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Capacity of black soldier fly and house fly larvae in treating the wasted rice in Malaysia.

Song-Quan Ong[1,5*], Bui-Bui Lee[2], Geok-Pin Tan[3] and Saravanan A/L Maniam[4]

[1,2,3,4]KDU Penang University College. 32, Jalan Anson 10400 Penang Malaysia

[5]School of Biological Sciences, Universiti Sains Malaysia 11800 Pulau Pinang Malaysia *corresponding author Email:songguan26@gmail.com

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ABSTRACT

Rice is the major source of carbohydrate in the world and also the common composition in avoidable food waste. Due to the rich food culture in Malaysia, different pretreated rice waste is generated and the pretreat-component may affect the outcome in managing the rice waste using fly larvae. In this study, black soldier fly larvae (BSFL; *Hermetia illucens*) and house fly larvae (HFL; *Musca domestica*) are introduced to four types of rice waste: 1) steamed white rice (WR), 2) rice with curry (CR), 3) rice with coconut milk (CCR), and 4) fried rice (FR). The reduction rate of rice waste and larval survival rate, and nutrient analysis were measured by prepupal stages of both fly larvae. BSFL showed no significant difference in the reduction for four types of rice wastes (p= 0.28) and significantly higher survival rate than HFL for the CR and CCR wastes; indicating better tolerant to the feeding substrate. Although BSFL has significantly greater reduction rate (3.03 – 3.26 g /10 larvae/ day) than HFL, but in a fixed timeframe (20-25 days) four batches of HFL were generated and therefore having significantly more mass production than BSFL (500g of substrates generating 11.96g of BSFL but 22.62g of HFL). Rice waste management using fly larvae is effective subjected to the needs and purpose; BSFL is more adaptive to different types of rice waste and high in fat content, whereas HFL is sensitive to the waste but high in protein content.

1. INTRODUCTION

The amount of food waste in Malaysia is estimated to increase to more than 6 million ton per day by 2020 [1]. Food waste in Malaysia was managed as the municipal solid waste (MSW) and avoidable trashed food was the dominant composition that has occupied almost 60% of the MSW [1]. Moreover, an internal study for food waste was conducted by School of Biological Sciences, Universiti Sains Malaysia (USM) and KDU Penang University College, which finds rice is the main disposed avoidable waste in five cafeteria of USM healthy campus. These food wastes may bring significant impact to the environment as they emitt greenhouse gasses that cause climate changes when decomposing at the landfill [2]. Transforming rice and kitchen waste into animal feed should be the most effective food waste management in terms of minimizing energy and heat loss; however this practice is restricted by the FEED ACT (Act 698) (Law of Malaysia, 2009) [3] in Malaysia which prohibits the use of food waste as animal feed.

The idea of converting food waste into fly larvae is commendable. As demonstrated by numerous studies [4, 5, 6], fly larvae such as black soldier fly larvae (BSFL) and house fly larvae (HFL) has significant role in reducing organic waste volume and biologically convert the waste into useful protein for livestock animal feed [6]. By using BSFL and HFL on the organic waste, it is crucial to understand the capacity and nutrition value of the larvae on the feeding substrate. As demonstrated by Ooninсx et al (2015) [7] their survival rate and growth rate were greatly affected when the BSFL grew on different culture environment and medium and (Cickova et al. 2012) [5] had shown that house fly preferred on relatively moister breeding medium. Most of the kitchen wastes in Malaysia were unsegregated and consisted of unknown pretreated components such as curry paste.Therefore in order to manage rice waste using fly larvae, the effect of the pretreated components and the capacity of the fly larvae in reducing rice waste have to be investigated.

2. Material & Methods

2.1 Fly culture

A population of black soldier fly, *Hermetia illucens* L., was maintained under a poultry house at Balik Pulau, Penang. The poultry house was placed on a meadow, exposed daily to direct sunlight for about 8 h. A black plastic foil-covered tray (2 m) containing organic waste was used to attract ovipositing females and eggs laying. Larvae hatched approximately 3 days after oviposition and the young larvae at an age of 4–6 days were then introduced into the pretreated rice waste.

For the house fly, *Musca domestica* L. the WHO/VCRU house fly strain was used in the study. The population was reared at 25±2°C and 67±5% relative humidity (RH) with a photoperiod of 8:16 (L:D) h in Insectarium II at the Vector Control Research Unit (VCRU) of University Science Malaysia. First to second instar larvae were identified by their body size (2-5mm) and introduced to the rice waste substrate.
Both pre-pupae of BSFL and HFL were identified with the presence of larvae in the drying agent and morphological identification: BSFL forming blackish exoskeleton and HFL forming reddish exoskeleton [8].

2.2 Rate of reduction and Survival rate

The experimental methods used in this study are slight modified from Nguyen et al. [6, 9]. Four types of wasted rice: 1) white rice (WR), 2) curry rice (CR), 3) coconut milk rice (CMR), and 4) fried rice (FR) were obtained mainly from kitchen and plate waste from the cafeteria in University Sains Malaysia. To keep the waste medium consistent, large quantity of rice waste was first grinded into a homogenous mixture, packaged and frozen for use throughout the experiment [9]. Briefly, fifty BSFL and HFL were placed in a container cup (16 oz) with 50g of medium and drying agent was placed externally for harvesting

the pre-pupae. The containers were monitored daily and the presence of larvae on the drying agent indicating the prepupae formation of the larvae [9], and the remaining waste medium was weighed and the time in days were recorded. Each waste medium repeated for 5 times and the control replicates were set up at the same time as described above but contained no larvae. This control was used to counter for any reduction in waste weight due to water evaporation and microbes decomposition, and the daily waste reduction from the larvae was corrected by this loss. As the pupa were collected, they were proceeded to the nutritional study.
Due to different development time for BSHL and HFL to pre-pupae stage (in this study BSHL takes 19.2±3.6 days; HFL takes 4.5±1.9 days) and therefore the reduction waste was measured as the mass reduced per larvae per days [9] and overall deduction percentage from initial 500g of rice waste. The capacity of BSFL and HFL in reducing four types of rice waste was compared using one-way analysis of variance (ANOVA) with post-hoc least significant difference (LSD) at level of $p<0.05$ in SPSS 17.0.

2.3 Nutrition analysis- proximate analysis The nutrition of pre-pupae of BSFL and HFL were determined according to the Association of Official Analytical Chemists International (2002) [10]. The moisture of the pre-pupae was conducted according to the AOAC (2002), Official Method 934.01, in which two subsamples of each sample weighing 2g respectively were placed in a crucible drying in an oven for 24 hours at 100ºC. The samples were weighed in an electrical balance before and after drying and moisture was indicated by the differences of mass and it was converted into percentages. As for the ash content, dry ashing method was used to determine the content. The samples were put in a preweighed ceramic cup and incinerated in a furnace. The crude protein content of the pre-pupae meal subsamples were determined by measuring the total nitrogen (N) content according to the method described by Association of Official Analytical Chemists International (2002), Official Method 4.2.07, Kjeldahl method. Approximately 0.10 g dried sample digested with concentrated sulfuric acid and catalyst in a Kjeldahl flask. The products were later cooled down at room temperature and sodium hydroxide was added into the flask. The flask was subjected to the distillation connection unit and the distillate was mixed with boric acid and few drops of methyl red. The distillate mixture was titrated with 0.40% hydrochloric acid and calculated the protein in percentage. The Crude Fat or Ether Extract (EE) content was determined by making use of the diethyl ether reagent method using the Tecator Soxtec System HT 1043 Extraction Unit according to Association of Official Analytical Chemists International (2002), Official Method 920.39. Two subsamples of each sample weighing 2g respectively were placed in a soxhlet fat beaker. Thereafter 50ml of diethyl ether was added to the subsample and placed into the Tecator Soxtec

System HT 1043 Extraction Unit. The subsamples were placed in a drying oven for 2 hours at 100ºC. The residue weight was the lipid and was expressed in the percentage. The total carbohydrate content in the samples was calculated by difference method. The food's constituents (protein, fat, water, ash) were determined individually, summed and subtracted from the total weight of the food. This is referred as the total carbohydrate by difference and it should be clear that the carbohydrate estimated in this method included fibers [17].

The nutritional contents were expressed in percentage and the value will be prior transformed by arcsine transformation and compared t-test in SPSS 17.0 at $p< 0.05$.

3. **Results & Discussion**

For the BSFL, no significant difference in reducing four types of waste, in which BSFL can reduced 3.03 – 3.48 g/ 10 larvae / day an overall 71.24 to 73.16% of rice waste. The BSFL reduction rate on rice waste was parallel to the studies of Diener et al. (2011) [4] and Oonincx et al. (2015) [7] that having the reduction rate ranged from 60 to 80% on the organic waste. The capability of BSFL in bioconversion of rice material had also been reviewed by Zheng et al. (2012) [11] in which applying BSFL and microbes Rid-X converting rice straw into larval grease that able to use as biodiesel. Comparing mass of the rice waste reduction rate per larvae between BSFL and HFL for all rice waste, BSFL showed significantly greater reduction rate than HFL ($P<0.05$, Table 1). This greater reduction rate could be explained by the nature of consumption of BSFL that having larger body size compared with HFL and required more calorie consumption [12] although digestive physiology and metabolism should also play a role in animal food consumption. In addition, BSFL demonstrated no significant difference on the survival rate for four rice waste; indicating growth of BSFL not influenced by the pretreated components of rice waste. Although BSFL showed promising reduction properties on four rice waste, but when comparing the mass conversion from rice waste to larvae mass, HFL generated significantly higher mass (22.62 ± 0.84g) compared to BSFL (11.96 ± 0.81g) in the experiment time frame (20-25 days). This is mainly attributable to the shorter development time taken by HFL in which it takes 4.5 days to become prepupae compared with BSFL which takes 19.2 days and in this study an average of 4 batches of house fly prepupae can be produced relative to one batch of BSFL, and therefore the mass of HFL is greater than BSFL.

Nevertheless, due to the concern of pest status, house fly seldom used as the food waste management agent and most of the HFL organic waste studies were focused on the animal manure [5, 13] to the best of our knowledge, this study is the first one reporting the use of rice waste as the growth substrate for HFL. The reduction and survival rate of HFL are showed in Table 1 in which HFL demonstrate relatively inconsistent reduction as the reduction rate for curry and coconut milk rice waste are significantly lower than the WR and FR ($p<0.05$, Table 1). This may be due to the presence of curry having certain amount of spices such as citronella that poses as a repellent of the insect [14]. The inconsistency in the survival rate of HFL also suggests the house fly growth is sensitive to the environment and pose challenges when applying HFL in the larvae management on the food waste with different pretreated components.

As for the nutritional analysis, prepupal of house fly significantly composed of high crude protein whereas prepupal of BSF significantly composed of high crude fat ($p<0.05$, Table 2). This study, HFL that feeds on the rice waste generated crude protein and fat percentage in the range as demonstrated by Pretorius (2011) [15] that using standard larvae meal as the substrate. BSFL consist of high level of crude fat but low protein content as showed in many studies [4, 16] and this was suggested as the adult black soldier fly do not eat and therefore, they usually need to build up a fat body that is necessary to complete development and survive as adults long enough to mate and lay eggs.

4. Conclusion

Rice waste management using fly larvae is effective subjected to the needs and purpose; BSFL is more adaptive to different types of rice waste and high in fat content, whereas HFL is sensitive to the waste but high in protein content.

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Table 1 Reduction and survival rate of BSFL and HFL on four different rice waste

		Reduction rate: Means mass ± S.E. (g) / 10 larvae / day	Reduction rate: Mean percentage ± S.E. (%)	Survival rate ± S.E. (%)
BSFL	WR	3.26 ± 0.06 ns	72.12 ± 2.85	82.80 ± 2.15 ns
	CR	3.03 ± 0.26 ns	69.68 ± 4.15	71.20 ± 3.08 ns
	CMR	3.48 ± 0.21 ns	73.16 ± 1.90	78.40 ± 1.72 ns
	FR	3.21 ± 0.21 ns	71.24 ± 2.58	78.00 ± 4.69 ns
HFL	WR	0.89 ± 0.03a	79.60 ± 0.82	70.50 ± 6.74a
	CR	0.58 ± 0.06b	48.56 ± 0.28	46.30 ± 1.95b
	CMR	0.66 ± 0.02c	60.73 ± 1.20	68.50 ± 0.02c
	FR	0.88 ± 0.04a	74.76 ± 2.03	81.80 ± 0.77d

BSFL black soldier fly larvae; HFL house fly larvae; WR white rice; CR curry rice; CMR coconut milk rice; FR fried rice; SE standard error; Ns no significant difference; Means followed by the same letter are not significantly different ($P>0.05$).

Table 2 Nutritional value of prepupae of BSFL and HFL and total mass generated from 500g of rice waste

	CP	CF	Moisture	Ash	Carbohydrate	Total mass ± S.E.
BSFL	41.02± 0.81	32.78± 0.81*	4.02	10.63	11.55	11.96 ± 0.81
HFL	60.87± 0.81*	16.64± 0.81	1.59	6.92	13.98	22.62 ± 0.84

BSFL black soldier fly larvae; HFL house fly larvae; CP crude protein; CF crude fat; SE standard error *significantly different at $P<0.05$

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