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RESEARCH ARTICLE

Spirulina and Azolla as a dietary supplement for enhancing growth and productivity of silkworm (*Bombyx mori* L.)

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Abstract

Silkworm *(Bombyx mori L.)* is a pivotal insect in sericulture, with its development and output highly reliant on nutrition. This study evaluated the potential of *Spirulina platensis*, *Azolla pinnata*, and their mixture as dietary supplements to enhance silkworm growth and economic traits. Mulberry leaves were treated with alcoholic extracts of each alga at concentrations of 1%, 3%, and 5%. The results highlighted the positive impact of both Spirulina and Azolla supplementation. A 3% Spirulina treatment significantly increased silk gland weight ($P<0.05$) over the control. All Spirulina treatments improved silk gland growth, with comparable effects recorded for 3% Azolla and the 3% mixture. Although cocoon weight showed some variation, no significant differences were found. Nevertheless, the 3% Spirulina, 5% Azolla, and 3% mixture groups exhibited the highest cocoon weights. While, filament weight was significantly higher in all algae-treated groups compared to the control ($P \leq 0.003$). The 3% Spirulina supplementation led to positive increases in the weights of larvae (2.65 ± 0.02 g), silk glands (0.690 ± 0.07 g), cocoons (1.02 ± 0.12 g), pupae (0.808 ± 0.10 g), and filaments (0.247 ± 0.01 g). Consequently, this study indicates that Spirulina and Azolla, especially at particular concentrations, can significantly enhance larval growth, silk gland development, and key silk production characteristics.

***Keywords*:** Azolla, Spirulina, Nutrition, Silk production, Silkworm performance

1. Introduction

Sericulture has been a cornerstone of agricultural economies for millennia, particularly in countries with a rich tradition in silk. (Pavithra, *et al*., 2024). The economic importance of sericulture goes beyond the production of silk fibers, encompassing various by-products and contributing to rural employment and income generation. Sericulture is recognized as a vital agro-based industry, supporting rural livelihoods and enhancing socio-economic conditions (Reddy and Parasuramudu,2024). The silkworm (*Bombyx mori* L.) is a highly specialized insect whose life cycle is intricately linked to the mulberry plant (*Morus spp.*), its sole natural diet (Xin *et al.*, 2024). The growth, development, and overall productivity of silkworms are profoundly influenced by their nutritional intake. Enhancing the nutritional quality of the silkworm diet is therefore pivotal in improving larval growth rates, silk gland development, and cocoon quality (Moustafa and Soliman 2019). Despite the established benefits of Spirulina and Azolla in other agricultural contexts, their application as dietary supplements in sericulture remains limited. Both Spirulina, a blue- algae rich in protein, vitamins, and minerals, and Azolla, an aquatic fern known for its high nitrogen content and rapid growth, have demonstrated potential as valuable feed supplements in various agricultural systems. The potential of Spirulina in enhancing growth and immune functions in various agricultural applications have approved, (Hassan, 2025; El-daim *et al.*, 2021). Some preliminary findings suggest that Spirulina supplementation can improve nutritional indices and tissue production in

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silkworms, offering a promising avenue for enhancing silk yield (Kumar and Balasubramanian, 2016). The novelty of this study lies in its integrated approach to assessing the synergistic effects of Spirulina and Azolla on silkworm performance. While previous research has independently evaluated the benefits of these supplements, few studies have investigated their Mixture impact, which could potentially offer superior benefits through complementary nutritional and physiological mechanisms. (Abdelkhalek, *et al*. 2015; Al-Deriny, *et al*. 2020; El-daim *et al*. 2021). This study investigates the effects of incorporating Spirulina and Azolla and their mixture into silkworm diets on growth parameters, silk production, and overall productivity, aiming to establish a more sustainable and efficient feeding strategy for sericulture.

2. Materials and Methods

The experimental study was conducted during the spring of 2024 at the Sericulture Research Laboratory, Plant Protection Department, Faculty of Agriculture, South Valley University, Qena, Egypt. This facility is equipped with state-of-the-art sericulture infrastructure essential for controlled rearing and experimentation.

2.1. Insect Source and Laboratory Preparation:

Silkworm eggs were procured from the Sericulture Research Department, Plants Protection Institute, Agricultural Research Centre, Giza, Egypt. The source ensures high-quality genetic stock suitable for experimental consistency. Prior to the commencement of the experiment, all rearing trays were meticulously washed and treated with a 3% formalin solution to eliminate potential pathogens. Following thorough drying, the trays were prepared for breeding to prevent disease outbreaks. Additionally, the laboratory environment, including floors, walls, and all rearing tools, underwent disinfection with the same formalin solution. Post-disinfection, the laboratory was sealed for 24 hours to ensure complete eradication of contaminants, after which it was ventilated adequately. Incubators were sterilized using 70% ethanol to provide a germ-free environment for egg incubation.

2.2. Experimental Treatments and Design

The rearing process was maintained under controlled laboratory conditions, specifically at 25 °C and 90% relative humidity for egg incubation. Upon hatching, larvae were provided with thin strips of fresh mulberry leaves and subsequently transferred to small rearing trays in a process known as "pulling." These trays were covered with plastic frames and surrounded by wet sponge strips to sustain an optimal rearing environment of 26 ± 2 °C and 75-85% relative humidity. From hatching until the conclusion of the third larval instar, silkworms were exclusively fed clean mulberry leaves. The larvae were randomly assigned into two groups: control group and an experimental group. The experimental design employed a completely randomized design (CRD) with three replicates per treatment, each consisting of 50 larvae. The control group received standard fresh mulberry leaves, whereas the experimental group was fed mulberry leaves supplemented with extracts of Spirulina, Azolla, or their combination.

2.3. Preparation of Treatment Solutions:

Spirulina powder was sourced from the Algal Department, National Research Centre, Giza, Egypt. A 200 ml alcoholic Spirulina solution was prepared by dissolving 20 g of Spirulina powder in 200 ml of ethyl alcohol. The mixture was agitated for one hour to ensure homogeneity and subsequently concentrated by placing it in a water bath at 45 °C for 48 hours. From this concentrated solution, stock solutions of 1%, 3%, and 5% were prepared for experimental use. Also, Fresh Azolla was harvested from the Azolla farm at the Faculty of Agriculture, South Valley University. The collected Azolla was thoroughly washed with clean tap water, dried, and subsequently ground and sieved to obtain a fine Azolla powder. Similar to Spirulina, Azolla extracts were prepared in concentrations of 1%, 3%, and 5%. To formulate a Mixture treatment, 10 ml of each concentrated Spirulina and Azolla solution were mixed in a separate beaker. This mixture was utilized to

prepare the requisite concentrations for the experimental group.

3.4. Evaluation Criteria

At the 7 day of the fifth instar, fifteen mature 5^{th} instar larvae from each treatment were weighted and dissected to extract and weight the silk glands (g). The mean weight value was calculated for comparative analysis. Also, fifteen fresh healthy cocoons were collected from each treatment, and total and mean cocoon weights (g) per cocoon were calculated. The previous cocoons were cut, and opened and the pupae were weighed individually (g), and both total and mean weights per pupa and shell were determined and the shell ratio for each treatment was calculated. To extract the filament, cocoons are soaked in hot water to soften the sericin, a gum-like substance holding the filament together. Then, the filament is unwound onto a reel and dried at 70 Ċ for 24 hours.

3.5. Statistical Analysis

All data collected were subjected to statistical analysis using the Statistical Package for the Social (Version 25.0), IBM Corp. (2020). Descriptive statistics were computed, and analysis of variance (ANOVA) was performed to determine the significance of differences between treatment groups. A p-value of <0.05 was considered statistically significant. Data were presented as mean ± standard deviation to ensure clarity and reproducibility.

3. Results

3.1 Impact of Spirulina and Azolla on larvae of silkworm

***Larva Weight*:** Larval weight exhibited remarkable improvements, particularly with Spirulina at 3% (2.65± 0.02 g), followed by Spirulina at 5% (2.58± 0.15g) as compared to control group (2.50 ± 0.04 g). (Fig.1A). These findings imply that moderate supplementation with Spirulina can enhance larval growth, potentially through improved nutritional profiles or metabolic efficiencies.

Silk Gland Weight: A significant increase in silk gland weight ($p<0.05$) was observed with 3% Spirulina supplementation (0.69 ± 0.07 g) compared to the control group (0.306 ± 0.02 g), indicating a substantial improvement in the gland's capacity for silk production. Furthermore, all Spirulina treatment concentrations promoted silk gland growth above the control levels (Fig. 1B), Similarly, the most effective concentration in Azolla and Mixture groups was at 3% (0.560± 0.10g & 0.529± 0.04, respectively). All treatments resulted in significant increase in silk gland weight underscoring the efficacy of this dietary supplement in enhancing silk gland development and overall silk output.

Table 1. Impact of Spirulina, Azolla, and their mixture supplementation on larval growth in *Bombyx mori*

Treatments	Control	Parameters (mean ± SD)	
		Weight of larvae (g)	Silk gland weight (g)
Spirulina	1%	2.31±0.07	0.544± 0.05
	3%	2.65± 0.02	0.690± 0.07
	5%	2.58± 0.15	0.518± 0.09
Azolla	1%	2.38± 0.20	0.507± 0.05
	3%	2.28± 0.26	0.560± 0.10
	5%	2.50± 0.09	0.509± 0.01
Mixture (*Spirulina* and *Azolla)*	1%	2.44± 0.15	0.447± 0.03
	3%	2.43± 0.16	0.529± 0.04
	5%	2.49± 0.07	0.510± 0.02
Control		2.50± 0.04	0.306± 0.02
P value	-	0.371	0.000

Values are mean of observations; standard deviation and p-value for probability

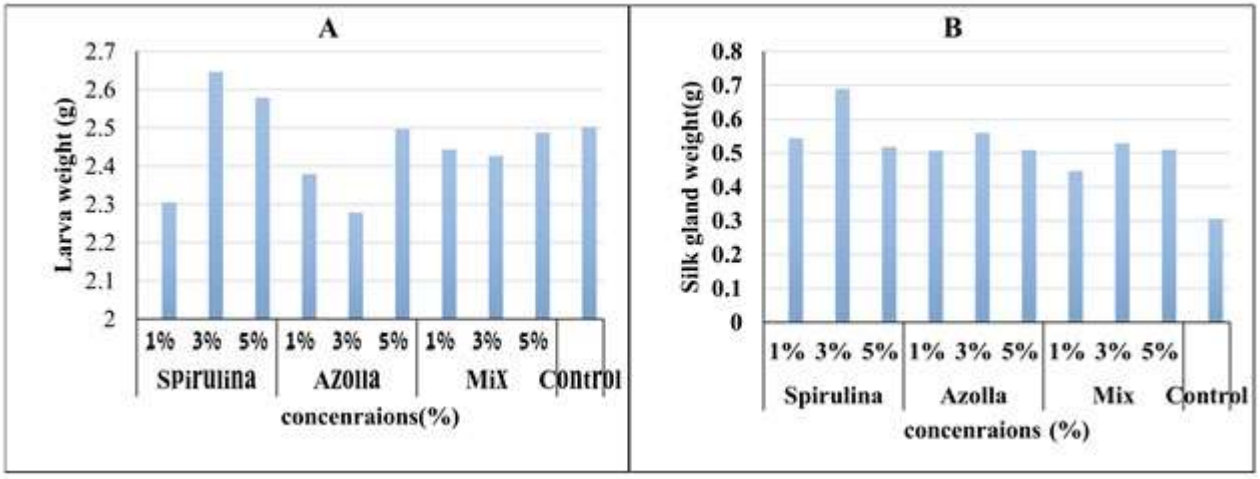

Figure 1. Impact of Azolla and Spirulina supplementation on larval weight (A), and silk gland weight (B) in *Bombyx mori larvae*

3.2. Impact of Spirulina and Azolla on Economic characteristics of the silkworm

The cocoon weight showed numerical fluctuations across the treatments, but these differences were not statistically significant ($P > 0.05$). In Spirulina group the highest cocoon weight was observed with 3% concentration (1.02± 0.12), while in Azolla group the 5% Azolla treatment resulted in the highest cocoon weight (0.975 ± 0.04 g), and the 3% mixture treatment resulted in highest weight (0.933± 0.02g) in the mixture group. (Fig. 2A). The increase in cocoon weight could be attributed to the enhanced nutritional availability provided by Spirulina, which promotes larval growth and development.

Pupa Weight: Pupa weight is a vital economic parameter, reflecting the overall health and development of the silkworm. Spirulina at 1% and 3% increased pupa weight (0.793 ± 0.05 g and 0.808 ± 0.10 g, respectively) compared to the control (0.620 ± 0.05 g). Azolla treatments, generally increased pupa weight more than control group, while, 1% Mixture exhibiting the lowest pupa weight (0.575 ± 0.14 g) (Fig.2B). These findings suggest that Spirulina may enhance larval health and subsequent pupa development, Pupa weight (g) showed a trend of variation, but the overall differences were not statistically significant ($P = 0.067$). The lower pupa weight in the mixture might be due to a competitive interaction between the two algae, or a poor balance of nutrients.

Shell Weigh: The 5% Azolla treatment exhibited the highest shell weight (0.280 ± 0.02 g), closely followed by the 1% Azolla group (0.26 ± 0.12 g), both showing marked increases compared to the control (0.202 ± 0.02 g). Conversely, Spirulina treatments generally resulted in reduced shell weights, with only the 1% Spirulina concentration showing a slight increase (0.213 ± 0.02 g). Remarkably, the mixture treatments demonstrated enhanced shell weight at 1% and 3% concentrations. (Fig. 2C). Based on these observations, Azolla appears to exert a more substantial positive influence on shell weight than Spirulina. As shell weight directly correlates with silk yield, the observed results highlight the superior performance of Azolla.

Shell Ratio: While numerical variations were observed in shell ratio the differences were not statistically significant ($P > 0.05$). The mixture treatments displayed higher shell ratios, with 1% Mixture reaching 28.99%, the highest among all treatments. (Fig. 2D). The shell ratio is a critical parameter reflecting the proportion of silk in the cocoon, and a higher ratio is generally desirable. The increased shell ratio and weight observed with Azolla might be attributed to its high protein content, which is essential for silk gland development and silk protein synthesis.

Filament Weight: A statistically significant difference ($P < 0.05$) was observed in filament weight across the treatments. The 1% Azolla treatment resulted in the highest filament weight (0.281 ± 0.01 g), indicating a potential positive impact of Azolla on silk fibroin production. Specifically, Azolla's rich nutrient profile, including essential amino acids, vitamins, and minerals, may have contributed to enhanced fibroin

synthesis. In Spirulina groups, 3% Spirulina increased filament weight (0.247±0.01g). While, mixture at 5% indicated significant increased with filament weight (0.279± 0.01g). The control group exhibited the lowest filament weight (0.208 ± 0.10 g) less than all treatments. (Fig. 2E).

Table 2. Impact of Spirulina, Azolla, and their mixture on Economic Parameters of *Bombyx mori*

Values are mean of observations; standard deviation and p-value for probability

Treatments	Control	Parameters (mean ± SD)				
		Cocoon Weight (g)	Pupa Weight (g)	Shell weight (g)	Shell ratio %	Filament Weight (g)
Spirulina	1%	0.965± 0.08	0.793 ± 0.05	0.171±0.04	17.39± 3.34	0.233±0.01
	3%	1.02± 0.12	0.808± 0.10	0.213± 0.02	20.74± 0.88	0.247±0.01
	5%	0.913± 0.03	0.734± 0.02	0.179± 0.04	19.62± 3.59	0.24± 0.02
Azolla	1%	0.973± 0.14	0.713± 0.07	0.26 ± 0.12	25.44± 7.84	0.281± 0.01
	3%	0.925± 0.06	0.738± 0.04	0.186± 0.03	20.16± 2.17	0.236± 0.01
	5%	0.975± 0.04	0.695± 0.03	0.280± 0.02	27.6± 1.05	0.231±0.002
Mixture	1%	0.794± 0.06	0.575± 0.14	0.23± 0.07	28.99± 10.63	0.23± 0.01
	3%	0.933± 0.02	0.704±0.06	0.229± 0.07	24.66± 7.50	0.232± 0.01
	5%	0.891± 0.02	0.714± 0.02	0.177± 0.01	21. 9± 0.77	0.279± 0.01
Control		0.822± 0.05	0.620± 0.05	0.202± 0.02	26.61± 3.08	0.208±0.10
P value		.135	.067	.553	.402	.003

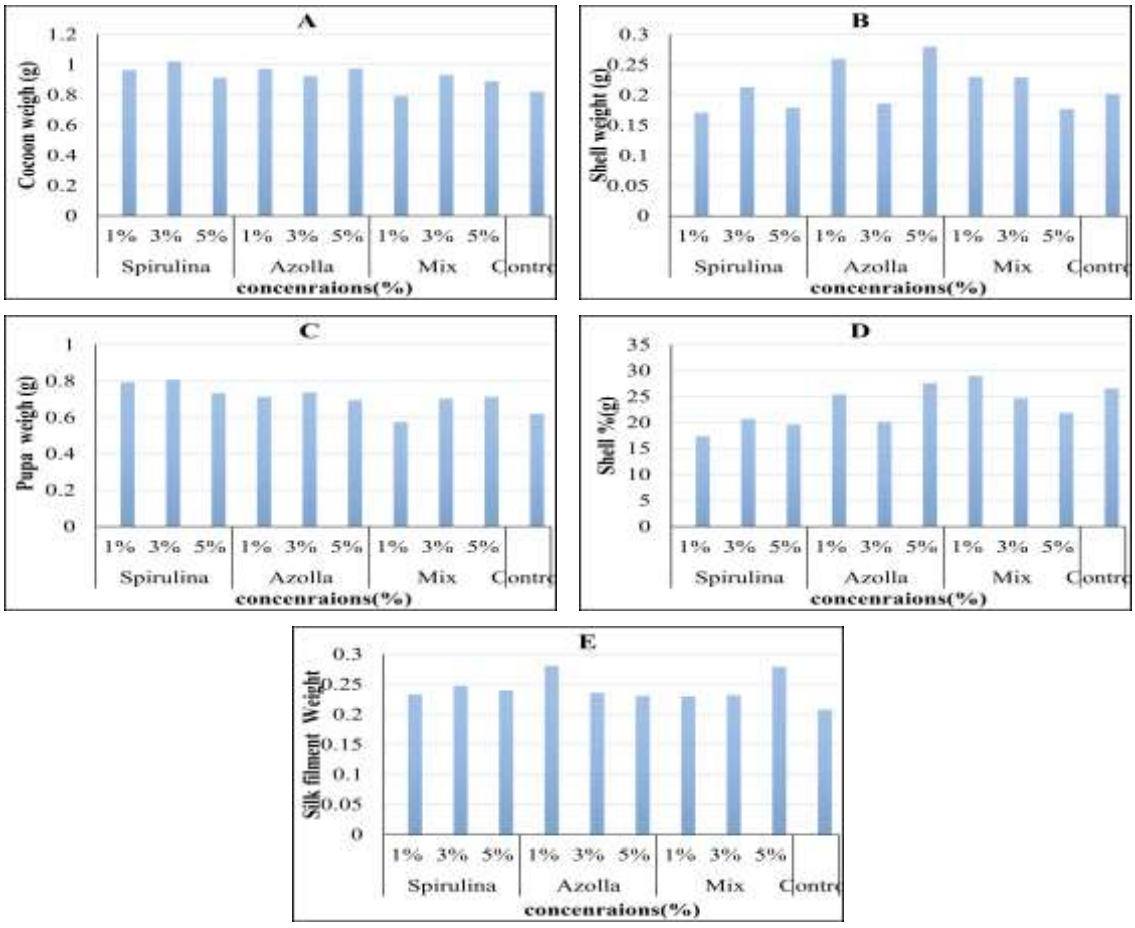

Figure 2. Impact of Azolla and Spirulina Supplementation on Cocoon Weight (A), Shell Weight (B), Pupa Weight (C), Shell Ratio (D) and Filament Weight (E) in silkworm.

4. Discussion

The present study aimed to evaluate the efficacy of *Spirulina platensis*, *Azolla pinnata*, or their combination, as dietary supplements to improve the growth and productive performance of silkworm larvae (*Bombyx mori L.*). The findings indicate that supplementation with Spirulina and Azolla significantly improved both biological and economical traits of silkworms compared to control group, with the most pronounced effects observed at a 3% concentration of Spirulina and 1% Azolla. These results not only align with previous research but also provide new insights into the optimal use of these supplements in sericulture. Consistent with the findings of (Saad *et al*., 2014) demonstrated that nutritional supplements could substantially enhance the growth parameters of silkworm larvae. corroborating our observation that a 3% concentration of Spirulina resulted increase in larval weight. The high protein and essential amino acid content in Spirulina likely contribute to this enhancement by providing the necessary nutrients for rapid growth and development (Karkos *et al*. 2011). Similarly, (Masthan *et al*., 2017) reported that Spirulina supplementation led to an increase in larval weight because of its high contain of protein and other nutrition contents. Moreover, (Moustafa 2024) highlighted the positive impact of algae probiotics, including Spirulina and Azolla, on silkworm growth and enzyme activity. Our study extends these findings by demonstrating that not only do these supplements improve growth rates, but they also enhance specific physiological parameters such as silk gland weight ($p<.05$). (Kumar and Balasubramanian 2014) reported that Spirulina supplementation positively affects lipid metabolism and enzyme activity in the silk glands of silkworms, which aligns with our observation of significant increase in silk gland weight at a 3% Spirulina concentration (0.690± 0.07g). Moreover, in economic traits, including cocoon weight, pupal weight, shell weight, shell ratio were improved with Spirulina and Azolla treatments and filament weight was boosted significantly ($p<0.003$). The increase in the cocoon weight with 3% Spirulina concentration followed by 5% Azolla, which is consistent with the results of (Maqbool *et al.* 2023), who reported positive impacts of Spirulina and thyroxine supplementation on cocoon parameters. Additionally, (Sujatha *et al*., 2015; Vijaykumar *et al.*, 2016) found that Azolla supplementation enhanced cocoon and shell weights, respectively, supporting our findings that Azolla contributes to improved silk production metrics. The differential effects of Spirulina and Azolla on the shell weight and ratio highlight the distinct roles these supplements on resource allocation within the silkworm larvae. While Spirulina supplementation reduced the shell ratio, Azolla at various concentrations maintained or slightly increased the shell ratio. at 5% concentration (27.6±1.05). This suggests that silkworms fed a diet supplemented with these organisms may exhibit improved nutrient conversion efficiency, leading to enhanced productivity and superior cocoon characteristics, in addition the nutrient combination in both of them can results in various effects on silkworm (Hassan, 2025). The physiological mechanisms underpinning these improvements are multifaceted. Spirulina is renowned for its high protein content, essential amino acids, vitamins, and minerals, which are critical for rapid larval growth and silk gland development (Karkos *et al.* 2011; Kumar, *et al*., 2019). These nutrients facilitate enhanced metabolic processes, enzyme activities, and cellular functions that contribute to increased larval weight and silk production. Azolla, a nitrogen-fixing aquatic fern, provides not only proteins but also phytonutrients and bioactive compounds that may enhance immune functions and metabolic efficiencies in silkworms. (Radhakrishnan *et al.* 2017; El-daim *et al*. 2021; Simon *et al*. (2024). The synergistic effects observed with Mixture supplementation suggest complementary nutritional and physiological roles, where Spirulina primarily boosts growth and silk production, while Azolla may enhance overall health and resilience. Also, the observed increase in filament weight with Spirulina supplementation is particularly

significant ($p<.003$) for sericulture, as filament weight directly correlates with silk quality. (Dharanipriya, 2019; Moustafa, 2024) emphasized the role of Spirulina's rich nutritional profile in enhancing silk protein synthesis, which our results substantiate. Enhanced filament weight not only improves the economic value of the silk produced but also reflects better larval health and metabolic efficiency. However, the impact of Azolla and Spirulina revealed that higher concentrations might have inhibitory effects on certain parameters, such as pupa weight. This aligns with the findings of (Jiang, 2019), who reported that excessive supplementation could negatively affect larval development, where higher concentrations caused toxicity and reduced growth and reproduction. The decrease in pupa weight at higher Azolla concentrations may be attributed to imbalanced nutrient intake or potential anti-nutritional factors present in Azolla, which could interfere with optimal metabolic processes during pupation (Pachiappan *et al.* 2021).By identifying optimal concentrations, particularly the 3% Spirulina and Azolla 1% supplementation, the study provides actionable insights for sericulture practitioners aiming to enhance silk yield and quality. This aligns with the broader objective of promoting eco-friendly and cost-effective agricultural practices that support rural livelihoods and economic sustainability.

5. Conclusion

This study evaluated the effect of dietary supplements containing Spirulina, Azolla, and their mixture on the main silk production parameters in silkworms. The results showed the positive impacts of Spirulina and Azolla on larval growth and cocoon production in silkworms; although, some differential effects were recorded among treatments and concentrations. Spirulina, especially at a 3% concentration, effectively enhanced larval weight, silk gland weight, and cocoon and pupal weights, while Azolla, at 1% and 5% concentrations, significantly increased filament weight and shell weight. The mixture treatments also showed positive effects, especially at a 5% concentration for filament weight and at 1% for shell Ratio, suggesting synergistic or complementary effects of the algal mixture. The study provides compelling evidence that Spirulina and Azolla are effective dietary supplements for enhancing the growth and productivity of *Bombyx mori*.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors of the manuscript have read and agreed to the publication

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no conflicts of interest.

Funding

Not applicable.

Authors' contributions

ROA, supervised the work and provided guidance throughout the research process. GAA, designed the study, conducted experiments, took the measurements, recorded the data and wrote the first draft. MAA, reviewed the first draft of the manuscript. SIAH, developed the work plan, followed up on the progress of the work, provided guidance throughout the research process, reviewed the research and wrote the final draft of the manuscript.

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