

using the mulberry fruit extract for improving silk production of mulberry silkworm, *bombyx mori* l.

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ABSTRACT

Mulberry are mainly used as food for the silkworms and they are eaten as vegetable or used as cattle fodder in different parts of the world. Mulberry fruit is well known as a good source of anthocyanins with many biological activities. Mulberry (*Morus nigra*) is a fruit not known only for its nutritional qualities and its flavour, but also for its traditional use in natural medicine as it has a high content of active therapeutic compounds. In this study, the effect of the *Morus nigra* L., black mulberry fruit extract supplement on the biology of mulberry silkworm was investigated. Fruits were extracted by cold-water method. The mulberry leaves were dipped in the fruit extract. The treated leaves were offered to the last larval instar of silkworm at concentrations of 25, 50 and 100%. The results indicated that mulberry fruit extract enhanced the biology of silkworm and silk production. The concentration of 50% of fruit extract gave the best results. Which improve the mean weight of mature larvae by 23%, fresh cocoon by 38.2%, pupae by 40.9% and cocoon shell by 20.9% over the control.

INTRODUCTION

The mulberry belongs to the *Morus* genus of the Moraceae family. There are 24 species of *Morus* with at least 100 known varieties (Orban and Ercisli, 2010). Mulberry is grown worldwide, and its leaves are used to feed the silkworms which in turn produce silk fiber. Sericulture is mostly practiced in China, India and Japan. In the rest of the world, mulberry is generally used as forage in animal production, or for other purposes. Besides using the leaves, mulberry bears are a sweet fruit (Singhal *et.al.*, 2010). Mulberry (*Morus nigra*) is a fruit not known only for its nutritional qualities and its flavour, but also for its traditional use in natural medicine as it has a high content of active therapeutic compounds. Mulberries are considered as valuable materials for pharmaceutical use because of bioactive compounds (Duyen *et al.*, 2013). In most countries, mulberries are grown for fruit production. Plants of the genus *Morus* are known to be a rich source of flavonoids including quercetin 3-(-malonylglucoside), rutin, isoquercitin (Katsube *et. al.*, 2006), cyanidin 3 rutinoside and cyanidin 3-glucoside (Chen *et. al.*, 2006 and Kang *et. al.*, 2006). The antioxidant potential of the extracts obtained from mulberry leaves and fruits have been investigated by several authors. Among the 28 fruits commonly consumed in China, mulberry pulp was characterized by one of the highest values of the ferric reducing antioxidant power (FRAP) at 4.11 mmol/100 g wet weight (Guo *et. al.*, 2003). Mahmoud (2013) studied the effects of different levels of black and white mulberry fruits on lipid profile and oxidative stress in hypercholesterolemic rats in Egypt. He suggested that consumption of black and white mulberry fruits

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may modify the risk of hypercholesterolemia and it has more potential as a health supplement rich in natural antioxidants. Moreover, mulberries are emerging as a dietary source of multiple compounds and nutrients, including anthocyanins, flavonoids, vitamins and fiber.

The present study was attempted to test the efficacy of mulberry fruit extract, supplemented to the mulberry leaves, for feeding the silkworm, *Bombyx mori* L. The effect of such treatments on some biological aspects of the silkworm was considered.

MATERIALS AND METHODS

1. Insects:

The mulberry silkworm *Bombyx mori* L., used in the present study, was the local hybrid Novi. The larvae were reared under the laboratory hygrothermic conditions of $25\pm 2^{\circ}\text{C}$ and 65-70% R.H., according to the conventional method, in trays and provided with suitable amounts of fresh mulberry leaves. The experimental study was performed on the last larval instar. All the larvae which molted to the last instar at the same time were grouped and used for the experimentation.

2. Mulberry fruits and Method of extraction:

The mulberry fruits were collected from the trees, then left to dry under the temperature of 60°C . The fruits were crushed in a stone mortar, then 200 g of crushed fruits were taken for extraction with 1000 ml of distilled water. The juice extracted through a cheese cloth was collected in amber coloured bottles and corked air tight then kept in the dark for 24 hours at room temperature. From the extract, 25%, 50% and 100% concentrations were prepared using distilled water as diluents. A distilled water control and another control without water were also maintained.

3. Method of Treatments:

Mulberry leaves were dipped in the fruit solution, shaded dried and offered to 20 larvae for feeding for each treatment, three times at first day of the last larval instar. Other feeds being untreated. The observations recorded were: weight of twenty mature larvae, twenty fresh pupae, twenty pupae and twenty of the shell weight of cocoons. The data was statistically analyzed by Statistics program version 9.0 by using one way analysis of variance (ANOVA) followed by LSD test.

RESULTS

1- Effect of mulberry fruit extract on the weight of mature larvae:

Weights of mulberry silkworm larvae fed on mulberry leaves supplemented with mulberry fruit extract during the last larval instar at three concentrations 25, 50 and 100% are presented in Table (1). The data showed an increase in the mean weight of mature larvae by 9.6, 23.0 and 3.0%, respectively. The concentrations of 50 and 25 % gave the significant increase compared to control. The highest significant increase in larval weights was gained when the larvae were fed on mulberry leaves supplemented with 50% of mulberry fruit extract.

Table (1): Mean weight of mature larvae fed on mulberry leaves supplemented with mulberry fruit extract.

Treatment	Mean Weight (g)±SD	Reaction %
Control	3.025 ± 0.331 c	-
25%	3.315 ± 0.468 b	+ 9.6
50%	3.750 ± 0.335 a	+ 23.0
100%	3.115 ± 0.166 bc	+ 3.0

In a column, means followed by the same letter are not significantly different, at the 5% level.

2- Effect of mulberry fruit extract on the weight of fresh cocoons:

Data represented in Table (2) showed that mulberry silkworm larvae fed on mulberry fruit supplement showed an increase in fresh cocoon weights at all concentrations. The highest and significant increase (38.2 %) was gained when larvae were fed on fruit supplement of 50%, followed by 16.8 % at the concentration of 100%. Another increase, but insignificant, in fresh cocoon mean weight (11.5%) was found at the concentration of 25%

Table (2): Mean weight of fresh cocoon after feeding the larvae on mulberry leaves supplemented with mulberry fruit extract.

Treatment	Mean Weight (g)±SD	Reaction%
Control	1.224 ± 0.215 c	-
25%	1.365 ± 0.161 bc	+ 11.5
50%	1.691 ± 0.353 a	+ 38.2
100%	1.430 ± 0.341 b	+ 16.8

In a column, means followed by the same letter are not significantly different, at the 5% level.

3- Effect of mulberry fruit extract on the weight of pupae:

Mulberry fruit extract enhanced the mean weight of pupae at different concentrations (Table 3). The mean weights of pupae increased significantly over the control by about 23.2 and 40.9% when larvae were fed on fruit supplement at concentrations of 100 and 50%, respectively. Another increase

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over untreated larvae (12.1%) at the concentration of 25% was also observed but was insignificant.

Table (3): Mean weight of pupae after feeding larvae on mulberry leaves supplemented with mulberry fruit extract.

Treatment	Mean Weight (g)±SD	Reaction%
Control	0.996 ± 0.178 c	-
25%	1.116 ± 0.176 bc	+ 12.1
50%	1.403 ± 0.363 a	+ 40.9
100%	1.227 ± 0.338 ab	+ 23.2

In a column, means followed by the same letter are not significantly different, at the 5% level.

4- Effect of mulberry fruit extract on the weight of cocoon shell and cocoon shell ratio:

The obtained data in Table (4) present significant increase in cocoon shell, by about 20.9% over control when larvae were fed with 50% of mulberry fruit supplement. Another increase of weight of cocoon shell (4.2%) was observed at the concentration of 25% when larvae were fed with mulberry fruit supplement but was not significant. However, the concentration of 100% gave a significant decrease in cocoon shell. On the other hand, the cocoon shell ratio was higher in untreated larvae than in treated ones.

It could be concluded that mulberry fruit extract enhances the biology of silkworm and silk production. At the concentration of 50% of fruit extract, there are increases in the mean weight of mature larvae by 23% , fresh cocoon by 38.2%, pupae by 40.9% and cocoon shell by 20.9% higher than control.

Table (4): Mean weight of Cocoon Shell after feeding larvae on mulberry leaves supplemented with mulberry fruit extract.

Treatment	Mean Weight (mg)±SD	Reaction %	Cocoon Shell Ratio %
Control	239 ± 5.3 b	-	19.5
25%	249 ± 1.5 b	+ 4.2	18.2
50%	289 ± 4.1 a	+ 20.9	17.1
100%	203 ± 5.0 c	- 15.1	14.2

In a column, means followed by the same letter are not significantly different, at the 5% level.

DISCUSSION

Mulberry fruit are valuable food supplies that are consumed in various ways by humans. The fruit of *Morus spars* are slightly acidic, and have a quite dark color. It was mentioned that the origin of this color is anthocyanins that has an immense role in food industry (Jia *et al.*, 1999). Some

researchers, in their previous studies, have reported that mulberry fruit have antioxidant and free radical scavenging activity in considerable level (Jia *et al.*, 1999; Naderi *et al.*, 2004; Suh *et al.*, 2003, 2004; Arfan *et al.*, 2012; Mahmoud, 2013). It has been detected that in mulberry fruit, besides anthocyanins, there are other polyphenolic components such as rutin, resveratrol (Aydin *et al.*, 2011), myricetin and naringenin (Hakkinen *et al.*, 1999). It is known that naturally found flavonoids have extensively scavenging effect on oxygen radicals in vivo and vitro (Kravchenko *et al.*, 2003; Seyoum *et al.*, 2006). In a study by Ercişli and Orhan (2007), they stressed that there is a correlation between *M. nigra* fruits' total phenolic ingredients and antioxidant activity. It has been found that the mulberry fruit that was used in our study enhances the biology of silkworm and silk production. This maybe due to that black mulberry juice is highly nutritious and is full of anthocyanins, it has high amounts of zinc and iron. The black mulberry juice is also rich in ascorbic acid (23.45 mg/100 g (khalid *et al.*, 2011).

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إستخدام مستخلص ثمار التوت لتحسين إنتاج الحرير لدوده الحرير التوتية

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يستخدم التوت أساساً لتغذية ديدان الحرير وقد يستخدم كخضار أو علف للماشية في بعض مناطق العالم. وكما هو معروف أنه مصدر جيد من الأنتوسيانين وله بعض الخواص البيولوجية. ولا تتميز ثمار التوت الأسمر بقيمتها الغذائية فقط بل أيضاً في استخدامها في الطب البديل نظراً لاحتوائها على نسبة عالية من المركبات العلاجية النشطة. وقد تم في هذه البحث دراسة تأثير تغذية العمر الأخير لدوده الحرير التوتية على أوراق توت معاملة بمستخلص ثمار التوت الأسود على بيولوجي دوده الحرير التوتية. تم استخلاص الثمار مانياً على البارد وتم إضافتها إلى أوراق التوت المقدمة لتغذية دوده الحرير التوتية بتركيزات ٢٥، ٥٠، ١٠٠ % . أوضحت النتائج تحسن الحالة البيولوجية للحشرة حيث أعطى التركيز ٥٠% أفضل النتائج حيث زاد متوسط وزن اليرقة الكاملة النمو بمعدل ٢٣% وكذا الشرائق الطازجة بنسبه ٣٨,٢% والعذراء بنسبه ٤٠,٩٥% وأخيراً قشره الشرنقة بنسبه ٢٠,٩% مقارنة بالكنترول.