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Population Management of *Samia cynthia ricini* Boisduval (Lepidoptera: Saturniidae) by Eggs Refrigeration and Its Effect on Hatchability, Development, and Fecundity

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ABSTRACT

Egg viability, hatchability, and development are significant factors in the natural silk industry. Life cycle modification is an important aspect that must be considered to perform effective and efficient mass-rearing and quality control. This study aimed to improve the mass rearing efficiency of *Samia chintya ricini* through a life cycle modification technique by refrigeration. The study was conducted by storing *S. c. ricini* eggs in the refrigerator at a temperature of 5°C for one to seven days. Observations were made on the hatchability, development time, cocoon shell weight, and adult female fecundity. The results showed that the egg refrigeration period affected the hatchability of the eggs. A more extended period of egg refrigeration significantly decreased hatchability. The eggs could develop into adult insects after being stored for one to five days. Meanwhile, none of the eggs hatched successfully after going through a cold storage period of six and seven days. Furthermore, the egg refrigeration period affected the development time of the egg, larvae, pupae, and adult. A more extended period of egg refrigeration results in slower egg and larval development time. But a faster cocoon development time. The refrigeration period did not significantly affect the cocoon shell weight. However, it was affecting adult female fecundity. Total fecundity decreased as the refrigeration period increased with different daily oviposition patterns. This study implies that when the egg population is excessive, the life cycle of *S. c. ricini* can be delayed for 1-5 days. This method can be implied as a simple life cycle modification technique for eri silkworm cultivation.

1. Introduction

Eri silkworm *Samia cynthia ricini* Boisduval (Lepidoptera: Saturniidae) is an insect native to the Brahmaputra Valley of India (Jolly *et al.* 1979). The distribution of this insect has spread to China and Japan (Peigler 1989; Singh and Benchamin 2002). This silkworm is relatively new in Thailand and Indonesia (Tungjitwitayakul and Tatun 2017; Trisnawati and Nurkomar 2020). *Samia cynthia ricini* is an insect that feeds on more than 29 plants (Reddy *et al.* 2002). This silkworm primarily feeds on castor leaves and can produce heavier cocoon shells; thus, more silk can be produced (Devaiah *et al.* 1985).

Samia cynthia ricini develops over ±50 days from egg to adult (Shifa *et al.* 2014). One adult female can lay 300-500 eggs (Das and Das 2018). Many eggs will be an obstacle for small-scale farmers like Indonesia. Many eggs require more resources, such as the availability of spaces, equipment, labour, and diet used in the rearing process. Excess eggs are usually discarded, so the rearing process cannot be carried out optimally (Trisnawati and Nurkomar 2020). Therefore, insect population management through modification of the life cycle by refrigeration can be done as a step to delay the development of immobile phases such as eggs or pupae at a specific temperature so that eggs can be stored and reared at different times.

Refrigeration is a method for modification of the life cycle widely used in the cultivation of silkworms. Benchamin *et al.* (1989) reported that

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refrigeration of newly hatched larvae affected the survival rate, growth, and egg production of *Bombyx mori*. Refrigeration of cocoons also affected the fecundity and egg hatchability of *B. mori* (Gaurav *et al.* 2015; Upadhyay *et al.* 2006). Refrigeration was also reported in *S. c. ricini* eggs with a limited impact on their hatchability (Somaprakash and Prasad 2009; Vishwakarma 1982).

This study was carried out to determine the effects of the egg refrigeration period on the egg hatchability, development time, cocoon shell weight, and adult female fecundity of *S. c. ricini*. Also, this study aimed to determine an appropriate egg refrigeration period to delay the life cycle of *S. c ricini* that applicable to all stakeholders.

2. Materials and Methods

2.1. Insect Diet

Castor (*Ricinus communis*) was used as the best diet to support the development of *S. c. ricini* (Devaiah *et al.* 1985). The leaves were collected from the surrounding area of Universitas Muhammadiyah Yogyakarta's campus in Bantul, Yogyakarta, Indonesia. Before being used, the leaves were washed with tap water and dried using sterile tissue paper. The diet was given in different quantities based on the development of larvae because each instar has a different morphological characteristic and feeding behavior. The diet was given once per day (in the morning) for the first and second instar larvae, twice per day (in the morning and afternoon) for the third and fourth instar larvae, and three times per day (in the morning, afternoon, and evening) for the fifth instar larvae.

2.2. Effect of Egg Refrigeration on Hatchability, Development, and Adult Female Fecundity

The research was started by storing 1-day-old *S. c. ricini* eggs at a temperature of 5°C for 1-7 days as a treatment, respectively. These eggs were obtained from a breeder at PT. Jantra Mas Sejahtera in Kulon Progo, Yogyakarta, Indonesia. There were eight treatments for the egg refrigeration period, including control at a room temperature. Each treatment consisted of four replications, resulting in 32 experimental units. Fifty eggs of *S. c. ricini* were used in each treatment. In total, there 1,600 eggs were used in this study. Each treated egg per replication was placed on a Petri dish (86 × 13 mm). After treatment, the eggs were kept in a rearing room under laboratory conditions at (25±1°C, 80±10% r. h., and 16:8 light) until they hatched.

After hatching, the first instar larvae were reared in a plastic container (16 × 12 × 5 cm) until they developed to the third instar. The fourth instar larvae were then transferred into a tray (32 × 25 × 5 cm) and placed inside an adult rearing cage (37 × 30 × 33 cm) until the formation of a cocoon. The rearing container needs to be adjusted because the larva's body will enlarge with time. When the cocoon formed, each cocoon was hung inside the cage using a thread in the anterior-posterior (sagittal) position to facilitate the adult emergence process. Adults were kept inside the cage until they died.

Observations were made daily to record each stage's development time, including eggs and their hatchability, larvae, pupae, and adults. The cocoon shell was weighed when the pupa had formed. Adult female fecundity was recorded by calculating the number of eggs laid every day until the female died.

2.3. Data Analysis

All data sets recorded were subject to stepwise simplifications to determine the suitable model using the lowest AIC number. The final model was analyzed using one-way ANOVA, except adult development time was analyzed using a general linear model (GLM) with Gamma family and identity link function. The cocoon shell weight and total development time data were also analyzed using GLM with Gaussian family and log link function. The mean difference between treatments was further tested using Tukey's HSD multiple comparisons with Holm's adjustment (Hothorn *et al.* 2008). A simple regression and correlation analysis was also performed to analyze the relationship between the egg refrigeration period and the development time of *S. c. ricini*. Statistical analysis was carried out using R Statistics v 4.2.1 (R Core Team 2015), and the graph was visualized using the ggplot2 package (Wickham 2009). The data from the 6-day and 7-day refrigeration periods were discarded from the analysis because no eggs developed in each treatment.

3. Results

The egg refrigeration period significantly affected the hatchability rate (lm: $F_{5,18}$ = 34.5, dF= 5, P Value = 1.297e-08); 87-100% eggs can still hatch after refrigerated for 1-2 days. The hatchability rate decreased to 45-58% when the eggs were refrigerated for 3-4 days, and the hatchability of eggs decreased to 15% when refrigerated for five days. Eggs could not hatch after being refrigerated for six to seven days (Figure 1). In addition, the refrigeration period also significantly affected the development time of *S. c. ricini* eggs (lm: $F_{5,18}$ = 2471.1, P Value = 6.274 e-16). Under room temperature (±26-27°C), *S. c. ricini* eggs developed in six days. The development time of eggs increased by 2-3 days when refrigerated for 1-2 days and and five days when refrigerated for 3-4 days respectively. After refrigerated for five days, the eggs could develop for up to 13 days, seven days longer than the usual egg development time (Table 1). Regression analysis showed that the longer refrigeration period egg experienced a longer development time (R^2 = 0.991, P-value = 2.2e-16).

When the egg developed into a larva, the development time of the larva was also significantly affected by the egg refrigeration period (lm: $F_{5,18}$ = 15.57, P Value = 5.427 e-06). Usually, the development time of a larva from 1st to the 5th instars was 20 days. The development time of larvae increased by 4-6 days when the eggs were refrigerated for 1-4 days and increased by eight days when the eggs were refrigerated for five days (Table 1). Regression analysis also showed that the longer refrigeration period larvae experienced a longer development time (R^2 = 0.825, P-value = 0.00091).

The egg refrigeration period affected the development time of the pupae (lm: $F_{5,18}$ =3.317, P Value = 0.0268) Interestingly, the pupa developed faster by 1-2 days compared to the control (Table 1). Regression analyses showed a significant inverse relationship between the egg refrigeration period and development time of pupae (R^2 = 0.827, P-value = 0.00083). Nevertheless, there was no significant effect of the egg refrigeration period on the cocoon shell weight (lm: $F_{5,18}$ = 1.943, dF= 5, P Value = 0.136). Cocoon shell produced ranged from 0.25–0.35 g (Figure 2).

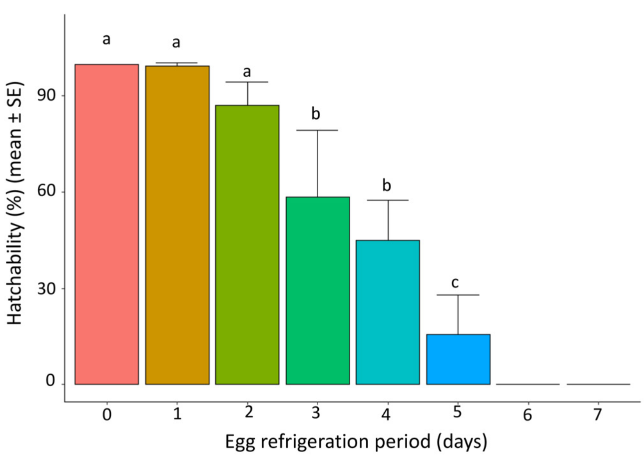

Figure 1. Effects egg refrigeration on hatchability rate of *Samia cynthia ricini* egg. Means with different letters are significantly different by Tukey HSD Test (α = 0.05). SE: standard error

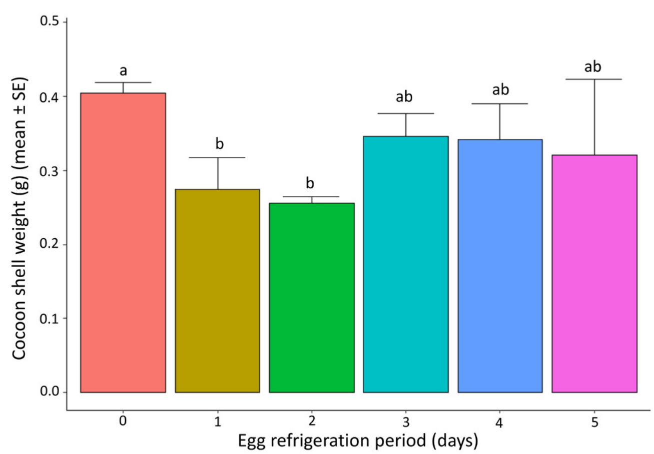

Figure 2. Effects of egg refrigeration on *Samia cynthia ricini* cocoon shell weight. Means with different letters are significantly different by Tukey HSD Test (α = 0.05). SE: standard error

Table 1. Effects of egg refrigeration on the development time of *Samia cynthia ricini*

Egg refrigeration period	Development time (days) (Mean ± S.D.)				
	Egg	Larva	Pupa	Adult	Total
0 day	6.000±0.000[f]	20.908±0.362[c]	18.941±0.172[a]	4.641±0.125[b]	50.491±0.544[a]
1 day	8.125±0.118[e]	26.577±1.094[ab]	17.906±1.236[ab]	5.765±0.226[ab]	58.374±0.364[c]
2 days	9.595±0.081[d]	24.862±1.379[b]	17.822±0.561[ab]	7.013±0.776[a]	59.294±1.325[c]
3 days	11.122±0.720[c]	25.220±1.035[b]	16.834±0.767[ab]	6.003±0.657[ab]	59.181±0.814[c]
4 days	11.710±0.299[b]	24.924±1.884[b]	17.341±1.018[ab]	6.429±0.857[ab]	60.406±0.769[c]
5 days	13.205±0.180[a]	28.209±1.116[a]	16.392±1.526[b]	6.035±1.853[ab]	63.843±2.145[b]

*Means with different letters in a column are significantly different by Tukey HSD Test (α = 0.05)

The egg refrigeration period also affected the development time of adults (glm: $F_{5,18}$ = 0.9542, P Value = 0.03019). Adult longevity lasts 5-7 days, 1-3 days longer than control (Table 1). However, some moths would live for 9-10 days (max) in all treatments. There was no correlation between the egg refrigeration period and adult longevity (R^2 = 0.665, P-value = 0.0862). Nonetheless, the egg refrigeration period significantly affected the number of eggs laid by the adult female (lm: $F_{5,18}$ = 12.49, P Value = 2.476 e-05). The number of eggs laid by females decreased by ±29% compared to the control when the egg was refrigerated for 1-5 days (Figure 3). Interestingly, the female's daily oviposition pattern differs (Figure 4). Under room temperature, adult females have a predictable pattern of egg production, with an increase in eggs at first and a decline as they age until they die (Figure 4A). Meanwhile, the egg refrigeration alters the pattern of oviposition. At the 1-2 days egg refrigeration period, females laid more eggs on the first day of emergence (Figure 4B-C). Females from the eggs refrigerated for 3 days showed a fluctuated oviposition pattern (Figure 4D). Meanwhile, females from 4-days refrigeration period start to lay the eggs on day 4, with the peak on day 7 (Figure 4E). Females from the 5-day refrigeration period only lay eggs in the last three days of their lives in quite lower

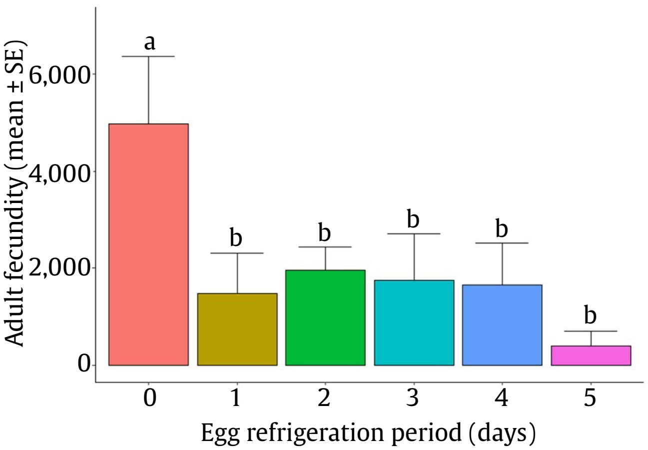

Figure 3. Effect egg refrigeration on adult female fecundity of *Samia cynthia ricini*. Means with different letters are significantly different by Tukey HSD Test (α = 0.05). SE: standard error

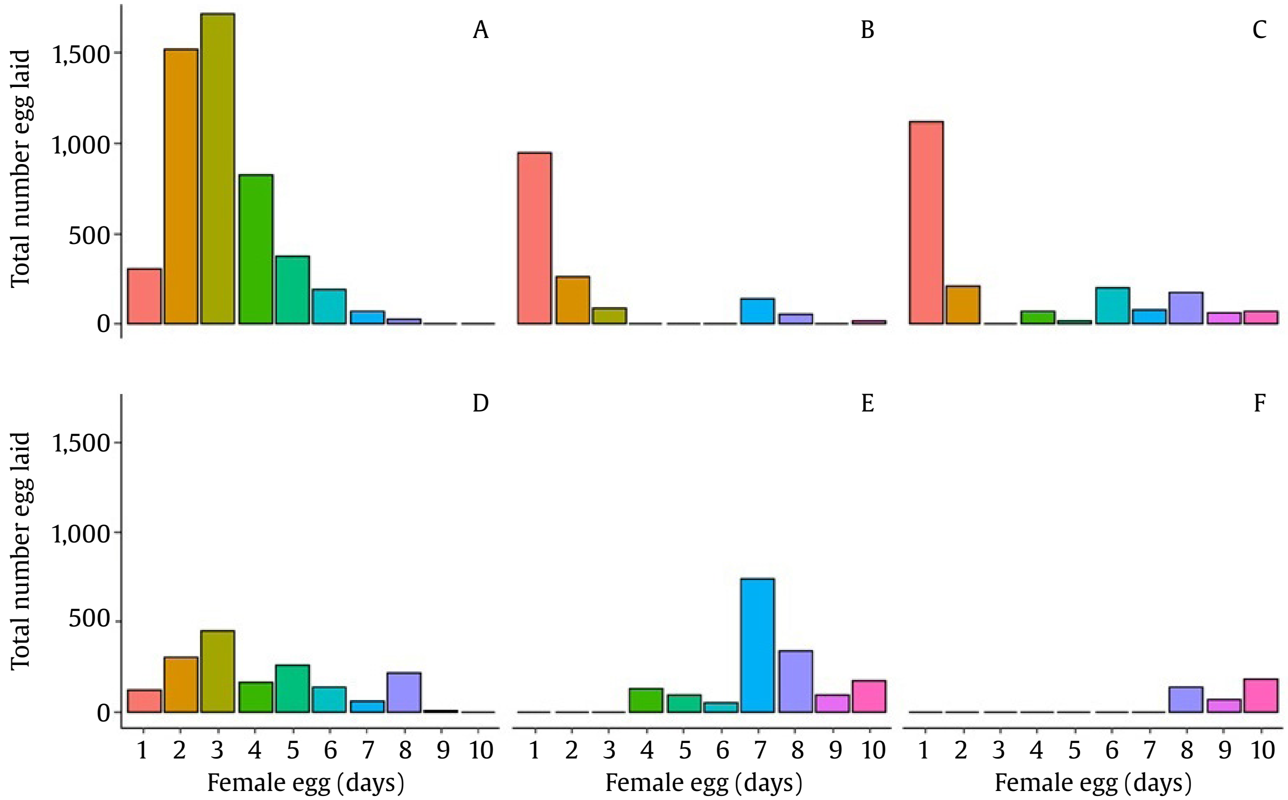

Figure 4. Daily oviposition pattern of adult female of *Samia cynthia ricini* on the different egg refrigeration. A. 0 days; B.1 day; C. 2 days; D. 3 days; E. 4 days; F. 5 days refrigeration period

numbers than in other treatments (Figure 4F). Finally, the egg refrigeration period significantly affected the total development time of *S. c. ricini* (glm: $F_{5,18}$ = 68.773, P Value = 4.294 e-11).

4. Discussion

A delay in insects' growth and development through egg refrigeration could slow down their life cycle. This is a strategy to keep the number of insects within the population stable and avoid overpopulation. Thus, insects' population regulation within the species can be performed effectively and efficiently. The results of this study showed that the egg refrigeration period affected most of all observed variables, such as hatchability, development time from egg to adult, and fecundity. Meanwhile, there was no significant effect on the cocoon shell weight as an essential component in the cultivation of silkworms.

This study showed that *S. c. ricini* eggs could survive at a temperature of 5°C for five days. Similarly, Somaprakash and Prasad (2009) reported that a one-day-old *S. c. ricini* egg could be refrigerated for five days at the same temperature. Meanwhile, two days-old eggs could be refrigerated at a temperature of 15°C for 5-10 days without affecting their hatchability. Interestingly, this study found that the longer the refrigeration period egg experienced, the lower the hatchability rate. However, the refrigeration period significantly affected the eggs' development time of this moth. In conditions without treatment (control), eggs developed in six days at room temperature. Temperature and humidity greatly affected the development of eggs. Our previous studies observed that eggs also developed in six days (Nurkomar *et al.* 2022a, 2022b). However, other studies reported that *S. c. ricini* eggs could develop in 8-9 days (Kavane 2014), ten days, and 14-15 days in the winter (Das and Das 2018).

The longer the egg refrigeration period, *S. c. ricini* also experiences a longer development time, this may occur due to the accumulation of cold temperatures in the egg's body, so its development time becomes longer. Vaidya *et al.* (2014) reported that temperature and humidity affected *S. c. ricini* rearing performance. Other conditions were also reported in other insects. The pre-ovipositional period of bamboo borer Dinoderus minutus (Coleoptera: Bostrichidae) decreased as the relative humidity increased (Norhisham *et al.* 2013). The development time of larvae, pupae, pre-oviposition, and adults of Heliothis viriplaca (Lepidoptera: Noctuidae) was also reported to decrease with increasing temperatures (Cui *et al.* 2018).

However, as for the effect on the larval development time, the refrigeration period increased the larval development time (6-8 days), which has implications for increasing the cost of the larval diet. The time required for the maintenance of larvae will result in the expenditure of production costs. Rustiono and Trimurti (2015) recorded that the longer the production time required by the insect, the more cost production needs to be spent.

Another interesting finding was also observed in effect on the pupal development time, in which the longer the egg refrigeration period, the pupa experienced the shorter development time. The refrigeration period of 1-4 days could accelerate the development of the pupa within one day. Meanwhile, the refrigeration period of 5 days could accelerate the development of the pupa within two days, which has implications for faster cocoon shell harvest times and can indirectly reduce production costs and labor. The refrigeration period will also not cause losses economically because there was no significant influence on the weight of the cocoon developed. The cocoons shell weighed the same as the weights of *S. c. ricini* cocoons reported in our previous studies (Nurkomar *et al.* 2022b) and others (Tungjitwitayakul and Tatun 2017).

Finally, the most crucial factor is adult fecundity because the number of eggs produced will determine the sustainability of the subsequent cultivation of silkworms. Although the adult female fecundity decreases with the more extended the egg's refrigeration period, the female is still capable of laying eggs in a high number. Endrawati *et al.* (2006) stated that the maternal effect on females influenced fecundity, such as size, age, condition of the female, and embryo characteristics after fertilization. In addition, we assumed that temperature had a significant effect on the fecundity of females. Kumar and Elangovan (2017) reported that low temperatures and humidity during the winter had a more significant effect on *S. c. ricini* fecundity than in the Spring. Hussain *et al.* (2011) recorded that temperature and humidity affected the fecundity and fertility of *B. mori.* Wanule and Balkhande (2013) added that temperature also affected reproductive behavior, site selection for oviposition, and the longevity of adult *B. mori.*

In conclusion, refrigeration effectively delays the development of *S. c. ricini* eggs for 1-5 days. However, to maintain the quality of rearing performance, the refrigeration can be carried out for a maximum of 1-2 days at a temperature of 5°C, so those parameters remain high.

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