LIFE CYCLE AND SURVIVORSHIP OF ERI SILKWORM, *Samia cynthia ricini* BIOSDUVAL (LEPIDOPTERA: SATURNIIDAE) ON THREE DIFFERENT CASSAVA LEAVES DIET

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ABSTRACT

Samia cynthia ricini, Eri silk worm is an oligophagous insect that consume several plant leaves, including cassava. However, it is not sure whether all cassava leaves can be used as a diet for *S. cynthia ricini*. This research was conducted to study the life cycle and survivorship of *S. cynthia ricini*, which was given three kinds of *Manihot* spp. leaves diet i.e., *Manihot glaziovii*, *M. esculenta*, and *M. utilissima*. The research was conducted by rearing silkworms in these three kinds of leaves diet. The development time of each stage, pupa and cocoon shell weight, and daily fecundity of adult of *S. cynthia ricini* were observed in this research. The results showed that *S. cynthia ricini* completed their life cycle when fed on *M. glaziovii* and *M. esculenta* leaves diet only. There was no difference in the development time of larvae when fed on *M. glaziovii* (28.15 days) and *M. esculenta* (28.19 days) leaves diet. However, the development times of pupae (17.99 and 18.21 days) and adult (3.96 and 3.62 days) of *S. cynthia ricini* were different when fed on *M. glaziovii* and *M. esculenta* leaves diet, respectively. The weight of *S. cynthia ricini* cocoon shell fed on *M. esculenta* (0.42 g) leaves diet was heavier than *M. glaziovii* (0.35 g), but the pupal weight of *S. cynthia ricini* was heavier when fed on *M. glaziovii* (1.90 g) than *M. esculenta* (1.77 g) leaves diet. There was no difference in adult fecundity among the two leaves diet tested. Adult of *S. cynthia ricini* produced 1214.89 eggs when the larvae reared on *M. glaziovii* and 1150.30 eggs on *M. esculenta*. We concluded that *M. esculenta* is the best alternate diet source for *S. cynthia ricini*.

Keywords: *Samia cynthia ricini*, life cycle, *Manihot glaziovii*, *Manihot esculenta*, *Manihot utilissima*

ABSTRAK

Samia cynthia ricini, ulat sutera Eri adalah serangga oligofagus yang memakan beberapa spesies daun tanaman, termasuk ubi kayu. Namun, tidak dapat dipastikan sama ada semua daun ubi kayu boleh dijadikan makanan untuk *S. cynthia ricini*. Penyelidikan ini dilakukan untuk mengkaji kitar hidup dan kemandirian *S. cynthia ricini* yang diberikan tiga jenis diet, iaitu daun *Manihot glaziovii*, *M. esculenta*, dan *M. utilissima*. Penyelidikan dilakukan dengan memelihara *S. cynthia ricini* dalam ketiga jenis diet daun ini. Masa pertumbuhan setiap peringkat hidup, berat pupa dan cengkerang kokun dan kesuburan harian *S. cynthia ricini* diperhatikan dalam penyelidikan ini. Hasil kajian menunjukkan bahawa *S. cynthia ricini* melengkapkan kitar hidup apabila diberi makan daun *M. glaziovii* dan *M. esculenta* sahaja. Tidak ada perbezaan waktu pengembangan larva ketika diberi makan menggunakan *M. glaziovii* (28.15 hari) dan *M. esculenta* (28.19 hari). Walau bagaimanapun, masa perkembangan pupa (17.99 dan 18.21 hari) dan dewasa (3.96 dan 3.62 hari) *S. cynthia ricini* berbeza ketika diberi makan menggunakan *M. glaziovii* dan *M. esculenta*. Berat cengkerang kokun *S. cynthia ricini* yang diternak menggunakan diet daun *M. esculenta* (0.42 g) lebih berat daripada *M. glaziovii* (0.35 g), tetapi berat pupa *S. cynthia ricini* lebih berat ketika diternak menggunakan diet daun *M. glaziovii* (1.90 g) lebih berat daripada *M. esculenta* (1.77 g). Tidak ada perbezaan dalam kemampuan pembiakan *S. cynthia ricini* antara dua diet daun yang diuji. *Samia cynthia ricini* menghasilkan 1214.89 telur apabila larva diternak pada *M. glaziovii* dan 1150.30 telur pada *M. esculenta*. Hasil penyelidikan ini menyimpulkan bahawa *M. esculenta* adalah sumber makanan pilihan terbaik untuk *S. cynthia ricini*.

Kata kunci: *Samia cynthia ricini*, kitaran hidup, *Manihot glaziovii*, *Manihot esculenta*, *Manihot utilissima*

INTRODUCTION

Silkworms are several insect species that belong to Lepidoptera order. These insects have high economic value for humans because, in the final phase of its larval stage, they can form cocoon (Nursita 2011), a silky web spun around the larvae body as a protective layer of pupa (Nuraeni & Putranto 2007) that can be used as raw materials for silk thread production. Silkworm cultivation plays an important role in the economy of a country like Indonesia. Cocoon production can generate income for those who cultivate it (Giacomin et al. 2017; Goswami & Bhattacharya 2013; Siddappaji et al. 2014). The worldwide silk market is expected to be worth USD 16.94 billion by 2021, with 7.8% compound annual growth rate from 2016 to 2021 (Markets and Markets Research 2017).

Over the years, the most cultivated silkworms in Indonesia are *Bombyx mori* (Lepidoptera: Bombycidae) (Atmosoedarjo et al. 2000). Recently, *S. cynthia ricini* (Lepidoptera: Saturniidae) is a new type of silkworm industry developed in Indonesia. *Samia cynthia ricini* is an oligophagous insect that can consume castor leaves as the primary diet (Kedir et al. 2014), and cassava (*Manihot esculenta*) (Sirimungkararat et al. 2005) as the second food. These two plant species can be found throughout the area in Indonesia. Thus, the mass rearing of *S. cynthia ricini* has a good opportunity to be developed in Indonesia (Handoro 1997).

Previous studies have shown that although the development and fecundity of *S. cynthia ricini* are better when reared using castor than cassava leaves diet, this suggests that cassava leaves can be used as an alternative diet for *S. cynthia ricini* (Tedy 2021). However, the use of kinds of cassava leaves as a food source for *S. cynthia ricini* remain widely unknown. Several studies on the use of cassava leaves in the mass rearing of *S. cynthia ricini* have been carried out, such as Setiyawan and Fitasari (2018) who examined the consumption and feed conversion rates of *S. cynthia ricini* on several types of cassava leaves, and Bili et al. (2018) who investigated the effect of cassava leaf species on larval morphometric characteristics and cocoon weight of *S. cynthia ricini*. In this study, research was conducted to study the life cycle and reproductive ability of *S. cynthia ricini* reared on three kinds of cassava leaves diet.

MATERIALS AND METHODS

Preparation of Leaves Diet

The leaves diet used were *Manihot glaziovii* which known as singkong karet, *M. esculenta* which known as singkong manggu, and *M. utilissima* which known as singkong sayur (Figures 1a-c) in Indonesia. *Manihot glaziovii* and *M. esculenta* were obtained from the area near of Universitas Muhammadiyah Yogyakarta campus, Kasihan, Bantul, Yogyakarta, Indonesia while *M. utilissima* is obtained from the market. The leaves diet was previously cleaned by washed using tap water and then dried by wiped using a clear cloth. Leaves diets are given based on larval development. During the 1st and 2nd instars larvae, the leaves diet was given once (one leaf) a day because the level of consumption was still low. Leaves diet was given twice a day after the larvae changes to 3rd and 4th instar larvae, and after the larvae changes to 5th instar, the leaves diet was given three times a day due to the higher level of consumption.

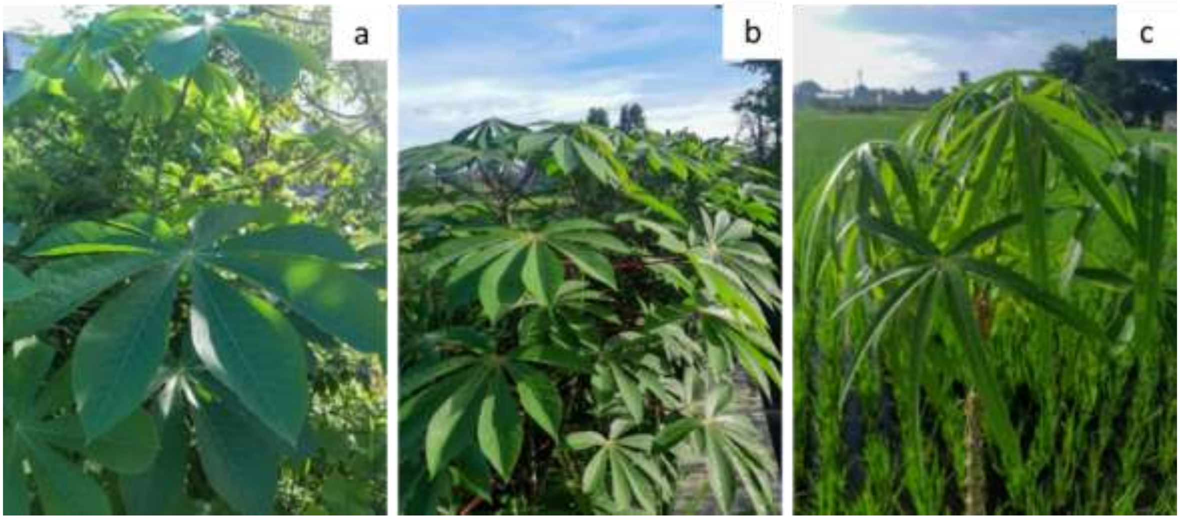

Figure 1. Kind of cassava leaves used as diet for *Samia cynthia ricini*. a) *Manihot glaziovii*, b) *M. esculenta*, and c) *M. utilissima*

Species Identification

Samia cynthia ricini was identified based on the morphological characters and the type of host plant eaten by the larvae. Identification is done with reference to Kalshoven (1981).

Effect of Kind of Cassava Leaves Diet on the Biology and Reproductive Capacity of *Samia cynthia ricini*

The experiment was conducted by testing three kinds of cassava leaves diet against *S. cynthia ricini* larvae. Each treatment was repeated in five replications, in total of 15 experimental units. Each treatment was tested using 30 individual silkworms, and 450 individual silkworms observed.

The eggs of *S. cynthia ricini* used were obtained from PT. Jantra Mas Sejahtera, a medium enterprise of silkworm in Pengasih village, Kulonprogo, Yogyakarta. The eggs used are from 14 generation of *S. cynthia ricini* reared with castor leaves. For the experiment, 30 eggs of *S. cynthia ricini* were reared in transparent plastic containers which covered by a perforated lid (W: 30 cm, L: 11.5 cm, H: 3.5 cm) which had been prepared according to the treatment tested. The eggs are incubated until they hatched into larvae and the rearing process was done under laboratory condition (25±1°C, 80±10% RH, and L16: D8 photoperiod). The 1st to 3rd instar larvae is reared in the same plastic containers as eggs. After the larvae changes to 4th instar, the larvae were moved to and reared in another transparent plastic container (W: 32 cm, W: 25 cm, H: 5 cm) which were placed in adult cages (W: 37 cm, W: 30 cm, H: 33 cm). This is done to facilitate the pupating process. During the pupal stage, the cocoon is attached to a thread (Figure 2) at the top of the adult rearing cage with the head facing up to help the moth during the eclosion process from pupae. Then, the moths are kept until they die in that cage.

Figure 2. *Samia cynthia ricini* pupa rearing method in the adult rearing cage

The parameters observed during this research included the development time of each stage (eggs, larvae, pupae and adults), pupa and cocoon shell weight, and reproductive capacity (adult fecundity). The development time of each stage was calculated and recorded. Adult fecundity was observed and recorded daily until the adults died by measuring the number of eggs laid by adult females of *S. cynthia ricini* using a hand tally counter.

Data Analysis

The data on the development time of each phase, the weight of the pupa, and the cocoons were analyzed using the Z-test. The data from *M. utilissima* leaves diet treatment were not included in the analysis because *S. cynthia ricini* did not develop well, but it was included in the results. Meanwhile, adult daily fecundity was analyzed using Analysis of Variance (ANOVA). The mean difference in daily fecundity was further tested with the Tukey HSD test. Data analysis was performed using Microsoft Office Excel 2019 software and SAS Statistical Analytical Software version 9.4.

RESULTS AND DISCUSSION

Diet is one of the important factors in insect biology (Kraus et al. 2019). Several studies have demonstrated the influence of diet on insect biology (de Castro-Guedes et al. 2016; Ganda et al. 2020; Mohamed et al. 2021; Zahari et al. 2019). However, research on the effect of diet on the biology of silkworms, especially *S. cynthia ricini* is still very limited. This research studies the biology of *S. cynthia ricini* in various types of cassava as a secondary host. The results showed that the kinds of cassava leaves diet used affected the development of *S. cynthia ricini*. There was a significant difference in the survival rate from eggs to adults of *S. cynthia ricini* fed on the three kinds of cassava leaves diet. *Samia cynthia ricini* larvae fed on *M. glaziovii* and *M. esculenta* leaves diet experienced a very high survival rate (above 90%) compared to that of *M. utilissima*. The survival rate of *S. cynthia ricini* larvae reared with *M. utilissima* leaves diet decreased 20% when the larvae changes to 3rd instar then continue until 90% when *S. cynthia ricini* larvae changes to pupal phase (Figure 3).

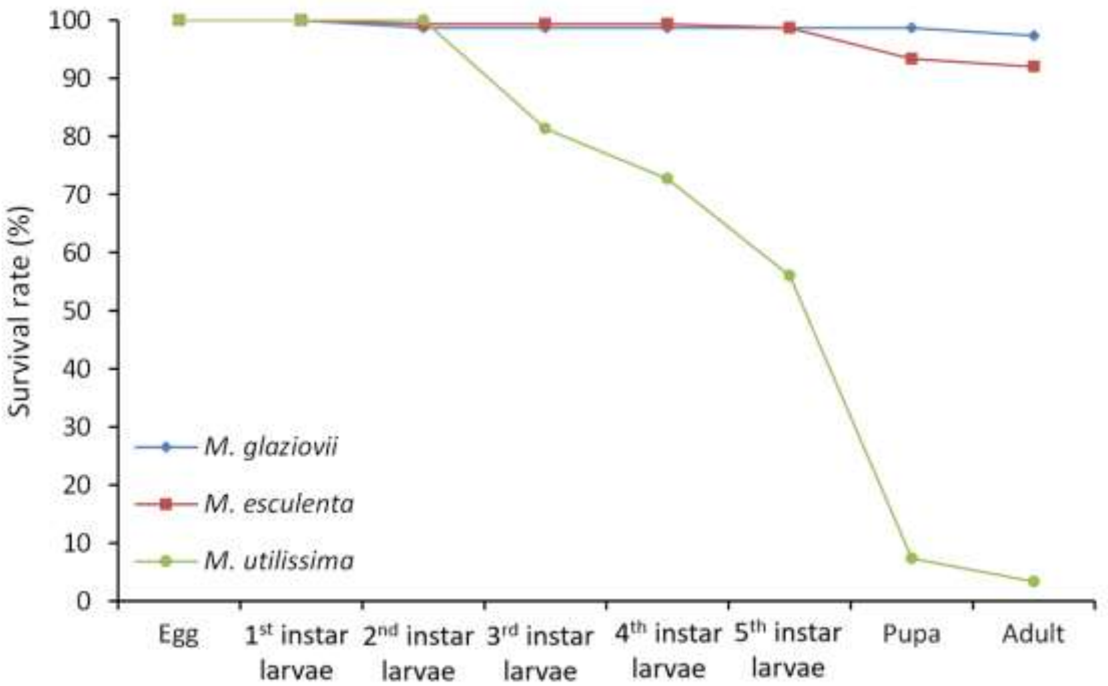

Figure 3. Survival rate of *Samia cynthia ricini* reared with different cassava leaves diet

Setiyawan and Fitasari (2018) stated that differences in cassava varieties will affect the nutritional composition and its characteristics. This will also affect the palatability of the larvae, which will indirectly affect their survival. Derara et al. (2020) also stated that different cassava varieties have significantly different mineral content such as nitrogen and other nutrients. Cassava leaves of the kello variety had a higher nitrogen content than cassava leaves of the qulle variety, resulting in a higher *S. cynthia ricini* survival rate of 92.66% when reared with cassava kello variety, and only 89.33% when reared with cassava qulle variety. Senoaji and Praptana (2015) stated that total N in plants is used as a precursor for the formation of enzymes and proteins needed by plants and insect pests. Several studies have shown an increase in insect growth, survival, reproduction rate, population density, and crop damage rates in response to increased nitrogen application (Altieri & Nicholls 2003). Further research needs to be carried out to observe the nitrogen content on those cassava leaves diets tested and its effect on the survival of *S. cynthia ricini*. Different species of cassava plants may have different nitrogen content, thus affecting the survival of *S. cynthia ricini*.

In addition, the development time of each phase of *S. cynthia ricini* reared with *M. glaziovii* and *M. esculenta* leaves diet also differed depending on the phase passed. The 1st, 3rd, and 5th instar larvae of *S. cynthia ricini* developed at relatively the same time (Table 1). Meanwhile, the 2nd and 4th instar larvae as well as the pupa and adult of *S. cynthia ricini* had quite different developmental times in the two kinds of leaves diet given. The 2nd instar larvae of *S. cynthia ricini* developed faster when fed on *M. glaziovii* leaves diet. But the 4th instar larvae of *S. cynthia ricini* developed faster when fed on *M. esculenta* (Table 1). The development time of the 1st to 4th instar larvae takes a shorter time than the development time of the 5th instar larvae because the late instar larvae require more feed consumption in their development. Samsijah and Kasumaputra (1975) said that the main needs of the 3rd instar larvae of *B. mori* are water and protein. While the 4th and the 5th instar larvae need more protein and carbohydrates, especially for the formation of silk glands. The number of leaves eaten by the larvae gradually increases according to the development of their life. Digested food and the proportion of the amount of food that is converted into body mass are metabolized to produce energy for growth and development (Scriber & Slansky 1981). The nutritional needs of each larval instar of *S. cynthia ricini* is another interesting thing that needs to be observed in further research.

The development time of *S. cynthia ricini* will affect production management. A long development time will cause an increase in production costs, and in this case, will reduce the value of profits and effectiveness. The mass rearing of *S. cynthia ricini* with a development time of larvae of 25 days had a benefit-cost ratio as a comparison between total operating income and total rearing costs of 1.84 (Rustiono & Trimurti 2015). If the required development time increases, the production costs will also increase. The development time of the 1st to 5th instar larvae of *S. cynthia ricini* from each treatment, both of *M. glaziovii* and *M. esculenta* lasted for 21 days. Similar findings were also reported by Venu & Munirajappa (2013) that the development time of larvae of *S. cynthia ricini* reared with *M. glaziovii* leaves was about 20 days. This study indicates that *S. cynthia* rearing with cassava leaves will shorten the larval development time which can indirectly reduce the Benefit-Cost Ratio as described above.

Table 1. Development time of *Samia cynthia ricini* reared with different cassava leaves diet

Stadia	**Development time (Mean±SD) (days)**			***P* value**
	M. glaziovii	***M. esculenta***	***M. utilissima***	
Eggs	6.00±0.00	6.00±0.00	6.00±0.00	-
1st instar larvae	4.00±0.00	4.00±0.00	4.00±0.00	-
2nd instar larvae	4.53±1.06	5.28±1.10	4.91±0.88	8.42 E-05*
3rd instar larvae	2.21±0.80	2.26±0.82	2.45±0.68	0.114
4th instar larvae	3.09±0.85	2.50±0.56	2.68±0.74	4.72 E-09*
5th instar larvae	8.56±0.89	8.32±1.49	4.07±3.02	0.154
Pupa	17.99±0.65	18.21±0.66	16.46±2.33	0.043*
Adult	3.96±1.08	3.62±0.95	3.4±1.51	0.014*
Total	49.76±4.75	48.51±6.96	43.88±1.07	0.035*

*Significantly different by Z-test (α=0.05%) between *M. glaziovii* and *M. esculenta* treatment

Cocoon weight is the most important parameter to determine silkworm productivity. The quality of the cocoons will affect the selling price in the market. Cocoon weight is an important commercial characteristic used to estimate the amount of raw silk that can be obtained. Indrawan (2007) stated that the heavier the weight of the cocoon of *Attacus atlas* moth, the more silk fibers produced. This is also possible with *S. cynthia ricini*. The results showed that the cocoon shell weight from *S. cynthia ricini* larvae reared with *M. esculenta* (0.42 g) was heavier than that of reared with *M. glaziovii* (0.35 g) leaves diet (Table 2). In contrast, the pupal weight of *S. cynthia ricini* from larvae fed on *M. glaziovii* leaves diet was heavier than that of the larvae fed on *M. esculenta*. There was no difference in total weight of pupa in the two treatments tested. The formation of cocoon shell of *S. cynthia ricini* affected by humidity condition. Lisa (2019) said that the weight of *S. cynthia ricini* pupae from larvae reared on cassava leaf diets was only about 1.65 g due to the lack of humidity conditions.

According to Patil et al. (1986), the weight of *S. cynthia ricini* cocoons is generally around 2.50 g when fed with a castor leaf diet. This is different from the average cocoon weight of *B. mori* which is only around 1.5-1.8 g. The number and types of leaves eaten by silkworms affect the weight of the cocoons shell formed. The more the number of leaves eaten, the heavier the weight of the cocoons produced because one of the constituents of nutrients in cocoons is protein, where protein is obtained from cassava leaves that are eaten. Cholifah et al. (2012) stated that the difference in cocoon weight can be influenced by the ability of larvae to accumulate silk fiber-forming proteins during the larval stage, especially in the 4th and the 5th instar larval stages. Protein and carbohydrates are very important for the 5th instar because at that stage the larvae from silk glands for cocoon formation. In addition to the number and kind of leaves, the kind of larvae, the environment such as temperature and humidity, the sex of the pupa, and the rearing method are other factors that affect the weight of the cocoons formed (Hartati & Umar 2012).

Table 2. Comparison of pupal and cocoon shell weight of *Samia cynthia ricini* reared with different cassava leaves diet

Parameter	**Mean±SD**			**P value**
	M. glaziovii	***M. esculenta***	***M. utilissima***	
Pupal weight (g)	1.90±0.39	1.77±0.54	1.09±0.27	0.02*
Cocoon shell weight (g)	0.35±0.05	0.42±0.27	0.35±0.31	2.087 E-05*
Total weight (g)	2.29±0.28	2.28±0.27	1.44±0.31	0.137

*significantly different by Z-test (α=0.05%) between *M. glaziovii* and *M. esculenta* treatment

Adults were able to live for 2-8 days. There were significant differences in daily fecundity of adults of *S. cynthia ricini* (F=12.71, dF=7, *P*<0.0001). Adult fecundity of *S. cynthia ricini* fluctuated in both treatments. The highest daily fecundity of adult of *S. cynthia ricini* when the larvae fed on *M. esculenta* leaves diet occurred on the 5th days old female, while the highest daily fecundity of adult of *S. cynthia ricini* when the larvae fed on *M. glaziovii* leaves diet occurred on the 3rd old days female (Figure 4). However, there was no difference in the total fecundity produced by the adult female of *S. cynthia ricini* in the two treatments tested (F=0.10, dF=1, *P*=0.76).

The previous study has shown a positive relationship between cocoon and pupal weight on the fecundity of *B. mori* (Singh & Kumar 1995). A high cocoon weight will produce more eggs and vice versa. However, this statement is not in accordance with the data obtained in this study. The weight of cocoons shell of *S. cynthia ricini* fed on *M. glaziovii* was higher than that of fed on *M. esculenta* leaves diet. Meanwhile, the number of eggs produced by an adult of *S. cynthia ricini* fed on *M. esculenta* was higher than that of fed on *M. glaziovii* leaves diet. Endrawati et al. (2006) said that the number of eggs produced was more influenced by the maternal effect or the nature of the adult female itself such as the body size, age, condition of the female that affects the egg and the growth and characteristics of the embryo after fertilization.

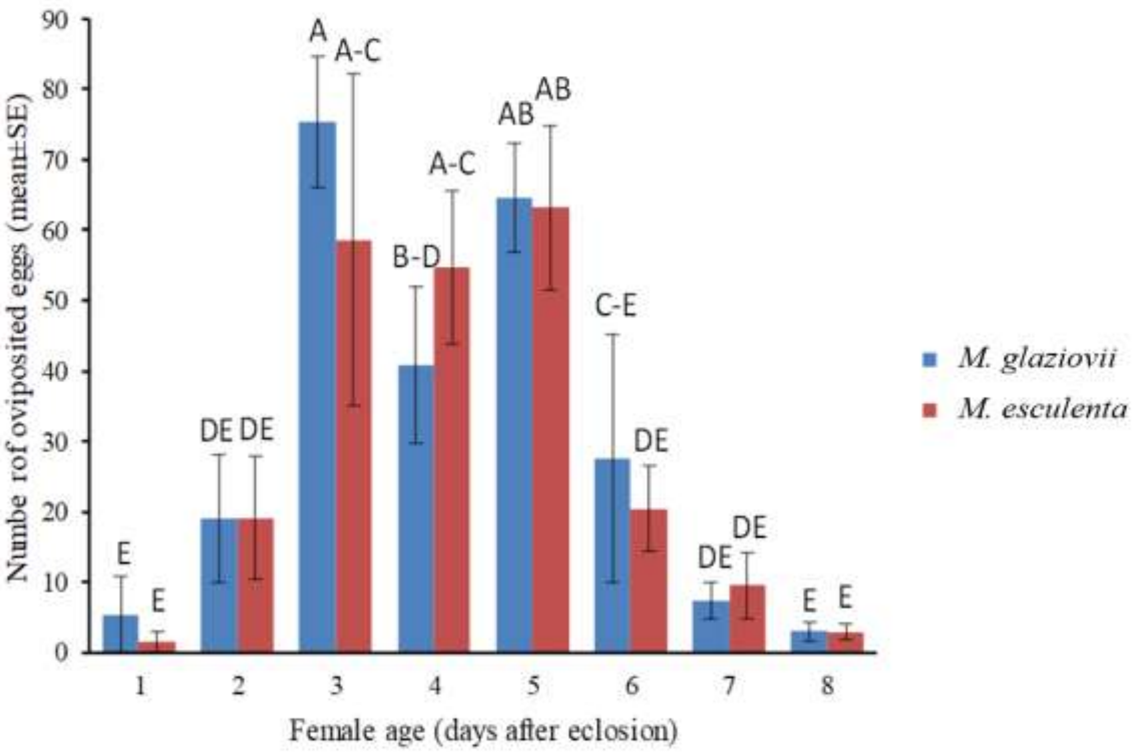

Figure 4. Daily adult fecundity of *Samia cynthia ricini* reared on *M. glaziovii* and *M. esculenta* leaves. Means followed by different letters are significantly different (Tukey HSD Test, $\alpha = 0.05$).

Lastly, in addition to the development time of *S. cynthia ricini* at certain phases, the total development time of *S. cynthia ricini* reared in the two kinds of cassava leaves diet above was also significantly different (Table 1). Differences in development time may be influenced by the characteristics of cassava itself. Each cassava species has a different nutritional content. In a study conducted by Amarullah et al. (2016), each variety of cassava has its characteristics and advantages that have an impact on the content of tubers and plants. Varieties of course will also affect the nutritional value of the leaves which may have an impact on the performance of larvae.

CONCLUSION

The use of *Manihot glaziovii* and *M. esculenta* leaves was able to support the life cycle of *S. cynthia ricini* from egg to adult completely, and *M. utilissima* is not suitable for *S. cynthia ricini* rearing. *Samia cynthia ricini* experienced a high survival rate when fed on *M. glaziovii* leaves diet, but *S. cynthia ricini* develop faster when fed on *M. esculenta* leaves diet. Moreover, *S. cynthia ricini* cocoon shell reared using *M. esculenta* leaves diet was heavier than that of *M. glaziovii*. There was no difference in adult fecundity of *S. cynthia ricini* when reared with both leaves diet. Therefore, *M. esculenta* is the best diet source for *S. cynthia ricini.*

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