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THE EFFECT OF HOMEOMEDICINE ALFALFA ON LARVAL GROWTH, SILK PRODUCTION AND ECONOMIC TRAITS OF *BOMBYX MORI*

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ABSTRACT

The impact of homeo medicine alfalfa on the silkworm, *Bombyx mori* was demonstrated with reference to its larval growth, silk production and economic parameters of sericulture. The alfalfa works well at a minimum concentration of 5% in distilled water. At this concentration it enhances growth rates in the larval body as well as silk gland during fifth instar development. It positively reinforces the day-to-day larval growth rate by 4.2 additional percentile points, silk gland growth rate by 18.37 additional percentile points and the gland-body ratio by 3.55 additional percentile points. It stimulates silk protein synthesis in all the three segments of the silk gland, viz., the anterior, middle and posterior parts. Under its influence, the silk gland protein profiles grew significantly by 177.16 additional percentile points in the anterior silk gland (ASG), minimally by 51.9 additional percentile points in the middle silk gland (MSG) and by 29.05 additional percentile points in the posterior silk gland (MSG). It also stimulates the core shell protein synthesis by ~16% and floss protein synthesis by ~2% in the three segments of silk gland. In doing so, it contributes to sericulture industry by causing improvements in gland-body ratio, cocoon weight, shell weight, raw silk weight, denier and renditta and floss. Thus, the alfalfa stimulates growth in both profit-making (shell protein and shell weight levels) and loss making (floss protein and floss weight) parameters of sericulture. Nevertheless, keeping in view its overall positive impact, this homeo is recommended as a profitable supplementary diet for silkworm.

Keywords: Alfalfa, *Bombyx mori*, Gland-body ratio, Larval growth, Silk proteins, Economic traits.

INTRODUCTION

The Alfalfa is a homeo medicine, obtained from the germinating barley seeds. Its commercial version, SBL Alfalfa is available in the form of delicious chocolate flavoured malt and has a high nutritional value conferred by about 60 natural ingredients, more than half of which are recognized as essential to life. Alfalfa is a potent energy stimulant enriched with carbohydrates vitamins (vitamin C, thiamine, niacin), minerals (Fe, Ca, Na, K,P), amino acids and potent herbs. Further, the SBL's Alfalfa malt possesses mother tinctures of *Alfalfa*, *Avena sativa* and *Ginseng*, *Cinchona afficinalis*, *Hydrastis Canadensis*, *Kalium arsenicosum*, *Kalium Phosphoricum*, *Ferrum acetium* and *Calcarea Phosphorica*. ***Alfalfa*** and ***Avena sativa*** contributes essential minerals and vitamins, whereas, ***Ginseng*** builds the much needed resistance to fight against infections[1]

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The growth and development of *B. mori* largely depends on its nutritional status[2]. Enriching the mulberry leaves with exogenous factors could provide a balanced diet for silkworm in terms of essential nutrients. In fact, such studies yielded useful gains for the sericulture industry[3-11]. A close review of literature reveals that the nutritional-enrichment studies were based largely on the application of commercial and expensive nutrients. The use of SBL Alfalfa, which is a homeo medicine-cum-nutrient has not been explored so far in this field. The present study intends to fill this gap by testing the efficacy of this homeo medicine on the growth and silk production and economic traits of ***Bombyx mori*** at its minimum effective concentration.

MATERIALS AND METHODS

The present investigation was carried out on Pure Mysore x CSR_2 hybrid strain of *Bombyx mori* reared under 28°C and 85% RH as per Krishnaswami[12]. After hatching, the worms were fed with M_5 variety of mulberry leaves at 6 AM, 10 AM, 2 PM, 6 PM and 10 PM, under normal 12 hr light and 12 hr dark conditions. After the third moult, the fourth instar larvae were

divided into five batches (one control and four experimental) of 100 worms each. While the control batch was fed normally, the experimental batches were fed with mulberry leaves dipped in 5% minimum effective concentration (MEC) of alfalfa at their 6 PM diet as recommended by Thulasi and Sivaprasad[10]. Before feeding, the alfalfa-fortified mulberry leaves were dried under cool weather conditions. The experiment was carried out on the fifth instar larvae on alternative days, i.e., on 1st, 3rd, 5th and 7th days of instar life. Since, the body weight is an index of maximal growth, the impact of alfalfa on larval and silk gland (SG) growth rates were determined by recording the mean body weight of 25 randomly selected silkworms and 5 pairs of silk glands in an electronic balance (ELICO; MODEL BL-22OH) and the same was expressed in grams. The impact of alfalfa on the growth of larval body and silk gland was determined by analyzing changes in the gland-body ratios throughout the fifth instar development, while its impact on silk production was determined by analyzing the total protein profiles in the anterior (ASG), middle (MSG) and posterior (PSG) regions of the silk gland.

The SG was isolated by mid-dorsal dissection of the larval body in the Silkworm Ringer[13] and separated into anterior (ASG), middle (MSG) and posterior (PSG) regions and their total protein levels were estimated in 1% homogenates by the method of Lowry *et al*[14]., and the same was expressed in mg protein /gram wet weight of tissue. Similarly, the protein content of cocoon was estimated in 1% homogenate in distilled water, after soaking the hard silk cocoon in diluted sodium hydroxide solution for a short while. Some important economic parameters of sericulture such as, gland- body ratio, green cocoon weight, shell weight, shell protein, floss weight, floss protein, pupal weight, raw silk weight, denier, renditta were analyzed as per the standard methods in vogue[8,9,11,15-17]. The experimental data were statistically analyzed online by using Graphpad (www.graph.pad.com/quickcalcs/indexctm) and percent change (www.percent-change.com)/index/php) packages and meaningfully interpreted by computing compound periodical growth rates (CPGR) as per Sivaprasad[18].

RESULTS AND DISCUSSION

SBL's alfalfa is a homeo-medicine. With its rich base of proteins, minerals, vitamins, carbohydrates and other nutrients, it has been recognized as an essential nutrient-cum-medicine[1]. At its MEC, alfalfa has significantly influenced larval growth, silk gland growth and silk production in *B. mori*. The findings are presented in Tables 1 to 3 and Figure 1.

Table 1: Effect of 5% alfalfa on larval body weight, silk gland (SG) weight and gland- body ratio (GBR) in Bombyx mori during fifth instar larval development

Day	Statistical tools	Control Batch			Experimental Batch (5% Alfalfa)		
		Body weight	Silk gland weight	Gland-body ratio	Body weight	Silk gland weight	Gland-body ratio
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Day-1	Mean S.D	0.56 ±0.0009	0.017 ±0.01	3.03 ±0.20	0.56 ±0.0009	0.017 ±0.01	3.03 ±0.20
Day-3	Mean S.D	1.30 ±0.0008*	0.090 ±0.0009*	6.92 ±0.01*	1.46 ±0.0008*	0.098 ±0.0005*	6.70 ±0.005*
Day-5	Mean S.D	2.18 ±0.0008*	0.179 ±0.002*	8.21 ±0.02*	2.32 ±0.04*	0.276 ±0.01*	11.89 ±0.05*
Day-7	Mean S.D	2.47 ±0.0005*	0.330 ±0.0008*	13.65 ±0.04*	2.68 ±0.02*	0.390 ±0.0005*	14.55 ±0.01*
OPC (%)		341.07	1841.17	350.49	378.57	2194.11	380.19
CPGR (%)		64.0	220.75	65.16	68.52	239.12	68.71

**** Statistically significant (P value < 0.001). **Statistically not significant***

Each value is a mean, ± standard deviation of four individual observations. The overall percent changes (OPCs) were calculated taking the control as the base value and the compound periodical growth rates (CPGRs) were computed on the basis of first and seventh day values as per Sivaprasad[18].

Alfalfa versus larval growth and gland-body ratio: The day-to-day changes in the growth rates of larval body vis-à-vis silk gland indicate positive trends under the influence of alfalfa-enriched mulberry diet (Table 1). At this concentration, this factor showed profound effect on larval body weight and silk gland weight vis-à-vis the gland-body ratio (GBR). Under natural conditions, the larval weight recorded an overall percent change (OPC) of +341% and a compound periodical growth rate (CPGR) of ~64% during fifth instar development (Col.3). Simultaneously, the silk gland (SG) grew maximally by an OPC of ~1841% and a CPGR of ~220.75% (Col. 4). With simultaneous growth in both body and silk gland, the GBR showed an OPC of +350% and a CPGR of ~65.16% (Col.5). Under the influence of 5% alfalfa, the body weight showed an OPC

of +378.57% and a CPGR of ~68.52% (Col. 6), while the SG weight grew by an OPC of 2194% and a CPGR of 239.12% (Col.7). Concomitant with gland growth, the gland- body ratio recorded an OPC of +380.19% and a CPGR of ~68.71% (Col. 8). Thus, alfalfa accelerates larval growth rate significantly during fifth instar development, probably through the phago-stimulant activity of vitamin C, the chief ingredient of alfalfa[19].

The gland- body ratio (GBR) is an important measurable parameter of silk production in *Bombyx mori*[16]. The present study demonstrates that alfalfa, at its minimum effective concentration could be used as a powerful modulator of GBR in silkworm. It has been widely demonstrated that higher growth rates in GBR could be achieved by enriching mulberry leaves with exogenous modulators and nutrients[10,11,17]. The analysis of present data in terms of CPGR and overall percent change (OPC) reflect positive gains in the GBR. Under the influence of 5% alfalfa, the CPGR grew by 4.52 additional percentile points (68.52 – 64.00%) in the body weight, 18.37 percentile points (239.12-220.75%) in SG weight and by 3.55 percentile points (68.71-65.16%) in the GBR (Table 1). Though, the present findings are not supported by previous studies, the impact of alfalfa on larval growth is attributable to its multiple vitamins such as ascorbic acid, thiamine, niacin and minerals such as Ca ,P, and Zn[5-7,9,20].

Alfalfa versus silk gland proteins: Under the influence of alfalfa, the protein levels recorded positive impact in all the three regions (ASG, MSG and PSG) of the silk gland (Table 2). The analysis of their growth trends of control and experimental batches, in terms of OPC indicate that their protein levels were elevated respectively by 33.79% and 210.95% in ASG (Cols. 3 and 4), ~220.73% and 272.63% in MSG (Cols 5 and 6) and by 150.63% and 179.68% in PSG (Cols 7 and 8). During the entire period of fifth instar, the respective CPGRs for total proteins the control and experimental batches were 10.19% and 45.96% in ASG, 47.47% and 55.03% in MSG and 35.84% and 40.89% in PSG (Table 2).

Table 2: Effect of 5% alfalfa on the total protein levels profiles of anterior (ASG), middle (MSG) and posterior (PSG) regions of the silk gland in the fifth instar larva of Bombyx mori

Day	Statistical tools	ASG		MSG		PSG	
		Control	5%Alfalfa	Control	5%Alfalfa	Control	5%Alfalfa
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Day-1	Mean S.D	10.77 ±0.13	10.77 ±0.13	19.48 ±0.30	19.48 ±0.30	17.97 ±0.70	17.97 ±0.70
Day-3	Mean S.D	12.12 ±0.37*	13.56 ±0.44*	28.79 ±0.51*	33.49 0.51*	24.63 1.15*	29.37 1.27*
Day-5	Mean S.D	12.83 ±0.13*	29.37 ±1.27*	42.83 ±1.72*	64.27 ±1.02*	35.88 ±0.86*	43.13 ±1.01*
Day-7	Mean S.D	14.41 ±0.21*	33.49 ±0.51*	62.48 ±0.40*	72.59 ±0.68*	45.04 ±0.62*	50.26 ±1.66*
OPC (%)		33.79	210.95	220.73	272.63	150.63	179.68
CPGR (%)		10.19	45.96	47.47	55.03	35.84	40.89

** Statistically significant (P value < 0.001). **Statistically not significant.*

Each value is a mean, ± standard deviation of four individual observations. The overall percent changes(OPCs) were calculated taking the control as the base value and the compound periodical growth rates (CPGR) were computed on the basis of first and seventh day values as per Sivaprasad[18].

The silk gland is the major site of silk proteins synthesis. Apart from two silk proteins (fibroin and sericin), it synthesizes and stores 91 other proteins involved in metabolism, immunity, heat-shock mechanism, cytoskeleton formation, protease inhibition, transport and transcription[21-26]. Under the impact of 5% alfalfa, the silk gland protein levels were reinforced in significant proportions in the three segments of the silk gland during fifth instar development (Table 2). Within the silk gland, the ASG represents a relatively inert region, while the MSG and PSG act as the chief protein repositories (Shimura, 1993). In terms of OPC, the protein levels were enhanced maximally by 177.16 percentile points (210.95-33.79%) in ASG, moderately by 51.9 percentile points (272.63 - 220.73%) in MSG and minimally by 29.05 percentile points (179.68 - 150.63%) in PSG (Table 2). Similarly, the analysis of their trends in terms of CPGR has shown that 5% alfalfa caused an elevation in synthesis by 35.77 percentile points (45.96 - 10.91) in ASG, 7.56 percentile points (55.03% - 47.47%) in MSG and by 5.05 percentile points (40.89 - 35.84%) in PSG. Obviously, the homeo medicine activates the ASG and stimulates silk protein synthesis more predominantly in this region compared to the other two regions (MSG and PSG). The positive impact of alfalfa on the protein content of silk gland is attributable to its mineral and vitamin deposits[27-29].

Alfalfa versus economic parameters: The positive impact of 5% alfalfa on larval growth and tissue proteins has been extended to the economic parameters of sericulture such as the cocoon weight,

shell weight, shell protein, floss weight, floss protein, pupal weight, raw silk weight, denier and renditta (Table 3). Under the influence of 5% alfalfa the green cocoon weight grew by ~31%, shell weight by ~8%, floss weight by ~6%, pupal weight by ~10%, shell protein by ~16%, floss protein by ~2% raw silk weight by ~49%, denier by ~13% and renditta by ~ -3% (Table 3).

Table 3: Effect of 5% alfalfa on the economic traits of Bombyx mori

Sl. No	Economic parameter	Statistical tool	Control	5% Alfalfa
1.	Green cocoon weight (g)	Mean S.D	0.83 ±0.008	1.09 (31.32) ±0.007*
2.	Shell weight (g)	Mean S.D	0.13 ±0.0009	0.14 (7.69) ±0.002*
3.	Shell protein (mg/g)	Mean S.D	29.03 ±0.05	33.60 (15.74) ±0.02*
4.	Floss Weight (g)	Mean S.D	0.018 ±0.0005	0.019 (5.55) ±0.0008**
5.	Floss protein (mg/g)	Mean S.D	8.27 ±0.01	8.43 (1.93) ±0.01*
6.	Pupal weight (g)	Mean S.D	0.70 ±0.001	0.77 (10.22) ±0.009*
7.	Raw silk weight (g)	Mean S.D	9.22 ±0.001	13.77 (49.34) ±0.001*
8.	Denier	Mean S.D	11.63 ±0.01	13.15 (13.06) ±0.10*
9.	Renditta	Mean S.D	7.11 ±0.01	6.91 (-2.81) ±0.008*

Statistically significant (P value < 0.001) **Statistically not significant. Each value is a mean ± standard deviation of four individual observations. The weights of the cocoon, shell and floss represent the mean of 25 individual cocoons. The values in parentheses represent the percent changes from the control value.

The impact of alfalfa on the economic parameters of sericulture is generally positive and it is more pronounced at its minimum effective concentration. The positive impact of alfalfa on larval growth observed during the fifth instar has been extended to the quiescent pupal stage with ~10% elevation (Table.3). Its effectiveness is attributable to its stimulatory role on the larval growth and silk protein synthesis in the silk gland. Similarly its elevatory effects on silk protein synthesis in ASG, MSG and PSG (Table 2) have contributed to the rise of green cocoon weight by ~31% and shell weight by ~8%. The homeo medicine also caused an increase ~49% in raw silk weight and ~13% in denier, but significantly lowered the renditta value (~3%) of the silk that reflects its positive effect on silk production. Surprisingly, the alfalfa has caused elevations in floss weight by ~6% and floss protein weight by ~2% and thus enhances the sericultural wastage in addition to its contribution to silk output (Fig.1). Nevertheless, its contribution to raw silk output, the renditta and denier values of the silk is immense. The rise in the levels of raw silk weight and denier value, coupled with decline in the renditta attracts the attention of sericultural scientists. The possibility of using this exogenous factor in the sericultural diet may be explored after conducting appropriate field studies.

Figure 1: The raw silk spun from 100 green cocoons produced by Bombyx mori in the control and alfalfa treated conditions. Note significant increase in the output of raw silk under treatment conditions

CONCLUSION

The homeo product alfalfa has shown promising gains for sericulture at its minimum effective concentration (MEC) of 5% in distilled water. It significantly influences not only the larval growth, but also the silk production in *Bombyx mori*. It has been identified as potent stimulator of protein synthesis, more particularly that

of fibroin (silk) and sericin (floss). Despite its contribution to floss, the sericultural wastage, it could be recommended as a supplemental diet for silkworm due to its gainful impact on silk protein synthesis.

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