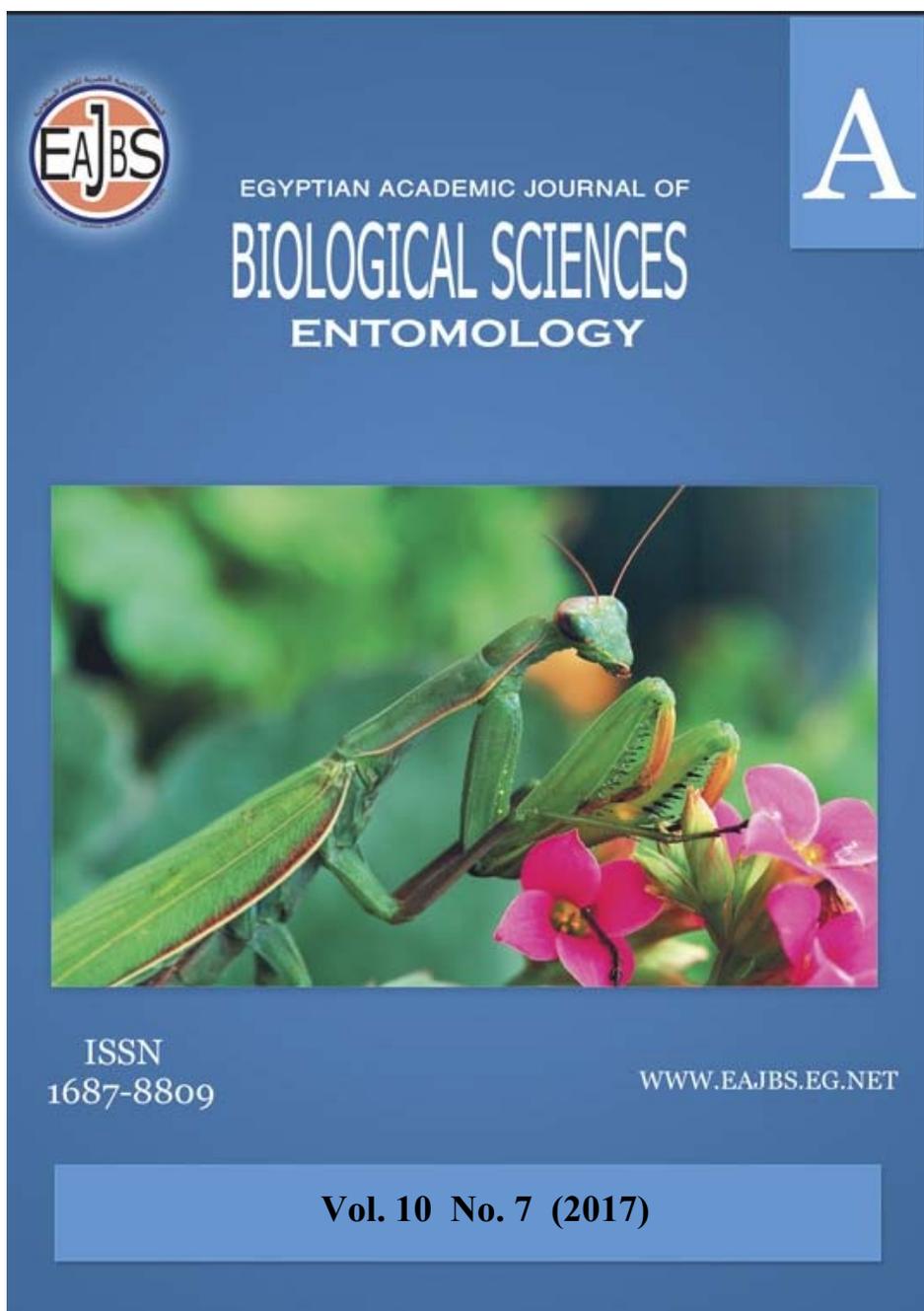


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**Assessment of Different Imported Hybrids of Mulberry Silkworm,
Bombyx mori L. in Egypt**

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ABSTRACT

Two different Thai hybrids (1 and 2) of mulberry silkworm, *Bombyx mori* L. were reared under the Egyptian climatic conditions. The fitness of imported hybrids was compared with Egyptian hybrid (as a control) in different aspects. Biological parameters *viz*; egg hatching percentages, larval weight, Effective Rate of Rearing (ERR), cocooning and pupation percentages, pupal weight and egg fertility were recorded. Economical parameters *viz*; cocoon, cocoon shell weights and cocoon shell ratio, as well as, technological parameters *viz*; filament length, weight, size and silk percentage were studied. Mature larval haemolymph total proteins, free amino acids, protease and transaminases (GOT and GPT) enzymes were measured colorimetrically. Imported hybrids were significantly higher than the local in most of the tested parameters. Hybrid 1 recorded a significant increase in: larval wt, cocoon shell %, randitta, as well as, filament length, size and silk percentage. While Hybrid 2 was significantly higher in hatching and pupation percentages. Haemolymph total proteins and free amino acids were significantly high in both hybrids 1 and 2 compared with local one. The same trend was observed for tested protease and transaminases enzyme activities. It may be recommended to use these two imported hybrids in the breeding programs as parents for the production of superior local hybrids.

INTRODUCTION

Sericulture is one of the most important industries in several countries, however, in Egypt is not well developed due to several factors; one of these is lack of good hybrid with high productivity. Therefore, it is necessary to evaluate imported hybrids to determine their suitability to our climatic condition. The recent increase of domestic and international markets for silk production combined to the deep-rooted national traditions as well as the favorable climatic condition gives a push for developing the sericulture in Egypt (Souad and Azza, 2008).

Widespread utilization of hybrids towards achieving sustainability and quality oriented increased production is well established in plants and animal where hybrids are used compulsory (Datta and Pershad, 1988). Effective Rate of Rearing (ERR) and cocooning percentage resistance and healthiness as well as economical characters: cocoon, cocoon shell weights and cocoon shell ratio are characters related to disease (Pershad *et al.*,1986). Physiological activity of insect depends on haemolymph, that is the only extra cellular fluid having diverse functions such as immunity, and as the reservoir for the products which are required for survival (Sowri and Sarangi, 2002) and protein have always been an interesting biochemical role in the development, morphogenesis and almost in all intermediary metabolic pathways of insects and since they are the key organic constituent, their role in the compensatory mechanism of silkworm is vital (Hiremath *et al.*, 2006 ; Ramakrishna, and Jayaprakash, 2007). 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 (Naguchi *et al.*, 1974). The main amino acid, glycine, alanine, serine and tyrosine are synthesized in the silk-gland cell by transamination process (Prudhomme *et al.*, 1985). Amino-transferases depend on the uptake of nitrogen from mulberry leaves by body tissues and silk glands and synthesis silk protein (Li and Zhu, 1985).

The aim of the study is to identify the high yielding hybrid that can be used in various breeding programmes as for the synthesis of superior hybrids due to shortage of pure lines of *Bombyx mori* L.

MATERIALS AND METHODS

Silkworm:

Two commercial Thai hybrids were imported from Thailand hybrid 1 and hybrid 2 and were evaluated comparing to Egyptian hybrid (as a control) and fed on Egyptian local mulberry leaves *Morus alba* var. Balady (native).

Bioassay:

The young larvae (1st~3rd instars) were reared at 27~28 °C , 85%~90% relative humidity(RH) and the late age larvae (4th and 5th instars) were maintained at 24~26 °C with RH of 70%~80%. Each hybrid was maintained in three replicates. At the beginning of 4th instar, 300 larvae were counted from each hybrid and retained for further studies. Rearing was carried out under hygienic conditions according to Krishnaswami (1978). Larval weights were recorded at 8th day of 5th larval instar but larval haemolymph samples were collected at 6th day for biochemical analysis. The spinning larvae were collected manually and mounted in plastic collapsible mountages. Observations on hatchability, Effective Rate of Rearing (ERR), pupal weight, pupation ratio, cocooning percentage, single cocoon and single shell weights, single cocoon shell ratio and single female moth fertility were recorded. Renditta which is the quantity of cocoons required for producing a kilogram of raw silk was estimated as suggested recently by The Central Silk Technological Research Institute (CSTRI) of India, by giving certain constants that can be used for estimating the renditta from the shell ratio.

$$\text{Renditta} = \text{Constant} / \text{shell ratio}$$

The constant is 165 for cocoon with shell ratio of 14-16%, and 150 for cocoon with shell ratio of 17-20% , 133 for cocoon with shell ratio of 21-23% .

Technological parameters were estimated such as; filament length, weight,

size and silk percent.

Larval haemolymph was centrifuged at 8000 rpm for 15 min at 5 °C in a refrigerated centrifuge. The supernatants were kept in a deep freezer till use. Double beam ultraviolet /visible spectrophotometer (Sectronic 1201, Milton Roy Co., USA) was used to measure absorbance of coloured substances .

Total proteins:

Total proteins were determined by the method of Bradford (1976). Protein reagent was prepared by dissolving 100mg of Coomassie Brilliant blue G-250 in 50ml 95% ethanol, then 100ml 85% (W/V) phosphoric acid were added. The absorbance was measured at 595 nm against blank prepared from 1 ml of phosphate buffer (0.1M, pH 6.6) and 5 ml protein reagent.

Free Amino acids:

Total amino acids were colorimetrically estimated by ninhydrin reagent according to the method described by Lee and Takabashi (1966). The reaction mixture consists of 1 ml sample and 1.9 ml ninhydrin-citrate buffer-glycerol mixture that consists of 0.5 ml of 1% ninhydrin solution in 0.5 M citrate buffer (pH 5.5); 0.2 ml of 0.5 M citrate buffer (pH 5.5) and 1.2 ml glycerol. The developed colour was read at 570 nm. Amino acids were expressed as ug alanine per gm body weight.

Protease enzyme:

Proteolytic activity was measured according to Tatchell *et al.*(1972) and Lee and Takabashi (1966). The developed color was read at 570 nm. D,L alanine was used as the standard and the amino acids were expressed as ug alanine /min/g.b.wt.

Transaminases (GOT and GPT):

Glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) were determined colorimetrically according to the method of Reitman and Frankle (1957). GOT transfer amino group from L-aspartate to α -keto acid (α -ketoglutaric acid), producing a new amino acid (L-glutamate) and a new keto acid (oxaloacetic acid).GPT transfer the amino group from D,L alalnine to α -keto acid (α -ketoglutaric acid), resulting in a new amino acid (L-glutamate) and a new Keto acid (pyruvic acid). Pyruvate or oxaloacetate reacts with 2,4-dinitrophenylhydrazine, formig pyruvate or oxaloacetate hydrazone which in alkaline medium form a brown color , which can be measured spectrophotometrically. The reaction mixture optical density was measured using spectrophotometer at 520 nm. The enzyme activity is expressed as U /gm body weight.

Statistical analysis:

Collected data were recorded and analyzed using statistical analyzing system version 9.1 program proc. GLM (SAS Institute, 2003).

RESULTS AND DISCUSSION

Biological characters

The mean values of biological parameters of Thai and Egyptian hybrids were tabulated in Table (1), hatching percentage was significantly higher in Hybrid 2 (99.33%) comparing with hybrid 1 (93.67%) and Egyptian hybrid (84.58%). While weight of larvae of 5th larval instar hybrid 1 increased significantly (2.74gm) comparing with hybrid 2 and the Egyptian hybrid (2.63 and 1.74 gm respectively). Also, pupation % exhibited the highest values with both imported hybrids 1, 2 (91% and 99%, respectively) compared with the Egyptian hybrid (76%). No significant differences between hybrid 1 and hybrid 2 in the other tested parameters were recorded. Values of these results are consistent with Souad and Azza (2008) who

found that the imported hybrids showed the best performance in most biological characters compared with the Egyptian hybrid. At the same trend, Prakash (1986) observed that, estimation of different biological characters especially the effective rate of rearing (ERR) and cocooning % characters were linked with disease resistance and healthiness.

Table (1): Biological characters of *Bombyx mori* L. different hybrids (Mean±SE)

Parameters	Egyptian hybrid	Thai hybrid 1	Thai hybrid 2	LSD ^{1%}
Hatching %	84.58±12.88 ^b	93.67±17.51 ^{ab}	99.33±10.15 ^a	10.86
5 th larval wt (gm)	1.74±0.10 ^c	2.74±0.15 ^a	2.63±0.06 ^b	0.28
ERR %	73±4.34 ^b	97±6.08 ^a	96±2.52 ^a	2.48
Cocooning %	81±7.28 ^b	93±2.84 ^a	95±1.52 ^a	11.51
Pupation %	76±10.87 ^b	91±5.22 ^{ab}	99±1.15 ^a	15.56
Single pupal wt (gm)	0.65±0.07 ^b	0.88±0.03 ^a	0.91±0.05 ^a	0.12
No. of fertile eggs/female moth	359±28 ^b	492±17 ^a	460±12 ^a	72.00

Economical Characters (Cocoon Characters):

Recommendation of superior hybrids of silkworm needs firstly to evaluate these hybrids before their release into the field. Cocoon and cocoon crop characters linked to the economic aspects were determined and registered in Table (2), Thai 1 showed significantly the highest cocoon shell ratio and randitta (19.49% and 7.67 kg, respectively) comparing to Thai 2 (18.01% and 8.30 kg, respectively) and Egyptian hybrid (16.75% and 8.89 kg, respectively). No significant differences between hybrid 1 and hybrid 2 in the other tested parameters. These results are supported by those of Souad and Ghazy (2005) who reported that, Thailand hybrids were good for breeding programs in Egypt, and they were superior for biological and cocoon characters

Table (2): Economical characters of *Bombyx mori* L. different hybrids (Mean±SE)

Parameters	Egyptian hybrid	Thai hybrid 1	Thai hybrid 2	LSD ^{1%}
Single cocoon wt(gm)	0.78±0.07 ^b	1.11±0.09 ^a	1.11±0.06 ^a	0.22
Single shell wt (gm)	0.13±0.06 ^b	0.21±0.02 ^a	0.20±0.01 ^a	0.04
Cocoon Shell %	16.75±0.27 ^c	19.49±2.59 ^a	18.01±1.19 ^b	0.91
Renditta	8.89±0.12 ^a	7.67±1.34 ^c	8.30±0.42 ^b	0.40

Technological characters:

As shown in Table (3), Hybrid 1 recorded significantly the highest filament length and silk% (779m and 26%, respectively) compared to Hybrid 2 (710m and 24.10%, respectively) and Egyptian hybrid (610.67m and 20.32%, respectively). No significant differences between hybrid 1 and hybrid 2 in the filament weight and size but still higher than Egyptian hybrid values.

This in accordance with Megalla *et al.* (1997) who reported that, significant positive variations were estimated among the different imported hybrids in biological and technological values compared with the Korean hybrid annually reared in Egypt.

Table (3): Technological characters of *Bombyx mori* L. different hybrids (Mean±SE)

Parameters	Egyptian hybrid	Thai hybrid 1	Thai hybrid 2	LSD ^{1%}
Filament length (m)	610.67±39.59 ^c	779±70.11 ^a	710±47.02 ^b	50.62
Filament wt (gm)	0.159±0.02 ^b	0.289±0.02 ^a	0.266±0.01 ^a	0.04
Filament size (Denier)	2.33 ^b	3.33 ^a	3.37 ^a	1.03
Silk %	20.32±2.75 ^c	26±1.75 ^a	24.10±2.05 ^b	1.95

Biochemical analysis:

Silkworm protein metabolism and enzymes were identified as an important markers for improving the viability in silkworm. As shown in Table (4) the biochemical parameters, it revealed significant variation between the imported hybrids and Egyptian one in the levels of free amino acids, GOT and GPT. While no significant variation was noticed in values of total protein and protease among the three hybrids.

These results are in agreement with Rajannan *et al.* (1994) who found that total protein concentration differed according to races.

Both Thai hybrids 1 and 2 showed good performance and remarkable superiority over the Egyptian hybrid therefore, it may be recommended to use these imported hybrids in the breeding programs for the production of superior local hybrids.

Table (4): Biochemical analysis of *Bombyx mori* L. different hybrids (Mean±SE)

Parameters	Egyptian hybrid	Thai hybrid 1	Thai hybrid 2	LSD ^{1%}
Total protein (mg/ml)	95±0.87 ^a	129.66±30 ^a	126.33±32.52 ^a	64
Free amino acid (mg D,L-alanine /ml)	2.73±0.21 ^b	9.07±2.63 ^a	9.03±2.82 ^a	5.58
Protease (ug D,L-alanine/min/ml)	19.7±2.4 ^a	20.5±2.4 ^a	21±3.9 ^a	7.48
GOT(U/L)	157±6.81 ^b	246.5±0.5 ^a	255.5±5.5 ^a	12.68
GPT(U/L)	33.33±2.89 ^b	45±5 ^a	46.5±5.5 ^a	11.53

REFERENCES

- Bradford, M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72: 248-254.
- CSTRI : The Central Silk Technological Research Institute (CSTRI) of India
- Datta, R.K. and Pershad, G.D. (1988): Combining ability among multivoltine X bivoltine silkworm, *Bombyx mori* L. hybrids. *Sericologia*, 28(1): 21-29.
- Hiremath, S.S.; Srikar, L.N.; Narayanaswamy, T.K.; Poddar, U.S. and Shankar, M.A. (2006): Biochemical constituents of silkworm, *Bombyx mori* L. as influenced by grown through organic based nutrient management. *Sericologia*, 46(3): 351-355.
- Krishnaswami, S. (1978): New technology of silkworm rearing. Central Sericulture Researches and Training Inst., Mysore Bull., 2:1- 10.
- Lee, Y.P. and Takabashi, T. (1966): An improved colorimetric determination of amino acids with the use of ninhydrin. *Anal. Biochem.* , 14 :71-77 .
- Li, X. Y. and Zhu, X.O. (1985): Mechanisms of silk increase in silkworm by N 15 urea administration. *Sci. Sericult.*, 9 (4): 209-213.

- Megalla, A. H.; Souad, M. Mahmoud and Zannon, A. A. (1997): Performance of different imported silkworm hybrids of *Bombyx mori* L. under the Egyptian conditions. Proc. 1st. Int. Conf. Of silk "ICSAI", 72-77.
- Naguchi, A.; Tokeshita, H. and Shigematsu, H. (1974): Interrelationship between the silk gland and other tissues in protein metabolism in the latest larval stages of the silkworm, *Bombyx mori* . J. Insect physiol., 20(9): 783-794.
- Pershad, G.D.; Datta, R.K.; Vijayakumar, H.V.; Bhargava, S.K. and Jolly, M.S. (1986): Performance of some multivoltine races of *Bombyx mori* L. Sericologia, 26(3): 295-301.
- Prakash Kumar R. (1986): A study on adoption of improved sericultural practices and labour utilization among big, small and tenant farmers of Ramanagara Taluk, Bangalore district. M.Sc. thesis, University of Agricultural Science, Bangalore, India.
- Prudhomme, J.C.; Couble, P.; Garel, J.P. and Daillie, J. (1985): Silk synthesis. In Comprehensive Insect Physiology, Biochemistry and Pharmacology. (ed. G.A. Kurkut and L.I. Gilbert), vol. 10, Pergamon Press, Oxford, New York, Toronto, Sydney, Paris, Frankfurt.
- Rajannan, K.L.; Pattarju, H.P.; Manjula, A.C.A. and Yadav, P.R. (1994): Biochemical differences among some selected lines of two different voltine races of *Bombyx mori* L. J. Entom. Res., 18(1):53-60.
- Ramakrishna, S. and Jayaprakash (2007): Shifts in protein metabolism in haemolymph and fat body of the silkworm, *Bombyx mori* L. in response to fuccid toxicity. Int. J. Entomol., 15(1): 59-68.
- Reitman, S. and S. Frankel (1957): Colourimetric method for aspartate and alanine transaminases. Amer. J. Clin. Pathol., 28 : 56 .
- Tatchell, R.J.; Araman, S.F. and Boctor, F.N. (1972): Biochemical and physiological studies of certain Ticks (Ixodoidea). Z. Parsitenk., 39: 345-350.
- SAS Institute (2003): SAS version 9.1. Cary, M. C.
- Souad, M. Mahmoud and Ghazy, M.M.U. (2005): Performance of two hybrids of silkworm, *Bombyx mori* L. in different locations. Egypt. J. Agric. Res., 83(3): 1039-1041.
- Souad, M. Mahmoud and Azza, T. Ashour (2008): Biometric and biochemical studies on different hybrids of the mulberry silkworm, *Bombyx mori* L. Bull. Ent. Soc. Egypt, 85: 73-83.
- Sowri, D. M. and Sarangi, S. K. (2002): Changes in protein and reducing sugar content of the haemolymph during v instar development of *Bombyx mori* L. Bull. Ini. Acad. Seric., 6(2): 103-106.

ARABIC SUMMARY

تقييم هجن مستوردة مختلفة من دودة الحرير التوتية *Bombyx mori* L. في مصر

رحاب حسني طه، إيمان محمود حسان، مروى نبيل مصطفى

معهد بحوث وقاية النباتات- مركز البحوث الزراعية

يهدف هذا البحث إلي تقييم هجن تايلاندية تحت الظروف المصرية. و مقارنة قوة هذه الهجن و أدائها مع هجين مصرى (ككتنرول) في نواحي مختلفة . الصفات البيولوجية تم قياس نسبة الفقس ، وزن اليرقات، كفاءة التربية، نسبة التشنق و التعذير، وزن العذراء و عدد البيض المخصب. في الصفات الإقتصادية تم قياس وزن الشرائق و غلاف الشرنقة و نسبة الغلاف إلي وزن الشرنقة كما تم قياس طول خيط الحرير وزنه و حجمه و نسبة الحرير في الشرنقة كصفات تكنولوجية. كما تم دراسة بعض الخواص البيوكيميائية في هيموليمف اليرقة الكاملة مثل كمية البروتين الكلية، و الأحماض الأمينية الحرة، و إنزيم البروتياز و الإنزيمات الناقلة للأحماض الأمينية (GPT, GOT). و قد أظهرت النتائج أن الهجن المستوردة كانت أعلى معنوية من الهجين المصرى في كل القياسات حيث سجل الهجين الأول زيادة معنوية في وزن اليرقة و النسبة المئوية لغلاف الشرنقة و الرينديتا كما سجل أعلى القياسات في طول خيط الحرير و حجمه و نسبة الحرير بينما أظهر الهجين الثاني زيادة معنوية في نسبة الفقس و التعذير. أما بالنسبة للبروتين و الأحماض الأمينية الحرة قد لوحظ نفس الشئ في البروتياز و نشاط الإنزيمات الناقلة للأمينات (GPT & GOT) . من كل ما سبق نستطيع أن نوصي بإستخدام الهجن التايلاندية Thai₁ & Thai₂ للإعتماد عليه في برنامج التربية في مصر.