



## Investigation on effects of delay and acceleration in round up and accumulation of matured silkworm larvae and transferring them to cocoon making frames

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### ABSTRACT

The aim of this experiment was investigation on effects of delay and acceleration in round up and accumulation of matured silkworm larvae in order to transferring them to cocoon making frames. Three different ways of silkworm making cocoon-starting time was studied. The larvae hatching and rearing was conducted based on standard and similar methods. Treatments were (1) treatment 1: the completion time of larvae feeding and larvae transfer from rearing tray to cocoon frame was standard and performed individually based on larvae mature time; treatment 2: the completion time of larvae feeding and larvae transfer time from rearing tray to cocoon frame was 12 hours before larvae mature time; treatment 3: the completion time of larvae feeding and larvae transfer time from rearing tray to cocoon frame was 12 hours after larvae mature. Production traits recorded and analyzed using generalized linear models procedure based completely randomized design model. From obtained results, it is showed that among studied methods, the highest level of best cocoon number belonged to 2nd treatment (80.50), and 1st treatment (74.75) remained at lower level than other methods ( $P>0.05$ ). Among studied methods, the highest level of best cocoon alive pupae number belonged to 3rd treatment (79.75), and 1st treatment (71.25) remained at lower level than other methods ( $P>0.05$ ). Among studied methods, the highest level of best cocoon weight belonged to 3rd treatment (147.88 gr), and 2nd treatment (124.32 gr) remained at lower level than other methods ( $P>0.05$ ). Among studied methods, the highest level of male Cocoon shell percentage belonged to 3rd treatment (24.11%), and 2nd treatment (23.44%) remained at lower level than other methods. Other methods were between these two groups ( $P>0.05$ ). Among studied methods, the highest level of male cocoon weight belonged to 3rd treatment (1.66 gr), and 1st treatment (1.64 gr) remained at lower level than other methods ( $P>0.05$ ). Among studied methods, the highest level of male cocoon shell weight belonged to some treatments (0.443 gr), and 2nd treatment (0.38 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

**Keyword:**

*mori*, Delay, Spin, Cocoon Frame, Quality, Quantity

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## INTRODUCTION

Silk is a kind natural yarn that is produced from silkworm cocoon. Silk is consisted based on protein largely. Silk heavy-duty textiles are weaved with silk yarns, one of the world's best and softest textiles. Due to the silk shine properties, silk woven textiles are like prism and if we look them from different directions, they have different colors. Silkworm is rearing in order to cocoon production. Cocoons are used to silk reeling. Silk is one of the things that produce textiles, carpets and other tissues. Today, silk is one of the major industrial export items in some of the countries. Silk have beautiful luminosity and these fibers are the best clothing for human. Approximately five thousand years ago, the Chinese human discovered the fact that the silk can be used for preparing of textile achieve (Bizhannia et al., 2007).

One of the most affected factors on cocoon production quantity and quality is cocoon mounting duration. In the industrial farms, due the non-automatic process in mature larvae gathering and bad timing situation in selection of grown and ready larvae for gathering and cocoon frame preparation, farmers lost large part of their production. Silkworm larvae are not ready for cocoon making as uniform and do not reach the stage of full development simultaneously. Therefore, if farmers gather larvae before larvae are ready for cocoon making, larvae cannot make cocoon fully. On the other hand, if farmers gather larvae after larvae are ready for cocoon making with delay, larvae lost silk fibers largely (Seidavi et al., 2006).

It is possible that if we gather silkworm larvae before they reach to final time for cocoon making, and put them in cocoon frames, somewhat we can reduce the level of silk production losses. On the other hand, it is possible that if we gather silkworm larvae after arrival time for cocoon making, and put them in cocoon frames, this method decrease labor costs for larvae gathering and silk production and is do not reduce production levels also. Therefore, aim of this experiment was investigation on effects of delay and acceleration in round up and accumulation of matured silkworm larvae in order to transferring them to cocoon making frames.

## Materials and Methods

In this study, three different ways of silkworm making cocoon-starting time was studied. The larvae hatch and reared based on ESCAP (1993) and performed equally. Silkworm egg production stages, egg wash, disinfect, maintenance of silkworm eggs, microscopic tests in order to review and remove contaminated samples against Pebrin pathogen, first to fifth larval instars rearing, cocoon production framework and cocoon recording was conducted based on standard guidelines and protocols, especially ESCAP (1993). Commercial hybrid silkworm egg was prepared from Iran Silkworm Research Center (ISRC). Hatching operations were conducted based on common standards of hatching eggs. In addition, hatching steps and coordinating of the appropriate time on embryonic development, according to the instructions of light regime was provided based on ESCAP (1993).

Three treatments were (1) treatment 1 (control): the completion time of larvae feeding and rearing and larvae transfer time from rearing tray to cocoon frame in this treatment was standard and performed individually based on larvae mature; treatment 2: the completion time of larvae feeding and rearing and larvae transfer time from rearing tray to cocoon frame in this treatment was 12 hours before larvae mature; treatment 3: the completion time of larvae feeding and rearing and larvae transfer time from rearing tray to cocoon frame in this treatment was 12 hours after larvae mature.

It was used rice straw as cocoon position (framework) in cocoon spinning stage separately for each replication. After completing of the pupa development (7 days after onset spinning of cocoons), it was collected total produced cocoons. Then, it was sorted and classified all cocoons based on appearance, hardness and softness, and cleanliness levels of cortex and outer cortex into four categories including good, moderate, low and double cocoons. Health situation of the cocoon pupae and the disease and mortality of pupae have been studied and it was calculated the percentage of pupa vitality for each replication separately. Also good and double cocoon weight in each replication was recorded. All recording steps was performed on the eighth day after the onset of cocoon spinning. Production records analyzed by statistical software SPSS using generalized linear models procedure (GLM), and after ensuring of data normality, the averages was compared using Duncan test. All the measured indices was compared between different treatments based completely randomized design model (CRD).

## Results and Discussion

Obtained results are summarized in Tables 1-6.

### Best cocoon number

From obtained results, it is showed that amount of best cocoon number in three studied methods included between 74.75-80.50. Among studied methods, the highest level of best cocoon number belonged to 2nd treatment (80.50), and 1st treatment (74.75) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

### Best cocoon alive pupae number

From obtained results, it is showed that amount of best cocoon alive pupae number in three studied methods included between 71.25-79.75. Among studied methods, the highest level of best cocoon alive pupae number belonged to 3rd treatment (79.75), and 1st treatment (71.25) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

### Best cocoon alive pupae percentage

From obtained results, it is showed that amount of best cocoon alive pupae percentage in three studied methods included between 95.42-99.98%. Among studied methods, the highest level of best cocoon alive pupae percentage

belonged to 3rd treatment (101.13%), and 1st treatment (95.42%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Best cocoon dead pupae number**

From obtained results, it is showed that amount of best cocoon dead pupae number in three studied methods included between 1.75-3.50. Among studied methods, the highest level of best cocoon dead pupae number belonged to 1st treatment (3.50), and 3rd treatment (1.75) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Middle cocoon number**

From obtained results, it is showed that amount of middle cocoon number in three studied methods included between 9.25-13.50. Among studied methods, the highest level of middle cocoon number belonged to 3rd treatment (13.50), and 1st treatment (9.25) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Middle cocoon alive pupae number**

From obtained results, it is showed that amount of middle cocoon alive pupae number in three studied methods included between 7.75-12.50. Among studied methods, the highest level of middle cocoon alive pupae number belonged to 3rd treatment (12.50), and 1st treatment (7.75) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Middle cocoon alive pupae percentage**

From obtained results, it is showed that amount of middle cocoon alive pupae percentage in three studied methods included between 83.32-90.20. Among studied methods, the highest level of middle cocoon alive pupae percentage belonged to 3h treatment (90.20), and 1st treatment (83.32) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Low cocoon number**

From obtained results, it is showed that amount of low cocoon number in three studied methods included between 0.00-1.50. Among studied methods, the highest level of low cocoon number belonged to 3rd treatment (1.50), and 1st treatments (0.00) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Low cocoon alive pupae number**

From obtained results, it is showed that amount of low cocoon alive pupae number in three studied methods included between 0.00-0.75. Among studied methods, the highest level of low cocoon alive pupae number belonged to 3rd treatment (0.75) and 1st treatment (0.00) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences

between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Low cocoon dead pupae number**

From obtained results, it is showed that amount of low cocoon dead pupae number in three studied methods included between 0.00-0.75. Among studied methods, the highest level of low cocoon dead pupae number belonged to 3rd treatment (0.75), and 1st treatment (0.00) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Double cocoon number**

From obtained results, it is showed that amount of double cocoon number in three studied methods included between 1.25-2.50. Among studied methods, the highest level of double cocoon number belonged to 2nd treatments (2.50), and 1st treatment (1.25) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Double cocoon alive pupae number**

From obtained results, it is showed that amount of double cocoon alive pupae number in three studied methods included between 1.50-4.50. Among studied methods, the highest level of double cocoon alive pupae number belonged to 3rd treatments (4.50), and 2<sup>nd</sup> treatment (1.50) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Double cocoon alive pupae percentage**

From obtained results, it is showed that amount of double cocoon alive pupae percentage in three studied methods included between 56.25-75.00%. Among studied methods, the highest level of double cocoon alive pupae percentage belonged to 3rd treatment (75.00%), and 2nd treatment (56.25%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Double cocoon dead pupae number**

From obtained results, it is showed that amount of double cocoon dead pupae number in three studied methods included between 0.00-0.50. Among studied methods, the highest level of double cocoon dead pupae number belonged to 3rd and 2nd treatments (0.50), and 1st treatment (0.00) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Cocoon number per one liter**

From obtained results, it is showed that amount of cocoon number per one liter in three studied methods included between 113.50-117.50. Among studied methods, the highest level of cocoon number per one liter belonged to 2nd treatment (117.50), and 1st treatment (113.50) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Cocoon weight per one liter**

From obtained results, it is showed that amount of cocoon weight per one liter in three studied methods included between 209.97-217.25. Among studied methods, the highest level of cocoon weight per one liter belonged to 3rd treatment (217.25), and 2nd treatment (209.97) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Best cocoon weight**

From obtained results, it is showed that amount of best cocoon weight in three studied methods included between 124.32-147.88 gr. Among studied methods, the highest level of best cocoon weight belonged to 3rd treatment (147.88 gr), and 2nd treatment (124.32 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Middle cocoon weight**

From obtained results, it is showed that amount of middle cocoon weight in three studied methods included between 14.85-31.51 gr. Among studied methods, the highest level of middle cocoon weight belonged to 2nd treatment (31.51 gr), and 1st treatment (14.85 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Low cocoon weight**

From obtained results, it is showed that amount of low cocoon weight in three studied methods included between 0.00-2.41 gr. Among studied methods, the highest level of low cocoon weight belonged to 3rd treatment (2.41 gr), and 1st treatment (0.00 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Double cocoon weight**

From obtained results, it is showed that amount of double cocoon weight in three studied methods included between 4.74-9.16 gr. Among studied methods, the highest level of double cocoon weight belonged to 2nd treatment (9.16 gr), and 1st treatment (4.74 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Mounting duration**

From obtained results, it is showed that amount of mounting duration in three studied methods included between 0.00-0.16 days. Among studied methods, the highest level of mounting duration belonged to 3rd treatments (0.16), and 2nd treatment (0.00 days) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant ( $P<0.05$ ).

#### **Female cocoon weight**

From obtained results, it is showed that amount of female cocoon weight in three studied methods included

between 1.99-2.13 gr. Among studied methods, the highest level of female cocoon weight belonged to 1st treatment (2.13 gr), and 2nd treatment (1.99 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant ( $P<0.05$ ).

#### **Female cocoon shell weight**

From obtained results, it is showed that amount of female cocoon shell weight in three studied methods included between 0.40-0.42 gr. Among studied methods, the highest level of female cocoon shell weight belonged to 1st treatment (0.42 gr), and 2nd treatment (0.40 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Female cocoon shell percentage**

From obtained results, it is showed that amount of female cocoon shell percentage in three studied methods included between 19.86-20.02%. Among studied methods, the highest level of female cocoon shell percentage belonged to 1st treatment (20.02%), and 2nd treatment (19.86%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Coefficient of variations for female cocoon weight**

From obtained results, it is showed that amount of coefficient of variations for female cocoon weight in three studied methods included between 6.85-9.14%. Among studied methods, the highest level of coefficient of variations for female cocoon weight belonged to 2nd treatment (9.14%), and other treatments (6.85%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P>0.05$ ).

#### **Coefficient of variations for female cocoon shell weight**

From obtained results, it is showed that amount of coefficient of variations for female cocoon shell weight in three studied methods included between 9.15-13.28%. Among studied methods, the highest level of coefficient of variations for female cocoon shell weight belonged to 3rd treatment (13.28%), and 2nd treatment (9.15%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were significant ( $P<0.05$ ).

#### **Coefficient of variations for female cocoon shell percentage**

From obtained results, it is showed that amount of coefficient of variations for female cocoon shell percentage in three studied methods included between 9.88-12.69%. Among studied methods, the highest level of coefficient of variations for female cocoon shell percentage belonged to 2nd treatment (12.69%), and 1st treatment (9.88%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant

( $P > 0.05$ ).

#### **Male cocoon weight**

From obtained results, it is showed that amount of male cocoon weight in three studied methods included between 1.64-1.66 gr. Among studied methods, the highest level of male cocoon weight belonged to 3rd treatment (1.66 gr), and 1st treatment (1.64 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P > 0.05$ ).

#### **Male cocoon shell weight**

From obtained results, it is showed that amount of male cocoon shell weight in three studied methods included between 0.38-3.43 gr. Among studied methods, the highest level of male cocoon shell weight belonged to some treatments (3.43 gr), and 2nd treatment (0.38 gr) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P > 0.05$ ).

#### **Male Cocoon shell percentage**

From obtained results, it is showed that amount of male Cocoon shell percentage in three studied methods included between 23.44-24.11%. Among studied methods, the highest level of male cocoon shell percentage belonged to 3rd treatment (24.11%), and 2nd treatment (23.44%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P > 0.05$ ).

#### **Coefficient of variations for male cocoon weight**

From obtained results, it is showed that amount of coefficient of variations for male cocoon weight in three studied methods included between 10.09-8.11%. Among studied methods, the highest level of coefficient of variations for male cocoon weight belonged to 1h treatment (10.09%), and 3rd treatment (8.11%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P > 0.05$ ).

#### **Coefficient of variations for male cocoon shell weight**

From obtained results, it is showed that amount of coefficient of variations for male cocoon shell weight in three studied methods included between 11.41-32.59%. Among studied methods, the highest level of coefficient of variations for male cocoon shell weight belonged to 3rd treatment (32.59%), and 2nd treatment (11.41%) remained at lower level than other methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P > 0.05$ ).

#### **Coefficient of variations for male cocoon shell percentage**

From obtained results, it is showed that amount of coefficient of variations for male cocoon shell percentage in three studied methods included between 9.68-11.18%. Among studied methods, the highest level of coefficient of variations for male cocoon shell percentage belonged to 2nd treatment (11.18%), and 1st treatment (9.68%) remained at lower level than other

methods. Other methods were between these two groups. Meanwhile statistical differences between studied methods for this trait were not significant ( $P > 0.05$ ).

There are different glands in insects for regulating of molting. These glands are responsible for Metamorphism (Gilbert et al, 1996; Gilbert et al, 2002). Prothoracicotropic hormone (PTTH) was the first insect hormone that was discovered. Originally described simply as "brain hormone", early workers such as Kopeć (1922), and Wigglesworth (1934) realized that ligation of the head of immature insects could prevent molting or pupation of the body region excluded from the head if the ligation was performed before a critical age in the lifestage was reached.

Prothoracicotropic hormone (PTTH), is a neuropeptide which is produced by the nerve- secretory cells of the brain (Bollenbacher and Granger, 1985; Gilbert et al, 2002). In the domesticated silkworm, endocrine activity influenced by evolutionary changes at the end of larval instars (Okuda et al, 1985; Gu et al, 1996; Gu et al, 1997). During the early larval ages, Prothorostatic glands are secrete steroids significantly and respond to the PTTH secretion hormone. However, these glands during the early stages of the fifth larval age cannot respond to these hormones (Gu et al, 2000; Gu and Chow, 2005a).

Endocrine mechanisms for regulating of cell prothorostatic growth not well be understood (Chen and Gu, 2006). Gu and Chow (2005b) had confirmed that the prothorostatic glands in insect *Samia Cynthia ricini* and *Antheraea polyphemus*, are responsible of DNA forming in fourth and fifth larval instars. In the domesticated silkworm has also been proven that the amount of DNA making in the endocrine cells Prothorostatic glands, in third, fourth and fifth larval instars are suffered under severe changes (Gu and Chow, 2001; Gu and Chow, 2005b). In addition, the obtained findings show that activation of making DNA in endocrine cells in the mid-fifth larval age, is related to nutrition and hungry of silkworm larvae during the initial three days of this instar, inhibited DNA making (Chen and Gu, 2006). However, metamorphosis is occurred when the fifth instar larvae be exposed under hungry, at the end of this instar (Chen and Gu, 2006). Therefore, it is necessary to study of growth and secretion pattern of prothorostatic glands in hungry larvae. Studies have shown that after starting the third day of hunger, increased protein levels of are less than the control group. However, these larvae glands, are produced the steroids equal to control larvae (Chen and Gu, 2006).

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