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Black Soldier Fly (*Hermetia illucens* L.*)* Larval Development as Affected by Different Substrates

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ABSTRACT

With the rising demand for sustainable protein sources and effective waste management solutions, Black Soldier Fly (BSF) larvae (*Hermetia illucens*) have gained attention for their ability to convert organic waste into high-protein biomass. This study investigates the effects of different organic substrates on the growth and nutritional quality of BSF larvae, aiming to identify optimal substrate types for enhancing protein production and supporting sustainable waste management. Conducted in Tomay, La Trinidad, Benguet from January to March 2022, the experiment compared the larval development duration, crude protein content, and associated rearing costs of BSF larvae fed with cabbage trimmings (control), banana peelings, and retrograded rice. The larvae's development period, from first to sixth instar, ranged from 15 to 17 days under room temperature conditions of 24°C to 30°C and relative humidity levels between 52% and 99%. Results showed that larvae fed on banana peelings achieved the highest crude protein content at 17.81% with a moisture content of 61.11%, followed closely by cabbage trimmings with 17.34% protein and 64.22% moisture, and retrograded rice with 16.82% protein and 63.96% moisture. These differences in protein and moisture content suggest that substrate type significantly influences the nutritional quality of BSF larvae, with implications for their use as a sustainable protein source. In terms of cost, rearing BSF larvae on banana peelings resulted in the highest expenses (PhP3,343.00), followed by cabbage trimmings (PhP3,104.00) and retrograded rice (PhP2,970.00), with total experimental costs amounting to PhP9,509.50. This study highlights that substrate choice affects not only the growth rate and nutrient content of BSF larvae but also the economic feasibility of BSF rearing.

Keywords: Biological control, entomopathogen fungi, oil palm, pest

INTRODUCTION

Black soldier fly (*Hermetia illucens* L.*)* (BSF) belongs to the insect order Diptera and family Stratiomyidae. This insect is useful in waste management. Its larvae are fast decomposers composting about five kilograms of waste per square meter of compost in a day if larval density is sufficient. Depending on the kind of garbage, this insect is capable of composting organic waste before rotting, averting the generation of greenhouse gases, thus, contributing in the mitigation of climate change. Furthermore, BSF larvae can reduce organic garbage up to 5% of its original state. This insect can also convert 20% of the weight of fresh food waste (Li et al. 2011; Ibadurrohman, 2020).

Some parts of the Philippines use BSF in managing organic wastes. Example of this is the Sustainable Feeds located at Nueva Ecija. According to the interview with Mr.James Bayang (2022), owner of the Sustainable Feeds, they collect biodegradable wastes such as vegetable and fruit trimmings from the market to feed to the BSF larvae. In doing so, the farm is also contributing in the waste management of the province. Pacleb (2021) mentioned that one significant issue of La Trinidad, Benguet is that the municipality's composting facilities exceeded their capacities can no longer accommodate waste from different establishment. This problem is further aggravated when farmers dump their produces increasing waste in the municipality. An average, 30-35 tons of garbage is being collected. This is separate from the daily collected vegetable waste at the La Trinidad trading post generating mean of five trucks every day with an average weight of four tons per truck or about 20 tons per day (Aro 2017 as cited by Pacleb 2021; Barragán-Fonseca, 2018).

Only few studies are conducted on the biology of BSF here in the locality with most of these studies using chicken dung as substrate in rearing the insect. The only work comparing different substrate is that of Biology of Black Soldier Fly (*Hermetia illucen* L.) on Different Substrate at La Trinidad, Benguet. Wherein it was observed that BSF reared on chicken dung has shorter larval stage duration while BSF larvae reared on rice bran + kitchen waste has longer larval stage duration but shorter pupal development. This study is conducted to further current understanding on the influence of substrate to BSF biology. Result of the study can also a baseline data in the formulation of suitable substrate for BSF production.

This study is in line with the Sustainable Development Goals (SDG) 2, 6 and 12 which focuses on zero hunger, clean water and sanitation, and responsible consumption and production. These could be achieved by practicing sustainable management, reducing waste, managing chemicals and waste in an environmentally responsible way (Candian, 2023.

Generally, the study aimed to assess the growth of BSF larvae in different substrate. Specifically, it aimed to determine the larval duration of the BSF larvae that fed on the different substrate; to determine the crude protein content of BSF larvae fed with different substrates; and to determine the cash expenses in rearing BSF using the different substrates.

METHODS

Collection of Black Soldier Fly Eggs

As a starting culture of the study, egg traps were placed inside the BSF rearing house of Cordillera Center for Animal Research & Development (CCARD) located at La Trinidad, Benguet. Laid eggs in the traps were collected and placed in another container. Collected eggs were placed on the top of substrate composed of 250g of rice bran. Two days old of the larvae were brought to Tomay and fed with rice bran + corn meal at 1:1 ratio. The top of the food substrate was daily sprayed with water to maintain its moisture. BSF remained in the said substrate until they reached the third larval instar, seven days from egg stage.

Rearing of the BSF Larvae on Different Substrates

Twelve pieces of 12-liters capacity mineral water gallons were assembled as experimental set-up. Five holes measuring 12mm were made at the bottom of each gallon to serve as exit point of leachate and facilitate ventilation. A cellophane was placed under the galloon to trap the leachate. An empty, uncovered, 1.5-L capacity plastic bottle was placed at the rear side of the gallon to serve as catch basin for the larvae when they self-harvest. Two stands measuring 152cm x 152cm served as shelves for the 12 containers.

Substrate Preparation

Forty to eighty grams (40-80g) BSF larvae were weighed and placed in the different substrates. The study of Pacleb (2021) served as the basis of the treatments. The treatments replicated four times and laid following Complete Randomized Design (CRD) were as follows:

Treatment	Substrate (1:1)
T_1 (control)	Cabbage trimmings + rice bran
T_2	Banana peeling (lakatan) + rice bran
T_3	Retrograded rice + rice bran

Cabbage trimmings were collected at Camp Dangwa, La Trinidad, Benguet from the farmers who delivered their crop at the market. Banana peelings were collected from the fruit vendors Camp Dangwa to Shilan, La Trinidad, while the retrograded rice gathered at the restaurants in Saddle, Atok, Benguet. The cabbage trimmings and the banana peelings were chopped into one to two inches length pieces. Each substrate was then weighed and fed to the larvae every two days. One-half kilogram of rice bran was added to the substrate to minimize the foul odor emitted by the organic matters.

Determination of the Crude Protein of the BSF Larvae

Using a digital weighing scale, 100g of BSF 6th instar larvae were weighed from each replication and mixed together to have 400g of BSF larvae per treatment.From this, 250g were obtained and brought to the Department of Agriculture for the crude protein and moisture content analysis.

Temperature and Humidity

A hydrothermometer was used to measure the temperature and humidity of the study site. This is to determine the effect of the said factors to the larval development of the BSF.

Data Gathered

1. Larval duration. The number of days of the larval development of BSF.

2. Larval weight (g). This refers to the change in the weight of the larvae before being fed with the different treatments and after they start to self-harvest from the substrates.

3. Protein content of the BSF larvae. This is the crude protein content of the BSF larvae fed with the different substrates.

4. Larval measurement (mm). This is the width and length of the larvae grown on the different substrates.

5. Percent Mortality (%). Percent mortality was computed using the formula of Kumar el at (2015).

$$\text{Percent Mortality} = \frac{\text{Total number of hatched to larvae}}{\text{Total number of eggs deposited}} \times 100$$

6. Cash Expenses. This is the cost in rearing BSF using the different substrates.

Data Analysis

One-way Analysis of Variance (ANOVA) and Tukey's test were used as tools in interpreting and analyzing the significance of each treatment.

RESULTS AND DISCUSSION

Larval Duration of BSF Fed with Different Substrates

The Black Soldier Fly (BSF) larvae in this study underwent six instars, with an average larval duration ranging from 15 to 17 days across different substrates (Fig. 1). Larvae fed with cabbage trimmings exhibited the longest larval duration, while those on retrograded rice had the shortest. These findings suggest that substrate composition, including nutrient content and physical properties, may play a significant role in influencing larval development time. Substrates with nutrient deficiencies, such as low protein or fat content, could contribute to prolonged larval development as larvae expend more energy to extract the necessary nutrients for growth (Beniers et al. 2019; Jalil et al. 2021).

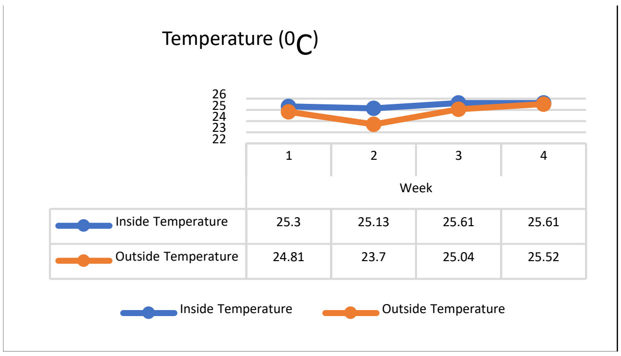
Temperature and humidity measurements were taken weekly, with ambient conditions ranging from 24°C to 30°C and relative humidity between

52% and 99%, aligning with optimal BSF growth conditions (Kimmy, 2020; Ikram et al. 2023). However, substrate temperatures, which can influence larval behavior and development, were not directly recorded. The accumulation of heat at the bottom of rearing containers, observed during the study, may have contributed to larvae burrowing toward the surface to avoid extreme temperatures, ultimately impacting their survival. Future studies could benefit from precise substrate temperature recordings to assess their effect on larval development more accurately (Qomi et al. 2021).

Table 1. Larval duration of BSF fed with different substrates

Treatments	1st	2nd	3rd	4th	5th	6th
Cabbage Trimmings	2.00^{b}	4.00^{b}	9.00^{a}	12.00^{a}	14.00^{a}	17.00^{a}
Banana Peelings	2.00^{b}	4.00^{b}	8.00^{b}	10.00^{b}	12.00^{b}	16.00^{b}
Retrograded Rice	2.50^{a}	4.500^{a}	6.50^{c}	8.50^{c}	11.50^{c}	14.50^{c}

The shorter larval duration observed here compared to previous studies, such as Bailey (2016), who documented 18 days with mixed vegetable and animal waste, and Pacleb (2021), who found durations of 36 to 45 days with various organic mixtures, may be partially attributed to the difference in substrate types. Higher ambient temperatures likely accelerated growth, consistent with findings from Duzzel (2020), who noted shortened BSF developmental periods at elevated temperatures (Kawasaki et al. 2019.



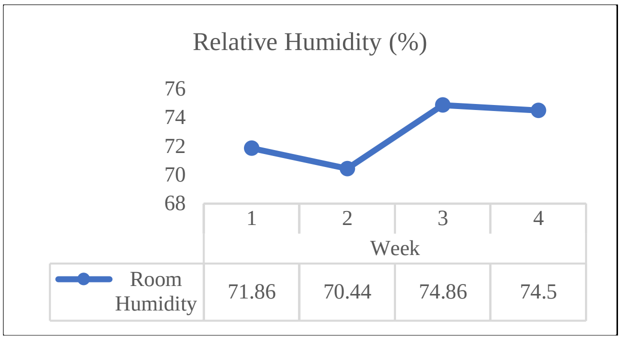


Figure 1. Weekly average of temperature and relative humidity

Mortality Rates and Substrate Influence

Mortality rates were notably high across all treatments, with values ranging from 78.38% to 82.35% (Table 2). Larvae mortality was characterized by a change in color to white or black, along with an elastic cuticle and a soft body texture. This high mortality rate could be attributed to several factors, including overcrowding and high substrate temperatures, which can cause heat buildup and stress. Applebaum and Heifetz (1999) and Esperk et al. (2021) report that crowding in larvae populations often leads to increased mortality, while substrate heat, as observed here, may further exacerbate stress, prompting larvae to surface in search of cooler areas and increasing their vulnerability to death.

Table 2. Percentage of Mortality of BSF Larvae.

Treatment	Mortality (%)
Cabbage trimmings	78.3
Banana peeling	82.35
Retrograded rice	79.93

The accumulation of heat within the substrate corroborates with Harnden and Tomberlin's (2016) findings, which showed increased mortality in response to high temperatures. Future studies should explore optimal container designs or substrate handling practices that allow for better heat dissipation, potentially reducing mortality rates.

Larval Growth and Nutritional Content

Larval growth, in terms of length and width across instars, was consistent across substrates, with no significant differences observed (Tables 3 and 4). While differences in protein and fat content between the substrates were minor (1.1g protein in cabbage trimmings, 1g in banana peelings, and 2.36g in retrograded rice), these levels likely did not meet the BSF's protein requirement of around 10% (Schillewaert et al., 2019), which could explain the limited variation in larval size.

Table 3. Mean length (mm) of the different larval stages of BSF fed with different substrates

Larval Stages	Substrate		
	Cabbage Trimming	Banana Peeling	Retrograded Rice
1st instar	2.06	2.04	2.36
2nd instar	3.02	3.79	6.92
3rd instar	13.41[a]	10.83[c]	12.10[b]
4th instar	17.02[a]	14.45[b]	14.45[b]
5th instar	17.30[b]	17.47[ab]	17.92[a]
6th instar	18.42[a]	18.13[ab]	18.67 [a]

*Means with no letter in a column are not significant at 5% level of (Tukey's)
*Means with the same letter in a column are not significant at 5% level of (Tukey's)

Table 4. Mean width (mm) of the different larval stages of BSF fed with different substrates

Larval Stages	Substrate		
	Cabbage Trimming	Banana Peeling	Retrograded Rice
1st instar	0.53	0.67	0.59
2nd instar	1.46	1.46	2.24
3rd instar	2.13[c]	3.52[b]	3.95[a]
4th instar	4.16[b]	4.43[a]	4.50[a]
5th instar	4.83[b]	5.13[a]	5.18[a]
6th instar	5.25[ab]	5.30[a]	5.24[b]

Protein content in BSF larvae fed on the different substrates ranged from 16.82% to 17.81%, with larvae fed on banana peelings achieving the highest protein content (Table 5). This finding suggests that substrate type and nutrient density can impact the protein composition of larvae. While banana peelings provided higher protein content, additional protein-rich supplementation could enhance larval protein levels further, potentially increasing their suitability as a high-quality feed ingredient. Nguyen et al. (2015) reported pre-pupal BSF larvae with protein content as high as 42% when grown on substrates richer in protein, indicating that the substrate's nutrient profile plays a crucial role in larval nutritional quality.

Table 5. Weight gain and percentage of crude protein and moisture content of BSF larvae

Substrate	Gain Weight (G)	Crude Protein (%)	Moisture Content (%)
Cabbage trimmings (control)	172.18	17.34	64.22
Banana Peeling	88.53	17.81	61.11
Retrograded Rice	193.50	16.82	63.96

Moisture Content and Implications for Nutritional Quality

Moisture content in the larvae varied by substrate, with cabbage trimmings-fed larvae exhibiting the highest moisture level (64.22%) and retrograded rice-fed larvae the lowest (63.96%). Moisture levels in larvae can impact their nutritional stability and shelf life, especially if the larvae are intended for drying and use in animal feed. Substrates with lower water content could reduce the moisture content of larvae, making them easier to process and store. Adjusting moisture levels in the rearing environment may also improve the efficiency of BSF rearing for commercial applications.

Cost-Benefit Analysis and Practical Implications for Substrate Selection

The economic analysis of substrate use showed that rearing BSF larvae on banana peelings incurred the highest cost at PhP3,343.00, followed by cabbage trimmings (PhP3,104.00) and retrograded rice (PhP2,970.00) (Table 6). The total cost for the study amounted to PhP

9,509.50, representing the potential expenses associated with mass rearing of BSF using these substrates. While banana peelings produced larvae with the highest protein content, the associated costs may not justify its use for large-scale rearing unless economic conditions favor its availability.

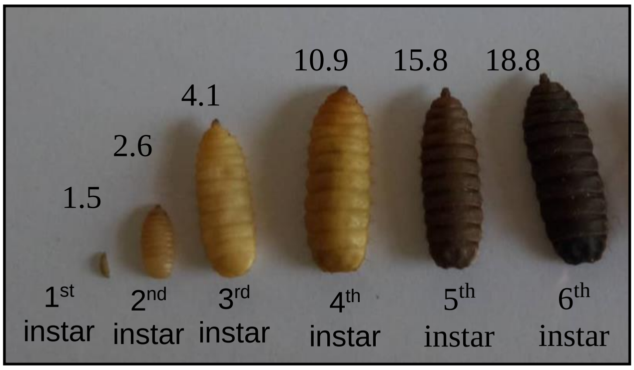


Figure 2. Measurement (mm) of BSF larvae

Figure 3. BSF larvae cadaver

From a cost-benefit perspective, retrograded rice, with its relatively lower expense and comparable protein yield, may be the most practical substrate for large-scale BSF rearing. However, if maximizing protein content is the priority, substrates or supplements with higher protein concentrations should be considered to enhance the larvae's nutritional value without significantly increasing rearing costs. Further research could explore alternative substrates that balance cost-effectiveness and nutrient density or investigate supplementation strategies that boost protein content economically (Cammack and Tomberlin, 2017).

Table 6. Total expenses in rearing BSF

Treatment	Total Cost (Php)
Cabbage Trimmings	3,104.50
Banana Peelings	3,434.00
Retrograded Rice	2,970.50
Grand Total	**9,509.50**

Comparison to Other Studies and Recommendations

Compared to other studies, the high mortality rate and lower protein levels in this study highlight the importance of substrate selection and environmental control in BSF rearing. For instance, previous research by Dwomoh et al. (2019) found that larvae reared on protein-rich food waste achieved lower mortality and higher nutritional yields, supporting the value of protein-dense substrates in promoting larval health and productivity (Kinney, 2022).

This study demonstrates that substrate composition has a marked effect on both larval development and nutritional quality, with banana peelings providing higher protein content but at a greater cost. Optimizing substrate choice based on a cost-benefit analysis could improve the feasibility of BSF larvae for use in protein production and waste management. Future research should consider exploring additional high-protein, low-cost substrates, implementing environmental controls to reduce mortality, and evaluating supplementation methods to maximize protein content in BSF larvae sustainably.

CONCLUSIONS

All of the substrate are suitable for BSF rearing as seen in the duration of BSF larval growth which is not significantly different from each other. Moreover, the shorter duration the better because more life cycle results to mass production. When it comes to crude protein content,the substrates are not suitable due to it's low protein content. Using the different substrate is sustainable and affordable

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