



## Optimization of egg laying and longevity of *Hermetia illucens* L (Diptera: Stratiomyidae) with different cage shading colors

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### Abstract

The increase in economic demand for black soldier fly larvae (*Hermetia illucens*) as a biological agent for controlling organic waste is not proportional to the quantity and quality of egg production which is still low. Lack of production and population is a problem. It is necessary to carry out technology and strategies for maintaining *Hermetia illucens* in bulk. Efforts have been made to increase egg productivity by increasing mating behavior and egg weight by providing different light spectra based on *Hermetia illucens* photoreceptors. Four color shades of plastic cages were tested, no color, yellow, red and blue in order to obtain maximum egg production. This research was conducted from 1 November 2022 to 7 January 2023 at the Laboratory of Animal Physiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Indralaya. The research stages consisted of providing test animals, observing mating behavior, calculating the number of mating pairs, duration of copulation, average egg weight and life span of *Hermetia illucens*. The shading color has an effect on stimulating mating behavior and increasing egg weight. The blue shading treatment gave the best results compared to other plastic shading colors. Blue shading can stimulate mating behavior with the highest number of mating pairs of  $7.71 \pm 1.38$  with an average copulation duration of  $33.47 \pm 1.56$ . Optimum increase in egg weight by  $0.25 \pm 0.12$  but has no effect on the life span of *Hermetia illucens*.

**Keywords:** Black soldier fly (*Hermetia illucens*), Light spectrum, photoreceptors, ommatidia

### Introduction

Black soldier fly (*Hermetia illucens* Linneus) is a tropical fly species with great economic benefits for processing various by-products into insect-based products. Larvae *Hermetia illucens* can be used as a biological agent to control organic waste by converting it into body biomass that has economic value. (Anggia and Eka, 2021)<sup>[1]</sup>.

Increasing demand for larvae economy *Hermetia illucens* from the animal feed industry is not proportional to the quantity and quality of egg production which is still low. Lack of production and population is also a problem. Therefore, it is necessary to develop technology and maintenance strategies *Hermetia illucens* en masse. Efforts are being made to increase egg productivity by increasing mating behavior and egg weight by providing different light spectra (Guinti *et al.*, 2018).

One way to increase population and mass production *Hermetia illucens* is to get the optimal marriage. *Hermetia illucens* very sensitive to light and attracted to direct sunlight for mating. Artificial light also has a significant effect on mating behavior. It was reported that sunlight, and temperature have a significant influence on mating success *Hermetia illucens* and production of fertilized eggs. This is due to the relationship between temperature and color wavelength. Temperature has an influence on mating behavior, egg weight and life span *Hermetia illucens* (Sakai *et al.*, 2002).

Light has different energies and wavelengths based on its spectrum. Energy from light will provide a different temperature or heat based on the color of the light. Temperature will accelerate the reproductive maturity phase, marked by male flies that are ready to ejaculate their

sperm and female flies that are ready to be fertilized (Rijal *et al.*, 2021).

While long waves in light have propagation power so they can enter the photoreceptors *Hermetia illucens* and stimulate sex hormones for mating. Adequate and appropriate light will also help maximize mating behavior and egg weight. Therefore, a technology strategy is needed for maintenance *Hermetia illucens*.

In previous studies, black soldier fly reproduction (*Hermetia illucens*) was successfully bred in a room with artificial temperature and lighting in the form of LED lights in subtropical regions (Oonincx, 2016.). However, this can result in a waste of energy. In this study, using the intensity of natural sunlight takes advantage of the geography of Indonesia which is in the tropics with full sun exposure. Shade treatment used in the form of plastic cages. Plastic can be an alternative. Various plastics can be used and found in the market easily. Each color of plastic reflects a different color of light. Each color of light has a different wavelength. The longer the wave, the smaller the energy it contains. Light energy from high to low is infra red (IR), red, orange, yellow, green, blue, violet, ultra violet (UV) (Noorbati *et al.*, 2012).

### Material and Method

#### Animal test

The larvae are reared in a dark room using a tub until the prepupa stage. The prepupae are placed in the pupation medium in the form of palm shells. Pupae before becoming adult flies are moved into the cage for imago emergence. Newly emerged flies were separated by sex and pupation time. Flies were caught manually using a clear cup and kept

in a love cage measuring pxlxt (30 cm x 30 cm x 30 cm) with a net (nylon size 200 × 300 mm, mesh size 2 × 2 mm). 10 pairs of black soldier flies were included with a male to female ratio of 1;1

### Cage Design

A rack-shaped cage frame was made with 4 levels of steel measuring pxlxt (220 cm x 40 cm x 190 cm). For each type of artificial light, a pxlxt (210 cm x 30 cm x 30 cm) cage is used and divided into 7 sub-enclosures (mating cages) pxlxt (30 cm x 30 cm x 30 cm) which are separated by a cage divider. This is shown in Figure 5. The design of the shelter was done by cutting colored plastic according to the size of the shade using a clear solution on the roof, right, left and back of the cage. The same treatment was carried out on the four cages with different plastic colors. The cage is placed under the roof and the walls are made using UV plastic and the sides of the cage are changed. This is so that all parts of the cage get full sunlight.

### Place for Making Eggs

Place for laying black soldier fly eggs (*H. illucens*) was made using pxlxh (18 cm, 3 cm, 0.5 cm) thin strips of wood. After that, 3 pieces of thin wooden planks were arranged and given a gap with small tacks. Then the end of the wood is tied using a rubber band. The prepared wood is placed on top of the fish feed media that has been provided. Then weighed to calculate the weight of black soldier fly eggs (*H. illucens*).

### Making Attractant Media

In this study using fish feed media in the form of pellets (Beauty PP812 Premium Patee) because it is easy to obtain and commonly used. According to Anggia and Ramadhani (2021) <sup>[1]</sup>, fish feed media is in the form of pellets (Beauty PP812 Premium Patee), which is similar to commercial chicken feed given during the process of growth and development of larvae. before pupation is the most preferred substrate of the black soldier fly (*Hermetia illucens*). Fish feed is dissolved in water in a 1:1 ratio. Then the media is put in each cage. Observation Number of Black Soldier Fly Pairs (*Hermetia illucens*) Married Observation of the black soldier fly (*Hermetia illucens*) done with qualitatively describe mating behavior, count the number of mating pairs and the duration of copulation per day.

As many as 10 pairs of male and female flies with a sex ratio of 1:1 were put into each cage with a different shade color. The treatment is placed in a room exposed to sunlight. Temperature measurements were carried out using a digital thermometer HTC-1 and sunlight intensity (Lux), using a Lux meter every 09.00,12.00 and 15.00 WIB in each test cage. Calculation of the egg weight of Black Soldier Fly Eggs (*Hermetia illucens*) In each cage, a place for laying fly eggs (ovitrap) is provided, namely wooden blocks (trap) measuring (18 in length, 3 in width, 0.5 cm in height). Each ovitrap consists of 3 pieces of wood arranged and given a gap with small tacks. Then on each wood tied using a rubber band. Media for laying eggs using a substrate. The substrate used is fish feed mixed with water.

Long Life Observation Long-lived observations of the black soldier fly begin when the pupa becomes an imago. Then observing the long life of flies black soldier (*Hermetia illucens*) is done by counting and recording the number of flies that die per day, until all the flies in the cage die.

### Data Analysis

Presentation of data on the number of mating pairs, duration of copulation, egg weight, and life span of the black soldier fly are presented in the form of tables, graphs, and documentation in the form of photos and videos. Analysis of black soldier fly egg weight data (*Hermetia illucens*) statistical analysis was performed using one way- analysis of variance (ANOVA) at the 95% confidence level ( $P < 0.05$ ) using SPSS 25.0 software. If each treatment shows a significant difference, it can be continued with further tests, namely the Ducan Multiple Range Test (DMRT) to determine the effect of each treatment on the parameters observed. measurement datfaactor environment using parameters: daily average temperature (EC), using a digital humidity thermometer HTC-1, and sunlight intensity (Lux), using a lux meter LM-3000.

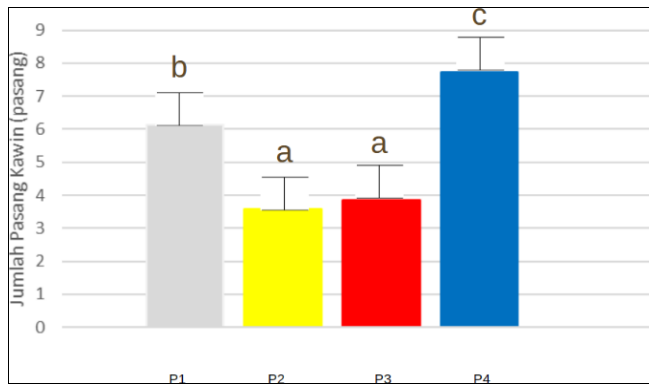
### Results And Discussion

This observation is presented in a manner Descriptively of mating behaviour test the black soldier fly (*H. illucens*) which occurred in several shelters (clear shade, yellow shade, red shade, and blue shade). Observations were made for 40 days under full sun exposure from 08.00 to 15.00 WIB. Black soldier fly mating (*Hermetia illucens*) occurs after the reproductive maturation phase. Female flies start to do flight activity, while there are other females that show no flight so they are unresponsive. The female's flight activity stimulates the male's approach response. The male approaching the female flies up, touches the female's chest with his foot and drives her partner down or towards the side wall. When falling and landing, the female stops moving her wings, while the male continues to flap the wings and how aedeagus. When landing, the male generally places himself on the female's back After landing, the male begins to perform wing fanning behavior while boarding the female. The duration of wing fanning reflects successful mating because shorter wing fanning is more successful than long ones.

After fanning his wings, the male moves backwards onto the female's body in the opposite direction trying to make sexual contact. During this process, the male touches the female's thorax with his front pair of legs, while he starts tapping her belly with the tarsi of his back legs. During the approach to sexual intercourse, the female may remain still, try to run, fly away, or dismount from the male by rapidly flapping her wings up and down to dodge. Receptive female accept position receptivity i.e. remaining still, with wings folded on ventral sides at a 60° angle, then male moves back and arches his abdomen downwards to achieve sex contact and copulation. 2Once copulation begins, the male rotates 180° in an inverted position with his head on the opposite side and makes genital contact. During copulation the female remains motionless and the male randomly performs antenna waving, moving the antennae alternately up and down, lasting synchronously for a few seconds or for a long time.

The minimum copulation duration in this study was 8 minutes and the maximum copulation duration reached 49 minutes. Copulation ends when the aedesatuguds plucks out and begins grooming activities before flying away. Mated females also show grooming activity on the ovipositor before leaving the location. This kind of behavior has been observed before by Ucu *et al.*, (2020).

**Number of Black Army Fly Mating Pairs (*Hermetia illucens*)**



**Fig 1:** Number of Black Soldier Fly Mating Pairs (*H.illucens*)

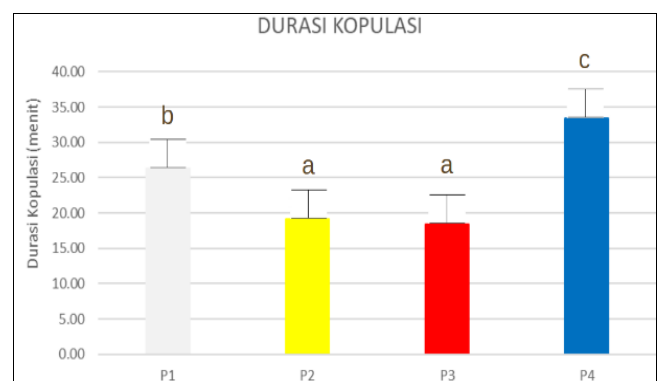
P4 (shading blue) has a more significant value, namely 7.71 pairs. This is caused by several factors, including temperature. The resulting temperatures from highest to lowest were P4 30°C, P1 34.35°C, P2 35°C and P3 36.40°C. Different colors in the shade of the cage will result in different temperatures. This is due to the temperature generated in each color of the shade of the cage which is absorbed by the black soldier fly (*Hermetia illucens*). The temperature that affects the number of mating pairs of black soldier flies (*Hermetia illucens*) ranges from 30°C-36.40°C. According to Singh *et al.*, (2018), approximately 99% of mating and oviposition occur in the temperature range of 27.5 to 37.5°C.

The temperature and light intensity are affected so by the intensity of light and dark light on the daily rhythm. Obtained temperature and the highest light intensity at 12.00 followed by 15.00 and 09.00 as the lowest temperature and intensity. The average copulation behavior in the four treatments occurred at 10.00 - 11.00 WIB, this is in accordance with previous research in April *et al.*, (2016), reported BSF mating activity generally reached its peak at 10.00. In this case, hormones are an important factor. Hormones that function in the reproductive process are sex pheromones. According to Giunty *et al.*, (2018), one of the factors that influence marriage is the behavior of male lekking which is influenced by sex pheromones. Blue light also has a light intensity of 450 nm which has the same preference as the black soldier fly photoreceptor organ (*Hermetia illucens*) and can increase the number of black soldier fly mating pairs (*Hermetia illucens*). According to Singh *et al.*, (2022), Ommatidia are the structural units of insect compound eyes and have photoreceptor cells for light waves. A suitable light source, which is between 450 nm - 700 nm wavelength, is ideal for reproductive activity in black soldier flies (*Hermetia illucens*) mature. P1 (clear shade) is the second highest average, namely 6.14. This is because the clear color is natural light for black soldier flies (*Hermetia illucens*) carry out the marriage process. Black soldier fly (*Hermetia illucens*) under the clear plastic receives a mixture of different wavelengths of sunlight. According to Heussler, *et al.*, (2018), black soldier fly reproduction (*Hermetia illucens*) occurs naturally depending on space and sunshine in sub-tropical temperate climates.

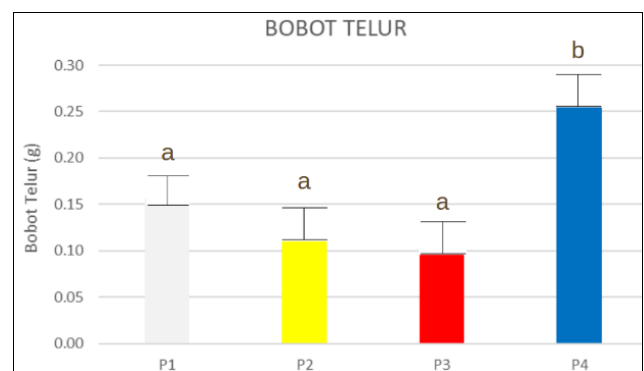
**The value of the number of mating**

pairs P2 (yellow shading) and P3 (red shading) showed the lowest average values of 3.57 and 3.85. This is because the extreme increase in temperature causes black soldier flies (*Hermetia illucens*) could not regulate the thermoregulator in his body. So that the black soldier flies (*Hermetia illucens*) runs out of energy before mating. Black soldier fly (*Hermetia illucens*) is not sensitive to yellow and red light, this is in accordance with Kluber's previous research *et al.*, (2020), based on the spectral sensitivity of the ommatidia photoreceptor compound eye of the black soldier fly (*Hermetia illucens*) can sense blue, ultraviolet, and green light which affect mating behavior.

The maturity of the reproductive organs is also an important factor affecting the number of mating pairs of black soldier flies (*Hermetia illucens*). According to Guitti *et al.*, (2018), females who avoid interaction with males and are unresponsive due to their physiological status, i.e. immature reproductive organs.



**Fig 2:** Black Soldier Fly Copulation Duration



**Fig 3:** Black Soldier Fly Egg Weight (*H. illucens*) (*H. illucens*)

P4 (blue shading) has a more significant value than the other treatments, namely 33.47 minutes. In the old copulation parameter, it is suspected that there is a lack of correlation between temperature and the duration of copulation. This is supported by the absence of reports in previous studies that there is a relationship between temperature and duration of copulation. But correlated with the number of mating pairs. This is because the more the number of mating pairs, the more time is added.

In this study, the shortest duration of copulation was only 8 minutes and the longest duration was 49 minutes, exceeding previous studies. According to Guitti *et al.*, (2018), states that the minimum duration of copulation is 20 minutes and the maximum duration of copulation is 42 minutes. fly body size *H. illucens*) male is thought to affect the duration of

copulation. In this study, the size of the black soldier fly (*Hermetia illucens*) of various males. The number of sperm produced is directly proportional to the body size of the black soldier fly (*Hermetia illucens*) male. The more sperm the black soldier fly produces (*Hermetia illucens*) male, the longer the duration of copulation. The duration of copulation will maximize the fertilization of eggs in females. According to Hariet *et al.*, (2016), Male flies that have large body sizes tend to be more fertile than those with small sizes.

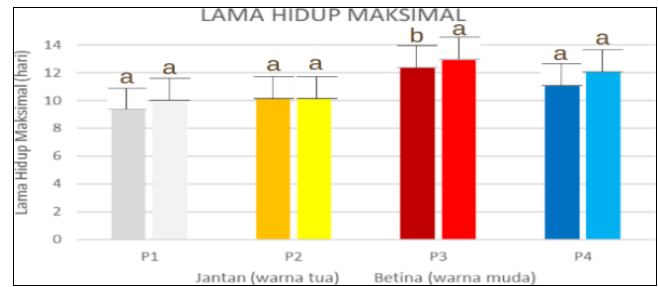
In the present study, it was found that no re-mating was recorded for any of the tested females during the observation period. Black soldier fly (*Hermetia illucens*) males and females are monogamous. Males will die after mating and females will die after laying eggs (synovigenic). This was also reported in previous studies Tomberlin *et al.*, (2002), that the black soldier fly (*Hermetia illucens*) females are reported to lay only one egg during their lifetime, after which they die.

P4 correlated with the duration of copulation seen from the most significant value of 0.25 g. In this study, the results of the appropriate correlation between copulation duration data and egg weight data were obtained. This matter caused ever copulation affect the number of sperm transferred by the black soldier fly (*Hermetia illucens*) male to female. The more sperm that are transferred, the more optimally the female egg will be fertilized. Black soldier fly body size *Hermetia illucens* female affects egg weight. According to Hariet *et al.*, (2016), The number of eggs produced by flies with large bodies is more than flies with small bodies.

Another factor that affects egg weight is temperature. The lowest average egg weight was found in treatment P3 (red shading), this was due to the effect of temperature on each treatment. The temperature measured in the shade of the red cage exceeds the optimal temperature of 36.6oC. According to Singhet *et al.*, (2022) has concluded that about 99% mating and oviposition occur at temperatures of 27.5 to 36.5°C. Temperature also plays a role in stimulating egg laying. Treatment with blue shading resulted in the highest egg weight of 0.26 g. This is due to the large number of mating fly pairs and the longest copulation duration at P4 (shaded blue). This is in accordance with Schneider's previous research (2020) [10], Blue light which has a wavelength of around 440 nm and green around 540 nm, and some of their ratios, has been shown to be very important for successful mating and egg production of black soldier fly (*Hermetia illucens*).

Oonincx *et al.*, (2016) also found that black soldier fly ommatidia (*Hermetia illucens*) contains photoreceptor cells that are sensitive to blue, green and UV light can significantly increase egg weight.

It was found that female flies only mate and lay eggs during their lifetime. It was also found that one female fly could produce more than 1 group of eggs in one oviposition indicating there was a difference in egg weight between the mating cages. In general, eggs began to be produced on the fourth or fifth data collection with the peak of egg production appearing on data collection on the 31st day reaching 0.56 g. According to Heussler *et al.*, (2018), The peak of oviposition ranges from four to eight days.



**Fig 4:** Longevity of Black Soldier Fly (*Hermetia illucens*)

The life span of females at P4 (blue shading) had a more significant value than the other treatments, namely 12.87 days. Meanwhile, the longevity of the black soldier fly (*Hermetia illucens*) The highest male) was at P3 (red shelter) which was 12.28 days followed by P4 (blue cage shelter), P2 (yellow cage shelter) then P1 (natural cage shelter) as the lowest mean value of 9.28 days.

The relationship between parameters in this study is the black soldier fly (*Hermetia illucens*) are stimulated using several colors of light in order to speed up the mating process. Each color of light will affect the temperature in the experimental cage. The optimal temperature accelerates mating. The sooner the first mating occurs, the shorter the life span of black soldier flies (*Hermetia illucens*). Male flies will die after mating, while females will die after laying eggs. This is supported by Macevei *et al.*, (2020), stated that the black soldier fly (*Hermetia illucens*) is known as a monogamous species that mate only once during its lifetime. P3 data (shading red) in males and females show no association with other parameters. This is due to the body temperature adaptation of the black soldier fly (*Hermetia illucens*) to the ambient temperature. According to Oliveira (2015), the ideal life cycle conditions for breeding are temperatures between 28-30 Celciusbut did not rule out the black soldier fly (*Hermetia illucens*) evolve and adapt to defend themselves.

One of the influencing factors is black soldier fly nutrition (*Hermetia illucens*) at the time of larvae. Wardhana *et al.*, (2016), reported that the quality of larval development media was positively correlated with larval length and the percentage of survival of adult flies. In this study, it was found that the black soldier fly (*Hermetia illucens*) females have the longest life time. This is directly proportional to P4 data (blue shading) on the number of mating pairs, P4 data (blue shading) on copulation duration, and P4 data (blue shading) on egg weight.

Black soldier fly (*Hermetia illucens*) male had the longest survival time at P3 (shading red). Apart from nutritional factors at the time of larvae, it can also be caused by black soldier flies (*Hermetia illucens*) who do not perform copulation due to extreme temperatures exceeding the optimal temperature, so that sperm release does not occur in males. This resulted in male flies having a longlife span and many individuals without partners who were unresponsive to mating, so they did not mate until they died.

This is according to Macavei *et al.*, (2020), In the tropics, the black soldier fly (*Hermetia illucens*) mate and lay eggs throughout the year, while in warm climates natural reproduction is limited to a few generations when above average temperatures prevent flies from mating. Other factors are also thought to affect male longevity at P3 (shade red) is not met with a responsive female resulting in a phenomenon called single flies. This has been reported in a previous study by Guinti *et al.*, (2018).

## Conclusion

Based on the research results that have been obtained, several conclusions are obtained as follows: stimulating mating behavior and increasing black soldier fly egg weight (*Hermetia illucens*). Blue shading treatment with an intensity of 12,000 lux and the lowest temperature is 30oC gives the best results compared to other plastic shade colors. Blue shading treatment can affect mating behavior and increase egg weight of black soldier fly imago (*Hermetia illucens*).

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