The Impact of Ascorbic Acid, Some Nanomaterials and Their Mixtures on Some Biological and Physiological Parameters of the Mulberry Silkworm *Bombyx mori* L.

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ABSTRACT

This study was carried out to determine some of the biological parameters and economic traits of the mulberry silkworm *Bombyx mori* after feeding of 5th instar larvae on mulberry leaves (*Morus alba*) leaves treated with different materials (ascorbic acid at concentration 1%, silver nanoparticales (AgNPs), Titanium oxide nanoparticales (TiO2NPs), Copper oxide (CuONPs), Zinc oxide (ZnONPs) at concentration 50µg/l and the mixtures of each nanoparticales and ascorbic acid in relation to some biological parameters such as larval weight, pupal weight and cocoon shell weight.

The present study revealed that the economic parameters were enhanced by 1% ascorbic acid and all tested nanomaterial at a concentration of 50µg/l than control. On contrast, the mixture of each nanomaterial and ascorbic acid didn't improve the economic parameters and have a negative effect on the biological parameters of silkworm larvae. This study was indicated that using ascorbic acid and AgNPs, TiO_2NPs, CuONPs, ZnONPs significantly improved silk production by about 42.3, 34.7, 20.86 and 25.29%, respectively.

Key Words: *Bombyx mori,* Vitamin C, silver nanoparticales, Titanium oxide nanoparticales, Copper oxide nanoparticales, Zinc oxide nanoparticales Growth rate, silk production.

INTRODUCTION

Sericulture is a traditional industry in many countries all over the world and the life of many people is depended on it. Increases of larval growth and cocoon quality and quantity may give better economics for this industry and meet the production needs. Consequently, the enrichment of mulberry leaves by supplementary compounds with the aim of increasing the production of cocoon is a very important goal. Many investigations have been published on this topic (Legay 1958; Eteberi 2002; Etebari *et al.,* 2004; Islam *et al.*, 2004).

Ascorbic acid has many important functions in the animal body. It is a powerful antioxidant, protecting against oxidative damage to DNA, membrane lipids and proteins. Antioxidant activity of ascorbic acid decreases reactive oxygen species and oxidative pressure, and, as a result, the absorption of nutritious substances in the midgut would increase (Felton and Summers, 1993). The relationship of ascorbic acid supplementation and growth of silkworm was recorded by (Ito, 1978).

Many research demonstrated the phagostimulatory effect of ascorbic acid for insects (El Arakasy and Idriss, 1990; Gad, 1996 and Balasundaram *et al.*, 2008).

Recently, inorganic nanoparticles (NPs) have been added as a food additives to mulberry leaves to produce silk with special features. For example, TiO2 NPs have been added for high ultraviolet-resistant silk fibers (Cai *et al.*, 2015).

Silver Nanoparticles (AgNps) can be utilized as an ancillary complex which can boost up the growth and development of the larvae and also the quality and quantity of the cocoon (Balasundaram *et al.*, 2012). Moreover, research on silkworm, *B. mori* clearly demonstrates that nanoparticles could stimulate more production of fibroin protein which can help in producing carbon nanotube in future (Bhattacharya *et al.*, 2008). Similar study was recorded by Wang *et al.*,(2016) who confirmed the presence of carbon nanomaterial in silk fibers and silkworm excrement by Raman spectroscopy after raising silkworms with a diet of mulberry leaves and single-walled carbon nanotubes and graphene.

In addition, Li *et al.*, (2016) demonstrated that silkworm growth and feed efficiency are affected by modified diets of different TiO2 NP concentrations.

Cu NPs and Ag NPs are highly conductive, and they are also thought to have significant and broad-spectrum bioactivity, such as antimicrobial activity against bacteria, fungi, and viruses (Bondarenko *et al.*, 2013 and Ingle *et al.*, 2014).

Akhtar and Asghar, (1972) have found that vitamins and mineral salts played an important role in the nutrition of silkworm. Keeping the importance of vitamins and other compounds like silver nanoparticles on silkworm nutrition are very effective (Ponraj *et al.*, 2011).

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The present study was designed to evaluate the effect of mulberry leaves supplemented with ascorbic acid or AgNPs, TiO_2NPs, CuONPs, ZnONPs and their mixtures with ascorbic acid on some biological and physiological parameters of silkworm *B. mori*.

MATERIALS AND METHODS

Chemicals

Nanomaterials (Silver nanoparticales (AgNPs), Titanium oxide nanoparticales (TiO2NPs), Copper oxide (CuONPs), Zinc oxide (ZnONPs) and Ascorbic acid Vitamin C) purchased from Sigma–Aldrich, MO. and used at the highest purity offered by commercial sources.

Silver nanoparticales AgNps were prepared from an aqueous 50ml solution of 0.001 M AgNO3 (Sigma-Aldich) according to Rai *et al.*, (2009).

CuONPs, TiO_2NPs and ZnONPs (1.0g) of each nanomaterials were mixed with 100ml of d- ionozed water separately, treated by an ultrasonic homogenizer (amp: 60%, 15 min for the dispersion, stabilization) and used for preparation of the stock solution.

Insect Strains

Larvae of *B. mori* (strain: Jingsong × Haoyue) were maintained in our laboratory at department of Applied entomology and zoology and reared on mulberry (*Morus alba*) leaves under a 12-h light/12-h dark cycle and 26Cº. The larvae were fed three times per day.

Study design

The study was designed to examine the effect of nanomaterials Ag NPs, TiO2NPs, CuONPs, ZnONPs at concentration of 50µg/l, Vitamin C at concentration of 1% and its mixtures on the 5th instar larvae of *B. mori*. The larvae were divided into two groups (Control and Treated). The treated group divided into nine sub groups (T_1, T_2, T_3….and T_9) these sub groups were treated with Vitamin C (1%), tested nanomaterials at concentration 50µg/l and their mixtures. The control and treated larvae were daily fed on mulberry leaves supplemented with tested materials.

The efficacy of tested materials on weight of larvae, pupae and cocoon shell was recorded and compared with control.

Experimental Groups

Bombyx mori 5th instars larvae were fed on the following mulberry leaves. Control group (C) larvae were fed normal mulberry leaves, group T_1 larvae were fed on 1% Vitamin C, group T_2 larvae fed on mulberry leaves treated with Ag NPs at 50 µg/l, group T_3 larvae fed on mulberry leaves treated with TiO_2NPs 50 µg/l, T_4 larvae fed on mulberry leaves treated with CuONPs 50 µg/l and group T_5 larvae fed on mulberry leaves treated with ZnONPs 50 µg/l and group T_6 (a mixture of vitamin C and Ag NPs), group T_7 (a mixture of vitamin C and TiO2NP), group T_8 (the mixture of vitamin C and CuONPs 50 µg/l) and group T_9 (a mixture of vitamin C and ZnONPs 50 µg/l).

Fresh mulberry leaves were separately soaked with each tested materials and their mixtures for 15 minutes and then were dried in air for 10 minutes. The Vitamin C treated leaves were used for feeding 5th instar larvae of silkworm *B. mori* (Gad, 1996).

Mortality were recorded daily, and the weight of treated larvae were recorded before the cocoon spinning.

Haemolymph preparation:

The haemolymph samples from *B. mori* 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 analysis

All data obtained were expressed as the mean ± standard error. Statistical analyses were performed with one-way analysis of variance followed by unpaired two-tailed Student's t-test. A p-value < 0.05 was considered to be significant. (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Larval and cocoon traits:

The effect of tested nanomaterials at a concentration of 50 µg/l, ascorbic acid at a concentration of 1% and their mixtures on larval growth and some economic parameters of silkworm, *Bombyx mori* are presented in (Figure 1 to 3).

The present results revealed that *B. mori* larvae which fed on mulberry leaves treated with ascorbic acid significantly increased the larval weight, cocoon weight, pupal weight and shell weight by about 49.81, 36.9 and 42.3, respectively. The maximum increase in the larval weight was observed after *B. mori* larvae fed on mulberry leaves treated with ascorbic acid (T_1) about 49.8% followed by AgNPs (T_2) about 36.08% more than control.

Among the other treatments, the least weights of larvae, pupae and cocoon shell were recorded after larvae fed of mulberry leaves treated with the mixture of TiO_2 NPs and VC (T_7) to be .711, 0.546, and 0.1511g, respectively.

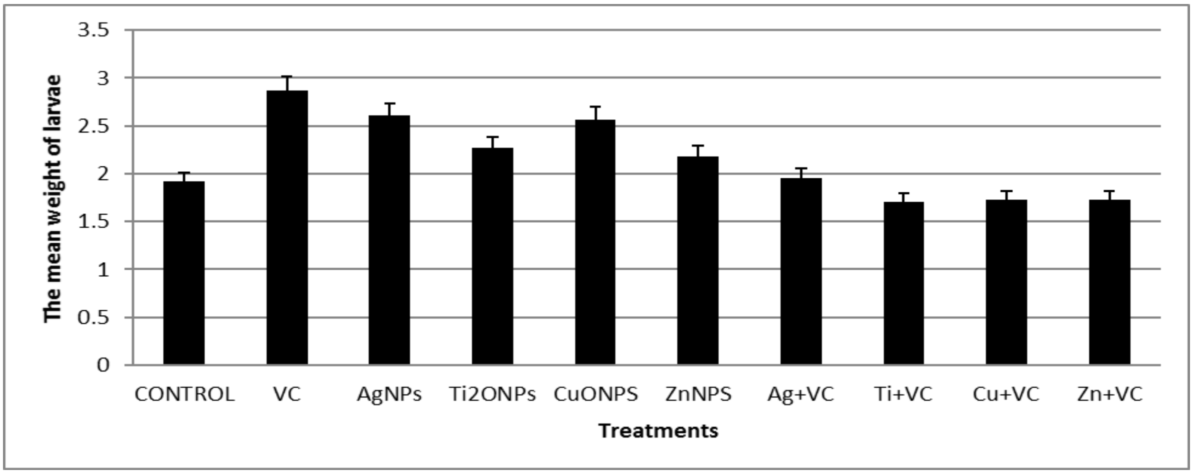

Figure 1. The effect of Ascorbic acid, tested nanomaterials and their mixtures on the weight of 5th larval instar of silkworm *B. mori.*

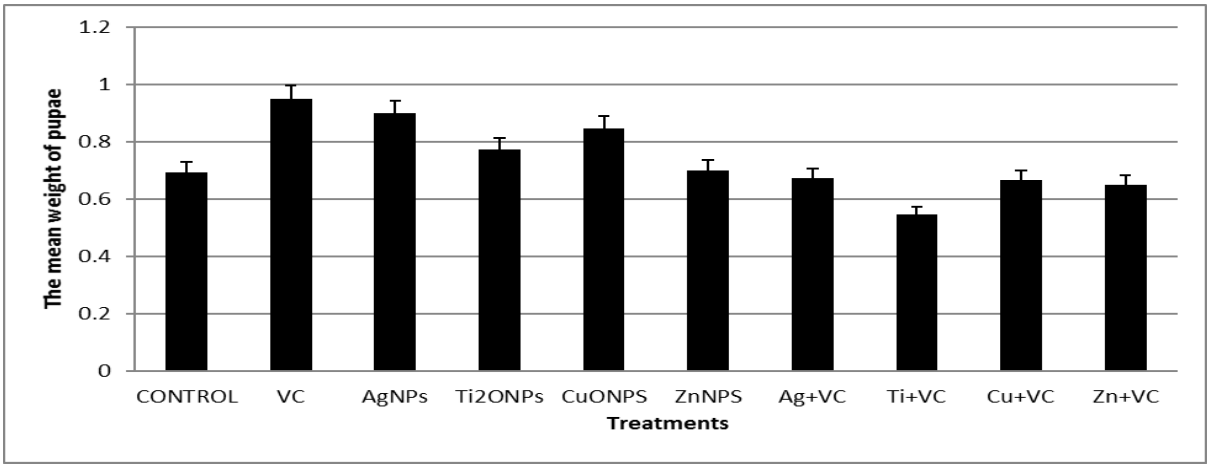

Figure 2. The effect of Ascorbic acid, tested nanomaterials and their mixtures on the pupal weight of silkworm *B. mori.*

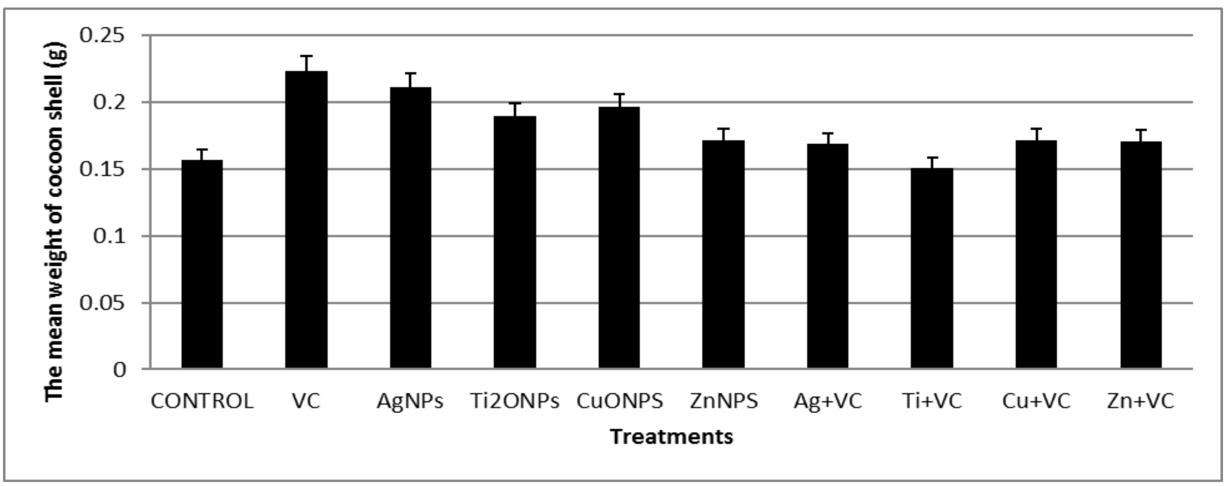

Figure 3. The effect of Ascorbic acid, tested nanomaterials and their mixtures on the cocoon shell weight of silkworm *B. mori.*

Feeding 5th larval instar of *B. mori* with ascorbic acid (T_1) increased the pupal weight and cocoon shell weight by about 36.97 and 42.26 %, respectively more than control. Followed by AgNPs (T_2) which significant increased the pupal weight about 29.53 % and cocoon shell weight about 34.71 % more than control.

The present results are in agreement with the observations of earlier workers (Kl-Karkasy and Idriss, 1990; Ramadevi *et al.*, 1992; Chenthilnayaki *et al.*, 2004; Balasundaram *et al*., 2012; Ponraj *et al.*, 2011; Panraj *et al.*, 2012).

Balasundaram *et al.* (2008) revealed that the weight of cocoon was maximum in silkworm larvae when fed with vitamin C treated mulberry leaves than MR2 leaf variety. Similar trend was observed by Udupa, (1986) and Tayade, (1987), on *B. mori* in relation to heterosis effect on economic traits of new hybrids. Bose *et al.* (1995) have reported that succulent mulberry leaves with less fiber and higher mineral contents presumably stimulated the metabolic activities in silkworm, resulting in qualitative improvement of cocoon and silk. Sengupta *et al*. (1972) have reported that silk production increased with 1% ascorbic acid in the diet of silkworm. On the other hand, Pandiarajan *et al.* (2016) found that AgNPs at the concentration 100 ppm were able to produce lethal effects on pupation and adult development, with accumulative hazard in silkworm. However, Ponraj *et al.* (2011) suggested that AgNps stimulated silkworm to feed more amount of nutrients intake than the control. This work is corroborated with Kl-Karkasy and Idriss, (1990), suggested that this enhancement in larval and cocoon length, width and weight related to phagostimulation of ascorbic acid.

Furthermore, Feeding of 5th instar larvae of silkworms with the mixture of nanomaterials and VC (T_6, T_7, T_8 and T_9) increased the larval duration by about 12 ± 1days while in the case of other treatments (T_1, T_2, T_3, T_4 and T_5) the larval duration were 10± 1 days as compared with the control which was 9± 1days.

Determination of Haemocyte Types

The light microscopic observations of the stained larval hemolymph revealed the presence of four morphologically distinct types of heamocytes. Prohaemocytes (Pr), Granulocytes (Gr), Plasmatocytes (Pl) and Oenocytoides (Oe).

All treated groups clearly affected the differential haemocytes counts of 5th instar larvae of *B. mori.*

With regard to the tested nanoparticle treatments and their mixtures, a reduction in the number of the prohaemocyte was observed after (T_2, T_3, T_4 and T_5) to be 4.3, 5.6, 5.2 and 4.0 cell, respectively. Furthermore, ascorbic acid treatment (T_1) caused a significant increase in the number of prohaemocyte to be 6.3 cells while control was 4.5 cells (Table 1). However, a reduction was observed in the number of granulocyte after treatment with (T_2, T_3, T_4 and T_5) to be 4.3, 5.6, 5.2 and 4.5cell. The same trend was observed in the number of granulocyte to be 33.3, 32.5, 33.7 and 33.4cells, respectively while control was 35.3 cells (Table 1).

Table 1. Effects of Ascorbic acid, tested nanomaterials and their mixtures on the different haemocyte count in 5th larval instar of *B.mori.*

Concentrations		Pr	Gr	Pl	Oe
Ascorbic acid (1%)	T1	6.3 ± 0.5^a	45.4 ± 0.5^a	39.5 ± 1.2^a	9.6 ± 0.5^a
AgNPs (50 µg/ml)	T2	4.3 ± 0.5^b	33.3 ± 1.3^c	33.5 ± 0.9^a	5.8 ± 0.2^d
TiO2(50 µg/ml)	T3	5.6 ± 0.4^a	32.5 ± 1.6^b	32 ± 1.2^a	5.6 ± 0.6^a
CuO (50 µg/ml)	T4	5.2 ± 0.2^a	33.7 ± 1.9^c	34.2 ± 1.7^c	5.3 ± 0.3^b
ZnNPs(50 µg/ml)	T5	4.0 ± 0.2^b	33.4 ± 1.7^c	31.2 ± 1.5^c	5.1 ± 0.6
AgNPs+VC	T6	3.6 ± 0.2^c	28.2 ± 1.5^d	26.2 ± 0.5^c	3.7 ± 0.4
TiO2+ VC	T7	3.2 ± 0.2^c	22.3 ± 0.9^e	24.2 ± 0.7^c	3.5 ± 0.4
CuO+ VC	T8	3.6 ± 0.2^c	25.1 ± 1.3^d	22.2 ± 0.4^c	3.7 ± 0.3
ZnNPs+VC	T9	3.5 ± 0.2^c	23.4 ± 0.7^e	21.2 ± 0.6^c	3.4 ± 0.3
Control		4.5 ± 0.3^b	35.3 ± 0.6^b	38.1 ± 1.3^b	6.2 ± 0.2^c

-Pr: Prohaemocyte, Gr: Granulocyte, Pl: Plasmatocyte Oe: Oenocyte.

- Mean in same column followed by the same letters is not significant.

-Probability level at 0.05.

Moreover, the recorded showed that the number of plasmatocyte significantly decreased being 33.5, 32.1, 34.2 and 31.2 cell, after treatment with (T_2, T_3, T_4 and T_5), respectively while control was 35.3cells. The same trend was observed in the number of oenocyte. On contrast, ascorbic acid treatment (T_1) significantly increased the number of granulocytes, plasmatocytes and oenocytoides to be 45.4, 39.5 and 9.6 cells, respectively while control were 35.3, 32.1 and 6.2 (Table 1). On the other hand, all mixtures treatments (T_6, T_7, T_8 and T_9) significantly decreased the different haemocyte counts. The present results in agreement with Li *et al*. (2017) who observed that injection the last larval instar of *B.mori* with Cadmium Telluride Quattro Dots(CdTe QDs), Silica nanoparteciales (SiNPs), or Citric acid nitrogen depending carbon dots (C–NCDs) exerted significantly decrease the number of silkworm hemocytes.

CONCLUSION

In the present study, the enrichment of mulberry leaves treated with AgNps, TiO_2NPs, CuONPs, ZnONPs at concentration 50µg/l and ascorbic acid (VC) at concentration 1% separately increased the weight of larvae, pupae and cocoon shell.

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.

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الملخص العربي

تأثير حامض الأسكوربيك وبعض المواد النانوية وخلائطها على بعض الخصائص البيولوجية والفسيولوجية لدودة القز

احمد سليمان، عبير جاد

أجريت هذه الدراسة لتحديد تأثير بعض المواد النانوية مثل الفضه واكسيد التيتانيوم واكسيد النحاس واكسيد الزنك بتركيز ٥٠ ميكروجرام/ لتر وكذلك حامض الأسكوربيك بتركيز ١٪ وخلائط هذه المواد مع الأسكوبيك اسد علي بعض الخصائص البيولوجية والفسيولوجية لدودة الحرير التوتية كوزن اليرقات، ووزن العذاري، ووزن قشرة الشرنقة (جم).

أوضحت الدراسة أن الخصائص الاقتصادية لدودة الحرير قد تم تحسينها عند استخدام حامض الأسكوربيك بتركيز ١٪ وجميع المواد النانوية المختبرة بتركيز ٥٠ ميكروجرام/ لتر وذلك عند المقارنه بالكنترول، وعلى النقيض من ذلك، لم يحسن خليط اي من المواد النانويه المختبره وحامض الاسكوبيك اي من الصفات الاقتصادية لدودة الحرير.

وقد اشارت هذه الدراسه ان استخدام المواد النانويه المختبره او حامض الاسكوربيك بشكل منفصله يمكن ان يزيد من انتاج الحرير الطبيعي.