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Influence of Species and Different Varieties of Mulberry Trees on Consumption and Nutritional Efficiency of The Silkworm Larvae *Bombyx mori* L. Under Temperate Climate of Egypt

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ABSTRACT

Silkworm larvae were fed on the leaves of four mulberry trees*)Morus nigra* and three different varieties of *Morus alba* (Indian, Balady and Romi)) soon after hatching (newly hatched larvae) and during the larval stage. The results revealed that the maximum body weight of larvae occurred in larvae fed on leaves of *M. nigra* being 3.071 g. The larval duration was prolonged to 29 day in larvae fed on *M. nigra* and *M. alba* Indian, leaves. While the larval duration was 27 and 25 day in larvae fed on *M. alba* Balady and *M. alba* Romi, respectively. The maximum total weights of consumed and digested leaves by larvae (third, fourth and fifth instar) were 18.104g and 6.451g when larvae fed on leaves of *M. nigra,* respectively. The maximum efficiency of conversion of ingested and digested food to body substance in fifth instar larvae was 21.24 and 66.47% when larvae fed on leaves of *M. alba* Romi, respectively. On the other hand, The minimum efficiency of conversion of ingested and digested food to body substance in fifth instar occurred in larvae fed on leaves of *M. nigra,* (14.87% and 42.95 %, respectively). The maximum weight of fresh and dry cocoons was found in larvae fed on leaves of *M. nigra,* (1.449 and 1.305 g, respectively). The maximum length and weight of silk filament occurred in larvae fed on leaves of *M. nigra,* (1146 m and 0.23 g, respectively). The highest number of laid eggs was 420.7 occurred larvae fed on leaves of *M. nigra*. According to the chemical analysis, Percentage of total carbohydrate, protein, phenols as gallic acid and crude fibers (3.1916%, 21.00%, 1.168 % and 13.74%, respectively) were higher in leaves of *M. nigra,* than leaves of *M. alba*.

Keywords: mulberry trees – consumed – digested – silkworm – *Bombyx mori*

INTRODUCTION

Silkworm larvae, *Bombyx mori* L. dependant on mulberry leaves in rearing (monophagus), so that it must be improved and selected a better leaves to feed larvae to produce and obtain good yield of cocoons and raw silk. Mulberry trees in Egypt suffering from insects, careless and the road dust polluted mulberry leaves which affected the food consumption (Ahanger *et al.*, 2014) so that it must be care and grow many of mulberry trees from different species and varieties to give chance to rear silkworm and produced raw silk. The species and spacing of mulberry trees have significant effect on growth parameters of silkworm larvae (Eltayb *et al*., 2013). The leaves of mulberry trees mainly contain proteins, carbohydrates, vitamins, sterols and minerals. Such nutritional requirement in food consumption have direct effect on all genetic characteristics such as larval and cocoon weight, quantity of raw silk, pupation and reproductive traits (Purusothaman *et al*., 2012; Sabhat *et al*., 2011 and Ramesha *et al*., 2010).

The aim of the work is to study the effect of four different of mulberry trees on weights of larvae, instar duration, weight of consumed and digested leaves, efficiency of conversion of digested and ingested food to body substance, weight of cocoons, length and weight of silk filament and fecundity and fertility of eggs. In addition, chemical analysis to estimate the percentage of total carbohydrate, protein, chlorophyll, (Xanthophyll and Caroteins), phenols as gallic acid and crude fibers in leaves of four mulberry trees.

MATERIALS AND METHODS

Laboratory experiments were conducted on the monovoltine hybrid imported race of *B. mori* obtained from Sericulture Research Department, Agriculture Research Centre, Ministry of Agriculture in form of eggs and reared under laboratory conditions of 25 ± 3 0C, 72 ± 2 % R.H. and 15:9 daily L:D. Just before starting experiments, the implements and laboratory were disinfected by used formalin 3%.

Soon after hatching, larvae were divided into four groups, each group (25 larvae) divided into five replicates (5 larvae), which were supplied with sufficient amount of one of the four kind of mulberry leaves, (Morus nigra, Morus alba (Indian, Balady and Romi)) daily. The leaves were always cleaned and washed from dust, and were given to the different larval instars. The larvae were reared under standard rearing conditions (Krishnaswami., 1983).

Larvae were offered known weights of fresh mulberry leaves. To estimate the natural loss of water from the food, similar weights of leaves were kept in a separate box without larvae to calculate the evaporation rate of leaves under the same laboratory condition. The faecal pellets were sifted and the remaining dry leaves were daily collected and weighed. Larvae were also weighed at the beginning and at the end of each instar (3^{rd}, 4^{th} and 5^{th} instar) to determine the increase of weight in each instar larvae.

To estimate the mean weight of fresh consumed and assimilated food by each instar larvae fed on four different of mulberry leaves, the following equations suggested by Waldbauer (1964) was used:

- Weight of consumed food (C) = weight of fresh leaves offered to larvae – weight of fresh remnants.
- Weight of fresh remnants = weight of dry remnants x blank.
- Blank = total weight of fresh leaves in the control box / total weight of dry leaves in the control box after three day.

Weight of digested food (D) = weight of consumed food (C) – weight of faecal pellets.

- Efficiency of conversion of ingested food to body substance (E.C.I.) = (increase of weight in larva / C) x 100.
- Efficiency of conversion of digested food to body substance (E.C.D.) = (increase of weight in larva / D) x 100.
- Increase of weight in larva =weight of larva at the beginning of instar – weight of larva at the end of instar.

In addition, weight of fresh and dry cocoons, length and weight of silk filament, number and fertility of laid eggs/female was determined.

Chemical analysis

The four different leaves of mulberry trees were collected at the morning and washed by water then dried until constant weight in laboratory to estimate Percentage of total carbohydrate according to A.O. A.C., (1990), Proteins concentration was quantified in the crude extract by the method of Bradford, (1976) and Lowry et al. (1951), Chlorophyll a, b and carotenoids were extracted and estimated according to A.O. A.C., (1990), Determination of total soluble phenols (gallic acid) was performed using method described by Shahidi and Naczk (1995) and crude fibers at the Laboratory Center in Faculty of Agriculture, Ain Shams University.

RESULTS AND DISCUSSION

Weight of larvae and instar duration:

As shown in Table 1 the mean weight of third and fourth instar larvae was not affected by the kind of mulberry leaves, while the mean weight of fifth instar was affected. The higher weight of larvae gained when larvae fed on leaves of *M. nigra* mulberry (3.071g), while the lighter weight of larvae happened when larvae fed on *M. alba* Balady mulberry (2.828g).

Data presented in Table (1) indicated that the larval duration was affected by different varieties of mulberry leaves. The larval duration reached the maximum when larvae fed on leaves of *M. nigra* and *M. alba* (Indian) (29 day), while the minimum larval duration occurred when larvae fed on *M. alba* (Romi) (25 day).

This could be explained the higher weight of larvae when fed on leaves of *M. nigra* than when fed on *M. alba* (Romi) due to the larval duration prolonged in *M. nigra* four days more than that fed on *M. alba* (Romi). Accordingly the larvae when fed on *M. nigra* produced good yield of cocoons. Subhan *et al.* (2013) found that the larvae which fed on *M.nigra* leaves gained more body weight and gave better cocoon characters compared with leaves of *M. alba.*

Table 1 Mean weights of larvae at the end of the third, fourth and fifth instar larvae (g) fed on leaves of *Morus nigra* and *Morus alba* (Indian, Balady and Romi).

Mulberry trees	Weights of larval instars/g			larval duration/day
	3rd	4th	5th	
M. nigra	0.184 ±0.0273	0.712±0.0026	3.071^{a} ± 0.0045	29
M. alba (Indian)	0.218±0.0277	0.717±0.0033	2.835^{b} ±0.0286	29
M. alba (Balady)	0.157±0.0530	0.774±0.0665	2.828^{b} ±0.0510	27
M. alba (Romi)	0.189±0.0163	0.675 ±0.0184	2.988^{a} ±0.0702	25

***:means with the same superscript in the same column were not significantly different (p<0.05)**

Weight of consumed and digested mulberry leaves by silkworm larvae.

The obtained results in Tables 2 and 3, showed that the mean weights of consumed and digested leaves by larvae was highly significant affected by the different varieties of mulberry leaves in fourth and fifth instars but these weights did not significantly affected in third instar larvae. The maximum total weights of consumed and digested leaves by larvae of third, fourth and fifth instars were 18.104g and 6.451 g in larvae fed on leaves of *M. nigra*, respectively. The minimum total weights of consumed leaves by larvae of third, fourth and fifth instars were occurred in larvae fed on leaves of *M. alba* (Balady and Romi) (12.809g and 12.576g, respectively). The minimum total weights of digested leaves by the larvae of third, fourth and fifth instars were found in larvae fed on leaves of *M. alba* (Balady and Romi) (4.4811g and 4.9219g, respectively).

This could be explained that the total weights of consumed leaves by larvae fed on *M. nigra* was higher than that fed on *M. alba* (Romi) because the duration of larvae fed on leaves of *M. nigra* was longer than larvae which fed on leaves of *M. alba* (Romi). Sabhat *et al.* (2011) reared the larvae of mulberry silkworm on three different mulberry varieties. They found that the combined effect of the leaf quality and rearing season was significantly affected growth rate and consumption of the larvae.

Table 2 Mean weights of consumed mulberry leaves (g) by the third, fourth and fifth instar larvae fed on leaves of *Morus nigra* and *Morus alba* (Indian, Balady and Romi).

Mulberry trees	Weight of consumed leaves/g/larva			Total weight
	3rd	4th	5th	
M. nigra	0.4197 ±0.0678	1.8253^{a} ±0.0154	15.859^{a} ±0.0694	18.104
M. alba (Indian)	0.5106 ±0.0766	1.6944^{b} ±0.0373	11.459^{b} ±0.1277	13.664
M. alba (Balady)	0.3299 ±0.0600	1.7342^{b} ±0.0272	10.745^{c} ±0.0310	12.809
M. alba (Romi)	0.4272 ±0.0765	1.2577^{c} ±0.0229	10.891^{c} ±0.0400	12.576

***:means with the same superscript in the same column were not significantly different (p<0.05)**

Table 3 Mean weights of digested mulberry leaves (g) by the third, fourth and fifth instar larvae fed on leaves of *Morus nigra* and *Morus alba* (Indian, Balady and Romi).

Mulberry trees	Weight of digested leaves/g/larva			Total weight
	3rd	4th	5th	
M. nigra	0.2157 ±0.0726	0.7423[a] ±0.0860	5.493[a] ±0.0318	6.451
M. alba (Indian)	0.3386 ±0.1131	0.8604[a] ±o.o161	5.218[a] ±0.0289	6.417
M. alba (Balady)	0.1709 ±0.0364	0.8572[a] ±0.0268	3.453[c] ±0.1861	4.4811
M. alba (Romi)	0.2612 ±0.0652	0.5647[b] ±0.0205	4.096[b] ±0.1649	4.9219

***:means with the same superscript in the same column were not significantly different (p<0.05)**

Efficiency of conversion of ingested and digested food to body substance by silkworm larvae.

Data arranged in Tables 4 and 5, clearly showed the efficiency of conversion of ingested and digested leaves of four different varieties mulberry trees to body substance of the third, fourth and fifth instar larvae of *Bombyx mori*. The efficiency of conversion of ingested and digested food to body substance of the larvae was affected by the different varieties of mulberry leaves. The maximum efficiency of conversion of ingested and digested food to body substance was obtained in fifth instar larvae fed on *M. alba* (Romi) leaves (21.24 and 66.47), respectively. Meanwhile the minimum efficiency of conversion of ingested and digested food to body substance occurred in fifth instar larvae fed on *M. nigra* leaves (14.87 and 42.95), respectively.

This could be explained that although, the weights of consumed and digested leaves by the third, fourth and fifth instar larvae fed on leaves of *M. nigra* was very higher than the weights of consumed and digested leaves by larvae fed on leaves of *M. alba* (Romi) and weights of larvae at the end of fifth instar fed on leaves of *M. nigra* was higher than the weights of larvae at the same age and fed on leaves of *M. alba* (Romi) but this increase in weight of larvae was little due to the minimum efficiency of conversion of ingested and digested food to body substance was obtained in fifth instar larvae fed on *M. nigra* leaves Sabhat *et al.* (2011) found that the efficiency of conversion of ingested and digested food to body substance by the larvae of silkworm was affected by the different leaves of mulberry trees.

Table 4 Efficiency of conversion of ingested food to body substance by the third, fourth and fifth instar larvae fed on leaves of *Morus nigra* and *Morus alba* (Indian, Balady and Romi).

Mulberry trees	Efficiency of conversion of ingested food to body substance %		
	3rd	4th	5th
M. nigra	35.73 ±1.0002	28.93[c] ±0.6613	14.87[c] ±0.5348
M. alba (Indian)	34.86 ±0.5307	29.45[c] ±0.9063	18.48[b] ±0.6042
M. alba (Balady)	36.97 ±2.0984	35.58[b] ±0.6573	19.11[b] ±0.6327
M. alba (Romi)	33.00 ±2.0454	38.64[a] ±0.4205	21.24[a] ±0.4246

***:means with the same superscript in the same column were not significantly different (p<0.05)**

Table 5 Efficiency of conversion of digested food to body substance by the third, fourth and fifth instar larvae fed on leaves of *Morus nigra* and *Morus alba* (Indian, Balady and Romi).

Mulberry trees	Efficiency of conversion of digested food to body substance %		
	3rd	4th	5th
M. nigra	69.52[a] ±0.9553	71.13[b] ±0.6899	42.95[d] ±0.7348
M. alba (indian)	52.57[b] ±2.4699	57.99[c] ±1.7799	46.51[c] ±1.0040
M. alba (balady)	71.36[a] ±0.9185	71.98[b] ±0.8981	59.48[b] ±0.8205
M. alba (romi)	53.98[b] ±0.6940	77.05[a] ±0.6409	66.47[a] ±0.7062

***:means with the same superscript in the same column were not significantly different (p<0.05)**

Weights of fresh and dry cocoons, length and weight of silk filament/cocoon and fecundity and fertility of eggs/female of mulberry silkworm fed on different varieties of mulberry trees.

As shown in Table 6, the mean weights of cocoons, length and weight of silk filament and fecundity was affected by different varieties of mulberry trees. The maximum weights of fresh and dry cocoons (1.449 and 1.305 g/cocoon, respectively), the maximum of length and weight of silk filament (1146.8m and 0.23 g/cocoon, respectively) and the maximum of fecundity and fertility of eggs occurred (420.7/adult and 99.5%, respectively)were occurred in larvae fed on *M. nigra*. On the other hand, the minimum of weights (fresh and dry) cocoons, length and weight of silk filament and fecundity and fertility of eggs were occurred in larvae fed on *M. alba* (Indian), (0.737g, 0.550g, 639.3m, 0.15g/cocoon, 208.3/adult and 94.5%, respectively).

Subhan *et al.* (2013) found that the larval growth by weight was greatly influenced by the nutritive value of different mulberry leaves. The maximum cocoon weight of 1.34 g was recorded when larvae fed on leaves of *M. nigra* while minimum was 1.035 g when fed on leaves of *M. rubra*. The larvae which fed on leaves of *M.nigra* showed maximum cocoon shell weight of 0.55 g. Maximum shell ratio of 26.49% was recorded when larvae fed on leaves of *M.nigra* while minimum shell ratio 23.43% was recorded in larvae which fed on leaves of *M.alba*. These variations could be attributed to the nature of nutritive value in the different species of the mulberry leaves.

Table 6 Mean weights of fresh and dry cocoons, length and weight of silk filament/cocoon and fecundity and fertility of eggs/female of mulberry silkworm fed on different varieties of mulberry leaves.

Mulberry trees	Wt. cocoons (g)		Silk filament/cocoon		Eggs/adult	
	Fresh/cocoon	Dry/ cocoon	Length (m)	Weight (g)	fecundity	Fertility %
M. nigra	1.449[a] ±0.018	1.305[a] ±0.016	1146.8[a] ±9.720	0.23[a] ±0.005	420.7[a] ±3.29	99.5[a] ±0.16
M. alba (Indian)	0.737[d] ±0.013	0.550[d] ±0.008	639.3[d] ±10.200	0.15[c] ±0.012	208.3[d] ±6.24	94.5[c] ±0.69
M. alba (Balady)	1.100[c] ±0.023	0.961[c] ±0.013	883.4[c] ±10.332	0.18[b] ±0.005	305.3[c] ±4.49	96.6[b] ±0.99
M. alba (Romi)	1.284[b] ±0.012	1.077[b] ±0.021	934.5[b] ±13.730	0.21[a] ±0.008	352.0[b] ±9.09	99.7[a] ±0.42

***:means with the same superscript in the same column were not significantly different (p<0.05)**

Chemical analysis of four different leaves of mulberry trees.

As shown in Table 7, the percentage of total carbohydrate, protein, chlorophyll, (Xanthophyll and Caroteins), phenols as gallic acid and crude fibers differed in the different leaves of mulberry leaves varieties.

The maximum percentage of total carbohydrate occurred in leaves of *M. nigra* and *M. alba* (balady) (3.1916 and 3.0385 %, respectively) but the minimum percentage of total carbohydrate occurred in leaves of *M. alba* (Indian) and *M. alba* (Romi) was (2.2521 and 2.0496 %, respectively). The maximum percentage of total protein was found in leaves of *M. nigra* and *M. alba* (Romi) (21.00 and 19.69 %, respectively) but the minimum percentage was found in leaves of *M. alba* (Balady) and *M. alba* (Indian) (11.69 and 10.31 %, respectively). The maximum of pigment as chlorophyll and Xanthophyll + Caroteins occurred in leaves of *M. alba* (Romi) (1.1199 and 4.477 %, respectively) but the minimum one was found in leaves of *M. nigra* (0.4421 and 2.359%, respectively). The maximum percentage of phenols as gallic acid occurred in leaves of *M. nigra* and *M. alba* (Romi) (1.108 and 1.240 %, respectively) but the minimum one occurred in leaves of *M. alba* (Balady) and *M. alba* (Indian) (0.334 and 0.058 %, respectively). The maximum percentage of crude fibers occurred in leaves of *M. nigra* and *M. alba* (Romi) (13.74% and 12.82%, respectively) but the minimum one occurred in leaves of *M. alba* (Balady) and *M. alba* (Indian) (7.50% and 8.07 %, respectively).

Subhan *et al.* (2013) found that the maximum protein (21.06%) and carbohydrate (37.25%) were found in *M.nigra* leaves compared with *M. alba* leaves. Hamamura *et al*. (1966) found that the silkworm larvae reared on the artificial diet which did not contain gallic acid did not reach the 2nd instar in 6 to 9 days after hatching. on the other hand, when larvae reared on the diet contain gallic acid reached the 2nd instar in only 4 or 5 days after hatching, and their growth was similar to that of those fed on untreated fresh mulberry leaves.

Table 7 Percentage of total carbohydrate, protein, chlorophyll, (Xanthophyll + Caroteins), phenols as gallic acid and crude fibers in different leaves of varieties of mulberry trees.

Mulberry trees	Carbohydrate %	Protein %	Chlorophyll %	(X+c) %	phenols as gallic acid %	Crude fibers %
M. nigra	3.1916	21.00	0.4421	2.359	1.108	13.74
M. alba (Indian)	2.2521	10.31	0.5900	2.653	0.058	8.07
M. alba (balady)	3.0835	11.69	0.6618	4.190	0.334	7.50
M. alba (Romi)	2.0496	19.69	1.1199	4.477	1.240	12.82

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تأثير الأنواع والأصناف المختلفة لأشجار التوت على الاستهلاك والكفاءة الغذائية ليرقات دودة الحرير التوتية تحت الظروف المناخية فى مصر

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تم تغذية ديدان الحرير على أربع أنواع من أورق شجر التوت(التوت العمانى(الاسود) والتوت الابيض (الهندى والبلدى والرومى ( ) بعد فقس البيض من بداية العمر اليرقى الأول حتى نهاية العمر الخامس. وكانت النتائج المتحصل عليها هى : كان أقصى وزن لليرقات ٣.٠٧١ جرام فى اليرقات التى تغذت على التوت العمانى,وسجلت مدة العمر اليرقى عند تغذية اليرقات على كل من التوت العمانى والتوت الهندى ٢٩ يوم بينما سجلت ٢٧ يوم فى اليرقات التى تغذت على التوت البلدى ثم ٢٥ يوم فى اليرقات التى تغذت على التوت الرومى. وكان أقصى وزن لأوراق التوت التى تناولتها والمهضومة فى الطور اليرقى (عمر ثالث ورابع وخامس) هى ١٨.١٠٤ و٦.٤٥١ جرام عند تغذيتها على أوراق التوت العمانى ,على التوالى. وأقصى كفاءة لتحويل الغذاء المتناول والمهضوم الى مواد جسمية فى العمر الخامس لليرقات هى ٢١.٢٤ و ٦٦.٤٧ % وذلك فى اليرقات التى تغذت على أوراق التوت الرومى, على التوالى. وأقل كفاءة تحويل للغذاء المتناول والمهضوم الى مواد جسمية فى العمر الخامس كانت فى اليرقات التى تغذت على أوراق التوت العمانى (١٤.٨٧ و ٤٢.٩٥ %, على التوالى). بالأضافة الى ذلك كان أقصى وزن للشرانق الطازجة والجافة لليرقات التى تغذت على أوراق التوت العمانى (١.٤٤٩ و ١.٣٠٥ جرام , على التوالى).وكذلك اعطى أقصى طول ووزن للخيط الحريرى للشرنقة (١١٤٦ متراً و٠.٢٣ جرام , على التوالى). وبالنسبة الى الفراشات التى تغذت يرقاتها على أوراق التوت العمانى أعطت اعلى كفاءة تناسلية حيث قدر عدد للبيض ٤٢٠.٧ بيضة/ أنثى. وعضدت التحليلات الكيميائية فى ذلك حيث كان نسبة الكربوهيدرات والبروتين والفينولات ممثلة فى حمض الجاليك الموجودة فى التوت العمانى وهى ٣.١٩١٦ و ٢١.٠٠ و ١.١٦٨ %, على التوالى أعلى من أوراق التوت الأخرى.