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Effect of Ascorbic Acid in Artificial Diets on the Development of *Spodoptera litura*

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The growth and development of insects are based on what they consume. Vitamin C or ascorbic acid is an essential element in artificial diets that stimulates the metabolic activity during the development of *Spodoptera litura* (*S. litura*). By manipulating the amount of ascorbic acid in artificial diets, the growth and development of *S. litura* can be regulated and controlled. The present study was carried out to determine the optimum amount of ascorbic acid concentration in the artificial diet of *S. litura* and it's effects towards morphology (length & weight), larval growth index and total development index. An artificial diet containing ascorbic acid (20 g, 14 g, 7 g and 0 g) was given to *S. litura* larvae. The larval growth and development were assessed on a daily interval and were analysed by using ANOVA. The result revealed that 14 g of ascorbic acid showed the highest weight with 1.16 g ± 5.0E-02 with length of 4.15 cm ± 0.109. The total development index of 2.38 and the total larval growth index of 0.83 were significantly higher compared to the larvae population in 0 g, 7 g and 20 g of ascorbic acid. Thus, 14 g of ascorbic acid was the suitable amount to be added in artificial diet in assisting *S. litura* to grow better and healthier.

Keywords: *Spodoptera litura*; artificial diet; ascorbic acid; rearing

I. INTRODUCTION

Vegetable pests do not only damage the crops but also affect the economy as they give huge impacts on the products and cost of compensation. Because of that, insect rearing is important in order to successfully implement the pest management strategy. In research, to control the key insect pest, it is a must to investigate pest using high a number of individuals for evaluating all potential control methods. Nevertheless, mass rearing of insects is one of the important part of conducting research to achieve the best results. Polyphagous insect pest like *S. litura* has been reported to cause extensive losses to plenty of commercial crops in India, China, Pakistan and Bangladesh as it feeds on buds, flower, legume pods and cotton bolls. *S. litura* or it's common name cluster caterpillar or armyworm, is an order from Lepidoptera. The eggs cluster laid on leaves contain 100 to 300 eggs that hatch in 3 to 6 days and covered with hair scales for protections. There are six larval instars and the pupal stage survived about 12 days. The larvae of *S. litura* depend on food plants, temperature and develop to pupae stage in 15 to 21 days. Temperature, photoperiod, diet quality and quantity, rearing density, and humidity play major roles in determining the number of instars in many types of insects (Mo *et al.*, 2013) and a very important for insects as they succeed in pupation (Lee, 2010). The nutrient such as ascorbic acid plays a major role in biological characteristics of *S. litura,* as it is important to maintain the physiological functions of the insects. Ascorbic acid deficiency in the artificial diet was found not to affect the first and second instar of the larvae but the mortality of the larvae increased at the third instar when compared with the positive control (Cappeloza *et. al.*, 2005; Goggin *et al.*, 2010). As ascorbic acid

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is an essential element in artificial diets towards the growth of *S. litura,* rearing it by using different amount of ascorbic acid might produce different growth rate. Hence, this study was conducted to determine the suitable amount of ascorbic acid in the artificial diets by measuring the length, weight of the larvae, mortality and survival rate of the *S. litura* which can be a great deal of information in insect rearing. The Outcome of this study can contribute to the agricultural industry, especially on pest management control. Insect mass rearing is very crucial for expansion of efficient and appropriate pest control strategy.

II. MATERIALS AND METHOD

A. Preparation for S. litura Eggs

The eggs were collected from the Entomology Department of Malaysian Agricultural Research & Development Institute (MARDI) and they were placed at room temperature in a beaker. The eggs attached to aluminium foil were immersed in 3% Minncare for about 10 minutes in a fume cupboard for sterilisation. Next, the eggs were rinsed with running water at room temperature for about 30 minutes. The eggs were then immersed in water bath at 43.3 °C for 30 minutes. Afterwards, the eggs were drained and dried in a laminar flow cabinet. The eggs were placed into a polystyrene cup that contained prepared artificial diets. This preparation followed the Seth and Sharma recipe (2002) with a slight modification. Temperature factor was one of the challenges for hatching the eggs, as at this stage they were sensitive to temperature. The change of colour of the eggs from yellowish to dark brown indicated that they were matured and ready to hatch. Eggs were hatched after three days. 24 °C to 27 °C was the favourable temperature range for *S. litura* egg hatchability until they reached to 4th instar, pupae and adult emergence (Thakur *et al*., 2017). The eggs of *S. litura* did not successfully hatch at first, as the temperature had inadvertently become high due to an air conditioner breakdown in the laboratory. After the experiment was repeated with the temperature of 25 °C, the eggs succeed in hatching stages.

B. Preparation for Artificial Diets

In order to determine the optimum ascorbic acid required for *S. litura* growth and development, various combinations of ingredients were evaluated. The diet recipe according to Seth and Sharma's study (2002) followed with a slight modification was dependent on the ingredients' availability and MARDI's protocols (Table 1). Ingredients A were mixed and cooked until boiled with medium heat. Then, Ingredients B were added and stirred thoroughly followed by ingredients C, D, E, F and G. Then, the mixture was poured into container and left for solidification. The solidified diet was cut into 2 x 2 cm sizes and transferred into polystyrene cups and then introduced to the *S. litura* larvae. 0 g, 7 g, 14 g, and 20 g of ascorbic acid were used in the experiment as treatment groups.

Table 1. The modified ingredients of artificial diets for rearing of *S. litura*.

Ingredients	Amount
Ingredients A	
Agar	96 g
Filtered water	2500 ml
Ingredients B	
Casein	125 g
Sucrose	135 g
Wheat germ	175 g
Ingredients C	
Ice cube	1 tray = 7 cubes (200 ml)
Ingredients D	
Wesson's salt	36 g
Potassium sorbate	4 g
Cellulose	25 g
Methyl paraben	5.4 g
Ingredients E	
Linseed oil	26 ml
Fabco	0.3 g
Ingredients F	
Cabbage powder	30 g
Ingredients G	
Vitamin mix	36 g
Fumadil	0.9 g
Aeromycin	4 g
Ascorbic Acid	0 g, 7g, 14 g, 20 g (manipulated)

C. S.litura Rearing

Each cup contained only a single larva. The slices of 2 x 2 cm of artificial diets were replaced every other day. Each group contained 5 cups (n=5) and this was replicated three times. Total number of cups was 20.

D. Evaluation of S. litura Morphology and Life Cycle

The growth and larval development were assessed every day.

The parameters used in this experiment were length and weight of the larvae. The length of the larvae was measured by using Olympus SZX10 Research Gate microscope and ruler while the weight of the larvae was measured by using GR-200 analytical electronic balance. The mortality and survival rate of the larvae in completing their life cycle were evaluated using a formula given by Sreelakshmi & Mathew (2017) that are shown below. Briefly, the larval growth index was calculated by measuring how many larvae succeeded in becoming pupae over the time taken. Meanwhile, the total development index was determined by measuring the numbers of larvae that survived in becoming adult per total time taken.

$$\textit{Larval growth index:} \quad \frac{\textit{\% pupation}}{\textit{Larval period days}} \qquad (1)$$

$$\textit{Total Development index:} \quad \frac{\textit{\% survival}}{\textit{Total development period days}} \qquad (2)$$

E. Statistical Analysis

Mean of the *S. litura* larvae weight, pupal weight, and the length of the larvae from each treatment was analysed by one-way ANOVA. All data were expressed as means ± standard error of means. The data were analysed by using SPSS version 21.0.

III. RESULTS AND DISCUSSION

A. Optimum Concentration of Ascorbic Acid in the Artificial Diet

Different ascorbic acid amounts (0 g, 7 g, 14 g, and 20 g) were used to observe their effects on the morphology, mortality and successful rate of *S. litura* larvae. Five (n = 5) larvae were reared in the polystyrene cups that contained 2 x 2 cm artificial diets. Toward the end of this study, 14 g and 20 g of ascorbic acid succeeded in promoting the hatching of *S. litura* eggs until adults. 14 g of ascorbic acid bore the optimal result because all 15 larvae successfully emerged into adults. Meanwhile, in 20 g of ascorbic acid, only 6 out of 15 larvae emerged to adult. All the larvae reared in the 14 g of ascorbic acid survived and completed the life cycle. However, the larvae reared in 0 g and 7 g ascorbic acid died before the pupation stages completed which was on week 5.

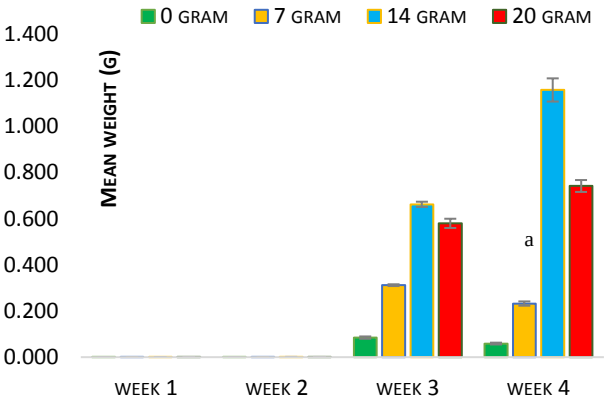

Figure 1. Effect of ascorbic acid in artificial diet on growth of *S. litura* larvae. [a] indicated significant different at $p<0.05$

From Figure 1, the mean of the larvae's weight on week 1 and week 2 did not change much. According to Goggin *et al.* (2010), ascorbic acid that derived from the parent insects made the larvae to appear normal until the second instar. All the larvae started to grow from the first instar to the second instar on week 1 and week 2. In contrast, Ohila and Asiya (2016) mentioned that the effect of ascorbic acid on the growth, development and performance of the larvae were directly seen from the first to the fifth instar period of rearing. Nevertheless, the 14 g ascorbic acid showed the highest weight of larvae. The mean difference from week 1 to week 3 was 0.66 g. Whereas the larvae that were reared in 0 g ascorbic acid showed the lowest weight compared to other diets with 0.08 g ($p<0.05$). All the larvae from week 3 in all treatment groups succeeded in developing to the next instars even though the weight of each larva was different. The larvae at the third instar had the ability to convert ingested food into growth by which they gained their weight from (Martinez & Van Emden, 1999). Next, the larvae's weight of 14 g ascorbic acid at week 4 was significantly the highest compared to other treatment which were 1.16 g ($p<0.05$) followed by 0.74 g, 0.23 g, 0.06 g on 20 g, 7 g, and 0 g ascorbic acid, respectively. Most of the larvae from 0 g and 7 g ascorbic acid died at week 4. The present study was in line with Chippendele (1975) and Carruthers's (2012) which reported that 87% of the larvae died before reaching maturity in ascorbic-free diets, thus showing that the ascorbic acid-free diet did not support the

species. Still, the 5th and 6th instars larvae were the sub-optimal instar for the *S. littoralis* development to the pupal stages, thus results would affect the mass rearing programs (Hegazi & Khafagi, 2005). It was reported that larvae reared on mulberry leaves with high concentration of ascorbic acid had an increase in overall growth of the larval tissues, which was reflected in healthier and heavier larvae (Rawgol *et al.*, 2011).

B. Length of the Spodoptera litura

Figure 2 below shows that the length of the larvae was not much different across all groups on week 1 and week 2, even though all larvae's lengths were increased. According to Sintim et al. (2009), larval period especially the first two instars was a crucial period for immature *S. litura* in which the diet utilisation, growth and development can be observed. Then, in week 3, results showed that the highest length was at 14 g ascorbic acid with 3.22 cm and was significantly higher than other diets. In the meantime, larvae reared in 0 g of ascorbic acid showed the lowest length which is only 1.43 cm. A previous study had mentioned that there was a significant difference in the development time on the third to fourth instars of *S. exigua* larvae based on the food that they consumed (Naghdi & Bandani, 2013). Moreover, larvae reared in 14 g ascorbic acid was found to be the highest length on week 4 with 4.15 cm ($p<0.05$). However, the lowest length was measured on 0 g ascorbic acid with 0.66 cm. According to Goggin *et al.* (2010) the larvae reared in ascorbic acid deficient diet showed a pathological effect when they become reduced in size, turned yellowish green in colour, started to shrivel and eventually died. This proved that manipulating concentrations of ascorbic acid also affect the length of *S. litura*.

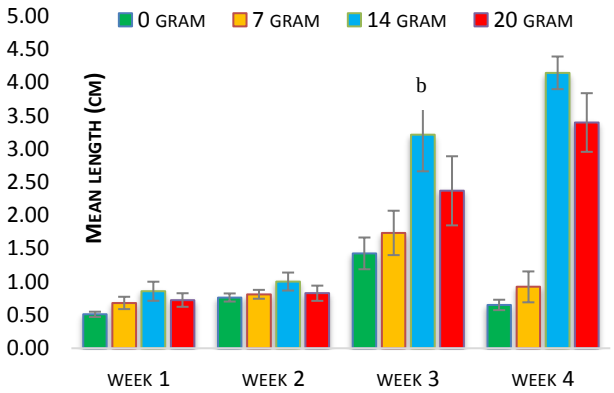

Figure 2. Effect of ascorbic acid in artificial diet on length of *S. litura* larvae. [b] significantly higher with $p<0.05$

C. Total Development Index and Larval Growth Index

Table 2 shows the larval growth index, percentage pupation and total development index for all groups tested. The larval stages took about 28 days to change from larvae into pupae, whereas to complete the life cycle it took about 42 days. All 15 larvae in 14 g of ascorbic acid survived until adults, whereas only 6 out of 15 larvae survived in 20 g of ascorbic acid. Meanwhile, the total development index for 0 g and 7 g concentrations of ascorbic acid was 0 as they died before completing the life cycle. Hence, we can assume that 14 g ascorbic acid was suitable to use for mass rearing of the *S. litura*. A study by Giongo *et al.* (2015) stated that diets that were introduced with nutritional indices and offered to fourth instar larvae until the pupal stage could verify effects on their growth and survival, thus affecting the larval growth. Hence, food sources like ascorbic acid gives effect on the survival and life cycle of *S. litura* (Silva *et al.*, 2017).

Table 2. Total development index and larval growth index of *S. litura* larvae

Amount ascorbic acid/ indices	**0 g ascorbic acid**	**7 g ascorbic acid**	**14 g ascorbic acid**	**20 g ascorbic acid**
Larval growth index (28 days)	0	0	3.57	1.42

Percentage pupation (larvae)	0/15	0/15	15/15	6/15
Total development index (42 days)	0	0	2.38	0.5

IV. CONCLUSION

In conclusion, an addition of 14 g of ascorbic acid into the artificial diets was found appropriate for the *S. litura* larvae to grow and mature until adult when compared to other treatment groups. It showed positive results in mass rearing of *S. litura* that had been carried out for 6 weeks. The weight of the larvae was the highest from week 1 till week 4 and the length of the larvae increased starting from week 1 till week 4. These results indicated that 14 g of ascorbic acid seem to affect the morphology of this pest including the weight and length of the larvae. Additionally, 14 g of ascorbic acid also improved the larval growth index and the total development index of economic importance in insects rearing. It is recommended to continue further research on DNA level in insects rearing and bioassay studies. It is also necessary to continue further research on standardisation of effective rearing techniques for mass rearing of the *S. litura*. Even though it is a pest, it is important to know the behavioural pattern of the insects in order to control their population.

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