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Effect of Lemon Juice Enriched Mulberry Diets on Economic Parameters of Silkworm, *Bombyx mori* L.

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Abstract:
The domesticated mulberry silkworm, *Bombyx mori* L. is a popular laboratory model organism utilized in various experimental studies. In the present investigation, the silkworm bivoltine double hybrid FC1×FC2 was reared on mulberry leaves fortified with lemon juice at different concentrations *viz.*, 1.0, 2.0 and 3.0% during the 4thand 5thinstar to record its impact on economic parameters of silkworm. The larvae fed on mulberry leaves enriched with 3.0% lemon juice significantly increase economic traits such as larval weight, cocoon weight, pupal weight, shell weight, shell percentage, filament length and filament weight compared to other treatments as well as control batch. Additionally, larvae supplemented with 3.0% lemon juice recorded enhanced reproductive parameters such as fecundity and hatching percentage. These findings suggest that fortification of mulberry leaves with lemon juice can positively influence the larval growth, improved cocoon quality and enhanced reproductive parameters.

Keyword: *Bombyx mori*, Lemon juice, Economic parameters, Fecundity, Hatching percentage

Introduction

The silkworm *Bombyx mori* L., is a monophagous insect that primarily feeds on mulberry leaves (*Morus spp.*), which are the sole natural source of nutrition for its growth and silk production. Over the years, considerable research has been undertaken to improve the nutritional quality and rearing efficiency of silkworms through the use of artificial and supplemented diets. Artificial diets have successfully addressed many nutritional deficiencies inherent in mulberry leaves and have provided certain practical advantages. These include reduced dependence on mulberry cultivation, lower maintenance costs and the possibility of rearing silkworms independent of seasonal constraints, thereby improving productivity and economic efficiency (Ovesenska *et al.*, 2000). Moreover, silkworms reared on artificial diets under laboratory conditions have been shown to yield cocoons of good quality and demonstrate higher economic traits such as growth rate, pupation percentage and cocoon yield (Nikolova, 2020). However, despite these advantages, the relatively high cost of artificial diets limits their large scale and continuous use in commercial sericulture.

Natural mulberry leaves, though nutritionally suitable, often fail to provide a completely balanced diet for the silkworm. This nutritional imbalance compels the larvae to engage in compensatory overfeeding, which has little impact on silk yield or quality (Etebari, 2002; Rahmathulla *et al.*, 2007). To overcome these limitations, a third feeding approach have been explored known as exogenous nutrient enriched

mulberry diet**,** which involves fortification of mulberry leaves with natural or synthetic supplements to enhance nutritional balance (Murugan *et al.*,1998). In this method, mulberry leaves are treated with nutrient solutions containing bioactive factors and modulators at optimal concentrations to promote better larval growth and silk production (Thulasi and Sivaprasad, 2015; Madhavi and Sivaprasad, 2020, 2022). This exogenous nutritional approach has proven effective in improving both biological and economic parameters of silkworms, including cocoon quality, shell ratio, filament length and raw silk yield (Rahmathulla *et al.,* 2007).

In the recent year, different type of supplementing agents was used so as to enhance larval and commercial parameters of the silkworm which includes proteins, carbohydrates, vitamins, amino acids, sterols, hormones, antibiotics, salts and other chemicals (Chowdhury *et al.,* 1996). Mulberry leaves enriched with nutrients such as sugars, potassium, glycine, alanine and soya milk has been shown to significantly improve larval weight, silk gland development and cocoon yield (Sarkar *et al.,* 1995; Sengupta *et al.,* 1972). Honey enhance the larval growth, silk gland development and protein metabolism at different concentrations (Thulasi and Sivaprasad, 2014a; 2015; Bhatti *et al.,* 2019). Similarly, combined treatment with sericin and honey during the fifth instar led to significant improvements in larval weight and biological traits (Shahzadi *et al.,* 2022).

Among various natural supplements, lemon juice has gained particular attention due to its rich nutritional profile and beneficial effects on silkworm growth and silk production. Lemon (*Citrus limon*), a member of the family Rutaceae, is a rich source of vitamin C (ascorbic acid), minerals and trace elements such as calcium, iron, magnesium, phosphorus, potassium, zinc, and vitamin A (Gonzalez *et al.,* 2009). Supplementation of mulberry leaves with lemon juice at different concentration has been reported to enhance larval weight, growth rate, and protein metabolism in the silk gland (Thulasi and Sivaprasad, 2014). Similarly, larvae supplemented with lemon juice enhances the rate of food consumption, silk gland activity and overall economic traits (Etebari *et al.,* 2004; Walaa *et al.,* 2018). Moreover, key economic parameters such as larval weight, cocoon weight, shell weight, shell percentage, filament length, filament weight, denier and renditta were significantly higher in lemon juice treated group (Thulasi and Sivaprasad, 2013). Even good number of works has been reported on lemon juice enhancing the larval and cocoon parameters in combination with other supplementing agents. However, reports available on fortification of lemon juice alone on silkworm rather scanty. Keeping this in view present study was undertaken.

Materials and methods

The present experimental analysis was carried out in the Department of Studies in Sericulture Science, University of Mysore, during 2024-25. The popular silkworm double hybrid FC1 × FC2 was used and rearing was conducted according to the standard method recommended by Krishnaswami (1986). The worms were fed with healthy mulberry leaves of S36 variety during chawki stage and with V1 variety leaves during later instars.

Experimental design and feeding patterns

After the third moult, silkworm larvae were divided into four different groups with three replications of 100 silkworms each maintained and labeled as Distilled water fed experimental, 1.0% Lemon juice fed experimental, 2.0% Lemon juice fed experimental and 3.0% Lemon juice-fed experimental batches. The silkworm larval groups were fed with two types of mulberry diets such as normal diet and nutrient

enriched diet. The nutrient solutions were prepared in distilled water using the minimum effective concentrations of lemon juice (Thulasi and Sivaprasad, 2014), determined as per standardized procedures in our laboratory. Accordingly, the nutrient enriched mulberry diets were prepared by soaking mulberry leaves 1.0, 2.0 and 3.0% lemon juice solutions and dried under cool weather conditions and fed to healthy silkworm of experimental groups, once in the day at 10 am. Twenty larvae from each treatment were used in three replications to record the economic traits, namely cocoon weight, shell weight, shell ratio, filament length, filament weight, denier, renditta, effective rate of rearing (ERR), fecundity and hatching percentage. The data obtained from the experiment were subjected to statistical analysis using standard deviation method.

The parameters, namely shell ratio, filament length, denier, renditta, effective rate of rearing and hatching percentage was computed using the following formulae.

$$\text{Shell ratio (\%)} = \frac{\text{shell weight}}{\text{cocoon weight}} \times 100$$

Filament length (m): L= R×1.125

R = Number of revolutions recorded by an eprouvette.

1.125 = Circumference of eprouvette in meter.

$$\text{Denier} = \frac{\text{filament weight}}{\text{filament length}} \times 9000$$

It indicates thickness of the silk filament.

$$\text{Renditta} = \frac{\text{weight of cocoons reeled}}{\text{weight of raw silk obtained}}$$

Unit quantity of cocoons required to produce one unit of raw silk.

$$\text{Effective rate of rearing (\%)} = \frac{\text{Total number of larvae brushed}}{\text{Total number of cocoons harvested}} \times 100$$

$$\text{Hatching percentage (\%)} = \frac{\text{Number of eggs hatched}}{\text{Total number of eggs}} \times 100$$

Results and discussion

Influence of mulberry leaves supplemented with lemon juice on economic parameters.

1. Larval weight and cocoon weight

Silkworm larvae reared on mulberry leaves enriched with lemon juice at different concentrations shows significant impact on larval weight and cocoon weight. The worms treated with lemon juice at 3.0% concentration registered maximum larval weight of 4.84 g and cocoon weight of 2.27 g compared to control batches (4.28g and 2.12 g respectively) (Table 1). The increase in larval and cocoon weight might be due to utilization of additional nutrients that are present in lemon juice. It is evident that lemon juice is a richest source of ascorbic acid which boost the immune system of the larva which enhance larval weight as well as cocoon weight. These findings are also supported by the observation of Thulasi

and Sivaprasad (2014) who have reported that larvae reared on mulberry leaves supplemented with 3.0% lemon juice enhance larval weight and cocoon weight compare to control. A similar trend was also observed with amla juice, folic acid and honey (Divya and Patil, 2016; Rahamathulla *et al.,* 2007; Bhatti *et al.,* 2019).

2. Shell weight and pupal weight

Silkworms nourished with mulberry leaves extra foliated with lemon juice at varied concentrations exerted significant influence on shell weight and pupal weight. The worms supplemented with lemon juice at 3.0% concentration registered higher shell weight and pupal weight of 0.505g and 1.77g, respectively. However, control batch recorded 0.460g and 1.66g, respectively (Table 1). It is presumed that the silkworm larvae absorbed and utilized the nutrients found in lemon juice for the synthesis of silk protein and also enhances body weight. These results are in conformity with earlier findings of Sayyad (2020) who have observed that silkworms fed with amla (2.0%) and lime (3.0%) fruit extract showed increase in shell weight and pupal weight. The trend was same with 2.0% ascorbic acid (EI-Karaksy and Idriss, 2009).

3. Shell percentage and filament length

The worms reared on mulberry leaves fortified with 3.0% lemon juice scored maximum shell percentage of 22.20% and longer filament length of 1380.09m as against respective control batches (21.66% and 1142.87m) (Table 1). These findings are in agreement with the earlier observations of Walaa *et al.,* (2018) who have noticed that the silkworm larvae treated with 4.0% lemon juice scores significant increase in shell percentage and filament length. This trend was also noticed with some other supplementing agents *viz.,* sugars, proteins, amino acids, vitamins and ascorbic acid (Sengupta *et al.,* 1972; Etebari *et al.,* 2004).

4. Filament weight and denier

The worms treated with lemon juice at 3.0% concentration shows increased filament weight and lowest denier of 0.524g and 2.98respectively, over control (0.383g and 3.02) (Table 1). The increase in filament may be due to higher utilization of lemon juice for the synthesis silk proteins. The denier important quality trait which donates thickness of the silk filament. These results are in accordance with those of Thulasi and Sivaprasad (2014) who have reported that larvae supplemented with lemon juice at the rate of 3.0% registered higher filament weight and denier when compare to control. Similarly, larvae administrated with camphor honey bee (5.0%) and camphor oil (1.0%) exhibited higher filament weight and denier (Saad *et al.,* 2014).

5. Renditta and effective rate of rearing

Silkworms nourished with mulberry leaves fortified with spirulina at different concentrations registered variations with respect to renditta and effective rate of rearing (ERR). Larvae reared on mulberry leaves enriched with lemon juice at 3.0% concentration shows decreased renditta of 5.20 and 84.15% of effective rate of rearing when compare to control (5.28 and 80.13%, respectively) (Table 1). According to Kiran Kumar *et al.,* (2024), larvae fed on mulberry leaves extra foliated with 0.2% amla juice registered lowest renditta when compared to remaining treatments as well as control batches. Similar trend was also reported with some nutritional supplements such as extracts of *Altthaea sp., Azolla sp.* and *Aloe vera* (Oukasha *et al.*, 2025)

6. Fecundity and hatching percentage

Larvae reared with mulberry leaves supplemented with spirulina at different concentrations registered significant influence on Fecundity and hatching percentage. The worms treated with lemon juice at 3.0%

concentration shows enhanced fecundity (883) and hatching percentage (97.24%) against control batches (757 and 94.56%, respectively) (Table 1). Lemon juice rich in vitamin C which improve egg development and vitellogenesis, leading to improved fecundity (Chauhan and Kamala, 1992; Ganesh Prabhu *et al.,* 2013). The hatching percentage remained high across all groups (~96–97%), showing no adverse impact of foliar additives on embryonic development and instead, maintaining egg viability under enhanced nutritional regimes (Etebari *et al.,* 2004).

Impact of mulberry leaves supplemented with lemon juice at different concentrations on economic parameters of silkworm *Bombyx mori* L.

Foliar additives	Larval weiwei (g)	Cocoon weight (g)	Shell weight (g)	Pupal weight (g)	Shell percentage (%)	Filament length (m)	Filament weight (g)	Denier (d)	Renditta (kg)	ERR (%)	Fecundity (no)	Hatching percentage (%)
Distilled water (control)	4.28 ±0.07	2.12 ±0.045	0.460 ±0.013	1.66±0.033	21.66 ±0.149	1142.87±30.374	0.383 ±0.007	3.02 ±0.033	5.28 ±0.066	80.13 ±1.285	757± 15.535	94.56 ±0.977
1.0% Lemon juice	4.50 ±0.06	2.18 ±0.015	0.477 ±0.006	1.70±0.017	21.93 ±0.338	1259.68±19.250	0.424 ±0.012	3.03 ±0.053	5.23 ±0.085	81.12 ±1.311	798± 20.033	94.72 ±0.810
2.0% Lemon juice	4.65 ±0.08	2.23 ±0.021	0.493 ±0.008	1.73±0.013	22.14 ±1.171	1374.44±27.484	0.457 ±0.011	2.99 ±0.018	5.22 ±0.051	82.13 ±1.216	817± 8.544	96.04 ±0.263
3.0% Lemon juice	4.84 ±0.08	2.27 ±0.021	0.505 ±0.006	1.77±0.015	22.20 ±1.075	1380.09±21.967	0.524 ±0.012	2.98 ±0.027	5.20 ±0.091	84.15 ±1.049	883± 12.220	97.24 ±0.903

Conclusion

The silkworm larvae nourished with mulberry leaves treated with lemon juice at 3.0% concentration enhance the larval, cocoon and reproductive parameters due to utilization of nutrients by the silkworm. Lemon juice rich in vitamin C and other nutrients, which could have positive effects on the silkworms and leads to achieve good quality cocoons as well as enhanced reproductive parameters. Hence, lemon juice could be used as supplementing agent for silkworm rearing after conducting certain field trails.

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