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Biological and technological effects of mulberry varieties and nutritional additives on silkworm *Bombyx mori* development

Abdel-Hamid AI Zannoon [1], Eman MM Hassan [1*], Somia S El-Akkad [2], Ismail M Abdel-Nabi [3] & Samy M Zalat [3]

1 - Plant Protection Research Institute, Agriculture Research Center, Cairo, Egypt.
2 - Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt.
3 - Zoology Department, Faculty of Science, Suez Canal University, Ismailiya, Egypt.

Abstract

The efficiency of two varieties of mulberry leaves, *Morus alba* var. Kokuso-27 and *Morus indica* var. Kanva-2, was investigated and compared with *Morus alba* var. Balady (native), the common local variety in Egypt. Mulberry varieties were offered to silkworm larvae in two, three or four feeds per day. Rearing with Kokuso-27 leaves, especially with the feeding schedule of four feeds per day, exhibited significantly shorter larval duration, lower larval mortality rate and increased larval fitness, cocooning percentage, fecundity and hatchability. It also yielded higher cocoon weight, silk content ratio and silk filament size. Kanva-2 showed the lowest performance. Kokuso-27 leaves were enriched with nutritional additives such as vitamins C (1%) and B (0.2%), and three kinds of honey (from clover, cotton and citrus, 50%) and offered once per day. Significant enhancement occurred in all tested groups compared with the control, especially for vitamin C and clover and citrus honey, in most variables except larval duration and fitness.

Keywords: *Morus alba* var. Kokuso-27, *Morus indica* var. Kanva-2, *Morus alba* native, Vitamin C, Vitamin B, Bee-honey.

Introduction

The quality of mulberry leaves plays an important role in the success of the sericulture industry and directs its economics (Choudhury *et al*. 1991), and hence much effort and research have been carried out to improve the quality and quantity of mulberry-leaf production for silkworm rearing and then cocoon production. Some varieties of mulberry leaves appeared to be superior to others (Raman *et al*. 1995). It is well-known that the amount and quality of mulberry leaves affects growth rate, developmental period, body weight, and survival rate of larvae, as well as influencing the subsequent fecundity, longevity, movement and competitive ability of the adults (Parra 1991). The nutritional status of mulberry leaves can be improved by enriching them with extra nutrients to increase larval growth and improve cocoon characteristics (Sengupta *et al*. 1992). Mulberry leaves immersed in solutions of 0.5, 1.0 and 1.5% ascorbic acid (vitamin C) solution significantly increased the weights of both larvae and cocoons of silkworm compared with untreated leaves (Miranda *et al*. 1998). Suprakash & Pal (2002) supplemented fresh mulberry leaves with 3 levels of vitamin B complex by dipping them in 0.5, 1.0 and 1.5% solution, and fed the dried leaves to larvae of various *Bombyx mori* races: the 0.5% level increased the weights of larvae and cocoons, and the shell ratio. Treatments of *B. mori* larvae with propolis extracts (a honeybee product) yielded heavier cocoons and cocoon shells, a higher silk content, and females laid more eggs than controls (Nour *et al*. 1997). Zannoon (1994) reported that, Various solutions of bee honey increased the mature weight of the larvae, silk gland, fresh cocoon and cocoon cortex, the number of deposited eggs per female, gave heavier, longer filaments of reeled cocoons, but did not affect cocoon silk content ratio and filament size.

We evaluate here the efficiency of two varieties of mulberry leaves (*Morus alba* var. Kokuso-27 and *Morus indica* var. Kanva-2) compared with *Morus alba* var. Balady (native), and determine the best feeding schedule. We then study the effects of fortification of mulberry leaves by vitamin C, vitamin B and three kinds of bee-honey on the biology and silk production of mulberry silkworm *Bombyx mori*.

* Address for correspondence

Materials & Methods

Widely cultivated in Japan, *Morus alba* var. Kokuso-27 was generated by an artificial hybridization between Naganuma (♀) × Kairyonezumigaeshi (♂). Its leaves are characterized by their shiny dark green colour, by remaining soft for a long time, and by tolerating drought. It can also be grown under wet conditions. It has high water (76%), crude protein (23%), and total sugar 14% content (Minamizawa 1997). *Morus indica* Kanva-2 is diploid, and widely cultivated in southern India, a result of selection from a natural population from Mysore. It has lower leaf moisture (70%), protein (21%) and sugar (11.5%) content, but a high rooting ability (80%) and great adaptability. It is resistant to leaf spot, and moderately resistant to leaf rust and powdery mildew (Datta 2000).

The rearing of silkworm (*Bombyx mori* 9F 7X- Chinese hybrid) was carried out under laboratory conditions (26±2 °C, 70±5% relative humidity), according to the technique of Krishnaswami (1978). The experiment had two parts. Firstly, larvae were reared on fresh mulberry leaves of three varieties (*Morus alba* var. Kokuso-27, *Morus indica* var. Kanva-2 and the native *Morus alba* var. Balady) under three different feeding schedules (two, three or four feeds per day). Second, larvae were reared on the variety of mulberry identified as the best one from the first part (Kokuso-27), enriched with 1% vitamin C, 0.2% vitamin B or one of three kinds of bee-honey (clover, cotton and citrus, each at 50%: each was compared with the control group. Mulberry leaves were dipped in each material for one minute and left to dry, and fed to larvae once per day; control leaves were dipped in distilled water. Each group was replicated three times.

The following biological variables were recorded. During development, larvae need large amounts of mulberry leaves costing effort and money, and hence the duration of the larval and moulting period (D) was measured for each tray. During each of the fourth and fifth larval instars, the daily number of dead larvae was recorded from each tray, and larval mortality rate (m) calculated as a percentage of the total number of larvae alive at the beginning of the 4th instar. The cocooning percentage was calculated as the number of fresh cocoons produced as a percentage of the total number of larvae alive at the beginning of 5th instar. Six pairs of adult moths from each tray were paired separately in paper cages, and the total number of deposited eggs per female were counted as a measure of fecundity (V). The hatchability percentage was the total number of hatched eggs per female as a percentage of the total number of eggs laid. Individual fitness (r) was calculated as a integrated performance measure by combining development time (D), survival (m) and fecundity (V) via the equation:

$$r = [Ln (m \times V)] / D \quad \text{(McGraw \& Caswell 1996)}$$

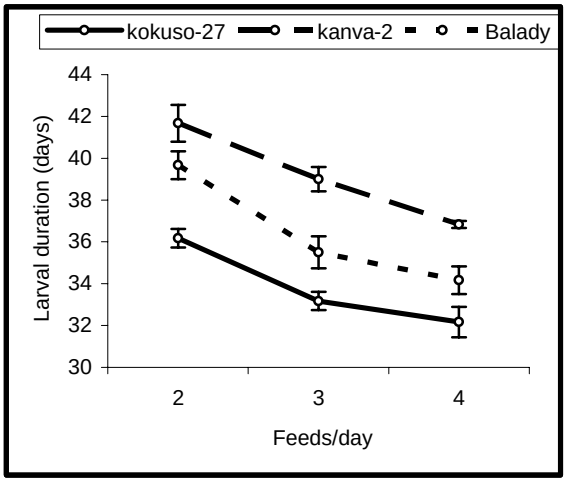
where Ln = natural logarithms, m = survival probability, V = fecundity, D = duration.

A number of technological variables were also recorded. For coccon weight, fifty cocoons from each group were collected randomly soon after pupation, cleaned and weighed separately (in g). The silk content ratio (net silk yield) was measured from the weight of the cocoon shell (weighed after removing the internal pupa) as a percentage of the weight of the fresh cocoon (Tanaka 1964). For the size of a reelable filament (dn), fifty cocoons from each group were collected, oven dried and reeled by an individual reeling machine, and the reelable filament length and weight recorded; the size of reeled silk filaments was calculated (Tanaka 1964) as the weight of the silk filament (in mg) divided by the length (in m) and multiplied by 9000 (the result is in 'denier').

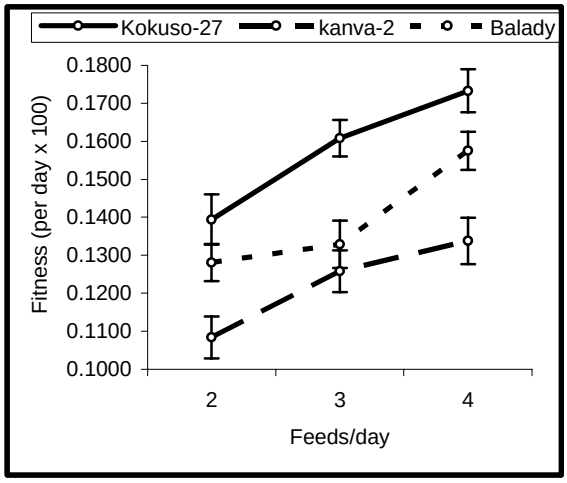
Biological and technological data were statistically analyzed (Little & Hills 1975) using Anova implemented by the Coastat software program.

Results

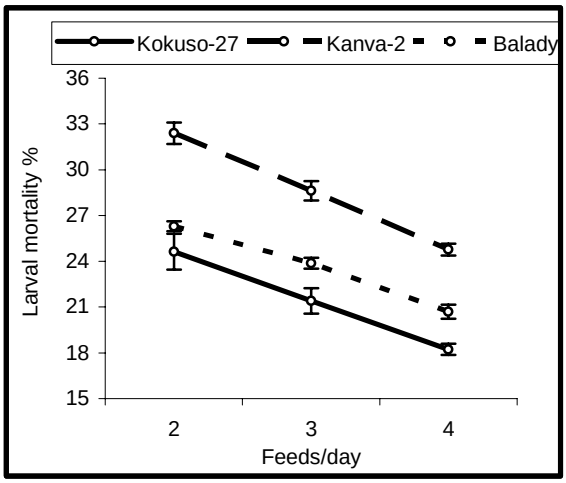
Of the three mulberry varieties tested, larvae fed on Kokuso-27 with feeding schedule four feeds per day showed the shortest total larval duration ($F_{2,18}$ = 54.4, p<0.001) the lowest total mortality rate ($F_{2,18}$ = 98.7, p<0.001), and the highest larval fitness ($F_{2,36}$ = 29.4, p<0.001) (see Fig. 1a,b,c). Similarly, when fed Kokuso-27 with four feeds per day, a significant increase in cocooning percentage ($F_{2,18}$ = 145.3, p<0.001), fecundity ($F_{2,45}$ = 43.2, p<0.01) and egg hatchability ($F_{2,45}$ = 92.7, p<0.001) resulted (Fig. 1d,e,f). In virtually all cases the Balady variety was intermediate and the Kanva-2 variety poorest. The analysis of interaction between effects of mulberry varieties and feeds number/day revealed non significant variation (p>0.05) within biological characters except fecundity ($F_{4,45}$ = 2.45, p<0.05) and hatchability ($F_{4,45}$ = 3.33, p<0.05).



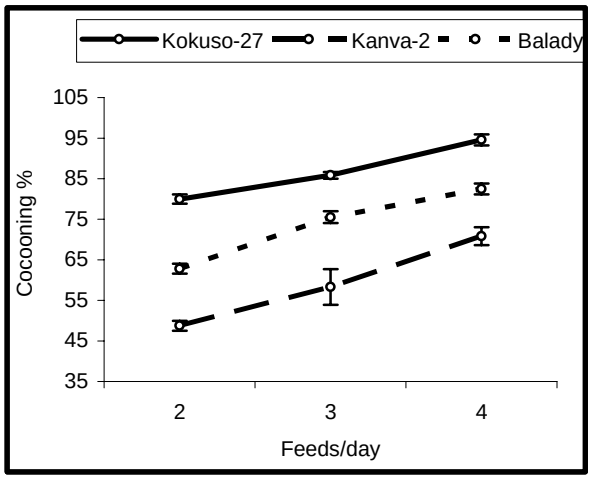
(a) Larval duration (days)



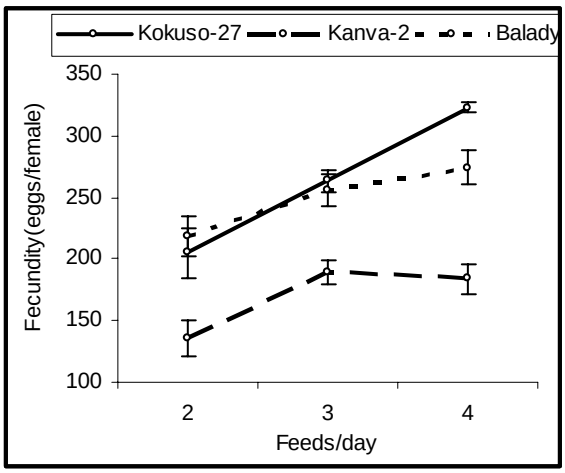
(b) Fitness (per day)



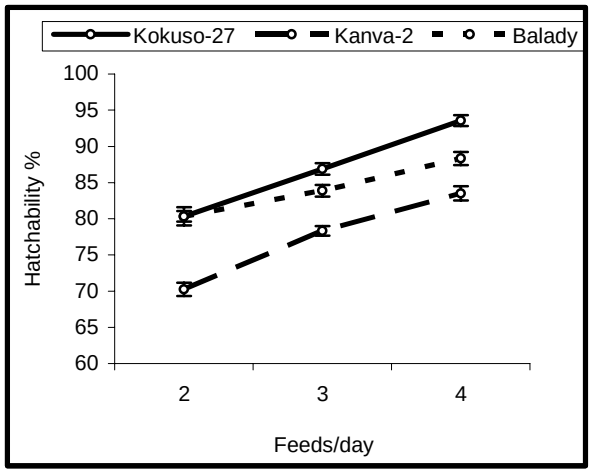
(c) Larval mortality %



(d) Cocooning %



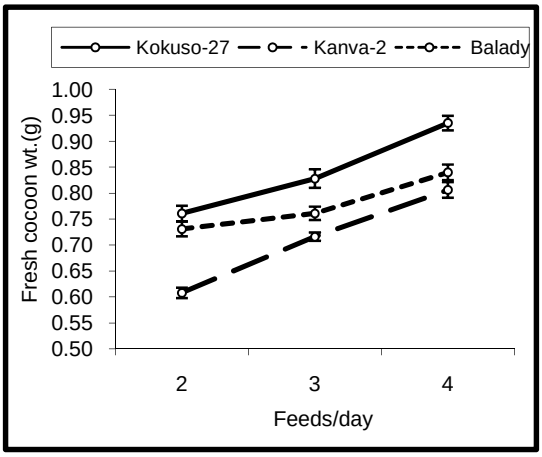
(e) Fecundity (eggs/female)



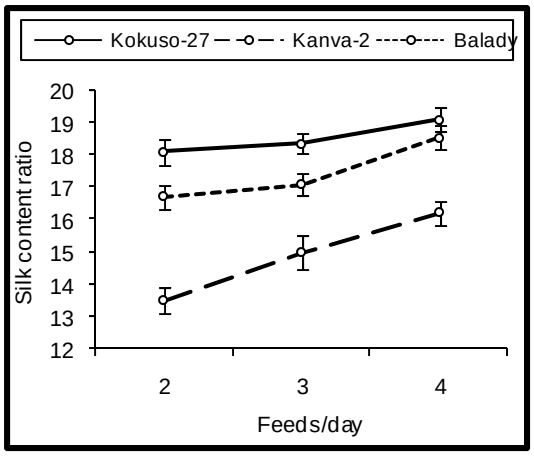
(f) Hatchability %

Fig. 1: Effect of mulberry varieties and number of feeds per day on biological characters of *B. mori* (mean ± SE).

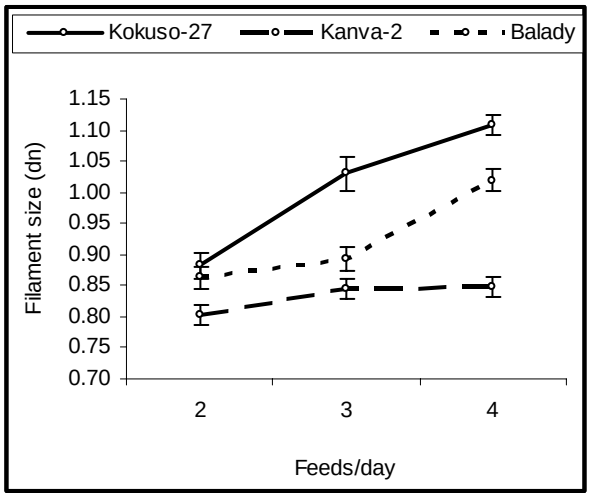
There were significant increases in fresh cocoon weight ($F_{2,441} = 66.6$, $p<0.01$), silk ratio ($F_{2,441} = 70.3$, $p<0.01$) and filament size ($F_{2,441} = 5.8$, $p<0.01$) in larvae reared on Kokuso-27, with those fed four feeds per day having the highest values (Fig. 2 a,b,c). As before, larvae fed on Kanva-2 recorded the poorest values for cocoon and filament characters, with Balady usually in between. There were significant variation ($p<0.05$) within means of technological characters as response to mulberry varieties, feeds number/day and the interaction between them ($F_{4,441} = 3.31$, $p<0.05$ & $F_{4,441} = 3.69$, $p<0.01$), except the analysis of interaction of filament size revealed non significant variation ($p>0.05$) .



(a) Cocoon weight (g)



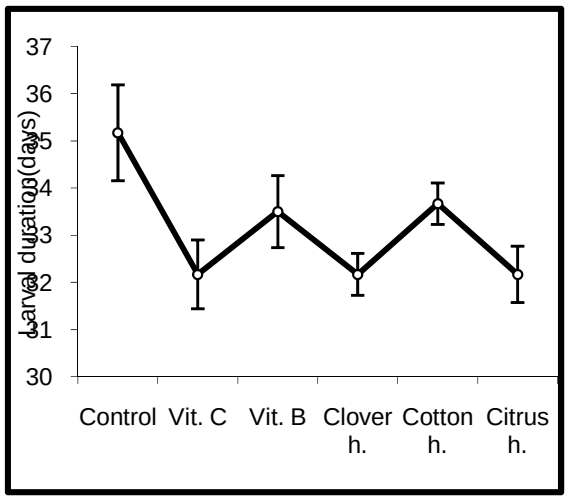
(b) Silk content ratio



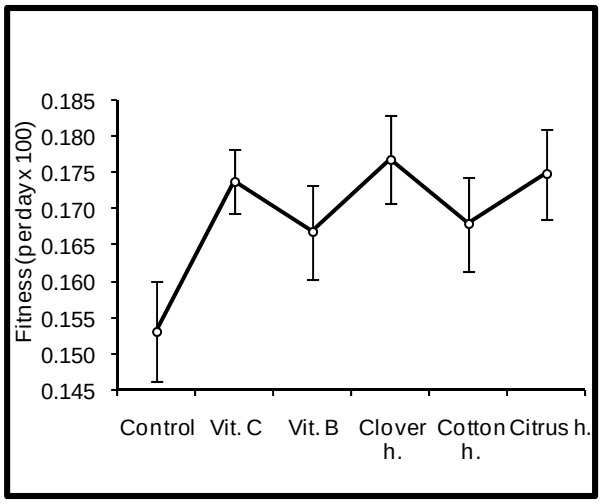
(c) Filament size (dn)

Fig. 2: Effect of mulberry varieties and number of feeds per day on technological characters of *B. mori* (mean ± SE).

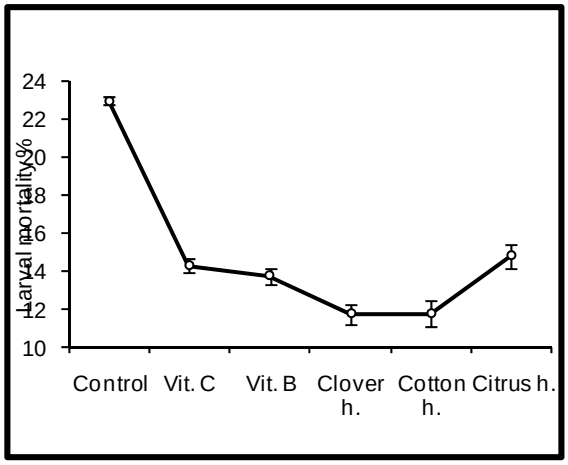
Feeding larvae with mulberry leaves enriched with additive compounds improved the biological measures in all tested groups compared with the control (Fig 3). There was a significant lowering in larval mortality rate compared to the control ($F_{5,12} = 68.6$, $p<0.001$), especially in clover and cotton honey groups (Fig 3c). Vitamin C, clover and citrus honey groups showed a significant enhancement in cocooning percentage (Fig 3d: $F_{5,12} = 68.0$, $p<0.001$). All test groups recorded higher fecundities (especially high in the bee-honey groups, Fig 3e: $F_{5,24} = 4.3$, $p<0.01$) and hatchability percentages (Fig 3f: $F_{5,24} = 6.1$, $p<0.001$) than the control, but there were no significant differences in larval duration and fitness (Fig 3a,b).



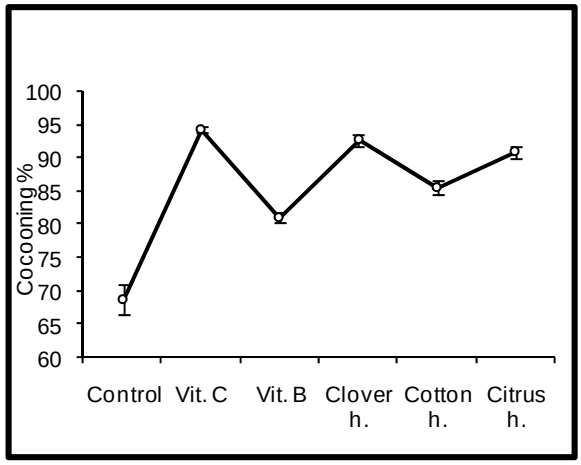
(a) Larval duration



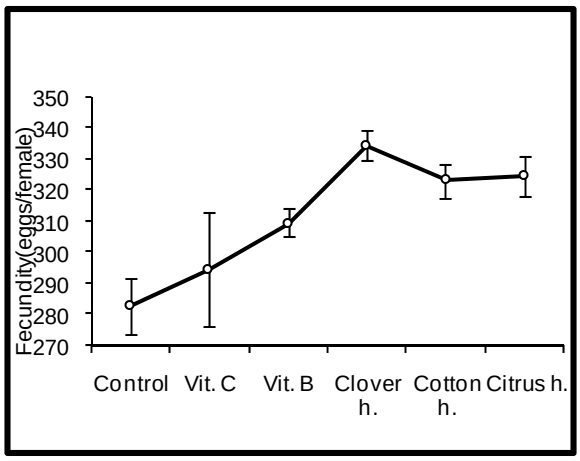
(b) Fitness



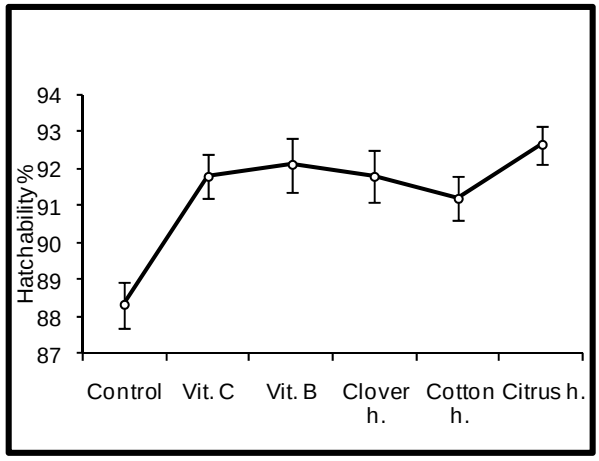
(c) Larval mortality %



(d) Cocooning %



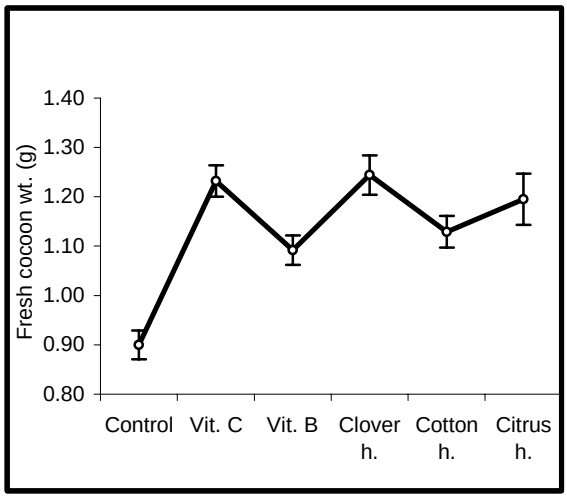
(e) Fecundity



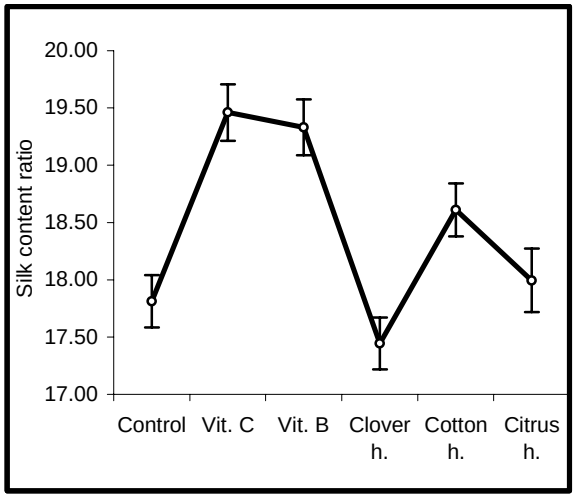
(f) Hatchability %

Fig. 3: Effect of some nutritional additives on biological characters of *B. mori* (mean ± SE). Vitamin C concentration= 1.0 %, vitamin B concentration= 0.2 %, bee-honey (clover, cotton & citrus) concentration= 50 %.

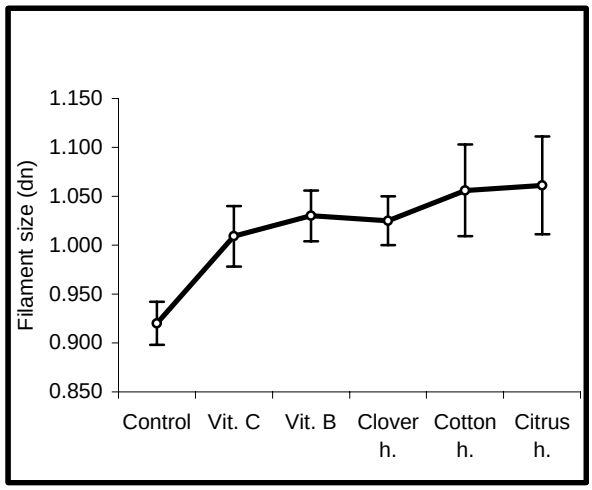
In technological measures, there were a significant increases in cocoon weight (Fig 4a: $F_{5,144} = 12.2$, $p<0.001$) and silk ratio (Fig 4b: $F_{5,144} = 11.7$, $p<0.001$) in all tested groups, especially vitamins C, B and clover honey compared with the control. The reeled silk filaments of all tested groups exhibited higher filament size than the control group (Fig 4c: $F_{5,144} = 2.6$, $p<0.05$).



(a) Cocoon weight



(b) Silk content ratio



(c) Filament size

Fig. 4: Effect of some nutritional additives on technological characters of *B. mori* (mean ± SE). Vitamin C concentration= 1.0 %, vitamin B concentration= 0.2 %, bee-honey (clover, cotton & citrus) concentration= 50 %.

Discussion

Rearing *Bombyx mori* on *Morus alba* var Kokuso-27 leaves with four feeds per day clearly improved the rearing and silk productivity of the tested larvae compared with the control, whilst rearing larvae on the Kanva-2 variety had a deleterious effect. The superiority of Kokuso-27 might be explained by its increased amounts of crude protein, soluble sugar and starch content in their leaves, which might improve the health and growth of larvae, perhaps by increasing the rates of the digestive and oxidizing enzymes which help in utilizing food and increasing food consumption (Mahmoud 2000, Ashour 2005). Zannoon (1999) reported that rearing grown larvae with Kokuso-27 led to shorter larval durations and lower larval mortality rate relative to feeding on the native Balady variety. The improvement in cocooning percentage, fecundity and hatchability in the Kokuso-27 treatment might be referrable to haemolymph protein improvement and an increase in storage proteins (Seo *et al*. 1985). This improvement might also be due to levels of bombyxin (an insulin-like protein) in the haemolymph. This hormone is modulated by the brain in response to variation in nutrition, and forms part of the mechanism coordinating the growth of internal organs with overall somatic growth; it also plays a role in carbohydrate metabolism (Nijhout & Grunert 2002).

In technological measures, the Kokuso-27 treatment increased fresh cocoon weight, silk content ratio and silk filament size, especially with four feeds per day. The Kanva-2 again exhibited the poorest results. The increase may be due primarily to the promotion of silk protein synthesis by increased enzymatic activity of aminotransferase, involved in the uptake of nitrogen from leaves by body tissues and the silk gland: Machii & Katagiri (1991) found a high correlation between leaf protein levels and the production efficiency of the cocoon shell (i.e. cocoon shell weight relative to total consumption). Dai *et al.* (1995) indicated that ecdysone plays a significant role in nucleic acid metabolism and the related protein synthesis in silkworm: it induces both growth and silk production. Ashour (2005) tested differences among three different mulberry varieties (Kokuso-27, Morittiana and Rosa), and also showed that Kokuso-27 had the most efficacious effect on silkworm production.

Enriching mulberry leaves with additives led to an obvious improvement in several measures, lowering larval mortality rate and elevating cocooning percentage, fecundity and hatchability, but not affecting larval duration or fitness. The enhancement of larval

development may be due to the action of vitamin C and B as coenzymes in amino-acid metabolism and antioxidant agents, which may increase amino-acid concentrations in larval tissues, leading to improvements in productivity. This hypothesis coincides with that of Babu *et al*. (1992), who observed that leaf consumption was greater in ascorbic-acid-fortified leaves as compared to control: tissues and body fluids contained amounts of vitamin C dependent upon the amounts ingested in food. Suprakash & Pal (2002) also found that vitamin B complex significantly improved growth and development, with beneficial effects on the economic characteristics of the cocoon. The improvement of cocoon and silk characters in this experiment may be attributed to improvement of the efficiency of conversion of dietary nitrogen into the cocoon shell. In other words, the increase in the cocoon and filament characters might be due to increased protein conversion efficiency of the silk glands as a result of increased availability of vitamins and the positive antibacterial effect of honey on the growth of many kinds of bacteria. This is supported by Sarker *et al*. (1995), who reported that supplementation of mulberry leaves offered to silkworm with ascorbic acid (1%) and vitamin B complex (0.5%) improved cocoon yield and silk filament quality. Basson *et al*. (1994) assumed that the antibacterial activity of honey was attributed to multiple factors such as pollen present in honey which could be the source of the antibacterial aromatic acids. The hypertonic sugar concentration may also play an important role in this activity. Nafea (2004) reported that clover and cotton honey show antibacterial activity: clover and citrus honey were better than cotton honey, probably because of the lower levels of fructose and glucose in cotton honey (or possibly botanical or soil factors affecting nectar quality of nectar: Nour *et al*. 1992; El-Atabany 1992).

According to the overall results of this study, the mulberry variety Kokuso-27 is highly recommended for rearing silkworm in Egypt, and a feeding rate of four feeds per day is also recommended, because of its beneficial effects on silkworm biological and economic productivity. Fortification with either vitamins or bee-honey is also highly beneficial, improving rearing and the quality of the silk filament.

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References

Ashour A (2005) Silkworm feeding trials for evaluating the varietal effect of three mulberry leaves on the silkworm growth and cocoon yield quality. *Egyptian Journal of Agricultural Research* 83(3): 1043-1049

Babu M, Swamy MT, Rao PK & Rao MS (1992) Effect of ascorbic acid-enriched mulberry leaves on rearing of *Bombyx mori* L. *Indian Journal of Sericulture* 31(2): 111-114

Basson NJ, du Toit IJ & Grobler SR (1994) Antibacterial action of honey on oral streptococci. *Journal of the Dental Association of South Africa* 49(7): 339-341

Choudhury PC, Shuka P, Chosh B & Sengupta K (1991) Effect of spacing, crown height and method of pruning on mulberry leaf yield, quality of cocoon yield. *Indian Journal of Sericulture* 30(1): 46-53

Dai YJ, Li R & Zhang YQ (1995) Moulting hormone and silk protein synthesis: effect of ecdysone on nucleic acid synthesis in the posterior portion of silk gland in *B. mori*. *Acta Entomologica Sinica* 28(1): 8-14

Datta RK (2000) Mulberry for animal production: mulberry cultivation and utilization in India. *FAO Animal Production & Health Paper* 147. *Central Sericultural Research & Training Institute, Central Silk Board, Srirampura, Mysore. Agriculture Department, Mysore, India*

El-Atabany AI (1992) Biochemical studies on royal jelly. MSc Thesis, Faculty of Veterinary Medecine, Zagazig, University, Egypt.

Krishnaswami S (1978) New technology of silkworm rearing. *Bulletin of the Central Sericulture Research & Training Institute, Mysore* 2: 1- 10

Little TM & Hills FJ (1975) Textbook of statistical methods in agricultural research. University of California at Davis, California, USA.

Machii H & Katagiri K (1991) Varietal differences in nutritive values of mulberry leaves for rearing silkworms. *Journal of Agricultural Research Quarterly* 25: 202-208

Mahmoud S (2000) Feeding effect of different mulberry varieties on silkworm, *Bombyx mori* L. *Egyptian Journal of Applied Science* 15(6): 253-261

McGraw JB & Caswell H (1996) Estimation of individual fitness from life-history data. *American Naturalist* 147: 47-64

Minamizawa K (1997) *Moriculture: the science of mulberry cultivation*. CRC Press, Boca Raton, Florida, USA.

Miranda JE, Takahashi R, Silva AF & da Silva AF (1998) Ascorbic acid supplement in the food of *Bombyx mori* L. (Bombycidae: Lepidoptera). *Revista de Agricultura Piracicaba* 73(3): 367-372

Nafea AE (2004) The biological effect of honeybee products as environmentally safe substances against some pathological microorganisms. PhD Thesis, Department of Agricultural Science, Institute of Environmental Studies & Research, Ain Shams University, Cairo, Egypt.

Nijhout HF & Grunert LW **(**2002**)** Bombyxin is a growth factor for wing imaginal disks in Lepidoptera. *Proceedings of the National Academy of Sciences USA* 99(24): 15446-15450

Nour ME, Abou-Zaid MM & Selim HA (1992**)** Effect of long-term storage on Egyptian honey quality. *Journal of Agricultural Sciences, Mansoura University* 16(11): 2691-2698

Nour ME, El-Maasarawy SA & Mahmoud S (1997) Propolis in the nutrition of the silkworm *Bombyx mori* L.: influence on biology, silk yield and biochemical changes in haemolymph. *Proceedings of the 1st International Conference on silk "ICSAI" 1997.*

Parra JRP (1991) Consumoe utilizaçào de alimentos por insetos. In: Panizzi AR & Parra JRP (eds) *Ecologia nutricional de insetos e suas Implicaçòes no manejo de pragas. 359 p. Manole, Sào Paulo.*

Raman KVA, Mala VR, Magadum SB & Datta RK (1995) Effect of seasons and mulberry varieties on the feed conversion efficiencies of different silkworm hybrids of *Bombyx mori* L. *Uttar Pradesh Journal of Zoology* 15(3): 157-161

Sarker A, Haque M, Rab M & Absar N (1995) Effect of feeding Mulberry (*Morus* sp.) leaves supplemented with different nutrients to silkworm (*Bombyx mori* L.). *Current Science* 69(2): 185-188

Sengupta K, Singh BD & Mustafi JC (1992) Role of vitamins in silkworm nutrition. *Indian Journal of Sericulture* 11(1): 11-19

Seo EW, Yun CY, Kang CS & Kim HR (1985) A study on the protein pattern of haemolymph during last instar larval and pupal stages of *Bombyx mori***.** *Entomological Research Bulletin* 11: 153-163

Suprakash P & Pal S (2002) Effect of vitamin B-complex supplementation on the growth and development of mulberry silkworm, *Bombyx mori* L. *Insects & Environment* 8(4): 150

Tanaka Y (1964) Manual of sericology. *Central Silk Board, Bombay 95*(*B*): 216-220

Zannoon AA (1994) Effect of some nutritional additives on certain biological and productivity characters of mulberry silkworm, *Bombyx mori* L. *Journal of Productivity & Development (Agricultural Research)* (Institute of Efficient Productivity, Zagazig University, Egypt). 1: 19-24

Zannoon AA (1999) Effect of alternative feeding on mulberry leaves of different varieties on the silkworm, *Bombyx mori* L. *Journal of Productivity & Development (Agricultural Research)* (Institute of Efficient Productivity, Zagazig University, Egypt). 4(2): 1-7

الملخص العربي

التأثيرات البيولوجية والتكنولوجية لبعض أصناف ورق التوت وبعض الإضافات الغذائية علي ديدان الحرير "*بومبيكس موراي*"

عبد الحميد على إبراهيم زنون[١]، إيمان محمود محمد حسان [١]، سمية صلاح الدين العقاد [٢]، إسماعيل محمد عبد النبي [٣]، سامى محمد زلط[3] .

١ - معهد بحوث وقاية النباتات، مركز البحوث الزراعية، القاهرة، مصر.

٢- قسم النبات، كلية العلوم، جامعة عين شمس، القاهرة، مصر.

٣- قسم الحيوان، كلية العلوم، جامعة قناة السويس، الإسماعيلية، مصر.

تم تقييم صنفين من أصناف ورق التوت وهما؛ الصنف الياباني (Kokuso-27) والصنف الرومي (Kanva-2) ومقارنتهما بالصنف المحلي المنتشر في مصر (Balady) وتعيين الصنف الأفضل وكذلك نظام أو عدد الوجبات اليومية الأكثر فاعلية في تغذية اليرقات. حيث تم تقسيم اليرقات ثلاث مجموعات، وتم تغذية كل مجموعة بعدد وجبات مختلف (مرتان و ثلاثا و أربع مرات يوميا) وذلك لكل صنف طوال العمر اليرقي. وقد أظهرت الدراسة تحسن الصفات البيولوجية و التكنولوجية لليرقات التي تغذت علي الصنف الياباني مقارنة بالأصناف الأخرى وخاصة عند إتباع النظام الغذائي أربع مرات يوميا، حيث قلت مدة العمر اليرقي وانخفضت نسبة الوفيات كما أرتفع مستوى الحيوية عند اليرقات وكذلك نسبة التشرنق، كما زادت نسبة الخصوبة وكذلك معدل فقس البيض. وقد أنتجت هذه اليرقات شرانق ذات جودة عالية من حيث وزن الشرنقة، نسبة المحتوى الحريري فيها وكذلك حجم الخيط الحريري. بينما أظهرت نتائج الصنف الرومي أقل النتائج بين الأصناف قيد الدراسة. وقد تم أيضا دراسة تأثير إضافة بعض الإضافات الغذائية مثل: فيتامين سي بتركيز ١%، وفيتامين بي المركب بتركيز ٠.٢% وثلاثة أنواع من عسل النحل (عسل البرسيم والقطن والموالح) كل نوع بتركيز ٥٠% إلى أوراق التوت الياباني علي بعض الصفات البيولوجية و التكنولوجية لديدان الحرير التوتية، على أن تتم التغذية بهذه الإضافات مرة واحدة يوميا. وقد أظهرت الدراسة تحسنا واضحا في نفس الصفات البيولوجية و التكنولوجية قيد الدراسة في جميع المعاملات مقارنة بمجموعة الكنترول التي تغذت علي ورق التوت الطازج بدون إضافات وخاصة فيتامين سي وعسل البرسيم والموالح. بينما لم تتأثر مدة العمر اليرقي ومستوى الحيوية عند اليرقات.