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## Biology and Behavior of Mulberry Silkworm (*Bombyx mori*) in Northern Iraq

### Abstract

Domestic silk worms were reared in Iraq during the 1970s and 1980s of the last century, but unfortunately they completely disappeared after 1990 till now due to the unstable situations and economic problems. This study was aimed to revive the neglected valuable insect in Iraq after its absent for three decades, and obtaining a large number of silkworm eggs for continuous rearing and experiments in the next years. *Bombyx mori* (Bursa White; 'Bursa Beyazi') were imported from Sericulture Center in Bursa, Turkey. Incubated eggs were hatched into larvae  $7.00 \pm 1.00$  days after incubation. Hatching percentage was  $97.75\% \pm 3.11$ . The durations of larval instars (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>) were  $3.60 \pm 0.55$ ,  $3.75 \pm 0.55$ ,  $5.12 \pm 0.73$ ,  $5.98 \pm 0.51$ ,  $7.72 \pm 0.55$  days respectively. The total larval period was  $26.17 \pm 1.50$ . mature larvae spent  $46.00 \pm 14.50$  hours for spinning cocoons. Weight of a single cocoon was 1.7 to 2.5 gm and the weight of the cocoon shell was 0.45 g. The pupal period lasted for 10 to 12 days. The completion of the cocoon spent  $46.00 \pm 14.50$  hours. Adults were emerged after  $10.60 \pm 1.50$  days. Adult male longevity was  $115.00 \pm 5.00$  hours, while adult female longevity was  $135.50 \pm 7.50$  hours. Fertilized female laid more than 500 eggs in the form of clusters and the average weight of these eggs per a single female was  $250 \pm 30.00$  mg. Average weight of 4<sup>th</sup> and 5<sup>th</sup> instars larvae were  $3.45 \pm 0.90$  g and  $5.25 \pm 1.25$  g respectively. It can be concluded that successful rearing can be performed during April -May and September - October due to the moderate temperature in these months.

## INTRODUCTION

Domestication is one of the most important developments in human history. Sericulture has served the humanity by providing natural animal silk for centuries. Silk is produced naturally by the larva of the silkworm moth (*Bombyx mori* L. 1758; Lepidoptera, Bombycidae), it is produced in more than 60 countries across the world (Datta and Nanavaty, 2007). Silk production and trade have long been important activities in the Middle East and Central Asia and have been linked with the Silk Road for hundreds of years (Yilmaz and Wilson, 2017). Silkworm pupae are insects that are beneficial to human health, due to their high nutritional value and various biomedical functions (Zhou et al., 2022). Silk is called as the queen of all fabric materials over thousands of years (Saha, 2022). Sericulture industry has not been able to flourish in most part of the world mainly due to its great dependence on mulberry as sole food, environmental concerns, silkworm diseases and lack of training facilities for the silkworm farmers. The success of this industry is based on availability of high yielding mulberry varieties, rearing of silkworm larvae for cocoon production under prevailing favorable environment and marketing facilities (Hussain et al., 2011a). *Bombyx mori* L. commonly known as mulberry silkworm reared on mulberry plant which used on the large scale for silk production (Mahmoud, 2013) and monophagous species, with complete metamorphosis, eating only mulberry leaves during its larval stage (Ravikumar et al., 2019). The most important characteristics of the silkworm is its ability to transform the plant proteins into animal proteins (Nair and Kumar 2004). *Bombyx mori* has lost the ability to fly and survive under extreme environmental conditions. (Torres McCook et al., 2021). It is well known that the amount and quality of leaves affects their growth, developmental period, body weight and survival rate of larvae, as well as influencing the subsequent fecundity,

longevity and movement (Para, 1991). The worms in the fifth instar ingest more than 88 % of mulberry, and it reaches its maximum weight one or two days before starting to spin the cocoon. When they finish their development and stop eating, larval integument appears transparent. At this stage, they are called mature worms, and begin to form the cocoon for two or three days (Cifuentes and Sohn 1998). Mulberry leaves provide proteins, vitamins and other nutrients from which silk proteins are synthesized. Quality and quantity of mulberry leaves along with environmental factors affect production of raw silk spun by larvae before pupation in the form of cocoons. It is an important contributor to household economies and to the income of women in many of these countries. Many other countries, including Turkey, China, and Egypt are known to have an array of silkworm genetic resources (Sekhar et al., 1991). Domestic silk worms were reared in Iraq during the 1970s and 1980s of the last century, but unfortunately they completely disappeared after 1990 till now due to the unstable situations and economic problems. Therefore, the main aims of the current study were to revive the neglected valuable industry (Sericulture) in our area and obtaining a large number of eggs for continuous rearing and experiments in the next years, to study some biological aspects of mulberry silkworms as well as to observe the behavior of the silkworm during their rearing in the laboratory.

## MATERIALS and METHODS

The experiment was performed in the growth chamber at the Department of plant protection, College of Agricultural Engineering Sciences, University of Duhok, Northern Iraq; from middle of September to the end of October 2021. The disease-free eggs of *Bombyx mori* (Bursa White; 'Bursa Beyazi') were obtained from Sericulture Center in Bursa, Turkey. These imported eggs were dark gray in color but they changed to light creamy then to transparent eggs after incubation. Silkworm rearing was

carried out in a growth chamber where temperature, relative humidity and photoperiod were controlled. Five experimental groups (replicates) of silkworms were reared and fed with mulberry (*Morus nigra*) leaves.

#### **Incubation and hatching**

The eggs were subjected to incubation ( $27 \pm 1$  °C and 85 to 90 % RH with 16 h light: 8 h of darkness) according to Hussain et al. (2011a).

#### **Larval Rearing**

Early larval instars (1st to 3rd) were reared at  $27 \pm 1$  °C temperature and RH conditions of 85 to 90 %, whereas 4th and 5th larval instars were reared at  $25 \pm 1$  °C temperature and 70 to 80 % RH, (Rahmathulla et al., 2002); Rahmathulla 2012). During observations, larvae of 4th and 5th instars were reared in plastic containers (Prabu, 2012). Each container included thirty larvae.

#### **Mounting and Cocoon Spinning**

The 5th instar mature larvae when stopped feeding and searching for place to pupate were handpicked and transferred for cocoon spinning under controlled conditions ( $25 \pm 1$ °C and  $75 \pm 5$ % RH). After the completion of cocoon construction by pupating larvae, fresh cocoons were harvested on 8<sup>th</sup> day of spinning to allow uniform cocoon crop (Hussain et al., 2011). The dimensions of eggs and each larval instar were measured under microscope with the help of ocular micrometer with micrometer slide, in addition to the weight of fourth and fifth instar larvae. The total larval period was calculated from the date of egg hatching to the date of formation of pre-pupa. The duration between formations of pupa to the emergence of adult was considered as pupal period. After formation of cocoon, the length and width were measured, it was cut and a pupa was taken out from it. Measurement regarding length and width of pupa were also recorded.

#### **Biological parameters during larval stage**

The duration of 4<sup>th</sup> and 5<sup>th</sup> instars larvae. Length, width and weight of 4th and 5th instars larvae.

#### **Economic traits**

The economic traits like cocoon parameters (length, width and weight), cocoon shell weight was measured by using scales and digital balance.

#### **Weight of cocoons**

Five fresh cocoons from each replicate were cut and the whole cocoons were weighed separately.

#### **Cocoons shell weight**

After removing the pupae from the previous cocoons as mentioned, and cleaning them from exuviae, then were weighed (Kamel et al., 2016). Copulation period and the weight of eggs per a single female were also measured.

## **RESULTS**

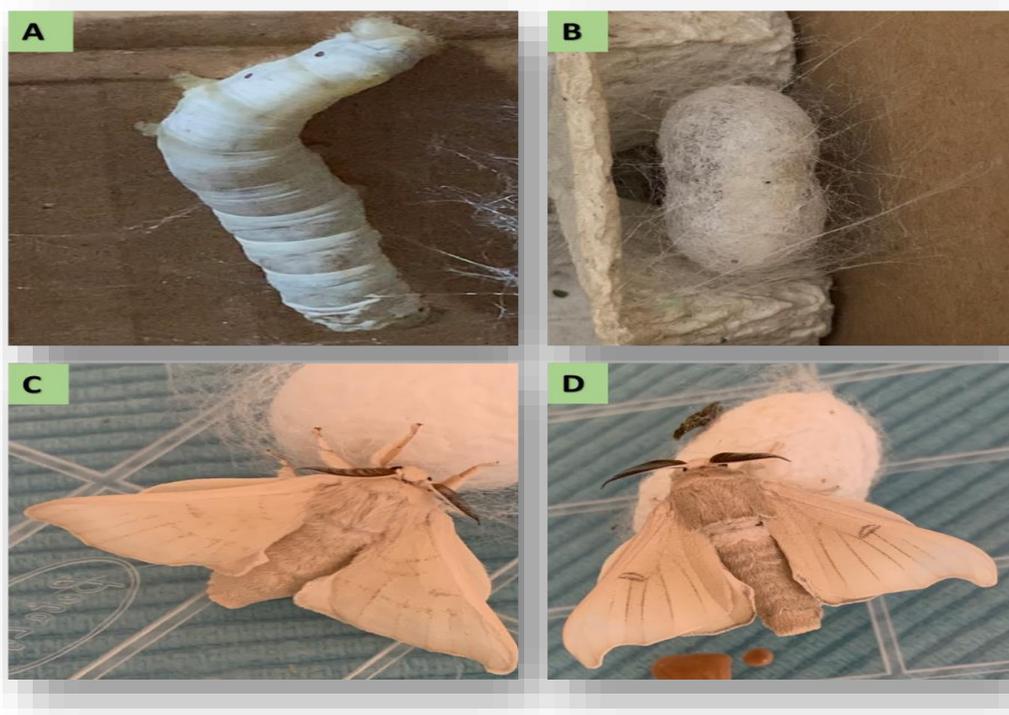
The incubated eggs were hatched into larvae  $7.00 \pm 1.00$  days after incubation. The newly hatched larvae were dark in color. The hatching percentage was  $97.75\% \pm 3.11$  and the durations of larval instars (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>) were  $3.60 \pm 0.55$ ,  $3.75 \pm 0.55$ ,  $5.12 \pm 0.73$ ,  $5.98 \pm 0.51$ ,  $7.72 \pm 0.55$  days respectively, while the total larval period was  $26.17 \pm 1.50$  days (Table 1). The mature larvae stopped feeding and moved towards the corners then secreted a sticky fluid through their mouth parts. The mature larvae spent  $46.00 \pm 14.50$  hours for spinning cocoons. The fluid secreted out from the mouth parts of the mature larvae in the form of fine filament of silk; hardened when exposed to the air then wrapped around the body of the mature larva in the form of protected covering. All produced cocoons were elliptic and white in color. The weight of a single cocoon was 1.7 to 2.5 gm and the weight of the cocoon shell was 0.45 gm (Table 2). The pupal period lasted for 10 to 12 days. Cocoons were white in color and the completion of the cocoon was varied from 32 to 60 hours in average of  $46.00 \pm 14.50$  hours as shown in Table 1.

The silk worm within the cocoon secreted a yellow fluid and opened their way to the outside. The adults were emerged after  $10.60 \pm 1.50$  days, then immediately copulated and separated after  $12.00 \pm 7.00$  hours. Adult male longevity was  $115.00 \pm 5.00$  hours, while adult female longevity was  $135.50 \pm 7.50$  hours and the females spent  $20.50 \pm 7.55$  hours of their short life in egg laying (Table 1). The fertilized female laid more than 500 eggs in the form of clusters and covered with gelatinous secretion for the proper attachment. The new generation of eggs were rounded and white creamy in color at the beginning then changed to darker and the dimension of eggs were  $1.26 \pm 0.05$  mm length and  $1.00 \pm 0.04$  mm width (Table 3). The average weight of

these eggs per a single female was  $250 \pm 30.00$  mg. as shown in Table 2. According to the tables 2 and 3; the average weight of 4<sup>th</sup> instar larva was  $3.45 \pm 0.90$  g with length of  $3.6 \pm 0.95$  cm and width of  $0.55 \pm 0.05$  cm. While the average weight of 5<sup>th</sup> instar larva was  $5.25 \pm 1.25$  g with length of  $5.80 \pm 0.90$  cm and width of  $0.75 \pm 0.04$  cm. The average dimensions of adult female were  $1.85 \pm 0.14$  cm length and  $0.55 \pm 0.05$  cm width and all were pale creamy to white in color. The entire body was covered with scales. The males had longer antennae and narrow abdomen and more active than females, while the females had small antennae, large and flat abdomen. These adult moths were not fed during their very short life period (Figure 1 and 2).



**Figure 1.** Larval development of silk worm, A; Newly hatched larvae, B; 2<sup>nd</sup> instar, C; 3<sup>rd</sup> instar, D; 4<sup>th</sup> instar



**Figure 2.** Stages of silk worm, A; Mature larvae, B; Incomplete cocoon, C; Adult female, D; Adult male

**Table 1.** Duration of different stages of development of silkworm (*Bombyx mori*) reared in the laboratory

Parameters	Duration			No. observations
	Min.	Max.	Av. $\pm$ SD	
Incubation period (days)	6.00	8.00	7.00 $\pm$ 1.00	700
Hatching percentage (%)	94.06	99.13	97.75 $\pm$ 3.11	700
1 <sup>st</sup> instar larva (days)	3.00	4.00	3.60 $\pm$ 0.55	100
2 <sup>nd</sup> instar larva (days)	3.50	4.00	3.75 $\pm$ 0.55	100
3 <sup>rd</sup> instar larva (days)	4.50	5.50	5.12 $\pm$ 0.73	100
4 <sup>th</sup> instar larva (days)	5.25	6.50	5.98 $\pm$ 0.51	100
5 <sup>th</sup> instar larva (days)	7.50	8.00	7.72 $\pm$ 0.55	100
Total larval period (days)	23.75	28.00	26.17 $\pm$ 1.50	100
Spinning (hours)	32.00	60.00	46.00 $\pm$ 14.50	90
Pupa (days)	8.00	11.00	10.60 $\pm$ 1.50	80
Female longevity (hours)	110.60	140.50	135.50 $\pm$ 7.50	55
male longevity (hours)	98.75	112.00	115.00 $\pm$ 5.00	35
Copulation (hours)	2.50	15.00	12.00 $\pm$ 7.00	50
Egg laying (hours)	18.00	30.00	20.50 $\pm$ 7.55	30

**Table 2.** Weight of 4<sup>th</sup> and 5<sup>th</sup> instars larvae, cocoons and the eggs laid by a single female

Parameters	Weight (gm)			No. observations
	Max	Min	Average $\pm$ SD	
4 <sup>th</sup> instar larva (g)	3.00	3.80	3.45 $\pm$ 0.90	30
5 <sup>th</sup> instar larva (g)	4.20	6.00	5.25 $\pm$ 1.25	30
Fresh cocoon (g)	1.70	2.50	2.15 $\pm$ 0.25	20
Cocoon shell (g)	42	50	0.45 $\pm$ 0.06	20
Weight of eggs per a single female (mg)	225	280	250 $\pm$ 30.00	15

**Table 3.** Measurements of different stages of development of silkworm (*Bombyx mori*) reared in the laboratory

Stage of development	length			Width		
	Min.	Max.	Average $\pm$ SD	Min.	Max.	Average $\pm$ SD
1 st instar larva (mm)	3	3.8	3.5 $\pm$ 0.15	3.88	0.91	0.89 $\pm$ 0.01
2nd instar larva	9	9.5	9.25 $\pm$ 0.50	0.98	1.11	1.00 $\pm$ 0.09
3rd instar larva	12.5	17	14.50 $\pm$ 1.55	1.85	3.58	3.00 $\pm$ 0.45
4th instar larva (cm)	3.45	3.9	3.6 $\pm$ 0.95	0.45	0.56	0.55 $\pm$ 0.05
5th instar larva (cm)	5.5	8.6	5.80 $\pm$ 0.90	0.7	0.85	0.75 $\pm$ 0.04
Pupa(cm)	1.75	2.3	1.95 $\pm$ 0.11	0.55	0.8	0.75 $\pm$ 0.06
Cocoon (cm)	3	3.6	3.45 $\pm$ 0.11	1.42	1.6	1.50 $\pm$ 0.09
Adult female (cm)	1.7	2	1.85 $\pm$ 0.14	0.45	0.65	0.55 $\pm$ 0.05
Adult male (cm)	1.55	1.5	1.65 $\pm$ 0.07	0.4	0.65	0.52 $\pm$ 0.07
Eggs (mm)	1.18	1.5	1.26 $\pm$ 0.05	0.95	1.1	1.00 $\pm$ 0.04

## DISCUSSION

Although there are no uni-cultivar mulberry farms in Iraq, but there are a large number of mulberry trees distributed in all parts of the country. Hatching was the most important and more sensitive phase of silk moth life. Many attempts of incubation and hatching inside the rearing room were failed during July and beginning of August, therefore we used the controlled incubator machine. Because of the high temperature and highly dry environment of northern Iraq during September, the laboratory was provided with air conditioning machine for increasing the humidity inside the rearing room. Differences with the results of researchers from neighboring countries regarding the life cycle and larval development were not expected, because of all rearing stages were conducted inside controlled room with optimum conditions.

Some biological parameters like hatching percentage, larval duration (Zannoon, 2012), weight of 4<sup>th</sup> and 5<sup>th</sup> instar larvae, in addition to the weight of a single cocoon were not differed from results of other researchers (Raghuvanshi et al., 2019; Hassan et al., 2020). Relative humidity was changed from 70-80% in rearing room for all instar stages (Hussain *et al.*, 2011 a, b and c); therefore, in this study relative humidity of 5<sup>th</sup> instar stage in rearing room was about 75%.

## CONCLUSIONS

Based on the results of the current study, summer season (June, July and August) in Iraq was not suitable for silkworm rearing, except for research purposes inside laboratories, while successful rearing can be performed during

April -May and September – October due to the moderate temperature in these months.

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