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Aabid Khaliq Tantray
Govt Degree College Sopore, J&K, India

Correspondence
Aabid Khaliq Tantray
Govt Degree College Sopore, J&K, India

Influence of single booster dose of botanical based and synthetic antiscorbutic factor on larval growth and silk gland tissue somatic index of the silkworm, *Bombyx mori* L

Aabid Khaliq Tantray

Abstract
Two commercial silkworm hybrids viz., CSR2 x CSR4 and PM x CSR2 were used for the larval growth pattern and silk gland tissue somatic index study after dietary supplementation of botanical based and synthetic ascorbic acid. Marked influence on the larval growth rate pattern and silk gland tissue somatic index was observed by supplementing the newly ecdysed 5th instar larvae with a single booster dose of both the ascorbic acid sources. The treatments induced significant change in 5th instar silk gland weight than in larval weight. The 5th instar larval weight increased only to the tone of 4.7- 9.86% in both the hybrids (CSR2 x CSR4 and PM x CSR2) whereas, the daily silk gland weight and tissue somatic indices of the hybrids increased significantly from 19.74 to 23.96% and 9.59 to 12.75% respectively, giving an impression that the ascorbic acid exerts its impact more on silk gland than the whole larval body.

Keywords: Growth pattern, tissue somatic indices, *Bombyx mori*

1. Introduction
An active development and growth of the silkworm *Bombyx more* L., like that of other insects, from one generation to another, is a continuous process for its survival. Its developmental and biological phases extend from the egg-hatch to the adult emergence, where afterwards, the productive phase follows. Various changes, in this phase, are generally expressed in the form of food consumption, coefficient of food utilization, larval body weight, larval body length, larval duration, moth eclosion, mating duration and many other related characters. A better growth-index of silkworm is not only important for a better larval rearing, but also for a healthy and good cocoon harvest. This requires an unabated and continuous supply of the various nutrients, including the antiscorbutic factor called vitamin C (VC) or ascorbic acid, necessary to keep it in a proper physiological condition. Any short-fall in their supply is likely to put further growth into despair. Larval stage is the only period wherein food elements are transformed into body elements [5]. Although the mulberry leaves are complete diet for silkworm [16], it is possible that some deficiencies occur for different reasons. In tropics, the quality of mulberry leaf with respect to soluble sugars, proteins, vitamins, sterols etc., gets affected mainly due to high temperature, inadequate agronomical inputs to mulberry garden and low rainfall. Further, the quality of mulberry leaf under rainfed conditions is far more inferior. Thus, the hypothesis that develops from the facts laid down above is that, there should exist a strong relationship between various nutritional packages and silk production.
A galore of researchers have worked out that nutritional status of the mulberry leaves could be improved by enriching them with different nutrients. Etebari *et al*., (2004) [5] have reported on different aspects of mulberry leaf supplementation with various nutritional compounds in sericulture. Fortification of mulberry leaves for enhancing the economic traits of *B. mori* with different compounds like minerals [7, 11, 10], amino acids and proteins [16, 9, 8] and sugars [16]. There are fewer reports available regarding the effect of ascorbate on growth pattern and almost no report on the silk gland-tissue somatic index of the silkworm and therefore, the present study was conducted to evaluate the comparative effects of the botanical based and synthetic ascorbic acid on growth rate pattern and silk gland tissue somatic index of the silkworm, *Bombyx mori* L.

2. Materials and Methods

The silkworm crop was raised from the basic stock maintained at Silkworm Physiology and Product Diversification Research Centre of C.S.R. & T.I. Mysore and rearing was conducted as per the standard method [12]. Two commercial silkworm hybrids viz., CSR2 x CSR4 and PM x CSR2 were used for the Growth Pattern and Silk Gland Tissue Somatic Index study. In this bioassay only the doses which were identified as the best doses during the large-scale bioassays of crude and synthetic vitamin C (VC) were used [1 2]. Accordingly, 0.05% of crude Amla-based VC (C), 0.50% of purified (P) and synthetic VC (S) were prepared in water and used for CSR2, CSR4 and CSR2 x CSR4 hybrid. Similarly, for PM x CSR2 hybrid, 0.30% VC in crude extract (C), 3.00% of synthetic (S) and purified (P) were used.In case of purified VC, the dose equivalent to synthetic VC was prepared. All the treatments were given only once at 0h of 5th instar since this time of application resulted in significant improvement in economic traits of silkworm during large-scale bioassay [1]. The treatments were sprayed to mulberry leaves @ 60 ml/ 200g for 100 larvae and the latter were kept under shade for 15 minutes to remove the excess moisture and fed *ad libitum* to 5th instar larvae. Treatments were preferably given with night feeding among the three feedings per day to ensure the proper ingestion of the leaves.

The growth rate pattern of silkworm hybrids as influenced by treatment of VC-quantified crude plant extract (C), purified VC (P) and synthetic VC (S) at effective dose and application time was studied. Five male and five female larvae were weighed daily. Larvae were sex wise dissected and silk glands separated. Water was drained out using tissue paper and the weight of paired silk gland was recorded. The procedure was continued till the onset of spinning activity. Apart from recording the growth rate pattern of larvae and silk gland, the silk gland tissue somatic index (SGTSI) was determined in respect of each treatment and control using the following formula [13].

$$\text{SGTSI (\%)} = \frac{\text{Weight of silk gland (g)}}{\text{Weight of larva (g)}} \times 100$$

The experiments were repeated thrice and the data statistically analyzed by ANOVA through Statistical Package for Social Science, SPSS 7.5 for Windows [3].

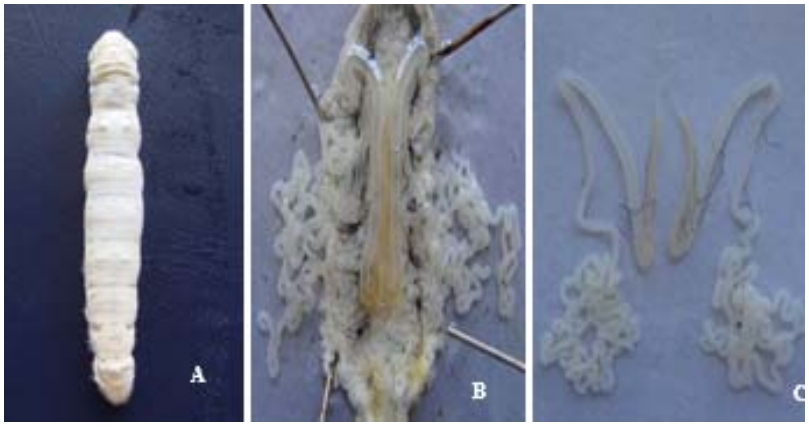

Plate 1: Process of dissection for separation of silk gland (A-C)

3. Results and Discussion

3.1 Daily larval weight gain

Tables, 1-2 summarize the data on the effect of vitamin C-quantified crude plant extract (C), purified vitamin C (P) and synthetic vitamin C (S) treatment on daily increase in 5th instar larval weight of different silkworm hybrids. In bivoltine hybrid, CSR2 x CSR4 increment in body weight of 5.42, 5.55 and 4.7% was observed with C, P and S treatments respectively as compared to control (Table 4), whereas, in multivoltine x bivoltine hybrid, PM x CSR2, same treatments were capable of bringing out the improvement to the extent of 9.86,7.69 and 7.74% over the control (Table 5).

3.2 Daily silk gland weight gain

In bivoltine hybrid, CSR2 x CSR4, respective treatments were capable of bringing out the increase in silk gland weight to the extent 9.94, 10.06 and 9.99 times as compared to that of the control (8.11 times). Improvement over the control was to the tone of 22.53, 23.96 and 23.22% respectively (Table 3). Respective treatment in PM x CSR2, posed 13.15,13.21 and 13.19 times increase in silk gland weight compared to that of the control (10.98 times) with the percentage improvement of 19.74, 20.33 and 20.11% (Table 4).

3.3 Silk gland tissue somatic index (SGTSI)

SGTSI of the treated and control silkworm has been summarized in Tables, 5 and 6. Generally in all hybrids silk gland weight was more in females than males and the SGTSI had an opposite pattern, more in males than females. In treated groups (C, P and S) of CSR2 x CSR4 hybrid, an average increase in SGTSI was to the extent of 12.53, 12.75 and 12.73% as compared to control (Table 5). PM x CSR2 hybrid treated with C, P and Shad increase in SGTSI to the tone of 10.37, 9.59 and 9.90% over the control (Table 6).

In the present study it was interesting to note that three different forms of VC *viz.,* VC-quantified crude plant extract, (C), purified VC, (P) and synthetic VC (S) in their respective doses were equally effective thereby exerting almost similar influence on larval and silk gland weights. It was observed that the treatments induced prominent change in 5th instar silk gland weight than in larval weight. The 5th instar larval weight increased only to the tone of 4.7- 9.86% in both the hybrids (CSR2 x CSR4 and PM x CSR2) whereas increase in silk gland weight, 13.58 to 17.22% and 19.74- 23.96% increase was observed respectively (Table 4 & 5). The study gives an impression that VC exerts its impact mainly on silk gland than whole larval body. The VC is believed to have played its role on the activation of protein synthesis (Walingo, 2005) in silk gland. There is no report available with regard to the impact

of VC on silkworm silk glands. However, a lot of work has been done in higher animals. Sodek *et al*., (1982) reported that VC stimulated the protein synthesis and improved their hydroxylation in adult mouse periodontal tissues. Supplementation of mulberry leaves with vitamin B_{12} which is not present in the leaves could increase the synthesis of nucleic acid and proteins in the silk gland of silkworm, *B. mori* (Das and Medda, 1988).

Besides silk gland weight, daily silk gland tissue somatic index was recorded in order to have a clear idea of the present supplementation. As discussed above, the silk gland was bigger in female than males in all the three sources of VC (C, P and S). But the opposite trend was observed in case of tissue somatic index. Male had more tissue somatic index than the females in both hybrids. There is no report available regarding the effect of VC on silk gland-tissue somatic index. It has been observed that silk gland as an organ grows at a much faster pace than the general body does (Reddy and Bechamin, 1989; Reddy *et al*., 1995 & 1996). Fayard (1978) reported that there is only a low correlation between silk production and silk gland-tissue somatic index. It can therefore be surmised that activity of the silk gland is determined primarily by its own characteristic and remains largely independent of other organs. This indicates that the increased larval weight is not always necessarily prerequisite for the increased silk production.

Table 1: Daily record of larval weight (g) of silkworm hybrid, CSR2 x CSR4, treated with vitamin C-quantified crude plant extract, purified vitamin C and synthetic vitamin C

5th instar daily larval weight (mg/larva)							
	0	**1**	**2**	**3**	**4**	**5**	6
Treat. C ♂♂	1.199	2.318*	2.613*	3.019*	3.131*	4.044*	3.985*
Treat. P ♂♂	1.202	2.334*	2.616*	2.991*	3.212*	4.109*	3.974*
Treat. S ♂♂	1.202	2.338*	2.622*	3.017*	3.183*	4.047*	3.964*
Control ♂♂	1.201	2.180	2.335	2.807	3.020	3.742	3.875
Treat. C ♀♀	1.340	3.304*	4.054*	4.414*	4.595*	5.142*	5.107*
Treat. P ♀♀	1.344	3.342*	4.183*	4.466*	4.591*	5.137*	5.011*
Treat. S ♀♀	1.339	3.333*	4.128*	4.450*	4.603*	5.104*	5.023*
Control ♀♀	1.324	3.199	3.971	4.103	4.303	4.497	4.841
Avg. treat. C	1.270	2.811*	3.334*	3.717*	3.863*	4.593*	4.546*
Avg. treat. P	1.273	2.838*	3.399*	3.728*	3.901*	4.623*	4.493*
Avg. treat. S	1.271	2.836*	3.375*	3.733*	3.893*	4.575*	4.494*
Avg. Control	1.262	2.690	3.153	3.455	3.661	4.120	4.181
SE±	0.001	0.002	0.002	0.003	0.002	0.002	0.002
CD 5%	0.003	0.006	0.007	0.008	0.007	0.007	0.007

C: crude vitamin C, **P**: purified vitamin C, **S**: synthetic vitamin C and ***** significant at 5%

Table 2: Daily record of larval weight (g) of silkworm hybrid, PM x CSR2 treated with vitamin C-quantified crude plant extract, purified vitamin C and synthetic vitamin C

5th instar daily larval weight (mg/larva)							
	0	**1**	**2**	**3**	**4**	**5**	6
Treat. C ♂♂	0.978	1.674*	2.260*	2.852*	2.955*	3.952*	3.466*
Treat. P ♂♂	0.980	1.682*	2.294*	2.797*	3.020*	3.952*	3.423*
Treat. S ♂♂	0.982	1.685*	2.287*	2.837*	2.999*	3.895*	3.454*
Control ♂♂	0.979	1.596	2.048	2.588	2.774	3.439	3.144
Treat. C ♀♀	1.002	2.561*	3.279*	3.673*	4.355*	5.010*	4.847*
Treat. P ♀♀	1.019	2.581*	3.370*	3.660*	4.332*	4.916*	4.832*
Treat. S ♀♀	1.009	2.582*	3.336*	3.678*	4.354*	4.942*	4.827*
Control ♀♀	1.009	2.482	3.200	3.381	4.084	4.750	4.575
Avg. treat. C	0.990	2.118*	2.769*	3.263*	3.655*	4.481*	4.156*
Avg. treat. P	0.999	2.132*	2.832*	3.229*	3.676*	4.434*	4.128*
Avg. treat. S	0.995	2.133*	2.811*	3.257*	3.677*	4.419*	4.141*
Avg. Control	0.994	2.039	2.624	2.985	3.429	4.095	3.859
SE±	0.001	0.002	0.002	0.002	0.002	0.002	0.002
CD 5%	0.003	0.006	0.007	0.007	0.007	0.007	0.007

C: crude vitamin C, **P**: purified vitamin C, **S**: synthetic vitamin C and ***** significant at 5%

Table 3: Daily record of silk gland weight (g) of silkworm hybrid, CSR2 x CSR4, treated with vitamin C-quantified crude plant extract, purified vitamin C and synthetic vitamin C

5th instar daily silk gland weight (g/larva)							
	0	**1**	**2**	**3**	**4**	**5**	**6**
Treat. C ♂♂	0.1230	0.4626*	0.6799*	0.9218*	1.0043*	1.4322*	1.5890*
Treat. P ♂♂	0.1209	0.4633*	0.6799*	0.9216*	1.0143*	1.4319*	1.5988*
Treat. S ♂♂	0.1218	0.4629*	0.6800*	0.9682*	1.0243*	1.4322*	1.5976*
Control ♂♂	0.1232	0.3956	0.5787	0.8284	0.9306	1.129	1.231
Treat. C ♀♀	0.2068	0.6600*	0.8919*	1.1327*	1.2473*	1.4590*	1.6891*
Treat. P ♀♀	0.2061	0.6558*	0.8940*	1.1328*	1.2474*	1.4590*	1.6892*
Treat. S ♀♀	0.2070	0.6410*	0.8890*	1.1310*	1.2473*	1.4590*	1.6892*
Control ♀♀	0.2099	0.5860	0.7890	1.0024	1.1351	1.230	1.471
Avg. treat. C	0.1649	0.5613*	0.7859*	1.0272*	1.1258*	1.4456*	1.6390*

Avg. treat. P	0.1635	0.5595*	0.7869*	1.0272*	1.1309*	1.4454*	1.6440*
Avg. treat. S	0.1644	0.5520*	0.7845*	1.0496*	1.1358*	1.4456*	1.6434*
Avg. Control	0.1665	0.4908	0.6839	0.9154	1.0328	1.1796	1.3507
SE±	0.0017	0.0016	0.0022	0.0017	0.0016	0.0017	0.0017
CD 5%	0.0050	0.0047	0.0066	0.0050	0.0048	0.0050	0.0049

C: crude vitamin C, **P**: purified vitamin C, **S**: synthetic vitamin C and ***** significant at 5%

Table 4: Daily record of silk gland weight (g) of silkworm hybrid, PM x CSR2, treated with vitamin C-quantified crude plant extract, purified vitamin C and synthetic vitamin C

5th instar daily silk gland weight (g/larva)							
	0	**1**	**2**	**3**	**4**	**5**	**6**
Treat. C ♂♂	0.080	0.2765*	0.4405*	0.6367*	0.7837*	1.1646*	1.3544*
Treat. P ♂♂	0.079	0.2771*	0.4424*	0.6360*	0.7889*	1.1604*	1.3543*
Treat. S ♂♂	0.080	0.2762*	0.4409*	0.6590*	0.7933*	1.1641*	1.3577*
Control ♂♂	0.080	0.239	0.385	0.566	0.717	0.963	1.104
Treat. C ♀♀	0.130	0.3836*	0.5547*	0.7505*	0.9836*	1.2285*	1.4127*
Treat. P ♀♀	0.130	0.3816*	0.5578*	0.7499*	0.9817*	1.2300*	1.4079*
Treat. S ♀♀	0.130	0.3736*	0.5537*	0.7488*	0.9811*	1.2305*	1.4118*
Control ♀♀	0.132	0.342	0.498	0.662	0.885	1.064	1.224
Avg. treat. C	0.105	0.3300*	0.4976*	0.6936*	0.8837*	1.1965*	1.3836*
Avg. treat. P	0.105	0.3293*	0.5001*	0.6929*	0.8853*	1.1952*	1.3811*
Avg. treat. S	0.105	0.3249*	0.4973*	0.7039*	0.8872*	1.1973*	1.3847*
Avg. Control	0.106	0.290	0.441	0.614	0.801	1.014	1.164
SE±	0.0015	0.0017	0.0024	0.0018	0.0018	0.0018	0.0018
CD 5%	0.0043	0.0048	0.0070	0.0051	0.0052	0.0051	0.0052

C: crude vitamin C, **P**: purified vitamin C, **S**: synthetic vitamin C and ***** significant at 5%

Table 5: Daily record of silk gland tissue somatic index (%) of silkworm hybrid, CSR2 x CSR4, treated with vitamin C-quantified crude plant extract, purified vitamin C and synthetic vitamin C

5th instar daily silk gland tissue somatic index (%)							
	0	**1**	**2**	**3**	**4**	**5**	**6**
Treat. C ♂♂	10.255	19.953*	26.017*	30.530*	32.079*	35.412*	40.721*
Treat. P ♂♂	10.062	19.845*	25.990*	30.810*	31.582*	34.844*	40.230*
Treat. S ♂♂	10.132	19.796*	25.930*	32.090*	32.178*	35.389*	40.300*
Control ♂♂	9.987	18.144	24.785	29.513	30.815	30.161	35.172
Treat. C ♀♀	15.433	19.975*	21.998*	25.662*	27.144*	28.376*	33.075*
Treat. P ♀♀	15.333	19.620*	21.374*	25.368*	27.171*	28.401*	33.710*
Treat. S ♀♀	15.467	19.231*	21.534*	25.418*	27.097	28.588*	33.628*
Control ♀♀	15.748	18.316	19.870	24.432	26.380	27.361	30.406
Avg. treat. C	12.844	19.964	24.008	28.096	29.611	31.894	36.898
Avg. treat. P	12.698	19.733	23.682	28.089	29.376	31.622	36.970
Avg. treat. S	12.799	19.514	23.732	28.754	29.638	31.988	36.964
Avg. Control	12.868	18.230	22.327	26.972	28.597	28.761	32.789
SE±	0.121	0.048	0.028	0.023	0.026	0.022	0.020
CD 5%	0.352	0.139	0.082	0.068	0.077	0.064	0.057

C: crude vitamin C, **P**: purified vitamin C, **S**: synthetic vitamin C and ***** significant at 5%

Table 6: Daily record of silk gland tissue somatic index (%) of silkworm hybrid, PM x CSR2, treated with vitamin C-quantified crude plant extract, purified vitamin C and synthetic vitamin C

5th instar daily silk gland tissue somatic index (%)							
	0	**1**	**2**	**3**	**4**	**5**	**6**
Treat. C ♂♂	7.721	16.519*	21.465*	27.070*	26.525*	32.340*	39.180
Treat. P ♂♂	7.611	16.474*	21.513*	27.147*	26.321*	31.935*	38.704
Treat. S ♂♂	7.649	16.399*	21.417*	28.012*	26.599*	32.308*	38.796
Control ♂♂	7.567	14.948	20.732	25.879	25.279	28.391	35.132
Treat. C ♀♀	11.724	15.385*	17.597*	20.430*	25.518*	24.519*	29.146*
Treat. P ♀♀	11.662	15.191*	17.328*	20.491*	25.505*	24.768*	29.140*
Treat. S ♀♀	11.674	14.896	17.351	20.361	25.455	24.727	29.245
Control ♀♀	11.879	14.325	16.055	19.564	24.607	23.570	26.778
Avg. treat. C	9.723	15.952*	19.531*	23.750*	26.022*	28.429*	34.163*
Avg. treat. P	9.636	15.833*	19.420*	23.819*	25.913*	28.351*	33.922*
Avg. treat. S	9.662	15.647*	19.384*	24.186*	26.027*	28.518*	34.020*
Avg. Control	9.723	14.637	18.393	22.722	24.943	25.980	30.955
SE±	0.1278	0.0669	0.0784	0.0392	0.0330	0.0266	0.1373
CD 5%	0.3729	0.1952	0.2287	0.1143	0.0964	0.0778	0.4008

C: crude vitamin C, **P**: purified vitamin C, **S**: synthetic vitamin C and ***** significant at 5%

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