as chlorophylls and carotenoids (Podgórska-Kryszczuk, I. 2024; Spínola, M. et al., 2024). Similar benefits have been reported in mulberry silkworms (*Bombyx mori*), where Spirulina supplementation improved cocoon weight, shell ratio, and filament length (Kumar et al., 2009) and the present findings extend its applicability to non-mulberry sericulture.

Among the tested concentrations, the reduced performance observed at the 1:10 *Spirulina* concentration may be attributed to decreased consumption and assimilation efficiency at higher supplementation levels, as previously

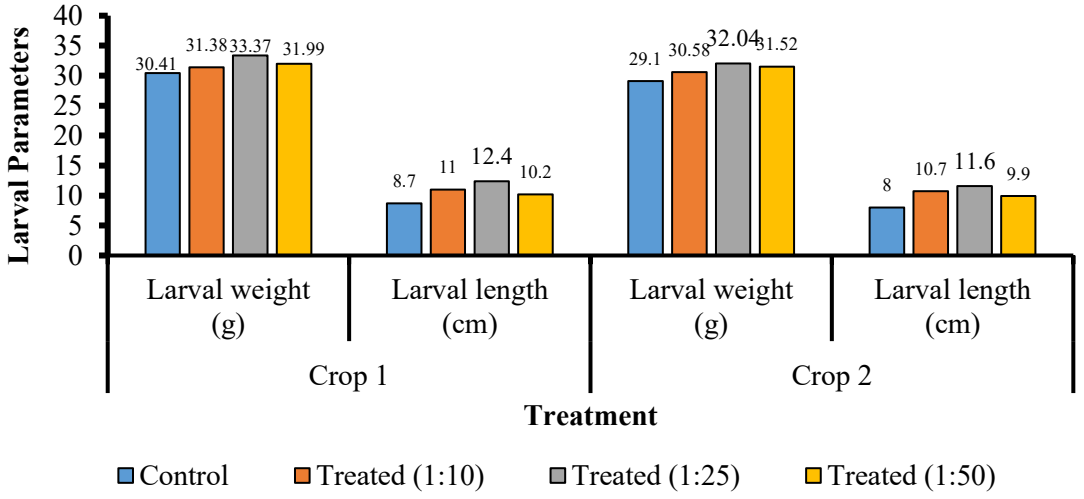

Fig 4. A graph on larval parameters of crop-1 and crop-2 of Tasar silkworm, *Antheraea mylitta* Drury.

Table 2. Post-cocoon parameters of control and *Spirulina*-treated groups (1:10, 1:25, and 1:50) of Tasar silkworm (*Antheraea mylitta* Drury). Values are expressed as mean ± SD. Asterisk (*) indicates values that are significantly different from the control group ($p < 0.05$; highly significant at $p < 0.001$).

Cocoon Category		Cocoon Weight (gm)	Pupal weight (gm)	Cocoon shell weight (gm)	Shell ratio (%)	Cocoon width (cm)	Cocoon length (cm)	Filament length (m)	Reliability (%)	Denier
Crop-1										
Control		10.11±0.80	7.54± 0.30	0.98±0.027	14.0	1.4	3.5	845	8.4	9.7
Treated	**1:10**	10.97 ±0.70	9.58 ±0.33	1.004±0.079	13.0	1.5	3.8	822	7.90	9.05
	1:25	*13.38±0.59	*11.36±0.61	*1.79± 0.14	*15.2	*1.8	*3.9	*869	*8.7	*9.0
	1:50	10.46 ±0.40	10.21± 0.54	1. 23 ±0.25	11.9	1.7	3.8	790	7.88	9.4
Crop -2										
Control		9.13 ± 0.42	7.49 ±0.37	0.814±0.021	14	1.5	3.3	838	8.2	10.0
Treated	**1:10**	10.44 ±0.28	8. 38± 0.50	1.024±0.021	12.09	1.4	3.6	789	7.83	9.3
	1:25	*12.13±0.68	*11.03±0.67	*1.50 ±0.37	*13.8	*1.8	*3.7	*857	*8.5	*9.2
	1:50	9.30± 0.34	8.29 ± 0.25	0.914±0.030	11	1.7	3.5	747	6.53	9.5

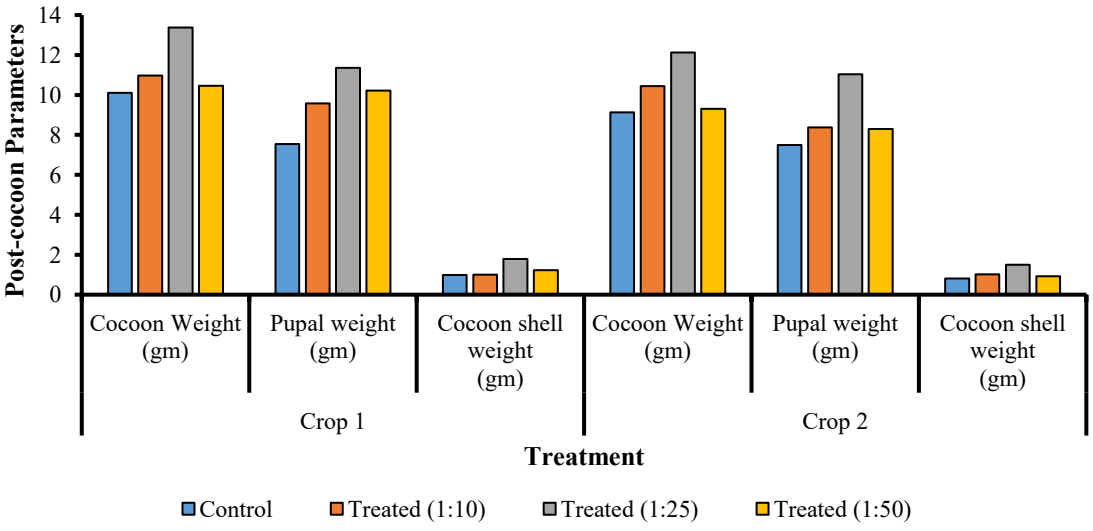

Fig 5. A graph on Post-cocoon parameters of crop-1 and crop-2 of Tasar silkworm, *Antheraea mylitta* Drury

reported by Kumar et al. (2015). In contrast, the 1:50 *Spirulina* concentration may reflect nutrient under-utilization due to insufficient supplementation. Previous studies have indicated that moderate levels of *Spirulina* enhance enzyme activity and nutrient assimilation in silkworms (Dharanipriya & Thangapandiyan, 2019), supporting the present finding that the 1:25 concentration was optimal for larval growth and silk productivity. Seasonal differences were also observed, with the August crop outperforming the October crop, likely due to rainfall and humidity affecting leaf quality and larval development (Suryanarayana & Srivastava, 2005). However, Spirulina-fed groups consistently outperformed controls across both seasons.

The improvement in reelability and filament length in *Spirulina*-treated groups may be attributed to its rich biochemical composition, including proteins, essential amino acids, vitamins, minerals, and antioxidant compounds such as phycocyanin and β-carotene. (Spínola, M. et al., 2024). These nutrients enhance larval metabolism and silk gland activity, thereby promoting fibroin and sericin synthesis responsible for silk filament formation (Kumar, K., et al., 2015). Similar findings have been reported where *Spirulina* or protein-enriched diets improved silk yield, filament quality, and overall growth performance in *Bombyx mori* and other insects (Dharanipriya & Thangapandiyan, 2019).

Comparable trends have been observed with other bioactive supplements. In earlier studies, Lattala et al. (2014) reported that spermidine supplementation enhanced silk production in mulberry silkworms by improving larval growth and post-cocoon parameters. While spermidine, a polyamine, enhances metabolic activity at the cellular level, Spirulina offers the advantage of being a natural, protein-rich microalga with diverse bioactive compounds. The parallel outcomes of both studies highlight that different nutrient sources, whether biochemical (spermidine) or biological (Spirulina)-can stimulate silk gland activity and improve cocoon traits. Importantly, Spirulina is readily available, eco-friendly, and cost-effective, making it a particularly promising candidate for sustainable sericulture.

Although biochemical analysis was not performed in the present study, previous findings by Lattala (2014) indicated that spermidine (Spd) can enhance protein synthesis by interacting with promoter sequences such as fibroin, leading to increased silk production. This suggests that bioactive compounds in *Spirulina* may similarly influence silk gland metabolism and protein expression.

Overall, spirulina supplementation, particularly at 1:25 concentration, effectively enhanced cocoon weight, shell weight, and filament length. These improvements have significant economic relevance, as higher silk yield directly benefits tribal farmers and contributes to the sustainable growth of Tasar sericulture.

4. Conclusion

The present study indicates that *Spirulina platensis* supplementation can positively influence the growth and post-cocoon performance of *Antheraea mylitta*. Among the tested concentrations, 1:25 appeared most effective, showing notable improvements in cocoon weight, shell weight, and filament length. These results suggest that *Spirulina* has potential as an eco-friendly, natural supplement for enhancing Tasar silk production

Conflicting Interests

The authors have declared that no conflicting interests exist.

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