

Effect of 12 hours delay and anticipation from traditional practice in silkworm mounting to spinning frames on economic traits of *Bombyx mori* (Lepidoptera: Bombycidae) cocoons

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ABSTRACT

In this study, the aim was to determine the effect of 12 hours delay and time advancement of *Bombyx mori* larvae transfer to spinning frames on the economic traits of harvested cocoons. Our experience focused on the transfer of three lots of silkworm mature larvae to cocoon frames with 12 hours delay or advancement from usual spinning time. Commercial silkworm eggs were obtained and three replications of 100 larvae per each treatment were used. The larvae and cocoon conditions of hatching and rearing, feeding conditions, silkworm egg production stages were performed based on standard protocols. Rice straws were used as cocoon position (framework) in the cocoon spinning stage separately for each replication. After completion of the pupa development, total cocoon production was collected and classified based on appearance, hardness, softness, and cleanliness levels of the cortex and outer cortex into four categories, viz. good, moderate, low and double cocoons. Economic characteristics were calculated and compared between treatments using the Duncan test at $p < 0.05$. The comparison of economic traits of cocoons produced in perfected treatments showed no significant difference between treatments and the control. This result suggests that the 12 hours' time anticipation or delay of larva transfer to cocoons building frames has no effect on the quantity or the quality of produced cocoons. Therefore, it is possible to shorten the rearing duration of *Bombyx mori* larvae by 12 hours without affecting the cocoon economic traits, and this could improve cocoon production and generate an important economic advantage in commercial sericulture.

Keywords: *Bombyx mori*; Cocoon traits; Sericulture; Silkworm mounting; Spinning

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Introduction

The commercial production of natural silk is monopolized by the *Bombyx mori* (Lepidoptera: Bombycidae) moth larvae which make it one of the most beneficial insects to mankind (1-2). This species is the most widely domesticated, intensively studied and most developed in terms of rearing techniques (3). For many centuries, natural silk represented a precious commodity. As the most elegant and luxurious fiber, the natural silk maintained its value as the “Queen of textiles” in the world of textile since immemorial time. Recently, despite the competition from synthetic fibers, the natural silk maintained its appeal and its value and continues to play an important economic role. Indeed, at the economic level, it still retains its place as one of the major industrial export items (4). In addition to its historical value, the position of natural silk as biomaterial is increasingly highlighted in many biomedical and biotechnological (pharmaceutical and cosmeceutical) applications (5-6).

The sericulture is an agro-based cottage industry, a technique that deals with silk production dating for approximately 5000 years in China and known as the industry of poor (6). This activity involves mulberry (*Morus* spp., Moraceae) cultivation (moriculture), silkworm rearing to produce cocoons and post cocoon activities to produce silk yarn. Despite its low investment requirements, sericulture plays a central socio-economic role in sustainable development and anti-poverty programs to improve the living standards of the people in large rural and semi-urban zones in Asia and many other countries over the world (7). This activity provides important economic gainful because it generates considerable opportunities and contributes actively to women employment. Therefore, it will contribute to the reduction of rural poverty and in the stabilization of indigenous populations for the low potential area (8).

Bombyx mori is a holometabolous insect that completes its life cycle in four distinct instars: egg, larva, pupa, and adult (9). The silkworm larval life has five instars. The final larvae molt produces a mature or 5th instar larva. In general, the 5th instar larvae of the sixth and seven days is ready to spin when silkworm

shows morphological signs that indicate the onset of the spinning event. In this stage, the larvae become tinged with pale yellow slightly translucent with pigmentation on spinneret organ and a net wandering activity. Indeed, these signs are among the first signs that mark the onset of the spinning period event (10). At this stage, mature silkworms are transferred to separate frames for cocoons building. Silkworm *Bombyx mori* has long been a model system for fundamental and practical studying to improve silk productivity. Recently, numerous efforts have been made to develop many biotechnological aspects of *Bombyx mori* to improve both the quantity and quality of silk (11-15).

The present investigation sought to determine the effect of 12 hrs delay and anticipation from traditional practice in silkworm mounting to spinning frames on cocoons economic traits of *B. mori*. The aim of this study is to examine and compare the effect of specified treatment on characters that interest quantity, quality, and survival of harvested cocoons. In practical scale, the eventual reduction of the mounting time of mature silkworms to cocoon frames could significantly reduce larval rearing duration, labor costs and eventually can improve performance in sericulture technology. The subsequent investigations on silkworm mounting time to spinning frames and their effect on characteristics of harvested cocoon might determine the optimal time to be capitalized without affecting economic traits of produced cocoons.

Materials and methods

To compare the difference between biological and economic traits of produced cocoons by *Bombyx mori* silkworm, three treatments were conducted depending on the transfer time of mature larvae to cocoon frames:

- Treatment 1 (control): the silkworms transfer time from rearing trays to cocoon frames were standard and performed individually based on the onset of natural spinning marked by the appearance of mounting signs naturally as usual practices done in traditional and artisanal sericultural techniques (temperature: 25°C, relative humidity: 70-80%, light: 24 hrs darkness, ventilation: normal ventilation).
- Treatment 2: silkworms transfer time from rearing

tray to cocoon frame was performed 12 hrs before the appearance of natural spinning signs. To determine the batch of larvae to keep in treatment 2, batches of silkworms were marked and transferred to the cocoon frames at every hour during the period following the cessation of larval feeding. Just with the appearance of the first spinning signs, the lot taken before 12 hrs was preserved to perform the further experimental tests and cocoon comparison with the other treatments.

- Treatment 3: the silkworm transfer time from rearing tray to cocoon frame was performed at 12 hrs after the appearance of spinning signs.

Commercial silkworm eggs were obtained from Iran Silkworm Research Center (ISRC, Rasht). In every treatment, four replications of 100 larvae were used. The larvae and cocoon conditions of hatching and rearing were performed especially based on standard experimental guidelines and protocols detailed in ESCAP (Economic and Social Commission of Asia and the Pacific) (16). These experimental manipulations include feeding conditions, silkworm egg production stages, egg washing, disinfection, maintenance, and microscopic tests in order to review and remove contaminated samples against Pebrin pathogen (16).

In our experience, rice straws were used as cocoon position (framework) in cocoon spinning stage separately for each replication. After completion of the pupa development (7 days after onset spinning of cocoons), total cocoon production was collected. Then, for each treatment, all cocoons were sorted and classified based on appearance, hardness, softness, and cleanliness levels of the cortex and outer cortex. Cocoons were arranged into four categories including good, moderate, low and double cocoons based on ESCAP (16). The cocoon traits comparison among studied treatments is considered to be influenced by the mature larva mounting conditions. The comparison between treatments for traits with economic interest considered to be influenced by the mature larvae mounting conditions.

The studied traits include: 1- quantitative traits of productivity (cocoons number, cocoon weight, cocoon shell weight, cocoon shell percentage), 2- survival of cocoon pupae has been analyzed and the percentage of cocoon alive pupa for each replication was calculated

separately, 3- cocoon size expressed by cocoons number and cocoons weight per one liter was compared particularly between perfected treatments.

Cocoons were collected and fresh weight were determined. In addition, shell was obtained by subtracting the pupa weight from the cocoon weight, to calculate the shell to cocoon ratio as an economic trait. All weights are referred to wet weights. To advance comparison between treatments, the cocoon traits were compared between classified categories of cocoons and on the other hand among male and female harvested cocoons. Cocoon weight were recorded using a A&D GF-300 digital scale balance (310 gr × 0.001 gr, A&D Weighing Design and Manufacture, San Jose, CA). In addition, the coefficient of variation is calculated for quantitative cocoons traits.

All recording steps were performed on the eighth day after the onset of cocoon spinning. Production records were analyzed by statistical software SPSS, using generalized linear models procedure (GLM). After ensuring data normality, the averages were compared using Duncan test at $p < 0.05$. All the measured indices were compared between different treatments based on randomized design model (CRD).

Results

Economic traits of harvested cocoons were compared between perfected treatments and control. Results are summarized in Figures 1-2 and Tables 1-2.

Comparison of the productivity and survivability traits of cocoon between cocoon categories in studied treatments

Cocoon is the principal raw material used for reeling raw silk. The cocoon weight is the most significant commercial feature in sericulture. The number, weight, and survival of harvested cocoons can be considered as quantitative characters of cocoons that can be influenced by the spinning conditions. To more advance comparison, harvested cocoons were classified visually based on their quality to four categories (best, middle, low and double cocoons) and cocoon data were taken globally in all perfected tests of comparison based on ESCAP (16).

Figure 1 highlights the pairwise comparison of traits of yield interest and survival traits (cocoon number,

cocoon weight, and cocoon alive pupae percentage) between the same categories classified in studied treatments. The main obtained results were:

- The number of cocoons obtained in 'best category' is the most important and represents almost all produced cocoons in the same way in all studied treatments with an average of 74.5 and 80.5 and 79 cocoons harvested in treatment 1, 2 and 3, respectively.
- The number of cocoons produced in 'medium category' is relatively low in all treatments with only 13.5, 13 and 2.25 cocoons, respectively in treatments 1, 2 and 3. In the 'low' and 'double' cocoon categories, the cocoon number is the least numerous with an average of 1.25, 2.5 and 1.75 for low cocoons and 0.5, 1.5 and 0.5 for double cocoon category, respectively in treatments 1, 2 and 3 of both categories.
- The number and percentage of alive pupa from cocoons in different categories show that the best cocoon category gives the largest number of live pupae with value percentages of 99.9%, 96.19% and 95.42% for treatments 1, 2 and 3, respectively.
- The middle cocoon category gives a low number of live pupae with percentage values of 90.2%, 89.5% and 83.32% for treatment 1, 2 and 3, respectively.
- For middle and double cocoon categories, the percentage of alive pupa obtained was the lowest with a mean value of 84.13% for 'middle cocoon category' and 78.4% for double cocoon category.

Using pairwise comparisons at $\alpha=0.05$, produced cocoon show no significant difference between the same cocoon categories in all treatments concerning cocoon number, cocoon weight, and cocoon alive pupae percentage traits.

Cocoon size comparison

In sericulture, breeders attribute great importance to cocoon size because it is a critical trait when evaluating the raw silk quality. Differences in size are considered to be attributed to the environmental and harvesting characteristics during the cocoon formation. The cocoon size is expressed by the cocoons number and cocoon weight per one liter (l^{-1}) traits. In our study, no significant difference was observed between sizes of cocoons obtained between control and perfected treatments.

Comparison of economic traits in male and female cocoons among studied treatments

Cocoon weight, cocoon shell weight, and cocoon shell percentage are the most important economic traits to correlate with rearing conditions and yield performances of the silkworms. For more precision, comparison of quantitative traits that may affect the cocoon weight, shell weight and shell percentage were examined among the three treatments in male and female cocoons. Obtained results point out that the male cocoons weight (g) is 1.64, 1.66, and 1.64, while the cocoons female weight (g) is 2.13, 1.99 and 2.08 for treatment 1, 2 and 3, respectively (Fig. 2). The female cocoon shell weight (g) is 0.42; 0.4 and 0.42 and the male cocoon shell weight (g) is 0.38, 0.43 and 0.38 in treatment 1, 2 and 3, respectively. The shell percentage (%) is of 20.02, 19.86 and 20.02 for male cocoons and of 23.17; 25.9 and 23.1 for female cocoons, in treatments 1, 2 and 3, respectively.

To evaluate the precision of cocoons trait values, cocoon weight and cocoon shell weight for male and female coefficients of variation (c.v.) were calculated for each treatment (Table 2). Results show that c.v. was between 6.85% and 10.09% for cocoon weight and between 3.25% and 9.15% for cocoons shell weight in male and female cocoons for all treatments. The c.v. values do not exceed 10% for cocoon weight and 9.15% for cocoon shell weight in male and female cocoons for all treatments. This finding envisages that repeatability of tests and estimation of results were accurate and values of mean traits are similar because no significant difference in the c.v. of considered cocoons traits was observed in all treatments at $\alpha=0.05$. In this comparison, cocoon weight, cocoon shell weight, and cocoon shell percentage traits showed no significant difference between male cocoons in perfected treatments and the control. Similarly, the same result was noted between the same female cocoon traits. However, in this comparison, a significant decrease of 6.5% in the weight of female cocoons was noted between the treatment 2 and the control (Fig. 2). Nevertheless, when data were taken in their entirety without referring to the cocoon sexes, cocoon weight comparison showed no significant difference between female or male cocoons in all treatments with the control.

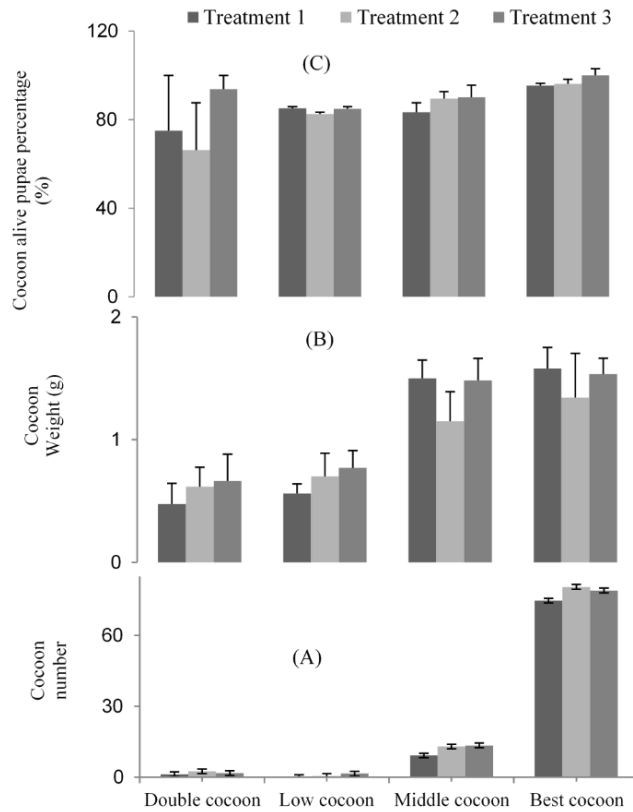


Figure 1. Pairwise comparison of cocoon number (A) cocoon weight (B), and cocoon alive pupae percentage (C) (\pm SEM) between cocoon categories (best, middle, low and double cocoon) classified in perfected treatments. In tested treatments, no significant difference was registered between the same categories of cocoons using Duncan test at $\alpha=0.05$

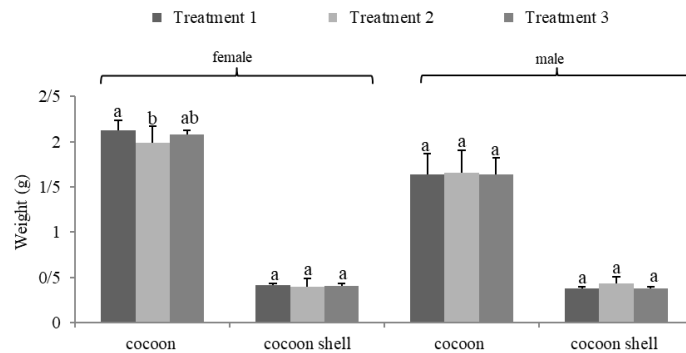


Figure 2. Comparison of male and female cocoon weight and cocoon shell weight (\pm SEM) in studied treatments. Histograms with the same letters are not significantly different using multiple comparison with Duncan test at $\alpha=0.05$.

Table1. Comparison of cocoon weight per one liter (g) and cocoon number per one liter (\pm SEM) in studied treatments.

Treatment	cocoon weight per one liter (g)	cocoon number per one liter
1	211.36 \pm 3.86 ^a	113.50 \pm 1.71 ^a
2	209.97 \pm 4.92 ^a	117.50 \pm 2.63 ^a
3	217.25 \pm 6.03 ^a	117.25 \pm 2.14 ^a

Means values in each column followed by the same letters are not significantly different at $\alpha=0.05$

Table 2. Coefficient of variations comparison of male and female cocoon weight and cocoon shell weight (\pm SEM) in studied treatments.

Coefficient of variations				
Treatment	Female cocoon shell weight	Female cocoon weight	Male cocoon shell weight	Male cocoon weight
1	9.88 \pm 0.88 ^{ab}	6.85 \pm 0.64 ^a	12.76 \pm 1.46 ^a	10.09 \pm 1.16 ^a
2	9.15 \pm 0.63 ^b	9.14 \pm 0.35 ^a	11.41 \pm 1.30 ^a	8.58 \pm 0.86 ^a
3	13.28 \pm 0.88 ^a	6.85 \pm 0.64 ^a	32.59 \pm 22.04 ^a	8.1 \pm 1.24 ^a

Mean values followed by the same letters within the same column are not significantly different at $\alpha=0.05$

Discussion

Our results show that delaying or advancing by 12 hrs the time of mature larva transfer time to cocoon frames do not affect cocoon survival and productivity of *Bombyx mori*, silkworm. In fact, almost all produced cocoons in perfected treatments were counted in the 'Best cocoons category' (77%) and defective cocoons (low and double cocoons) were poorly represented (1.5%).

The results also clearly indicate that all compared cocoon traits that affect productivity, survivability, and size of cocoons (cocoon number, cocoon weight, and cocoon alive pupae percentage and cocoon size) in classified categories, and in male and female harvested cocoons show no significant difference between perfect treatments and the control. This point out that studied traits of produced cocoons by larva transferred to cocoon frames at the onset of natural spinning (control), and at 12 hours before or after the appearance of the first spinning signs show no significant difference. This suggests that a delay or an advance of 12 hrs from the time of the onset of the natural spinning process in larval transfer to cocoon frames was tolerated by larva to produce cocoons without significant effects on considered cocoons characteristics. This means that the *Bombyx mori* larvae spinning behavior are not significantly disturbed after changing the time of their transfer to cocoon frames by 12 hrs interval. In fact, it is generally known in insect species that the passage from one developmental stage to the next is principally controlled by ecdysteroid and juvenile hormone (17). These hormones induce physiological regulations that control growth, larval-larval and larval-pupal development (17-18). Under combined

action of these hormones, larvae have the option of remaining a larva, becoming a pupa or developing to become an adult. Indeed, certain authors stated that with lower amounts of juvenile hormone, ecdysteroid that is known more as molting hormone (19) accelerates the maturation event and promotes the appearance of the spinning signs (20). Other studies emphasized that more than the titer; a hormonal balance of these hormones governs the timing of the developmental pattern in transitions between instars in insect species (18, 21).

In *Bombyx mori*, Akain and Kobayachi in 1971 reported that the larval-pupal transformation and the initiation of the spinning event are delayed by the injection or topical application of juvenile hormone at the early fifth instar larvae without affecting the quality of cocoons (22). Moreover, Gu in 1997 reported that the application of juvenile hormone during the first four days of the fifth instar provoked a significant delay in pupation of mature larvae (23).

The present study shows that 12 hrs delay or advance in larvae natural transfer time induce no perturbation in harvested cocoons economic traits. In fact, the appropriate conditions and equipped spinning frames might stimulate the response of larvae to anticipate its spinning behavior. This response might be the physiological adaptive nature that involves the hormonal balance of ecdysteroid and juvenile hormone because of the title role of these hormones in developmental transitions control in insects' life cycle. Further studies are indispensable, to confirm the physiological nature and to elucidate the mechanism of this probable adaptive response in *Bombyx mori* silkworm.

In the practical level, this study predicts the possibility to manage early pupation of larvae by

anticipating by 12 hrs transfer of the fifth instar larvae to spinning frames without effects on productivity and economic characters of the obtained cocoon. Despite the short period of 12 hours retained in this investigation, it is a significant task to anticipate the spinning event and to shorten its usual duration which represents a decisive factor to reduce input labor charges in commercial sericulture.

This study highlights also the possibility of delaying by 12 hrs in the silkworm transfer to cocoon frames without affecting the economic traits of harvested cocoons. This outcome might be perceived as a potential physiological adaptation in response to appropriate conditions in frames equipped to build cocoons. To elucidate operating mechanisms of this physiological approach in silkworm larvae is of crucial

importance to manage transfer duration and pupation stage of silkworms without affecting economic traits of harvested cocoons. In subsequent studies, it will be important to determine maximal time susceptible to be capitalized by anticipating silkworms transfer to reduce input labor and associated losses in silkworm rearing, because in sericulture the silkworm rearing input labor is a crucial factor which cost more than 50 percent of the expence of silkworm rearing (24-25).

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Editorial Note

Volume 7, issue 2 of Progress in Biological Sciences was initially scheduled to be published in December 31, 2017. However, some administrative changes led to a major delay in processing of the manuscripts. This issue is actually published in May 1, 2020. Editor-in-chief apologizes deeply for any inconvenience caused especially to the authors.

