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Research Article

Ovitrap Preference in the Black Soldier Fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae)

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Abstract

Background and Objective: The adult female of the Black Soldier Fly (BSF) usually performs oviposition near decaying organic matter in cracks found in dry areas. In mass-rearing facilities or on a laboratory scale, females usually lay eggs on the provided ovitrap which is made of various types of materials. This study was aimed to observe the female preferential behaviour for ovitrap types during the oviposition period. **Materials and Methods:** The study was conducted in a semi-outdoor screen house with direct sunlight. The ovitrap materials used were: Dry leaves, wood, cardboard and plastic (infraboard). The parameters calculated in this study were ovitrap preference, oviposition duration, the number and weight of the eggs, fertility of eggs, development time and mortality of offspring. **Results:** The BSF females preferred the wood ovitrap to other ovitrap types. This was indicated by the high number of females visits, the number of egg-laying females and the total number of eggs on the wood ovitrap. Eggs found in the plastic ovitrap had the highest mortality compared to eggs found in the other ovitraps. **Conclusion:** The difference of ovitrap material affected female visitation preferences, the oviposition frequency and the duration of egg-laying of female BSF but did not affect offspring development time in any of the stages (egg-adults).

Key words: Black soldier fly, *Hermetia illucens*, eggs, fertility, ovitrap, oviposition, preferential behaviour

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The Black Soldier Fly (BSF), *Hermetia illucens* (L.) (Diptera: Stratiomyidae), is one beneficial insect species which applicable to fulfill some of the human economic needs. The larval phase applicable as a decomposition agent for various organic wastes¹⁻⁵ and furtherly can be used as an additional nutrient for fish and livestock⁶⁻⁹ and Pre-pupal biomass can be converted into biodiesel¹⁰. In the area of organic waste management, the presence of BSF can reduce the growth of *Escherichia coli*¹¹, during the adult phase, BSF can inhibit and control the oviposition and development of houseflies, *Musca domestica*¹². In forensic entomology, information about the development of BSF (eggs to adults) can be used to help estimate PMI (Post-Mortem Intervals) from human corpses¹³⁻¹⁶.

The adult BSF is commonly found in the wild, especially the males while the females (usually those who are ready for oviposition or adults that have just emerged from pupae) are commonly found near farm facilities or other areas with a large amount of organic matter^{1,17}. Bonebrake *et al.*¹⁸ argued that the female insect will search for and prefer places that fully support the development and survival of their offspring. The ability of organisms to select suitable environments for the survival of their offspring can have a profound influence on their immediate fitness and potential for further evolution¹⁹. Studies showed that some environmental factors may influence the oviposition behaviour of BSF such as relative humidity²⁰, temperature²¹ and light intensity^{22,23}. Furthermore, other than environmental factors, the profile of the substrate and the area around potential substrate for larvae may affect the egg-laying oviposition behaviour²⁴.

The BSF has high economic potential which attracts many researchers to develop research and the general public to study and do mass-breeding in artificial rearing systems. To ensure the sustainability of the BSF population, both for research and large-scale production, rearing methods that resemble their natural conditions are needed. To develop such rearing methods, it is important to know the oviposition behaviour, especially to increase egg collection effectivity and the rearing of BSF throughout the year. In this study, the female preference behaviour to ovitrap types during the oviposition period to determine the optimal type of ovitrap material for collecting eggs during the post-mating oviposition period under semi-outdoor artificial rearing conditions was observed.

MATERIALS AND METHODS

Study sites: This research was conducted for 2 months, from May-June, 2019, in a Semi-outdoor screen house in the integrated garden of the Faculty of Science and Technology, UIN Sunan Gunung Djati, Bandung, West Java, Indonesia.

Rearing conditions and experimental design: The BSF used in this study were obtained from a colony that was reared in a chicken feed medium during the larval period. The observation cage was 60×60×60 cm, covered with nylon net and placed inside a screen house to obtain sunlight. Four types of ovitraps were placed inside the cage: dry banana leaves, cardboard, wood and plastic (infraboard), all about 20 cm long. Each type of ovitrap had an approximate thickness of 5 cm, was tied with rubber and placed in the corner of a tray/container containing a chicken feed medium mixed with milk (60% humidity) as the organic medium attractant which would stimulate the female to perform oviposition (Fig. 1). The container of the organic medium was placed in the middle of the cage. Fifty pairs of one-day-old adult virgin fly having the homogeneous size and weight were selected from pupa stock and put in the observation cage. This treatment was repeated five times. Observation of the ovitrap type preference behaviour lasted from 06.00-18.00 hrs for 14 days. The parameters observed in this study include:



Fig. 1: Experimental design comparing egg-trapping efficiency on different types of ovitraps
(a) Dry leaves, (b) Wood, (c) Cardboard, (d) Plastic (infraboard) and (e) Organic attractant (chicken feed+milk)

- **Ovitrap preference:** Determine the most preferred ovitrap type by calculating the number of females visiting and performing the process of laying eggs (oviposition) on each type of ovitrap and outside the ovitraps (those who did not choose any ovitrap)
- **Oviposition duration/Egg-laying duration:** The length of time needed by females for laying eggs on each type of ovitrap
- The number and weight of the eggs found in each type of ovitrap. The trapped eggs were identified by their morphological appearance and matched with BSF egg characteristics, according to Oliveira *et al.*²⁵
- **Fertility:** The number of eggs that were successfully fertilized and that hatched into larvae. Fertility percentage was determined by first observing 100 eggs that originated from each type of ovitrap. After that, the average percentage of successfully hatched eggs from three repetitions was calculated
- **Development time of offspring:** The development time (time required) for each phase of the BSF (eggs to adults), originating from each type of ovitrap treatment
- **Mortality:** The number of dead flies in each phase (egg-larva-prepupa-pupa-adult) of each treatment

The eggs laid on each type of ovitrap were collected using a fine brush and then weighed using an analytical scale. The number of eggs was calculated to determine the fecundity. Hatching eggs were counted from the first day eggs that were obtained in each ovitrap. A hundred eggs with three replications for each type of ovitrap were kept in a hatching media made from chicken feed mixed with water in a cup with a diameter of 8 cm and covered with a cloth. After the eggs hatched, the length of life and mortality rate of all stages up to pupae was observed. Adult BSF was kept in a rearing cage with an iron frame covered by a nylon net to obtain the mean of adult age.

Measurement of environmental factors: To determine the effect of environmental factors during observation of the ovitrap preference behaviour, we measured the daily mean temperature (°C), relative humidity (%) and light intensity (Lux) of the screen house once every hour from 06.00-18.00 hrs. The equipment used included a digital room thermo-hygrometer (HTC-1, China) to measure temperature and humidity, a Lux meter (LT Lutron, LX-101 A, Taiwan) to recorded light intensity in the screen house. Also, an infrared thermometer (Fluke 62 MAX, Taiwan) was used to measure the internal temperature/inside the ovitrap.

Statistical analysis: The analysis of variance (ANOVA) was applied to determine the differences in the total number (frequency) of visiting females, total number of egg-laying females, total eggs oviposited in each ovitrap type, development time and mortality rate among treatments. When the results of ANOVA were significant, the means were separated using Duncan's test and compared using the "F" test at a 5% probability. Pearson's correlation was used to determine the relationship between the total number of visiting females, the total number of egg-laying females and total eggs oviposited with significant values of 95% ($p = 0.05$). All statistical analyses were performed using SPSS 25.0.

RESULTS

Frequency of female BSF visits to each ovitrap type: The female started to visit all the types of ovitrap on the first day after emerging from their pupae. The frequency of visits on the wood ovitrap was significantly higher than other ovitrap types ($p < 0.05$) while the lowest visitation rate was recorded on the plastic ovitrap (Fig. 2a-b).

Egg-laying duration and daily oviposition frequency: The average Egg-laying duration of females ranged approximately 6.5-16.2 min which the duration on the wood and cardboard ovitrap significantly longer than others ($p < 0.05$) while the shortest recorded on the dry leaf (6.5 min) which not significantly different with plastic ovitrap (Fig. 3a). Both wood and cardboard ovitraps also received significantly more visits than others ($p < 0.05$) (Fig. 3b). The oviposition period lasted for 5 days, started evenly at day 4 and continuing to day 8 and reaching the oviposition peak on the fifth day after emergence or on average, two days after mating activity (Fig. 3c). For the daily oviposition time, female flies lay their eggs in the middle of the day, around 11.00 am-3.00 pm (Fig. 3d).

Total number of eggs, egg fertility and the total weight of eggs: The highest weight and number of eggs were found in the wooden ovitrap (total weight of 227.7 mg from 9,262 eggs) while the lowest egg weight recorded on the plastic ovitrap (total weight of 61.8 mg from 2,742 eggs) (Fig. 4a). The highest number of eggs laid daily in each ovitrap, except plastic ovitrap, was recorded on the 5th day after emergence (Fig. 4b) or 2 days after the peak mating activity which related to the peak of their oviposition period on the 5th day after emerging (Fig. 3c).

On the other hand, the highest egg fertility was recorded on the eggs laid on cardboard ovitrap (85%), followed by eggs

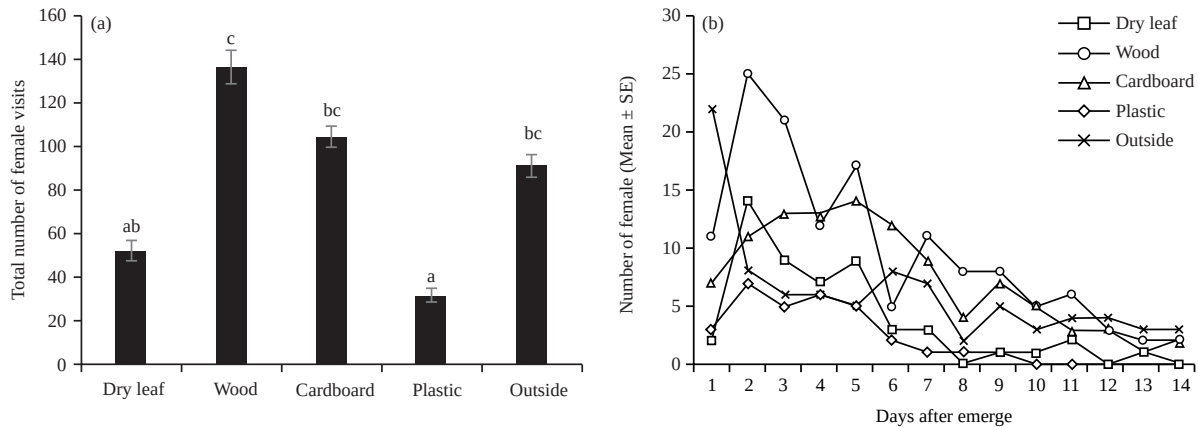


Fig. 2(a-b): Frequency of female visit on ovitraps

(a) Total number of female visits on each type of ovitrap. Bars topped with the same letter are not statistically different ($p>0.05$), (b) Daily observation of female visits to each ovitrap type

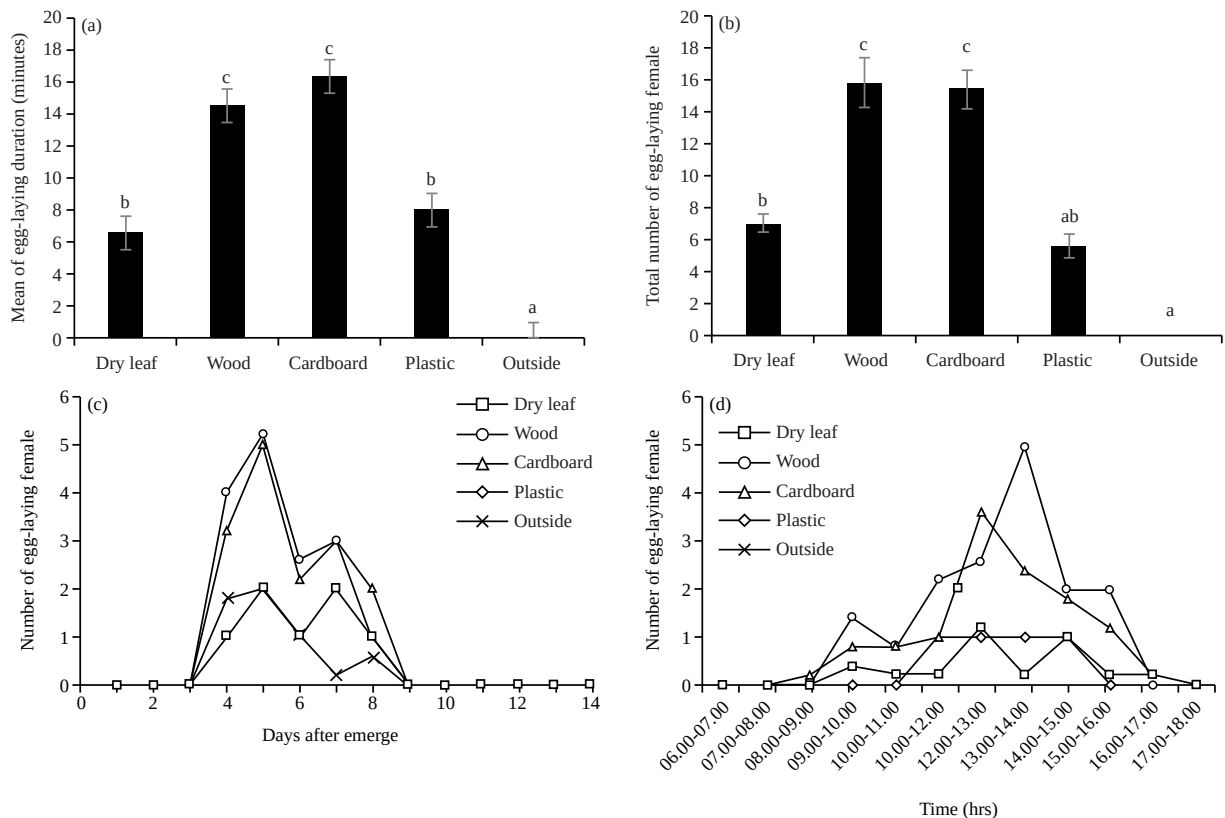


Fig. 3(a-d): Oviposition frequency of female BSF on each ovitrap

(a) Mean ($\pm$ SE) of egg-laying duration on each ovitrap type, (b) Total number ($\pm$ SE) of Egg-laying females on each ovitrap type. Bars topped with the same letter are not statistically different ($p>0.05$), (c) Comparison of daily oviposition frequency on each ovitrap type, (d) Comparison of mean oviposition frequency per hour on each ovitrap type

in the wood ovitrap (83.4%), the dry leaf ovitrap (79.2%) and the plastic ovitrap (65.5%) (Fig. 4c).

We found a strong positive correlation between the total number of Egg-laying females and the total number of eggs oviposited ($r = 0.959$, $p<0.01$) and between the mean of

Egg-laying duration and the total number of eggs oviposited ($r = 0.760$, $p<0.01$). However, there was only a weak positive relationship between the total number of female visits and the total number of Egg-laying females ($r = 0.342$, $p<0.01$).

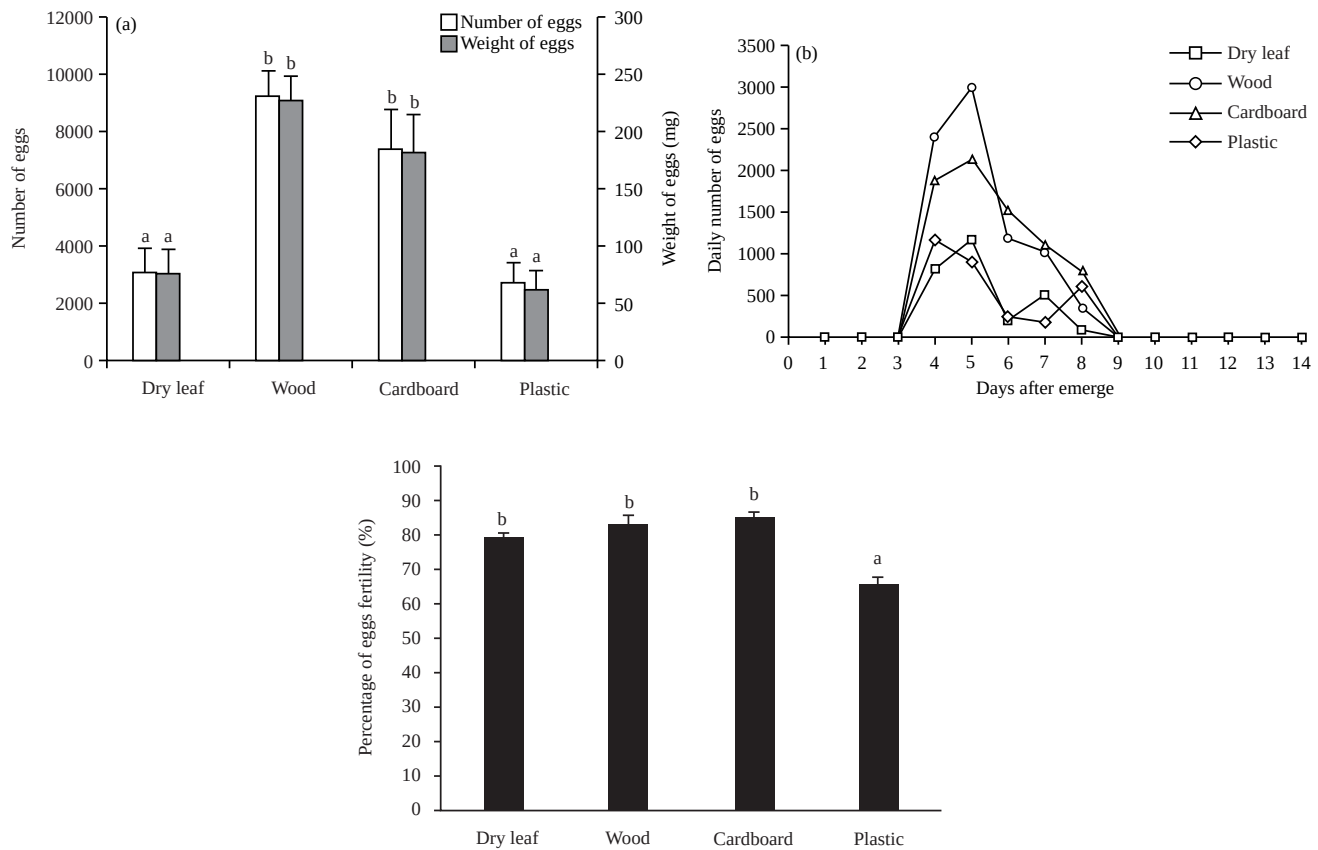


Fig. 4(a-c): Number of eggs, eggs weight and fertility of eggs

(a) Total number of eggs and total weights of eggs laid in each of ovitrap type, (b) comparison of daily number of eggs laid in each of ovitrap type, (c) percentage of eggs fertility (the number of successfully hatched eggs into larvae). Bars topped with the same letter are not statistically different ($p > 0.05$)

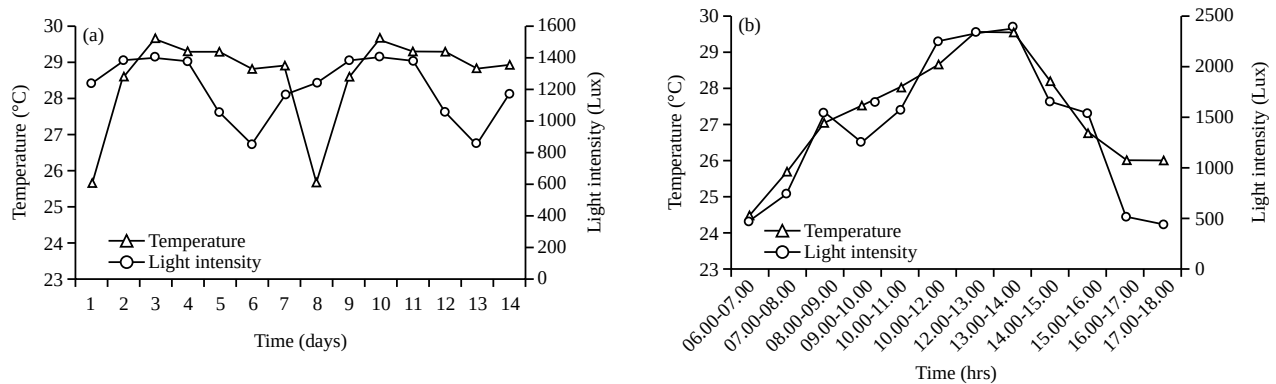


Fig. 5(a-b): Environmental factors recorded during the study inside the screen house

(a) Daily temperature and light intensity during observation time, (b) Temperature and light intensity per hour during observation time

Daily environmental observation: Based on environmental factors observed during the study inside the screen house, the daily temperature mean was 28.8°C, the relative humidity was 78.3% and the average light intensity was 1382 Lux (Fig. 5a).

It was observed that females performed oviposition generally from 11.00-15.00 hrs (Fig. 5b), when the temperature and daily light intensity were at their highest range, with temperatures of 30.4-33°C and the range of light intensity at 1600-2240 Lux.

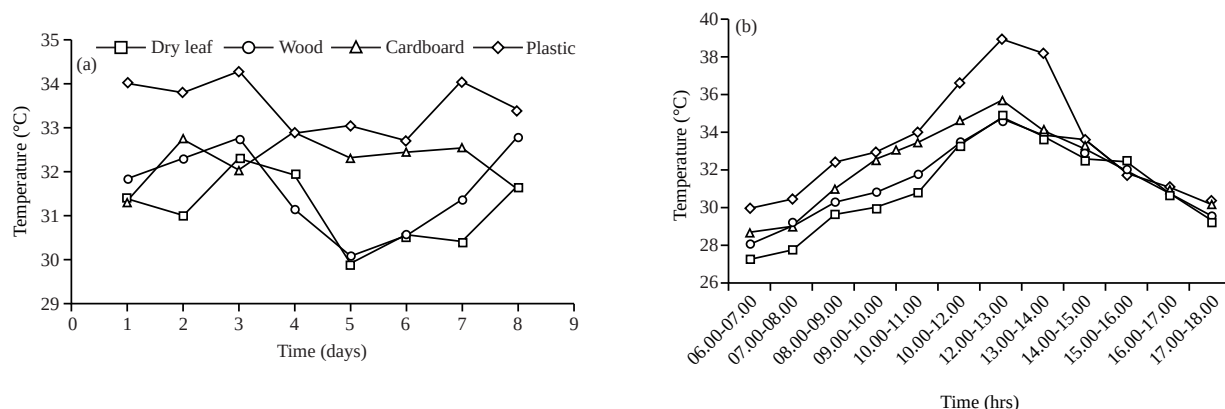


Fig. 6(a-b): Internal temperature of ovitraps

(a) Daily internal temperature of each ovitraps type during observation time, (b) Daily internal temperature of each ovitraps type per hour

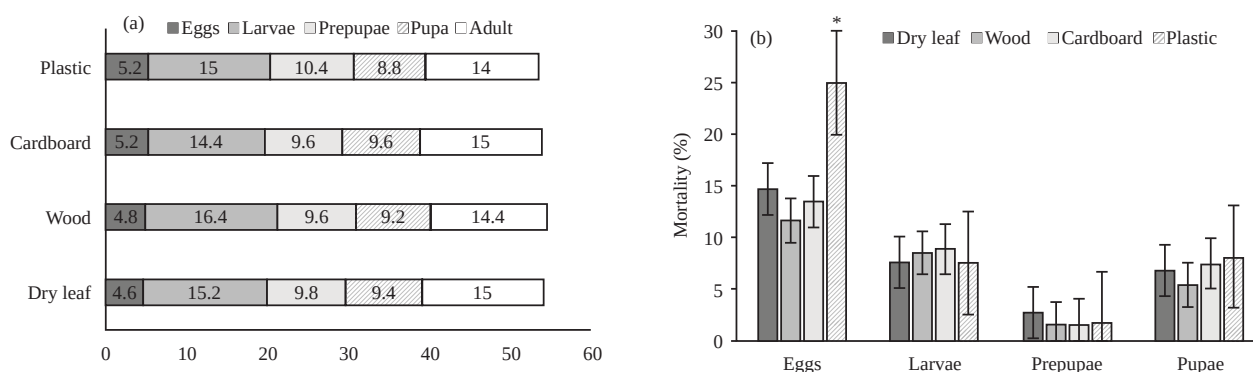


Fig. 7(a-b): Development time and mortality of offspring

(a) Influence of different ovitraps types on the development time of offspring, (b) Mortality of offspring at each stage and each ovitraps type. The asterisk represents significant differences ($p < 0.05$) in egg mortality between ovitraps types

This condition was the most optimum condition for female flies to perform oviposition activities. The measurement of the average temperature of the gaps where eggs were laid showed that plastic ovitraps had the highest temperature (33-34°C) than other trap types (Fig. 6a). On the other hand, the daily pattern of the temperature of the gaps was relatively similar among traps. The range of the temperatures of the gaps was ranged at 32.8-33.5°C in the dry leaf ovitraps, 33.6-34.9°C in the wood ovitraps, 33.3-35.9°C in the cardboard ovitraps and 33.8-39.2°C in the plastic ovitraps (Fig. 6b).

Time development and mortality of offspring: The difference in ovitraps type did not affect the average of offspring development time as the egg phase lasted for 4-5 days, the larval phase for 14-15 days, the pre-pupa phase for 9-10 days, the pupa phase for 8-9 days and the adult phase for 14-15 days (Fig. 7a). The mortality rate of eggs laid on

plastic ovitraps was significantly higher than other groups while the mortality rate of larval, pre-pupa and pupa phases was similar among groups (Fig. 7b).

DISCUSSION

This study demonstrated that the different types of ovitraps influenced the female BSF preference behaviour in determining the material for laying eggs. The highest total number of eggs was recorded on wood and cardboard ovitraps. However, eggs were found in all types of ovitraps, indicated that the BSF did not distinguish between natural and artificial ovitraps when laid their eggs as long as the ovitraps were dry, had gaps and placed close to decaying organic matter which confirmed the report of Zhang *et al.*²².

In Egg-trapping techniques, bait and trap material are the important components. These affect the BSF mass production because the determination of oviposition sites by BSF females

is considerably affected by the availability of food sources for their offspring²⁴ and the suitable area for egg attachment to ensure the survival of both eggs and offspring's²⁶. In nature, BSF females lay eggs at some crevices near moist and decaying organic matter which was intended as a food source for when the eggs hatch into larvae^{21,27,28}. The suitable oviposition sites in insects are usually determined by sensory devices that are found in the ovipositor^{29,30}.

Higher preference of females to lay eggs on wood and cardboard may be related to the profile of the ovitrap as the cardboard and wood ovitraps tend to be dry with a rough texture and the gap visible as well as having warm temperatures. This finding confirmed the previous study which reported that wood and corrugated cardboard are materials that are best suited to be used in designing the oviposition³¹. Female BSF presumably preferred the wood ovitrap when considering the comfort and protection of their eggs during and after oviposition. The process of Egg-laying by several female insects of Diptera is influenced by a combination of environmental stimuli and chemical stimuli or oviposition pheromones. Oviposition pheromones are produced when female insects lay their eggs to protect the eggs from dryness so that the eggs remain fresh while also stimulating other female insects to lay their eggs in these locations communally³²⁻³⁴. This may explain the higher Egg-laying visits on the wood ovitrap by different females.

During the observation times, most females performed oviposition during the day until late afternoon. This is in line with the research conducted by Booth and Sheppard²⁹ and Tomberlin and Sheppard²³. On the other hand, our study found that one female BSF took about 6.5-16.2 min to lay its eggs with a total egg production of between 450-600 eggs with an average unit weight of the eggs being 0.023-0.036 mg. The level of egg production was relatively in the range of the healthy egg production rates of 412-1,060 eggs³⁵ and the average weight of 0.028 mg^{29,36}. In this study, the fertility rates of eggs were different, which may have been influenced by the type of ovitrap material. The lower egg fertility of the plastic ovitrap was caused by the tendency of the material to absorb heat from the environment compared to the other types of ovitrap, which may have caused egg deaths.

Environmental conditions around the ovitrap also affected the number of eggs laid by the female. BSF can successfully mate and oviposit at warm temperatures above 26°C and relative humidity above 60%²³, which was recorded in this study. The difference in ovitrap types did not affect the development time of BSF offspring at all stages. These findings supported the study that reported the development time of larvae and quality is more likely to be influenced by

environmental conditions and the availability of feed in the larval stage^{23,29,37,38} regardless of the source of the eggs.

Overall, the results of this study suggest that the differences in ovitrap material significantly affect the oviposition preference behaviour of BSF females and egg viability. Wood and cardboard ovitraps are the most preferred for BSF females to lay eggs, thus recommended to apply in the BSF rearing system. However, further research is needed to ascertain the effectiveness of wood and cardboard ovitraps for eggs collecting in BSF indoor rearing systems without significant changes in environmental factors.

CONCLUSION

Based on the result, it can be concluded that the material applied of ovitrap significantly influenced the behaviour (Egg-laying duration and number of eggs laid) and outcome (egg fertility) of egg-laying BSF females. By considering all observations, wood and cardboard ovitraps are highly recommended to be used in BSF rearing systems, both for mass production or on a laboratory scale.

SIGNIFICANCE STATEMENT

This study revealed the ovitrap preference in Black Soldier Fly. The outcome of this research can be used to increase the egg-trapping efficiency of BSF which is needed for further experimentation and application, particularly in improving the reproductive process in captivity.

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