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Effect of Substrate and Strain on the Mortality Rate of the Early Larval Stage of Black Soldier Fly (*Hermetia illucens*)

Ramadhani Eka Putra[1*], Nadia Fairuz Aprilia[1] and Ida Kinasih[2]

[1]*School of Life Sciences and Technology, Institut Teknologi Bandung, 40132 Bandung, West Java, Indonesia*

[2]*Department of Biology, Islamic State University Sunan Gunung Djati Bandung, 40614, Bandung, West Java, Indonesia*

Corresponding author: *ramadhani@sith.itb.ac.id*

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ABSTRACT

Black Soldier Fly (BSF) larvae (*Hermetia illucens* (L., 1758)) is recently applied as a bioconversion agent to manage organic wastes. However, one major limiting factor to the application on a large scale is the high mortality rate of young larvae. Studies showed that the genetic makeup and nutrition could influence the mortality rate of insect larvae. In this study, the effect of strains and substrate on the mortality rate of the early larval stage of BSF was observed. Black soldier fly eggs originated from 4 locations in Indonesia (Bogor, Ciamis, Sidoarjo, and laboratory colony). They were kept on the five substrates (fish fed, fish mill, tofu dreg, fruit, and vegetables). The total number of larvae after seven days of feeding treatment was observed. This study showed that the BSFL group fed with vegetables had the lowest survival rate (2.6 %) while the highest was found for fish feed (12.5 %). On the other hand, larva hatched from Ciamis and Sidoarjo showed the lowest survival rate (less than 5 %) while Bogor showed the highest (more than 13 %). A production system using more nutrition balance and environmentally suitable strains could lower early larva stage mortality, which improves the economic viability of BSFL production.

Keywords: *Black soldier fly, Larvae, Mortality, Strains, Substrate*

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INTRODUCTION

One significant and growing environmental problem is waste produced from economic activities. Organic wastes are considered the most dominant wastes that mostly end in landfills or water bodies and are burned [1, 2]. However, most of these methods produce a significant environmental problem due to the release of greenhouse gases and carbon, which are also responsible for altering the ecosystem balance, i.e. producing alga bloom [3, 4]. Traditional efforts to reduce organic waste include composting through composting, aerobic and anaerobic fermentation and animal feed. However, there are concerns about the rate and final products produced, legal aspects, and sustainability of the activities [5-9].

In recent years, another attempt to upcycle the organic waste has been made by applying the bioconversion concept by microfauna and macrofauna as decomposers [10, 11]. Black soldier fly (BSF) (*Hermentia illucens* (L., 1758) Diptera: Stratiomydae) is known worldwide as one of the biological agents applied to convert the biodegradable wastes into biomass high in protein and lipid [12-15], which is applicable as part of animal feed and another industrial need [16, 17] and the process is relatively inexpensive and straightforward [18-20]. Unlike other insect species, this species can be kept in a container and reared in a closed ecosystem, making it one of the conversion processes of biodegradable wastes by BSF larvae that applies from small-scale to an industrial level [21, 22].

One of the most critical but neglected factors related to the continuity of bioconversion activities is the mortality rate of the early larval stage. Most studies focused on the later stage of larval mortality rate (starting from 7-days old), which is affected by substrate, rearing, and environmental condition [23, 24]. Lack of information on the BSF neonate (newly hatched) larva mortality may be responsible for miss judgment on the larval density and rate of the substrate application that affects the productivity of BSF larvae biomass production [25, 26]. On the other hand, studies on other insect species showed that substrate and genetic makeup affect the neonate mortality rate [27, 28]. Based on this information, the study was designed to assess the influence of substrate and egg origin on the mortality rate of BSF neonates.

EXPERIMENTAL

Study Area

The study was conducted at the Laboratory of Entomology of Ganesa Campus of Institut Teknologi Bandung, Indonesia. Specimen kept in the room with RH between 70-90 %, temperature between 28-32 ^{o}C (measured by digital Thermometer and Hygrometer on a daily based), and photoperiod 12:12 hours.

Black Soldier Fly

The black soldier fly used in this study originated from four areas, (1) the population originated from the Bandung area kept in the Laboratory of Entomology, School of Life Sciences and Technology, Ganeca

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Campus of Institut Teknologi Bandung (Google map -6.889044145118191, 107.60947700000001) (2) commercial eggs provided by a private company located in Bogor, West Java, (3) commercial eggs provided by small scale BSF farm in Ciamis West Java (Google map -7.118014655991642, 108.20904567732289), and (4) commercial eggs provided by a private company (Waste4Change) located in Sidoarjo area, East Java (Google map -7.36896138701221, 112.68480775397467) (Figure 1)

Figure 1: Location of Black Soldier Fly egg origin used in the present study

Substrate

Neonates (less than one-day-old larvae) were provided with five types of the substrate as feeding material, (1) Commercial fish feed, (2) Fish meal purchased from a local pet shop, (3) Tofu dreg provided by a local tofu maker, (4) Fruit (Papaya) purchased from the local market, and (5) Vegetables (Cabbage) purchased from the local market. All substrates except tofu dreg and fish meal were mashed by a food processor and mixed with water to the moisture level of 70 % (W/W). On the other hand, tofu dreg and fish meal were mixed directly with water to a moisture level of 70 % (W/W). The nutrient content of each substrate was based on information on the package and available literature (Table 1).

Table 1: Nutrient content of substrates

	Protein (%)	Carbohydrate (%)	Fat (%)	Fibre (%)	Reference
Vegetable (Cabbage)	2.86	4.67	0.42	3.2	USDA, 2018
Commercial fish feed	31	N/A	5	6	Factory label
Fish meal	64.1	8.5	6.5	0.8	Milamena et al. 2002
Fruit (Papaya)	0.47	10.28	0.26	1.7	USDA, 2018
Tofu dreg	32	26.92	5.54	16.53	Nugrahani, 2018

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Substrate Application

Fifteen grams of prepared substrate were placed inside a petri dish. Approximately ten thousand BSF eggs (± 0.5 gram), based on the individual weight of the egg of 0.02 mg [29], were placed on the tissue paper, which was separated by a small piece of plastic (2 x 2 cm) from the substrate. All Petri dishes were kept inside the large plastic tray and covered with nylon fabric to prevent an infestation from other insects (Figure 2). This procedure was replicated three times for each batch of eggs (depending on the population source).

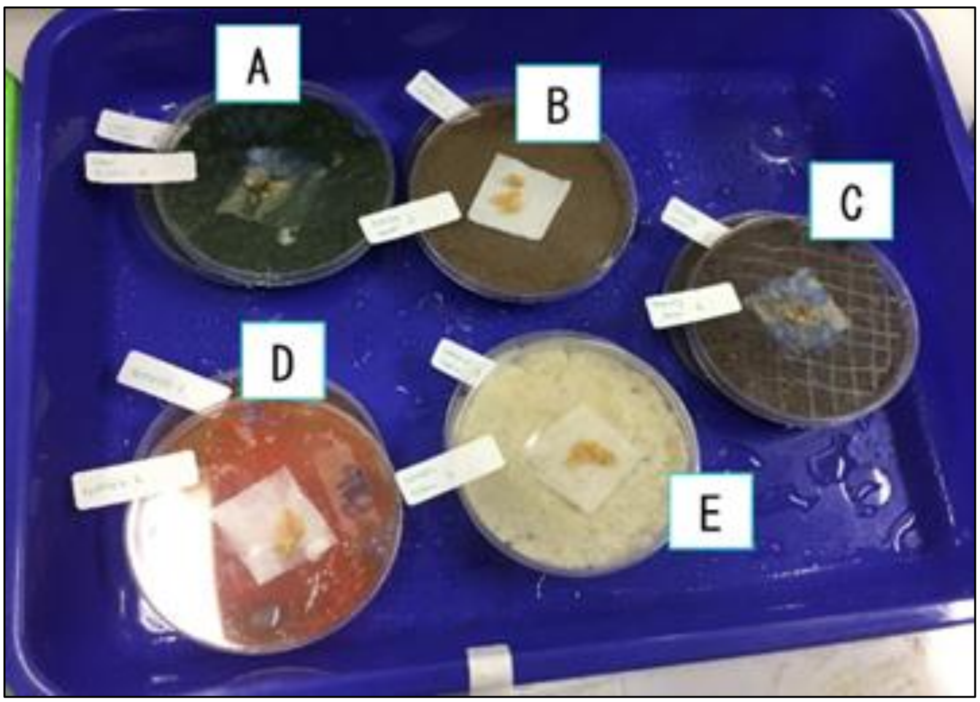

Figure 2: Five different substrates for BSF neonate larva to feed on. (A) Vegetables, (B) Commercial fish feed, (C) Fish meal, (D) Fruits, (E) Tofu dreg

Larva Mortality Rate

Larvae were separated by washing the substrate and sieving them by a tea sieve on the seventh day after substrate application. Survivorship of larvae was defined as the total number of first instar larva that hatched and survived after one week [29] and calculated by the formula in Equation 1 as follows:

$$Larva\ survivorship = \frac{number\ of\ larva}{Total\ number\ of\ eggs} \times 100\% \qquad \text{Equation 1}$$

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Data Analysis

Analysis by *the Kolmogorov-Smirnov* normality test showed a non-normal data distribution for the survivorship of BSF larvae. Thus, the effect of hatching medium and egg origin on neonate survivorship was tested by *the Kruskal-Wallis test* followed by the *Mann-Whitney U test* with a significant $P < 0.05$.

RESULTS AND DISCUSSION

Effect of Substrate to Survival Rate of Early Larvae of Black Soldier Fly

The highest early larva survival rate was recorded on the eggs reared from commercial fish feed (12.53 %). In comparison, larva fed on cabbage minced had a significantly lower survival rate than other groups (2.67 %) (Kruskal-Wallis, $P = 0.002$) (Figure 3).

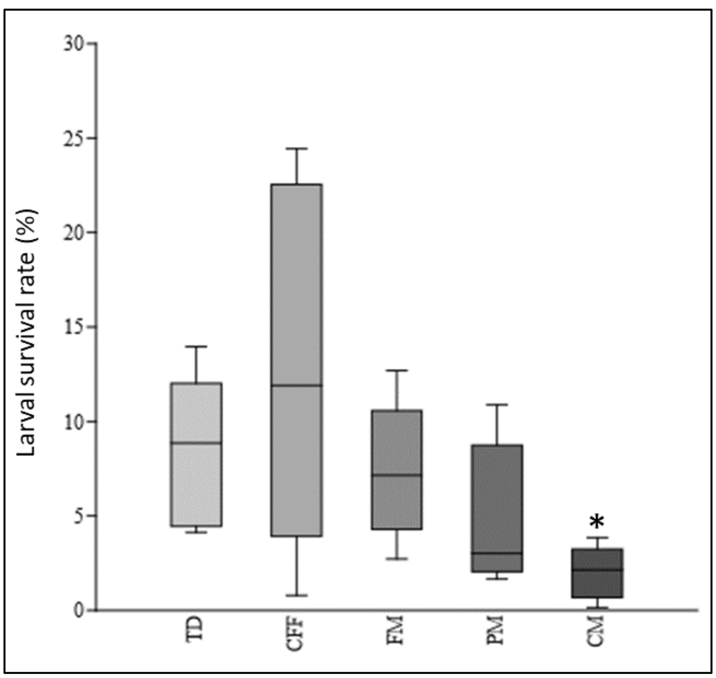

Figure 3: Survival percentage of 1 week old age BSF larvae (n ~ 10,000 eggs) feed on different substrates. TD = Tofu Dreg, CFF = Commercial Fish Feed, FM = Fish Meal, PM = Papaya Minced, CM = Cabbage Minced. (*) significant different on $P < 0.05$

The low number of larvae that survived after one week could be explained by egg hatchability which is related to the total number of surviving larvae [29-31]. Some studies reported the possible effect

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of the environment on the egg hatchability, especially temperature and humidity. The best temperature to achieve the highest egg hatchability was between 27 – 30 ºC [24, 31, 32], while humidity above 60 % [33] will ensure the best hatching rate. However, since the environmental condition of our study site was according to the best environmental condition to achieve the highest hatchability, we hypothesised that other factors might explain a low number of surviving larvae related to the substrate.

In general, insect eggs contain nutrition for the embryogenesis process to produce larvae, and the newly hatched larvae depend on the lipid and yolk in the eggs. Access to nutrition from reserve yolk, substrate, and environmental conditions is necessary for newly hatched larvae [18, 25, 34, 35]. Although newly hatched larva showed a high preference for soft material with high protein and carbohydrate content, the young larvae are relatively unable to create a pore to breathe when the substrate has high water content [36, 37], as shown by papaya minced (PM) and cabbage minced (CM) that quickly developing sticky and wet substrate due to the natural decaying process. This may explain the low survival rate of fish meal (FM) with relatively unbalanced nutrition, while PM and CM had sticky and wet properties, suffocating young larvae.

Another factor that influenced the larval survival rate was the infestation of the substrate by fungi and other microbial communities (Figure 4). Fungi may compete with newly hatched larvae for substrate and infested the eggs for nutrition [38]. Also, fungi and microbial communities infection has been reported as a critical factor for larval mortality [30, 39, 40].

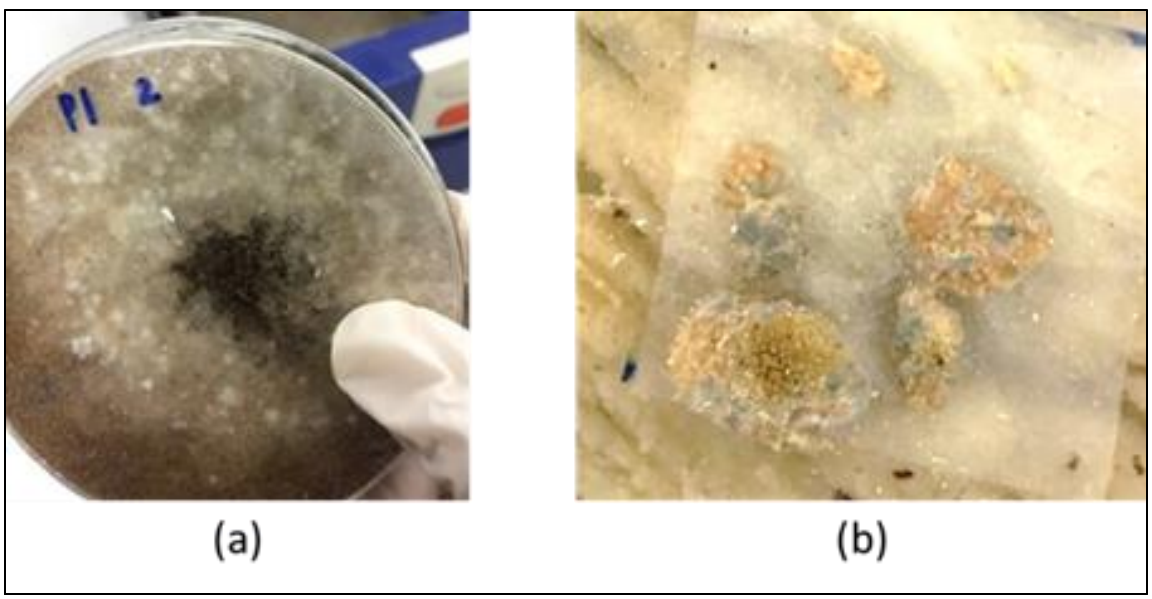

Figure 4: Infestation of fungi on the (a) substrate and (b) eggs of black soldier fly

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Effect of Strain on the Survival Rate of the Early Larva of the Black Soldier fly

Larva from egg originated from Bogor, and the lab colony had a similar survival rate as larva fed on commercial fish feed (CFF), which was higher than other groups. On the other hand, both Ciamis and Sidoarjo strains showed a similar pattern of substrate's effect on larva survival rate. Bogor strains generally had better fitness levels than other strains (*Kruskal-Wallis*, $P = 0.003$) (Figure 5).

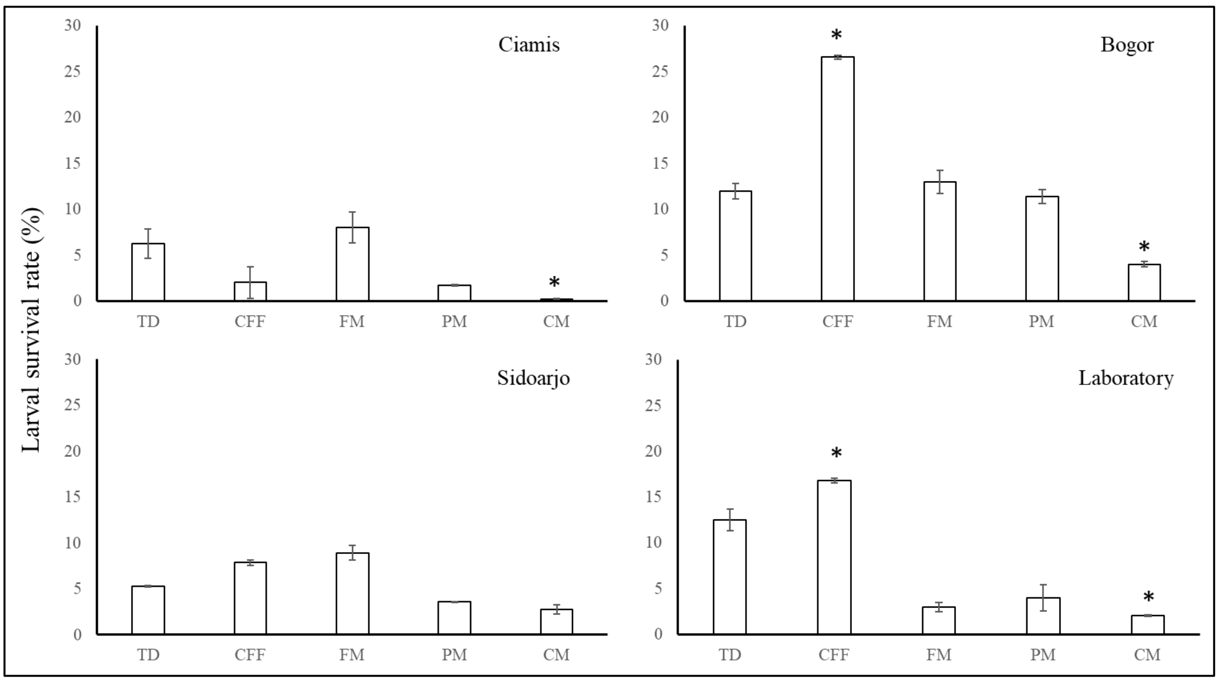

Figure 5: Percentage of larvae reached 1 week old age (n = 10,000 eggs) from four origin fed on different substrates. TD = Tofu Dreg, CFF = Commercial Fish Feed, FM = Fish Meal, PM = Papaya Minced, CM = Cabbage Minced. (*) significant different on $P < 0.05$

A study by Zhan et al. [41] showed the high genetic diversity of black soldier fly larvae and created strains with differences in physiological ability [42,43,44]. These differences further translate into specific nutritional needs utilised to determine each strain's physiological and morphological development. This study found each strain's different responses on a specific substrate related to larval diet, as previously reported [45]. Larva of Bogor and laboratory strains showed a high survival rate when fed on commercial fish as they originated from a larva that fed on high protein substrate (palm kernel mill and commercial chicken feed, respectively). On the other hand, both Ciamis and Sidoarjo strains that fed on household wastes dominated with vegetables and fruits also varied in content and showed relatively indifferent survival rates among treatment groups. This condition indicated some genetic selection led by the substrate. It could help new industries and practitioners select the eggs' origin or design a group of BSF optimised for a particular type of feed.

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CONCLUSION

This study showed the significant impact of substrate and strain on the production of black soldier larvae. Applying substrate with balanced nutrition and water content while preventing the infestation by fungi and microorganisms may reduce the mortality of young larvae. Reducing the rate of young BSF larva mortality for implementation of mass rearing would require further studies on diet and genetic strain.

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AUTHOR’S CONTRIBUTION

Ramadhani conceptualised the central research idea, supervised research progress and funding, and wrote and revised the article. Nadia carried out the research and wrote an early manuscript. Ida Kinasih provided the theoretical framework and the article submission.

CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted without any self-benefits, commercial or financial conflicts and declare the absence of conflicting interests with the funders.

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