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Photoperiodic Influence on Larval Morphogenesis in Superworms (*Zophobas morio*) of the Coleoptera Order: A Tenebrionidae Investigation

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Abstract

Superworms have gained attention as an alternative protein source for animal feed due to their nutritional value and easy cultivation in organic settings. A study conducted in Sultan Kudarat State University, using in a Complete Randomize Design (CRD) with five treatments replicated four times. These treatments involved different durations of light exposure (24, 18, 12, 6, and zero hours) to investigate their impact on superworm development. The results showed that the response of superworms to light exposure varied at different stages of development. Exposure to light for 24 hours was found to be beneficial for the weight and size of pupa and adult superworms. However, it also led to higher mortality, severe deformities, and the presence of undeveloped larvae. Less light exposure resulted in significant abnormalities, but the effects were less severe compared to prolonged light exposure. In summary, the study highlights the complex relationship between light exposure and various aspects of superworm development. Understanding these dynamics could contribute to better practices for rearing superworms as a sustainable protein source for animal feed.

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Introduction

Insects are increasingly acknowledged as integral components within the realms of both alimentation and animal nutrition. Consequently, there exists a paramount imperative to unravel the nuanced implications of light exposure on their multifaceted biological facets encompassing growth, development, behavior, and overall physiological fitness. Projections from the Food and Agriculture Organization (FAO, 2019) for the year 2050 anticipate a global population surpassing 10 billion. This demographic trajectory precipitates an acute necessity to recalibrate conventional practices in land utilization and resource allocation, particularly in the context of animal feed. The strategic reduction of these resources, entailing a concomitant optimization for sustainable animal protein production, becomes pivotal for long-term viability. The exigency for an alternative and sustainable protein source, capable of meeting the escalating nutritional requisites of the burgeoning global populace, is underscored by these circumstances. Insects, emerging as a prospective solution, warrant heightened attention in this pursuit. Simultaneously, the animal feed industry is actively engaged in the quest for avant-garde protein sources to alleviate the prevailing dependence on conventional constituents such as soy and fishmeal in livestock production. This imperative shift aligns with broader objectives aimed at enhancing the ecological sustainability and efficiency of the animal protein supply chain.

Several insect species have been identified as alternative protein sources to be included in human food and animal feed over the last decade due to their numerous benefits (Van Huis, 2013). The superworm, or giant mealworm, is an insect species with great potential as both food and feed that has been overlooked by researchers and insect producers (Coleoptera; Tenebrionidae). The species *Zophobas morio* belongs to the order Coleoptera, which includes beetles known as dark beetles in the case of this species (Kulma *et al.*, 2020). It is commonly raised as a food source for birds, reptiles, and fish. As food, along with giant mealworms of *Tenebrio molitor*, superworms are commonly found in the pet animal industry. Humans can consume superworms as "chicha-worms" – crunchy treats packaged as "exotic" treats, considered one of the delicacies in Bohol, Philippines.

Due to their preference on the environments, *Zophobas morio* requires additional lighting during rearing. The aims to investigate the effects of varying light exposure on the larval morphism of superworms. The objectives of the study are to ascertain: The effect of different light exposures on the number of days required for pupation into an adult. The effect of different light exposures on the mass and size of pupa and adult. The occurrence of deformities in adults. The survival and mortality rates of superworms affected by different light exposures. The number of undeveloped larvae under five light treatments.

Material and methods

The investigation was conducted in Entomology Laboratory, Sultan Kudarat Kudarat State University, employing a Completely Randomized Design (CRD). The experimental framework comprised five distinct treatments, rigorously replicated four times, with a precise allocation of 50 larvae per treatment. To ensure methodological precision, each larva was individually placed in dedicated containers, facilitating meticulous control over experimental conditions. The treatments spanned diverse durations of light exposure: 24 hours, 12 hours, 8 hours, and 6 hours, complemented by a control group devoid of light exposure. This approach adheres to rigorous standards, emphasizing precision in design and execution for a nuanced exploration of the effects of varying light exposure on larval development.

All larvae of *Zophobas morio* were purchased from Polomolok, South Cotabato. Upon arrival, the larvae were placed at room temperature to acclimate to their new environment, which plays an important role in their growth and development. They were also provided with Integra 1000 as a feed substrate, along with chayote to serve as a source of moisture, in order to minimize cannibalistic behavior. A total of 200 superworms were utilized in the study, with 50 superworms per treatment, and replication carried out four times. The experiment employed isolation cups, cut cartoons as receptacles, and a lighting source setup. The lighting was controlled by placing a 5-watt white LED bulb, emitting 455 lumens, at a height of 2.5 meters in the room, ensuring consistent exposure for all the larvae used. After one week or less, if the larvae were ready to pupate (2 inches or more in length), they were carefully handpicked to prevent damage from the mixed feed and then isolated. Each larva was individually placed in rearing cages and subjected to five treatments: 24 hours of light, 18 hours of light, 12 hours of light, 6 hours of light, and no light. After the completion of each light treatment, the individual container was covered. Each rearing cage was monitored daily, and any changes were recorded. The larvae were reared until pupation and the adult phase. Pupae duration, as well as the survival and mortality of newly emerging beetles, were diligently recorded.

Data gathered

Days to morphism

Assessment for metamorphosis stages was done such as defining adult emergence as the moment when an adult beetle fully extends its wings and becomes actively mobile. A record-keeping to log daily observations, noting, for instance, the day an adult beetle emerges from the pupal stage.

Mass (grams) of pupa and adult

Record the weight of pupae just before adult emergence for accurate comparisons. The insects are measured in the same life stage and at the same time of day.

Sizes of pupa and adult (millimeters)

Employ a calibrated digital caliper to measure sizes consistently, like measuring the length from the head to the ovipositor. Take measured when insects are in a natural, extended position, e.g., measuring the length of an adult beetle when its wings are fully expanded.

Survival rates and mortality rates

Assessment was done daily counting, noting survived and dead insects. For example, count the number of surviving larvae each morning. Record environmental factors influencing survival, e.g., temperature fluctuations or any changes in food availability.

Deformities of adult

A standardized checklist for identifying deformities, such as misshapen wings or abnormalities in leg structure. Use imaging technology, like a microscope, to capture and analyze deformities for more precise documentation.

Fail to morph

Assessment was done by identifying larvae that fail to morph, for instance, larvae showing no signs of pupation by a specified day. Regularly inspect and document the status of each larva, noting instances where larvae remain in the larval stage beyond the expected morphing period.

Statistical analysis

All collected data were undergo statistical analysis using One-Way Analysis of Variance (ANOVA) in a Completely Randomized Design (CRD). A test for significant differences among treatment means were conducted using the Statistical Tool for Agricultural Research (STAR)

Result and discussion

Days of morphism from pre-pupa, pupa and adult

During the 30-day observational period, the majority of larvae undergoing metamorphosis into pre-pupae exhibited no significant interactions across different light exposures ($F=0.70$, $Pr > 0.05$). Notably, larvae subjected to 24 hours of light exposure recorded the lowest mean of 8.25, followed by those in complete darkness (0), and 12, 18, and 6 hours of light exposure, with mean values ranging from 9.50 to 10.50. The initiation of pupation in pre-pupae was observed to commence with the lowest average of 4.50 days under no light exposure, followed by 12, 24, 18, and 6 hours of light exposure, with mean durations of 4.75, 5.0, and 5.25 days, respectively.

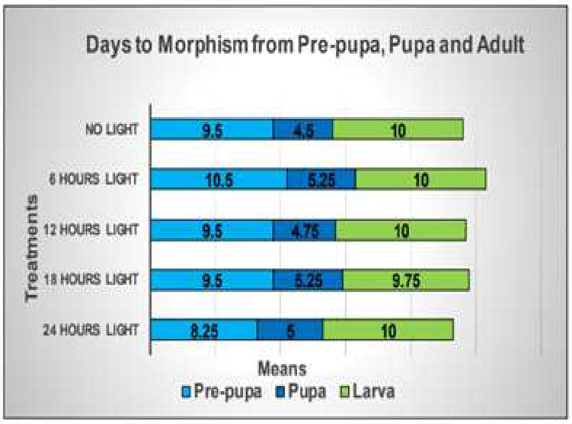

Fig. 1. Development period of Superworm morphism as influenced by different light exposure.

The pupation process, however, exhibited no significant variation when subjected to different light conditions, as indicated by a non-significant (F=1.21, Pr > 0.05). Analysis of superworm larvae exposed to no light unveiled the lowest weighted mean, signifying an accelerated development time from pre-pupa to pupa compared to the other five treatments.

These findings corroborate prior research by Ferdousi and Sultana (2021), which asserted that black soldier fly pre-pupae pupate more rapidly under 0 hours of light compared to alternative conditions. Conversely, the study by Holmes *et al.* (2017) presented divergent findings, asserting that Black Soldier Flies exhibit the shortest pupation duration in 12 hours of light exposure and the longest duration under no light exposure.

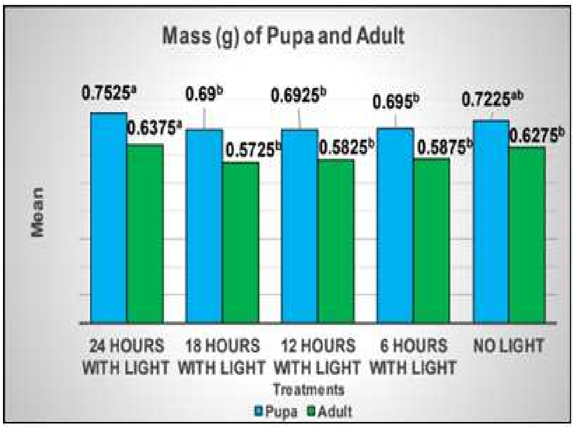

Fig. 2. Mean of pupa and adult mass (grams) during development of Superworm at 24, 18, 12, 6 and 0 hours of light.

Irrespective of light exposure, the mean duration for adult emergence from pupa was consistent at 10 days (F=1.00, Pr > 0.05). Notably, superworms exposed to 18 hours of light displayed a mean emergence time of 9.7 days, indicating the fastest development among the various light exposures. Despite numerical comparability, no statistically significant differences were observed. In line with this, Holmes *et al.* (2017) contended that the emergence percentage of Black Soldier Fly adults (Coleoptera: Scolytidae) is notably influenced by light, with a higher emergence percentage observed under no light conditions compared to 12 hours of light exposure (Figure 1).

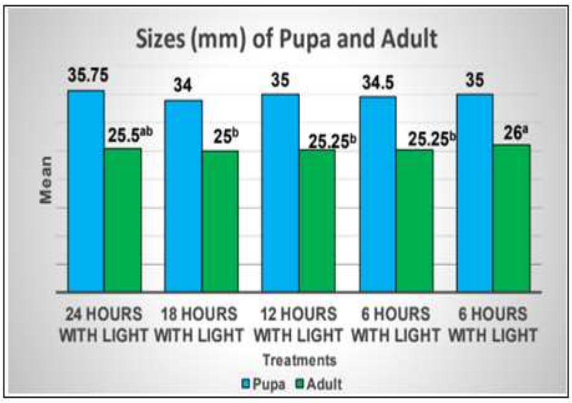

Fig. 3. Mean of pupa and adult size (mm) during development of Superworm at 24, 18, 12, 6 and 0 hours of light.

Mass (grams) of pupa and adult

The final mass had a significant impact on the pupa (F= 4.12, Pr < 0.05). The weighted mean of 24 hours of light exposure and no light was comparable, with means of 0.75 and 0.72, respectively. Eighteen hours with light, 12 hours with light, and 6 hours with light showed comparable means of 0.69, 0.69, 0.69, and 0.70, respectively. The mass of newly emerged adults under 24 hours of light exposure was significantly higher than the mass from other periods of light exposure (F-value of 0.00 and Pr < 0.05). Twenty-four hours of light exposure recorded the lowest mean of 0.85 during the larval phase, increasing its weight throughout the pupa (0.75) and adult (0.63) stages. On the other hand, 12 hours of light exposure had the highest mean of 2.88 during the larval stage but experienced declines, reducing its pupa (0.69) and adult (0.58) weights (Figure 2). According to a study conducted by Koojiman *et al.* (2015), increasing the daily hours of light exposure in mice elevated body adiposity by decreasing brown adipose tissue (BAT) activity, a crucial contributor to energy expenditure. Similarly, Molcan *et al.* (2019) found that exposure to unnatural light cycles is increasingly associated with obesity and metabolic syndrome. While no research has directly evaluated the effect of constant light exposure on the weight gain of insect pupae, it is feasible that the findings from these studies can be generalized to insects.

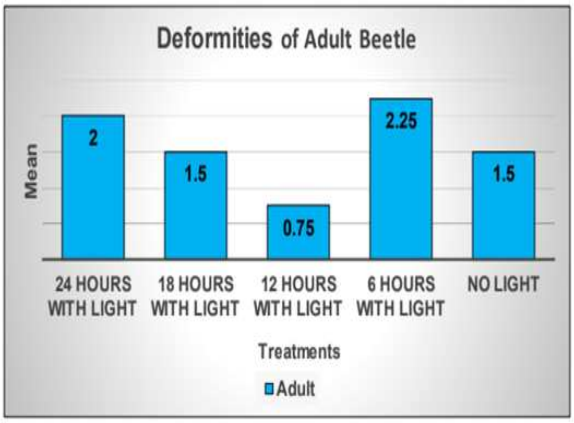

Fig. 4. Mean of adult deformities of Superworm expose at different light Exposure.

Therefore, persistent light exposure can lead to considerable weight gain in insect pupae. Coomans, *et al.* (2013) also subjected C57Bl/6J mice to an LL or an LD cycle as a control. In vivo recordings revealed that LL caused a rapid drop in SCN amplitude, which stabilized at 44% after three days. Furthermore, LL increased weight growth even before the high-fat diet did. This suggests that when rearing superworms and aiming for desired weight, it is advantageous to use 24-hour light exposure. However, health implications may arise due to disrupting the circadian rhythm.

Sizes (mm) of pupa and adult

The size of the pupa revealed no significant difference (F=1.85, Pr > 0.05). Across all treatments, 24 hours of light revealed the highest mean of 35.75, followed by no light and 12 hours of light exposure with a mean of 35, followed by 6 hours of light exposure with a mean of 34.50, and 18 hours of light exposure revealed the lowest mean of 34. The mean adult size differs from the pupa mean size, showing a significant influence with an F-value of 3.45 and a Pr value < 0.05. No light and 24 hours of exposure had comparable means of 26 and 25.50, respectively, whereas 12, 6, and 18 hours had mean values of 25.25, 25.5, and 25, respectively. Consequently, when rearing the superworm, it was much more advantageous to rear them in 24-hour light when they morphed into pupa and provide no light during the adult phase to obtain the desired size (Figure 3).

Deformities of adult beetle

The deformities observed in adults exhibited no statistically significant differences (F= 2.65, Pr > 0.05). However, numerically, the highest mean of 2.25 was evident after 6 hours of light exposure, followed by 24 hours of light exposure, 18 hours of light exposure, and no light exposure, and 12 hours of light exposure (Figure 4) with means of 2, 1.5, 1.5, and 0.75, respectively. Twelve hours of light exposure in adult deformities were predominantly observed on their wings, resulting in the exposure of their hindwings and underdeveloped elytra. Adult wing deformities were commonly noted and reported at 6 hours of light exposure, followed by 24 hours of light exposure. At the 24-hour mark of exposure, wing abnormalities were less distorted.

Fig. 5. Deformities of adult super worm. (a)1st deformed wings of adult beetles during 18th day; (b,c) deformed wings of adult beetle at 24 hours light; (d) deformed wings of adult beetle at 6 hours light.

The severity of deformities after 24 hours of light exposure was substantially higher than after 6 hours of light exposure, which had the greatest number of deformities. The findings of the present study align with the research conducted by Fan *et al.* (2019), which investigated the impact of light exposure duration on the development and morphology of *Tenebrio molitor*, a yellow mealworm. Reduced light had a significant effect on both adult beetle development and morphology. Beetles exposed to the least amount of light (8 hours per day) exhibited a high number of abnormalities, such as shorter antennae and wings and a reduced body size. Furthermore, these beetles took longer to mature into adults compared to other beetle types. This suggests that adult abnormalities are more prevalent when exposed to 6 hours of light.

Fig. 6. Survival and mortality rates of superworm morphism under 5 different treatments (24, 18, 12, 6 and 0 Hour of light).

Survival and mortality rates

Survival and mortality rates among superworms were investigated under five distinct light exposures, revealing no significant impact on their mortality and survival rates, F=2.89, Pr > 0.05 for both (Figure 6). Over a 30-day period, superworms exposed to varying light conditions consistently exhibited high survival rates, with mortality rates remaining below 5%. Specifically, 18 hours and 6 hours of light exposure yielded the highest survival rate at 10.00, followed by 12 hours of light exposure and no light with a mean of 9.75, while 24 hours showed the lowest survival rate at 9.00. Notably, there were no recorded mortality rates at 18 hours and 6 hours of light exposure. Conversely, 12 hours of light exposure and no light had a mortality mean of 0.25, and 24 hours had the highest mortality rate with a mean of 1.0. Furthermore, the optimal light exposure for the survivability of superworms was identified as 18 hours. In contrast, 24 hours of light exposure exhibited the least favorable outcomes. Additionally, Bes *et al.* (2018) subjected arthropods to varying light durations during a 5-day observation period, revealing that prolonged exposure (more than 12 hours per day) adversely affected survival rates. While scrutinizing deceased larvae samples under a stereoscope (Figure 6), a few mites and eggs were uncovered, prompting doubts about the role of light exposure in insect mortality. Posited that insect mortality could be ascribed to parasites rather than light exposure, suggesting an increase in prevalence and intensity in response to light. They cautioned against overemphasizing the influence of light exposure on insect mortality based on past studies, emphasizing the imperative for further research to better comprehend the role of parasites in insect population dynamics. This implies that, depending on insect requirements, both light and darkness are essential for survival and development, and additional factors may contribute to insect mortality rates, necessitating further investigation.

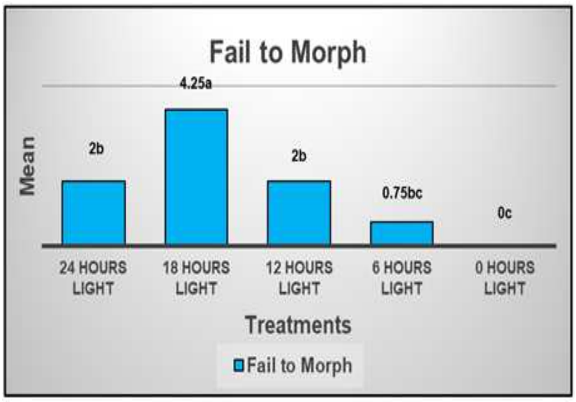

Fig. 7. Unsuccessful Morph of superworm morphism.

Unsuccessful morphs

The analysis of unsuccessful morphs observed over a 30-day period reveals a notable impact on the larval development (F= 6.13, Pr < 0.05). Notably, the highest mean of underdeveloped superworms, recorded at 4.25, occurred after 18 hours of light exposure. This was followed by 24 hours of light exposure and 12 hours of light exposure, which had means of 2.00 and 0.75, respectively. In contrast, 6 hours of light exposure resulted in a lower mean of 0.75, and no light exposure demonstrated a 100% morphism rate with a mean of 0 (Figure 7).

Wise's (2007) findings align with our observations, as reported tadpoles did not undergo metamorphosis when exposed to white light. This suggests a potential suppression of the hormone responsible for metamorphosis or a disruption in the metabolic pathway of the metamorphosis hormone. However, Xu *et.al* (2020) study on *Portunus trituberculatus* contradicts this, revealing that constant darkness prevented metamorphosis, resulting in larval mortality in various stages. The observed variations in the response to light exposure among superworm larvae, especially during diapause, suggest that the treatment at 24 hours may induce diapause and subsequent development. This highlights the species-specific nature of responses to external factors, particularly during critical developmental stages such as diapause.

Conclusion

In conclusion, Investigation into the impact of light exposure on different developmental stages of superworms has revealed varied responses. Prolonged exposure to light over a 24-hour period demonstrated positive outcomes in terms of size and weight, particularly in the pupa and adult stages. However, this benefit comes at the cost of disrupting the circadian rhythm, potentially leading to adverse consequences such as extreme obesity and associated health issues. Conversely, limited light exposure resulted in increased abnormalities, albeit less severe than those observed with prolonged light exposure, and a higher incidence of mortality among undeveloped larvae. While the findings advocate for the consideration of light exposure, especially the 24-hour cycle, in commercial superworm production to enhance size and weight during the pupa stage, the economic feasibility of continuous 24-hour light usage raises concerns.

Recommendations (S)

Therefore, exercising caution in implementing constant 24-hour light exposure due to its potential economic impracticality and the associated risks of abnormalities and mortality. Ongoing monitoring of these adverse effects is crucial for producers utilizing extended light exposure. Moreover, future research should delve deeper into the impact of light exposure on insect growth, particularly in the context of dark-loving species like the superworm. Investigation into overlooked factors contributing to mortality, diapause, and abnormalities is essential for a comprehensive understanding and improved cultivation practices in commercial superworm production.

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