

## Original Research Article

# Ovipositional Preferences of Suitability of different ovipositional substrates for Black Soldier Fly (*Hermetia illucens*) on Different Ovitrap Materials

### ABSTRACT

The Black Soldier Fly (*Hermetia illucens*) has garnered significant attention as a sustainable and nutrient-rich option for poultry feed, owing due to its high protein and fat content. This study delves into the intriguing oviposition preferences of female black soldier flies, utilizing an array of ovitrap substrate materials like cardboard, wood, corrugated plastic tubes, and an outside area within a controlled environment. Employing a completely randomized block design, we assessed a range of parameters, including ovitrap substrate selection, oviposition duration, egg count, fertility, offspring development, and mortality rate. The findings revealed that plastic tubes were not only the most favored ovitrap substrate, attracting 142.67 female visits, but they also boasted with an impressive average egg-laying duration of 13 minutes and an egg fertility rate of an outstanding 85.33% egg fertility rate. This Such preference suggests a unique compatibility between may be due to the optimal microenvironment the plastic material provides and the flies' oviposition of the Soldier fly females needs, possibly due to the optimal microenvironment it provides. Cardboard traps, though less popular, still garnered 122.67 visits, with a notable 10.67-minute egg-laying duration and a fertility rate of respectable 72.67% fertility rate. Interestingly, wood traps, despite their natural appearance, were the least attractive, with only 52.33 visits, the shortest egg-laying duration (6 minutes), and a disappointing fertility rate of 33.67% fertility rate. These results highlight the unexpected yet demonstrate the significant role that material choice plays in the reproductive success of *Hermetia illucens*. The study underscores that corrugated plastic tubes create an almost ideal setting for egg-laying and subsequent development, making them an invaluable tool are the best oviposition substratum for the efficient mass-rearing of black soldier flies in for both research and practical applications commercial requirements.

Keywords: Black Soldier Fly, *Hermetia illucens*, Ovitrap, Ovipositional substratum, 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, has ~~have~~ successfully colonized regions including Australia, India, Africa, and Europe, and ~~its~~ ~~their~~ ability to withstand various environmental conditions such as light, temperature, and humidity [4]. The ~~larvae of the~~ BSF ~~larvae~~ effectively break down organic matter ~~and~~ which ~~making~~ ~~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 ~~maintain the sustainability of the~~ ~~mass produce~~ the BSF population, both for research ~~purposes~~ and ~~large-scale production~~ ~~commercial purposes~~, it is essential to employ rearing methods that mimic their natural environment. In this context ~~to~~ 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 ~~maintanace~~ ~~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 ~~are, one~~ ~~One~~ reason is to lay eggs at locations with a narrow separation between them, while the other ~~reason~~ is to deposit eggs at sites ~~where there is~~ ~~with the presence of~~ organic material ~~present~~. 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 ~~ovitraps~~ ~~ovipositional substrata~~ ~~materials~~ 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 ~~was~~ were placed inside the cage to provide light and dark condition for 12hrs. In this research three types of ~~ovitraps~~ substrates viz., Cardboard, wood and corrugated plastic tubes were placed in the ~~insect rearing~~ insect-rearing cage. Interestingly the outer side of the substrate was also considered as one of the oviposition sites. Each ~~ovitraps~~ ovipositional substrate, was approximately 10 cm in length, uniform thickness for cardboard and wood around 5 cm. They were secured with rubber bands or banana fibre-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 ~~the~~ fruit waste was placed in the ~~centre~~ center of the cage (Fig. 1).

**Comment [GN1]:** How many replications were made?



**Fig 1.** Ovipositional Preference studies of Black Soldier Fly on different ~~ovitraps~~ ovipositional substrates

### 2.2 Conditions for selection of effective ~~ovipositional substrate~~ ovitraps for BSF

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#### 2.2.1 Egg-laying duration of BSF

The duration taken by female insects to lay eggs on each variant of the ~~ovitraps~~ ovipositional substrate was taken. The quantity and number of eggs laid in each type of ~~ovipositional substrate~~ ovitraps ~~was~~ were counted. The eggs captured were identified based on their physical characteristics and compared to the typical features of BSF eggs [7].

**Comment [GN2]:** How many females were allowed for egg laying/replicate/treatment? What was the age of the females?

**Comment [GN3]:** Please indicate the time intervals at which the observations were taken.

### 2.2.2 Assessment of ~~Ovitrap~~Ovipositional Substrate Inclination-inclination

The preferred ~~ovitrapp~~ovipositional substrate was identified by assessing the number of females visiting and engaging in the egg-laying process (oviposition) on each type of ~~ovitrapp~~ovipositional substrate as well as outside the ~~ovitrapp~~ovipositional substrates [8].

**Comment [GN4]:** Please indicate the time allowed for the female visits.

### 2.2.3 Fruitfulness of female-FemaleBSF

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~~egg-laying capacity of the BSF ~~in-with~~the respect to ~~the~~ type of ~~ovitrapp~~ovipositional substrate is important for maintainingBSF in large ~~quantity~~quantities throughout the year.

**Comment [GN5]:** What was the total period allowed for egg-laying? How many females were allowed for egg laying. What was the age of the BSF females?

### 2.2.4 Developmental period of BSF

The life cycle of the black soldier fly is summarized ~~in-into~~five phases: egg, larvae, prepupae, pupae, and adult.(1) The egg hatches in about four days, (2) the larval stage spans five instars ~~over-a~~period ~~of-for~~ 13 to 18 days, (3) the prepupal stage, which is the sixth and final instar, lasts for approximately seven days, (4) the pupal stage varies from 10 to 30 days under favourable conditions before transforming into an adult, and (5) the adult stage.~~Among the four selected~~The ~~best~~ovitrappovipositional substrates, ~~which one highly influence to hold with the a~~greater number of egg clutches ~~with-in~~within a short ~~time-period~~period followed by ~~speedup-faster~~growth of the other life stages of BSF~~was-were assessed~~assessed.

**Comment [GN6]:** How does the ovipositional substrate influence the developmental time?

### 2.2.5 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, China) was utilized to monitor the internal temperature within the BSF ~~ovitrapp~~ovipositional substrates.

**Comment [GN7]:** Please delete as no such assessments were made during this study.

### 2.3 Statistical analysis

~~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[25]. All statistical analyses were performed using SPSS 25.0.

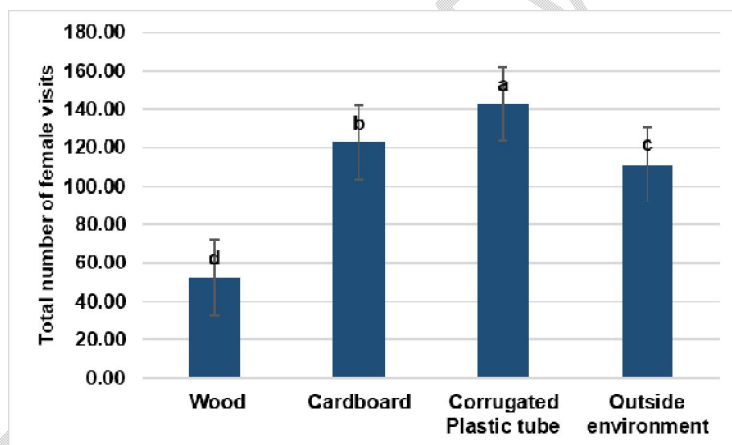
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## 3. RESULTS AND DISCUSSION

Based on the study, the preference and reproductive success of the Black Soldier Fly (*Hermetia illucens*) were evaluated using various ~~ovitrapp~~ovipositional substrates, 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 substrate 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 [ovitrapovipositional substrate](#) (9,262 eggs), while the plastic [ovitrapovipositional substrate](#) 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.

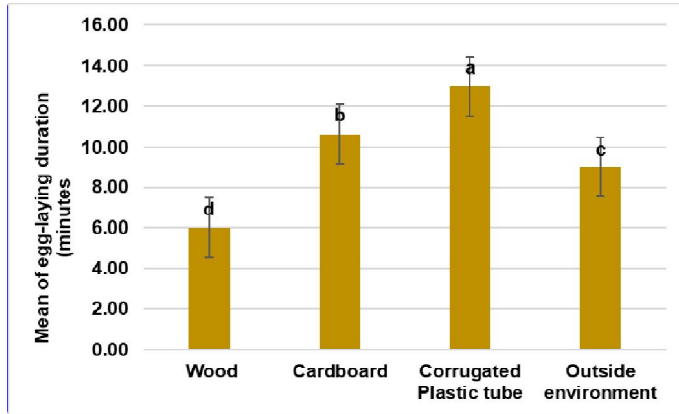


**Fig 2. Total number of female BSF (Black Soldier Fly) visits on different [ovitrapovipositional substrates](#)**

Total number of female visits on each type of [ovitrapovipositional substrate](#) (Cardboard, wood, plastic tube, and outer side). Bars topped with the same letter are repeated means statistically non-significant ( $p > 0.05$ )

The study revealed significant differences in the mean egg-laying duration across various [ovitrapovipositional substrates](#) 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 ~~a duration of~~ 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. [10], who noted that egg-laying conditions could significantly influence reproductive success.

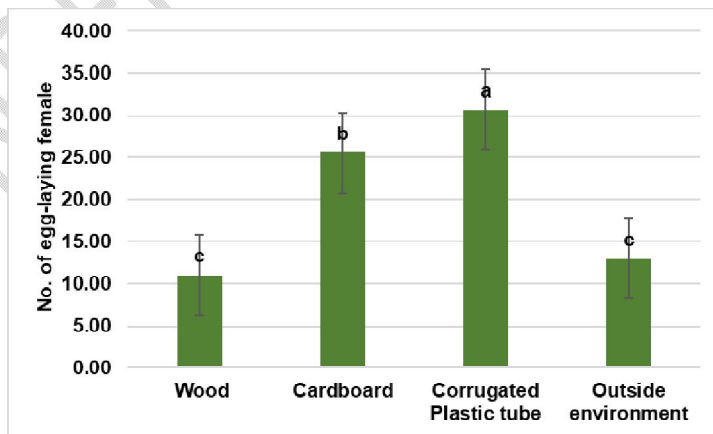


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**Fig 3. Mean of egg-laying duration of BSF (minutes)**

Mean ( $\pm$ SE) of egg-laying duration on each [ovitrapovipositional substrate](#) 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 [11]. Similar trends have been observed in other studies, where material properties significantly influenced oviposition behavior[12, 13].



**Fig 4. Number of egg-laying BSF females on different [ovitrapovipositional substrates](#)**

Total number ( $\pm$ SE) of Egg-laying females on each [ovitrapovipositional substrate](#) type. Bars topped with the same letter are not statistically different ( $p>0.05$ ).

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.

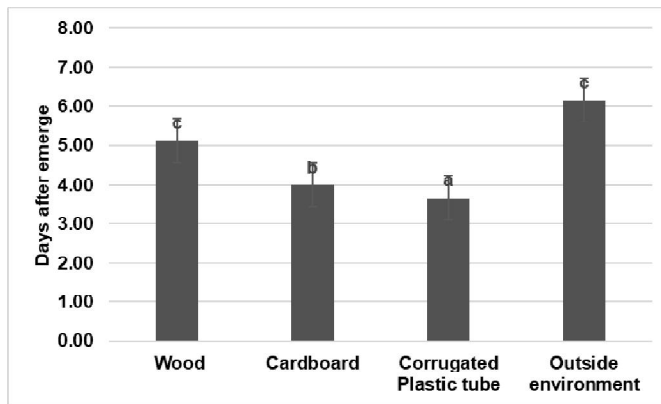


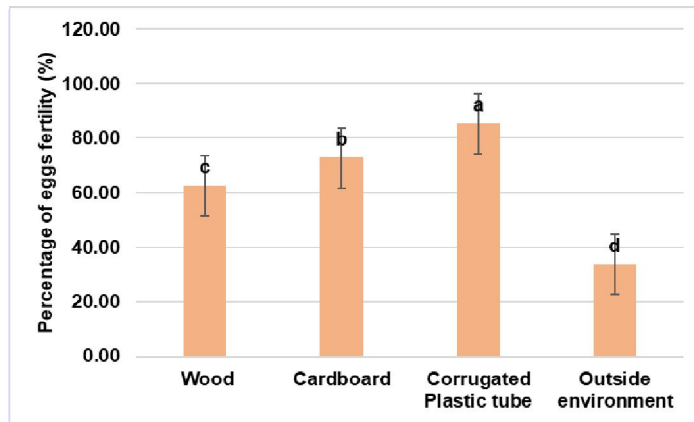
Fig 5. Days after [the emergence](#) of BSF on different [ovitrapovipositional substrates](#) (pre-ovipositional period)

Comparison of daily number of eggs laid in each of [ovitrapovipositional substrate](#) type, Bars topped with the same letter are repeated means statistically non-significant ( $p>0.05$ ).

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 [ovitrapovipositional substrates](#) 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. [14], who demonstrated that substrate choice could impact both oviposition and larval development.

**Comment [GN10]:** This should be ( $p\leq 0.05$ ) as it indicates significance of non significance of differences among treatments at 5% probability levels or lesser.

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Comment [GN12]: egg fertility

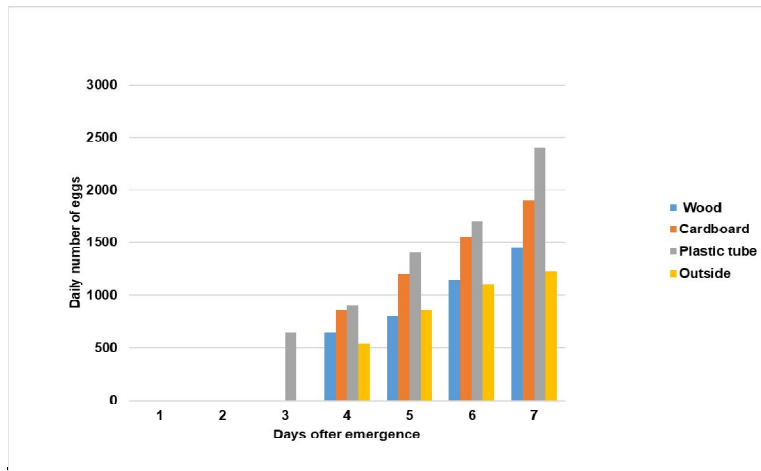
**Fig 6. Percentage of eggs fertility of BSF (Hatchability) on different ovitrapovipositional substrates**

Percentage of eggs-eggfertility (the number of successfully hatched eggs into larvae). Bars topped with the same letter are not statistically different ( $p>0.05$ )

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 ovitrapovipositional substrates. Their study also noted that wood and cardboard ovitrapovipositional substrates 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 behavior may be influenced by environmental factors such as temperature and humidity, as noted in studies by [15, 16].

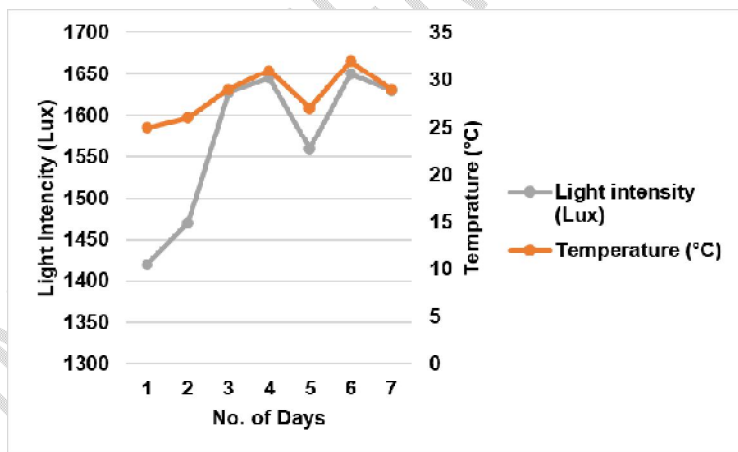
The highest number of eggs, totalling 2,400, was found in the corrugated plastic tube, while the fewest, just 550 eggs, were recorded in the external environment. The peak daily egg count in each ovitrapovipositional substrate, excluding the external environment, was observed two days after peak mating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

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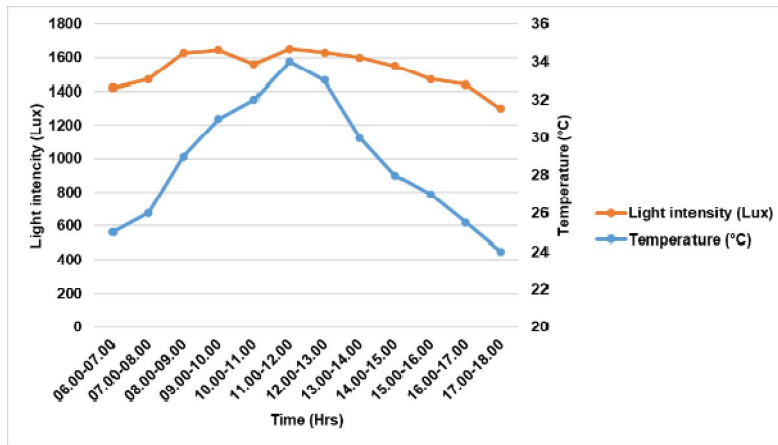


**Fig 7. Total number of eggs laid in each of the ovitrapovipositional substrates**

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).



**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

The environmental conditions surrounding the [ovitrapovipositional substrate](#) 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% [14]. Our findings are consistent with these observations, as these conditions were recorded during the study.

Additionally, the variation in [ovitrapovipositional substrate](#) 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 [17].

According to Sheppard et al. [26], 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 molting 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. [18] and Cammack & Tomberlin [19], who emphasized the importance of environmental conditions in determining developmental rates.

Furthermore, studies by Park et al. [20] and Nguyen et al. [27] have highlighted the role of material choice and environmental conditions in enhancing the reproductive success of BSF. For instance, Park et al. [20] found that certain materials could significantly improve egg adhesion and moisture retention, [the](#) crucial factors for egg viability. Nguyen et al. [27] also reported that the chemical composition of substrates could influence female oviposition preference, supporting the observations in the current study.

~~Other studies, such as those by~~ Liu et al. [21] and Ooninx et al. [22], have shown that the nutritional quality and microbial composition of the oviposition substrate can affect the fertility and viability of eggs. ~~This underscores the importance of substrate choice in optimizing BSF breeding programs.~~ Additionally, the findings by Bonelli et al. [23] and Leong et al. [24] further confirm the critical role of

environmental parameters in BSF oviposition ~~behavior~~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 study's results ~~underscore~~ establish the importance of selecting appropriate ovitrapovipositional substrate materials to maximize reproductive success and egg viability in BSF.

#### 4. CONCLUSION

This study demonstrates that corrugated plastic tubes are the most effective ovitrapovipositional substrate 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 ovitrapovipositional substrate 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.

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