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Foliar Fertilizers Impact on Biochemical Analysis of Silkworm, *Bombyx mori* L.

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Abstract: Mulberry silkworm, *Bombyx mori* L. used for produce natural silk from incident time. Mulberry plant is the soul plant for feeding silkworm larvae, quality of mulberry leaves effect on silkworm growth and cocoon quality. Experiment made by using three foliar fertilizers to improver mulberry leaves. Concentrations of 0.1 %, 0.3% and 1.3 % were prepared from three foliage fertilizers Ascobein, Novatrin and Citreen. Leaves of mulberry were immersed in each concentration for ten minutes. Silkworm *B. mori* L. Larvae were divided into two groups, first group, treated from first instar to end of the third instar (young instars), second group treated from first instar until spinning (whole instars). Blank treatment was immersed in distilled water and control treatment kept with any application. Different instars of silkworm, *B. mori* L. haemolymph were analyzed to estimate of total protein, carbohydrate and lipids content. Data revealed that, treatment T_2 (Novatreen) has best results for total content of protein, lipids and carbohydrates. Highest concentration is better results than others for the same parameters. Foliage fertilizer Novatreen with high concentration or Ascobein and Citreen can used for improved the silk production, in case of poor quality of mulberry leaves, shortage in fertilizers and in reclaimed lands.

Key words: Foliar fertilizers · Total protein · Total carbohydrates · Total lipids · Silkworm · *Bombyx mori* L.

INTRODUCTION

The silkworm, *Bombyx mori* L. (Order: Lepidoptera, Family: Bombycidea) is a monophagous insect feed only on mulberry leaves to convert leaf protein into silk. Silk activities are one of the agricultural and industrial projects that plays an effective role in improving the economics for some countries. Quality of silk is depending on many factors include; nutrients in the mulberry, *Morus alba* L. biochemical status; hormonal level in the larvae and conditions of environment [1]. Proteins, carbohydrates, lipids found in mulberry leaves play an essential role in the biochemical process of insect growth and development [2]. Deficient of mulberry leaves elements is affecting the quality and quantity of cocoon [3, 4]. Scientists have demonstrated a lot of efforts to enhance silk production through improve the quality and quantity of mulberry leaves such as hormones, plant extracts, antibiotics, vitamins, mineral, soya protein, herbal products, vermicompost, and fertilizers [5-10].

Mulberry leaves, *M. alba* (L.) should be supplemented with various nutrients to help productivity of silk quality and quantity [11, 12]. Foliar nutrients are frequently applied topically to increase production, improve quality, and make up for trace element deficits. Mulberry plants respond quickly to foliar nutrients, Protein and carbohydrate activity changes in silkworm *B. mori* haemolymph after treatment foliar applicant [13].

All living organisms need mainly carbohydrates, which serve as an energy source for all essential processes. Carbohydrates play an effective role as energy source and protecting silkworm during adverse condition [14]. Later stages (fourth and fifth instars) of development's haemolymph, which contains a higher concentration of carbohydrates, may be used to provide the energy needed for the activities that result in the construction of the cocoon, the pupal and adult cuticles, and other developmental structures [15-17].

Lipids are important and has an effective role in chitin synthesis [18]. Furthermore, lipids are energy reserves in the fat body that used during moulting, oogenesis and embryogenesis [19]. Lipids, known as fats, are a heterogeneous group and naturally occurring edible fats [20-21]. Womeni *et al*. [22] explained that, insects and their larvae are potential sources of lipids. The growth, development and economic characters are influenced to

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a great extent by the nutritional content of the mulberry leaf [23]. The present experiments were an attempt to enhance the quality of mulberry leaves using some foliage fertilizers on the different instars of silkworm, *B. mori* (L.) through evaluate biochemical analysis.

MATERIALS AND METHODS

Silkworm Resource: Hybrid silkworm eggs, *B. mori* L. Giza A were collected from Research Department of Sericulture, Plant Protection Research Institute, Agriculture Research Center- Giza during Spring season.

Fertilizers Resource: Foliage fertilizers of Ascobein (T_1), Novatrin (T_2) and Citreen (T_3) were attained from General Organization for Agriculture Equalization Fund. Distilled water used for dilution as blank treatment (T_4) and control treatment kept with any treatment (T_5). Three concentrations for each treatment were prepared (0.1 %, 0.3% and 1.3 %) and coded as C_1, C_2 and C_3, respectively. Ascobein fertilizer consists of organic matter, Ascorbic and citric acid. Novatreen is containing macro and micro elements (Nitrogen, Phosphorus, Potassium, Iron, Manganese, Zinc, Boron, Molybdenum). Citreen is consisting of claw micro elements (Iron, Manganese, Zinc).

Rearing Technique: The rearing laboratory and equipments were thoroughly disinfected with 5% formaldehyde solution. Newly hatched *B. mori* larvae were brushed in plastic trays (60 x 90 x 7 cm). Room temperature was 24.9°C ± 0.118°C and relative humidity was 72.02% ± 5.693. Fresh mulberry leaves of *M. alba* var Canava-2 were offered four times daily to fed larvae. Polythene sheets as bottom and cover and wet foams used for young instars [24]. Disinfectants were applied by using muslin cloth on the larvae body and beds in the determined days as described by Hosny *et al.* [25]. Collapsible frame was used for mountage mature larvae.

Treatments and Concentrations: Silkworm larvae *B. mori* L. were divided into two groups, first group, treated from brushing to end of the third instars (young instars). Second group treated from first instar until spinning (whole instars). Leaves of mulberry were immersed in each concentration for ten minutes. The treated mulberry leaves were drained and then used for fed to the larvae of silkworm. Blank treatment was immersed in distilled water and control treatment kept without any application. Seventh day of fifth instar larvae were used for collecting the heamolymph by cutting the abdominal legs and collect in eppendorf tubes with small amount of phenyl thiourea (phenyl thiourea prevent melanization). Biochemical analysis was estimated in Unite of Fine Chemical Analysis Pest-Physiology Research Department Plant Protection Research- Agriculture Research Center. Total proteins were determined by the method of Bradford [26]. Total carbohydrates were estimated in acid extract of sample by the phenol-sulphuric acid reaction of Dubois *et al*. [27]. Estimation of total lipids was applied by the method of Knight *et al*. [28].

Statistical Analysis: All the Data collected in this experiment were subjected to ANOVA analysis of variance multi-way test to determine the significance between corresponding parameters of the treated groups by using SAS program [29].

RESULTS AND DISCUSSION

Data in Table 1, 2 and 3. showed the effect of foliar fertilizers on total protein content (mg/ml) of *B. mori* haemolymph between treatments, instars, treatments x instars, concentrations, interactions between concentrations x instars, treatments x concentrations x instars and treatments x concentrations.

Treatment T_2 is the best treatment followed by T_3 and T_1. Treatment T_2 for whole instars is superior to young instars. There were highly significant differences between concentrations, the best concentration was C_3 followed by C_2 and C_1. Highly significant differences appeared for the interaction between concentrations and instars. All concentrations are higher for treatment with whole instars than the young instars treatment. Also, highly significant differences were noticed for the interactions between treatments, concentrations and instars. Treatment T_2 is better for treated young instars. Also, is better for treatment young and grown instars for all concentrations. Highly significant differences were observed for the interaction between treatments x concentrations. Treatment T_2 represented high results for C_3, C_2 and C_1, respectively. This is may be due to foliage fertilizer enrich by macro and micro elements which enhanced silk production.

These results are in agreements with those found by Ito and Niminura [30] and Horie *et al.* [31] reported that, potassium, and some microelements are essential for the silkworm growth. So, leaves of mulberry were sprayed over with macro and micronutrients to fed the *B. mori* L., and notes that all silkworm growth parameters were found

Table 1: Effect of foliar fertilizers on total protein content (mg/ ml) of silkworm haemolymph between treatments, instars and treatments x instars

	Treatment									
Instars	T_1	T_2	T_3	T_4	T_5	Mean	F Instar	LSD 0.05	FTrt x Instar	LSD 0.05
Young Instars	57.650	58.450	57.850	42.100	42.100	51.630	3407.890**	0.423	111.890**	7.139
Whole Instars	68.217	72.483	62.317	58.800	58.050	63.973				
Mean (Trt)	62.933	65.4667	60.083	50.450	50.075					
F (Trt)					912.780**					
LSD 0.05					0.669					

where: Trt=treatments (T_1, T_2, T_3, T_4 and T_5), (*) significant at 0.05, (**) highly significant at 0.01

Table 2: Effect of foliar fertilizers on total protein content (mg/ml) of silkworm haemolymph between concentrations, concentrations x Instars and treatments x concentrations x instars

		Treatment										
Concentration		T_1	T2	T_3	T_4	T_5	Average Concx Instar	Mean Conc	FConc	LSD 0.05	F Concx Instar	LSD 0.05
C_1	Y	54.200	53.000	55.350	42.100	42.100	49.350	55.060	181.02**	0.518	6.45*	5.337
	W	62.000	65.300	59.700	58.800	58.050	60.770					
C_2	Y	59.400	59.000	59.150	42.100	42.100	52.350	58.515				
	W	68.750	74.900	62.900	58.800	58.050	64.680					
C_3	Y	59.350	59.050	63.350	42.100	42.100	53.190	59.830				
	W	73.900	77.250	64.350	58.800	58.050	66.470					
FTrt x Conc x Instar								4.49**				
LSD 0.05								11.616				

where: Trt=treatments (T_1, T_2, T_3, T_4 and T_5), Conc= concentrations (C_1, C_2 and C_3), Y= young instars, G=Whole instars (*) significant at 0.05, (**) highly significant at 0.01

Table 3: Effect of foliar fertilizers on total protein content (mg/ml) of silkworm haemolymph between treatments concentrations

	Treatment				
Concentration	T_1	T_2	T_3	T_4	T_5
C_1	58.100	59.150	57.525	50.450	50.075
C_2	64.075	66.950	61.025	50.450	50.075
C_3	66.625	70.300	61.700	50.450	50.075
F TrtxConc			39.59**		
LSD 0.05			11.616		

where: Trt=treatments (T_1, T_2, T_3, T_4 and T_5), Conc= concentrations (C_1, C_2 and C_3), (*) significant at 0.05, (**) highly significant at 0.01.

Table 4: Effect of foliar fertilizers on total lipids content (mg/ml) of silkworm haemolymph between treatments, instars and treatments x instars

	Treatment									
Instars	T_1	T_2	T_3	T_4	T_5	Mean	F Instar	LSD 0.05	FTrt x Instar	LSD 0.05
Young Instars	4.417	5.133	4.717	3.300	3.150	4.143	409.45**	0.107	42.55**	1.322
Whole Instars	5.983	7.017	6.150	3.850	3.150	5.230				
Mean	5.200	6.075	5.433	3.575	3.150					
F Trt						440.07**				
LSD 0.05						0.170				

where: Trt=treatments (T_1, T_2, T_3, T_4 and T_5), (*) significant at 0.05, (**) highly significant at 0.01

Table 5: Effect of foliar fertilizers on total lipids content (mg/ml) of silkworm haemolymph between concentrations, treatments x concentrations and treatments x concentrations x instars

		Treatment									
Concentration		T_1	T_2	T_3	T_4	T_5	Mean	FConc	LSD 0.05	FConcx Instar	LSD 0.05
C_1	Y	4.250	4.383	4.250	3.300	3.150	4.143	130.49**	0.132	33.50**	0.954
	W	4.550	5.750	4.800	3.850	3.150					
C_2	Y	4.350	5.517	4.550	3.300	3.150	4.712				
	W	5.850	7.150	6.250	3.850	3.150					
C_3	Y	4.650	5.500	5.350	3.300	3.150	5.205				
	W	7.550	8.150	7.400	3.850	3.150					
Ftrt x Conc x Instar							7.62**				
LSD 0.05							0.416				

where: Trt=treatments (T_1, T_2, T_3, T_4 and T_5), Conc= concentrations (C_1, C_2 and C_3), Y= young instars, G=Whole instars (*) significant at 0.05, (**) highly significant at 0.01

Table 6: Effect of foliar fertilizers on total lipids content (mg/ml) of silkworm haemolymph between treatments x concentrations

	Treatment				
Concentration	T_1	T_2	T_3	T_4	T_5
C_1	4.400	5.067	4.525	3.575	3.150
C_2	5.100	6.333	5.400	3.575	3.150
C_3	6.100	6.825	6.375	3.575	3.150
F Trt x Conc			23.07**		
LSD 0.05			1.653		

where: Trt=treatments (T_1, T_2, T_3, T_4 and T_5), Conc= concentrations (C_1, C_2 and C_3), (*) significant at 0.05, (**) highly significant at 0.01

Table 7: Effect of foliar fertilizers on total carbohydrates content (mg/ml) of silkworm haemolymph between treatments, instars and treatments x instars

	Treatment									
Instars	T_1	T_2	T_3	T_4	T_5	Mean	F Instar	LSD 0.05	FTrt x Instar	LSD 0.05
Young Instars	9.017	10.067	8.967	8.900	8.350	9.060	3059.21**	0.187	384.37**	3.149
Whole Instars	18.300	17.717	16.267	10.500	8.350	14.227				
Mean	13.658	13.892	12.617	9.700	8.350					
F Trt						565.79**				
LSD 0.05						0.295				

where: Trt=treatments (T_1, T_2, T_3, T_4 and T_5), (*) significant at 0.05, (**) highly significant at 0.01

Table 8: Effect of foliar fertilizers on total carbohydrates content (mg/ml) of silkworm haemolymph between concentrations, treatments x concentrations and treatments x concentrations x instars

		Treatment									
Concentration		T_1	T_2	T_3	T_4	T_5	Mean	FConc	LSD 0.05	FConc x Instar	LSD 0.05
C_1	Y	8.500	8.800	6.500	8.900	8.350	10.285	217.11**	0.229	38.23**	2.375
	W	16.250	16.500	10.200	10.500	8.350					
C_2	Y	9.400	10.500	10.500	8.900	8.350	12.130				
	W	19.050	18.750	17.000	10.500	8.350					
C_3	Y	9.150	10.900	9.900	8.900	8.350	12.515				
	W	19.600	19.850	19.860	10.500	8.350					
Ftrt x Conc x Instar							16.48**				
LSD 0.05							0.724				

where: Trt=treatments (T_1, T_2, T_3, T_4 and T_5), Conc= concentrations (C_1, C_2 and C_3), Y= young instars, G=Whole instars (*) significant at 0.05, (**) highly significant at 0.01.

Table 9: Effect of foliar fertilizers on total carbohydrates content of silkworm haemolymph between treatments x concentrations

	Treatment				
Concentration	T_1	T_2	T_3	T_4	T_5
C_1	12.375	12.650	8.350	9.700	8.350
C_2	14.225	14.625	13.750	9.700	8.350
C_3	14.375	15.275	14.875	9.700	8.350
F Trt X Conc			72.91**		
LSD 0.05			4.493		

where: Trt=treatments (T_1, T_2, T_3, T_4 and T_5), Conc= concentrations (C_1, C_2 and C_3), (*) significant at 0.05, (**) highly significant at 0.01

to be enhanced [32-34]. Foliar application of 'seriboost' significantly improved larval weight of silkworms [35]. Further, Bose *et al*. [36]. also cleared the improve total larval duration and larval weight. Ankalgi and Ansari [37] developed foliar spray of triacontonal and fasal which is effectively used in silkworm disease management. Nickel chloride can be used at low concentration for enhancing the economic character of silkworm, *B. mori* [38]. Furthermore, enrichment mulberry leaves with $ZnCl_2$ elevated the protein levels in silk gland and haemolymph [39].

Tables 4, 5 and 6 represented the effect of foliar fertilizers on total lipids content (mg/ml) of *B. mori* haemolymph between treatments, instars, treatments x instars, concentrations, treatments x concentrations and treatments x concentrations x instars. Highly significant differences were obtained for concentrations, the interaction between treatments x concentrations and treatments x concentrations x instars.

Highly significant differences were obtained between treatments, instars and the interactions between instars x treatments. Lowest results were found for control and blank treatments while T_2 has best results. Treated whole instars were better than young instars only. T_2 is the best treatment for young instars and whole instars.

Highest concentration of C_3 is better followed by C_2 and C_1. Treated whole instars is better for all concentrations and the higher concentration is the best concentration. Moreover, highly significant differences were obtained for the interaction between treatments and concentrations. T_2 is the highest treatment for all concentrations. C_3 is the highest concentration for all treatments followed by C_2 and C_1. Lipids content improved using foliar fertilizers may be due to the fertilizers containing macro and microelements. These results are accordance with findings of Choudhury *et al*. [40] showed the increased nutritional status and concentration of micronutrients in mulberry leaves obtained due to combined supplementation of nano ZnO + nano Cu might have stimulated the metabolic activities in silkworm resulting in better growth and development subsequently silk production.

Effect of foliar fertilizers on total carbohydrates content (mg/ml) of silkworm haemolymph between treatments, instars, treatments x instars, concentrations, treatments x concentrations and treatments x concentrations x instars. Differences between concentrations and the interactions between treatments x concentrations, treatments x concentrations x instars and treatments x concentrations were illustrated in Tables 7, 8 and 9.

Highly significant differences were noticed within treatments, instars and the interactions between treatments x instars. All treatments were better than blank and control treatments. Treatment with Novatreen fertilizer (T_2) is the highest treatment. Treated whole instars are better than treated young instars. Highest concentration C_3 is the best concentration followed by C_2 and C_1. Also, the same results observed for the interactions between concentrations and instars where, higher concentration C_3 is the best concentration for the young instars and whole instars treatments. Interaction between treatments, concentrations and instars raveled that, T_2 and concentration C_3 for whole instars is the best than other treatments. Treatments with foliar fertilizers lead to increase the content of carbohydrates this may be due to the foliar fertilizers consist of macro and micro elements.

The data are in agreement with the results collected by Bhattacharyya, A. and Medda [41], Bajapeyi *et al*. [42]. And Qader *et al*. [43]. who indicated that, zinc plays a vital role in the synthesis of lipids, protein and carbohydrates and in reducing the duration of larval. In addition, Prashanth Kumar and Umakanth [44]. showed that, the grown age of silkworm larvae accumulate higher carbohydrates compared to young age worms therefore there is a gradual increase in carbohydrate content with the advancement of the age of the larvae. Also in line with the previous finding by Bose *et al*. [45]. who proved that foliar micronutrients significantly increased the total carbohydrates in mulberry leaves, subsequently leads to increase the carbohydrate content in treated larvae. The raise may be attributed to the fact each micronutrient plays a significant role in physiological and biochemical process of mulberry and silkworm larvae.

Also, Kumar and Michael [46]. revealed that carbohydrates increased gradually from first day of fifth instar after applicant foliar fertilizer (green leaf) in the haemolymph of silkworm hybrid. Total sugar and reducing sugar had also shown similar trends in midgut and haemolymph. Foliar nutrients can play a significant role in sustainable improvement of mulberry plants and silkworm, *B. mori* (L.) productivity [47].

CONCLUSION

Foliar fertilizers play an important role in sustainability silkworm production. Three foliage fertilizers Ascobein, Novatreen and Citreen along with three concentrations for young or whole instars have better results than blank and control treatments. Therefore, significantly increased the total proteins, lipids and carbohydrates in haemolymph content of treated silkworm larvae, *B. mori* (L.). Treatment of Novatreen with high concentration during whole instars is superior treatment. Hence, data recommended that, foliage fertilizer Novatreen with high concentration or Ascobein and Citreen can used for improved the silk production, especially in case; paucity of mulberry leaves quality, shortage fertilization, plantation in reclaimed land.

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