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Impact of Medicinal Plant Extracts on Cocoon Traits of Silkworm *Bombyx mori* L.

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ABSTRACT: The study investigated the effects of fortifying mulberry leaves with medicinal plant extracts *viz., Aloe vera, Ocimum sanctum* and *Withania somnifera* on the cocoon parameters of the silkworm hybrid (CSR2 × CSR4). The experiment was conducted over two seasons (spring and autumn) from 2021 to 2023 under temperate climatic conditions of Kashmir. The research aimed enhance silkworm growth and silk productivity by enriching mulberry leaves with different concentrations (1%, 2% and 3%) of the plant extracts. The experiment was laid down under Completely Randomized Design (CRD) and 200 silkworm larvae per replication per treatment were reared to evaluate various economic traits such as cocoon weight, shell weight and shell percentage. The results revealed significant improvements in cocoon parameters, with *O. sanctum* (3%) producing the highest single cocoon weight (2.40 g) and shell weight (0.54 g) during the spring season. Similarly, *A. vera* and *W. somnifera* exhibited positive effects on these parameters over control. The findings suggest that the fortified mulberry leaves offer a promising strategy to enhance its nutritional quality and ultimately improve silk production.

Keywords: Mulberry, fortification, medicinal, cocoon.

INTRODUCTION

Sericulture, the practice of rearing silkworms for silk production, is a vital component of the agricultural economies of many developing countries. The domesticated silkworm, *Bombyx mori* L., is the primary species used in commercial silk production due to its efficient conversion of mulberry leaves into high-quality silk fiber. Since mulberry leaves serve as the exclusive diet of *B. mori*, their nutritional quality directly impacts silkworm growth, development and silk yield (Rafiqui *et al.*, 2023; Gautam *et al.*, 2022 Ayoub *et al*., 2023a). The mulberry varieties vary in the biochemical constitution of leaves viz., proteins, carbohydrates, amino acids, ascorbic acid etc which are needed for the proper growth and development of silkworms, hence the selection of quality mulberry plants is of prime importance to yield maximum benefits (Islam, 2023).

Nutrition plays a critical role in sericulture, influencing the health, development and feed conversion efficiency of silkworms. As monophagous insects, *B. mori* larvae derive all their required nutrients from mulberry leaves (Laskar and Datta 2000). However, variations in mulberry leaf quality due to environmental factors, pest infestation and cultivation practices can lead to nutritional deficiencies, potentially affecting silk production (Ayoub *et al.*, 2023b). Consequently, optimizing silkworm nutrition has become a major research focus in sericulture, with the goal of maximizing the silk yield (Gobena & Bhaskar 2015). In order to enhance silk productivity, recent research has explored the fortification of mulberry leaves with supplementary nutrients, including plant extracts to improve silkworm performance. Fortification of mulberry leaves using various nutritional supplement like carbohydrates, proteins, amino acids, vitamins and plant extracts like *Aloe vera*, Tulsi, etc. has been widely studied for its potential to enhance economic traits, disease resistance and overall silk yield in silkworms (Borgohain, 2015; Manjunatha *et al.*, 2017; Sathis & Sujatha 2020; Wani, *et al*., 2024; Hajam *et al*., 2024).

Among these fortification methods, plant-based fortification has gained attention due to their rich bioactive compounds that positively affect silkworm physiology (Rajashekaragouda *et al*., 1997). Extracts of *Zingiber officinale*, *Lantana camara* and *Vigna unguiculata* have been reported to enhance silk quality and yield when used as dietary supplements for *B. mori* (Saravanan *et al.*, 2011). Several other medicinal plants have demonstrated beneficial effects on key economic parameters of silkworms, including increased larval body weight, cocoon weight, shell weight and improved maturation rates (Pardeshi & Bajad 2014; Islam *et al.*, 2023; Singh & Saxena 2015; Ismail *et al.*, 2016; Saad *et al.*, 2019). Studies have shown that extracts from medicinal plants such as *Aloe vera, Ocimum sanctum,*

and *Withania somnifera* can enhance growth, cocoon traits and disease resistance in *B. mori* (Hajam *et al.*, 2024). *Aloe vera*, known for its high nutrient content, acts as an herbal tonic, improving silk gland development and overall yield (Park & Jo 2006; Balamurugan & Isaiarasu 2007). Similarly, *Ocimum sanctum* and *Withania somnifera* offer antimicrobial, anti-inflammatory and immunomodulatory benefits (Sen, 1993; Singh *et al.*, 2011), which can enhance silkworm health and silk production.

Given the critical role of mulberry leaf nutrition in silkworm growth and silk productivity, this study aims to evaluate the effects of *Aloe vera*, *Ocimum sanctum* and *Withania somnifera* extracts on cocoon parameters of *B. mori*. By enhancing the nutritional composition of mulberry leaves through plant extract supplementation, this research is an attempt to optimize silkworm nutrition and enhance silk productivity, ultimately provide sericulturists with effective strategies to overcome nutritional deficiencies and increase profitability of farmers.

MATERIAL AND METHODS

The present study was conducted at the College of Temperate Sericulture, Mirgund, SKUAST-Kashmir, over two rearing seasons (spring and autumn) from 2021 to 2023. The bivoltine silkworm hybrid (*Bombyx mori* L.) CSR2 × CSR4 was utilized and disease-free layings (DFLs) were reared by following the standard package of practices (Anonymous, 2003). Three plant extracts—*Aloe vera*, *Ocimum sanctum* and *Withania somnifera*, were procured from commercial sources and prepared in three concentrations (1%, 2%, and 3%) for fortification of mulberry leaves. The experimental design comprised nine individual treatment groups (T_1 to T_9), each representing a unique combination of plant extract and concentration. Additionally, three mixed treatments were included, in which all three extracts were combined at 1%, 2%, and 3% concentrations. Two control groups were established: an aqueous control and an absolute control.

The experiment was laid as a Completely Randomized Design (CRD) with three replications per treatment, comprising 200 larvae per replication. The plant extracts were dissolved in distilled water to achieve the desired concentrations and were applied to mulberry leaves using an atomizer at a rate of 60 ml per 200 g of leaf for every 100 larvae, following the method described by Jeyapaul *et al.* (2003). The treated leaves were air-dried for 15 minutes before being fed to silkworm larvae during the fifth instar on alternate days. Key parameters including cocoon weight, shell weight and shell percentage were evaluated to assess the impact of plant extracts on silkworm performance.

1. Single Cocoon Weight (g). From each replication, 20 cocoons (10 male and 10 female) were randomly selected and weighed using a digital balance to determine the single cocoon weight.

2. Single Shell Weight (g). The same cocoons used for cocoon weight measurement were carefully cut open to extract the shells. These shells were then weighed using a digital balance to determine the single shell weight.

3. Shell percentage. It was calculated by using the following formula:

$$\text{Shell percentage} = \frac{\text{Weight of single cocoon shell}}{\text{Weight of single cocoon}} \times 100$$

RESULTS

Single cocoon weight (g): The data with respect to the single cocoon weight showed that the fortification of *A. vera*, *O. sanctum*, *W. somnifera* and their mixtures significantly improved individual cocoon weight (g) of *B. mori* L. during spring 2022 and 2023 (Table 1) compared to controls. *A. vera* at concentrations of 1%, 2% and 3% improved single cocoon weight to 2.14g, 2.26g and 2.35g respectively. *O. sanctum* also showed favorable results with increased single cocoon weight of 2.21g, 2.28g and 2.40g at 1%, 2% and 3% supplementation. *W. somnifera* increased single cocoon weight in a dose-dependent manner to 2.12g, 2.26g and 2.36g at 1%, 2% and 3% concentrations respectively. The Mixture treatments further exhibited positive effects, increasing single cocoon weight to 2.12g, 2.18g and 2.25g. In contrast, the single cocoon weights for the aqueous and absolute controls were lower at 2.06g and 2.07g. The pooled data showed that across all concentrations the plant extracts outperformed the controls in augmenting the single cocoon weight. *O. sanctum* at 3% and *A. vera* at 3% were the optimal treatments, resulting in the highest single cocoon weight of 2.40g and 2.35g respectively (Table 1) (Fig. 1).

The analysis of the data pertaining to above parameter during autumn 2021 revealed that the highest single cocoon weight of 2.04g was recorded in T_6 (*O. sanctum* 3%). It was followed by T_5 (*O. sanctum* 2%) with 1.95g and T_9 (*W. somnifera* 3%) with 1.92g. The lowest single cocoon weight of 1.79g was observed in T_{13} (Aqueous Control), which was statistically at par with T_{14} (Absolute Control) at 1.80g, T_{10} (Mixture 1%) at 1.81g and T_{11} (Mixture 2%) at 1.83g. Among the plant extract treatments, the highest single cocoon weight was in T_6 (*O. sanctum* 3%) with 2.04g, while the lowest was in T_1 (*A. vera* 1%), T_7 (*W. somnifera* 1%) and T_{10} (Mixture 1%) with 1.81g (Table 1) (Fig. 1).

Single shell weight (g): The fortification of medicinal plant extracts to the mulberry diet significantly improved the single shell weight of silkworm cocoons (Table 1). The single shell weight varied from 0.42g in the control treatments (T_{13} and T_{14}) to 0.54g in T_6, which included *O. sanctum* at 3%, according to pooled data collected over two spring seasons. When compared to 1% concentration, higher concentrations of plant extracts produced increased shell weights. The treatments T_3, T_6 and T_9, which included 3% concentrations of *A. vera*, *O. sanctum* and *W. somnifera*, produced the significantly highest shell weights, ranging from 0.52 to 0.54g. This was followed by the 2% concentrations of the plant extracts in T_2, T_5, T_8 and T_{12}, which had shell weights of 0.48-0.50g. The remaining treatments were statistically similar with shell weights of 0.44-0.46g. The positive influence of these medicinal plant extracts on silkworm shell weight has important economic implications, as higher shell

weight directly translates to higher raw silk yield (Table 1) (Fig. 2).

The analysis of the data with respect to this parameter (autumn 2021) revealed that the highest single shell weight of 0.45g was recorded in T_6 (*O. sanctum* 3%). It was followed by T_3 (*A. vera* 3%) with 0.43g and T_5 (*O. sanctum* 2%) with 0.42g. The lowest single shell weight of 0.36g was observed in T_{10} (Mixture 1%), T_{11} (Mixture 2%), T_{13} (Aqueous Control) and T_{14} (Absolute Control). Among the plant extract treatments, the highest single shell weight was in T_6 (*O. sanctum* 3%) with 0.45g, while the lowest was in T_{10} (Mixture 1%) with 0.36g (Table 1) (Fig. 2).

Shell percentage (%): The fortification of medicinal plant extracts to mulberry leaves resulted in significant increases in shell percentage over two spring seasons (Table 1). The highest shell percentage of 22.40% was obtained in treatment T_6, with the addition of 3% *O. sanctum*. This was followed by T_3, T_9 and T_{12}, with shell percentages of 22.23%, 21.98% and 21.48% respectively. Treatments T_2, T_5 and T_8 with 2% plant extract concentration resulted in shell percentages ranging from 21.18-21.74%. The subsequent treatments exhibited statistically similar shell percentages ranging from 20.55-21.10%. The absolute and aqueous control treatments exhibited the lowest shell percentages, with 20.28% and 20.19%, respectively (Table 1) (Fig. 3).

Perusal of the data with respect to shell percentage during autumn 2021 revealed that the highest shell percentage of 22.32% was recorded in T_6 (*O. sanctum* 3%), which was statistically at par with T_9 (*W. somnifera* 3%) at 22.01% and T_3 (*A. vera* 3%) at 22.02%. It was followed by T_5 (*O. sanctum* 2%) with 21.75%. The lowest shell percentage of 20.02% was observed in T_{13} (Aqueous Control), which was statistically at par with T_{14} (Absolute Control) at 20.08% and T_{10} (Mixture 1%) at 20.19%. Among the plant extract treatments, the highest shell percentage was in T_6 (*O. sanctum* 3%) with 22.32%, while the lowest was in T_{10} (Mixture 1%) with 20.19% (Table 1) (Fig. 3).

Table 1: Influence of plant extract fortified mulberry leaf on the cocoon parameters of silkworm *Bombyx mori* L.

Parameters	Single cocoon weight (g)		Single shell weight (g)		Shell percentage (%)	
Season / Treatments	Spring	Autumn	Spring	Autumn	Spring	Autumn
T1 *A. vera*1%	**2.14^{b}**	1.81^{d}	**0.45^{d}**	0.37^{e}	**21.05^{b}**	20.62^{b}
T2 *A. vera*2%	**2.26^{ab}**	1.87^{c}	**0.49^{bc}**	0.40^{cd}	**21.54^{ab}**	21.21^{b}
T3 *A. vera*3%	**2.35^{a}**	1.93^{b}	**0.52^{ab}**	0.43^{ab}	**22.23^{a}**	22.02^{a}
T4 *O. sanctum* 1%	**2.21^{b}**	1.87^{c}	**0.46^{cd}**	0.40^{cd}	**20.74^{b}**	21.14^{b}
T5 *O. sanctum* 2%	**2.28^{a}**	1.95^{b}	**0.50^{b}**	0.42^{bc}	**21.74^{a}**	21.75^{a}
T6 *O. sanctum* 3%	**2.40^{a}**	2.04^{a}	**0.54^{a}**	0.45^{a}	**22.40^{a}**	22.32^{a}
T7 *W. somnifera*1%	**2.12^{bc}**	1.83^{d}	**0.44^{de}**	0.38^{de}	**20.76^{b}**	20.62^{b}
T8 *W. somnifera*2%	**2.26^{ab}**	1.87^{c}	**0.48^{c}**	0.40^{cd}	**21.18^{b}**	21.45^{ab}
T9 *W. somnifera*3%	**2.36^{a}**	1.92^{bc}	**0.52^{ab}**	0.42^{bc}	**21.98^{a}**	22.01^{a}
T10 Mixture 1%	**2.12^{bc}**	1.81^{d}	**0.44^{de}**	0.36^{e}	**20.55^{bc}**	20.19^{c}
T11 Mixture 2%	**2.18^{b}**	1.83^{d}	**0.46^{cd}**	0.37^{e}	**21.10^{b}**	20.52^{bc}
T12 Mixture 3%	**2.25^{b}**	1.85^{cd}	**0.49^{bc}**	0.39^{d}	**21.48^{a}**	21.11^{b}
T13 Aqueous Control	**2.06^{c}**	1.79^{d}	**0.42^{e}**	0.36^{e}	**20.19^{c}**	20.02^{c}
T14 Absolute Control	**2.07^{c}**	1.80^{d}	**0.42^{e}**	0.36^{e}	**20.28^{c}**	20.08^{c}
C.D ($p \leq 0.05$)	**0.132**	**0.0642**	**0.026**	**0.0198**	**1.184**	**0.945**

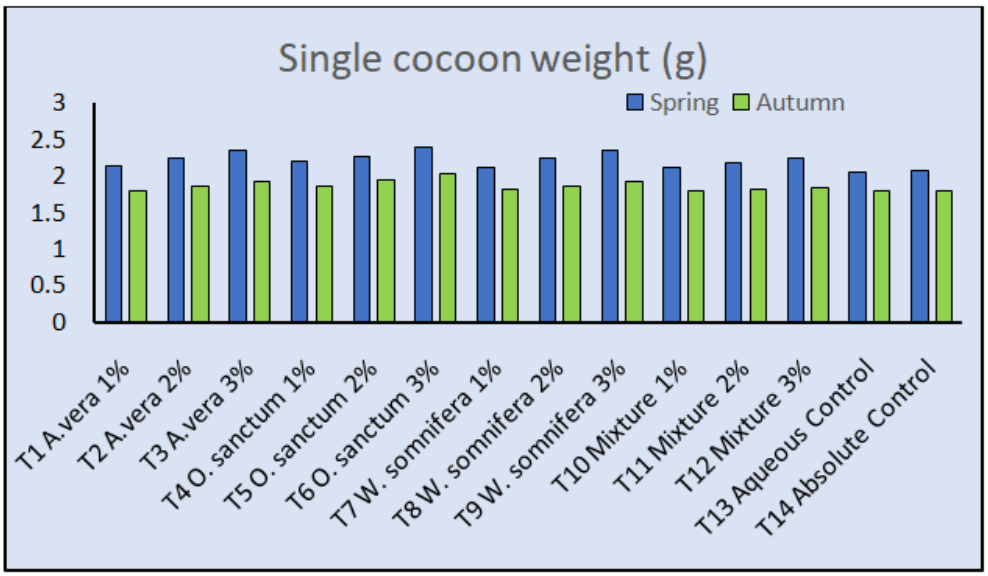

Fig. 1. Influence of Plant extract fortified mulberry leaf on Single cocoon weight (g).

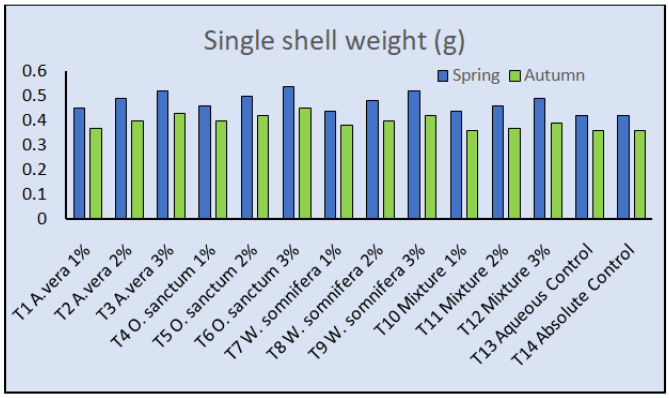

Fig. 2. Influence of Plant extract fortified mulberry leaf on Single shell weight (g).

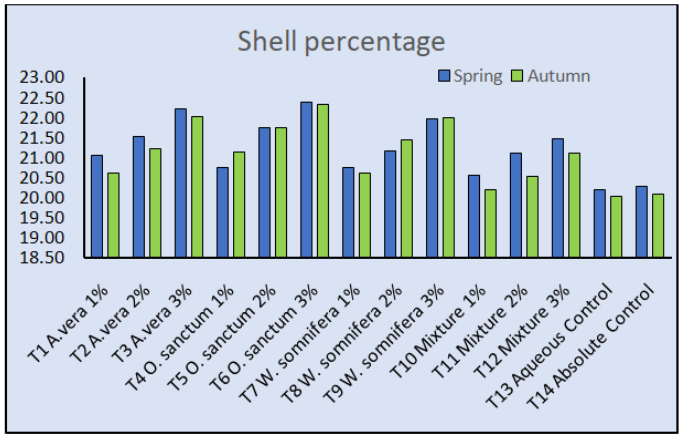

Fig. 3. Influence of Plant extract fortified mulberry leaf on Shell percentage.

DISCUSSION

The most significant commercial feature of cocoons is weight. Cocoons are sold in the marketplace based on weight as this index signals the approximate quantity of raw silk that can be reeled. The whole weight of a single cocoon is influenced by silkworm species, rearing season and harvest conditions. Cocoon weight, shell ratio and filament length are highly heritable traits and hold significant importance as they determine the quality, quantity and efficiency of the reeling process in sericulture (Nzyoki, 2020). Cocoon and shell weights are the primary traits evaluated for productivity in sericulture, with cocoon weight serving as a crucial commercial characteristic used to estimate the amount of raw silk obtainable. While shell weight provides a more accurate measure, it cannot be determined in commercial cultures without damaging the cocoon (Parvez *et al.*, 2024). During the current study in the spring seasons, *A. vera* supplementation at 3% concentration resulted in higher single cocoon weights of 2.35g and single shell weights of 0.52g. However, during autumn, the *A. vera* 3% treatment gave a single cocoon weight of 1.93g and single shell weight of 0.43g. The shell percentage was 22.02% in the *A. vera* 3% group during autumn. The findings of the present study are in conformity with previous studies that reported increased larval growth and cocoon weight when silkworms were fed with *A. vera* fortified mulberry leaves (Manimuthu & Isaiarasu 2010; Tiwari *et al*., 2014). *O. sanctum* supplementation at 3% led to the highest single cocoon weights of 2.40g and single shell weights of 0.54g during spring. It also produced the highest shell percentage of 22.40% in spring. In autumn, the *O. sanctum* 3% treatment again resulted in the maximum single cocoon weight (2.04g), single shell weight (0.45g) and shell percentage (22.32%). This is consistent with other studies that reported improved growth and commercial qualities of silk when silkworms were fed with *O. sanctum* fortified mulberry leaves (Devi & Ramani 2015; Sujatha *et al*., 2015). During spring, *W. somnifera* supplementation at 3% significantly improved single shell weight to 0.52g and shell percentage to 21.98%. While as in autumn, the *W. somnifera* 3% treatment gave a single cocoon weight of 1.92g, single shell weight of 0.42g and shell percentage of 22.01%. Although specific studies on the effects of *W. somnifera* on silkworms are limited, it is known that certain medicinal plants can improve the growth and development of silkworms, leading to better economic traits (Gobena & Bhaskar 2015). The data clearly shows that across both spring and autumn seasons, supplementation with 3% concentrations of these plant extracts, especially *O. sanctum*, resulted in marked improvements in single cocoon weight, single shell weight and shell percentage over the control groups. The improvements in these cocoon parameters can be attributed to the bioactive compounds present in these plant extracts. These compounds may have enhanced the development and functioning of the silk glands responsible for silk protein synthesis and deposition in the cocoon shell. This is supported by the fact that nutrition plays a crucial role in determining the quality of the cocoon crop. Higher cocoon weights, shell weights and shell percentages directly translate to increased raw silk production and quality, making these economically significant traits for the sericulture

industry. This suggests that the fortification of mulberry leaves with these plant extracts could be a viable strategy for improving cocoon crop quality and quantity. It is important to note that while these results are promising, further research is needed to identify the active principles in these extracts, standardize and formulate the botanicals and develop better administration techniques for commercial silkworm rearing (Manimuthu & Isaiarasu 2010; Gobena & Bhaskar 2015).

CONCLUSIONS

The present study demonstrates that fortifying mulberry leaves with medicinal plant extracts, specifically *Aloe vera*, *Ocimum sanctum* and *Withania somnifera*, significantly enhances the cocoon parameters of the silkworm, *Bombyx mori* L. The enriched diet improved key economic traits, including cocoon weight, shell weight, and shell percentage, with the highest performance observed at 3% concentrations of *Ocimum sanctum* and *Aloe vera*. These improvements can be attributed to the bioactive compounds present in the extracts, which enhanced larval growth, silk gland development, and overall silk production. The findings highlight the potential of using fortified mulberry leaves as a strategic nutritional intervention to optimize silkworm rearing, ultimately benefiting sericulturists by increasing raw silk yield and quality. This approach offers a sustainable and cost-effective means to overcome nutritional deficiencies in silkworm diets, promoting better commercial outcomes. Future research should focus on identifying the active principles in these extracts and refining their application methods to further enhance the efficacy and scalability of this fortification strategy in sericulture.

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