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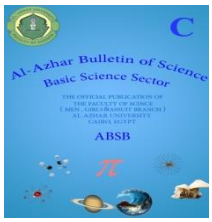

A COMPARATIVE STUDY ON TWO HYBRIDS OF SILKWORM *BOMBYX MORI* (L.) REARED ON MULBERRY LEAVES *MORUS ALBA* VAR. KANAVA-2.

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ABSTRACT

The recent investigation was carried out at the Department of Plant Protection, Faculty of Agriculture, Al-Azhar Univ., Nasr City, Cairo, Egypt. The comparative Study on two hybrids of silkworm *Bombyx mori* (HoxK1xR3xT3) from (Produced by sericulture and agriculture experiment station, Vra Tza, Bulgaria silkworm) *B.mori*, were obtained from the Sericulture Research Department of Plant Protection Research Institute, Agricultural Research Centre, Giza- Egypt, and (J444) native hybrid reared on mulberry leaves *Morus alba* var. kanava-2. silk production as well as quality of silk cocoon depends on the nutritional value of mulberry leaves and larval growth. Obtained results illustrated that feeding of silkworm larvae of imported hybrid previously highlighted by breeding programs for production of superior domestic hybrid for adoption in the breeding programs in Egypt presented higher and consistent growth rates than the native one for all the developmental stages, such as grown larvae weight, larval duration, fresh cocoon weight, cocoon shell weight and, cocoon shell ratio, pupa weights, pupation ratio, cocooning percentage and silk productivity. Moreover, technological parameters included filament length, weight and size (denier). Also, such feeding led to shorter larval duration and decreased larval mortality percentages.

Keywords: *Bombyx mori*; Growth parameters; Mulberry; *Morus alba*; Silkworm hybrid.

1. Introduction

The mulberry silkworm *B. mori* is a commercial exploited variety utilized on large scale production of silk [1, 2]. It is totally specialized insect as it feeds only on mulberry leaves to make its cocoon, producing the silk filament**.** Sericulture is one of the most a task industry in many countries of the world, especially India and China, India is the second largest producer of silk with 18475 MT (2006-07) and also the largest consumer of silk in the world (In India, mulberry silk is produced mainly in the states of Karnataka, Andhra Pradesh, Tamil Nadu, Jammu & Kashmir and West Bengal, while the no-mulberry silks are produced in Jharkhand, Chattisgarh, Orissa and north-eastern states [3], however, in Egypt is not well developed due to many reasons; one of these is shortage of good hybrid with high productivity. The development of silk industry largely based upon the use of developed breeds of silkworm, the production of quality leaves through better mulberry varieties and means of rearing [4]. Nutritional study on silkworms is an important requisite for its proper commercial exploitation and is lone factor which augment quality and quantity of silk. The main amino acid glycine, alanine, serine and tyrosine are made by in the silk gland cell by transfer process by Naguchi *et al*. [5] Mulberry leaves are rich in proteins and amino acids, amino-transferees based on the uptake of nitrogen from mulberry leaves by body tissues and silk glands and silk protein synthesis [6, 7] illustrated that, protein has a vital biochemical role in the development, morphogenesis and almost in all intermediary metabolic pathways of insects and since the key organic constituent, their function in mechanism of silkworm is essential. Hemolymph which is an important body fluid is imported for regulation of the homeostasis constitutes plasma proteins, which function in different activities similarity maintaining cell cultures and defense against microbial infection and in anti-apoptosis: and therefore, it is necessary to evaluation imported hybrids to determine their suitability to our climatic condition as suggested by Mahmoud and Ashour [8]. The present study aims to shed light on biological, economic and technological characteristics of the two hybrids of the silkworm under the Egyptian conditions that can be utilized in different breeding programmes as for the synthesis of superior hybrids due to lack of pure lines of silkworm.

2. Materials and Methodology

2.1. Silkworm rearing technique

Rearing was taking place under health conditions according to Krishnaswami [9]. Each hybrid was reared in three replicates (100 larva/ replicate), larvae were divided into two groups and each group was fed four times a day on the fresh

mulberry of *Morus alba* var kanava-2. The young larvae (1st to 3rd instars) were reared at 26 ± 2° C, 75 ± 5% (R.H.) and the late age larvae (4th and 5th instars) were maintained at 24 ± 2° C with R.H. of 70 ± 5%. Starting the fourth larval instar, 300 larvae were counted from each hybrid and retained for other studies. Larval weights of fifth instars were recorded at 7th day from the start 5th larval instar while larval hemolymph samples were picked up at 6th day for biochemical analysis by Ham and McKeehan, [10]. The spinning larvae were picked up manually and mounted in plastic collapsible frames montages. Hatchability, larval weights of grown instars, pupal weight, pupation ratio, larval mortality, cocooning%, single cocoon, weight single shell weights and cocoon shell ratio fecundity, fertility were estimated.

2.2. Biochemical analysis of silkworm

2.2.1. Total proteins were evaluated by the method of Bradford [11]. The results were expressed as (mg/mL).

2.2.2. Free amino acids: Total amino acids were colorimetricaly evaluated by ninhydrin reagent according to the means described by Lee and Takabashi [12]. Amino acids were expressed as mg D, L- alanine /ml.

2.2.3. Alkaline phosphatase (U/L)): Acid and alkaline phosphatases were estimated according to the method described by Powell and Smith [13]. The enzyme activity is expressed by unit (U/L).

2.2.4. Transaminases (GOT and GPT): Glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) were determined calorimetrically according to the method of Reitman and Frankel [14]. The enzyme activity is expressed as U/gm. body weight.

2.3. Statistical analysis: Biological, economic and technological parameters data were subjected to t-test by utilizing Costat software program [15] at probability level of P=0.05.

4. Results and discussion

4.1. Biological aspects

The mean values of biological aspects of the imported and native hybrids were tabulated in (Table1). Hatching percentage for imported hybrid was 94.19% or (94.19%) comparing with native hybrid (92.56%). While weight of larvae of 4th and 5th larval instar (immature "at the beginning of stage" and mature "at the end of stage") for imported hybrid were 2.004, 0.698 and 2.774gm. Comparing with native hybrid 1.424, 0.481 and 1.979gm., respectively. Pupal weight recorded 0.952, 0.797and 0.759g & 0.724g for female and male of imported and native hybrids, respectively, with high significant differences for female and non-significant for male pupal weight. Pupation ratio for both sexes for imported and native hybrids were 94.67, 92.33and 88.33, 87.67% for female and male, respectively, with high significant differences. However, such feeding lead to decrease of larval duration for the imported hybrid (26.5days) in imported compared to (28.2 days) in native- hybrids, furthermore, larval mortality% of the silkworm 4th and 5th instar recorded 2.40, 3.30% for imported and 3.30, 3.83% for native hybrid fed on the same mulberry leaves. While, no significant variation was noticed in values of larval mortality% and larval duration (day) among the two hybrids. Mean number of deposited eggs/female as reproductive index was varied between hybrids. The fecundity was 384with fertility373eggs/female in imported hybrid and 299 eggs with fertility 291egg/female for native hybrid with high significant in fecundity and fertility.

Similar results were reported by Taha *et al.* [16] and El-Saffany *et al.* [17] who recorded that the larval duration was non-significantly shorter in imported (H1xKKxG2xV2) hybrid as compared to local hybrid Giza B, and the mean weight of the 4th larval instar was significantly higher in Thai imported hybrid compared with local hybrid. The weight means of 5th larval larvae of imported hybrid was increased significantly as compared with native hybrid. Significant positive variations were estimated in imported including fecundity, fertility percentages, pupal weight and pupation % values as compared with the local hybrid annually reared under Egyptian conditions. Significant positive variations were estimated among the different two imported hybrids in biological and technological values as compared with five local hybrids annually reared under the Egyptian was reported by Fouad [18].

4.2. Economic aspects (Cocoon indices)

Data presented in (Table 2) show that the imported hybrid was better than the local hybrid in all economic aspects. A high significant variation of fresh cocoon weights (1.350 and 1.150g) in imported hybrid and1.070, 0.850g in native hybrid for female and male, respectively. The same trend for cocoon shell weights with no significant changes between imported and native hybrid were 0.311, 0.283g and 0.220, 0.187g for imported and native hybrid for female and male, respectively. Cocoon shell ratio was significantly different between imported and native for female (23.037, 20.561) and male (24.608, 22.000%), respectively. As represented in (Table 3) indicated that cocooning% showed significant differences in imported and native 95.66, 94.00 and 83.67, 81.50% for female and male, respectively. Silk productivity was 2.827, 2.572 & 2.00, 1.700 Cg/days for female and male of imported and native hybrids, respectively, with no significant differences between them.

Table 1: Certain biological aspects for two silkworm *B. mori* (L.) hybrids

Biological aspects 5th		Imported hybrid	Native hybrid	T value	P (t=0)
Hatching %		94.19	92.56	7.468	0.000**
Beginning of 4th instar (larvae / wt.10)		2.004	1.424	7.712	0.000**
5th instar larvae (One (larva/wt.)	Immature beginning (larva/wt.)	0.698	0.481	2.061	0.047*
	Mature end (larva/wt.)	2.774	1.979	11.691	0.000**
Pupa weight.	Female	0.952	0.759	7.638	0.000**
	Male	0.797	0.724	1.475	0.175ns
Pupation %	Female	94.67	88.33	11.355	0.000**
	Male	92.33	87.67	4.184	0.008**
Larval mortality	4th	2.40	2.30	1.713	0.147ns
	5th	3.30	3.83	1.947	0.109ns
Larval duration(day)		26.5	28.2	2.657	0.057ns
Fecundity		384	299	9.084	0.000**
Fertility		373	291	7.188	0.000**

Table 2: Certain economical characters for two silkworm *B. mori* (L.) hybrids

Silkworm hybrids	Mean of fresh cocoon weights/gm.		Mean of cocoon shell weights/gm.		Mean of cocoon shell ratio%	
	Female	Male	Female	Male	Female	Male
Imported hybrid	1.350	1.150	0.311	0.283	23.037	24.608
Native hybrid	1.070	0.850	0.220	0.187	20.561	22.000
P(t=0)	3.427	1.793	1.333	1.213	3.119	0.353
T value	0.0075**	0.007**	0.216ns	0.256ns	0.012*	0.733ns

Table 3: Certain economical characters for two silkworm *B. mori* (L.) hybrids

Silkworm hybrids	Cocooning percentage		Silk productivity% (Cg./days)	
	Female	Male	Female	Male
Imported hybrid	95.66	94.00	2.827	2.572
Native hybrid	83.67	81.50	2.00	1.700
P(t=0)	7.776	7.777	1.479	1.321
T value	0.016 *	0.016 *	0.173 ns	0.219 ns

These finding are in accordance with Sheidav [19] who found that fresh cocoon and cocoon shell weight are the important or main traits evaluated for productivity in sericulture and these parameters have been used for breeding for more than half a century**.** They also recommended the use of imported hybrids in the breeding programs as parents to produce superior local hybrids. In addition, the shell ratio varies according to age and breed of the silkworm. The reference range for hybrid silkworms is in between 19% -25%. These findings are also in accordance with El-Saffany *et al*. [17] who imported hybrids (HoxK1xR2xT2) which were good for breeding programs in Egypt, and they were superior for cocoon characters. Cocooning percentage exhibited the highest values with imported hybrids (HoxK1xR2xT2) compared with the native hybrid Giza B. Also, Tzenov *et al.* [20] tested 8 Indian silkworms, *B. mori*, hybrids, and the Bulgarian silkworm hybrid Super 1 x Hesa 2, used as a control. The Bulgarian silkworm hybrid Super 1x Hesa 2 manifests higher main biological characters values than the Indian silkworm hybrids tested.

4.3. Technological aspects

The data of imported hybrid gave the highest means for the mentioned technological parameters. The length, weight and size means were 763.000 m, 0.165wt and 1.940 denier, respectively, while the lowest ones were recorded in native hybrid 668.870 m, 0.121wt and 1.620 (denier), respectively with highly significant differences between them (Table 4). The obtained result also indicated that the filament denier, which is the size of silk in terms of its diameter. This is a pointer to the fact that the silkworm produced by this hybrid is the finest. This is in line with the findings of Rayar [21] observed that BL23×NB4D, BL24×NB4D2, MH1×NB4D2 and Pure Mysore×NB4D2. Pure Mysore×NB4D2 have filament length of 889.78 m and denier 2.87.

Table 4: Certain technological characters for two silkworm *B. mori* (L.) hybrids

Parameters	Imported hybrid	Native hybrid	P (t=0)	T value
Filament length (m)	763.000	668.870	9.038	0.000**
Filament wt. (gm.)	0.165	0.121	11.674	0.000**
Filament size (Denier)	1.940	1.620	9.329	0.000**

Table 5: Biochemical analysis for two silkworm *B. mori* (L.) hybrids

Parameters	Free amino acid(mg D,L-alanine /ml)	Total protein (mg/ml)	Alkaline phosphatase (U/L))	GOT (U/L)	GPT (U/L)
Imported hybrid	688.6	110.2	22.3	244.3	140.7
Native hybrid	425.5	108.3	12.4	220.0	107.7
T value	5.927	1.748	6.234	5.638	7.933
P (t=0)	0.0002 ***	0.114 ns	0.0002 ***	0.0002 ***	0.0002 ***

4.4. Biochemical analysis of silkworm.

Results in (Table 5) revealed that total protein, free amino acid, alkaline phosphates, GOT, and GPT defer between the two hybrids in *B. mori* hemolymph of fifth instar with no significant differences in total protein (110.2, 108.3 mg/ml) for imported and native hybrid.

4.4.1. Free amino acid recorded highly significant differences (688.6 and 425.5 mg D, L-alanine /ml) between imported and native hybrids, respectively.

4.4.2. Alkaline phosphates were 22.3 and 12.4 U/L for imported and native hybrids, respectively.

4.4.3. GOT and GPT were of highly significant differences and were of 244.3, 220.0 and 140.7, 107.7 U/L) for imported and native hybrid, respectively.

4.4.3. Total protein concentration differed according to races. Both Thai hybrids 1 and 2 showed good performance and remarkable superiority over the Egyptian hybrid.

4.4.4. Free amino acids determination: Silk protein composed of amino acids, during active feeding stage most of the amino acids transported from digestion of feeding materials to silk gland at the end of the larval life Amino acids are closely related to the biosynthesis of silk proteins as well as to the growth of silk glands of *B. mori***.** Taha *et al.* [16] Tzenov *et al.* [20] and El Saffany *et al*. [17] found that the hemolymph total proteins and free amino acids were significantly higher as compared with local one. They concluded that it may be recommended to use these two imported hybrids in the breeding programs as parents for the production of superior local hybrids.

4.4.5. Alkaline phosphates (U/L): Mahmoud [22] found that in the hemolymph and silk gland of *B. mori* and *Philosamia ricini*. Alkaline phosphatase was absent in the hemolymph of the two silkworm larvae**.** At the same trend amino transferase enzymes: GPT level of the enzyme activity was lower than that of GOT. Amino transferase was involved in the uptake of nitrogen from leaves by body tissues and silk gland which resulted in the subsequent promotion of silk protein synthesis by Rodwell [23]**.**

5. Conclusions

The imported hybrid is better than the native one in the biological, economic and technological aspects of the silk filament due to an increase in its productivity by 1.5 package cocoons, where a kilo of raw silk is extracted from 2.5 package cocoons. For each egg package (12gm silkworm eggs), this quantity increases with the increase in the number of egg package during the breeding season. A package of eggs produces 8-10 cocoons. The price of a kilo of silk is about 3500 EGP.

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Declaration of competing interest

The author declare that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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دراسة مقارنة على هجينين من دودة الحرير التوتية (L.) *(Bombyx Mori)* المرباه على أوراق التوت من الصنف الهندي

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الملخص

أجري هذا البحث في قسم وقاية النبات- كلية الزراعة - جامعة الأزهر الشريف- مدينة نصر بالقاهرة. لدراسة المقارنة على هجينين من دودة القز أحدهما هجين مستورد (HoxK1xR3xT3) والاخر محلي (J444). ربيت اليرقات على أوراق التوت من الصنف الهندى. أظهرت النتائج المتحصل عليها أن تغذية يرقات دودة القز من الهجين المستورد (الناتج من برنامج تهجين بمعهد بحوث الحرير- مركز البجوث الزراعيه- جيزة) مسبقًا لإدراجه ببرامج التربية لإنتاج هجين محلي متفوق لإعتماده في برامج التربية في مصر كان أعلى من الهجين المحلي فى جميع مراحل نموه وكانت قياسات الدراسه قد شملت: نمو اليرقات - وزن اليرقات - فترة العمر اليرقى - وزن الشرنقة الطازجة - وزن قشرة الشرنقة - نسبة الحرير الناتج - وزن العذارى - نسبة التعذير- نسبة الشرانق - ونسبه الحرير الناتج. بالإضافة إلى الصفات التكنولوجية وتشمل طول ووزن وحجم خيط الحرير الناتج.