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BIOLOGICAL ACTIVITY AND ENHANCEMENT OF SILK YIELD PRODUCED BY Bombyx mori L. LARVAE FED ON MULBERRY LEAVES FORTIFIED WITH ROYAL JELLY

Gomaa, H. A.
Plant Protection Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

ABSTRACT

Nutritional values of mulberry leaves greatly affected growth and development of the commercial silk worm, *Bombyx mori* L. larvae and in turn silk production. Therefore, larvae of the last two instars were fed on treated leaves by dipping them in royal jelly solution at the rate of 5 g in 200 ml distilled water before offering them to larvae as food. This treatment increased the weights of the fourth and fifth instar larvae, weights of fresh cocoons, pupae, cocoon shell and reeled silk filament. The weights of ingested and digested treated mulberry leaves as well as approximate digestibility and efficiency of conversion of ingested and digested food to body substances in the fourth and fifth instar larvae were also increased as compared with those of larvae fed on untreated mulberry leaves. Feeding the last two instar larvae on treated leaves elicits favourable response in improving the commercial quantities of silk fibre and can be used in Sericulture for yield enhancement.

Keywords: Feeding Efficiency, Silk Production, Mulberry Silk, Worm, Royal Jelly Treatment, Biological Aspects

INTRODUCTION

The mulberry silk worm, *Bombyx mori* L. is a monophagous insect, wghich survives on mulberry leaves. Such food plays an important role in nutrition of this beneficial insect and in turn affects silk production (Nagaraju, 2002). Fresh cocoon and cocoon shell weights are the major traits evaluated for productivity in Sericulture. According to Seidavi *et al.* (2005), substances of the mulberry leaves greatly affect growth and development of *B. mori* larvae and in turn silk production. Mulberry leaves of superior quality enhance the chances of good cocoon crop (Ravikumar, 1988). Nutrition of *B. mori* larvae has a direct influence on quality and quantity of silk production (Murugan *et al.*, 1998).

Recently, trials were made to improve the quality and quantity of produced natural silk through enhancing the leaves with nutrients (Hiware, 2006 and Nguku *et al.*,2007). On the other hand, the effects of vitamin-enriched food on the reproduction of *B. mori* female had been studied by Faruki *et al.* (1992) and Saha and Khan (1999). One of the most enriched food is royal jelly which is produced by the young (nurse) adult honey bee workers to feed brood and the queen. Such food is rich in nutrients and energy (Vitek,1995).

The present work aimed at studying the effects of feeding the late larval instars on mulberry leaves fortified with royal jelly on larval growth, silk production and feeding efficiency of *B. mori.*

MATERIALS AND METHODS

***B. mori* rearing technique**

Laboratory experiments were conducted on the monovoltine hybrid local race of *B. mori* obtained from Sericulture Research Department, Agriculture Research Centre, Ministry of Agriculture in form of eggs and reared under Laborat6ory conditions of 25±3 ºC, 72±2% R.H. and 16 : 8 hrs (L : D) daily photoperiodic regimes.

Soon after hatching, larvae were supplied with sufficient amounts of mulberry leaves, *Morus alba* two times daily on breeding frames (200 x 80 cm each). Leaves were given to the first and second instars as strips and the entire leaves were given to the other instars. Dried leaves and bellets of faeced were removed two times daily using perforated papers with small holes for the first three instars and with large holes for the fourth and fifth instars.

Preparation of royal jelly solution:

Royal jelly was obtained from the apiary of Faculty of Agriculture, Ain Shams University. *Apis mellifera* worker larvae were grafted at the age of 24 hrs. the royal jelly was harvested after 48 hrs and kept in deep freezer until used. The concentration of royal jelly used in the experiments was prepared by adding 200 ml of distilled water to 5 g of royal jelly. The entire mulberry leaves were dipped in this solution for few seconds and allowed to dry under laboratory conditions before offering them to larvae of the treated group. The remaining royal jelly solution was put in deep freezer and kept under laboratory conditions one hour before continuous use. Larvae of the control (untreated) group were simultaneously reared on fresh mulberry leaves dipped in distilled water only and dried under room conditions before offering them to larvae

Experimental work:

Soon after the third ecdysis, 100 newly moulted fourth instar *B. mori* larvae were divided into two groups. The first group of larvae were fed on on mulberry leaves fortified with royal jelly from the beginning of the fourth instar till the end of the last (fifth) instar, and larvae of the second group were fed on untreated leaves as control. Larvae of each group were divided into 10 subgroups (replicates), each contained 5 larvae and put in rearing box.

Four subgroup of larvae from either treated or untreated group were supplied with known amounts of mulberry leaves two times daily and the amounts of remaining leaves, the weights of faecal bellets as well as the daily larval weights were recorded. The indices of consumption, digestion and utilization of food suggested by Waldbauer (1964), modified by El-Shaarawy and Gomaa (1975) and applied by Gomaa (2009) were used.

Four days after spinning the cocoons, another four subgroups from each group were collected and weighed. The weights of pupae and cocoon shells were recorded the cocoon shell ratio (CSR%) were calculated according to the following formulation:

$$CSR\ (\%) = \frac{\text{single cocoon shell weight (mg)}}{\text{Single cocoon weight (mg)}} \times 100$$

Another two subgroups of cocoons from each group were taken, allowed to dry in an electric incubator at 60ºC for 6 hrs and reeled. The weight and length of silk filament per cocoon were obtained and recorded.

RESULTS AND DISCUSSION

Daily weights of *B. mori* larvae:

Soon after the third ecdysis, the newly moulted fourth instar larva fed on untreated mulberry leaves averaged 368.05 mg. this weight gradually increased as the age of larva progressed to reach the maximum (560.76 mg/ larva) at the end of the fourth instar (Table 1). In the seventh day, the same trend was found in the fourth instar *B. mori* larvae fed on mulberry leaves fortified with royal jelly. Means of 361.89 and 705.68 mg were recorded for the weights 0f one and seven day old larvae, respectively. On the other hand, larvae fed on treated leaves were always heavier than those of the corresponding age of larvae fed on untreated leaves. The difference between both means at any age was statistically significant and the "t" values emphasize the obtained results (Table 1).

Table (1): Daily weights of fourth instar *B. mori* larva (mg) fed on mulberry leaves fortified with royal jelly solution (Means ± S,E.)

Successive days during the 4th instar	Mean weight of larva fed on		"t" value	Rate of increment (%)
	Treated leaves	Untreated leaves (Control)		
1	361.89± 42.16	368.95±32.25	0.3261*	9.00
2	432.68± 40.78	405.61± 27.19	0.5107*	6.67
3	486.52± 51.29	450.48± 38.15	0.6263*	8.00
4	557.19± 44.48	491.52± 29.64	0.6719*	13.59
5	594.34± 52.20	522.90± 36.16	0.6698*	13.66
6	646.26± 39.86	541.89± 40.02	0.7012*	19.26
7	705.68± 56.05	560.76± 31.96	0.7303*	25.84
F between days	29.08**	18.44**		
L.S.D. at 0.05	36.64	28.15		

After the fourth ecdysis, the weight of one day old *B. mori* larva averaged 630.23 and 725.41 mg when it fed on untreated and treated mulberry leaves from the beginning of the fourth instar, respectively. The rate of increment was 15.10%. the weight of larva gradually inceased as its age progressed to reach the maximum in full grown larva. Means of 3267.27 and 3435.32 mg/ individual were recorded for the weights of 8 day old fifth instar larvae fed from the beginning nof the fourth instar on untreated mulberry leaves and leaves treated with royal jelly solution, respectively. The rate of increment due to leaf treatment was 5.14% (Table 2).

At any day within the last larval instar, the weight of *B. mori* larva fed on royal jelly treated mulberry leaves was heavier than that of larva fed on untreated leaves. The difference between both means proved to be

statistically significant and the "t" values emphasize the obtained results (Table 2).

According to Keizo *et al.* (1965), royal jelly contains acetylcholine which is required in silk worm diet for occurrence of normal larval moulting. In 1989, Saikatsu *et al.* found that the rate of growth and development accelerated by addition of royal jelly to the artificial diet of the silk worm larvae.

Table (2): Daily weights of fifth instar *B. mori* larva *(*mg) fed during fourth and fifth instars on mulberry leaves fortified with royal jelly solution (Means ± S,E.)

Successive days during the 5th instar	Mean weight of larva fed on		"t" value	Rate of increment (%)
	Treated leaves	Untreated leaves (Control)		
1	725.41±56.58	630.23±67.39	0.6262*	15.10
2	1128.64±62.35	964.82±71.62	0.7156*	16.98
3	1575.38±68.20	1296.11±63.08	0.7222*	21.55
4	2085.35±58.49	1772.94±59.25	0.7635*	17.64
5	2640.67±66.17	2272.58±69.54	0.7701*	16.20
6	2992.53±54.54	2674.86±77.71	0.7679*	11.88
7	3191.46±59.46.	3011.45±65.51	0.5373*	5.98
8	3435.32±62.62	3267.27±75.51	0.6148*	5.14
F between days				
L.S.D. at 0.05				

Weights of *B. mori* cocoons and pupae:

Four days after spinning the cocoons, the mean weight of fresh cocoon was 1328.57 and 1504.82 mg for that produced from larva fed on untreated leaves and that fed during the fourth and fifth instars on mulberry leaves fortified with royal jelly solution. The difference between both mean weights proved to be statistically significant (Table 3). The same trend could be applied for the weight of pupa as being affected by the royal jelly addition to larval food. Means of 1040.68 and 1216.15 mg/ pupa were recorded for mean weights of pupae fed as fourth and fifth instar larvae on untreated and treated mulberry leaves, respectively, with rate of increment of 16.86%. the difference between mean weights was statistically significant.

According to Nguku *et al.* (2007), feeding B. mori larvae on mulberry leaves fortified with royal jelly resulted in heavier pupae and cocoons.

Silk production and reeling silk filament:

The data given in table (3)clearly show that the mean weight of cocoon shell produced from *B. mori* larvae fed during the last two instars on mulberry leaves fortified with royal jelly (298.84 mg) was significantly heavier than that produced from larvae fed on untreated leaves (283.26 mg). the rate of decrement in this case was 5.50%. However, cocoon shell ratio (CSR) did not statistically affect by the royal jelly treatment offered to larvae. Means of19.86 and 21.32% were recorded for CSR when larvae of the last two instars were fed on treated and untreated mulberry leaves, respectively (Table 3).

It is well known that the use of high protein larval diet effectively increased the quality of silk shell produced by *B. mori* larvae. However,

contradicting results were given by Nguku *et al.* (2007), who found that no significant difference was noticed between shell weghts produced by larvae fed bon mulberry leaves fortified with royal jelly and those fed on untreated leaves.

Table (3): Characteristics of *B. mori* cocoon and pupa fed during the fourth and fifth larval instars on mulberry leaves fortified with royal jelly solution

Characteristics of Cocoons and pupae	Larvae fed on		"t" value	Rate of increment (%)
	Treated leaves	Untreated leaves		
Mean weight of fresh cocoon (mg)	1504.82 ±101.66	1328.57±111.36	0.6021*	13.26
Mean weight of pupa (mg)	1216.15±96.08	1040.68±85.78	0.5976*	16.86
Mean weight of cocoon shell (mg)	298.84. ±15.35	283.26±18.82	0.3547*	5.50
Cocoon shell ratio (%)	19.86	21.32		

After reeling the cocoons, the length and weight of silk filament were obtained and the data tabulated in table (4). From this table, it is obvious that thr length uf reeled filament did not statistically affect by the food treatment. Means of 978.37 and 950.46 meters were recorded for the length of silk filament obtained from cocoon fed as fourth and fifth larval instars on leaves fortified with royal jelly and on treated leaves, respectively, with the rate of increment of 2.94% only. However, the weight of produced filament was significantly affected by the larval food treatment, as 289.89 and 266.45 mg were given for dilk filament reeled from cocoons fed as larvae on treated and untreated mulberry leaves, respectively. With the rate of increment of 8.80% (Table 4).

According to Kamimura and Kiuchi (1998), the increase in silk filament length is the most important commercial character in the improvement of natural silk yield.

Table (4): Mean length and weight of silk filament reeled from cocoon fed as larva during the fourth and fifth instars on mulberry leaves fortified with royal jelly solution

Characteristics of cocoons and pupae	Larvae fed on		"t" value	Rate of increment (%)
	Treated leaves	Untreated leaves		
Mean length of silk filament (m)	978.37±64.12	950.46±56.26	0.3154	2.94
Mean weight of silk filament (mg)	289.89±2028	266.45±16.58	0.6031*	8.80

Consumption, digestion and utilization of food:

B. mori larva consumed and digested an average of 1076.55 and 665.28 mg untreated mulberry leaves during its whole fourth instar. Respectively. Treating the leaves with royal jelly solution, increased the weights of consumed and digested food. Being 1291.64 and 853.42 mg/ larva, with the rates of increment of 19.76 and 28.28%, respectively.

The calculated approximate digestibility (AD) as well as the efficiency of conversion of ingested (ECI) and digested food (ECD) to body substances had been calculated. AD measures the relation between the amounts of ingested and digested food in the larval alimentary canal based on the fresh weight. ECI measures the overall ability of larva to convert the ingested fresh food to body substances, i.e., body tissues and stored nutritional compounds in addition to energy production. ECD measures the assimilation of digested food into larval body substances. These parameters increased after feeding the fourth instar larva on treated mulberry leaves. Means of 61.68 and 66.07% for AD, 34.05 and 36.66% for ACI, and 45.95 and 49.72% for ACD were recorded when the fourth instar larva was fed on untreated and treated leaves, respectively. The difference between treated and untreated every criterion proved to be statistically significant and the "t" values emphasize the obtained results.

The same trend could be applied for the feeding efficiency of the fifth instar larva as being affected by treating the mulberry leaves with royal jelly and offering it to the last two instars. Larva consumed and digested an average of 7178.64 and 5073.34 mg untreated mulberry leaves during its whole fifth instar, respectively. Treating the leaves with royal jelly solution, increased the weights of consumed and digested food. Being 8915.53 and 6908,61 mg/ larva, with the rates of increment of 24.20 and 36.17%, respectively. The same findings were given recently by Nguku *et al.* (2007), who stated that royal jelly in larval diet of *B. mori* silk worm caused enhanced absorption of consumed food and better assimilation of digested compounds.

AD, ECI and ECD values. increased after feeding the fourth and fifth larval instars on treated mulberry leaves. Means of 70.67and 77.49% for AD,33.09 and 37.35% for ECI, and 45.68 and 52.02% for ECD were recorded when the fifth instar larva was fed on untreated and treated leaves, respectively. The difference between treated and untreated any tested criterion proved to be statistically significant and the "t" values emphasize the obtained results.

Table (5): Consumption and utilization of mulberry leaves fortified with ruyal jelly by the fourth instar *B. mori* larva

Nutritional aspects	Larvae fed on		"t" value	Rate of increment (%)
	Treated leaves	Untreated leaves		
Mean weight of consumed leaves (mg)	1291.64±86.12	1076.55±79.91	0.6016*	19.76
Mean weight of digested leaves (mg)	853.42 ± 61.44	665.28 ± 57.09	0.6469*	28.28
Mean approximate digestibility (AD) (%)	66.07	61.68	0.4097	
ECI * (%)	36.66	34.05	0.3631	
ECD ** (%)	49.72	45.95	0.3867	

***ECI, Efficiency of conversion of ingested food to body substances**
****ECD, Efficiency of conversion of digested food to body substances**

From the fore-mentioned results, it could be concluded that feeding the last two instars of silk worm larvae on mulberry leaves fortified with royal jelly elicits favourable response in improving the commercial quantities of silk fibre and can be used in Sericulture for yield enhancement

Table (6): Consumption and utilization of food by the fifth instar *B. mori* larva after feeding on mulberry leaves fortified with royal jelly during the fourth and fifth instars

Nutritional aspects	Larvae fed on		"t" value	Rate of increment (%)
	Treated leaves	Untreated leaves		
Mean weight of consumed leaves (mg)	8915.53±201.82	7178.64±211.08	0.6534*	24.20
Mean weight of digested leaves (mg)	6908.61±178.67	5073.34±165.59	0.8017*	36.17
Mean approximate digestibility (AD) (%)	77.49	70.67	0.5068*	
ECI * (%)	37.35	33.09	0.5007*	
ECD ** (%)	52.02	45.68	0.5022*	

***ECI, Efficiency of conversion of ingested food to body substances**
****ECD, Efficiency of conversion of digested food to body substances**

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النشاط الحيوى وتحفيز محصول الحرير الناتج من يرقات دودة القز المغذاه على أوراق توت معاملة بالغذاء الملكي

هانى أحمد على جمعه

قسم وقاية النبات – كلية الزراعة بجامعة عين شمس – شبرا الخيمة – القاهرة – مصر

من المعروف أن معدلات نمو وتطور يرقات دودة القز تتأثر يشدة بالمحتوى الغذائي لأوراق التوت المقدمة لها ، ويؤثر ذلك بالتبعية على كمية الحرير الطبيعي الناتج من هذه اليرقات ، ومن جهة أخرى فإن اليرقات ذات الأعمار الكبيرة وخصوصا في العمر الأخير (الخامس في حالة دودة القز) تعتبر شرهة لتناول الغذاء وتعبر بصدق عن أطوارها اللاحقة (العذراء والحشرة الكاملة) ، لذلك ففي الدراسة الحالية غذيت يرقات العمرين الأخيرين (الرابع والخامس) من دودة القز (السلالة المحلية) على أوراق توت من الصنف الأبيض سبق معاملتها بمحلول الغذاء الملكي بمعدل 5 جم غذاء ملكى لكل 200 مل ماء مقطر ، وقد أدت هذه المعاملة الى تحسين الصفات البيولوجية لليرقات والمواصفات الكمية للحرير الطبيعي الناتج منها.

ويمكن تلخيص أهم النتائج المتحصل عليها فيما يأتي :

1- زيادة الأوراق اليومية ليرقات العمرين الرابع والخامس ، وكذلك أوزان الشرنقة الطازجة والعذراء وقشرة الشرنقة والخيط الحريري الذي تم حله من الشرنقة ، بينما لم يتأثر احصائيا طول الخيط الحريري

2- زيادة أوزان أوراق التوت المعاملة المتناولة والمهضومة اليومية وكذلك معامل الهضم التقريبي ومعدلات كفاءة تحويل الغذاء المتناول والمهضوم لمركبات جسمية في يرقات العمرين الرابع والخامس وذلك بالمقارنة بالقيمة المتحصل عليها عند تغذية يرقات العمرين المذكورين على أوراق توت غير المعاملة بمحلول الغذاء الملكي.

3- تغذية يرقات العمرين اليرقيين الأخيرين لدودة القز على أوراق توت معاملة تسبب استجابة مناسبة فى تحسين وزن اليافع الحرير الطبيعى الناتجة من الناحية التجارية ، وبالتالي يمكن إستخدام هذه المعاملة في تحفيز كمية الحرير المفرزة من اليرقات اليافعة عند غزل الشرنقة وبالتالي وزن الخيط الحريري الناتج من حل هذه الشرنقة

قام بتحكيم البحث

أ.د / عبد البديع عبد الحميد غانم	**كلية الزراعة – جامعة المنصورة**
أ.د / محمد احمد احمد عيد	**كلية الزراعة – جامعة القاهرة**