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Biochemical analysis for mulberry silkworm *Bombyx mori* L. fed with treated leaves by some foliage fertilizers

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Abstract
Three foliar fertilizers viz., Ascobein, Novatreen and Citreen were tested for increasing mulberry quality and their effects on biochemical analysis of silkworm *Bombyx mori* L. Enzymes of alpha-esterase, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were estimated. Mulberry leaves dipped in three concentrations (1.33 g/L, 2.66 g/L and 13.33 g/L) of each fertilizer.
Highly significant differences observed between treatments. Both of control and blank treatments have lowest values. Treatment of Novatreen was the best one followed by Ascobein and Citreen treatments for alpha-esterase and transaminases (GOT) & (GPT) enzymes. So that, Novatreen, Ascobein and Citreen were recommended to use (Novatreen, followed by Ascobein and Citreen, respectively) in case of shortage of common fertilizers and in traditional rearing when leaves of mulberry trees wide speared on the canals and road sides. These trees dose not fertilized and the quality of leaves is very low.

Keywords: mulberry silkworm, *Bombyx mori*, foliage fertilizers, enzymes, micronutrients, macronutrients

1. Introduction
Mulberry silkworms produce the precious material in the world. Silkworm, *Bombyx mori* L., is monophagase insects [1]. The growth and development of silkworm are dependent on the mulberry leaves components. Mulberry leaf quality is dependent on the variety, agronomic practices, maturity, position of the leaf, and season. Feeding with good quality mulberry leaves that meet the requirements of silkworm larvae throughout the growing period results in high silk productivity [2]. A mulberry field with fertile soil, high planting density, luxuriantly growing branches and leaves with high productivity requires greater quantities of manure and fertilizers [3]. The quality of mulberry leaves alone contributes to the tune of 38.2 % [4]. Silk production affected by the quality of mulberry leaves. Many researchers over all the world attempt to increase the quality of mulberry leaves through food additives or using different kinds of fertilizers [5, 6, 7, 8, 9].

Silkworms as all insects need macro and micronutrients. As well as larvae needed appreciable amounts of potassium, phosphate and magnesium [10]. Larval nutrition and nutritive value of mulberry leaves is very essential for silk production which producing good quality cocoons [11]. Many of the heamolymph proteins are enzymes. It is play important role in the economy of insects [12]. Synthesis of silk amino acids is leading by transamination reaction, among the numerous enzymes, which reversibly catalyze the transfer of an amino group from an amino to an α- Keto acid, there are aspartate amino transferase (Glutamate Oxaloacetate Transaminase) and alanin amino transferase (Glutamate Pyruvate Transaminase) [13].

Main aims of these experiments are to improve the quality of mulberry leaves. Find best solution for traditional rearers who depended on spread trees over channels and road sides. Also, when the lack of common fertilizers.

2. Materials and methods
Experiments were design to raise the quality of mulberry leaves. Three foliage fertilizers Ascobein, Novatreen and Citreen were applied. It obtained from General Organization for Agriculture Equalization Fund. (G. O. A. E. F.) - Ministry of Agriculture. It was codes as A, N and C, respectively. Three concentrations were prepared (1.33 g/L, 2.66 g /L and 13.33 g /L) for all fertilizers. Lower concentrations was take number 1, middle 2 and higher one take 3. Distilled water used for dilution.

Ascobein fertilizer consists of organic matter, Ascorbic and citric acids. Novatreen is containing macro and micro elements (Nitrogen N, Phosphorus P, Potassium K, Iron Fe Claw, Manganese Mn Claw, Zinc Zn Claw. Boron B, Molybdenum Mo). Citreen is containing claw micro elements (Iron Fe, Manganese Mn, Zinc Zn).

Mulberry leaves were soaked in each concentration for five minutes. Also, some leaves were soaked in distilled water as blank treatment. While, control treatment kept without any application. Three replicates were conducted for each treatment, containing 300 larvae. Frist group was fed on treated leaves from first to the end of third instar (G_3). Second group fed from first to the end of fifth instar (G_5) with treated mulberry leaves of *Morus Alba* var Kokuso-27.

Imported hybrid of mulberry silkworm, *Bombyx mori* L., from Bulgaria (G_2 X V_2 X H_1X KK) was utilized for experiments. Treated leaves chopped during first to the third instar. Humidity was adjusted by wet foam and polyethylene sheets [14]. Whole leaves offered during fourth to fifth instar.

Room temperature and humidity were registered. Average of temperature and humidity were 25.133 ºC ±1.074 and 53.633 % ± 11.461.

Haemolymph collected by cut the abdominal legs of pre-mature larvae in abndrof tubes containing a small crystalline

of phenyl thiorea for preventing melanization and then freeze according to Takai and Tamashiro [15].

Biochemical analysis made in Unite of Fine Chemical Analysis Pest-Physiology Research Department-Plant Protection Research Institute-Agriculture Research Center-Ministry of Agriculture and Land Reclamations. Estimation of alpha-esterase, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) enzymes [16, 17].

Statistical analysis using SAS program was adopted for all estimated data [18].

3. Results & Discussion

Analysis of variance between treatments, instars, the interactions between both of treatments and instars, treatments with concentrations, treatments with concentrations and instars for alpha-esterase enzyme estimation were found in Table 1.

Table 1: Biochemical analysis of alpha-esterase enzyme (µg α-naphthol/min /ml) for different treatment of some foliage fertilizers.

Fertilizers		Treated instar G_3	Treated instar G_5	Average TR X CON	Average Treatments	F Between Treatments	LSD 0.05	F TRTXCON	LSD 0.05	F TRT X CONX INSTAR	LSD 0.05
Ascobein	A_1	561.500	675.500	618.500	690.917	2652.660**	5.437	100.990**	136.300	50.840**	13.319
	A_2	585.500	835.500	710.500							
	A_3	602.000	885.500	743.750							
	Mean	583.000	798.833								
Novatreen	N_1	631.500	726.500	679.000	751.417						
	N_2	665.000	857.000	761.000							
	N_3	675.500	953.000	814.250							
	Mean	657.333	845.500								
Citreen	S_1	622.500	636.000	629.250	662.083						
	S_2	630.500	695.000	662.750							
	S_3	669.500	719.000	694.250							
	Mean	640.833	683.333								
Blank		539.000	618.500	578.750	578.750						
Control		498.500	498.500	498.500	498.500						
Average of instars		583.733	688.933								
F Between Instar		3744.490**									
LSD 0.05		3.439									
F (TRT X Instar)		588.420**									
LSD 0.05		103.500									

Where: (TRT = treatments, CONC = concentrations 1, 2 and 3) & (G3 young instars, G5 Whole instars) (*) significant at 0.05, (**) highly significant at 0.01.

Highly significant differences observed between treatments. Both of control and blank treatments have lowest values. Treatment of Novatreen was the best one followed by Ascobein and Citreen treatments.

Same trend obtained between instars. Treated during whole instars (G_5) were better than treated the first three instars only (G_3).

High Concentration (N_3) of Novatreen was best value. As well as concentration three of Ascobein and citreen was better than others. Treated whole fifth instars (G_5) were better average for Novatreen, Ascobein and Citreen treatments than treated only first three instars (G_3).

Differences of interaction between treatments, concentrations and instars were highly significant. Treated whole instars by high concentration of Novatreen were the best.

Figure.1. showed the differences between concentrations and the interactions between instars and concentrations of estimated alpha-esterase enzyme. The high concentration is better than the others. High concentration of the groups (G_3 and G_5) is the best.

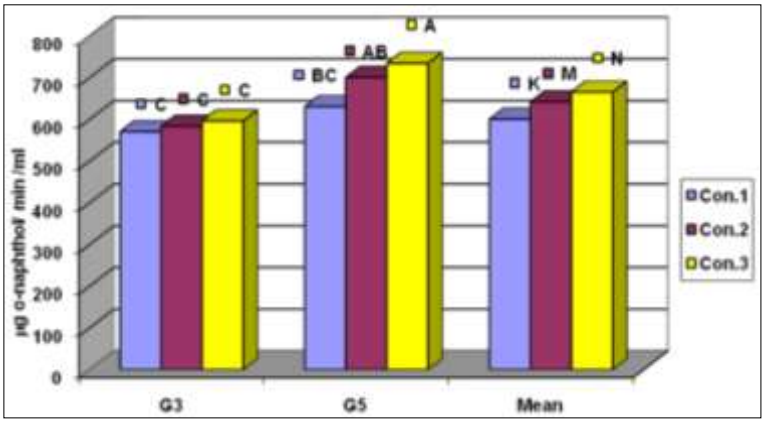

Fig 1: Estimation of alpha-esterase enzyme during third and fifith instars of silkworm Bombyx mori.

From the previous results, higher concentration of Novatreen fertilizer is the best, treated whole fifth instars is better values than others. Because of Novatreen contains macro and micro elements so that application of mulberry

leaves may be enhanced the quality. Similar results were obtained by Ito & Niminura and Hori *et al.* [19, 20]. Who reported that iron element is very necessary for mulberry silkworm larvae. It has been observed that iron has a potentially to enhance the larval development and cocoon yield.

Quality and quantity of mulberry leaves are raised by using macro and micro nutrients as well as cocoon yield [21, 22, 23]. Also, Lokanath *et al.* [24]. Recorded that, from the viewpoint of silkworm nutrition, it is seen that Mg, Fe, B and Mn have potential to enhance the larval development and cocoon yield.

As shown in table 2. Highly significant differences were appeared between treatments, instars, interactions between both of treatment with concentration, treatments with instars and treatments with concentrations and instars for glutamic oxaloacetic transaminase (GOT) assessment.

Table 2: Biochemical analysis of glutamic oxaloacetic transaminase GOT enzyme (mU /ml) for different treatment of some foliage fertilizers

Fertilizers		Treated instar		Average TR X CON	Average TRT	F Between TRT	LSD 0.05	F TRTXCON	LSD 0.05	F TRT X CONX Instar	LSD 0.05
		G_3	G_5								
Ascobein	A_1	158.000	188.500	173.250	390.500	9177.400**	4.303	1907.350**	259.570	1476.830**	10.539
	A_2	209.000	692.000	450.500							
	A_3	210.000	885.500	547.750							
	Mean	192.333	588.667								
Novatreen	N_1	191.500	251.000	221.250	458.167						
	N_2	235.000	857.000	546.000							
	N_3	266.000	948.500	607.250							
	Mean	230.833	685.500								
Citreen	S_1	159.000	201.500	180.250	305.667						
	S_2	179.000	270.000	224.500							
	S_3	189.000	835.500	512.250							
	Mean	175.667	435.667								
Blank		127.000	162.500	144.750	144.750						
Control		131.000	131.000	131.000	131.000						
Average of instars		171.367	400.667								
F (Between Instar)		28409.400**									
LSD 0.05		2.721									
F(TRT X Instar)		4586.530**									
LSD 0.05		225.750									

Where: (TRT = treatments, CONC = concentrations 1, 2 and 3) & (G_3 young instars, G_5 Whole instars) (*) significant at 0.05, (**) highly significant at0.01.

Similar trends obtained for the differences between concentrations and interactions between concentrations and Instars (groups) (figure 2).

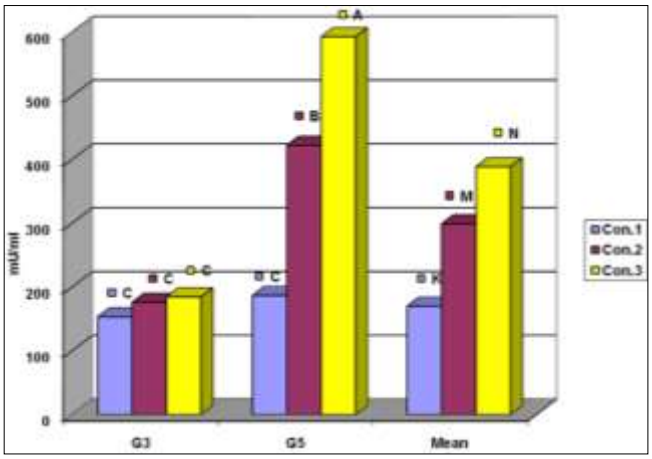

Fig 2: Estimation of glutamic oxloacetic transaminase (GOT) enzyme during third and fifith instars of silkworm *Bombyx mori*

All treatments of Ascobein, Novatreen and Citreen fertilizers enhanced average of GOT enzyme comparing with blank and control treatments. Concentration 3 was the best one.

A Noatreen has higher average followed by Ascobein and Citreen fertilizer. This may be due to the improved the mulberry quality by this fertilizer which contains macro, micro, citric and ascorbic acids.

These results are coincidences with those found by Bhattacharya and Kaliwal [25].

Who reported that zinc is more particularly mineral nutrition which has an essential role in lipids, proteins and carbohydrates synthesis? In addition K, Mg and Fe are essential for the silkworm and Mn and Co accelerate growth [19, 20].

Analysis of variances of glutamic pyruvic transaminase (GPT) enzyme was found in Table 3 and figure 3. Data reviled that, highly significant differences between treatment, concentration, instars (G_3 and G_5), and between interactions both of treatment with concentration, treatment with instars, concentration with instars and treatments with instars and concentrations.

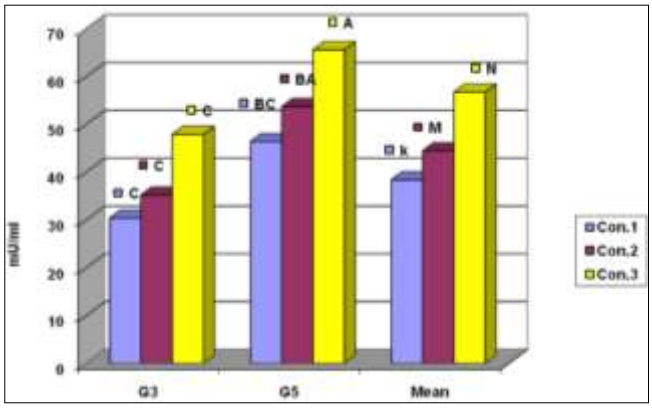

Fig 3: Estimation of glutamic pyruvic transaminase (GPT) enzyme during third and fifth instars of silkworm *Bombyx mori*

Table 3: Biochemical analysis of glutamic pyruvic transaminase GPT enzyme (mU /ml) for different treatment of some foliage fertilizers.

Fertilizers		Treated instar G_3	Treated instar G_5	Average TRT X CON	Average TRT	F Between TRT	LSD 0.05	F TRT X CON	LSD 0.05	F TRT X CON X Instar	LSD 0.05
Ascobein	A_1	27.500	32.000	29.750	53.833	1166.370**	1.921	137.720**	31.158	34.870**	4.705
	A_2	33.500	56.000	44.750							
	A_3	68.000	106.000	87.000							
	Mean	43.000	64.667								
Novatreen	N_1	35.000	92.500	63.750	80.000						
	N_2	50.000	104.500	77.250							
	N_3	89.000	109.000	99.000							
	Mean	58.000	102.000								
Citreen	S_1	41.000	57.000	49.000	49.333						
	S_2	44.000	57.500	50.750							
	S_3	34.000	62.500	48.250							
	Mean	39.667	59.000								
Blank		27.000	29.000	28.000	28.000						
Control		21.500	21.5000	21.500	21.500						
Average of instars		37.833	55.233								
F Between Instar		820.730**									
LSD 0.05		1.215									
F (TRT X Instar)		172.060**									
LSD 0.05		25.321									

Control followed by Blank treatment was lowest average of glutamic pyruvic transaminase (GPT) enzyme. Concentrations of Novatreen treatments were the best means. High concentration was better the others. These may be due to enhancement of mulberry leaves quality. Because of Novatreen contains macro and micro elements.

The previous results are agreements with the findings of Bhattacharya and Medda, Younus Wani *et al*. [26, 27] who registered that mineral nutrition plays vital role in lipids, proteins and carbohydrates synthesis.

4. Conclusions

Three foliar fertilizers (Novatreen, Ascobein and Citreen) with three concentrations were used for dipping mulberry leaves. Two groups of silkworm *Bombyx mori* L. were treated. For the first three instars (G_3) and the other group for whole fifth instars (G_5). Both of control and blank treatments have the lowest values of alpha-esterase, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) enzymes. High concentration of Novatreen is the best followed by Ascobein and Citreen. Treated whole instars G_5 were better than the first three instars.

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