EVALUATING THE GROWTH AND DEVELOPMENT OF BLACK SOLDIER FLY (BSF) (*Hermetia illucens* (L.) (DIPTERA: STRATIOMYIDAE) LARVAE REARED ON DIFFERENT AGRICULTURAL WASTE MATERIALS

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Submission: 25 March 2022; Acceptance: 27 December 2022

ABSTRACT

The black soldier fly (BSL), *Hermetia illucens* (L.), is a beneficial insect that helps manage high concentrations of animal manure and other biosolids in tropical and warm-temperate areas. Bioremediation with black soldier fly larvae (BSFL) can turn biosolids into high-value biomass (proteins, oils, lipids, and chitin). This study explored the effects of three agricultural wastes as feedstocks for the growth and development of BSFL. A total of 1000 7-day-old BSFLs were reared separately in containers containing either palm kernel extract (PKE), soybean waste (SW), or coconut milk waste (CMW). The effect of feeding substrate on larval growth and development was determined for ten days by daily measurements of the BSFL's length and weight. BSFL reared on PKE substrate resulted in the highest mean length (22.67±2.31 mm) and weight growth (0.27±0.02 g), followed by BSFL reared on SW (length: 18.67±1.53 mm; weight: 0.20±0.02 g) and CMW (length: 18.33±0.58mm; weight: 0.15±0.01 g). Bioconversion rate of BSFL was also found to differ across treatments; BSFL reared on

PKE grows and converts substrates twice as fast as those fed on SW or CMW. This work then examines the effects of several SW:PKE ratios (20:80; 80:20; 30:70; 70:30 and 50:50) on BSFL growth. The weight (0.28±0.04 g) and length (22.67±2.52 mm) of BSFL were highest when SW:PKE ratio of 80:20 is used. High content of SW resulted in a significant increase in BSFL length (6.76%-15.25%) and weight (23.54%-64.71%). Therefore, it can be concluded that substrate feed type has a substantial effect on BSFL's growth and development.

Keywords: Black soldier fly larvae, palm kernel extract, soybean waste, coconut milk waste, composting

ABSTRAK

Lalat Tentera Hitam (BSL), *Hermetia illucens* (L.), merupakan serangga yang bermanfaat yang dapat membantu dalam pengurusan najis haiwan dan lain-lain biopepejal di kawasan tropika dan beriklim panas. Bioremediasi menggunakan larva Lalat Tentera Hitam (BSFL) boleh menukar biopepejal menjadi biojisim bernilai tinggi (protein, minyak, lipid dan kitin). Kajian ini mengkaji kesan tiga sisa pertanian sebagai bahan mentah terhadap pertumbuhan dan perkembangan BSFL. Sejumlah 1000 BSFL berumur tujuh hari telah diternak secara berasingan dalam bekas yang mengandungi sama ada ekstrak isirong sawit (PKE), sisa kacang soya (SW) atau sisa santan (CMW). Kesan substrat pemakanan terhadap pertumbuhan dan perkembangan larva ditentukan selama 10 hari dengan melakukan pengukuran harian bagi panjang dan berat BSFL. BSFL yang diternak dalam substrat PKE menghasilkan purata pertumbuhan panjang (22.67±2.31 mm) dan berat (0.27±0.02 g) yang tertinggi, diikuti oleh BSFL yang diternak dalam SW (panjang: 18.67±1.53 mm; berat: 0.20±0.02 g) dan CMW (panjang: 18.33±0.58 mm; berat: 0.15±0.01 g). Kadar biokonversi BSFL juga didapati berbeza bagi semua ujikaji; BSFL yang diternak dalam PKE membesar dan menukar substrat dua kali lebih cepat daripada yang diternak dalam SW atau CMW. Kajian ini seterusnya mengkaji kesan beberapa nisbah SW:PKE (20:80; 80:20; 30:70; 70:30 dan 50:50) terhadap pertumbuhan BSFL. Berat (0.28±0.04 g) dan panjang (22.67±2.52 mm) BSFL adalah tertinggi apabila nisbah SW:PKE 80:20 digunakan. Kandungan SW yang tinggi menyebabkan peningkatan ketara bagi panjang BSFL (6.76% -15.25%) dan berat (23.54% - 64.71%). Oleh itu, dapat disimpulkan bahawa jenis makanan substrat mempunyai kesan yang besar terhadap pertumbuhan dan perkembangan BSFL.

Kata kunci: Larva Lalat Tentera Hitam, ekstrak isirong sawit, sisa kacang soya, sisa santan, pengomposan

INTRODUCTION

The increase in the human population has led to a high demand in the meat industry. Unfortunately, meat production is hampered by a paucity of protein-rich feedstuffs that can be fed to livestock (Tallentire et al. 2018). Fish and plant protein are two of the most used protein sources for livestock and aquaculture feed (Van der Spiegel et al. 2013). In current times, a large portion of by-products from the agricultural and food sector is used as livestock feed due to their high nutritional value. Utilizing by-products as feed has several advantages including reduced waste production, decreased competition for crops between animals and humans, and possible cost savings associated with feeding (Salami et al. 2019).

Malaysia has recently focused on the energy generation potential of five major agricultural residual biomasses: Rubber palm, oil palm, rice, cocoa, and coconut (Chuah et al.

2006). However, only a small percentage (27%) of these leftover biomasses are utilized as fuel, while the remainder are incinerated for disposal (Wong et al. 2020). Improving the efficiency of solid waste treatment is beneficial and critical for environmental protection, resource conservation, economic development, and human health protection (Chilakamarry et al. 2022). Utilization of residual biomasses as livestock feed is an excellent alternative to the costly and polluting incineration process.

Insect-rearing on by-products that would otherwise end up as waste is a fascinating example of a sustainable circular economy (Diener et al. 2011; Jucker et al. 2017; Meneguz et al. 2018). The use of insects in the management of various solid organic wastes such as food wastes, fruit wastes, poultry manures, palm kernels, and other types of organic matter is very much feasible and has attracted significant attention worldwide (Cai et al. 2019; Fowles & Nansen 2020; Mohd-Noor et al. 2017; Nguyen et al. 2018; Pleissner & Rumpold 2018; ur Rehman et al. 2017). In fact, biological conversion processes have long been recognized as a potential method for transforming raw materials into useful chemical feedstock and energy (Diener et al. 2011; Weerachanchai et al. 2012). Several insects have been identified for this purpose including mealworms (*Tenebrio molitor*), locusts (e.g., *Locusta migratoria* and *Schistocerca gregaria*), crickets (e.g., *Acheta domestica* and *Gryllodes sigillatus*), house flies (*Musca domestica*), and black soldier fly (*Hermetia illucens*; BSF) (Surendra et al. 2020). Different insect species (detritivores and herbivores) vary significantly in their capacity to use organic substrates as a food source (Fowles & Nansen 2020).

In recent years, there has been a spike in interest in fly larvae composting as a new and innovative waste management strategy (Čičková et al. 2015). The black soldier fly (BSF) is a focal species among farmed insects due to its larvae (BSFL) being able to thrive on organic waste streams (Lalander et al. 2019; Tomberlin & van Huis 2020) such as kitchen waste (Diener et al. 2011; Nguyen et al. 2015), dairy manure (Myers et al. 2014), chicken manure (Zhou et al. 2013) and human faeces (Banks et al. 2014; Lalander et al. 2013). Unlike regular houseflies, the adult BSF does not need food because all extra nutrients were acquired during the larval stage (Isa & Hassan 2021) and there have been no indication that adult BSF can propagate diseases (Bradley & Sheppard 1984). Furthermore, BSFLs have significant amounts of important micronutrients that make them valuable as animal feed (Spranghers et al. 2017; Tschirner & Simon 2015). Also, at the end of the larvae stage, BSF larvae have higher fat content compared to other fly larvae (Čičková et al. 2015). The main advantage of using BSFL for the bioconversion of organic waste is that no toxic chemicals are required or produced during the process (Ebeneezar et al. 2021). Therefore, BSFL is particularly appealing for use as a protein and fat source in animal feed.

The BSFL, like all living things, requires food to thrive. Both larval and adult BSF growth is influenced by the quality of the food they consume. The choice of food substrate is therefore crucial for maximizing BSFL bioconversion activity. BSFL can effectively decompose a wide range of organic material if there is appropriate quantities of protein and carbohydrates (Lalander et al. 2019). BSFL can be made into compost for field use or as a source of protein and fat in animal feed. The growth of the BSFL have been observed to be delayed when fed organic waste that is deficient in nutrients (Siddiqui et al. 2022). Therefore, nutrient-deficient wastes can be supplemented with low-cost high-nutritive substrates such as soybean curd residue to increase BSFL growth and development (Raksasat et al. 2020).

The primary objective of this study is to investigate the effect of different substrates on fly larvae growth and development. Three types of solid wastes were used as feed substrates in this study: Palm kernel extract (PKE, palm kernel meal), soybean waste (SW, soybean curd residue), and coconut milk waste (CMW, desiccated coconut waste). The agricultural by-product of Malaysian palm kernel meal (palm kernel residue after oil extraction) contains oil (7.9%), protein (14.8%), crude fibre (16.7%), moisture (6.4%), ash (3.9%), and carbohydrates (50.3%) (Ibrahim 2013). Meanwhile, soy-bean curd residue (okara) contains crude protein (23.24 %), crude fat (9.19%), crude fibre (22.18%), total carbohydrate (30.15%) and crude ash (4.33%) (Li et al. 2021). Desiccated coconut waste has 2.5 % moisture content, 58.0–69.0 % fats and oils, 5.5–6.5% sugars, 6.0–8.0% protein, 12.0–18.0% carbohydrates, 2.0–4.0% crude fibre, and 1.5–2.0% ash (based on dry-weight %) (Johari et al. 2013). This research aimed to determine the influence of SW:PKE ratio (w/w) on the growth and development of the BSFL.

MATERIALS AND METHODS

Adult Fly Colony

The primary black soldier fly (BSF) colony was sourced from Entomal Sdn. Bhd. Throughout the period of the study, the colony was reared and maintained in the UiTM Insectarium at room temperature (23 to 30°C) and relative humidity of 75 to 95%.

Diet Source

The agricultural wastes used in this study were palm kernel extract (PKE), soybean waste (SW), and coconut milk waste (CMW). The PKE and SW were sourced from Sime Darby Plantation. CMW was obtained from a local coconut milk shop.

Experimental Design

The BSF adults were reared in a 2×2×5 ft. cage which allowed for egg collection. After two days, the BSF eggs were collected and stored in a 0.1L plastic container until the BSF larvae emerged. The larvae were fed chicken bran up until day seven to produce a uniform size of 7-day-old (3rd instar) BSFL before undergoing treatment. Each treatment (with three replicates) contains 1000 BSFL and is fed one of the three types of agricultural wastes: PKE, SW, or CMW. The food rations have been prepared, weighed, and kept frozen for 24 h prior to use. The BSFL were placed on the prepared feed (1 kg per treatment) in a plastic container (1.30 × 0.36 × 0.98 ft.). Then a lid with a 10 × 10 cm hole that is covered with a polyester cloth is placed on top. This setup allows for air circulation through the top hole and the polyester cloth prevents the larvae from escaping. The feeding container is placed in a naturally ventilated and shaded environment for ten days, and the moisture content of all feed substrates was kept at 70% throughout the experiment. The initial and final weight of each waste was recorded at the beginning and end of the experiment. The mean larvae weight was determined by weighing three replications of 10 larvae with a precision scale. The BSFL samples were collected from each container on days 1, 2, 3, 4, 5, 6, 7, and 10 for the measurement of their length and weight. In a subsequent experiment, the procedure is repeated but the pure substrate is replaced with a mixture of SW and PKE (SW: PKE (w/w) ratio: 20:80; 80:20; 30:70; 70:30, or 50:50). The BSFL samples were collected at day 1, 3, 5, 7, 9, and 10 and their length and weight were measured.

Data Analysis

The growth performance of BSFL rearing on different diets and ratio were measured based on the optimum weight and length of the BSFL during the study period. The SPSS statistical software for Microsoft Windows (version 27, IBM Corp., Armonk, NY, United States) was used for the analyses. Shapiro wilk test was first performed to assess the normality of the samples and the result was not significant. One-Way ANOVA was used to assess the mean differences between the various types of diets and at different ratios (PKE, SW, CMW and SW:PKE ratio). Additionally, the substrate reduction (SR) and bioconversion rate (BCR) were used to assess the effectiveness of BSFL in reducing PKE, SW, and CMW as in equations 1 and 2 (Cai et al. 2019; Ebeneezar et al. 2021; Raksasat et al. 2021).

$$SR\ (\%) = \frac{S\ (g) - R(g)}{S} \times 100 \quad (1)$$

$$BCR = \frac{(Wl_f - Wl_0)}{S} \times 100\% \quad (2)$$

Where S is the total quantity of substrate fed to the BSFL throughout the experiment, R is the residue left after treatment, Wl_f is the larvae weight by the end of the experiment, and Wl_0 is the larvae weight at the start of the experiment.

RESULTS AND DISCUSSION

Comparison of Different Diets Towards the Growth and Development of BSFL.

BSFL go through six instars and range in size from 1.8 to 20mm. The BSFL 1st instar starts 1-2 days after hatching. The 4–5-day old larvae started their second period with a body length that was double that of their 1st instar. After growing for 6-7 days, the larva reaches a length of around 7 mm and becomes macroscopic in the 3rd instar. The 4th and 5th instar occurred in 11–12 and 16–20-day-old BSF respectively. After the 5th instar, the 6th instar of the BSFL, known as the prepupa, turned dark grey (Cai et al. 2022). In this study, the effects of various feed substrate types on BSFL growth and development were evaluated using larvae in their 3rd instar. This is due to the consumption rates of BSFL rising significantly starting the 3rd instar period (Liu et al. 2019). According to the study's findings, generally, the BSFL quickly consumed all the feed substrate during their 3rd instar, increased steadily in the 4th instar, and began to progressively decline after the 5th instar.

The effect of various dietary sources on BSFL length was depicted in Figure 1a. The initial average length of BSFL was only 7.0±0.0 mm. However, during the 3rd instar, (day 7 to day 11), a tremendous increase in the length of BSF was recorded in all types of diets. At the end of the 3rd instar, BSFL reared on SW displayed a 167% increase in length, reaching 18.67 ± 1.53 mm. BSFL reared on PKE and CMW also displayed the same trends but slightly lower length increments than SW which were 17.30±2.10 mm (148%) and 13.00±2.65 mm (86%), respectively. During the 4th instar (day 12 - day 14), The BSFL reared on all types of dietary sources displayed a slower increase in larval length (<36%). After reaching the 5th instar (day 17), the length increment in BSFL (<5%) is no longer significant.

The effect of various dietary sources on BSFL weight was depicted in Figure 1b. In general, the BSFL weight increment exhibits the same pattern as the length measurement. The weight of BSFL produced in all types of feed substrates rapidly increased during the 3rd instar phase, followed by a steady increase in the 4th instar and gradually decrease in the 5th instar. The BSFL used in this experiment had an initial weight of just 0.01±0.00 g. The larvae that were fed SW had a 20-time weight increment at the end of the third instar phase, followed by a 16-time increment in PKE (0.16±0.02 g, 15%), and a 7-time increment in CMW (0.07±0.02 g). The BSFL weight was steadily decreasing in SW while continuing to increase significantly in CMW (53%) and PKE (41%) throughout the fourth instar period.

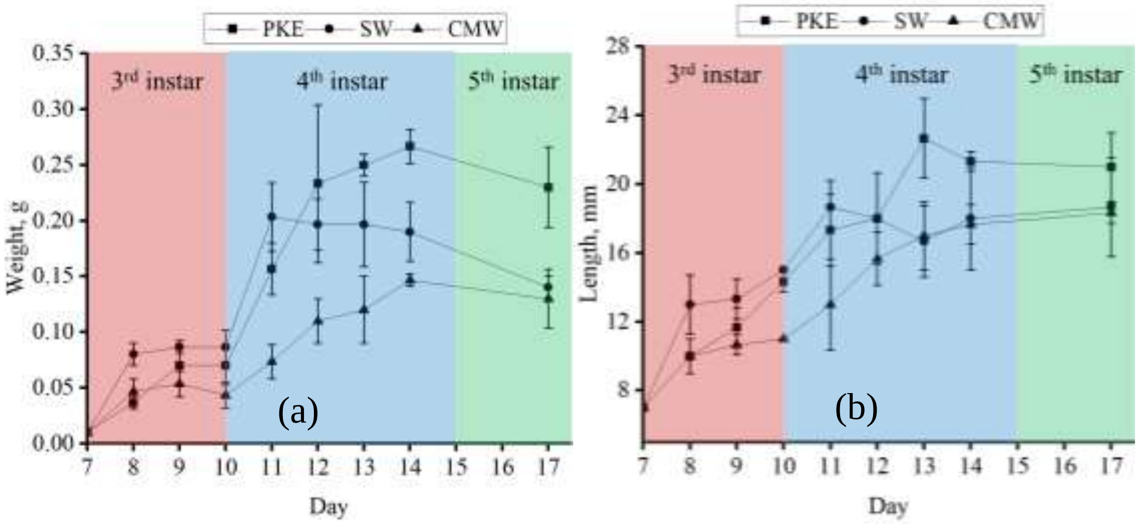

Figure 1. Effect of diet source towards (a) length (b) weight of BSF

Different feed substrates demonstrated varying rates of BSFL development. Larvae fed on unsuitable organic waste may develop at a slower rate due to poor nutritional balance in the diet (Kinasih et al. 2018). The different nutritional content between PKE, SW, and CMW feed results in the diverse growth rates of the BSFL. SW contained higher protein and other nutritional content as compared to PKE. Meanwhile, CMW has the lowest nutritional balance among the feed substrates. BSFL fed with SW was observed to grow more quickly than BSFL fed with PKE or CMW. This might be due to the BSFL obtaining its ideal nutritional content from SW. The lower protein and carbohydrate content of PKE and CMW resulted in the BSFL having to eat through more of the feed substrate to obtain its dietary requirements. BSFL fed on these substrates suffer a lower growth anyway despite eating more of the substrate (Lee et al. 2004; Nijhout 2003; Simpson et al. 2006; Wright et al. 2003). The slowest growth of BSFL was observed in CMW. A deficient amount of critical nutrients lengthened the feeding period for BSFL to reach their critical stage of development (Nijhout 1981). For insects, maximum larval weight is attained when they reach their critical stage of development (Nijhout & Williams 1974; Keena 2005). At this stage, a shift in hormonal level occurs which will induce further development (Raubenheimer & Simpson 1997).

A summary of the maximum BSFL average length and weight reared on PKE, SW, and CMW is tabulated in Table 1. BSFL had the longest length after being reared on PKE (22.67 ±2.31 mm). BSFL reared on CMW had the shortest larval length (18.33±0.58 mm) followed by SW (18.67±1.53 mm). The p-value of One-Way ANOVA's test was significant, indicating that length of BSFL was influenced by the types of feeding substrates $F_{2,6}$ = 6.54, P=0.031. Tukey post-hoc test was utilized to perform additional statistical analysis. The findings showed that the p-value obtained for PKE and CMW was significant (P=0.04), demonstrating a difference in the length of the larvae reared from both substrates. The same outcomes were obtained for the weight of the BSFL reared on PKE, CMW, and SW. There was a statistically significant difference of the BSFL weight reared on the four substrate types, $F_{2,6}$ = 27.028, P=0.001. Further statistical analysis using Tukey post-hoc test indicates that the p value of CMW, PKE and SW was less than 0.05 indicating the difference in larvae weight obtained from all the substrates. The highest BSFL weight was produced by PKE (0.27±0.02 g), while SW produced the second highest (0.20±0.02 g). CMW produced the lowest increment of BSFL weight (0.15±0.01 g).

Table 1. Maximum larval length and weight mean of BSFL reared on different agricultural waste for ten days (n=3)

Feeding Substrates	Maximum Larval Length (mm)		Maximum Larval Weight (g)	
	Mean	SD	Mean	SD
PKE	22.67	2.31	0.27	0.02
SW	18.67	1.53	0.20	0.02
CMW	18.33	0.58	0.15	0.01

Substrate Reduction (SR) and Bioconversion Rate (BCR) of BSFL

It appears in previous research that employing BSFL in a bioconversion system is more effective than composting. This process generates high-quality products while producing less organic waste in a shorter amount of time (Dortmans et al. 2021). The substrate reduction percentage (SR) and bioconversion rate (BCR) are frequently used as measures of the BSFL's efficacy in treating various types of waste. SR is a value that is calculated from a difference between the feed provided and residue and then divided by the feed provided during the experiment. Meanwhile, the BCR is calculated by dividing larvae weight gain (the final larval weight reduced by the initial larval weight) by the total feed provided (Gold et al. 2020). The SR and BCR of PKE, SW, and CMW were summarized in Table 2. The findings demonstrated that BSFL can drastically reduce all types of substrates in this study by up to 40%. SW was found to be the best-reduced substrate with very little residue (87.9%) followed by CMW (62%) and PKE (42%). These results were higher than the previously published study that used different organic waste, including restaurant garbage (37.9%), cow manure (12.7%), abattoir waste (46.3%), and digested sludge (13.2%) (Gold et al. 2020; Lalander et al. 2019).

Instead of SR, the efficiency of the BSFL in consuming a substrate can be gauged by evaluating its BCR. The BCR of BSFL is affected by several variables, including the environment's humidity, nutritional composition, physical characteristics, temperature, and oxygen content (Singh & Kumari 2019). In this study, the feed substrate of PKE produced the highest BCR (22±4%) followed by SW (13±1) and CMW (12±3). Although SW-fed BSFL had the highest SR value, the BCR was comparatively low when compared to PKE-fed BSFL. Similarly, CMW-fed BSFL with a relatively high SR% had the lowest value of BCR. Like all other living things, BSFL requires nutrients in order to grow. As a result, in order to increase bioconversion performance, BSFL must consume organic wastes that are high in easily digestible nutrients (Kinasih et al. 2020). It was also emphasised that BSFL can efficiently breakdown different kinds of organic waste if it includes enough protein and carbs (Lalander et al. 2019).

Table 2. Substrate reduction (SR) and bioconversion rate (BCR) of BSFL fed on PKE, SW and CMW

Feeding Substrate	Substrate Reduction (%)±SD	Bioconversion Rate (%)±SD
PKE	42.0±0.2	22±4
SW	89.7±0.1	13±1
CMW	62.0±0.2	12±3

Effect of Blended Feed (SW to PKE ratio) on the Growth and Development of BSFL

Further research was conducted employing the combination of SW and PKE due to their excellent performance in the earlier experiment as compared to CMW. The effect of various ratios of SW:PKE feed substrate (20:80, 30:70, 50:50, 70:30, 80:20) (w/w)) on the growth and development of BSFL is investigated and the maximum BSF larval length and weight obtained from this study were shown in Table 3. The BSFL initially have an average length of 7.0±0.0 mm and the maximum average length was achieved on day 7 of treatment for all diet ratios. The BSFL length was increased with the increasing ratio of SW to the PKE. The lowest BSFL was found in the SW: PKE ratio of 20:80 (19.67±1.15 mm) while the longest length of BSFL was found in the SW: PKE ratio of 80:20. However, the p-value of One-Way ANOVA's test was not significant indicating that length of BSFL was not influenced by the ratio of rearing substrates $F_{4,10}$ = 1.397, P=0.303. All the combination ratios of feed substrates produced ±20 mm of BSFL length during their optimum growth. In contrast to length, there was a statistically significant difference in BSFL weight rearing on the different ratios of SW: PKE $F_{4,10}$ = 13.473, P=0.01. Further statistical analysis was performed using the Tukey post hoc test. The result found that the p-value of all ratios was less than 0.05 indicating the difference in larvae weight obtained from all different ratios of SW: PKE. The highest BSFL weight was produced by the highest inclusion of SW to PKE 80:20 (0.28±0.24) followed by 70:30 (0.27±0.02), 50:50 (0.24±0.04) and 30:70 (0.21±0.02) and 20:80 (0.17±0.01) respectively. Numerous factors influence the growth and development of BSFL. When faced with limited access to feed, BSFL growth becomes dependent on the presence of critical nutrients (Lalander et al. 2019). Since SW has the highest nutrient composition as compared to PKE, the highest inclusion of this diet would improve the overall growth and development of the BSFL.

Table 3. The maximum larval length and weight mean of BSFL reared on different ratio of SW:PKE

Feeding Substrates	Maximum Larval Length (mm)		Maximum Larval Weight (g)	
	Mean	SD	Mean	SD
20:80	19.67	1.15	0.17	0.01
30:70	21.00	0.00	0.21	0.02
50:50	22.00	1.00	0.24	0.04
70:30	22.33	2.08	0.27	0.02
80:20	22.67	2.52	0.28	0.04

CONCLUSION

BSF technology has proven to be a feasible solution for organic waste treatment due to the insatiable appetite of larvae and the variety of feeding preferences. The present work demonstrates the ability of BSFL to successfully use PKE, SW, and CMW as feeding substrates that would otherwise be discarded. It is important to determine the benefits and trade-off of using agricultural waste as feed and its effect on BSFL's growth and development. The growth and development of BSFL have been shown to be highly favourable in PKE and SW substrates. Additional investigation employing various mixing ratios of these two feed substrates also shown improvement in BSFL growth and development. This proves that the growth and development of BSFL is influenced by the type of food substrate. The nutritional availability of feed substrates significantly influences the time required for BSFL to reach their ideal average length and weight. In conclusion, PKE, SW, and SW:PKE (80:20, w/w) are highly suitable substrates for BSFL composting. Additional analysis is required to ascertain the nutritional content of the feed substrates and the BSFL.

AUTHORS DECLARATIONS

Funding Statement
This study was supported by the Special Research Grant (600-UiTMSEL (PI.5/4)(023/2020)) from Universiti Teknologi MARA.

Conflict of Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics Declarations
No ethical issue required for this research.

Data Availability Statement
The manuscript has no associated data.

Authors' Contributions
Ahmad Razali Ishak, Pui Wee Hua, and Siti Norashikin Mohamad Shaifuddin conceived this research and designed experiments; Nazri Che Dom, Zulhisyam Abdul Kari, and Siti Rohana Mohd Yatim participated in the design and interpretation of the data; Fairuz Liyana Mohd Rasdi, performed experiments and analysis; Ahmad Razali Ishak, Edinur Hisham Atan, Megat Azman Megat Mokhtar, and Razi Ikhwan Md Rashid wrote the paper and participated in the revisions of it. All authors read and approved the final manuscript.

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