International Journal of Biology Research
ISSN: 2455-6548, Impact Factor: RJIF 5.22
www.biologyjournal.in
Volume 1; Issue 1; March 2016; Page No. 67-70

Source-Oriented differential impact of ascorbate fortification on popular *Bombyx Mori* hybrids

Aabid Khaliq Tantray

Govt Degree College Sopore, Jammu and K, India

Abstract
Comparative bioassay of ascorbate in three different sources viz., synthetic, ascorbate in crude amla extract and ascorbate purified from the amla fruit, were conducted on commercial traits of two popular silkworm hybrids viz., CSR2 x CSR4 and PM x CSR2. It was interesting to note that ascorbate-quantified crude amla extract induced significant improvement in economic traits at 10 times lower ascorbate doses (0.05% & 0.3%) as compared to synthetic ascorbate (0.5% & 3.0%) in both the hybrids. It is surmised that the improvement in silkworm at considerably lower doses (0.05% & 0.3%) can possibly be due to synergetic activity of ascorbate with other compounds in the vitamin C-complex that increase its bioavailability and efficacy. Further, no difference was observed in the efficacies exhibited by purified ascorbate from that of synthetic ascorbate.

Keywords: Amla-based ascorbate, silkworm, vitamin C

1. Introduction

The silkworm, *Bombyx mori* has been classified among the insects which are unable to synthesize ascorbate in their body and depend on exogenous supply to fulfill the requirement (Ito and Arai 1965) [1]. Neither D-glucunolactone nor D-gulonolactone replaced the ascorbate which thus supported the evolutionary theory on the absence of biosynthetic pathway of ascorbate in the silkworm, *Bombyx mori* (Ito, 1978) [2]. Several researchers have demonstrated phagostimulatory effect of ascorbate (Ito, 1961a, 1961b, 1978, Ito & Arai, 1965 and Dobzhenok, 1974) [1]. During the first instar, a stimulatory effect of ascorbate on silkworm voluntary feeding was postulated by Ito (1961) [3]. In silkworm a gustatory stimulating activity have been observed to some extent (Ito, 1961) [3]. Ascorbate has always been regarded as indispensable for the growth and development of the silkworm, *Bombyx mori* and its deprivation in the diet affected larval growth and cocoon production (Ito, 1961 & Cappellozza *et al*., 2005) [3]. Ascorbate usually has been added to silkworm food (enrichment) in a quantity generally varying from 1–2% of the dry weight of the artificial diet, which is considered as optimum content of this vitamin (Ito, 1978) [2]. In view of the conditions which affect the ascorbate content of mulberry leaf, inability of silkworm to synthesize it *de novo*, it becomes inevitable to fortify the mulberry leaves with ascorbate for normal growth and development, enhancement in qualitative and quantitative production of silk.

Emblica officinalis or amla is known since ancient times for its medicinal value and is commonly used in Ayurvedic medicine. It is also believed to be a rich source of ascorbate and is being considered as a good replacement for ascorbate. Native to India, this plum sized fruit is honored for thousands of years for its tonifying, anti-aging and immune enhancing properties. The natural ascorbate present in the fruit is synergistically enhanced by the bioflavonoids and polyphenols and protected by shield of tannins from being destroyed by heat (Bajaj, 2006 & Anonymous, 2001) [8, 9]. During the large scale bioassay, the Amla fruit was short listed as the potential botanical, and the effective dose and time of application for both synthetic and Amla-based ascorbate were determined (Tantray & Trivedy, 2008, 2011 and Tantray *et al*., 2009) [13]. Further, it was observed that the properties of ascorbate purified from the crude botanical extract were similar to synthetic ascorbate in respect of molecular weight as detected and proved through TLC and HPLC. Therefore, a study was conducted to evaluate the comparative effects of ascorbate-quantified crude plant extract, purified ascorbate and synthetic ascorbate on commercial traits of mulberry silkworm hybrids.

2. Materials and Methods

Two popular silkworm hybrids, CSR2 x CSR4 and PM x CSR2 were used for the comparative study. In this bioassay only the doses which were identified as the best doses during the large-scale bioassays of crude and synthetic ascorbate were used. In case of purified ascorbate (P), the dose equivalent to synthetic ascorbate (S) ascorbate i.e., 0.5% was prepared in water. While, in case of crude Amla extract a dose equal to 0.05% of ascorbate was prepared in water and used for CSR2 x CSR4 hybrid. Similarly, for PM and PM x CSR2 hybrid, 0.30% ascorbate in crude extract (C), 3.00% of synthetic (S) and purified (P) were used. All the treatments were given only once at 0h of 5^{th} instar since this time of application resulted in significant improvement in economic traits of silkworm during large-scale bioassay. 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 5^{th} instar larvae. Treatments were preferably given with night feeding among the three feedings per day to ensure the proper ingestion of the leaves. The larval weight was recorded on maximum growth on last day of the 5^{th} instar. When the larvae were ready for spinning, they were picked individually and mounted on plastic collapsible mountage. When about 75-80% worms were mounted, the remaining worms were also mounted at once. On 6^{th} day of mounting, the cocoons were harvested. Data with regard to larval weight, pupation

rate, single cocoon weight, single cocoon shell weight, cocoon shell percentage filament length, non-breakable filament length, denier, reelability, renditta, raw silk percentage, raw silk recovery and neatness were collected. The experiments were repeated thrice and the data statistically analyzed by ANOVA through Statistical Package for Social Science, SPSS 7.5 for Windows (Berkowitz and Allaway, 1998) [11].

3. Results and Discussion

3.1 Effect on CSR2 x CSR4

Table 01 embodies the data on the comparative effect of crude extract containing ascorbate (C), purified ascorbate (P) and synthetic ascorbate (S), on larval and cocoon traits of a bivoltine silkworm hybrid, CSR2 x CSR4. It was found that all the three sources of ascorbate at their respective doses were capable of inducing significant improvement in larval weight (12.83, 9.47 & 11.64%), pupation rate (5.91, 4.04 & 2.90%), cocoon weight (14.33, 13.66 & 13.11%) and cocoon shell weight (16.24, 14.87 & 14.47 as compared to control. No improvement was recorded in cocoon shell % in any of the three treatments. It could be noted from the Table 01 that crude extract of Amla containing ascorbate elicited the improvement at 10 times lower concentration compared to purified and synthetic ascorbate. However, purified and synthetic ascorbates were effective at same dosage but 10 times lesser than crude extract.

The data on the effect of the above mentioned sources of ascorbate on reeling traits of CSR2 x CSR4 silkworm hybrid are summarized in Table 02. Improvement in the cocoon traits by the three forms of ascorbate (C, P & S) was pertinently reflected in the significant improvement in reeling traits *viz*., filament length (14.50, 13.54 & 15.12%), non-breakable filament length (15.17, 13.95 & 14.66%), reelability (10.50, 9.00 & 11.00%), renditta (10.91, 8.74 & 10.48%), raw silk % (10.50, 13.22 & 14.12%) and raw silk recovery (11.50, 13.50 & 14.71%) respectively compared to control. Neatness was only improved with purified and synthetic ascorbate by 2.50 and 2.45% respectively. None of the forms of ascorbate could exert any change in the denier.

Table 1: Comparative effect of ascorbate-quantified crude plant extract, purified ascorbate and synthetic ascorbate on larval and cocoon traits of silkworm hybrid, CSR2 x CSR4.

Treat.	*L.wt. (g)*	*P.R. (%)*	*C.wt. (g)*	*S.wt. (g)*	*Shell %*
0.05% –C	50.996*	91.64*	2.108*	0.486*	23.06
0.50% –P	49.474*	90.03*	2.095*	0.480*	22.92
0.50% –S	50.457*	89.04*	2.085*	0.479*	22.96
Control	45.195	86.53	1.843	0.418	22.68
SE±	*0.565*	*0.667*	*0.007*	*0.004*	*0.161*
CD 5%	*1.842*	*2.175*	*0.024*	*0.014*	*0.526*

C: crude ascorbate, P: purified ascorbate and S: synthetic ascorbate

Table 2: Comparative effect of ascorbate-quantified crude plant extract, purified ascorbate and synthetic ascorbate on reeling traits of silkworm hybrid, CSR2 x CSR4.

Treat.	F.L (m)	N.B.F.L (m)	Den.	Reel. (%)	Ren. (kg)	R.S. (%)	R.S.R. (%)	Neat. (p)
0.30% − C	1150.39*	1002.95*	2.792	82.28*	5.356*	18.29*	83.57*	96.00
3.00% −P	1140.74*	992.39*	2.726	81.16*	5.487*	18.74*	85.07*	96.94*
3.00% − S	1156.65*	998.50*	2.753	82.65*	5.382*	18.89*	85.98*	96.89*
Control	1004.71	870.87	2.757	74.46	6.012	16.55	74.95	94.58
SE±	*4.859*	*9.740*	*0.039*	*0.523*	*0.137*	*0.565*	*0.613*	*0.514*
CD 5%	*15.845*	*31.764*	*0.126*	*1.706*	*0.448*	*1.841*	*1.999*	*1.675*

C: crude ascorbate, P: purified ascorbate and S: synthetic ascorbate. F.L: Filament length, N.B.F.L: Non-breakable filament length, Den.: Denier, Reel. Reelability, Ren.: Renditta, R.S.: Raw silk, R.S.R: Raw silk recovery and Neat.: Neatness.

3.2 Effect on PM x CSR2

Table 03 presents the data on comparative performance of three sources of ascorbate (C, P & S) on larval and cocoon traits of PM x CSR2 silkworm hybrid. As already established during the large-scale bioassay that the doses required for multivoltine hybrid, CSR2 x CSR4 are 6 times higher than the bivoltine hybrid, CSR2 x CSR4. The three sources of ascorbate were equally effective to bring out significant improvement in PM x CSR2 hybrid to the extent of 13.59, 12.30 and 13.40% in larval weight, 13.28, 13.81and 13.20% in cocoon weight, 15.45, 15.19 and 14.28% in cocoon shell weight compared to control. However, no improvement was observed in pupation rate.

As perusal of the Table 04, significant improvement was recorded in reeling traits *viz*., filament length (12.60, 13.40 & 15.10%), non-breakable filament length (13.60, 12.64 & 15.60%), raw silk % (13.60, 14.64 & 14.73%) and raw silk recovery (7.62, 9.64 & 11.00%) respectively compared to control. However, reelability was improved by 8.40% in synthetic ascorbate group only while renditta was improved in purified and synthetic ascorbate groups only by 8.00% and 10.00%. Denier remained at parity with control and neatness was improved with none of the forms of ascorbate.

One important and major feature regarding plant-based ascorbate is that, if present in its natural environment acts at considerably lower doses compared to the synthetic ascorbate (Eberhardt *et al*., 2000) [18]. This fact has been substantiated in the present investigation wherein ascorbate-quantified crude amla extract induced improvement in economic traits at 10 times lower ascorbate dose (0.05% & 0.3%) as compared to synthetic ascorbate (0.5% & 3.0%) in both the hybrids. This capacity of plant-based ascorbate, if carefully delineated towards the mulberry leaf fortification could form an easy means for improving the silkworm produce. Reddy and Urooj (2005) [19] evaluated three plants *viz.*, amla (*Emblica officinalis*), drumstick leaves (*Moringa oleifera*) and Raisins (*Vitis vinifera*) as sources of natural antioxidants. All the three extracts exhibited a high percentage of antioxidant activity. Extracts from drumstick leaves and amla were more effective in controlling lipid oxidation during storage.

Table 3: Comparative effect of ascorbate-quantified crude plant extract, purified ascorbate and synthetic ascorbate on larval and cocoon traits of silkworm hybrid, PM x CSR2.

Treat.	*L.wt. (g)*	*P.R. (%)*	*C.wt. (g)*	*S.wt. (g)*	*Shell %*
0.30% − C	48.931*	92.13	2.015*	0.370*	18.37*
3.00% −P	48.375*	91.39	2.024*	0.369*	18.24*
3.00% − S	48.849*	90.72	2.013*	0.366*	18.15
Control	43.076	91.16	1.778	0.321	18.02
SE±	*0.150*	*0.924*	*0.005*	*0.001*	*0.049*
CD 5%	*0.488*	*3.012*	*0.016*	*0.002*	*0.161*

C: crude ascorbate, P: purified ascorbate and S: synthetic ascorbate.

Table 4: Comparative effect of ascorbate-quantified crude plant extract, purified ascorbate and synthetic ascorbate on reeling traits of silkworm hybrid, PM x CSR2.

Treat.	*F.L (m)*	*N.B.F.L (m)*	*Den.*	*Reel. (%)*	*Ren. (kg)*	*R.S. (%)*	*R.S.R. (%)*	*Neat. (p)*
0.30% − C	883.01*	754.08*	2.506	84.41	8.708	16.45*	78.10*	83.40
3.00% −P	889.28*	747.71*	2.523	85.00	8.143*	16.61*	79.57*	85.21
3.00% − S	902.61*	767.35*	2.520	86.14*	7.966*	16.62*	80.55*	83.67
Control	784.20	663.80	2.513	79.46	8.852	14.48	72.57	81.36
SE±	*19.290*	*17.372*	*0.012*	*1.734*	*0.067*	*0.108*	*1.406*	*0.975*
CD 5%	*62.909*	*56.653*	*0.039*	*5.655*	*0.220*	*0.352*	*4.586*	*3.178*

C: crude ascorbate, P: purified ascorbate and S: synthetic ascorbate. F.L: Filament length, N.B.F.L: Non-breakable filament length, Den.: Denier, Reel. Reelability, Ren.: Renditta, R.S.: Raw silk, R.S.R: Raw silk recovery and Neat.: Neatness.

Research findings show that amla is 12 times more assimilable than synthetic ascorbate. Just 8.7mg of natural ascorbate complex from amla is equivalent to 100 mg of the most commonly used synthetic ascorbate (Arora, 1985) [20]. The ascorbate in amla is bonded with tannins, preventing it from being destroyed by light and heat and better utilization within the body (Anonymous, 2001) [9]. In the present study ascorbate -quantified crude extracts of amla were found to be effective at 10 times lower doses (0.05 % and 0.3%). While, synthetic ascorbate has been reported by many authors to induce improvement in economic traits of the silkworm at comparatively higher doses *viz*., 1.5% (Babu *et al*. 1992) [14], 2 % (EI-Karaksy & Idriss, 1990 and Etebari *et al*., 2004) [15, 16], 2-3% (Prasad, 2004) [17] and 10 % (Chauhan and Singh, 1992) [21]. Eberhardt *et al*., (2000) [18] demonstrated that the total antioxidant activity of 5.7mg apple-based ascorbate was equivalent to about 1500 mg of synthetic ascorbate. Scartezzini *et al.,* (2005) [22] evaluated the antioxidant effects of amla in comparison to the real levels of ascorbate present by different antioxidant tests. It was shown that amla fruit ascorbate accounts for approximately 45-70% of the antioxidant activity. Vitamin C in amla is present in the form of a complex known as "Vitamin C-complex" with other compounds where ascorbate is only the anti-oxidant ring that surrounds the complex and is more effective when present in the same form (Bajaj, 2006) [8]. It is surmised that the improvement in silkworm at considerably lower doses (0.05% & 0.3%) can possibly be due to synergetic activity of ascorbate with other compounds in the vitamin C-complex that increase its bioavailability and efficacy. Bioflavonoids often found in ascorbate-rich fruits (especially citrus fruits), have been known to increase the bioavailability and efficacy of ascorbate (Johnston and Luo, 1994) [23].

The effective time of application is considered to be closely associated with the ascorbate dose to bring about significant improvement in silkworm. The application time effective for supplementation of ascorbate -quantified crude amla extract has been similar as that of synthetic ascorbate supplementation. To sum up, 0h application time has once again been proved as an effective application time to induce significant improvements in silkworm economic traits in case of crude amla extract at 0.05% & 0.3% ascorbate doses. There are a few workers who have reported the 48h of 5th instar as effective application time for some botanical supplements. Santoshkumar (1997) [24] reported the increased silk and egg yielding ability in multivoltine, bivoltine and cross breeds of mulberry silkworm when supplemental dusting of 5% *Lantana camara* and *Clerodendrum inermae* was done once at 48h of 5th instar. Mamadapur (1994) [25] reported the 6.23h prolonged larval duration in the larval batches treated with dust formulation of *Lantana camara* and *Clerodendrum inermae* at 48h of 5th instar. Similarly, Rajashekhargouda (1991) [26] could find that supplementation of crude petroleum ether extracts of *Tribulus terrestris* L and *Psoralea corylifolia* L at 48h of 5th instar increased larval duration of *Bombyx mori* to the extent of 12.5 to 23.5h and 10.0 to 28.0h respectively. The variation/contradiction between the effective time of application observed in present study and that observed by the workers mentioned above is attributed to the difference in the botanicals and extraction procedure used. It is inferred that time of application could vary according to the botanical extract utilized.

A perusal of the bioassay results of purified ascorbate from amla fruit pulp (P) makes it is adequately clear that there is no difference in the activities shown by purified ascorbate from that of synthetic ascorbate (S). Significant ($P<0.005$) improvement in economic traits was observed in both the forms of ascorbate (P & S) at same dosage (0.5 & 3.0%) and application time (T0). The present finding is in line with report of Gregory (1993) [27] who demonstrated that botanical-based ascorbate and synthetic ascorbate are chemically identical and there are no known differences in their biological activities or bioavailability. Bioflavonoids often found in ascorbate-rich fruits, especially citrus fruits have been reported to increase the bioavailability or efficacy of ascorbate (Johnston and Luo, 1994) [23]. Further, the present study substantiates the fact that plant-based ascorbate

in synergistic combination with other compounds present in its natural habitat shows highly increased efficacy at lower doses compared to the synthetic ascorbate (Arora, 1985 and Eberhardt *et al*., 2000) [20, 18].

4. References

1. Ito T, Arai N. Nutrition of silkworm *Bombyx mori* L. IX. Further studies on the nutritive effects of ascorbic acid. Bull. Seric. Expt. Statn. Japan. 1965; 20:1-19.
2. Ito T. Silkworm nutrition. In: The silkworm-An Important Laboratory Tool (eds Y.Tazima) Kodansha Ltd., Tokyo. 1978, 307.
3. Ito T. Nutrition on the silkworm, *Bobbyx mori* L. IV. Effects of ascorbic acid (English). Bull.Seric. Expt. Statn. 1961a; 17:134-137.
4. Ito T. Effect of dietary ascorbic acid on the silkworm, Bombyx mori. Nature. 1961b; 4806(192):951-952.
5. Dobzhenok NV. The effect of ascorbic acid on the physiological condition of codling moth and its resistance to fungus and bacterial infection, Zakhist. Roslin. 1974; 19:3-7.
6. Ito T. Nutritive values of carbohydrates for the silkworm, Bombyx-Mori. Nature. 1960; 187(4736):527-527.
7. Cappellozza L, Cappellozza S, Saviane A, Sbrenna G. Artificial diet rearing system for the silkworm *Bombyx mori* (Lepidoptera: Bombycidae): effect of vitamin C deprivation on larval growth and cocoon production. Appl. Entomol. Zool. 2005; 40:405-412.
8. Bajaj GJ. Benefits of amla powder, 2006. @ http://www.kanhaiyaamla.com.
9. Anonymous. Case Study on Amla-related patent, Technology Information, Forecasting & Assessment Council (TIFAC) Bulletin. 2001, 7-6.
10. Aabid KT, Trivedy K. Significance of application time for dietary vitamin C supplementation inthe silkworm, *Bombyx mori* L. Curr. biotica. 2011; 4(4):22-28.
11. Berkowitz D, Allaway A. Statistical package for social sciences (SPSS), Version 7.5 for Windows NT/Windows. 1998; 95:130-32.
12. Aabid KT, Kanika Trivedy, Nirmal Kumar S. Differential response of Bombyx mori larvae to vitamin C-quantified crude extract of *Emblica officinalis* G. Indian Journal of Sericulture. 2009; 48(2):145-149.
13. Aabid KT, Kanika Trivedy. Relative efficiency of some crude botanical extracts rich in vitamin C to improve economic parameters of the Silkworm, *Bombyx mori* L. Journal of Experimental Zoology. 2008; 11(2):1-8.
14. Babu M, Swamy MT, Rao PK, Rao MS. Effect of ascorbic acid- enriched mulberry leaves on rearing of *Bombyx mori* L. Indian J. Seric. 1992; 31:111-114.
15. El-Karaksy IA, Idriss M. Ascorbic acid enhances the silk yield of mulberry silkworm, *Bombyx mori*, L. J. App. Entomol. 1990; 109:81-86.
16. Etebari K, Edabi R, Matindoost L. Effects of vitamin C on biological, biochemical and economical characteristics of the silkworm, *Bombyx mori* L. Int. J Indust. Entomol. 2004; 8(1):81-87.
17. Prasad PR. Effect of fortification of ascorbic acid through mulberry leaf on cocoon traits of Pure Mysore race of silkworm *Bombyx mori* L. Indian J Entomol. 2004; 66(1):37-39.
18. Eberhardt MV, Lee CY, Liu RH. Nutrition: Antioxidant activity of fresh apples. Nature. 2000; 405:903-904.
19. Reddy V, Urooj A. Evaluation of antioxidant activity of some plant extracts and their application in biscuits. Food Chem. 2005; 90(1-2):317-321.
20. Arora BB. Development of Unani Drugs from Herbal Sources, Vitamin C from amla in equivalent to Vitamin C from Synthetic sources., 1985, 234.
21. Chauhan TPS, Singh K. Studies on the effect of ascorbic acid (vitamin. C) on the fecundity of mulberry silkworm, *Bombyx mori*, L. Sericologia. 1992; 32:567-574.
22. Scartezzini P, Antognoni F, Raggi MA, Poli F, Sabbioni C. Vitamin C content and antioxidant activity of the fruit and of the ayurvedic preparation of *Emblica officinalis* Gaertn. J Ethnopharm. 2005; 104(1-2):13-118.
23. Johnston CS, Luo B. Comparison of the absorption and excretion of three commercially available sources of vitamin C. J Am. Diet. Assoc. 1994; 94:779-781.
24. Santoshkumar GH. Large scale evaluation of insect growth regulatory activity of *Lantana camara* L. and *Clerodendrum inermae* G. on *Bombyx mori* L. M. Sc. (Agri) Thesis, University of Agricultural Sciences, Dharwad, 1997, 99.
25. Mamadapur BB. Botanicals with IGR activity on *Bombyx mori* L. M. Sc. Thesis, UAS, Dharwad, 1994, 84.
26. Rajashekhargouda R. Studies on the methods to increase silk yield of *Bombyx mori* L. Ph. D Thesis, TNAU, Coimbatore, 1991, 125-138.
27. Gregory JF. Ascorbic acid bioavailability in foods and supplements. Nutr. Rev., 1993; 51:301-303.