

## Journal of Plant Protection and Pathology

Journal homepage: [www.jppp.mans.edu.eg](http://www.jppp.mans.edu.eg)  
Available online at: [www.jppp.journals.ekb.eg](http://www.jppp.journals.ekb.eg)

### Effectiveness Vitamin C on different Characteristics of Silkworm, *Bombyx mori* L.

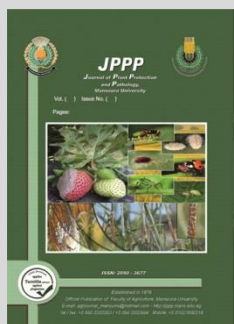
Rania. S. Gad<sup>1\*</sup> and Dina M. Fathy<sup>2</sup>

<sup>1</sup>Sericulture Research Department , Plant protection Research Institute , Agricultural research Center , Giza Egypt

<sup>2</sup>Economic Inscets Dept. Faculty of Agriculture, Mansoura University, Egypt.



Cross Mark



#### ABSTRACT

In order to investigate effectiveness vitamin C on different characteristics of silkworm, *Bombyx mori* L. Silkworm larvae through 4th to 5th instar were treated with dietary supplements of ascorbic acid 7000 (VC1) and 10000 (VC2) ppm . Leaves enriched with vitamin C were fed four times per day for the larvae. Results obtained that VC1 of vitamin C is more effective than VC2 and control. It had a significant increase in biological criteria s like larval weight and larval period while economical criteria s like cocoon weight, cocoon shell weight and pupal weight. They weren't shown any difference between VC1 and VC2 compared to control. Ascorbic acid (VC2) significantly increased the number of Prohaemocytes (Pr) 15, Granulocytes (Gr) 35.3, Plasmatocytes (Pl) 27.6 and Oenocytoides (Oe) 8 cells, respectively. This study was indicated that vitamin C has been effective on increasing the growth rate of larvae, weight of the cocoon and increasing the immunity of larvae. So, It is recommended to using it in the breeding of silkworms and production of silk commercially.

**Keywords:** Silkworm, Vitamin C, Haemocytes, Biological and economical criteria s.

#### INTRODUCTION

The production of high quality and quantity silk depends on healthiness of larvae and larval nutrition, which are depended on nutritive value of mulberry leaves (Ito, 1978). The silkworm needs certain essential sugars, proteins, amino acids and vitamins for normal growth, survival and increasing the silk production. The silkworm *Bombyx mori* mainly depends on mulberry leaves for its vitamin requirement since it is a monophagous insect (Horie, 1995).

It is generally accepted that all insects require vitamins, especially water-soluble vitamins such as ascorbic acid, thiamin, riboflavin, niacin, pyridoxine, pantothenic acid, biotin, folic acid and choline (Chapman, 1998). Growth retardation caused by lack of each one of these vitamins is rather small, but better growth is obtained by adding these vitamins to their diet (Horie,1995). The nutritive value of mulberry leaves can be raised by treating them with vitamins and nutritional supplements. Mulberry leaves were treated with complementary compounds had been increasing the larval growth and cocoon characteristics (Muniandy et al., 1995; Sarker et al., 1995; Nirwani and Kaliwal, 1996; Etabri, 2002, Etebari and Fazilati, 2003). Nirwani and Kaliwal (1996) showed that folic acid caused a significant increase in economical criteria s such cocoon weight, shell weight and others criteria s.

Ascorbic acid (C6, H6 is the active form of vitamin C and is a water soluble vitamin. The function of vitamin C is not fully known yet but it is assumed that like mammals, it involves in tyrosine metabolism, steroids synthesis, cametin synthesis, norumodealutory, feeding stimulatory,

immunity and detoxifications. It is assumed that ascorbic acid has an important role on ecdysis and formation of cuticle; Lindroth and Weiss, 1994). The necessity of ascorbic acid in insects diet becomes clarified. It has been identified as a powerful anti-oxidant, potential phage stimulant, efficient growth promoter and booster of silk production in the silk worm (Javed and Gondal, 2002; Hussain and Javed, 2002; Prasad 2004; Etebari et al., 2004, Etebari and Matindoost 2005).

Additional the authors have described that excessive amounts of Vitamin C supplementation on silkworm diet have negative effect, causing decrease in food consumption and cocoon characteristics due to hypervitaminosis (Tantray et al., 2011). Synthetic dietary supplementation and plant based on Vitamin C has been found to enrich the economic criteria s of the mulberry silk worm significantly (Tantray et al., 2016, Tantray and Trivedy 2016, Tantray, 2017). The highest concentration (4%) of ascorbic acid and lemon gave the highest mean of silk filament length. Lemon juice, sweet orange juice and ascorbic acid improved cocoon indices and silk filament characters as a profitable supplementary diet for silk worm *Bombyx mori* L (El-Santeel,Walaa, et al.,(2018)

Therefore, the present study was investigated the effectiveness of ascorbic acid on biological and economical criteria s of hybrid silkworm of *Bombyx mori* L.

#### MATERIALS AND METHODS

The present study was carried out during spring season, 2021 in silkworm laboratory on Entomology Department, Faculty of Agriculture, Mansoura University.

\* Corresponding author.

E-mail address: [raniagad9382@gmail.com](mailto:raniagad9382@gmail.com)

DOI: 10.21608/jppp.2021.203157

**Test species:** Mulberry silkworm, *Bombyx mori* L. (Q<sub>2</sub>\*V<sub>2</sub>\*H<sub>1</sub>\*UV).

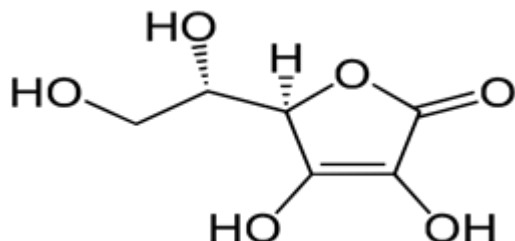
**Mulberry:** Native mulberry leaves *Morus alba* variety (Balady)

**Larval Instar:** 4<sup>th</sup> and 5<sup>th</sup> instar

**Duration of treatment:** 15 days

**Concentration: Vitamin C** 7000 ppm. and 10000 ppm.

**Chemical structure:**



2-oxo-L-threohexono1,4-lactone 2,3-enediol

**Preparation of stock solution:** each tablet contains: 1 gm ascorbic acid. The tablet was dissolved in 125 ml of distilled water. Therefore, 8 gm of vitamin c was dissolved in 1000 ml (1 L), which is as standard concentration of solution. For the Preparation of 7000 ppm concentration, 7gm was dissolved in 1000 ml of distilled water. Likewise, 10 gm was dissolved in 1000 ml of distilled water.

**Leaf treated method:** leaves have been soaked in different concentrations of vitamin C, T1 (7000 ppm), T2 (10000 ppm) and control for 15 minute.

**Treatments**

The larvae in Fourth instar were divided into 3 experimental groups, T1, T2 and control. Each group contained on 100 larvae with three replications. Fresh mulberry leaves were put in each concentration for 15 min and then were dried in air for 20 min. The supplementary leaves were fed to silkworm larvae from 4th to 5th instar every day

**Biological and economical criteria s**

After the treatments, the larval and cocoon criteria s were noticed. Weights of larvae were determined by weighing 30 larvae in different days of fourth and fifth larvae instars. The percentage of daily increase of weight (DIW%) was calculated for each group. Cocoon weight, cocoon shell weight, cocoon shell ratio and pupal weight were determined by using standard technique in sericulture that was described by (Nirwani and Kaliwal 1996).

The relative growth rate was [GR = G/ W.T], where G, the fresh weight gain was computed as the difference between the initial and final weight of larva in each

**Table 1. Effects of ascorbic acid on the larval criteria s of silkworm.**

Concen.	Larval weight				larval period	G/R
	4 <sup>th</sup> Instar		5 <sup>th</sup> Instar			
	1 day	5 day	1 day	8 day		
VC <sub>1</sub> (7000ppm)	0.183 a	1.160 a	1.139 a	4.073 a	8.50 c	4.015
VC <sub>2</sub> (10000ppm)	0.179 a	1.065 b	1.046 b	3.867 b	8.62 b	3.800
control	0.178 a	0.996 c	0.973 c	3.349 c	10.00 a	3.000

**Table 2. Efficacy of ascorbic acid on the silkworm cocoon criteria s**

Concen.	Cocoon criteria s			
	Cocoon weight (g)	Pupal weight (g)	C. shell weight(g)	C. shell ratio (%)
VC <sub>1</sub> (7000ppm)	3.104 a	2.350 a	0.7463 a	24.050 a
VC <sub>2</sub> (10000ppm)	3.07 a	2.331 a	0.7342 a	23.926 a
control	2.355 b	1.812 b	0.5168 b	22.174 b

**Cocoon characteristics:** As shown in (Table 2) Cocoon and pupal weight were significantly increased in VC<sub>1</sub> and

replication, W, the mean weight of the last instar feeding period. T, is the eating period of larva in last instar (Etebari et al., 2004).

**Cocoon shell ratio**

It is the ratio between the weight of the shell, and the whole weight of the cocoon expressed as percentage. It is calculated by using this formula (Rajitha & Savithri, 2015)

$$\text{Cocoon shell ratio} = \frac{\text{cocoon shell weight}}{\text{cocoon weight}} \times 100$$

**Haemolymph preparation:** The haemolymph samples from larvae 5th instars Larvae were taken after 72 hrs of treatment with all tested groups. To calculate the differential haemocytes count (DHC), 100 cells were identified to their typical haemocytes type after staining a smear of haemolymph with Wright's stain (Jones, 1962; Gad, 1996).

**Statistical calculations**

Collected data were subjected to statistical analysis of variance test to find out the low significant different between criteria s of control and treated groups. CoHort Software (2004) were used in all experiments. Data were statistically analyzed by One Way ANOVAs according to Duncan's Multiple Range Test.

**RESULTS AND DISCUSSION**

The effects of vitamin C (Ascorbic acid) on some biological, economical criteria s of silkworm are presented in the Table (1 and 2).

**Biological and economical criteria s**

**Larval weight:** The larvae weight significantly increased in the first concentration 7000 ppm in the larval instars (Table 1). The larvae weight under 7000 ppm was 1.016 in the last day of 4<sup>th</sup> instar of larvae while the larval weight was 4.073 in the last day 5<sup>th</sup>. It showed that the larval weight in VC<sub>1</sub> is increased compared to VC<sub>2</sub> ppm and control. They showed significant difference between VC<sub>1</sub> and VC<sub>2</sub> concentrations. The larval weight in VC<sub>1</sub> concentration during 5<sup>th</sup> instar was relatively good. The most highest weight increase was 4 % at VC1. The increase in the weight of larva was nearly the same as what we expected to have, *i.e.*, the increase of weight wasn't equivalent to whatever we obtained in 4<sup>th</sup> instar. The growth weight in control was less than VC concentration in 4<sup>th</sup> and 5<sup>th</sup> instar.

VC<sub>2</sub> of vitamin C. Cocoon weight was 3.104 and 3.07 in VC<sub>1</sub> and VC<sub>2</sub>, respectively. he heaviest weight of fresh

cocoon was gained from the larvae fed on mulberry leaves enriched with vc1 and vc2. Meanwhile, control cocoons recorded less of the weight. The increase in the weight of cocoon was nearly the same as what we expected to have. Also, the increase of weight of pupa was recorded 2.35 and 2.331 compared controlled pupae. It wasn't significant variation between VC<sub>1</sub> and VC<sub>2</sub> in the weight cocoon and the weight pupae.

**Cocoon shell weight (g):** The results in Table (2) obtained the heaviest cocoon shell weight was recorded for the cocoons spun by *B. mori* larvae fed during their 4th instar on mulberry leaves fortified with vc1 and vc2, recording 0.75 g and 0.73 in vc1 and vc2 compared to 0.52 g for the control cocoons. Respectively, VC1 and VC2 treatments proved to be the most effective. Moreover, all the tested additives improved these criteria s over the control.

**Silk cocoon ratio:** Obtained results in Table (2) showed that the highest silk content ratio was recorded for the cocoon spun by *B. mori* larvae fed in 4th instar on mulberry leaves treated with ascorbic acid (VC1), in VC1 treatments means the silk content ratio attained 24.050 %. Generally, the treatments of VC1 and VC2 proved to be the superior compared to control treatments. they were 24.05 % and 23.924%. On the other hand, control treatment recorded silk ratio of 22.17%.

There were different suggestions on ascorbic acid effects and taking in mind that the abundance of vitamin C in mulberry leaves has been reported by several authors, but the quantity of this vitamin is very variable in different conditions (Babu et al., 1992). Ascorbic acid significantly increased the weight of larvae and pupae of *B. mori* which similar to the findings of El-Karaksy and Idris (2009) and Gomaa et al. (1977) in this respect.

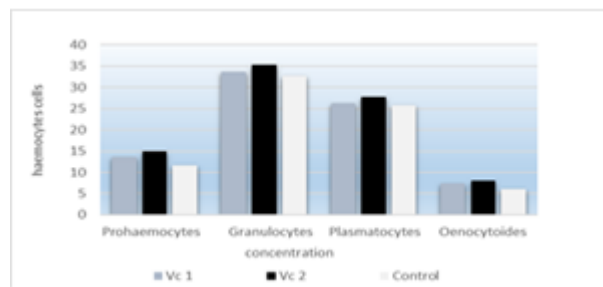
Several researchers proposed that larval weights had been enhanced related to phagostimulation of ascorbic acid (Balasundaram and Mathivanan. 2008 & El-Karaksy and Idris 2009). Also this results were similar with El-Santeel Walaa, et al.,(2018) the best result cleared that the highest concentration (4%), for lemon juice sweet orange juice and ascorbic acid gave the highest mean of fresh cocoon weight, shell cocoon weight and silk cocoon ratio.

#### **Determination of Haemocytes Types:**

The light microscopic observations of the stained larval hemolymph revealed the presence of four morphologically distinct types of haemocytes. Prohaemocytes (Pr), Granulocytes (Gr), Plasmacytes (Pl) and Oenocytoides (Oe). All treated groups clearly affected the differential haemocytes counts of 5th instar larvae of *B. mori*.

The differential haemocytes counts were affected with all treatments of vitamin c in 5th instar larvae of *B. mori*. The prohaemocyte was observed high counts in treatments (VC2 and VC1) to be 15 and 13.7 cell, respectively. Furthermore, Ascorbic acid treatment (VC2) caused a significant increase in the number of prohaemocyte to be 15 cells while control was 11.7 cells (Fig 1).

However, a reduction was observed in the number of Oenocytoides after treatments (VC<sub>2</sub> and VC<sub>1</sub>) with to be 8.01 and 7.33 cells., respectively while control was 6 cells.



**Fig. 1. Effects of ascorbic acid on the different haemocytes count cell in the 5<sup>th</sup> larval instar of *B. mori*.**

Moreover, the results showed that the number of plasmacyte weren't significant after treatment with (VC2 and VC1), respectively. It was recorded 27, 26 cells and control was 25.7 cells. The same trend was observed in the number of Granulocytes. the recorded of granulocytes was to be 35.3, 33.7 and 32.7 after treatment with VC2, VC and control, respectively.

Ascorbic acid treatment (VC2) significantly increased the number of granulocytes, plasmacytes, Prohaemocytes and oenocytoides to be 35.3, 27.7, 15 and 8.01 cells, respectively while control were 32.7, 25.7, 11.7 and 6 (Fig 1).

Furthermore, this results were agreements with Soliman and Gad (2020) The treatment of ascorbic acid (VC) at a concentration of 1% and AgNps at a concentration of 50µg/l have beneficial effects on the growth of *B. mori* silkworm larvae. Moreover, it increased the quantity of silk production by enhancing the feed efficacy than control.

Ascorbic acid concentrations were considered immunity booster to larvae of silkworm. Silkworm similar multivitamins effects have been proved by Saha and Khan (1996) as well as Etebari and Matindoost (2004). Etebari et al. (2004) Feeding by ascorbic acid at high concentrations decreased the weight of silkworm larval due to hypervitaminosis.

## **CONCLUSION**

These results recommended to use the recommended dose of vitamin C which is 7-8 tablets in feeding silkworm to increase the weight of larvae, cocoon, pupa, and increase the percentage of silk cocoon ratio, as well as increase the immunity of the larvae against diseases.

## **REFERNCES**

- Babu, M., M. T. Swamy, P. K. Rao and M. S. Rao (1992). Effect of ascorbic acid enriched mulberry leaves on rearing of Bom- bxv mori. Indian J. Seric. 31, 111-114.
- Balasundaram, Selvisabanyakam, D. and Mathivanan., V. (2008). Studies on comparative feed efficacy of mulberry leaves MR2 and MR2 treated with vitamin C on Bombyx mori (L.) (Lepidoptera: Bombycidae) in relation to larval criteria s. J. Curr. Sci., 12 (2) 31-35.

- Chapman, R. F. (1998) The Insects-Structure and Function, 4th edition, Cambridge University Press. Cambridge.
- CoHort Software, (2004). CoStat. www.cohort.com Monterey, California, USA.
- EL-Karakasy, I.A. and Idris, M. (2009). Ascorbic acid enhances the silk yield of the mulberry silkworm, *Bombyx mori*. *J. Appl. Entomol.* 109: 81-86.
- El-Santeel ,Walaa. S. F., EL-Sharkawy, H. and Saad, M. (2018). Technological effects of lemon, sweet orange juice and ascorbic acid as supplements additive on mulberry silkworm larvae, *Bombyx mori* L. *J. Product. & Dev.*, 23(1): 135-145.
- Etebari, K. and Fazilati, M. (2003). Effect of feeding on mulberry's supplementary leaves with multi mineral in some biological and biochemical characteristics of silkworm (*Bombyx mori*). *J. Sci. & Technol. Agric. & Natur. Resour.* 7, 233-244.
- Etebari, K. and Matindoost, L. (2005). The effect of vitamin C on biological, biochemical and economical characteristics of the silkworm *Bombyx mori* L. *J. Asia-Pacific. Entomo.*; 8(1):107-112.
- Etebari, K., Ebadi, R. and Matindoost, L. (2004). Effect of feeding mulberry's enriched leaves with ascorbic acid on some biological, biochemical and economical characteristics of silkworm *Bombyx mori*. *Int. J. Indus. Entomol.* 8: 81-87.
- Gad, A. A. (1996) Physiological studies on Lipedopterous insects. M.Sc., Thesis, Fac. Agric. Alex. Univ. Alexandria, Egypt.
- Gad, A. A. (2006). Effects of honey bee products on some biological and physiological criteria s of mulberry silkworm *Bombyx mori*. *The Egypt. Sci. Mag.* 3 (2): 27-32.
- Gomaa, A. A., EL-Shaarawy, M.F., Salem, Y.S. and Rizk, M.A. (1977). Effect of dietary constituents on the biology of silkworm *Bombyx mori* L. II. Vitamins. *Z. Ang. Ent.* 64 (2): 231-240
- Horie Y. and Ito, T. (1965) Nutrition of the silkworm, *Bombyx mori* -X. Vitamin B requirements and the effects of several analogues. *J. n. vecf. Physiol.* 11, 1585-1593.
- Horie, Y. (1995) Recent advances of nutritional physiology and artificial diet of the silkworm, in Japan. *Korean J. Seric. Sci.* 37, 235-243.
- Hussain, M. and Javed, H. (2002). Effect of 0.2% N with various combinations of ascorbic acid on growth and silk production of silkworm, *Bombyx mori*. *Asian Journal of Plant Science* 1(6) 650-651.
- Ito, T. (1978). Silkworm Nutrition; in *The Silkworm an Impotent Laboratory Tool*. Tazima, Y. (ed.), 121-157, pp. Kodansha Ltd, Tokyo.
- Javed, H. and Gondal, M. H. (2002) Effect of food supplementation of ascorbic acid on larval mortality of the silkworm, *Bombyx mori*. *Asian J. Plant Sci.*, 2002; 1(5):556-557.
- Jones, J. C. (1962). Current concepts concerning insect hemocytes. *American Zoologist.* 2: 209-246.
- Lindroth R. L. and Weiss, A. P. (1994) Effects of ascorbic acid deficiencies on larvae of *Lymantria dispar* (Lepidoptera: Lymantriidae). *Great Lakes Entomologist* 27, 169-174.
- Muniandy, S., Sheela, M. and Nirmala, S. T. (1995). Effect of vitamins and minerals (Filibon) on food intake, growth and conversion efficiency in *Bombyx mori*. *Environ. Ecol.* 13, 433-435.
- Nirwani, R. B. and Kaliwal, B. B. (1996) Effect of folic acid on economic traits and the change of some metabolic substances of bivoltine silkworm, *Bomb5x mori* L. *Korean J. Seric. Sri.* 38, 1 18-1 23.
- Prasad P. R. (2004). Effect of fortification of ascorbic acid through mulberry leaf on cocoon traits of Pure Mysore race of silkworm, *Bombyx mori* L. *Indian J. Entomo.* 66 37-39.
- Rajitha, K., & Savithri, G. (2015). Studies on symptomological and economic criteria s of silk cocoons of *Bombyx mori* inoculated with *Beauveria Bassiana* (Bals.) Vuill. *International Journal of Current Microbiology and Applied Sciences*, 4(2), 44-54.
- Sahab., n. and Khan, A.R. (1996). Effect of dietary supplementation of vitamins and minerals on the growth and development of *Bombyx mori* L. *Bangladesh . J. Zool.* 24(2): 125-131.
- Sarker, A., M., Haque, M. Rab and Absar , N. (1995) .Effects of feeding mulberry (*Morus* sp.) leaves supplemented with different nutrients to silkworm (*Bombyx mori*) L. *Curr. Sci.* 69,185-188.
- Soliman, A.M. and Gad, Abir A. (2020). The Impact of Ascorbic Acid, Some Nanomaterials and Their Mixtures on Some Biological and Physiological Criteria s of the Mulberry Silkworm *Bombyx mori* L.
- Tantray, A. K. (2016). A review on attributes of Vitamin C with particular reference to the silkworm, *Bombyx mori* L., *Inter. J. Zoolo. Studies*, 1(5):45-49
- Tantray, A. K. (2017). Growth studies of popular silkworm, *Bombyx mori* L. breeds influenced by dietary supplementation of synthetic and phyto ascorbate. *Inter. J. Chem. Studies*, 5(1):222- 226.
- Tantray, A. K. and Trivedy, K. (2016). Areview on attributes of vitamin C with particular reference to the silkworm, *Bombyx mori* Linn. *International Journal of Zoology Studies*,1(5): 45 - 49.

### فعالية فيتامين ج علي مختلف الخصائص البيولوجية والاقتصادية لديدان الحرير. رانيا صلاح جادا<sup>1</sup> و دينا مندوه فتحي<sup>2</sup>

اقسم بحوث الحرير -معهد بحوث وقاية النبات - معهد البحوث الزراعية - الجيزة  
اقسم الحشرات الاقتصادية - كلية الزراعة - جامعة المنصورة

اجريت هذه الدراسة من اجل تقييم فعالية فيتامين ج علي الخصائص المختلفة لودة القز ، حيث تم تغذية يرقات *Bombyx mori* L خلال الطورين اليرقيين الرابع والخامس علي المكملات الغذائية من حمض الاسكوربيك وهي معاملتين 7000 جزء من المليون VC1 و 10000 جزء من المليون VC2 ليرقات دودة القز. وتمت التغذية علي الاوراق الغنية بفيتامين ج اربع مرات في اليوم. ووضحت النتائج التي تم الحصول عليها الى ان التركيز الاول VC1 من فيتامين ج اكثر فعالية من التركيز الثاني VC2 والكتنترول . واطهر زيادة معنوية في الخصائص البيولوجية مثل وزن اليرقات وفترة الطور اليرقي بينما لم تظهر تأثيرا مختلفا علي وزن الشرنقة ووزن العنزة في التركيزين الاول والثاني مقارنة بالكتنترول . كما عمل التركيز الثاني من حمض الاسكوربيك الزيادة بشكل كبير في عدد الخلايا الأولية (Pr) 15خلية ، والخلايا الحبيبية (Gr) 35.3 خلية ، والخلايا البلازمية (PI) 27.6 خلية و Oenocytoides (Oe) 8 خلية ، على التوالي. وقد اوضحت هذه الدراسة ان فيتامين ج (ج) يظهر وجود نشاط محفز للنمو لليرقات وزيادة مفاعلتها لذلك يوصي باستخدامه لزيادة فعالية التغذية في تربية دودة القز وانتاج الحرير تجاريا .