Impact of Nutritional Composition of Wild and Cultivated Mulberry Varieties on Productivity and some Biochemical Parameters of Silkworm *Bombyx mori* L. (Bombycidae: Lepidoptera)

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Abstract: Two mulberry varieties, the cultivated variety, *Morus alba* var. Morittiana and the wild variety, *M. laevigata,* grafted on *M. alba* branches were investigated to assess the effect of their nutritional components on larval haemolymph biochemical constituents and economic traits of silkworm, *Bombyx mori* L. Results indicated that there were significant differences ($p \leq 0.05$) between the two tested mulberry varieties leaves in their nutritional composition. Protein content, free amino acids and nitrogen were significantly higher in the cultivated variety *M. alba* var. Morittiana, while reducing sugars content was significantly higher in *M. laevigata* with no significant differences between both varieties. Interestingly, total proteins and total carbohydrates were significantly high in haemolymph of larvae fed on *M. laevigata* leaves, while free amino acids were significantly high in haemolymph of larvae fed on *M. alba* var. Morittiana leaves, no significant difference was registered between total lipids content in haemolymph of larvae fed on both mulberry varieties. There were no significant differences between male full grown larvae fed on both mulberry varieties, while female full grown larvae weights were significantly high in larvae fed on *M. laevigata* leaves. No significant differences were found between cocoon incidences produced from larvae fed on the both mulberry varieties.

Keywords: *Bombyx mori*, mulberry varieties, M*orus alba,* M*orus laevigata*, biochemical study.

INTRODUCTION

In sericulture, the mulberry variety plays an important role and has long been cultivated for silkworm rearing. Their leaves are the exclusive source of nutrition for growth and development of silkworms (Tang *et al*., 2006). Mulberry leaves contain a rich source of protein and amino acids. However, the nutritional composition of mulberry leaves are ascertained by their biochemical constituents and vary with different cultivars that influences growth and cocoon production (Subhan *et al.,* 2013; Babu *et al*., 2014 ; Manjula and Vijaya Kumari, 2015 ; Prieto-Abreu *et al*., 2016).

Silk production in Egypt should have special attention because the local production does not meet the domestic consumption requirements although dominant climate conditions are proper (Diab *et al.,* 2009). Mulberry production alone need more than 60% of the total cost of sericulture and cocoon production, so maximum attention has been given for the improvement of mulberry in terms of both quality and quantity (Bongale and Chaluvachari, 1995**)**. In India, *Morus* is represented by four species *i.e.*, *M. indica* L., *M. alba* L., *M. laevigata* Wall. and *M. serrata* Roxb. (Tikader and Dandin, 2005). Japan is the only sericulturally advanced country where the wild mulberry, *M. laevigata* genetic resources have been exploited for developing new improved mulberry varieties (Naik *et al*., 2015**)**.

The performance of the *Bombyx mori* L. was evaluated on different mulberry cultivars based on economic, biological characters and productivity in silkworm rearing (El-Banna *et al*., 2013; Subhan *et al*., 2013; Babu *et al*., 2014; Kumar *et al*., 2014; Prieto-Abreu *et al*., 2016). However the biochemical contents were studied in different varieties of the cultivated mulberry leaves and silkworm haemolymph in relation to silk production (Zannoon *et al*., 2008; Yogananda Murthy *et al*., 2013), meanwhile the effects of the wild mulberry variety on silk production in comparison to the cultivated varieties was studied by Mahmoud Soad *et al.* (2013).

Therefore, the present study aims to evaluate two mulberry varieties, the cultivated one *Morus alba* var. Morittiana and the grafted wild one *M. laevigata* to assess their feeding effect on some biochemical and economical traits of the silkworm, *Bombyx mori* L.

MATERIALS AND METHODS

Silkworm rearing

Experimental silkworm eggs, the four-way hybrid (KK × Hesa 1) × (Vesletz 2 × Gergana 2), were imported from Sericulture and Agriculture Experiment Station, Vratza, Bulgaria, reared in the laboratory of Plant Protection Department, Faculty of Agriculture, Suez Canal University at 25 ±1ºC and 75 ± 5% RH from hatching till cocoon formation according to Mohamed (2000). Rearing was carried out during two successive spring seasons of 2015 and 2016. Removal of fecal matter, diseased larvae and bed cleaning was done regularly. Larvae of each two groups were fed on one of two chosen mulberry variety leaves four times a day, each group containing 3 replicates.

Mulberry varieties

Mulberry leaves were collected from healthy plants at the Faculty of Agriculture Farm, Suez Canal University, Ismailia Egypt, *Morus alba* var. Morittiana and *Morus laevigata* which was grafted on *M. alba* var. Morittiana branches.

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Biochemical analysis

Mulberry leaves preparation and biochemical analysis

Leaves samples were collected from medium portion of mulberry branches as pooling sample with three replicates, weighed (0.1-0.4 g) and stored at -20˚c, then processed as described by Ni *et al*. (2001). Enzymes were extracted from the frozen plant samples using cold potassium phosphate buffer. An aliquot (1.5 ml) of the extract was centrifuged for 10 min at 4ºC.

Total Protein content of leaves was assessed by method of Lowry *et al.* (1951). Free amino acids were colorimetrically assayed by ninhydrin reagent according to the method described by Lee and Takabashi (1966). Reducing sugars were extracted by weighing 100 mg of the sample then adding hot 80% ethanol twice (5 ml each). Carbohydrates were determined by estimating the reducing sugars content, while nitrogen in protein is converted to ammonium sulphate by H_2SO_4 during digestion (Sadasivam and Manickam, 1992).

Larval haemolymph preparation and biochemical analysis

Haemolymph of larvae fed on the two selected mulberry leaves were collected separately in glass tubes by punctured pro-abdominal leg of larvae at the 7th day of the fifth instar, each tube contains haemolymph of 10 larvae, as pooling samples, with three replicates for each mulberry variety then deep freezed at -20°C. Haemolymph samples were centrifuged (2500 rpm for 6 minutes).

Total proteins were determined using the method of Bradford (I976) while total lipids were estimated according to the method of Knight *et al.* (1972). Total carbohydrates were extracted and prepared for assay according to Crompton and Birt (1967). Assessment was conducted in acid extract of each sample by the phenol-sulphuric acid reaction (Dubois *et al*., 1956). A modified ninhydrin reagent for the photometric determination of free amino acids was used according to Moore and Stein (1968).

Economic traits of silkworm

At the end of the 5th instars, the weights of 30 individuals' male and female full grown larvae, cocoons, pupae and cocoons shell from three replicates of each group were recorded separately, the mean weights and cocoons cortex percentages were calculated.

Statistical analysis

Data collected from the experimental observations were statistically analyzed using Independent Samples t-test to compare means of both treatments in relation to the recorded traits using the statistical software SPSS. Statistical significance was assigned as ($p \leq 0.05$). In all the cases experimental work was carried out for three replicates.

RESULTS

Biochemical analysis

Mulberry leaves

Nutritional analyses of mulberry leaves are presented in Table (1). Results indicated a high level contents of total proteins in *M. alba* var. Morittiana (19.83 mg/gdw) while it was (15.43 mg/gdw) in *M. laevigata* leaves. Reducing sugars were 103.03 mg/gdw and 115.83 mg/gdw. Free amino acids registered 3.63 mg/gdw and 1.72 mg/gdw, while nitrogen were 3.16 mg/gdw and 2.69 mg/gdw in *M. alba* var. Morittiana and *M. laevigata* leaves, respectively.

Larval haemolymph

The influence of the mulberry varieties on the biochemical constituents in the haemolymph of larvae was shown in Table (2). The total protein content, total carbohydrates, total lipids and free amino acids were 24.10, 4.35, 1536.70 and 13.17 mg/ml in comparison to 30.21, 6.71, 1563.30 and 10.30 mg/ml in haemolymph of larvae fed on *M. alba* var. Morittiana and *M. laevigata* leaves, respectively.

Interestingly, the present larvae biochemical haemolymph analysis showed that total proteins and total carbohydrates were significantly higher in haemolymph of larvae fed on *M. laevigata* leaves. On the other hand, haemolymph of larvae fed on *M. alba* var. Morittiana exhibited highly significant differences in their content of free amino acids, while no significant difference was recorded between total lipids content in haemolymph of larvae fed on both mulberry varieties.

Table (1): Nutritional analysis of *M. alba* var. Morittiana and *M. laevigata* mulberry varieties leaves

Mulberry varieties	Total proteins	Reducing sugars	Free amino acids	Nitrogen
	mg/gdw			
***M. alba* var. Morittiana**	19.83± 0.55	103.03± 2.58	3.63±0.12	3.16± 0.09
M. laevigata	15.43± 0.34	115.83±4.69	1.72±0.12	2.69±0.11
Sig.	*	ns	*	*

Each value represents the mean ± SEM, n=3.
* Significant differences between means in the same column ($p \leq 0.05$).
ns ($P > 0.05$) no significant between means in the same column.

Table (2): Mean of biochemical constitutes in haemolymph of larvae fed on *M. alba* var. Morittiana and *M. laevigata* mulberry leaves

Mulberry varieties	Total proteins	Total carbohydrates	Total lipids	Free amino acids
	mg/ml			
***M. alba* var. Morittiana**	24.10± 1.05	4.35± 0.45	1536.70±156	13.17± 0.50
M. laevigata	30.21± 0.90	6.71±0.48	1563.30±163	10.30±0.67
Sig.	*	*	ns	*

Each value represents the mean ± SEM, n=3.
* Significant differences between the same column ($p \leq 0.05$).
ns ($P > 0.05$) no significant between the same column.

Silkworm economic traits

Mean weights of male full grown larvae fed on the grafted wild and the cultivated mulberry leaves and cocoon incidences are shown in Table (3). Full grown larvae weights registered 1921.2 and 1913.9 mg. Weights of pupae, cocoons and cocoons cortex that produced from larvae fed on *M. alba* var. Morittiana and *M. laevigata* leaves were 705 & 716 mg, 913 & 945 mg and 208 & 230 mg, respectively. Cocoons cortex percentages registered 22.71% and 24.35% in cocoons produced from larvae fed on *M. alba* var. Morittiana and *M. laevigata* leaves, respectively.

Data in Table (4) shown that the females full grown larvae weights were 2288.3 and 2418.5 mg, while pupal weights were 850 and 883 mg. Cocoon weights were 1065 and 1113 mg and cocoon cortex weights were 209 and 230 mg, while cocoon percentage registered 19.50% and 20.71% in cocoons produced from larvae fed on *M. alba* var. Morittiana and *M. laevigata* leaves, respectively.

Data in (Tables 3 and 4) showed that there were no significant differences ($p > 0.05$) between full grown larvae of males fed on both mulberry varieties, while there were significant differences between full grown larvae of females. No significant differences were recorded between weights of cocoons, cocoons cortex and pupae produced from larvae fed on both mulberry varieties in both sexes.

Table (3): Mean of male larvae, pupae and cocoon, weights which fed on *M. alba* var. Morittiana and *M. laevigata* mulberry varieties

Mulberry varieties	Full Grown Larvae	Pupae	Cocoon	Cortex	Cortex %
	Weights (mg)				
***M. alba* var. Morittiana**	1921.2± 17.91	705±17.80	913±23.16	208± 7.14	22.71%
M. laevigata	1913.9± 32.41	716±24.79	945±22.05	230± 15.30	24.35%
Sig.	ns	ns	ns	ns	-

Each value represents the mean ± SEM.
ns ($P > 0.05$) no significant between treatments.

Table (4): Mean single weight of female larvae, pupae and cocoon traits which fed on *M. alba* var. Morittiana and *M. laevigata* mulberry varieties

Mulberry varieties	Full Grown Larvae	Pupae Weights	Cocoon Weights	Cortex	Cortex (%)
	Weights (mg)				
***M. alba* var. Morittiana**	2288.3± 36.42	850±25.47	1065±33.80	209±10.05	19.5%
M. laevigata	2418.5± 51.48	883±23.10	1113±26.95	230±7.58	20.71%
Sig.	*	ns	ns	ns	-

Each value represents the mean ± SEM.
* Significant differences between the same column ($p \leq 0.05$).
ns ($P > 0.05$) no significant between treatments.

DISCUSSION

Silk cocoon yield mainly depends on the quality of mulberry leaves, which differ considerably from variety to variety in their chemical composition and nutritive value, like protein and carbohydrate contents (Jyothi *et al*., 2014; Manjula and Vijaya Kumari, 2015).

The carbohydrates, proteins and lipids play an important role in the biochemical process underlying growth and development of insects (Murthy, 2015), thus changes in the composition of haemolymph reflect the physiological and biochemical transformations taking place in the insect tissues (Brennan and Anderson, 2004).

Present results concluded that *M. alba* var. Morittiana leaves had a significantly high content of total proteins, free amino acids and nitrogen, while *M. laevigata* had high content of reducing sugars only. On the contrary, haemolymph biochemical analysis indicated that larvae fed on *M. laevigata* leaves recorded more total proteins, carbohydrates and lipids. However, larvae fed on *M. laevigata* leaves have unexpectedly gained more full grown larvae weights and cocoon incidences. Present results are in agreement with Subhan *et al*. (2013) Manjula and Vijaya Kumari (2015) who stated that **c**arbohydrates particularly the sugar content in mulberry leaves is closely related to the health of the silkworm. Likewise, Mahmood *et al*. (1987) who indicated that larvae fed on *M. laevigata* leaves gained more body weight in all the instars and gave better cocoon yield comparing to which larvae fed on *M. alba* variety leaves.

On the other hand, these results disagreed with Subhan *et al*. (2013), Kumar *et al*. (2014), Babu *et al*. (2014) and Bhojne *et al*. (2014) who found that mulberry leaves are rich in protein content, total carbohydrates, nitrogen and amino acids, and also had a highly significant positive association with production efficiency of the cocoons, cocoon weight, shell weight and shell ratio. Also the 5th larval weight was the lowest in larvae fed with *M. alba* var. Laevigata comparing to the other four studied varieties (El-Banna *et al.,* 2013).

The present results open a new venue of studies that focus on the nutritional value of mulberry leaves which may not depends only on the biochemical composition, but also on physiological profile of silkworm. Hence while *M. laevigata* leaves had the lowest nutritional contents, larvae fed on its leaves converted the low obtained proteins and carbohydrates to high value in its haemolymph which was considerably converted to larval weights and high cocoon incidences. Such process that is due to other larval endogenous factors which may need more studies like haemolymph enzymes.

Amino acids are the building blocks of the proteins. Obviously, the amino acid pool in silkworm is derived both from proteins through histolysis and from non-protein sources like carbohydrates and lipids through de novo synthesis. Continuous increase in the levels of free amino acids is attributable to the synthesis of amino acids from non-protein sources like glucose and fatty acids (Bose *et al.,* 1989). Transamination reactions play a leading role in the synthesis of silk amino acids. Meanwhile elevations in activities of Glutamate Oxaloacetate Transaminase and Glutamate Pyruvate Transaminase in the haemolymph of silkworm are sound indicators of the active transportation of amino acids, which increases synthesis of silk fibers (Saravanan *et al*., 2011).

Transaminases enzymes were high in larvae fed on *M. alba* var. Laevigata leaves compared to other mulberry varieties leaves as reported by Mahmoud Souad *et al*. (2013). This may explain that larvae produced more proteins in haemolymph and cocoons while obtained low protein content in mulberry leaves due to transaminases enzymes activity which play a vital role in the synthesis of silk protein and they are concerned with amino acids group transfer.

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تأثير المكونات الغذائية لصنفي التوت البري والمنزرع على إنتاجية و بيوكيميائية ديدان الحرير التوتيه

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تم دراسة صنفي التوت المنزرع (الرومي) والبري (العماني) لتقييم تأثير مكوناتهم الغذائية على التركيب البيوكيميائي لسائل الجسم والصفات الاقتصادية لدودة الحرير التوتية. أشارت النتائج إلي انه يوجد فروق معنوية بين صنفي التوت قيد الدراسة في مكوناتهم الغذائية. محتوى البروتين، الأحماض الأمينية الحرة، والنيتروجين كانت قيمتها أعلى معنويا في أوراق صنف التوت المنرزع (الرومي) بينما كان محتوى السكريات المختزلة معنويا أعلى في أوراق صنف التوت البري (العماني). ومن المثير للاهتمام أن محتوى البروتين الكلي والكربوهيدرات كان معنويا أعلى في سائل الجسم لليرقات التي تتغذى على أوراق التوت البري (العماني) بينما كان محتوى الأحماض الأمينية الحرة معنويا أعلى في سائل الجسم لليرقات التي تتغذى على أوراق صنف التوت المنزرع (الرومي). لم تسجل فروق معنوية بين محتوى الدهون الكلية في سائل الجسم لليرقات التي تغذت على أوراق صنفي التوت قيد الدراسة. لم يكن هناك فروق معنوية في أوزان يرقات الذكور مكتملة النمو التي تغذت على كلا صنفي التوت بينما كانت أوزان اليرقات الإناث مكتملة النمو معنويا أعلى عندما تغذت على أوراق الصنف العماني مقارنة بتلك التي تغذت على أوراق الصنف الرومي. لم توجد فروق معنوية بين أوزان الشرانق والعذارى الناتجة من اليرقات التي تغذت على كلا صنفي التوت قيد الدراسة.