Larval Growth of Great Mormon Butterfly (Papilio memnon memnon) Fed with Citrus aurantiifolia Leaves

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Abstract. Papilio memnon memnon is one of butterflies that has a specific host plant to lay eggs and for its larval growth, one of the host plants is a Citrus. Citrus leaf plays an important role for the growth of butterflies during the larval phase. The aim of this research is to know nutritional composition of C. aurantiifolia leaves, the growth of Papilio memnon memnon larvae which is fed with lime leaf (Citrus aurantiifolia) on Laboratory scale and other factors that influences its growth. The research method used was experiment, design of Completely Random Design with larvae feed as independent variable and larvae growth as dependent variable. The data were analyzed descriptively including body length, larvae body weight, and head capsule width followed by ANOVA and t-test. The results showed that the feeding of C. aurantiifolia leaves had no significant effect on body weight or body length of P. m memnon in all larval phases, from instar 1 to instar 5, but significantly affect the width of larval capsule cap at instar 4 and instar to 5. In the development phase, P. m. memnon larvae found symptoms of Nuclear Polyhedrosis Virus (NPV) disease that causes the death of larvae.

1. Introduction
Insects are the most numerous in number compared to other classes of Arthropod phylum. The number of identified species of insects is estimated to be close to one million, with thousands or possibly millions of other species never found and classified [1]. Among the types of insects, the best known are butterflies [2]. Butterflies have the greatest number of other insects, ranging from lowlands to high altitudes with altitude of 1500-1800 m above sea level [3]. The diversity of Indonesian butterfly species is estimated to reach 2,500 species [4]. To maintain the resources of butterfly species that exist, conservation efforts can be done with conservation strategies, one of which ex-situ preservation, namely captive breeding.

Butterflies have 3 - 4 plant species that are in the same or different family as host plants [5]. Some of these host plants have suitable chemical content for butterfly larvae such as Papilio memnon memnon larvae found in Citrus aurantiifolia plants. With the corresponding host plant, cultivation of butterflies can be done, one of them on a laboratory scale in captivity [6].

The breeding process of insect larvae in the laboratory scale must meet several requirements, among them are easy to maintain, easy to mate, and easily separated from the environment. Butterfly breeding on a laboratory scale can be accounted for because of the controlled environment and feeding [7].
host plant, whose leaves are edible for the butterflies, is a very important source for the growth and development of butterflies during its life cycle [8] (Tambaru 2015). So far, the research type of P. m. memnon associated with the growth of larvae is still very scarce and the species of Citrus host effective for the growth of P. m. memnon larvae is still not known. Therefore it is necessary to do research to know the growth of P. m. memnon larvae fed with Citrus aurantiifolia leaves at the laboratory scale in the breeding area as the basic for the management of biological resources especially for butterflies.

2. Experimental Methods
The research was conducted in the breeding laboratory of Curug Tujuh Cilember, Puncak, Cisarua, West Java, Indonesia, from June to August 2016. The tools used in this experiment includes: fine brush, petri dish, scissors, jars of larval breeds 11x11x12 cm, thermometer, hygrometer, slide bars, supporting wire, Nikon Coolpix S6300 camera, Samsung Ace 3 camera and data tabulation. Materials used include: larvae P. m. memnon feed (C. aurantiifolia leaves), cottons, gauzes, and tissues.

The experiment was conducted with experimental method of Completely Randomized Design, with the independent variable of Citrus leaf C. Arurantifolia while the dependent variable is the growth of larva, in the form of body weight, body length, and width of head capsule of P. m memnon larval. There were 5 treatments in this study, in each of the treatment, 20 larvae of P. Memnon memnon was assigned. Each Citrus leaf consists of five larval phases, so there are 100 experimental units. The instar larva 1 was obtained from P. memnon's successfully hatching eggs. Larva 1 is then prepared towards larva 2, and so on. Individual data taken were larvae that made it through the larval stage until the final instar.

Research procedure starts from the preparation phase covering six steps, namely preparation of tools and materials in reproduction laboratory, preparation of leaf sample of feed plant, proximate analysis of leaf sample of feed plant and egg sample preparation from oviposition (egg laying) offemale P. m. memnon on Citrus sp. which is inside the cage. Sample eggs were hatched in a petri dish. After the eggs hatch, the sample preparation of the larvae is starting and after that it went into the implementation stage.

Experiment stage consists of five steps, namely the first the breeding of larvae in glass container size 11x11x12 cm conditioned to be filled with air for circulation. Second, Citrus leaves as larvae feed was placed on top of the buffer wire that is in the glass container and given in the morning at around 6:00 to 7:00. Third, the measurement of larval growth begins when the larvae enter the instar larvae stage 1 to the instar larvae stage 5, the method of measuring the growth of the larvae is using a modified procedure from Helmiyetti et al[9]. Fourth is by measuring growth including body length, capsule width head and body weight of the larvae. Fifth, physical parameters were measured that affects the growth of larvae in reproduction laboratory in the temperature range of 18.9 °C - 24.2 °C and at 80% - 98% humidity.

The data were analyzed quantitatively including data on larvae body weight, larval body length, and width of head capsule of P. m larvae. memnon. Analysis of whether or not the feeding Citrus affects the growth of P. m larvae. memnon was performed using ANOVA (Analysis of Variance) and independent t-test (independent sample t-test) techniques to compare the mean values obtained from the measurements of the samples relating to each other.

3. Results and Discussions
After setting up the experiment tools, we conducted the experiment of nutritional composition of C. aurantiifolia leaves, weight of P. m. memnon larva, larval length and head capsule width, and other factors that affects the growth of the larva.

3.1. Nutritional composition of C. aurantiifolia leaves
Based on the results of proximate analysis of C. aurantiifolia leaves at the Laboratory of Research Center for Biological Resources and Biotechnology (PPSHB) Bogor Agricultural University. The
results of the feed proximate analysis contained some of the nutritional value required by the butterfly larvae (Table 1).

| Larvae feed | Composition (%) |
|-------------|-----------------|
| Leaves of *C. aurantiifolia* | Water | Carbohydrate | Crude fiber | Protein | Fat | Ash |
|             | 74.36 | 11.26 | 5.78 | 5.29 | 1.29 | 2.04 |

The nutritional value of the plant depends on its protein (nitrogen), water, and allelochemical content. Most plants have between 1% to 7% protein content [10]. Proteins are required during growth times aside carbohydrates. Proteins (nitrogen) and essential amino acids are used larvae during larval, pupation and adulthood [11].

Allelochemistry derived from plants in the form of terpenes, alkaloids, phenolics, and various proteins that can stimulate or even inhibit larvae to eat [10]. During the larval phase, feed plants play an important role. Feed plants are the place where larvae obtain essential nutrients and necessary chemicals from the larval stage to the imago [12].

### 3.2. Weight of *P. m. memnon* Larva

The growth of the larvae includes body weight, body length, and width of the larval head capsule, indicating a significant increase in body size at each instar. The range of larval body weight in every instar is shown in Table 2:

| Larvae stage | Weight range (gr) | Average ± SD |
|--------------|------------------|--------------|
| Instar 1     | 0.002 - 0.007    | 0.005 ± 0.002|
| Instar 2     | 0.025 - 0.047    | 0.035 ± 0.007|
| Instar 3     | 0.093 - 0.173    | 0.134 ± 0.031|
| Instar 4     | 0.523 - 0.695    | 0.620 ± 0.058|
| Instar 5     | 1.799 - 3.226    | 2.627 ± 0.507|

Larvae of *P. m. memnon* shows increased in body size and larval performance changes as the larvae grow. Larvae fed with *C. aurantiifolia* leaves are shown in Figure 1.
The result of t-test showed that there was no significant effect between *C. arantifolia* leaves feeding to the growth of *P. m. memnon* larvae in instar phase 1, 2, 3, 4, and 5 (p ≥ 0.05).

The general structure of the larvae can change dramatically from one instar to the next. Based on observations, the instar of *P. m. memnon* also has different morphologies. The larvae instar 1 is slightly pointed, with the color of the grayish white and dark brown bodies in the lateral part and an initial body length of about 4 mm. In instar larvae 2, the body segment is becoming more obvious. In instar larvae 3, the color of the larvae becomes dark brown to slightly yellowish green. Instar larvae 4 look like bird droppings, dark green mixed with white stripes and many spots on the head of the larvae. Instar larvae 5 change dramatically, there are 2 eye places on the third thorax segment, white band across the abdomen and a smooth green body.

The new adult insect emerging from the egg is called instar 1 [13]. The larva enters the next instar after the old outer shell changes with the new outer shell, and the larval body size will increase [10]. The body of the larvae is covered by a cuticle consisting in a mixture of protein and chitin polysaccharides. This layer becomes peeled during normal activity, thus it must be generated periodically [14].

The larval stage is a growth phase in which the body mass of larvae can reach more than 1,000 times [15]. The growth rate of larvae is strongly influenced by the nutrient quality of feed plants [10].

### 3.3. Larval length and width of head capsule

Larval length and width of head capsule during the growth *P. m. memnon* larvae for each stage is shown in Table 3 and Table 4.

| Larva stage | Length range (mm) | Average ± SD |
|-------------|-------------------|--------------|
| Instar 1     | 3.91 - 6.01       | 5.33 ± 0.69  |
| Instar 2     | 9.22 - 11.94      | 10.67 ± 1.11 |
| Instar 3     | 15.26 - 19.90     | 17.28 ± 1.67 |
| Instar 4     | 28.94 - 33.40     | 30.71 ± 1.54 |
| Instar 5     | 45.97 - 59.52     | 53.99 ± 5.02 |

The result of t-test showed that there was no significant effect between *C. arantifolia* leaves feeding to the growth of *P. m. memnon* larvae in instar phase 1, 2, 3, 4, and 5 (p ≥ 0.05).

| Larva stage | Width head capsule range (mm) | Average ± SD |
|-------------|-------------------------------|--------------|
| Instar 1    | 1.20 - 1.38                   | 1.28 ± 0.06  |
| Instar 2    | 1.60 - 1.87                   | 1.73 ± 0.10  |
| Instar 3    | 2.34 - 3.04                   | 2.64 ± 0.23  |
| Instar 4    | 3.73 - 4.18                   | 4.03 ± 0.15  |
| Instar 5    | 5.46 - 5.67                   | 5.57 ± 0.08  |

The result of statistical analysis showed no effect between feeding of *C. arantiiifolia* leaves to growth of capsule width of *P. m memnon* larvae in the instar larval phase 1, 2, and instar 3 (p ≥ 0.05). In contrast, instar 4 and instar 5 t-test results show that there is an influence between feeding of *C. arantifolia* leaves to the growth of head capsule width of *P. m memnon* larvae (p ≤ 0.05).

Towards the the final instar, the morphology of the larval body begins to change. The skin of the larvae begins to dry, looking like it will peel off and the skin tone is getting different. In the final instar, larvae prepares themselves for the pupa phase, the body size of the final instar larvae will shrink, then the pre-pupa hangs on the substrate. The last exfoliation occurs in the late 5th instar larvae. As the pre-pupa hangs on the substrate, the pre-pupa will shake its body until the outer skin of
the larvae changes with the new pupa skin. The transformation from the final phase of instar 5 larvae (pre-pupa) into the pupa phase is shown in figure 2.

![Figure 2](image)

**Figure 2.** The process of transition of the 5th instar larvae (pre-pupa) into pupa (left to right).

During the study, there were several disturbances that occurred both in the egg phase also in the larval phase. In the egg phase, a selection process was undertaken. Bad egg seeds will not be collected to proceed to the next stage. Healthy eggs are pale creamy during the first day, then the next day the color of the egg becomes dark, clear, up to one to two days before the egg is hatched [15]. The success of egg hatching is influenced by several factors such as temperature. Generally, the cooler the temperature the slower the growth rate of eggs to the larvae will be [10]. In addition to temperature, humidity is also a factor that will affect the study. If the weather is too moist it will cause mold on the eggs [16].

3.4. **Other factors affecting the growth of larvae**
In addition to physical factors, it is known there are several predators and pathogens that attack eggs, larvae, and imago of *P. m. memnon*. Predators have the characteristic of a larger body size compared to its prey, while pathogens are a group of microorganisms that live in the body of insects and cause diseases. Microorganisms that can cause disease (pathogen) in insects includes bacteria, fungi, viruses, protozoa, rickettsia and nematodes [13]. Symptoms of Nuclear Polyhedrosis Virus (NPV) disease in the larvae were found during the study, characterized by a hanging position, curved like a reversed V and ultimately causing death. Symptoms of NPV diseases is shown in figure 3.

![Figure 3](image)

**Figure 3.** Nuclear Polyhedrosis Virus Disease (NPV) in *P. m memnon* larvae

Usually the disease is not only found in the egg phase alone but the larval phase can also be exposed to bacterial and viral diseases [16]. About 40% of viruses are known to attack insects, including NPV. NPV most commonly affects insects from the Lepidoptera order (86%), and some attack the Hymenoptera order (7%) and the Diptera order about 3% [13]. NPV disease causes major mortality in larvae. The larvae infected with NPV will hang, forming an inverted V letter, melt, quickly disintegrate, and will die [17].
4. Conclusion
Nutritional composition of *C.aurantifolia* contained of water, carbohydrate, crude fiber, protein, fat and ash. *C. aurantifolia* leaf feeding had no significant effect on body weight and body length of *P. m. memnon* larvae in each instar phase of larvae (instar 1 to instar 5), but significantly affect the width of larval capsule cap at 4th instar and 5th instar. In the growth phase of *P. m. memnon* larvae, the symptoms of Nuclear Polyhedrosis Virus (NPV) disease was found.

Suggestion
Butterfly cultivation requires a well-controlled environmental conditions. Thus, factors such as physical factors (temperature and humidity) and biological factors (predators and pathogens) should be controlled as effectively as possible for growth to take place properly. In addition, it is necessary to consider the time and effect of ecdisis and diapause during the life cycle of butterflies.

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