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

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**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.


**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*.

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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:

\[ r = \frac{\text{Ln} (m \times V)}{D} \]

where \( \text{Ln} \) = natural logarithms, \( m \) = survival probability, \( V \) = fecundity, \( D \) = duration.

A number of technological variables were also recorded. For cocoon 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 %
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 ($p>0.05$).
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 ($F_{5,12} = 68.0$, $p<0.001$). All test groups recorded higher fecundities (especially high in the bee-honey groups, $F_{5,24} = 4.3$, $p<0.01$) and hatchability percentages ($F_{5,24} = 6.1$, $p<0.001$) than the control, but there were no significant differences in larval duration and fitness (Fig 3a,b).
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: $F_{5,144} = 2.6$, $p<0.05$).
**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 referable 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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الملخص العربي

التأثيرات البيولوجية والتكنيولوجية لبعض أصناف ورق التوت وبعض الإضافات الغذائية على ديدان الحرير "يوبيمكس مورياي".

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تم تقسيم أصناف ورق التوت ورصاص الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الرياحي الри