GROWTH, NUTRITIVE VALUE AND BIOCONVERSION EFFICIENCY OF PRE-PUPAL BLACK SOLDIER FLY FED ON URBAN HOUSEHOLD AND MARKET WASTE

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ABSTRACT

Globally, solid waste disposal is ranked second after unemployment as the most serious environmental problem in urban areas of low and middle-income countries. Poor collection and disposal of organic wastes from markets, households, and industries cause clogging of drainage systems during flood rains, formation of habitats and breeding grounds for pathogens and disease vectors, acidification and eutrophication of water bodies, local nutrient overloads, and sometimes climate change. Therefore, there has been a need not only to manage waste but also to add value to it using the Black Soldier Fly (*Hermetia illucens*) (BSF) on various organic substrates. This study aimed to evaluate the growth performance of the BSF fed on two different locally available Market Waste Substrates (MWS) and Household Waste Substrates (HWS). The study investigated the pre-pupal growth (length (cm) and weight (g), substrate reduction, bioconversion rate (BR), feed conversion rate (FCR) during the 45 days continuous feeding. Organic wastes (household and market waste) were introduced into rectangular basins (60 x 30 x 15 cm) with 20g of BSF eggs per rectangular basin with 500g of continuous feeding. From this study, MWS recorded higher pre-pupal yield of 3154.9 ± 0.219g (p>0.05), a pre-pupal weight of 0.2357± 0.219g (wet weight), while HWS registered a better pre-pupal length of 2.283±0.362. Under monitored favorable conditions (diet, temperature, humidity, and light), household waste recorded a better substrate reduction of 83%, while market waste registered a high bioconversion rate of 17 and feed conversion rate of 5.1. The nutritional profile for the two substrates was not significantly different P>0.05 with market waste CP 40.55% and household waste 41.21%. The nutritional values confirmed the great potential to reduce waste amounts significantly, minimize possible pollution and improve environmental sanitation as well as generate income while contributing to the saving of Earth's resources. This study confirmed the great potential of BSFL as a component of waste management.

Keywords: *Hermetia illucens*, bioconversion rate, waste substrate

INTRODUCTION

Globally aquaculture industry accounts for a massive (68%) of global fishmeal consumption (Naylor *et al.,* 2009). Fish meal is a major conventional ingredient in many aqua-feeds (El-Sayed, 2004) but is an expensive macro-feed ingredient (Tacon *et al.,* 2008). The capture of wild fish used to feed cultured fish is unsustainable (Naylor *et al.,* 2000). Developing countries such as Kenya are faced with shortages of animal protein. Therefore, prices of the few animal proteins in the market have soared beyond the reach of many smallholder farmers (Schönfeldt and Gibson Hall, 2012). This has led to a reduced intake level of protein countrywide, which results in incidences of protein-energy malnutrition and diseases (M'mboga, 2009).

Worldwide, poor solid waste disposal is ranked as the second most serious environmental problem in urban areas of low and middle-income countries after unemployment (UNDP, 1997; Diener *et al.,* 2009; Hoornweg and Bhada-Tata, 2012). The management of solid waste has undergone an evolution from practices of open dumping and burning to programs that emphasize the reduction of consumption, reuse, and recycling of resources (Mutafela, 2015). Overwhelming amounts of organic waste are generated by the rapidly increasing human population from diverse sources among them agricultural farms, municipal markets, households, supermarkets, industries, animals, and human settlements (van Huis *et al.,* 2013).

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The persistent increase in organic wastes generated worldwide is regarded as an emerging threat not only to humans but also to biodiversity and the ecosystem. Poor collection and disposal of organic wastes from markets, households, and industries cause clogging of drainage systems during flood rains, formation of habitats and breeding grounds for pathogens and diseases vectors such as rats, squirrels, wild dogs, and other pests, acidification and eutrophication of water bodies, local nutrient overloads and sometimes climate change (UN-HABITAT, 2010; Hoornweg and Badha-Tata, 2012, Gabler and Vinneras, 2014). In addition, the natural rotting of the waste generates harmful and noxious gases such as ammonia, methane, hydrogen sulphide and carbon dioxide, which have negative impacts on human health (Taiwo and Otoo, 2013)

Though waste substrate contains a lot of useful and recyclable nutrients, this form of waste is often considered unworthy and only about 5% is re-used majorly via composting and production of biogas energy (Zurbrugg, 2002; Diener, 2010; UN-HABITAT 2010; Hoornweg and Bhada-Tata, 2012). Transportation costs of the waste to rural areas where it can be used as organic fertilizer or animal feed discourage this option (Zurbrügg *et al.,* 2012).

Thus, there is a need to identify simple and affordable solutions appropriate to reducing consumption, embracing reuse and recycling of resources, and incorporating value addition in the management of generated waste (Van Huis *et al*., 2013). A good example is the use of bioconversion process that involves use of biological organisms such as redworms, micro-organisms and insect's larvae (Van Huis *et al*., 2013).

In Kenya, urbanization and increasing population are the main drivers for waste generation (NEMA, 2015). Urbanization has attracted a huge population of both informal settlement dwellers and the middle class. The Kenyan government has laws and policies guiding waste management, although there is sluggishness in prioritizing waste management, weak implementation practices which have led to towns and cities being overwhelmed by their waste (NEMA, 2015). For that reason, there is a need to identify simple and affordable solutions appropriate to African conditions to embrace reuse and recycling of resources and incorporate value addition in the management of generated wastes (van Huis *et al.,* 2013). For instance, through the bio-conversion process that involves the use of biological organisms ubiquitous in nature such as insect larvae (van Huis *et al.,* 2013; Nguyen *et al.,* 2015).

Quite a lot of insects have the potential to decompose organic waste and convert it into biomass Cickova H., Newton G.L., Lacy R.C., Kozanek M, (2015). One of the most interesting insects to use as a professional decomposer is the black soldier fly (BSF), *Hermetia illucens* (Diptera: Stratiomyidae). It lives in the tropical zone (Sheppard *et al.*, 2002), is not a disease vector, and is not harmful to humans or animals (Wang and Shelomi, 2017). The BSF larvae are largely considered an important candidate species to be used for animal and aquatic feeds (Cammack and Tomberlin, 2017). The BSF larvae have gained popularity both for their ability to decompose waste and serve as a source of protein for domestic livestock (Canary, 2012, Bullock *et al.,* 2013, Banks *et al.,* 2014). This insect technology has been used in management of waste with reduction values in the range of 50-79% being reported on municipal and household waste (Diener *et al.,* 2009; Lalander *et al.,* 2013; Banks *et al.,* 2014). In the process of transforming waste substrate, the larvae likewise eliminate bad odors and methane gas that is produced under anaerobic decomposition of organic wastes (Barry, 2004). Vahuis *et al*., 2013 reported BSF larvae having high protein content (≈ 40% of dry weight) and balanced profile of essential amino acids (AA) (Henry *et al.,* 2015; Liland *et al.,* 2017; Wang and Shelomi, 2017). They also have several macro and micro nutrients which are comparable to fishmeal and soyabean (Yu and Chen, 2009). The insects (BSF larvae) also have been known to convert the waste substrate to useful manure (Myers *et al*., 2008)

Although in nature BSF larvae consume a wide range of organic materials ranging from plant, animal and industrial wastes, not all the materials provide suitable diet for BSF larvae in confinement and commercial production. This is because of development time, fecundity, consumption, feed conversion efficiency, mortality, pupal weight and nutritional values of resultant biomass which all depends on diet (Tomberlin *et al*., 2002). Therefore, BSF larvae rearing could be a solution to managing and upcycling organic waste in an environmentally friendly and economically sustainable way. Thus, this study aimed to evaluate the performance of different organic waste substrates for BSF production as a key ingredient for climate smart aqua-feed. This is likely to improve food and protein security for the population (Gabriel *et al.,* 2007).

MATERIALS AND METHODS

Description of the Study Area

The study was carried out from November 2021 to February 2022 in Mtwapa Nyamone Farm. The farm is located along the Mombasa - Malindi highway at latitude 3.9364° S and Longitude 34° 16' 9.98" E and is characterized by generally high temperatures with a mean minimum of 24.7 ^{0}C, maximum 30.2 ^{0}C and high humidity (76%). The area receives mean annual rainfall of 1200 mm per annum in two rainy seasons (Kenya Meteorological Department, 2012)

Pre-pupae and waste substrate sourcing

Pre-pupae colony samples were sourced from Vicky farm in Karen End, Nairobi County. The farm is located about 50 km Southeast of Nairobi County, a mainly cold and highly populated area. The waste substrate collection was conducted in five villages along Mtwapa town Kilifi County, Kenya (3° 57' 0 S, 39° 45' 0 E), bordering Mombasa and Kilifi Counties to the south and north respectively. The distance from one household to another was approximately 100 meters. Mtwapa was considered due to its high human population of 20,677 people, Potential touristic development and presence of a nearby market (Government of Kenya, 2010**)**. Ten households were identified and trained on domestic waste selection and sorting. Two containers (150 l each) were allocated to a household for sorting and waste collection. The sorted and collected waste was weighed after every two days and transported to the BSF larvae breeding site. Market waste substrate (MWS) was sourced from Mtwapa market center in Mtwapa, Kilifi County. The waste substrate of fruits such as; mangoes, oranges, bananas, and vegetables were collected by gathering along the market and later transported to the experimental site. The waste substrate was chopped into small pieces using a knife and passed through a hollow tray to remove water. The selected substrates were majorly found and used in households and market waste form within the selected study site. Then six experimental trays (90 x 60 x 90 cm) were selected and clearly labelled according to the intended respective feeding treatment.

Experimental design for culturing of black soldier fly larvae

Considering the biological behavior of the BSF, a calm and quiet area was selected to avoid the disturbance. The experimental site was surrounded by different types of trees which makes the environment natural. A greenhouse structure (6 x 4 x 4 m) was used to construct for love-cage (where mating and laying eggs takes place) and another dark cage (6 x 4 x 4 m) where hatching of eggs and larvae growth takes place. Six rectangular containers (60 x 30 x 15 cm) were assigned in two treatments and three replicates.

Production of black soldier fly larvae

Love cage

A small greenhouse house (6 x 4 x 4 m) was constructed as a love cage for mating and laying eggs. The lower part of the house was fenced with hardboard and upper part and the roof with transparent polythene paper (for illumination of light), and maintain the right humidity and temperatures as recommended by Mutafela, (2015), also to prevent house flies and rainwater from getting inside during breeding. A pre-pupal amount of 5 kg each was placed in three different rectangular basins (60 x 30 x 15 cm) containing sawdust inside a nylon white cage net (1.8m x 2m x 1.5m) (Figure I1 b) as mentioned by Zhang *et a*l (2010) to contain the BSF in one position for easy eggs laying and collection. Since female BSF customarily lay their eggs in cracks and crevices, corrugated sheets were improvised to provide artificial cracks and crevices.

A bunch of corrugated sheets 2-3 inches long was tied together and hung over the waste substrates for laying eggs as BSF does not lay eggs on the waste (Figure 1 a). After 5-8 days the adult emerges from the pupal and are ready for mating and lay eggs. The adults BSF does not feed but only drink sugary water that is provided inside the nylon cage net. Wet fish and goat offal were placed inside a container (30 x 40 cm) to produce a putrid odor once rotten, to act as an attractant for female BSF to enter the bins and lay eggs in the corrugated sheets hanging over the waste substrate. Mating was observed between the adults BSF (Figure 1 b), where they were in contact, after 4-5 days cream eggs were observed hanged on corrugated sheets. The eggs were transferred to a dark cage for hatching and growth at humidity greater than 50% as mentioned by (Craig Sheppard *et al*., 2002).

Dark cage

A small dark cage house (6 x 4 x 4 m) was constructed for the hatching and growth of BSF eggs. The dark cage house had one rack (3 x 2 x 2 m) with the stands

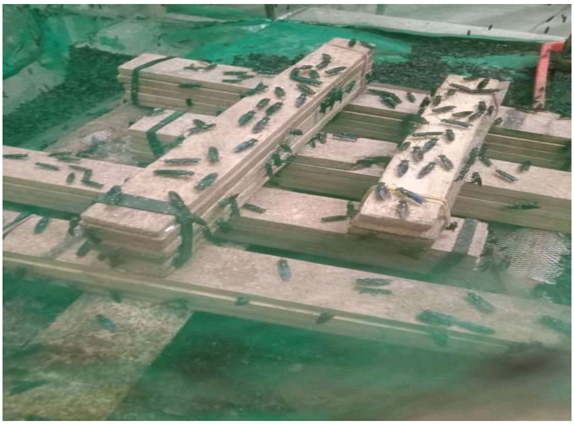

Figure 1 (a). Love cage BSF laying eggs on bundles **(b)** Adult BSF and mating and laying eggs

inserted in a container containing oil to prevent ants and other insects from entering the BSF house. The dark cage was covered entirely to enable eggs to hatch and grow as well as the newly emerged larvae effectively conserve the waste substrate provided.

Inoculation and hatching inside dark cage

The harvested eggs (Figure 2b) were placed over an open hatchling container with a high-quality food source (wheat bran or chicken feed for starter mixed with 70% water). The light creamed colored larvae hatched from the eggs after 4-5 days in optimum temperatures (26-30^0 C) and humidity (above 60%-70%) as reported by Zheng *et al.* (2012a) and larvae began falling from the eggs into the hatchling container below where they began feeding immediately. The larvae remained feeding in the same hatchling containers for five days after hatching.

100g of eggs were weighed (approximately 2000 larvae of the same size and age) and transferred into each of the six experimental containers. The retained larvae were placed into a nursery container where they continuously fed with both market and household waste until they transformed into prepupae within two weeks. The prepupae (Figure 2 d) started to leave the food source in search for a more suitable dry location to pupate. The larvae started to leave into a small dull colored collecting bucket placed directly under the exit ladder.

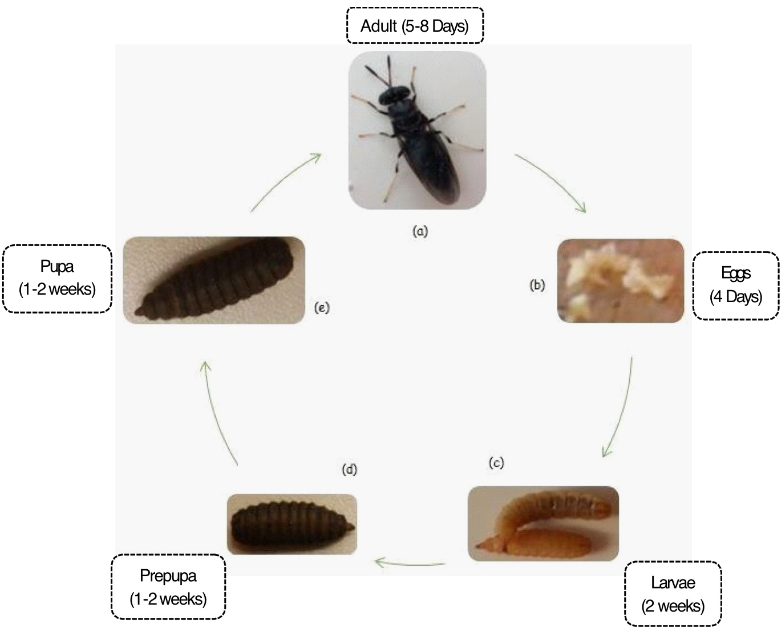

Figure 2. Life Cycle of *Hermetia illucens* (BSF)

Collection of Pre-pupae

When the larvae attained the pre-pupal stage (Figure 2 d), they stopped feeding and emptied their digestive tracts (Banks, 2014; Dortmans *et al.,* 2017). The pre-pupae used their hook-shaped mouthparts to emerge from the food source and reach a dry, dark and protected location to pupate into an adult fly (Figure 2 c) (Diener, 2010). At this stage the pre-pupae are found on top of the dry waste, and were handpicked (Figure 3) as others self-harvest. mass. The effect of different treatments was analyzed quantitatively in terms of weight gain and larvae growth rate, using a weighing scale and a ruler to sample 50 pre-pupae in each treatment (Figure IV a and IV b).

The effect of the different treatments on the nutrient content of pre-pupae was ascertained by proximate nutrient analysis as per procedures described in (AOAC, 2003). The dried BFL was ground into a powder form using kitchen blender. Analysis was done on dry matter basis using

Figure 3. Handpicking of pre-pupae black soldier fly larvae

The sampled pre-pupae were returned to love cage for continuous laying of eggs. However, 30% of the collection from both household and market waste were taken for proximate nutrient analysis (Table III). Upscaling of black soldier larvae using two wastes kept on course in both love and dark cage.

Sampling

Using a formula of $N = z^2.p.q.\ N/\ e^2\ (N-1) + z^2.p.q$: where, N = 10,000 (population of larvae in a container) z= 1.96 (desired confidence level is 95% and value obtained from table) p = 0.05 (sample proportion). q= 0.5 {(1-0.5) i.e., 1-p} e = 10% or 0.1 (approximate precision rate or acceptable error) (Kothari, 2004), 10 larvae were sampled after 30 feeding days from each container as per Tomberlin *et. al.* (2002). The total weight obtained was divided by the number of larvae weight. Food was continuously supplied until the first pupae were obtained, the addition of fresh food was stopped and larvae were allowed to feed for five more days to enable feed to dry up for easier sieving both larvae biomass and frass standard methods of the Association of Official Analytical Chemists (AOAC, 2003). Analysis of dry matter was done by drying pre-weighed samples in an oven at 105 ^{0}C for 16 hours to reach a constant weight. Nitrogen was analyzed using the Kjeldahl method. Crude protein, lipids and fiber were determined using procedures outlined by (AOAC, 2003). Ash content was determined by burning the samples in a muffle furnace at 550^{0}C for 4 hours.

Material consumption and a reduction efficiency

The efficiency of the BSFL to consume and reduce organic matter content in the fed substrate was determined by calculating waste reduction efficiency, feed conversion into increased body mass efficiency (feed conversion rate, FCR), and bioconversion rate (Banks *et al.,* 2014; Diener *et al.,* 2009):

Substrate reduction = Total feed added-residue feed after treatment/ Total feed added *100

Food conversion ratio= Feed consumed/ Total pre-pupal biomass (Zhou et al., 2013, Banks *et al*., 2014)

Bioconversion rate (%) = Total pre-pupal mass/ Total feed added *100

Statistical analysis

Data were collected and recorded in tables and computed into averages using Microsoft Excel for all parameters measured. Analysis of variance (ANOVA) was used to determine significant difference between feeding regimes. A student's t-test was used to compare parameters such as pre-pupal yield and waste reduction between treatments at p=0.05 significance level. All statistical analyses were conducted in Excel and data presented in form of graphs and tables

RESULTS

Breeding conditions

The optimal breeding conditions that were monitored included temperature, humidity, light, and diet, (Table I). Eggs performed better at temperatures of 28^{0}C-30 ^{0}C and humidity of >76% with no diets and in the dark with 0-50% daily light, juvenile larvae had lowest temperatures 23-33 ^{0}C, with no diet, and in darkness. The adults performed better during morning sunlight with sugary water and humidity of > 60%. Larvae over 4 days old had better growth when subjected to a special diet in darkness and temperature of 24-33^{0}C with humidity of 65-70%. Pre-pupae on the other hand needed no diet for growth, with a temperature of 24-33^{0}C and humidity 60-70% and performed better in darkness.

Substrate reduction, bioconversion rate and growth of larvae fed on organic waste substrates

There was no difference (P>0.05) in substrate reduction and bioconversion efficiency between household waste and market waste. Households waste recorded higher substrate reduction rate (83%) than market waste (73%). Larvae fed on HWS had higher length and width than those on MWS (P<0.01) but their fresh weight were similar (P>0.05). The bioconversion rates were nearly the same (16% vs 17%), respectively. The FCR was higher for MWS than HWS (5.1 vs 4.2). (Table II).

TABLE I- OPTIMAL BREEDING CONDITIONS FOR BLACK SOLDIER FLY LARVAE PRODUCTION.

Lifecycle stage	Temp	Humidity	Light	Diet
Eggs	28 ^{0}C-30 ^{0}C	>76%	Dark with 0-50% daily light	Diet
Juvenile larvae (4-6) days	23-33 ^{0}C	65-70%	Dark	None
Larvae over 4 days old	24-33 ^{0}C	65-70%	Dark	Species diet
Pre-pupae/pupae	24-33 ^{0}C	60-70%	Dark	None
Adults	25-32 ^{0}C	>60%	Morning sunlight	Watery sugary

TABLE II- SUBSTRATE REDUCTION, BIOCONVERSION RATE, GROWTH, LENGTH, WIDTH AND FEED CONVERSION RATE OF BLACK SOLDIER FLY LARVAE FED ON DIFFERENT ORGANIC WASTES

	Substrate/Feed resource		
Evaluation parameter	Household waste (HWS)	Market waste (MWS)	LSD
Substrate reduction (%)	83	73	
Bioconversion (%)	16	17	
Feed conversion ratio (FCR)	4.2	5.1	
Larvae length (cm)	2.28	2.03	0.2
Larva width (cm)	0.43	0.38	0.03
Larvae weight (g)	983.3	1151.00	1014.2

Market waste fed pre-pupae recorded similar (P>0.05) proximate compositions as compared to household waste fed pre-pupae with 40.55% crude protein, 13.32% crude fiber, 15.01% ash, and 33.41% fat content while household had 41.21%, 13.12%, 15.22%, and 34.12%, respectively of the same (Table III). ANOVA analysis of pre-pupal means per treatment showed no significant difference at the 95% confidence level (Table III).

TABLE III - PROXIMATE COMPOSITION OF BSF LARVAE FED WITH WASTES DURING 45 DAYS

*Parameters	BSF larvae substrate	
Proximate Analysis	**MWS**	**HWS**
Crude protein (%)	40.55±03	41.21±24
Crude fiber (%, $^{w}/_{w}$)	13.32± 22	13.12±31
Ash (%, $^{w}/_{w}$)	15.01±12	15.22± 17
Fat (%, $^{w}/_{w}$)	33.42±20	34.12±46

MWS-Market waste substrate, HWS-Household waste substrate. * Nutrient content was based on dry matter

DISCUSSION

Black soldier fly (BSF) larvae have the capability to convert various organic waste substrates into more valuable and less harmful biomass while emitting relatively fewer GHGs and little ammonia (Van Huis *et al.,* 2013 owing to their unique composition of gut microbiota. From the current study BSFL was able to handle a wide range of different waste substrates (household and market waste substrates) and thus reducing the biomass quantity as they converted it into manure. Studies by Newton *et al.,* (2005) and Barry (2004) have shown different biomass quantity reductions of 50%, Diener *et al* (2011) reported 65-75%, and Li *et al.*, (2011) reported 78% reduction, as compared with our study which recorded 83 and 73% household and market waste respectively.

In this study, the amount of pre-pupal harvested increased from day 5 of hatching until day 30 of the harvesting period. Likewise, as the number of adults produced increased so does the eggs in the love-cage. There were no foreign flies observed during the study period and this was supported by previous studies by Choi *et al.* (2009) and Park *et al.* (2014) who reported that BSF larvae produce antimicrobial compounds which discourage other fly species from laying eggs on the same medium (Zheng *et al.,* 2013). From the present study, maturity of larvae to pre-pupae was observed after 18 and 24 days in contrast with the reported average period of two weeks (Tomberlin *et al.,* 2009). Veldkamp *et al.,* (2012) suggested that poor environmental conditions such as temperature, relative humidity and poor diet can affect the life cycle of BSF larvae by less days or more. Thus, there are other factors affecting the normal period of maturation of larvae to pre-pupae.

The values for bioconversion rates in this study (16 to 17%) were found to be relatively similar to the findings of many workers (Banks *et al.* 2014, Diener *et al.,* 2011 and Newton *et al.* 2005) where bioconversion ranged from 11.7 to 20.8%. Initially, waste substrate produced a smelly rotten odour before BSF larvae were introduced, with time the smelly vanished in both study sites. This was in agreement with Diener *et al*. (2011) and Van Huis *et al.* (2013) who confirmed the ability of BSF larvae to reduce odour in wastes. This is achieved by making waste materials be processed extremely fast due to its voracious appetite.

In this study, higher production was observed in the substrate consisting of market waste as compared to household waste. From the previous studies, it has been reported that the growth of BSF larvae is better when fed on plant materials (St-Hilaire *et al*., 2007; Tomberlin *et al.*, 2009). This can be attributed to the superior nutritional quality of the substrate especially in terms of protein, carbohydrate, metabolizable energy, and fat contents when compared to the content of fruit and vegetable substrates. However, performance in terms of the total yield of pre-pupae was comparable to that of household waste substrates which had relatively high crude protein and carbohydrate content respectively (Arukwe *et al*., 2012).

The present study is in agreement with previous studies (Rehman et al., 2017; Herranz *et al.,*1983) that recorded poor BSF larvae consumption of plant materials such as stem, grasses, and mature leaves from the market due to the inability of BSF larvae to digest high lignin and cellulose content. This study indicates that lignin and cellulose content in a substrate is an important factor to consider in selecting suitable BSF larvae growing substrate.

The crude protein and lipid content of the pre-pupae from different substrates reflected the nutrient content of the feedstock used. The values in this study were comparable with fishmeal and soybean values as reported by St-Hilaire *et al.* (2007). The link between composition and diet suggests the possibility of growing BSF larvae on specialized diets to customize resultant biomass nutrient content to meet the needs of targeted animals (St Hilaire *et al.,* 2007). All substrates in this study yielded pre-pupal products that had high protein content. This indicates that BSF larvae are efficient at bioconversion as they are able to convert low nutrient feedstocks into high-value biomass with sufficient nutrient quantities. The technology of using waste substrate is expected to highly contribute to the reduction of production costs and make products more available in the market as, as well as reduce pollution.

CONCLUSION

This study was designed to evaluate growth, nutritive value and bioconversion efficiency of pre-pupal Black Soldier Fly fed on urban household and market waste. The findings established that BSF larvae could be used to recycle organic waste in small and medium scales. The quality and quantity of the larvae produced depend highly on the substrate used as feedstock and environmental conditions. Wide-scale application of this approach would greatly reduce the ecological and economic footprint of feed, thereby contributing to more sustainable animal husbandry systems. Furthermore, it can provide valuable ecosystem services through bioconversion of municipal (market) and household wastes into bio-compost. The production of BSF larvae can provide small fish farms with significant amounts of cheap but high-quality proteins. The technology is therefore recommended for adoption especially by smallholder farmers.

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