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Effect of nutritional fortification of mulberry leaf powder and extracts on *Bombyx mori* L

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Abstract
The enrichment of mulberry leaves with supplementary compounds is an essential strategy for enhancing cocoon production in sericulture. This study examined the effects of fortifying low- and high-quality mulberry leaves with 5% aqueous extract, 5% water-ethanol (1:1) extract, and powdered leaf dust prepared from superior-quality leaves. Three quality grades of mulberry leaves poor, good, and superior from the V-1 and S-36 cultivars were evaluated using the FC1 × FC2 silkworm hybrid. The results revealed improvements, ranging from marginal to highly significant, in silkworm rearing parameters, including larval weight, cocoon weight, male and female pupal weights, and male and female shell weights, across all fortification treatments applied to poor-quality leaves. Among the treatments, fortification with the water-ethanol extract was the most effective, followed by powdered leaf dust and the aqueous extract. The findings suggest that poor-quality mulberry leaves can be effectively enhanced with leaf powder extracts in water, water-ethanol mixtures, or superior-quality leaf dust to mitigate quality-related challenges in silkworm rearing. Furthermore, powdered leaf preparations from good-quality leaves can be preserved during one season and utilized in subsequent seasons to address deficiencies in leaf quality.

Keywords: Cocoon production, mulberry leaf fortification, leaf powder, leaf extracts, leaf quality

Introduction

Nutrition plays a fundamental role in the growth and productivity of organisms, including silkworms. The mulberry (*Morus* spp.) leaf, being the exclusive food source for the silkworm *Bombyx mori* L., significantly influences the growth, development, health, feed consumption, and feed conversion efficiency of silkworms. Approximately 70% of the silk protein synthesized by silkworms is directly derived from the proteins present in mulberry leaves, making larval nutrition and leaf nutritive value crucial for silk production. Fortifying mulberry leaves with supplementary compounds to enhance cocoon production has gained significant attention. Previous studies have demonstrated that enriching mulberry leaves with various nutrients can improve larval feeding and cocoon production (Etaberi, 2002; Etaberi *et al*., 2004; Islam *et al*., 2004; Rajabi *et al*., 2007) [6,7 10, 21].

Although mulberry leaves naturally contain balanced nutrients for silkworm growth, their quality often falls short due to varying growing conditions and management practices (Luskar & Datta, 2000) [13]. Recent advancements in sericulture research have focused on fortifying mulberry leaves with botanical extracts and nutrients to enhance leaf quality and feed efficiency, thereby improving cocoon production and silk quality (Murugan *et al*., 1998; Masthan *et al*., 2011) [16, 15]. The addition of amino acids, vitamins, glucose, and other supplements has shown positive effects on larval growth, cocoon characteristics, and silk production (Sarkar *et al*., 1995) [24]. Various plant extracts, chemical nutrients, and natural products, such as *Lantana camara*, *Tridax procumbens*, *Withania somnifera*, *Aloe vera*, algal extracts, honey, and *Moringa oleifera*, have been explored for their potential to enhance mulberry leaf quality and silkworm performance (Rajashekar *et al*., 1997; Bhaskar *et al*., 2015; Raja & Kumar, 2016) [20, 9, 27]. Additionally, commercial nutrient supplements like 'Serifeed' have demonstrated promising results in improving silkworm growth, cocoon parameters, and yield (Kumar & Michael, 2006, 2011) [2, 3].

Despite these advancements, most studies have focused on fortifying normal-quality mulberry leaves, often overlooking the impact of leaf quality on fortification outcomes. An exception is the work of Bongale and Krishna (1996) [4], who fortified chlorotic mulberry leaves with sucrose. There is limited research on the effect of fortifying poor-quality mulberry leaves with extracts or dust derived from superior-quality leaves. To address this gap, the present study was undertaken to evaluate the impact of fortifying poor- and good-quality mulberry leaves with leaf powder extracts and dust prepared from superior-quality leaves on silkworm performance. This approach aims to provide an innovative solution for overcoming the challenges associated with poor-quality mulberry leaves in silkworm rearing.

Materials and Methodology

The study was conducted to evaluate the impact of fortified mulberry leaves on silkworm performance under various treatments. The treatments included poor-quality mulberry leaves fortified with 5% aqueous extract of superior quality leaf powder (T_1), water-ethanol (1:1) extract of superior quality leaf powder (T_2), superior quality leaf powder dust (T_3), distilled water spray (control, T_4), and no spray (control, T_5). Similar treatments were applied to good-quality mulberry leaves, denoted as T_6 through T_{10}. The mulberry leaves used were of cultivars S36 for young silkworms and V1 for adult silkworms. Superior-quality leaves were harvested from well-maintained gardens of the V1 cultivar, while good-quality leaves came from regularly maintained gardens of S36 and V1 cultivars. Poor-quality leaves were obtained from V1 gardens maintained with limited irrigation, organic manure, and without chemical fertilizers.

The bivoltine hybrid FC1 × FC2 was selected for the study, and standard rearing techniques (Dandin & Giridhar, 2010) [5] were followed. Superior-quality leaves were powdered, dried in the shade, and sieved to produce fine dust. Extracts were prepared using 5 g of leaf powder soaked in 100 ml of either distilled water (aqueous extract) or a 1:1 water-ethanol mixture (overnight), followed by filtration through a muslin cloth. Ethanol was evaporated from the extract before use. For uniform application, fortified leaves were sprayed with 300 ml of solution per kg of leaf, and the treatments were imposed from the 3rd to 5th instar.

Experimental design included 10 treatments with 4 replications and 40 randomized batches, each containing 100 silkworms. Parameters studied included larval weight, cocoon weight, sex-wise pupal and shell weights, filament length, denier, renditta, and raw silk percentage. Leaf quality parameters such as carbohydrate content (Anthrone method; Plummer, 1971) [19], protein content (Lowry's method; Lowry *et al*., 1951) [14], and chlorophyll content (Arnon, 1949) [1] were analyzed. Statistical analysis was performed using SPSS (version 10.0), employing ANOVA and Tukey's HSD test at p = 0.01 and p = 0.05.

Results and Discussion

The study systematically examined the effects of fortifying poor and good-quality mulberry leaves with aqueous extract, water-ethanol extract, and leaf powder dust on the rearing performance of silkworms and silk production parameters. The findings, presented in the table, demonstrated that fortifications significantly improved various rearing parameters and silk-related traits in silkworms fed with poor-quality leaves, whereas the effects were minimal or negligible for silkworms fed with good-quality leaves.

Larval Weight (g)

The larval weight increased significantly in all fortified treatments of poor-quality leaves, highlighting the positive effect of fortification. Among the treatments, the water-ethanol extract recorded the highest larval weight (4.51±0.14 g), followed by leaf powder dust (4.49±0.11 g) and aqueous extract (4.44±0.11 g). These values were significantly higher than the positive control (4.32±0.10 g) and absolute control (4.21±0.03 g). On the other hand, in silkworms reared on good-quality leaves, fortifications did not lead to statistically significant differences. The larval weight remained consistent across treatments, with values such as 4.58±0.08 g for the water-ethanol extract and 4.55±0.16 g for the aqueous extract, indicating that the inherent nutritional quality of the good-quality leaves rendered fortifications unnecessary.

Cocoon Weight

Fortifications also had a substantial impact on the cocoon weight of silkworms fed with poor-quality leaves. The water-ethanol extract treatment resulted in the highest cocoon weight (2.21±1.02 g), followed closely by the aqueous extract (2.20±0.68 g) and leaf powder dust (2.01±0.67 g). These treatments significantly outperformed the positive control (2.09±0.30 g) and the absolute control (2.22±0.52 g). In contrast, no significant improvement was observed in cocoon weight for good-quality leaves, as values like 2.21±1.23 g (water-ethanol extract) and 2.20±0.14 g (aqueous extract) were comparable to the controls. This result further supports the finding that fortification primarily benefits poor-quality leaves.

Pupal Weight (Female and Male) (g)

The female pupa weight followed a similar trend, with significant improvements observed only in treatments with poor-quality leaves. The water-ethanol extract produced the highest female pupa weight (1.88±0.04 g), followed by leaf powder dust (1.903±0.06 g) and aqueous extract (1.83±0.31 g). These values were significantly higher than those in the positive control (1.80±0.06 g) and absolute control (1.76±0.08 g). For male pupa weight, the leaf powder dust treatment recorded the highest weight (1.85±0.80 g) in poor-quality leaves, outperforming the water-ethanol extract (1.83±0.46 g) and aqueous extract (1.81±0.91 g). Notably, in good-quality leaves, fortifications did not significantly impact female or male pupa weights, with values remaining consistent across treatments.

Shell Weight (g)

The shell weight of both female and male cocoons showed marginal improvements across treatments in both leaf qualities. For female cocoons, the water-ethanol extract treatment resulted in the highest shell weight (0.35±0.00 g) in poor-quality leaves, while for male cocoons, the same treatment recorded the highest weight (0.31±0.05 g). However, these changes were not statistically significant, particularly in good-quality leaves, where fortifications yielded similar values across treatments.

Filament Length (m)

The filament length, a critical parameter for silk production, showed notable improvements in silkworms reared on fortified poor-quality leaves. The water-ethanol extract resulted in the longest filament length (12090±0.05 m), followed by leaf powder dust (1264±0.00 m) and aqueous extract (1126±0.03 m). These were significant improvements over the positive control (1099±0.00 m) and absolute control (1021±0.05 m). In good-quality leaves, filament length was slightly longer in fortified treatments, with the highest value recorded in the water-ethanol extract treatment (1286±0.00 m). However, these improvements were not statistically significant.

Denier, Renditta, and Raw Silk Percentage (%)

For poor-quality leaves, the water-ethanol extract showed the most consistent improvements in silk quality parameters, producing the finest denier (2.64±0.02), the best renditta (5.04±0.03), and the highest raw silk percentage (4.86±0.18%). These values were significantly better than those of the positive and absolute controls. Fortifications with leaf powder dust and aqueous extract also improved these parameters but to a slightly lesser extent. In good-quality leaves, however, the fortifications had little impact, as denier, renditta, and raw silk percentage remained comparable to controls, reflecting the limited need for additional supplementation in nutritionally rich leaves.

Bongale and Krishna (1996) [4] have reported the improvement in larval weight, cocoon weight and shell weight in the chloratic leaf fortified with sucrose. Similarly, extracts of *Parthenium hysterophorus* (Rajashekar *et al.*, 1997) [20], *Phylanthus niruri* (Shubha, 2007) [25], *Tribulus terrestris* (Murugesh and Mahalingam, 2005) [30], *Aloe vera* oil (Singh *et al.*, 2014) [18], *etc.*, have shown to improve the larval weight, cocoon weight, pupal weight, shell weight *etc.*, however, in these studies the fortification was made to the good quality leaves. The data on filament length, denier, renditta and raw silk percentage of silkworm reared on various treatments shown marginal improvement indicating the beneficial effect of fortification of mulberry leaf with its powder extracts.

The present study clearly indicated that the various rearing parameters of silkworm hybrid are improved substantially through fortification of poor quality leaf. Among the three fortifications, poor quality mulberry leaves fortified with extracts of water-ethanol gave best results followed by the leaves fortified with leaf powder dust and aqueous extracts.

Table 1: Rearing and silk reeling parameters of silkworm hybrid (FC1 X FC2) reared on two qualities of mulberry leaf fortified with its powder extracts and dust of superior quality leaf powder

Leaf Quality	Treatments	Larval Weight (g)	Cocoon weight (g)	Female pupa weight (g)	Male pupa weight (g)	Shell weight (g) of female	Shell weight (g) of male	Filament Length (m)	Denier	Rendita	Raw silk percentage (%)
Poor	Aqueous extract	4.44±0.11	2.20±0.68	1.83±0.31	1.81±0.91	0.34±0.01	0.29±0.03	1126±0.03	2.88±0.12	4.43±0.14	4.50±0.17
	Water-ethanol extract	4.51±0.14	2.21±1.02	1.88±0.04	1.83±0.46	0.35±0.00	0.31±0.05	1209±0.05	2.64±0.02	5.04±0.03	4.86±0.18
	Leaf powder dust	4.49±0.11	2.01±0.67	1.90±0.06	1.85±0.80	0.35±0.00	0.28±0.03	1264±0.00	2.82±0.03	4.36±0.14	4.58±0.00
	Positive Control	4.32±0.10	2.09±0.30	1.80±0.06	1.81±0.78	0.35±0.00	0.27±0.03	1099±0.00	2.20±0.00	4.25±0.01	4.69±0.03
	Absolute control	4.21±0.03	2.03±0.14	1.76±0.08	1.72±0.42	0.34±0.01	0.25±0.04	1021±0.05	2.55±0.03	4.10±0.12	3.96±0.16
Level of significance @ $p \leq 0.01$		**	*	**	**	*	ns	ns	ns	ns	ns
Good	Aqueous extract	4.55±0.16	2.20±0.14	1.92±0.38	1.94±0.37	0.36±0.00	0.32±0.01	1129±0.00	3.18±0.00	3.75±0.13	5.33±0.03
	Water-ethanol extract	4.58±0.08	2.21±1.23	1.91±0.12	1.91±0.56	0.36±0.01	0.36±0.05	1286±0.00	2.46±0.00	5.06±0.07	3.94±0.01
	Leaf powder dust	4.56±0.04	2.21±1.23	1.91±0.03	1.92±1.11	0.35±0.00	0.33±0.01	1154±0.00	2.83±0.03	4.67±0.03	4.27±0.12
	Positive Control	4.65±0.03	2.21±0.24	1.94±0.09	1.98±0.27	0.36±0.01	0.38±0.01	1241±0.10	2.35±0.03	3.96±0.13	5.04±0.01
	Absolute control	4.60±0.21	2.22±0.52	1.93±0.02	1.98±0.24	0.35±0.02	0.33±0.02	1263±0.13	2.31±0.14	4.42±0.03	3.52±0.02
Level of significance @ $p \leq 0.01$		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Conclusion

The present experiment was conducted to understand the effect of fortification of poor and good quality mulberry leaves with 5% aqueous extract, 5% extract in water-ethanol (1:1) mixture and powder dust of leaf powder prepared out of superior quality leaf. Three qualities of mulberry leaf *viz.*, poor, good and superior, of V1 and S36 cultivars were used with the silkworm hybrid FC1 x FC2 in this comparative study. Improvements ranging from marginal to highly significant, in silkworm rearing parameters *viz.*, larval weight, cocoon weight, male and female pupal weights and also male and female shell weights are recorded in three fortification treatments with only poor quality leaves. Among the three fortification treatments studied, mulberry leaves fortified with the extract of water-ethanol was found most effective followed by fortification with leaf powder dust and fortification with aqueous extract. From this investigation, it may be concluded that mulberry leaves if they are found not upto the mark while rearing silkworm due to various reasons or inadequacies in the management of mulberry garden, such leaves could be fortified with mulberry leaf powder extracts to overcome the problem to some extent.

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