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Suitability of Different Ovipositional Trap for Black Soldier Fly (*Hermetia illucens*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The Black Soldier Fly (*Hermetia illucens*) has garnered significant attention as a sustainable and nutrient-rich option for poultry feed, due to its high protein and fat content. This study assesses the oviposition preference of female black soldier flies among different ovipositional traps like cardboard, wood, corrugated plastic tubes, and an outside area within a controlled environment.

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Employing a completely randomized block design, we assessed a range of parameters, including ovipositional trap selection, oviposition duration, egg count, fertility, offspring development, and mortality rate. The findings revealed that plastic tubes were the most favoured ovipositional trap, attracting 142.67 female visits, with an impressive average egg-laying duration of 13 minutes and an egg fertility rate of 85.33%. Such preference may be due to the optimal microenvironment plastic material provides for oviposition of the Soldier fly females. Cardboard traps garnered 122.67 visits, with a notable 10.67-minute egg-laying duration and a fertility rate of 72.67%. Interestingly, wood traps were the least attractive, with only 52.33 visits, the shortest egg-laying duration (6 minutes), and a fertility rate of 33.67%. These results demonstrate the significant role that material choice plays in the reproductive success of *Hermetia illucens*. The corrugated plastic tubes are the best ovipositional traps for the efficient mass-rearing of black soldier flies for both research and commercial requirements.

Keywords: Black soldier fly; Hermetia illucens; ovipositional traps; oviposition; insect rearing; egg fertility.

1. INTRODUCTION

The black soldier fly (BSF), *Hermetia illucens* (L.), can be found across tropical and warm temperate regions globally [1]. The BSF originates from the Americas and is prevalent across a vast area extending from Argentina to the central USA, spanning tropical and temperate zones [2]. This insect is primarily located near or within areas such as cattle sheds, manure sheds, refuse sites and food waste disposal areas [3]. These insects transported by human activity, have successfully colonized regions including Australia, India, Africa, and Europe, and their ability to withstand various environmental conditions such as light, temperature, and humidity [4]. The BSF larvae effectively break down organic matter which makes them useful for converting organic waste into valuable fertilizer. Additionally, prepupae of the BSF are suitable as feed for various animals, including fish and pigs [5,6]. The BSF has two important roles in the ecosystem, one is organic waste management and another is a potential source of valuable nutrition. Over the past few years, edible insects have garnered significant interest as a viable protein source for both humans and livestock, owing to their abundant nutritional value and minimal environmental impact. To mass produce the BSF population, both for research and commercial purposes, it is essential to employ rearing methods that mimic their natural environment. In this context understanding the most favourable conditions for mass rearing of BSF is very important. The ovipositional site is playing a significant role in the BSF life cycle and colony maintenance. This fly is predominantly located near and within structures such as cattle sheds, manure sheds, disposal sites for living waste, and disposal sites for food waste. The presence of organic matter at oviposition sites played a crucial role in attracting gravid female black soldier flies. Gravid black soldier flies engage in scattering behaviour for two potential reasons. One reason is to lay eggs at locations with a narrow separation between them, while the other reason is to deposit eggs at sites with the presence of organic material. When the specified conditions are not fulfilled, the gravid black soldier fly refrains from laying eggs [3]. To establish effective rearing techniques, understanding the oviposition behavior of black soldier flies is crucial for improving egg collection efficiency and enabling year-round rearing. This study examines female preference for various ovipositional trap during the post-mating oviposition period in artificial rearing conditions, providing valuable insights for researchers in this field.

2. MATERIALS AND METHODS

2.1 Rearing Conditions and Experimental Design

The BSFs utilized in this research were sourced from a colony raised in a fruit waste substrate during their larval stage. The observation cage was 75x75x75 cm, rearing cage and substrate were placed inside the cage to provide light and dark condition for 12hrs. In this research three types of ovipositional traps *viz*., Cardboard, wood, and corrugated plastic tubes were placed in the insect-rearing cage. Each was replicated four times. Interestingly the outer side of the substrate was also considered as one of the oviposition sites. Each ovipositional trap was approximately 10 cm in length, uniform thickness for cardboard and wood around 5 cm. They were secured with rubber bands or banana fiber thread and which was positioned in the corner of a tray. These containers were filled with a mixture of fruit waste and pineapple or dried fish (maintaining 60 to 70% humidity), serving as the best attractant to induce oviposition in the females. The container of fruit waste was placed in the center of the cage (Fig. 1).

2.2 Conditions for Selection of Effective Ovipositional Traps for BSF

2.2.1 Egg-laying duration of BSF

The duration taken by female insects to lay eggs on each variant of the ovipositional trap was taken. Three days old BSF, with an average of 150 females were used and replicated four times for each ovitrap. The observations were taken between 11 AM and 3 PM. The quantity and number of eggs laid in each type of ovipositional traps were counted. The eggs captured were identified based on their physical characteristics and compared to the typical features of BSF eggs [7].

2.2.2 Assessment of ovipositional traps Inclination

The preferred ovipositional trap as identified by assessing the number of females visiting and engaging in the egg-laying process (oviposition) on each type of ovipositional traps as well as outside the ovipositional traps [8]. The egg clutch count was taken two days after releasing the BSF onto the ovitrap.

2.2.3 Fruitfulness of Female BSF

The quantity of eggs that were effectively fertilized and subsequently developed into larvae was counted. Female black soldier flies mate once and have a single oviposition event in their lifetime. After mating, they selectively lay between 320 to 620 eggs in dry crevices near a moist food source, typically about two days after successful copulation [9]. Studying the egg-laying capacity of the BSF with the respect to the type of ovipositional traps is important for maintaining BSF in large quantities throughout the year.

2.2.4 Assessment of environmental conditions

To assess the impact of environmental factors on the oviposition preference behaviour of the BSF, we recorded the daily mean temperature (°C), relative humidity (%), and light intensity (Lux) in the rearing cage at hourly intervals from 06:00 to 18:00. A digital room thermo-hygrometer (HTC-1, China) was used to measure temperature and humidity, while a Lux meter (LX-101A, India) recorded light intensity. Additionally, an infrared thermometer (IR-580MC, Chaina) was utilized to monitor the internal temperature within the BSF ovipositional traps.

Fig. 1. Ovipositional preference studies of black soldier fly on different ovipositional traps

2.3 Statistical Analysis

Mean differences of the treatment were evaluated with ANOVA at a significant level ($P < 0.05$), and means were compared by Duncan's Multiple Range Test [10]. All statistical analyses were performed using SPSS 25.0. The percentage data (Egg fertility) is used to the arc sign transformation.

3. RESULTS AND DISCUSSION

Based on the study, the preference and reproductive success of the Black Soldier Fly (*Hermetia illucens*) were evaluated using various ovipositional traps, including cardboard, wood, corrugated plastic tubes, and an outside area of the cage for collecting egg clutches. The results indicated that the total number of female visits varied significantly across different trap types. Corrugated plastic tubes received the highest number of visits (142.67), possibly due to the smooth surface and optimal microenvironment they provided, making them more appealing for egg-laying. Cardboard traps had the second highest number of visits (122.67), likely because their textured surface offered a more natural ovipositional trap for oviposition. The outside area attracted 111.33 visits, which might be attributed to the variety of environmental cues available in this setting. In contrast, wood traps had the fewest visits (52.33), possibly due to their less favorable surface texture or scent (Fig. 2).

Interestingly, despite the lower number of visits, Julita et al. [8] reported that the highest number of eggs were found in the wooden ovipositional traps (9,262 eggs), while the plastic ovipositional traps recorded the lowest number of eggs (2,742 eggs). This discrepancy suggests that factors other than surface preference, such as the material properties affecting egg adhesion or moisture retention, may play a significant role in reproductive success.

The study revealed significant differences in the mean egg-laying duration across various ovipositional traps for the Black Soldier Fly (*Hermetia illucens*). The longest duration was observed in plastic tubes (13 minutes), suggesting that the material may facilitate extended periods of egg-laying, potentially due to better support and stability (Fig. 3). Cardboard traps had a mean duration of 10.67 minutes, while the outside area showed 9 minutes. Wood traps had the shortest mean duration (6 minutes), possibly indicating discomfort or a lack of suitable features for prolonged egg-laying. These findings align with previous observations by Ewusie et al. [11], who noted that egg-laying conditions could significantly influence reproductive success.

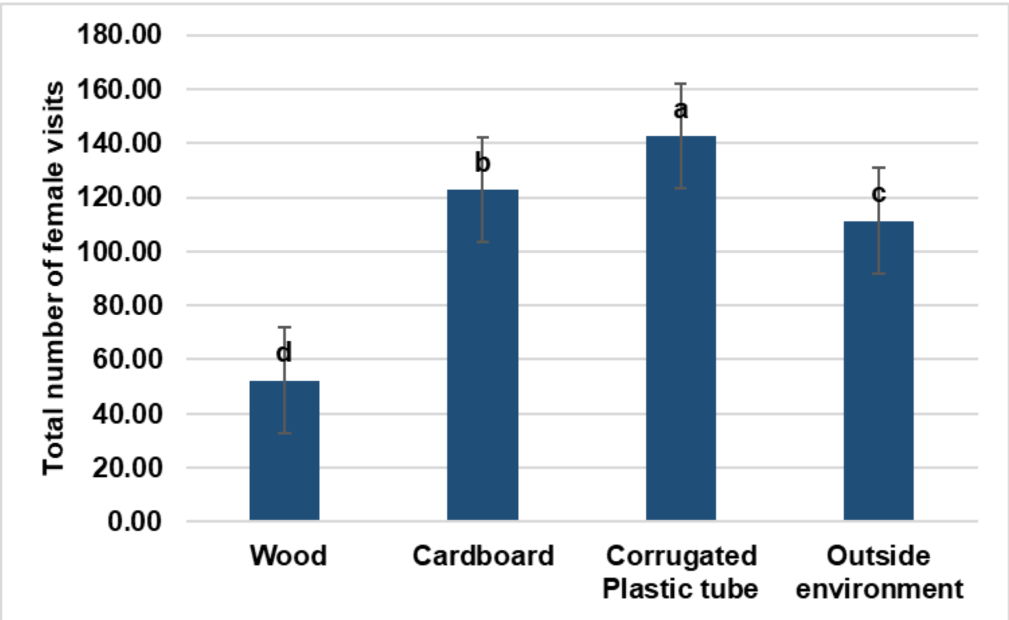

Fig. 2. Total number of female BSF (Black Soldier Fly) visits on different ovipositional traps
Total number of female visits on each type of ovipositional traps (Cardboard, wood, plastic tube, and outer side). Bars topped with the same letter are repeated means statistically non-significant (p<0.05)

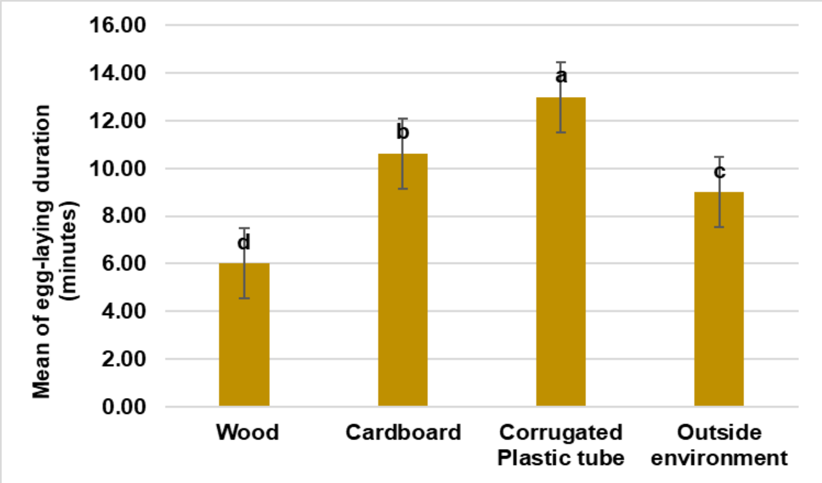

Fig. 3. Mean of egg-laying duration of BSF (minutes)

Mean (±SE) of egg-laying duration on each ovipositional traps type (Cardboard, wood, corrugated plastic tube and outer side)

The total number of egg-laying females also varied, with plastic tubes leading with the highest count (30.67), followed by cardboard (25.67), the outside area (13), and wood traps (11) (Fig. 4). The preference for plastic and cardboard traps could be attributed to a combination of physical and chemical cues provided by these materials, which may attract more females for oviposition [12]. Similar trends have been observed in other studies, where material properties significantly influenced oviposition behaviour [13,14].

Notably, the days after emergence (pre-ovipositional period) before egg-laying also showed variation, with the shortest duration in plastic tubes (3.67 days) (Fig. 5). This suggests that females may find these traps suitable shortly after emergence, likely due to favourable environmental conditions. In contrast, the outside area had the longest duration (6.17 days), possibly due to the less controlled conditions.

Regarding egg fertility, corrugated plastic tubes showed the highest percentage (85.33%), indicating that this material not only attracts more females but also provides an optimal environment for viable egg development. Cardboard traps had a moderate fertility rate (72.67%) (Fig 6), while wood traps and the outside area had significantly lower fertility rates (33.67%). These results highlight the superior performance of corrugated plastic tubes as ovipositional traps for BSF females, likely due to favourable physical characteristics and possibly lower contamination risk. This observation is consistent with the findings of Tomberlin et al. [15], who demonstrated that ovipositional trap choice could impact both oviposition and larval development.

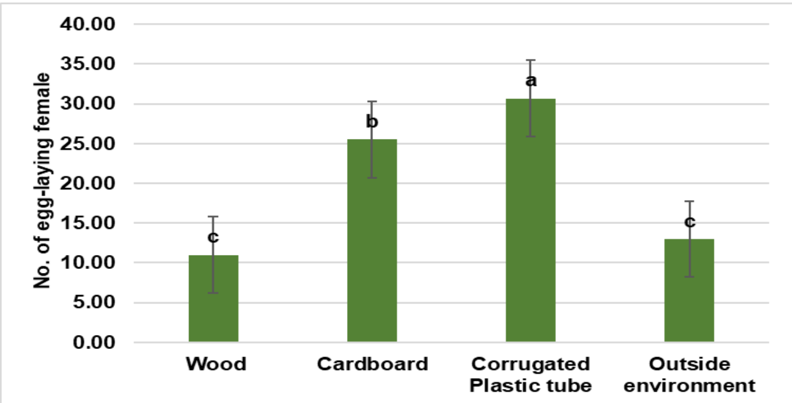

Fig. 4. Number of egg-laying BSF females on different ovipositional traps

Total number (±SE) of Egg-laying females on each ovipositional traps type. Bars topped with the same letter are not statistically different (p>0.05)

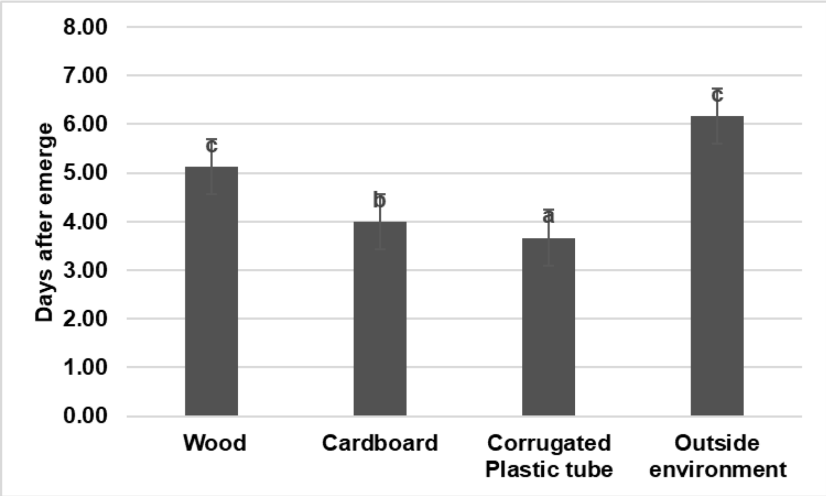

Fig. 5. Days after the emergence of BSF on different ovipositional traps (pre-ovipositional period)

Comparison of daily number of eggs laid in each of ovipositional traps type, Bars topped with the same letter are repeated means statistically non-significant (p≤0.05)

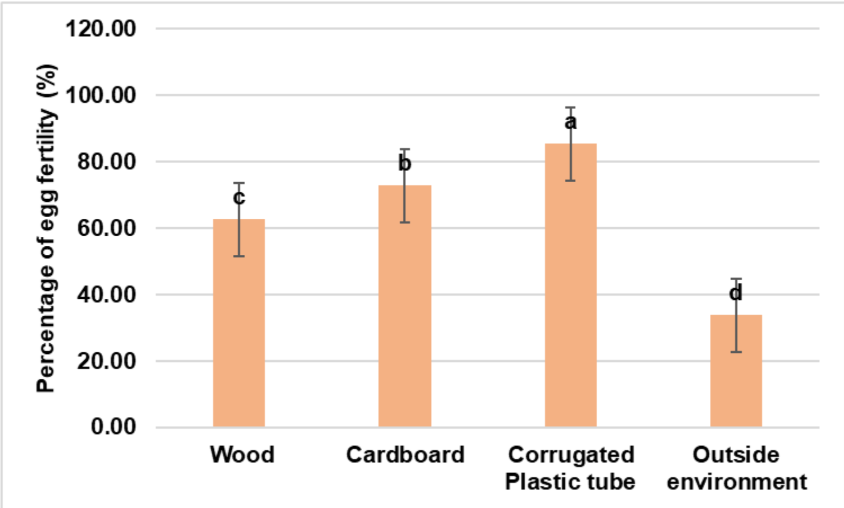

Fig. 6. Percentage of egg fertility of BSF (Hatchability) on different ovipositional traps

Percentage of egg fertility (the number of successfully hatched eggs into larvae). Bars topped with the same letter are not statistically different (p≤0.05), Values are arc sign transformed

In comparison, Julita et al. [8] found that the average egg-laying duration for female flies ranged from 6.5 to 16.2 minutes, with longer durations recorded on wood and cardboard ovipositional traps. Their study also noted that wood and cardboard ovipositional traps received significantly more visits than others, with the oviposition period lasting for 5 days, beginning on day 4 and peaking on day 5 after emergence. This period coincided with the highest activity, roughly two days following mating. They observed that female flies generally laid eggs around midday, between 11:00 AM and 3:00 PM. This time-specific behaviour may be influenced by environmental factors such as temperature and humidity, as noted in studies by [16,17].

The highest number of eggs, totalling 2,400 per BSF, was found in the corrugated plastic tube, while the fewest, just 550 eggs per BSF, were recorded in the external environment. The peak daily egg count in each ovipositional trap, excluding the external environment, was observed two days after peak mating activity, aligning with the peak of their oviposition period (Fig. 7).

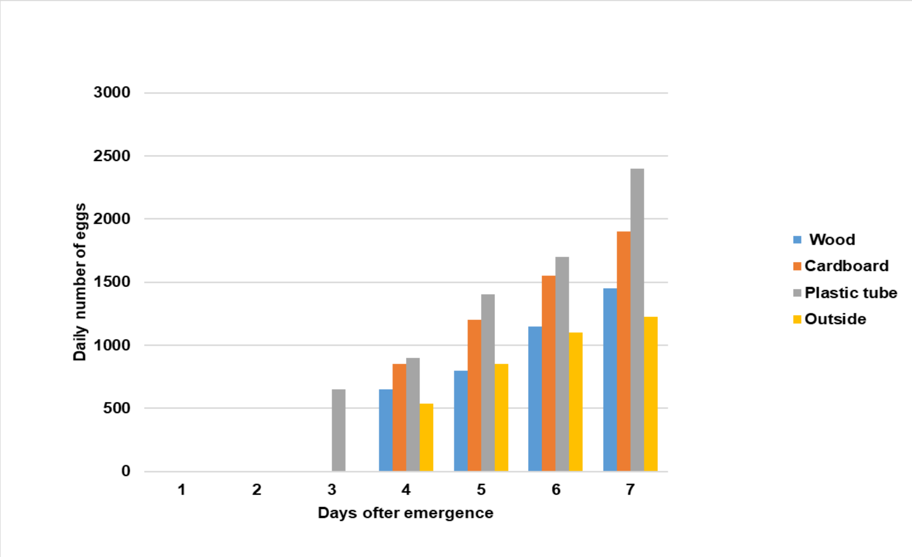

Fig. 7. Total number of eggs laid in each of the ovipositional traps

During the study, the monitored environmental conditions within the screen house revealed a daily mean temperature of 24.2°C, an average relative humidity of 72.4%, and an average light intensity of 1,500 Lux. Female oviposition predominantly occurred between 11:00 am and 3:00 pm, which coincided with the highest temperature and light intensity of the day. During this period, temperatures ranged from 30.4 to 33.8°C, and light intensity varied between 1,400 and 1,620 Lux (Fig. 8 and 9).

The environmental conditions surrounding the ovipositional trap significantly influenced the number of eggs laid by female BSF. Previous studies have shown that BSF can effectively mate and lay eggs at temperatures exceeding 26°C and relative humidity levels above 60% [15]. Our findings are consistent with these observations, as these conditions were recorded during the study. Additionally, the variation in ovipositional trap types did not impact the development time of BSF offspring at any life stage. These results are in agreement with previous research, which suggests that the development time and quality of BSF larvae are primarily influenced by environmental conditions and the availability of feed during the larval stage [18].

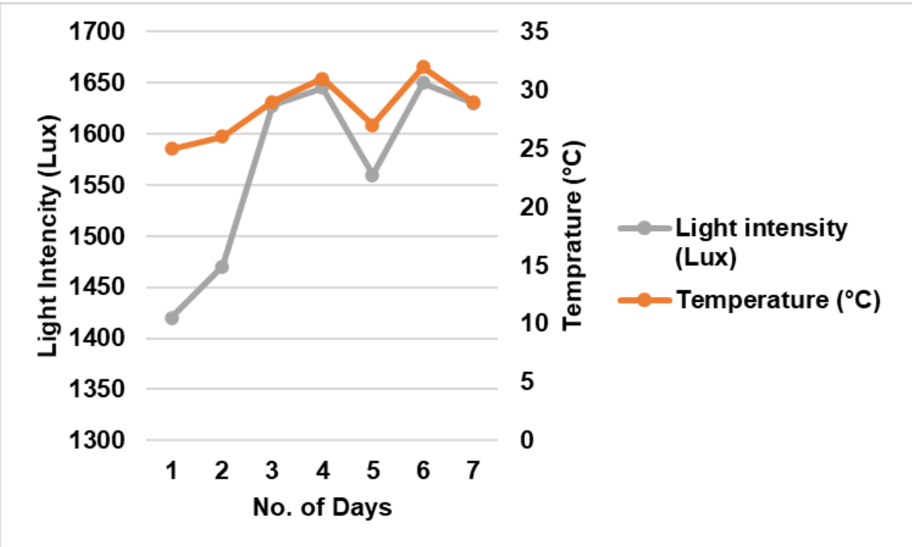

Fig. 8. Daily temperature and light intensity during observation time

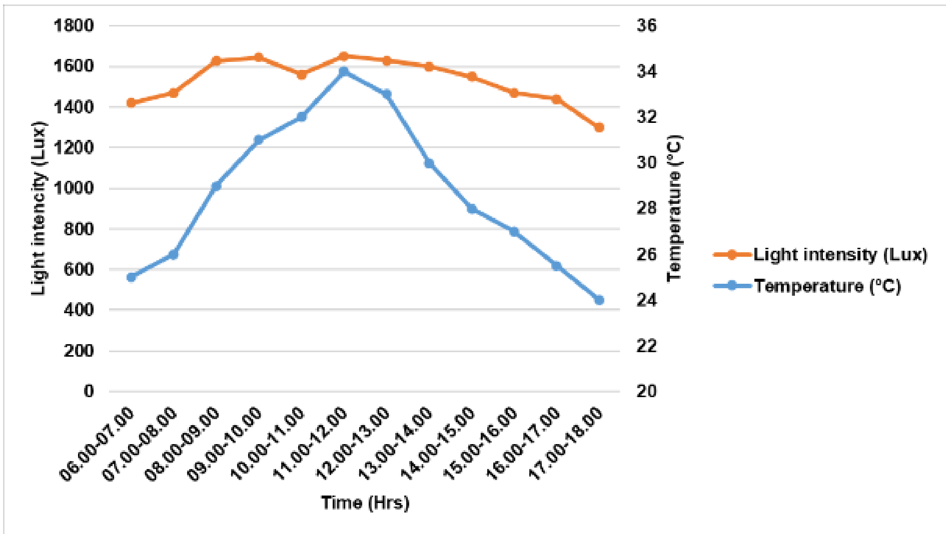

Fig. 9. Temperature and light intensity per hour during the observation time

Environmental factors recorded during the study inside the rearing cage

According to Sheppard et al. [19], the larval phase of BSF spans 22 to 24 days at a temperature of 27°C, after which the larvae transform into prepupae. These prepupae then move to a dry and sheltered location for pupation, undergoing moulting to emerge as adult flies. Once the adult females reach sexual maturity, they can mate and lay eggs. This lifecycle duration and behavior are consistent with findings by Fatchurochim et al. [20] and Cammack & Tomberlin [21], who emphasized the importance of environmental conditions in determining developmental rates.

Furthermore, studies by Park et al. [22] and Nguyen et al. [23] have highlighted the role of material choice and environmental conditions in enhancing the reproductive success of BSF. For instance, Park et al. [22] found that certain materials could significantly improve egg adhesion and moisture retention, the crucial factors for egg viability. Nguyen et al. [23] also reported that the chemical composition of substrates could influence female oviposition preference, supporting the observations in the current study.

Liu et al. [24] and Oonincx et al. [25], have shown that the nutritional quality and microbial composition of the oviposition substrate can affect the fertility and viability of eggs. Additionally, the findings by Bonelli et al. [26] and Leong et al. [27] further confirm the critical role of environmental parameters in BSF oviposition behaviour and reproductive success. These findings suggest that corrugated plastic tubes provide an optimal environment for both attracting females and ensuring high egg fertility, making them a preferable choice for egg clutch collection in research and practical applications. The current results establish the importance of selecting appropriate ovipositional trap materials to maximize reproductive success and egg viability in BSF.

4. CONCLUSION

This study demonstrates that corrugated plastic tubes are the most effective ovipositional trap material for black soldier fly oviposition, providing a favourable environment for egg laying and high fertility rates. The results underscore the importance of selecting appropriate ovipositional trap materials to optimize egg collection and support large-scale BSF rearing. This information is vital for enhancing the efficiency and sustainability of BSF-based protein production, offering a promising solution for the poultry feed industry. Further research could explore the impact of additional environmental factors on oviposition behaviour to refine mass-rearing practices.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. James MT. The genus Hermetia in the United States (Diptera: Stratiomyidae). Bulletin of the Brooklyn Entomological Society. 1935;30(4):165-170.
2. Sheppard DC, Newton GL, Thompson SA, Savage S. A value-added manure management system using the black soldier fly. Bioresource Technology. 1994;50(3):275-279. Available:https://doi.org/10.1016/0960-8524(94)90102-3
3. Kim JI. Newly recording two exotic insect species from Korea. Journal of Korean Biota. 1997;2:223-225.
4. Gujarathi GR, Pejaver MK. Occurrence of black soldier fly Hermetia illucens (Diptera: Stratiomyidae) in biocompost; 2013.
5. Bondari K, Sheppard DC. Soldier fly larvae as feed in commercial fish production. Aquaculture. 1981;24:103-109. Available:https://doi.org/10.1016/0044-8486(81)90047-8
6. Newton GL, Booram CV, Barker RW, Hale OM. Dried Hermetia illucens larvae meal as a supplement for swine. Journal of Animal Science. 1977;44(3):395-400. Available:https://doi.org/10.2527/jas1977.443395x
7. Oliveira FR, Doelle K, Smith RP. External morphology of Hermetia illucens Stratiomyidae: Diptera (L. 1758) based on electron microscopy. Annual Research & Review in Biology. 2016;9(5):1-10.
8. Julita S, Kompiang IP, Irawan B. Comparative study on oviposition preferences and egg production of Hermetia illucens in different oviposition substrates. Journal of Entomology and Zoology Studies. 2021;9(5):312-318.
9. Tomberlin JK, Sheppard DC. Factors influencing mating and oviposition of black soldier flies (Diptera: Stratiomyidae) in a colony. Journal of Entomological Science. 2002;37(4):345-352. Available:https://doi.org/10.18474/0749-8004-37.4.345
10. Gomez KA, Gomez AA. Statistical Procedures for Agricultural Research. John Wiley & Sons; 1984.
11. Ewusie EA, Kankam F, Torto B. Factors affecting the oviposition and larval development of Hermetia illucens. Journal of Insect Science. 2019;19(2):10.
12. Gligorescu A, Grumezescu AM, Grumezescu V, Dragostin O, Chifiriuc MC. Black Soldier Fly (Hermetia illucens) larvae as a sustainable protein source: A review. Insects. 2018;9(3):114.
13. Wang YS, Shelomi M. Review of Black Soldier Fly (Hermetia illucens) as animal feed and human food. Insects. 2017;8 (4):95. Available:https://doi.org/10.3390/foods6100091
14. Diener S, Zurbrügg C, Tockner K. Bioaccumulation of heavy metals in the black soldier fly, Hermetia illucens and effects on its life history traits. Ecotoxicology and Environmental Safety. 2015;132:1-8. Available:https://doi.org/10.3920/JIFF2015.0030
15. Sheppard DC, Tomberlin JK, Joyce JA, Kiser BC, Sumner SM. Rearing methods for the black soldier fly Diptera: Stratiomyidae). Journal of Medical Entomology. 2002;39(4):695-698. Available:https://doi.org/10.1603/0022-2585-39.4.695
16. Holmes LA, Vanlaerhoven SL, Tomberlin JK. Relative humidity effects on the life history of Hermetia illucens (Diptera: Stratiomyidae). Environmental Entomology. 2016;45(3):765-770. Available:https://doi.org/10.1603/EN12054
17. Barragan-Fonseca KB, Dicke M, van Loon JJA. Influence of larval density and dietary nutrient concentration on performance, body protein, and fat contents of black soldier fly larvae (Hermetia illucens). Entomologia Experimentalis et Applicata. 2020;168(1):47-55. Available:https://doi.org/10.1111/eea.12716
18. Gligorescu ASH, Toft NJA, Hauggaard S, Axelsen N. Achim. Development, metabolism and nutrient composition of black soldier fly larvae (Hermetia illucens, Diptera: Stratiomyidae) in relation to

temperature and diet. J. Insect. Food Feed. 2018;4:123-133.
Available:https://doi.org/10.3920/JIFF2017.0080

19. Tomberlin JK, Sheppard DC, Joyce JA. Selected life-history traits of black soldier flies (Diptera: Stratiomyidae) reared on three artificial diets. Annals of the Entomological Society of America. 2002; 95(3):379-386.
Available:https://doi.org/10.1603/0013-8746(2002)095[0379:SLHTOB]2.0.CO;2
20. Fatchurochim S, Geden CJ, Axtell RC. Filth fly (Diptera) oviposition and development in poultry manure at high-rise and manure-belt caged-layer houses. Journal of Agricultural Entomology. 1989;6 (2):127-136.
Available:https://doi.org/10.18474/0749-8004-24.2.224
21. Cammack JA, Tomberlin JK. The impact of diet protein and carbohydrate on select life-history traits of the black soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae). Insects. 2017;8(2):56.
Available:https://doi.org/10.3390/insects8020056
22. Park SI, Kim JW, Yoe SM. Purification and characterization of a novel antibacterial peptide from black soldier fly (Hermetia illucens) larvae. Developmental & Comparative Immunology. 2018;77: 141-148.
Available:https://doi.org/10.1016/j.dci.2015.04.018
23. Nguyen TTX, Tomberlin JK, Vanlaerhoven S. Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. Environmental Entomology. 2015; 44(2):406-410.
Available:https://doi.org/10.1093/ee/nvv002
24. Liu X, Chen X, Wang H, Yang Q, Ur Rehman K. Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. PLoS ONE. 2015;14(8).
Available:https://doi.org/10.1371/journal.pone.0215827
25. Oonincx DG, van Huis A, van Loon JJA. Nutrient composition of insect species used for human food and animal feed. Journal of Insects as Food and Feed. 2015;1(2):61-78.
Available:https://doi.org/10.1371/journal.pone.0144601
26. Bonelli M, Bruno D, Brilli M, Gianfranceschi N, Tettamanti G. Black soldier fly larvae gut microbiota and its effect on the biodegradation of different substrates. PloS ONE. 2020;15(5)
27. Leong SY, Kutty SRM, Malakahmad A, Tan CK. Feasibility of utilizing black soldier fly larvae for organic waste treatment and biodiesel production. Environmental Progress & Sustainable Energy. 2016;35 (3):816-823.

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