RESEARCH ARTICLE

BIOCONVERSION PERFORMANCES OF BLACK SOLDIER FLY LARVAE (Hermetia illucens L. DIPTERA: STRATIOMYIDAE) ON SELECTED ORGANIC SUBSTRATES

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Received: 01 July 2023, Accepted: 31 August 2023, Published: 30 September 2023

Abstract

Black Soldier Fly Larvae (BSFL; Hermetia illucens L.) is a bioconversion agent of organic substrates and its proven ability to degrade and biotransform of waste strengthens organic waste management and waste recycling strategies. BSFL have the advantages of rapid development and very efficient conversion of various organic wastes into body biomass. This process reduces the disposal costs of organic waste as well as the use of energy providing a sustainable solution to the treatment of organic waste. In addition, it provides valuable products and fertilizers for agriculture, potentially reducing crop production costs for farmers. The current study investigated the potential live biomass production of BSFL using different organic substrates available locally while understanding their decomposition ability. Seven treatments were compared (T₁- equal proportion of poultry manure and rice bran, T_2 - equal proportion of goat manure and rice bran, T_3 - rabbit droppings, T_4 - soybean meal, T₅- coconut scrapings, T₆- equal proportion of vegetable and fruit waste and T₇- cow dung) and the experiment was set up as completely randomized design with three replicates. The study results examined the ability of BSFL to reduce the weight of the end-substrate and promote the degradation of organic waste materials. The live biomass production of larvae, changes in live biomass production, substrate consumption rate, the weight of the end-substrate and substrate conversion efficiency were significantly different among treatments. The greatest live biomass production of BSFL was recorded in coconut scrapings and vegetables & fruit waste mixture while the lowest value was observed in cow dung. Coconut scrapings reduced most of their weight at the end of the study indicating the highest substrate consumption rate and the total weight of the end-substrate consisted of \leq 2mm particle in size. However, the highest substrate conversion rate was observed in cow dung. Aqueous solutions prepared from the end-substrates varied widely in pH, electrical conductivity, total dissolved solids, and salinity. In addition, significant differences were found among treatments for the colour and texture of the end-substrate. However, the appearance and odour did not differ significantly. According to the results of the present study, it can be concluded that the most efficient media for BSFL was coconut scraping though; it cannot be used as organic fertilizer as it exceeded the pH value of the SLS recommendation for compost. Overall, BSFL can be recognized as an effective and environmentally friendly tool to use in the biotransformation process of locally available organic waste materials.

Keywords: Bioconversion, Black Soldier Fly, Larvae, Organic waste

INTRODUCTION

Organic waste materials are converted into valuable resources using Black Soldier Fly Larvae (BSFL). Living organisms that consume organic waste, including earthworms, larval stages of various insects and microorganisms receive nutrition from the organic matter through the process of bioconversion.

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This process has several economic and environmental advantages. However, many fly and worm species require specialized feeding to be efficient bioconverters and are unable to metabolize a variety of organic wastes (Morales & Wolff 2010). Black soldier fly larvae (BSFL; *Hermetia illucens* in Diptera: Stratiomyidae) can be utilized to produce feed for animals, fertilizer and even methane energy (Liu *et al.* 2022).

Composting, landfill and incineration are often used for the treatment of organic waste materials (Kinasih et al. 2020). However, the bioconversion process reduces the amount of organic waste sent to landfill, reducing disposal costs and landfill methane emissions. Furthermore, the conversion of organic waste into BSF larvae also unlocks new values that can be generated from organic waste by-products. For example, it is possible to produce animal feed as larvae-based products (Siddiqui et al. 2022) and organic fertilizers and be able to generate revenue for companies involved in the biotransformation process. In this way, by converting organic waste into valuable resources as part of value-added activities, it is possible to reduce the cost of procuring raw materials and the burden of waste disposal. The larvae raised on waste feedstock gain weight while absorbing nutrients and reduce the waste mass. The robust digestive system and polyphagous nature of the larvae allow them to eat a variety of decaying organic materials that are both of animal and plant origin. In contrast, the larvae's voracious appetite makes it possible for them to consume large quantities of organic waste throughout their life cycle (Mutafela 2015). Therefore, one of the most effective and sustainable ways to manage organic waste is to use the bioremediation capability of BSFL (Zheng et al. 2013).

In addition to providing a better approach to reintegrating vital resources into the food chain, BSFL also offers many other advantages. Reducing odours and volume of biomass, restricting pollution of the environment, enhancing the nutritional value of animal feed while contributing to sustainable energy generation are some of the advantages of BSFL (Diener et al. 2011; Li et al. 2011; Popa & Green 2012). The body of BSFL is made of approximately about 30% of fat and 40% protein (Craig Sheppard et al. 1994), therefore BSFL protein can be an alternative source to fish meal in animal food that contributed significantly to the costs of feed and require large number of fish for its supply (Erickson et al. 2004). Beyers et al. (2023) reported that BSF

larvae can be used to produce insect protein which has the ability to replace the soybean meal and fishmeal requirement in rations (Mohan *et al.* 2022).

According to Van Huis (2013), this BSFL bioconversion is more environmentally friendly than conventional waste conversion and handling methods since the larval digestion improves the quality of the waste while providing additional value. The garbage would otherwise contribute to environmental pollution and increase the health risk of people, animals and the environment.

Black soldier flies are relatively large insects found mainly in North America. Its larvae feed on diverse types of organic wastes, including food waste, animal manure and plant debris, and are therefore often used as bioconverters (Erickson 2004). BSFL can digest up to 55% of his body weight a day as waste. This is up to five times more efficient than earthworms. In addition, BSFL grows faster than other organisms commonly used for composting, such as earthworms, which means that biomass can be produced in a much shorter amount of time. Biomass is also a source of organic compost and nutrient-rich soils that provide beneficial plant parts and microorganisms to improve soil fertility (Barry 2004). Additionally, the Black soldier fly is not recognized as a pest or a disease carrier to people or animals (Erickson et al. 2004) compared to some insects and microorganisms that spread disease to humans (Sasaki et al. 2000).

The overall objective of the present study was to investigate the organic waste recycling using bioconversion performances of BSFL. There are two specify objectives; (1) examine the live biomass production of BSF larvae using different locally available organic substrates and (2) the decomposition potential of the same organic substrates.

MATERIALS AND METHODS Collection of Pupal Stage and Eggs

The present study was conducted at the research laboratory of the Department of Crop Science, Faculty of Agriculture, University of

Ruhuna from June to October 2022. A household compost pile near the faculty was the source of a collection of pupae of the black soldier fly. A wooden cage with the dimensions of 1.5 x 0.8 x 0.6 m covered by white colour nets and black polythene was used to rear adult flies for two months. Substrates which have a strong odour were prepared by mixing rotten fruits and food wastes and placed inside the cage with pre-collected pupae. After 4-5 days, the adult black soldier flies emerged from the pupal case and freely moved inside the cage. The blocks of wooden pieces stuck together by rubber bands and several pieces of a corrugated plastic tube with a series of parallel ridges and grooves on its surface were kept on top of the container having the substrate to attract adult females for laying eggs. The petri dish was placed inside the cage with wet cotton wool to maintain the moisture content. The flies were supplied with water to prolong their life. The eggs were extracted from the blocks of wood and plastic tubes with a pen knife. Using a scale, the eggs were weighed. The process

was repeated until there were enough eggs. Black soldier fly eggs were collected in two days intervals and introduced to the hatching media prepared by rice bran and ripe bananas with a 60% of moisture level. Newly hatched larvae were fed with rice bran until they were 4 days old in order to speed up their growth as mentioned in Liland et al. (2017). The larvae were sieved through a mesh screen with a diameter of 1.2 mm on the fifth day and those that passed were deemed to have the same weight and size. Twenty grams of 5-day-old larvae were added into each treatment as the first batch and after 14 days 20 g of 5-day-old larvae were added into each treatment as a second batch.

Preparation of Organic Waste as Treatments

The organic wastes including cow dung, goat manure, rabbit droppings, coconut scrapings, poultry manure, rice bran, vegetable and food wastes and soybean residuals were all sourced locally. Cow dung, goat manure and poultry manure were collected respectively from cat-



Plate 1: Different stages of the life cycle of black soldier fly (A) adult black soldier fly (B) egg mass (C) 2nd instar (D) 3rd instar (E) 4th & 5th instar (F) prepupae (G) pupae

tle shed, goat shed and poultry pens of the faculty farm. Vegetable and food waste was collected from a market nearby the faculty. Coconut scrapings were collected from the faculty canteen. Rice bran was collected from a rice mill near the faculty while rabbit dropping was collected from a house near to faculty. Soybean meal was collected from Soy Sauce Production Company. All types of organic wastes were allowed to air dry for seven days in a shaded place. The soybean meal was washed 5 times to remove the salt before air drying. Then air dried waste materials were crushed manually. There were seven treatments as follows; T1- equal proportion of poultry manure and rice bran, T₂- equal proportion of goat manure and rice bran, T₃- rabbit droppings, T₄- soybean meal, T₅- coconut scrapings, T₆- equal proportion of vegetable and fruit waste mixture and T₇- cow dung.

Experimental Design and Replicates

Plastic trays (40 x 30 x 7 cm) were used to conduct the experiment. The trays were placed above a wood table to prevent invasion by predators like ants and rats. A mosquito net was placed above the tray to control other insects' intrusion such as house flies. The experiment was set by using a Completely Randomized Design with three replicates. Therefore, there were 21 experimental units. Treatments were allocated randomly within the experiment. Five hundred grams of different organic waste materials were added to the bottom of 21 trays. Twenty grams of neonatal BSFL was placed carefully on top of the substrate in each of the trays. Experimental units were placed at room temperature under the relative humidity (RH) of $75 \pm 5\%$. In order to maintain moisture content around 65-70%, the distilled water was sprayed on the substrate. All substrates were replenished after two weeks with fresh 20 g of neonatal BSFL.

Data Collection

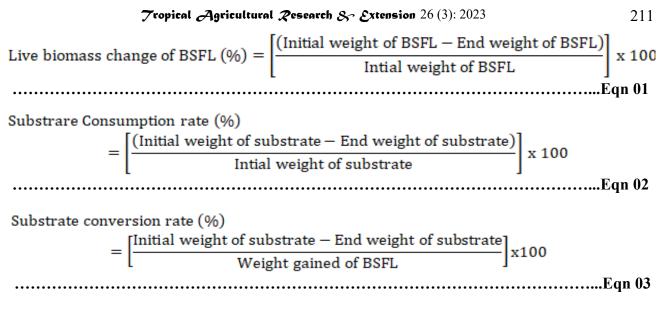
After 14 days, the first batch of insects was harvested and a second batch of 20 g of 5-day -old larvae was added to each treatment. Harvested pre-pupae were allowed to become flies inside the cage. The end-substrate was harvested 28 days after the establishment of the study and air died weight was taken. After that, air-dried samples of the end-substrate of 21 reactors were sieved using a 2 mm sieve and recorded the weight. Finally, pre-pupae were separated and reported the fresh live biomass of BSFL.

Aqueous solutions of the end-substrates of different treatments were prepared by mixing air-dried end-substrate (5 g) of each reactor with 100 ml of distilled water. Then samples were allowed to settle for 10 minutes and pH was measured using EXTECH pH meter. Salinity, EC and TDS of each sample were measured using WalkLAB conductivity, TDS and Salinity meter. Qualitative parameters of colour, odour (Wijeysingha and Fernando 2021), texture and appearance were evaluated according to the scale presented in the Table 1 for the end-substrate having $\leq 2mm$ particle size.

Live biomass change of BSFL (%), substrate reduction or consumption rate (SR) (%) (Tschirner *et al.* 2015) and substrate conversion rate (SCR) (Zhou *et al.* 2013, Banks *et*

Scale	Color	Odor	Texture	Appearance
1	Brown	Extremely bad odor	Very rough	Very bad
2	Dark brown	Moderately bad odor	Moderately rough	Bad
3	Light black	Bad odor	Rough	Neutral
4	Black	Odorless	Fine	Good
5	Dark black	Earthy smell	Extremely fine	Very good

Table 01: Description and scoring of qualitative parameters of the end-substrate



Where; Weight gained of BSFL = End weight of BSFL-Initial weight of substrate

al. 2014) on fresh matter basis were calculated using the equation 1, 2 and 3, respectively. Statistical analysis was done at 0.05 levels by means of one-way ANOVA using SAS Software for the quantitative data. Treatment comparisons were performed using Duncan's Multiple Range Test (DMRT). The qualitative data were analyzed using Kruskal-Wallis test with the aid of Minitab17 software. Descriptive data were presented using charts, graphs etc.

RESULTS AND DISCUSSION

There was a significant difference (P < 0.0001) in live biomass production of BSFL among treatments of the present study. According to Table 2, coconut scrapings were the most suitable substrate and recorded the highest growth as the biomass of the 1st batch of BSFL (936.65 g). Further, the lowest live biomass of the 1^{st} batch of BSFL (67.33 g) was recorded in cow dung compared to other treatments. This may be due to less palatability and limited availability of nutrients in cow dung for larval growth. However, the live biomass of the 2nd batch of larvae was greater in soybean meal and vegetable & fruit wastes while the lowest value was observed in coconut scraping. The greater live biomass increment was observed when feeding them with coconut scrapings in 1st batch (936 %) and vegetable & fruit waste (326%) and soybean substrate (363%) in 2nd batch (Table 1). The reason for the reduction of biomass production of the 2nd batch of larval biomass could be the unavailability of available nutrients such as fat and protein in coconut scrapings compared to all other waste materials for larval growth, as 1st batch of larvae utilized almost all of the nutrients available in the coconut scraping (Table 2). However, when we consider soybean meal and vegetable & fruit waste, the availability of nutrients for long utilization for larval growth is high compared to other substrates hence, 2nd batch of larvae utilized those available nutrients for their growth.

The BSFL transformed organic substrates into BSFL frass which is considered an organic fertilizer. Therefore, BSFL is used as a bioconversion agent to convert different types of organic waste materials into valuable organic fertilizers (Amrul et al. 2022; Lopes et al. 2022; Liu et al. 2022). Larval biomass is produced as a result of this bioconversion process, and it can be used for other waste valorization applications (Kim et al. 2021). BSFL has the potential to transform different organic waste materials into more beneficial and less destructive biomass while producing substantially fewer greenhouse gases and minimal ammonia (Van Huis 2013). Due to the makeup of their gut microbes, BSFL is able to consume a variety of dietary sources, including fresh human faeces, animal and human carcasses, decaying vegetables, animal manure, palm kernel meal, municipal organic

waste, and pit latrine faecal sludge, dramatically lowering the biomass's volume as they transform it (Banks *et al.* 2014).

Total live biomass production of BSFL $(P \le 0.0001)$ and live biomass change at the end of the study (P<0.0001) were significantly different among treatments (Table 2). The highest total live biomass production and live biomass change were recorded in coconut scrapings and vegetable & fruit waste while the lowest values for these two parameters were recorded in cow dung substrate. These differences are similar to those demonstrated by (Nguyen et al. 2013), who observed faster development of larvae in higher protein diets and slower development in lower protein diets. Therefore, the highest biomass change was observed in the reactor which contains coconut scraping and vegetables and fruits mixture may be attributed to the higher availability of readily available nutrients during the feeding process that enhances the growth of BSFL. Black soldier fly larvae must build up a huge fat mass in their larval stage because adults do not eat, but need energy until they complete their life cycle and survival of the adults (Craig Sheppard et al. 1994). BSFLs require more time to reach full maturity if their diets have low availability of fat (Nguyen et al. 2013). In contrast, Spranghers et al. (2016) reported that the development time of BSFL was not impacted by the waste they consumed, but the larval biomass production was almost 40% lower when reared on easily digested waste materials compared to complex and difficult-to-undigested waste.

It was observed that the volume and weight of waste is reduced throughout the experiment. There were significant differences among treatments on the substrate consumption rate (P<0.0001), the weight of the end-substrate (P<0.0001), the particle size of the end-substrate (P<0.0001), the particle size of the end-substrate (P<0.0001) and substrate conversion rate into BSFL live biomass (P<0.0001). The highest substrate consumption rate was observed in coconut scrapings (80.9%) while the lowest value (4.9%) was observed in poultry manure and rice bran mixture (Figure 1A). The highest substrate consumption rate of coconut scrapings may be associated with the

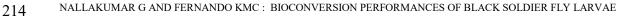
availability of easily decomposable materials for BSFL compared to other types of organic waste materials used in the experiment. On the other hand, the lowest weight of the endsubstrate (95.4 g) was recorded in coconut scrapings as most of the waste biomass was digested by BSFL and converted into live biomass (Figure 1B). The lowest substrate consumption rate was observed in poultry manure and rice bran mixture. This may be associated with the presence of complex structures of nutrients and the palatability of the BSFL. Isibika et al. (2019) revealed that total nitrogen, tannin and phenolic compounds present in the substrate negatively affected the size and weight of the BSFL and finally the biomass conversion ratio. In the present study, it was observed that 100% of the end-substrate of the coconut scrapings was less than or equal to 2 mm in particle size. However, the lowest percentages of the end-substrate having a particle size of $\leq 2 \text{ mm}$ was about 34% in soybean meal and 41% in poultry manure & rice bran mixture (Figure 1C). According to that BSFL was able to break down coconut scrapings into small pieces during their digestion compared to other organic substances used in the study. The range of the substrate conversion rate of the present study was 20% to 389% where the greatest value was recorded in cow dung and the lowest value was observed in poultry manure and rice bran mixture (Figure 1D). The lower substrate conversion ratio reflects the high substrate conversion efficiency. As Nana et al. (2018) mentioned that the substrate or feed conversion rate varies with the feeding ratio (weight/larvae/day). Further, they observed that the chicken had the highest SCR while kitchen waste reported the lowest at a feeding ratio of 220 mg/larvae/day.

There was a significant difference in pH among the aqueous solutions of treatments (P<0.0001) and ranged from 4.35 to 8.73 (Table 3). The lowest values of pH were reported by end-substrates of coconut scrapings and poultry manure and rice bran mixture while the highest value was reported by vegetables and fruits waste mixture. According to the standards used in Sri Lanka, the acceptable pH range of compost is 6.6 to 8.5 (Sri Lanka Standards 1635, 2019). Therefore,

Treatment	Initial live biomass of BSFL	Final live bio- mass of BSFL in 1 st batch (g)	Live biomass change of BSFL in 1 st batch (%)	Final live bio- mass of BSFL in 2 nd batch (g)	Live biomass change of BSFL in 2 nd	Total live biomass of BSFL at the	Live biomass change at the end of the
	(1 st batch & 2 nd batch) (g)				batch (%)	end of the experiment (g)	experiment (%)
1	40	115.33°	476.65°	49.33°	146.65°	164.66°	311.65°
2	40	128.00°	540.00°	60.67 ^b	203.35 ^b	188.67°	371.68°
3	40	121.33°	506.65°	60.67 ^b	203.35 ^b	182.00°	355.00°
4	40	129.33°	546.65°	92.67 ^a	363.35 ^a	222.00 ^b	455.00 ^b
S	40	207.33 ^a	936.65 ^a	48.33°	136.65°	256.66 ^a	541.65 ^a
9	40	169.33 ^b	746.65 ^b	85.33 ^a	326.65 ^a	254.66 ^a	536.65 ^a
7	40	67.33 ^d	236.65 ^d	50.00^{bc}	150.00^{bc}	117.33 ^d	193.32 ^d
<i>P</i> value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

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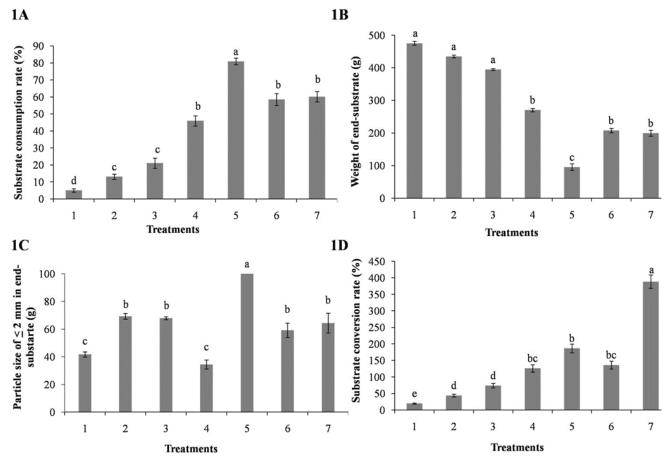


Figure 1: Effect of different treatments on (A) Substrate consumption rate (%) (B) Weight of the end-substrate (C) Percentage of particle size of $\leq 2 \text{ mm}$ in end-substrate (g) (D) Substrate conversion rate (%).

Mean values in each column followed by the same letter are not significantly different at 5% probability level according to DMRT. Error bars represent the standard error of means

when considering the pH value, only cow dung end-substrate could be used as compost before further processing. As BSFL is capable of reducing acidity, it can be effectively used for compost production with highly acidic substrates. pH of the compost depends on the acidic content of the substrate materials and different intermediate compounds of the substrate (Mutafela 2015). Therefore, pH of the compost may vary according to the substrate used for Black Soldier Fly composting.

EC was significantly different among treatments (P<0.0001) (Table 3). The final EC was in the range of 9.61 to 0.87 Ms/cm. As organic matter decomposes, the formation of soluble salts implies that exchangeable minerals including calcium, total phosphorus, and potassium are released into the reactors, enhancing the EC value of the compost. Additionally, the reactors with greater mineral concentrations, such as calcium, total phosphorus, and potassium, directly correlate to an increase in EC in the compost (Giannetto *et al.* 2020). Due to the additional increase in EC by BSFL, many restrictions may occur when compost is created from raw materials with high EC. However, BSFL may raise EC to a certain degree; it can be advantageous for substrates with very low EC values (Silva *et al.* 2015).

Total dissolved solids (TDS) of the aqueous solutions of different treatments were significantly different among treatments (P<0.0001). It ranged from 0.44 to 5.11 ppt (Table 3). Total organic and inorganic substances present in a suspension are indicated by the TDS value. Therefore, the reactor which contains vegetable and fruit waste mixture may be response.

sible for the higher availability of organic and inorganic compounds of the BSFL compost as it recorded the highest TDS value of the present study. The salinity of the BSF compost has a significant difference between all the treatments (P<0.0001). The treatment which used Food and vegetable waste was shown the highest salinity among other treatments. Salinity indicates the amount of salts dissolved in a suspension. Therefore, Food and vegetable waste was responsible for the high amount of salts during the BSF composting process. In addition, substrate reduction decreased with increasing salt concentrations. Several research studies have been conducted to examine the impact of the salt content of the medi-

Table 3: pH, electrical conductivity, total dissolved solids and salinity of the aqueous solution prepared from the end-substrates of the different treatments

Treatment	pH value	Electrical Conduc- tivity (EC) (mS/cm)	Total dissolved sol- ids (TDS) (ppt)	Salinity (ppt)
1	5.71 ^d	1.61 ^d	0.81^{d}	1.25 ^b
2	6.16 ^c	1.36 ^d	0.68^{d}	0.86 ^c
3	6.20 ^c	2.28 ^c	1.15 ^c	1.62 ^b
4	6.29 ^c	3.09 ^b	1.56 ^b	2.23 ^b
5	4.35 ^e	0.88 ^e	0.44 ^e	0.56 ^c
6	8.73 ^a	9.61 ^a	5.11 ^a	7.31 ^a
7	7.00^{b}	1.61 ^d	0.81 ^d	1.10 ^b
P value	< 0.0001	< 0.0001	<0.0001	< 0.0001

Remarks: Mean values in each column followed by the same letter are not significantly difference at 5% probability level according to DMRT

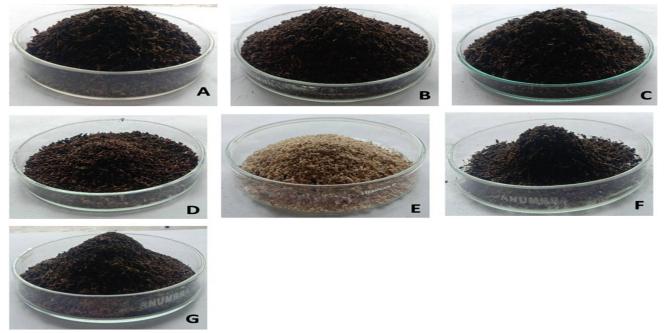


Plate 2: Appearance of the end-substrate of organic waste materials used in the study (A) poultry manure and rice bran mixture (B) goat manure and rice bran mixture (C) rabbit droppings (D) soybean meal (E) coconut scrapings (F) vegetable and fruit waste mixture (G) cow dung

um on BSFL. The rate of growth and maturation of the larvae, as well as the rate of decomposition of the medium, were reduced when BSFL were fed food waste that included NaCl compared to food waste without NaCl. This could be attributed to the suppression of BSFL growth caused by NaCl (Kim *et al.* 2021).

The colour and Texture of the end-substrate were significantly different among treatments. Colour and texture vary with the composition of the organic substrates used in the study. However, odour and the appearance of the end- substrate were not significantly different among treatments.

CONCLUSIONS

According to the findings of the present study it can be concluded that different types of locally available organic substances such as coconut scrapings, vegetable & fruit wastes, goat & poultry manure, rabbit's droppings, soybean meal and cow dung can be used as growth substrates for BSFL. Coconut scrapings reduced their weight rapidly compared to other organic substrates recording the highest total live biomass production of BSFL. However, cow dung reported the greatest substrate conversion rate among all organic substances used in the experiment. Therefore, coconut scraping and vegetable & fruit waste mixture are the most suitable substrates for growth of BSFL. This may be due to readily available nutrients which enhance the growth of larvae. However, the pH of the aqueous solution of coconut scrapings exceeded the SLS recommended value for compost. Other organic matter used in the study does not provide sufficient nutrients for larval growth. Due to its high rate of waste reduction, quick composting duration, and low level of odor generation, BSFL can be suggested as an acceptable alternative for management and recycling of selected organic substrates of coconut scrapings, vegetable & fruit wastes, goat & poultry manure, rabbit's droppings, soybean meal and cow dung. Furthermore, using BSFL over other composting techniques is recommended because of their advantages in the social and environmental spheres.

AUTHOR CONTRIBUTION

KMCF conceptualized & designed the study. GN performed the experiment & analyzed data. KMCF supervised the study. KMCF drafted the manuscript & critically revised the manuscript.

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