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## Effect of Feeding Frequency on Larval Metamorphosis of Black Soldier Fly (*Hermetia Illucens* L.)

KPOSSOU Nicolas<sup>1</sup> · YABI Biau Francis<sup>1</sup> · DJOSSA Agossou Bruno<sup>1</sup> · TONI Hermann Cyr<sup>1</sup> · KPOSSOU Sébastien<sup>2</sup>

<sup>1</sup>Forestry and Bioresource Conservation Research Unit (LaFCBio), school of Tropical Forestry, National University of Agriculture, BP 43, Kétou, Bénin

<sup>2</sup>Unité de Recherche en Aquaculture et Gestion de Pêche (URAGeP), Université Nationale d'Agriculture (UNA), BP 43, Kétou, Bénin.

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### Abstract

Breeding *Hermetia illucens* requires significant attention from the farmer, particularly during egg harvesting and metamorphosis phase. A crucial aspect of this breeding process is the management of feeding and the insectarium. This study aims to evaluate the influence of different feeding frequencies on molting, metamorphosis, and lifespan in black soldier flies, with the goal of optimizing rearing conditions for sustainable production. Five basins of 1125 cm<sup>2</sup> and 13,500 cm<sup>3</sup> each were randomly filled with 10,000 six-day-old larvae and 2.5 kg of feed. The basins were subjected to five feeding frequencies: T<sub>1</sub>: daily feed until metamorphosis signs appeared; T<sub>2</sub>: feeding every three days until effective metamorphosis indicators were observed; T<sub>3</sub>: daily feeding during the first week after three-day incubation; T<sub>4</sub>: weekly feeding after three days; and T<sub>5</sub>: feeding twice weekly after the incubation. Each treatment was replicated five times, and 900 samples were randomly selected and reintroduced to the basins covered with mosquito nets. The results indicated that feeding duration significantly affected the biological and physiological processes of the fly's life cycle ( $D = 0.9529$ ,  $p < 2.2e-16$ ). Statistical significance was noted across the number of pupae molting for different treatments ( $p < 0.001$ ). Insectariums for T<sub>1</sub>, T<sub>2</sub> and T<sub>5</sub> can be refilled every seven days to maintain a stable population, whereas T<sub>3</sub> and T<sub>4</sub> required refilling every four days. This study highlights the importance of feeding frequency in the development and longevity of *H. illucens*, addressing a significant gap in current research and optimizing conditions for sustainable insect farming.

**Keywords:** *Hermetia illucens*, Animal feeding, Black soldier flies, Molting, Metamorphosis

### Introduction

Animal feeding poses a significant constraint in animal husbandry, challenging the fundamental aspects of the livestock sector and hindering efforts to combat food insecurity, poverty, and job creation (Charman, 2006; Chen et al., 2020; Chen et al., 2024; Kashyap et al., 2024; Sjikwe

et al., 2024; Traore et al., 2024). Rising costs of protein sources, concerns regarding the safety of animal feed and environmental pressures exacerbate the challenges faced by livestock producers (Glencross et al., 2024; Traynor et al., 2024). Furthermore, the increasing human population and growing demand for protein are driving prices for both animal and plant-based protein sources (Nadathur et al.,

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2024; Phillips, 2024; Hadidi et al., 2024). To address these issues, various authors advocate for insect-based diets (Wardhana, 2016; Raman et al., 2022; Facey et al., 2024; Fricke et al., 2024). Among these, the black soldier fly (*Hermetia illucens*) stands out as a viable ecological solution due to its nutritional and immune-boosting properties (Wardhana, 2016; Siddiqui et al., 2024). The larvae of *H. illucens* contain 40-50% protein and essential amino acids making it a promising alternative to fish-based diets and soybean-based diets and in animal feed formulations (Diola et al., 2024; Sardi et al., 2023; Bonomini et al., 2024; Shafa et al., 2024). Additionally, these larvae are rich in antimicrobial peptides, which have been shown to enhance growth and immune responses in poultry, and positively influence the cecal flora of poultry (Gao et al., 2023; Linh et al., 2024; Szczepanik et al., 2024). The production of black soldier flies plays a critical role in mitigating pollution linked to agricultural waste (Diener et al., 2009; Gao et al., 2019; Lalander et al., 2019). This is emerging as a valuable asset in addressing global health challenges. The black soldier fly possesses a remarkable ability to effectively convert organic waste Elsayed et al. (2024) and Tirtawijaya et al. (2024), limiting the time required for specific pathogens to reach ecological conditions conducive to their development (Erickson et al., 2004; Lieberman et al., 2006; Kenis et al., 2018). Moreover, BSF production contributes significantly to climate change adaptation and mitigation (Kalogiannidis et al., 2024; Toromade et al., 2024), representing an effective strategy for ecological transition and sustainable development (Auzins et al., 2024). Insect farming requires fewer chemical inputs, leading to lower greenhouse gas emissions (Elsayed et al., 2024; Lehmad et al., 2024) and occupies less space compared to traditional agricultural practices (Rumpold & Schlüter, 2013). Its production does not require extensive resources (FAO, 2013), making it crucial for biodiversity conservation compared to conventional plant protein production (Franco et al., 2021; Gasco et al., 2024; Gliogrescu et al., 2024).

✉ YABI Biau Francis.: [francisyaabi@yahoo.fr](mailto:francisyaabi@yahoo.fr)

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Despite the recognized nutritional benefits of BSF, gaps remain in our understanding of how feeding frequency affects larval development and adult lifespan. Therefore, this study aims to; i) assess the influence of different feeding frequencies on molting and metamorphosis, and ii) assess

the impact of feeding frequencies on the adult lifespan of *H. illucens*.

## Materials and Methods

### Location and Timeline

This experiment was conducted in the municipality of Kétou, where the ambient temperature ranged from 25.4°C to 33°C and humidity levels fluctuated between 54% and 95% over a period of 41 days

### Larvae Culturing

To obtain the larvae necessary for our experiment, eggs of *Hermetia illucens* were collected from the insectariums and incubated on a substrate composed of 70% wheat bran and 30% toasted soybean flour. The eggs, laid in the interstices of stacked plates placed over attractants inside the insectarium, were collected at the start of the experiment. A thermo-hygrometer was used to record the ambient temperature and humidity at the site during the experiment. A total of 61.2 g of eggs were collected. These eggs were then placed on a substrate consisting of 2000 g of wheat bran and soybean flour for an incubation lasting 6 days. After the incubation period, 10 samples of 200 larvae each were taken and weighed and mean was calculated and extrapolated to estimate the total weight for 10,000 larvae, using a rule of three. The obtained weight was then used to select the 10,000 larvae required to inoculate each of the 25 basins in order to assess the effect of feeding frequency on the lifespan of the flies.

### Experimental design

For this experiment, five basins, each with an area of 1,125 cm<sup>2</sup> (45 cm x 25 cm) and a volume of 13,500 cm<sup>3</sup> (45 cm x 25 cm x 12 cm), were arranged randomly. Each basin received a population of 10,000 six-day-old larvae. Five treatments corresponding to different feeding frequencies were applied to these basins, with five repetitions. Thus, each treatment is represented by five basins, totaling 25 basins for the entire experiment. The larvae were kept at 26°C, the humidity was 100%, the internal oxygen level of the substrate was 0.9, and the pH was 8.7. Five treatments related to the frequency of feed refills were applied to these five basins with five replications T<sub>1</sub> = larvae were provided with daily feed until signs of successful metamorphosis appeared, at which point feeding was discontinued; T<sub>2</sub> = larvae received feed every three days until indicators of effective metamorphosis were observed, after which feeding was terminated; T<sub>3</sub> = larvae were fed daily during the first week; T<sub>4</sub> = larvae were fed once a week until signs of

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successful metamorphosis appeared; T<sub>5</sub> = larvae were fed twice a week until signs of successful metamorphosis appeared. For each treatment, 2.5 kg of feed per basin was utilized throughout the experiment. Once the larvae exhibited clear signs of successful metamorphosis, feeding was discontinued for treatments T1, T4, and T5 because adult BSF acquired all extra nutrients during the larval stage (Isa & Hassan 2021). Upon the emergence of the first flies, the remaining production residues, including pupa, were removed from each basin. Subsequently, 900 flies were randomly selected from each treatment group and reintroduced into the same basin, which was then covered with mosquito nets.

### Data collection

To measure the effect of feeding frequency on molting and metamorphosis in pupae, as well as on the biological cycle of black soldier flies (*Hermetia illucens*), the number of molting pupa was recorded throughout the experiment, which lasted 22 days. For each treatment, the daily count of molting pupa was recorded in each basin. To this end, the mean of the daily number of molting pupa was calculated for the five basins per treatment over the entire duration of the experiment. This average represents the daily number of molting pupa in a population of 900 pupa for each treatment. At the end of the experiment, the sum of the means was calculated for each treatment to estimate the total average number of molting pupae. Subsequently, this figure was extrapolated using a rule of three to estimate the number of molting pupae in a population of 10,000 larvae produced with 2,500 g of food. This 2,500 g of feed is used per basin. The choice of 900 pupae per basin is based on a power analysis, which demonstrates that a sample size of 900 pupae is adequate for the statistical tests. These pupae were selected through stratified random sampling based on their length, and distributed as follows: 300 pupae of the same age were randomly selected from the length category of 0 to 1.7 cm, 300 from the category of 1.7 cm to 2.1 cm, and 300 from the category of 2.2 cm to 3 cm. The total number of flies observed for each treatment was determined by conducting a bi-daily count (at 8 a.m. and 6 p.m.) of the dead flies recorded throughout the experiment, until the end of their biological cycle. At each count, the number of dead flies were removed from each basin to avoid any double counting between treatments. The total number of emerging flies per treatment was calculated by taking the mean of the number of dead flies counted in each of the five basins for each treatment. This average per treatment was then used to extrapolate from the initial 10,000 larvae to estimate the

number of pupae transforming into flies, considering that the larvae were fed a substrate composed of 70% wheat bran mixed with 30% soy flour, in basins with specific characteristics.

### Estimation of Retention Duration

The number of days the flies, emerging from eggs collected on the same day remain in the insectarium, depending on the type of diet, is estimated from histograms illustrating the effect of feeding frequency on the metamorphosis of flies for each of the treatments performed.

### Data Analysis

Data were organized and computed using MS Excel. Statistical analyses were conducted using R software, version 4.5.1. To evaluate the impact of feeding frequency on molting and metamorphosis, Pearson's chi-square tests was performed, analysis of variance (ANOVA) and Tukey's post hoc test, and Kruskal-Wallis's rank was conducted to compare the mean of molted pupae and emerged flies across different treatment groups. Significant levels were considered at  $p < 0.05$ .

## Result

### Effect of the feeding frequency on pupae molting

The Figure 1. Indicate that treatments T1 and T2 exhibited approximately 60 molts out of a total of 900 pupae before completing their life cycles. This pattern was not observed in other treatments, particularly T3 and T4. In all treatments, the number of pupae that began to molt started on the second day, with a count of 132. This number increased exponentially, reaching a maximum of 721 by the 11th day in T3 and T4, respectfully. In treatment T5, the molting process initiated within the first few days, also showing an exponential increase, approaching approximately 500 molts. A significant difference was observed in the mean number of molted pupae among different treatments ( $F = 15.52$ ,  $p < 0.001$ ). Conversely, significant differences were found in the molting counts between treatments T1 and T3 ( $p < 0.05$ ) and between treatments T5 and T1 ( $p < 0.05$ ). There was no significant difference in the number of pupae molting between treatments T4 and T3 ( $p > 0.23$ ), nor between treatments T1 and T4 ( $p = 0.07$ ). Importantly, the frequency of food supply significantly influenced biological and physiological processes, such as molting and metamorphosis, throughout the fly's life cycle, as demonstrated by Pearson's chi-square tests ( $r = 0.9529$ ,  $p < 0.05$ ).

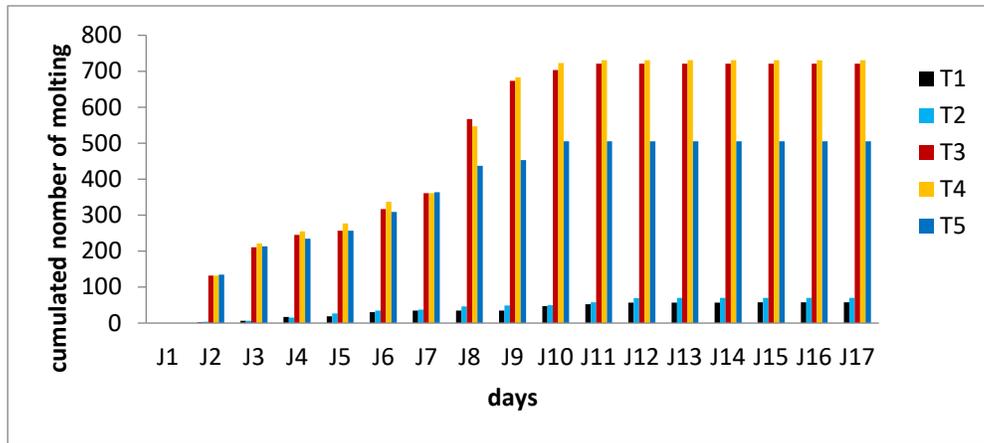


Figure 1: Cumulate Black soldier flies pupa molting by treatment

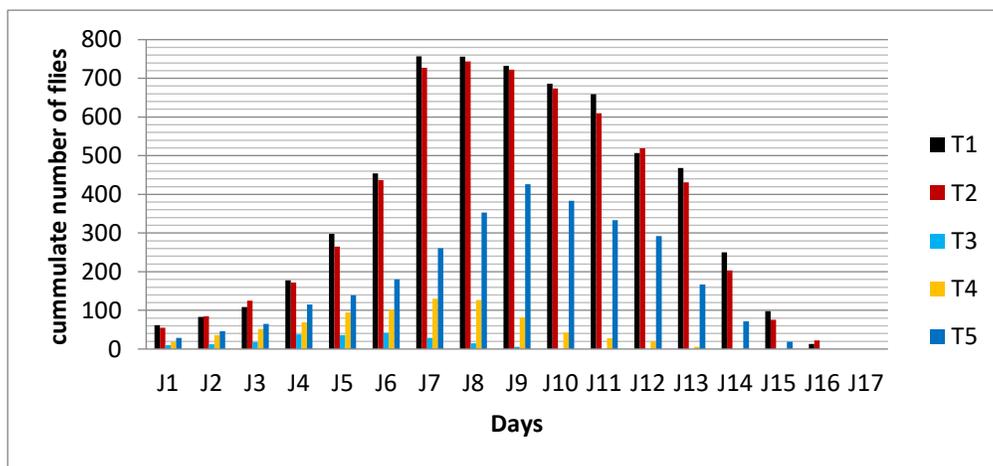


Figure 2: Cumulative population trends of flies according to the feeding frequency

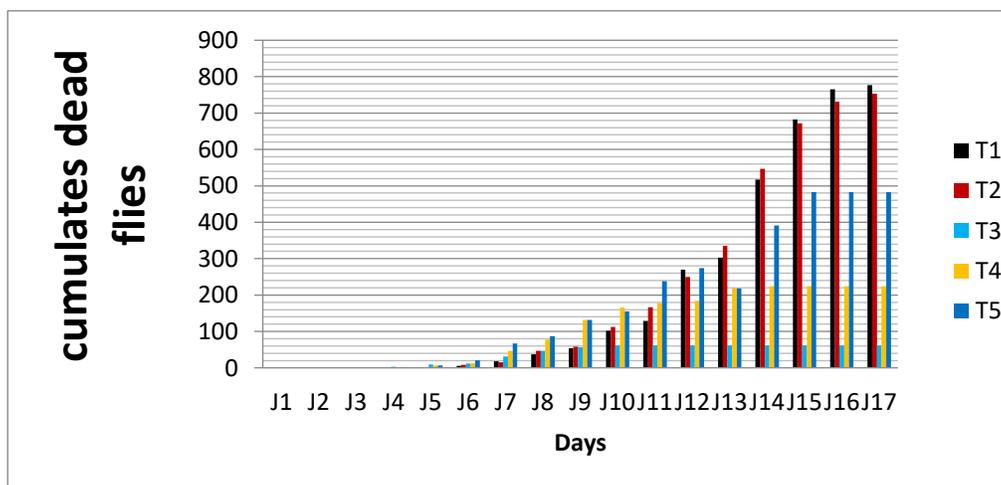


Figure 3: Cumulative mortality of flies according to feeding frequency

**Impact of feeding frequency on metamorphosis and lifespan of *H. illucens*.**

According to Figure 2, black soldier flies (BSF) were observed in the insectariums for up to 17 days for

treatments T1, T2, and T5, whereas this was not the case for treatments T4 and T3. When the feeding was distributing every day or three days over time, pupae derived from eggs collected on the same day yielded flies

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that inhabited the insectariums for an average of 16 days. In contrast, treatment T4 resulted in from occupancy of 12 days, while treatment T3 showed a shorter occupancy of 9 days. The frequency of food supply during larval rearing had a significant effect on the number of *H. illucens* pupae that successfully metamorphosed ( $F = 12.4$ ,  $p < 0.006$ ).

### Effect of Feeding Frequency on *H. illucens* Fly Emergence

A significant difference was observed in the cumulative daily number of live *Hermetia illucens* flies among the five treatments ( $F = 15.53$ ,  $p < 0.019$ ). As shown in Figure 2, the population of black soldier flies increased exponentially after their initial appearance, peaking at 757 for treatment T1 and 744 for treatment T2 after seven days. This peak remained relatively stable from days 7 to 10, followed by an exponential decline until the seventeenth day. Importantly, a statistically significant difference was found in the daily number of flies between treatments T1 and T2 ( $F = 8637.52$ ;  $p < 0.05$ ).

Pupae derived from eggs collected on the same day as they were laid did not emerge as flies on the same day, regardless of diet type. There was a significant difference in the average number of flies produced across different treatments ( $F = 12.4$ ;  $p < 0.05$ ). Most flies emerged by the 7th day for treatments T1 and T2. As seen in Figure II, pupae shells could be removed from the insectarium as early as the 10th day following the appearance of the first flies, facilitating space for treatments T1 and T2. Conversely, for those larvae fed twice a week, a waiting period of 14 days was necessary. This is supported by the observed decline in fly numbers on the 10th day, followed by an increase on the 11th day, indicating that emergence is not complete for this dietary group.

In contrast, treatment T3 showed fly presence for only 9 days, with numbers gradually increasing from 10 on the first day to 42 by the sixth day, followed by a decline until the 10th day. Additionally, larvae that were fed daily during the first week, after three days of incubation, did not accumulate the necessary nutritional reserves to fully express all genes related to organogenesis in the pupae. Thus, distributing food in a manner that allows larvae to access it once a week yields more favorable outcomes than providing all feed during the first week. However, this method still limited the number of larvae that matured into flies to no more than 4 or 5 (Figure 2).

## Discussion

### Effect of the feeding frequency on pupae molting

The adults of the black soldier fly (*Hermetia illucens*) are non-biting and non-stinging. They primarily consume water without feeding on solid food, and they do not vector or disseminate any known disease. Black soldier fly larvae (BSFL) are reported to consume a wide variety of organic materials and have been effectively utilized in small-scale waste management. Our study demonstrates that the frequency of feeding significantly influences molting, metamorphosis, and lifespan in *H. illucens*. We observed that pupae are likely to experience molting difficulties when faced with inadequate feeding and hydration conditions. When larvae were fed daily or every three days with a substrate consisting of 70% wheat bran and 30% soy flour until the 18th day, the cumulative total of pupae that molted did not exceed 70. In contrast, larvae fed exclusively during the first week or those fed once a week after a three-day incubation period exhibited a cumulative sum of molts ranging from 720 to 735 by the 17th day.

Additionally, larvae fed twice a week-four feedings of 200g each resulted in only 502 molts. The observed low number of molts in larvae fed more frequently can be attributed to their ability to store necessary nutritional reserves, which enables the expression of the species' genes without excessive molting. Therefore, a tank with an area of 1,125 cm<sup>2</sup> is sufficient for rearing approximately 10,000 larvae with a feeding regimen of 200g of the 70% wheat bran and 30% soy flour substrate every three days until the 18th day.

Interestingly, this reduced molting frequency suggests that larvae are able to accumulate reserves essential for the progression of other biological processes critical to their development. This finding highlights the importance of optimizing feeding frequency to enhance the overall productivity and viability of *H. illucens* in sustainable rearing systems.

### Effect of feeding frequency on pupae's metamorphosis and lifespan on *H. illucens*.

The larvae of *Hermetia illucens* successfully evolve to produce flies under various feeding frequencies; however, the emergence rates differ significantly between treatments. The observed decrease in the number of flies on the 10th day, followed by an increase on the 11th day for larvae fed twice a week, suggests that this feeding strategy extends the emergence time for larvae of the same age (Hans et al., 2021; Lehman et al., 2024; Grimenstein

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et al., 2024; Garriga et al., 2024). The consistent decline in fly counts each day until the 16th day, when numbers fell below 100, indicates that factors beyond mere food availability influence fly lifespan. Although the substrate was moistened prior to each feeding, the lack of water intake during later stages of larval development is a critical factor. This was especially evident when larvae fed only in the first week showed no emergence from the 9th day onward, likely due to hydration difficulties. The cumulative totals of 760, 743, and 413 flies observed for the cases where larvae were fed daily, every three days, and twice a week, respectively, highlight the significance of consistent feeding. This finding corroborates previous studies by Koné (2020), Savio et al. (2023), and Martin (2025), which emphasize that the relative humidity of the production substrate significantly impacts the life stages of *H. illucens*. Our results indicate that the strategic distribution of feed throughout the larval growth period sustains metabolic activity, facilitating the physiological maturity necessary for the expression of specific species characteristics. Notably, there are no distinct physical differences between the pupae of *H. illucens* and those of *Musca domestica* or other similar flies, underscoring the need for optimal nutritional strategies to ensure robust development.

The low fly counts noted when larvae were fed daily during the initial week or weekly until the 18th day may also be attributed to inadequate water availability. As larvae deplete their energy reserves through movement, their mobility becomes restricted. Additional factors, such as the substrate's contact area and environmental conditions—like high afternoon temperatures and low relative humidity can significantly affect water retention in the substrate. Previous studies have shown that both abiotic factors and developmental stages substantially influence the life cycle of larvae (Idris et al., 2011; Bragd, 2017; Belperio et al., 2024; Dörper et al., 2024). To further clarify these dynamics, future studies should investigate the relationship between water availability, substrate quantity, and larval activity. Understanding the interplay of these factors will enhance our knowledge of the optimal conditions necessary for effective larval production. Additionally, careful monitoring of larvae will facilitate improved management practices, ultimately simplifying the producer's workload in an industrial setting

### Effect of feeding frequency on *H. illucens* adult lifespan

The larvae feeding every day or every three days with this substrate made of 70% wheat bran and 30% soy flour until

the 18th day, allows pupae to undergo metamorphosis to transform into flies. Flies resulting from this feeding frequency for this substrate have an average lifespan of 7 days. This is not the case for cases where the larvae was feed daily during the first week and fed once a week until the 18th day after incubation. For this purpose, it is appropriate to say that this quantity of 200g of feed composed of 70% of its wheat mixed with 30% of soy flour provides 100 to 160 mg of feed per day to these 10,000 larvae after humidification when it is applied at a frequency of 3 days (Diner et al., 2009; Parra et al., 2015). In addition, this device allows keeping more larvae (8.9 larvae/cm<sup>2</sup>) than the maximum density of 6.7 larvae/cm<sup>2</sup> mentioned by Ducarme et al., (2023).

### Conclusion

The cultivation of black soldier flies represents an innovative approach that has demonstrated effectiveness in substituting protein sources in livestock feed. Gaining insights into how feeding influences their biological and physiological processes will aid in refining research initiatives focused on this species. This understanding is essential for optimizing production and guiding *H. illucens* producers in managing insectariums to enhance maggot output. Within a population of maggots of the same age, those that undergo molting after the initial emergence of flies have a significantly reduced likelihood of transforming into flies. Conversely, a consistent feeding every three days enables them to sustain metabolic activity during the critical feeding period necessary for the expression of the flies' distinctive traits. This regular feeding is beneficial for managing the aviary every 12 days, facilitating effective insectarium space management while ensuring a sufficient supply of eggs to enhance system profitability. In addition, the insectariums can be replenished every 7 days to keep the fly population relatively stable for in the case where the larvae with was daily feed until the signs of successful metamorphosis appeared, and in the conduction where the larvae received feed every three days until the indicators of effective metamorphosis were observed, after which feeding was halted as well as the cas where larvae were fed a twice per week following a three-day incubation period. Unlike the case where, the larvae were fed daily during the first week following a three-day incubation period as also the case where the larvae was feed once a week after a three-day incubation time; when require refilling every 4 days. But of all them, feeding larvae every three days until the indicators of effective metamorphosis suggested for

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insectariums frequency refill for maggots production in basin.

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### Data availability

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### Consent For Publication

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### Competing interests

*The authors declare no conflict of interest*

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