

## **INFLUENCE OF FORTIFICATION OF MULBERRY LEAVES WITH NATURAL AND SYNTHETIC MULTIVITAMINS ON GROWTH AND DEVELOPMENT OF *Bombyx mori* L.**

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### **ABSTRACT**

Fourth instar of silkworm larvae, *Bombyx mori* L. were fed on mulberry leaves fortified with two different multivitamin sources, natural (bee honey) and synthetic (Pharovit iron). The impact of the larval, cocoon, shell and pupal weight, filament length and weight and the number of breaks during reeling (filament size) were examined. Different results on biological parameters were obtained based on used concentrations or treatments. All the tested concentrations of two multivitamins increased filament size than control except the least concentration of Pharovit iron. Results also established highly significant elevation in the total soluble protein and increased number of protein bands, while decreased significantly the activity of transaminase enzymes (AST and ALT) either in honey or Pharovit treatments comparing to control. Thus, fortification of mulberry leaves with honey enhance protein metabolism, consequently, improving the commercial qualities of filament size, that's very important in sericulture.

**Keywords:** multivitamins; growth; development; biochemistry; *Bombyx mori*

### **INTRODUCTION**

The mulberry silkworm, *Bombyx mori* L., (Bombycidae: Lepidoptera) is reckoned to be one of the commercial insects, producing the finest natural silk, that called the "Queen of textiles". Additionally, the silkworm larvae have a high medicinal value and are usually used to reduce blood pressure and heart problems (Fenemore and Prakash, 1992).

*Bombyx mori* is essentially monophagous and survives solely on mulberry leaves which play an important role in nutrition of the silkworms and in turn cocoon and silk production (Nagaraju, 2002). One of the alternative ways of improvement of larval feeding is enrichment of mulberry leaves with supplementary nutrients such as vitamins (Rajabi *et al.*, 2007). Sengupta *et al.*, (1972) showed that *B. mori* requires specific essential sugars, aminoacids, proteins and vitamins for its normal growth survival and for the silk gland growth. Also, Akhtar and Asghar (1972) found that vitamins and mineral salts played an important role in the nutrition of silkworm.

Wherever nutrition is an important growth-regulating factor in silkworm, hence this study was designed to compare the effects of two cheaper compounds, both of them enriched with vitamins and multi minerals, the first one is natural source (honey) while the other is artificial one (Pharovit iron) on the growth of silkworm, some biological parameters and subsequently, the silk filament. Furthermore, the relationship between proteins and silkworm development was investigated.

## MATERIALS AND METHODS

### 1. Tested compounds:

#### 1.1. Bee honey:

Honey was obtained from Plant Protection Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation in Giza, Egypt. Honey is composed of sugars and minerals like magnesium, potassium, calcium, iron sulphur and phosphate while copper, iodine and Zinc appear in small quantities. Besides the above mentioned, it contains vitamins; B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>5</sub> and C.

#### 1.2. Pharovit iron solution (Multivitamin supplement):

It contains vitamins; A, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, C, D<sub>3</sub> and E. in addition, nicotinamide, calcium pantothenate, calcium gluconate and ferrous gluconate produced by Pharaonia Pharmaceuticals (Pharo Pharma), New Borg El-Arab city, Alexandria, Egypt.

### 2. Rearing technique:

The mulberry silkworm, *B. mori* (Egyptian hybrid, Giza) was obtained from the Sericulture Research Department, Plant protection Research Institute, and reared in the laboratory with standard rearing technique (Krishnaswami, 1979) under 25°C with R. H. of 75±5%. The larvae were fed on mulberry leaves, *Morus alba* variety (Balady) up to the last instar.

### 3. Treatments:

Three different concentrations (1.0%, 3.0% and 5.0%) of both honey and Pharovit Iron were prepared. The fresh mulberry leaves were soaked in each concentration for 30 seconds, and then dried in air. The treated leaves were fed twice to the freshly molted fourth and 5<sup>th</sup> instar larvae (only the first feeding). The rest ones till spinning were fed on untreated leaves. Control insects were simultaneously reared on fresh mulberry dipped in distilled water and dried in air. Three replicates per each concentration and control containing 100 healthy larvae were maintained.

The growth of larvae was determined after attaining maturity. Larval mortality %, larval duration (day), pupation and cocooning percentages were recorded. After the formation of cocoons, ten randomly cocoons were harvested from each replicate and cut very carefully at one end obliquely with a sharp blade to observe and weight pupae individually on an electronic balance on milligrams. (Nirwani and Kaliwal, 1996). Silk content ratio was calculated according to Tanaka, (1994) Formula:

$$\text{Silk content ratio (\%)} = \frac{\text{Weight of fresh cocoon shell (mg.)}}{\text{Weight of fresh cocoon (mg.)}} \times 100$$

### 4. Reelable silk filament parameters:

Ten cleaned cocoons of each replicate were dried in an oven at 80 °C for 6 hrs, then reeled using individual reeling machine and filament length (m.) was recorded. Each reelable filament was dried and weighed (gm.) and its size (denier) was calculated according to the formula of Tanaka (1964):

Reelable filament size of cocoon (denier) =

$$\frac{\text{Weight of silk filament (mg.)}}{\text{Length of silk filament (m.)}} \quad \times 9000$$

## **5. Biochemical studies:**

### **5.1. Haemolymph preparation:**

Three random samples from 5<sup>th</sup> instar larvae in their 6<sup>th</sup> day were selected for each replicate and one of their prolegs was cut. The haemolymph was collected in microtubes and 1 mg phenylthiourea added immediately to prevent melanization. The samples were centrifuged at 14,000 rpm for 10 min. the supernatant was removed and kept in -20 °C for analysis.

### **5.2. Analysis of total soluble protein:**

Protein was measured as total soluble protein in assay kit (Diamond, DP International, Heliopolis, Cairo, Egypt) with bovine albumin primary as the standard protein, according to the method described by Gornall *et al.*, 1949.

### **5.3. AST and ALT enzymes:**

The activities of Aspartate transaminase (AST) and Alanine transaminase (ALT) were measured in serum using kits (Diamond, DP International, Heliopolis, Cairo, Egypt) according to the method of Reitman and Frankle (1957).

### **5.4. Polyacrylamide gel electrophoresis (PAGE):**

The method of Laemmli (1970) is used for monodimensional electrophoresis.

## **6. Statistical analysis:**

The significance of the main effects was determined by analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range test ( $p < 0.05$ ) (Snedecor and Cochran 1980). Data were subjected to statistical analyses using a software package CoStat<sup>®</sup> Statistical Software (2005) a product of Cohort Software, Monterey, California.

## **RESULTS AND DISCUSSION**

### **1. Larval characteristics:**

The larval duration in treated larvae showed varied results (Table, 1). Larvae fed on mulberry leaves fortified with 1.0, 3.0 and 5% concentrations of honey survived for  $10.92 \pm 0.107$ ,  $11.12 \pm 0.081$  and  $10.89 \pm 0.121$  days, respectively and were highly significant still feeding after the control insects went into the pupal stage ( $10.58 \pm 0.098$  days),  $P=0.000$ . In contrary, larvae fed with 1.0, 3.0 and 5.0% concentrations of multivitamin (Pharovit iron) caused highly significant decrease in the duration that lasted  $9.26 \pm 0.032$ ,  $9.19 \pm 0.026$  and  $9.70 \pm 0.061$  days, respectively,  $P=0.000$ .

Results indicated that all tested concentrations of Pharovit iron caused intensive highly significant mortality in the larval stage especially the highest one;  $33.0 \pm 2.082$ ,  $36.0 \pm 3.464$  and  $60.0 \pm 3.464\%$ , respectively,  $p=0.000$ . While the tested concentrations of honey caused  $14.0 \pm 1.155$ ,

15.33±1.453 and 15.33±0.667%, respectively. Control gave 7.0±0.577, Table (1).

**Table (1): Effect of mulberry leaves fortified with multivitamins on *B. mori*.**

Treatment	Conc. %	Larval duration (4 <sup>th</sup> and 5 <sup>th</sup> instars) /days	Larval mortality %	Pupation	Cocoon %
Control	-	10.58±0.098 <b>b</b>	7.00±0.577 <b>d</b>	93.00±0.577 <b>a</b>	91.33±0.667 <b>a</b>
Honey	1	10.92±0.107 <b>a</b>	14.00±1.155 <b>c</b>	86.00±1.155 <b>b</b>	85.00±1.155 <b>b</b>
	3	11.12±0.081 <b>a</b>	15.33±1.453 <b>c</b>	84.67±1.325 <b>b</b>	83.00±1.00 <b>b</b>
	5	10.89±0.121 <b>a</b>	15.33±0.667 <b>c</b>	84.67±1.325 <b>b</b>	82.33±0.882 <b>b</b>
Pharovit iron	1	9.26±0.032 <b>d</b>	33.00±2.082 <b>b</b>	67.00±2.082 <b>c</b>	66.33±1.667 <b>c</b>
	3	9.19±0.026 <b>d</b>	36.00±3.464 <b>b</b>	64.00±3.464 <b>c</b>	63.00±2.887 <b>c</b>
	5	9.70±0.061 <b>c</b>	60.00±3.464 <b>a</b>	40.00±3.464 <b>d</b>	38.00±2.309 <b>d</b>
<b>P value</b>		0.0000***	0.0000***	0.0000***	0.0000***
<b>L. S. D.<sub>-0.05</sub></b>		0.2502	6.5412	6.543	5.127

Data expressed as Mean ± S. E.

\*\*\*= p ≤ 0.01

Mean under each variety having different letters in the same column denote a significant different (p ≤ 0.05).

## 2. Cocoon characteristics:

The results of this experiment indicated that, all the tested larvae caused reduction in the pupation percentages than control, which recorded 93.0±0.577% pupation, (Table, 1). This decrease ranged between a minimum value of 84.67% for larvae fed on mulberry leaves fortified with honey at both concentrations 3.0 and 5.0% to a maximum value of larvae fed on Pharovit iron 40.0±3.464%. Additionally, Pharovit iron showed significant reduction in pupation percentages especially the higher concentration than the remaining treatment, P= 0.000.

The cocoon percentages were recorded 91.33±0.667% for control. The cocoon percentages were recorded (85.0±1.155, 83.0±1.00 and 82.33±0.882 %) for honey and (66.33±1.667%), (63.0±2.887 %) and (38.0±2.309 %) for Pharovit iron, respectively. Higher significant decrease in the cocoon percentages was observed by Pharovit iron with special regard to 5.0% concentration comparing to other treatments, P=0.000. The maximum fresh cocoon weight (FCW) that contain shell and pupae was recorded 1.1.306±0.02 gm for larvae treated with 1.0% honey, whereas, the minimum one gave 1.156±0.04 gm for lower concentration of Pharovit iron, (FCW) of control gave 1.21±0.017 (Tables,1& 2).

Generally, the data of (FCW) exerted significant improved performance using all the tested concentrations of honey and only 3.0% of Pharovit iron over the control, P= 0.0087.

As a general trend, the average weight of pupae developed from all used concentrations were significant higher than that of control especially honey at 1.0 % concentration (1.026±0.009 gm) except Pharovit iron at the least used concentration (0.880±0.04 gm). Control recorded 0.933±0.02 gm, P= 0.0189.

The cocoon shell weight elevated from 0.277±0.003 gm in the control to 0.280±0.012, 0.287±0.009 and 0.293±0.003 gm as affected by honey 1.0 and 3.0% and Pharovit iron at 3.0%, respectively. The rest used concentrations reduced weight comparing to control, such variation was statistically significant, P= 0.055 (Table, 2).

The observation resulted to Silk Ratio (SR %) in Pharovit iron at 1.0 and 3.0 % (23.48±0.829 and 23.44± 0.448 %) being significantly higher than other concentrations and control, P= 0.0449. Following by 22.89±0.581, 22.78±0.852, 21.95±0.205, 21.41±0.555 and 21.01±0.283% for control, honey at 3.0, 5.0 and 1.0 % concentrations and Pharovit at the higher concentration, respectively, (Table, 2).

**Table 2. Effect of mulberry leaves fortified with multivitamins on various cocoon characteristic of *B. mori*.**

Treatment	Conc. %	Fresh Cocoon weight	Shell Weight	Pupae Weight	Silk Ratio %
Control	-	1.210± 0.017 <b>bc</b>	0.277± 0.003 <b>ab</b>	0.933± 0.020 <b>bc</b>	22.89± 0.581 <b>ab</b>
Honey	1	1.306± 0.020 <b>a</b>	0.280± 0.012 <b>ab</b>	1.026± 0.009 <b>a</b>	21.44± 0.555 <b>bc</b>
	3	1.260± 0.009 <b>ab</b>	0.287± 0.009 <b>ab</b>	0.973± 0.017 <b>ab</b>	22.78± 0.852 <b>ab</b>
	5	1.230± 0.012 <b>b</b>	0.270± 0.000 <b>b</b>	0.960± 0.012 <b>ab</b>	21.95± 0.205 <b>abc</b>
Pharovit iron	1	1.150± 0.040 <b>c</b>	0.270± 0.012 <b>b</b>	0.880± 0.040 <b>c</b>	23.48± 0.829 <b>a</b>
	3	1.250± 0.035 <b>ab</b>	0.293± 0.003 <b>a</b>	0.957± 0.032 <b>b</b>	23.44± 0.448 <b>a</b>
	5	1.190± 0.012 <b>bc</b>	0.250± 0.006 <b>c</b>	0.940± 0.006 <b>bc</b>	21.01± 0.283 <b>c</b>
<b>P value</b>		0.087 **	0.0055**	0.0189*	0.0449*
<b>L S D<sub>0.05</sub></b>		0.0714	0.0187	0.0687	1.768

Data expressed as Mean ± SE

\*= p≤ 0.05 \*\*= p≤ 0.01

Mean under each variety having different letters in the same raw denote a significant different (p≤ 0.05).

### 3. Filament characteristic:

The filament obtained from cocoons produced by larvae fed on different concentrations of honey and Pharovit iron were significantly shorter than the filament obtained from control except at 1.0% concentration, (Table 3). This decrease ranged between a minimal value of 1561.10±26.038 m for 1.0% honey to a maximum value of 834±9.851m for Pharovit at 5.0%, control gave 1593.50±15.30 m, P= 0.0000.

Likewise, the mean filament weight developed from the control (0.2554±0.0131 gm) heavier than other treatments with the exception of honey at 1.0 % concentration, that recorded (0.2575±0.007 gm). The difference among the means revealed highly significant effect, P= 0.0000. Pharovit iron at 5.0 % gave the least significant effects that recorded (0.1326±0.003 gm), Table (3).

However, all the used concentrations of honey and Pharovit iron at 3.0% concentration increased the size of filament (denier). Honey at the highest concentration was the most potent causing highly significant increase in the size (1.7345±0.033 dn.). While, Pharovit iron at 1.0% concentration gave the least significant effect among the tested concentrations (1.3459±0.022 dn.), control recorded (1.4425±0.060 dn.), P= 0.0132.

**4. Biochemical characteristics of the haemolymph:**

**4.1. Effect on protein and enzymes:**

The changes in the level of total soluble protein and activity of transaminase enzymes; aspartate amino transferase (AST) and alanine amino transferase (ALT) in *B. mori* larvae were shown in Table (4).

All the biochemical characteristics that were measured except total soluble protein showed highly significant reduction either in honey or Pharovit iron treatments comparing to control, with qualitative differences among tested concentrations. Total soluble protein was recorded (54.40±3.40 g/ml) for control, the lowest increase was (55.20±3.00 g/ml) for bee honey at the least tested concentration, while the highest significant increase gave (78.20±1.30g/ml.) for Pharovit iron at the highest concentration, P= 0.0000.

**Table (3): Effect of mulberry leaves fortified with multivitamins on various filament characteristic of *B. mori*.**

Treatment	Conc. %	Filament Length (cm.)	Filament Weight (gm.)	Filament Size (denier)
Control	-	1593.50±	0.2554±	1.4425±
		15.300 a	0.013 a	0.060 bc
Honey	1	1561.10±	0.2575±	1.4845±
		26.038 a	0.007 a	0.016 bc
	3	1042.65±	0.1872±	1.6159±
		9.844 c	0.008 c	0.014 ab
	5	1085±	0.2091±	1.7345±
		9.528 c	0.002 bc	0.033 a
Pharovit iron	1	1381.50±	0.2066±	1.3459±
		8.372 b	0.002 bc	0.022 c
	3	1361.0±	0.2295±	1.5176±
		11.836 b	0.006 ab	0.026 bc
	5	834.0±	0.1326±	1.4309±
		9.851 d	0.003 d	0.011 bc
<b>P value</b>		0.000***	0.0000***	0.0132*
<b>L. S. D. <sub>0.05</sub></b>		42.992	0.0281	0.1852

Data expressed as Mean ± S. E.

\*\*\*= p≤ 0.01

Mean under each variety having different letters in the same column denote a significant different (p≤ 0.05).

**Table (4): Changes in the effect of total soluble protein (gm./ml.) and activities of transaminase enzymes (AST and ALT, µg pyruvate/L./min.) in 5<sup>th</sup> instar of silkworm fortified by honey and Pharovit iron.**

Treatment	Conc. %	Total Soluble Protein	AST	ALT
Control	-	54.40± 3.40 d	270.11± 0.597 a	55.944± 3.342 a
Honey	1	55.20± 3.00 d	225.278± 2.986 bc	38.963± 0.054 c
	3	59.50± 0.5 cd	263.214± 1.99 a	44.434± 1.906 b
	5	63.50± 1.20 bc	249.076± 0.199 b	51.793± 2.342 a
Pharovit iron	1	59.60± 1.20 cd	173.042± 3.284 f	15.566± 2.015 d
	3	67.80± 0.5 b	196.49± 1.094 d	17.548± 0.217 d
	5	78.20± 1.30 a	183.386± 6.271 e	13.396± 1.416 d
<b>P value</b>		0.0000***	0.0000***	0.0000***
<b>L. S. D.<sub>0.05</sub></b>		5.96	9.214	5.218

Data expressed as Mean ± S. E.

\*\*\*= p≤ 0.01

Mean under each variety having different letters in the same column denote a significant different (p≤ 0.05).

As for transaminase enzymes, control were recorded (270.110±0.597 & 55.944±2.342 µg pyruvate/ L./min.) for AST and ALT, respectively. The reduction in AST ranged between (263.214± 1.990) for honey at 3.0% concentration to 173.042± 3.284 for Pharovit iron at the least concentration, P= 0.0000.

The highest significant decrease 13.396±1.416 recorded by Pharovit iron at 5.0% concentration, whereas the least significant one gave 51.793±2.342 for the highest concentration of honey, P= 0.0000

#### **4.2. Effect on electrophoretic pattern of *B. mori* soluble protein:**

The electrophoretic pattern of haemolymph soluble protein of 5<sup>th</sup> instar silkworm larvae was presented in Fig. (1).

Fractionation of proteins exhibited obvious variations in the number and position of bands between treated and untreated larvae. Total soluble protein content in the haemolymph of treated larvae showed an increase in the number of bands comparing to control. The number of protein bands were more in samples treated with 3.0 and 5.0% of bee honey and 1.0 and 5.0% of Pharovit iron that exhibited (11.0, 10.0, 10.0 and 9.0 bands, respectively).

Control and other rest concentrations gave 8.0 bands. These numbers of protein bands coming down in all the samples. Thickly stained bands were observed in the samples treated with 3.0, 5.0% of Pharovit iron comparing to other samples, Fig. (1).

**Fig. 1. Poly acrylamide gel electrophoretic pattern of haemolymph protein of 5<sup>th</sup> instar of silkworm larvae.**

Multivitamins (Pharovit iron) at all the tested concentrations especially the higher one caused intensive mortality in the larval stage comparing to control and honey. Molting was disrupted in each stage and many larvae died. In the silkworm, Saha and Khan (1996) described the extensive effects of multi-vitamin compounds as diet factors in growth interruption. Excess amount of vitamins C in silkworm diet have negative effects and decrease feeding (Etebari *et al.*, 2004). Furthermore, Etebari and Matindoost (2004) found that the high quantity of vitamin B<sub>3</sub> administered in larva food leads to feeding interruption, accident in larvae development and to an increase mortality during shedding.

Similar fluctuation results in the larval duration of *B. mori* were observed by many authors. Saikatsu *et al.*, (1989) and Ebeid (1993) revealed that the growth and development speed of the silkworm were quickened by addition of royal jelly. On the other hand, Etebari and Matindoost (2004) recorded increase in the larval stage to 31 days and only a few larvae turned into pupae as affected by hypervitaminosis with vitamin B<sub>3</sub>.

In recent study, positive trend was noted between fresh cocoon weight and pupae weight, thus 1.0% concentration of honey gave the highest significant differences as opposed to the other treatments at both parameters. Whereas, 1.0% concentration of Pharovit iron caused the least significant effect. Additionally Pharovit iron at 3.0% and 5.0% concentrations recorded the highest and least significant difference in the shell weight, respectively. This observation resulted to the silk ratio % in 1.0% and 5.0% concentrations of Pharovit iron being significantly highest and lowest that of other treatments, respectively.



Cocoon weight is an important commercial characteristic used to determine approximately the amount of raw silk that can be obtained. Shell weight gives a better measure, but can not be determined in commercial cultures because it requires damaging the cocoon. The difference between the two measures is the weight of the pupa (Gaviria *et al.*, 2006). Also, Quader *et al.*, (1992) found that nutritional value of mulberry leaves was directly reflected on cocoon characters of *B. mori*, that's may be explained the difference in cocoon characteristic among the treatments. Nguku *et al.*, (2007) indicated that, the royal jelly-enhanced diet significantly increased cocoon and pupal weight, but had no significant effect on shell weights compared to control. Moreover, Bentea *et al.*, (2011) reported that the use of such substances (mineral and vitamin additives) improve the quality parameters of cocoon.

In spite of the filaments obtained from cocoons produced by larvae fed on honey and Pharovit iron were shorter and lighter than that obtained from control, the size of filaments (denier) increased at all used concentrations of honey and at 3.0% concentration of Pharovit iron, and consequently decreased reeling breaks of silk that are the most important commercial characters in the improvement of silk quality and yield (Kamimura and Kiuchi, 1998). Reversly, Nguku *et al.*, (2007) found that royal jelly affected on the length and weight of filaments without any effect on its size comparing to control.

The proteins play an important role in the haemolymph of insects not only in specific transport functions, but also in their enzyme action. Hurlimann and Chen (1974) reported that, the concentration of protein in haemolymph increases progressively during larval development and reaches maximum in the late fifth instar larvae. Krishnasawami (1978) observed that the protein concentration in the silkworm body after the fourth molt is due to regular feeding and substantial increase in the body weight by the time the larva attains spinning stage. It is also reported that, the concentration of protein in silkworms increase progressively during larval development and reaches maximum in the late fifth instar larvae, or attributed to the development of reproductive organs (Sinha and Sinha, 1994). The protein in the tissue of sericigenous insects is responsible for the formation of silk protein in the silk glands.

From the current study, all the used concentrations of both treatments, elevation total soluble protein comparing to control. Similar results were recorded by Raju *et al.*, (2012) when studied the effect of tumeric on protein metabolism of silk worm. On contrary, caused reduction in the amino transferase enzymes than control. The variation in the concentration of protein present in this study could be ascribed to the differential tested concentrations and also due to difference in the physiological activities of treatments.

The major transmission reactions are performed by alanine amino transferase and aspartate amino transferase (Lehninger, 1993). Alanine amino transferase recorded a higher activity compared to aspartate amino transferase all through the embryonic development in silk worms (Pant and Kumar, 1979). The inhibition occurred in transaminase enzymes may be

resulting from its consumed to produce amino acids which the key constituents of protein.

Fractionation of haemolymph soluble protein resulting from larvae reared on mulberry leaves enriched with multivitamins, either natural source (bee honey) or synthetic (Pharovit iron) were characterized by obvious variations in the number and position of separated soluble protein bands among concentrations of two tested treatments, these bands were absent in control. Moreover, Lokesh and Anantha Narayana (2011) when analyzed the protein contents during 5<sup>th</sup> instar of *B. mori* treated with Diethyl sulphate, found to be higher in treated sets compared to control, also showed variations among the treatment of different concentrations. This might be caused by the action of vitamins C and B that increase food consumption and conversion leading to increased amounts of protein amino acids and other nutrients contributing to growth of silk worm. The same conclusion was obtained by Sarker *et al.*, (1995) who found that silk content increase when ascorbic acid added to the diet. Additionally, vitamin C also aids in detoxification of various metabolic or tissue toxins and acts as strong antioxidants, increasing protein synthesis. Activity levels of enzymes in the midgut, fat body tissues and silk glands increase as levels of dietary vitamin B6 increase (Horie and Nakamura, 1986). About 40-65% of the total nitrogen in honey is protein and some nitrogen residues are amino acids (Lee *et al.*, 1990). Thus, fortification of mulberry leaves with multivitamins either bee honey or Pharovit iron enhance protein metabolism, that is very important in silk production. Furthermore, nutrition is an important growth regulating factor in silkworm, like in any other organisms. Various minerals take part in regulation of osmotic pressure of intra and extra cellular fluids in the maintenance of an anionic balance suitable for the activity of living cells and as co-factors in some enzyme systems and as integral part of other systems. The known essential trace elements for insects growth include iron, nickel, manganese, zinc and iodine that obtained in multivitamins (honey and Pharovit iron).

## **CONCLUSION**

In conclusion, it is noted that fortified the mulberry leaves with two different sources of multivitamin supplements, nature (honey) and synthetic (Pharovit iron) recorded radically different results on biological and biochemical effects based on the used concentrations or treatments they were administrated at. In addition, honey elicit favorable response in improving the commercial qualities of silk fiber (filament size) than Pharovit iron, thus can subsequently be used in sericulture for yield enhancement.

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### تأثير تدعيم أوراق التوت بالفيتامينات الطبيعية و المصنعة على نمو وتطور دودة القز

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غذيت يرقات العمر الرابع لدودة القز على أوراق نبات التوت المضاف اليها مصدرين من مصادر الفيتامينات العديدة، فيتامين طبيعى (عسل نحل) و فيتامين صناعى (فاروفيت آيرون) وتأثير ذلك على كل من اليرقات، الشرائق، غلاف الشرنقة، وزن العذارى، طول ووزن الخيط الحريرى وسمكه.

تباينت نتائج التأثيرات على القياسات البيولوجية باختلاف التركيز المستخدم من مصدرى الفيتامينات حيث أدت الى زيادة قياسات الخيط الحريرى باستثناء اقل تركيز مستخدم من (فاروفيت آيرون). كما سجلت النتائج زيادة معنوية فى البروتين الكلى الذائب وزيادة عدد شرائح البروتين، وعلى العكس حدث انخفاض معنوى فى الإنزيمات الناقلة لمجموعة الأمين لكل من عسل النحل و فاروفيت آيرون مقارنة بالكنترول.

وعليه فإن تدعيم أوراق التوت بالعسل تزيد من بناء بروتين جسم الحشرة و بالتالى زيادة مواصفات الجودة فى خيط الحرير الناتج و هو الهدف النهائى من نشاط إنتاج حرير القز.

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