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Full Length Article

Performance of Tropical Fruit Wastes as Oviposition Attractants and Growing Substrates in Rearing Black Soldier Fly (*Hermetia illucens*)

Resti Rahayu[1*], Ratih Rahmawati[1], Mairawita Mairawita[1], Dodi Devianto[2] and Ramadhani Eka Putra[3]
[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas, Padang, Indonesia*
[2]*Department of Mathematics, Faculty of Mathematics and Natural Sciences, Universitas Andalas, Padang, Indonesia*
[3]*School of Life Science and Technology, Institut Teknologi Bandung (ITB), Bandung, Indonesia*
[*]For correspondence: restirahayu@sci.unand.ac.id
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Abstract

This study investigated the effectiveness of several tropical fruit wastes as oviposition attractants of Black Soldier Fly (BSF) and evaluated different hatching substrates and growing substrates to BSF larval performance. There were three sets of experiments, including an oviposition attractant using three fermented fruit wastes (rotten orange, pineapple peel, and overripe jackfruit), which scored based on the quantity of BSF egg laid on the egg trap, a hatching substrate experiment using chicken feed and rice bran for the early larval stage, and a growing substrates experiment using banana peel and pineapple peel. The growing substrate was combined with hatching substrate experiments resulting in six unit combinations. The result showed that the highest score of BSF egg found was in rotten orange and pineapple peel. The chicken feed showed the highest larval performance, producing the maximum larval dry weight (53.42 ± 10.39 g) as the hatching substrate. Chicken feed-pineapple peel treatment exhibited the most productive result, which had the highest larval dry weight, substrate consumption, and waste reduction index in the 19-day experiment. The proximate analysis of three selected treatments revealed a high protein content, up to 61%. In the oviposition test, all the fermented fruit waste might serve as an attractant substrate for BSF, yet overripe jackfruit was less favorable than rotten orange and pineapple peel. BSF larvae performance was better in chicken feed as hatching substrates, while pineapple peel outperformed banana peel as a growing substrate in BSF larvae.

Keywords: Fruit waste; Growing substrate; Hatching substrate; *Hermetia illucens* L.; Oviposition attractant

Introduction

Black Soldier Fly (BSF; *Hermetia illucens* L.) has received widespread attention for its role in integrating environmental challenges caused by increased biowaste and providing affordable and sustainable protein sources. Implementation of BSF in managing biowaste for bioconversion has such excellent points due to its low risk of global warming (Salomone *et al.* 2017), high yield low-cost process (Salam *et al.* 2022) and converting diverse bio-waste into high-nutrient biomass efficiently (Gold *et al.* 2018; Lalander *et al.* 2019; Surendra *et al.* 2020; Siddiqui *et al.* 2022). BSF is also well known for its potential as a natural resource for various components such as enzymes (Kim *et al.* 2021), chitin (Purkayastha and Sarkar 2020), biodiesel (Park *et al.* 2022), antimicrobial peptide (Zhan *et al.* 2020), bioplastic (Barbi *et al.* 2019) and natural pigment (Ushakova *et al*. 2019).

Vegetables and fruit contribute to the most food waste, accounting for 60 percent (Sagar *et al.* 2018). Fruit waste is produced by rotten fruit, inedible parts, or as a byproduct of production, storage, distribution, or consumption. Tropical fruits such as oranges, pineapples and bananas generate a lot of waste (Leong and Chang 2022). Thus far, fruit waste has been studied as a source of various bioactive compounds (Gupta *et al.* 2019; Murakonda and Dwivedi 2021), biosorbents (Gupta *et al.* 2019), biocomposites and films (Gowman *et al.* 2019), bioethanol (Zanivan *et al.* 2021) and as the substrate for rearing BSF larvae as well (Meneguz *et al.* 2018; Lalander *et al.* 2019).

Exploring the utility of fruit waste in BSF rearing may facilitate in yielding a high-quality BSF as a protein source for human food security while also improving biowaste management. This study evaluated several tropical fruit wastes for their effectivity as attractant substrates and growing substrates for BSF oviposition and also evaluated different hatching substrates in BSF larvae performance. The experiment was divided into three sets: oviposition attractant, hatching substrate and growing substrate. This research hopefully provides further information on the use of tropical fruit waste to strengthen BSF bioconversion technology.

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Materials and Methods

Study site and BSF colony

The study was done at the MinaGot House, one of the BSF research and cultivation sites in Padang, West Sumatra, Indonesia. The BSF colony used was established from MinaGot House as well. The experiment was performed at a temperature of 29 ± 1°C and humidity 65 ± 5%.

Materials

The attractant substrates used were rotten oranges, pineapple peel, and overripe jackfruit. The hatching substrates used were rice bran and chicken feed. The growing substrates used were banana peel [the variety of Uli bananas (*Musa paradisiaca* L.) and Kepok bananas (*Musa acuminata* × *Musa balbisiana*)] and pineapple peel. All material fruit wastes were collected from a market city in Padang, West Sumatra, while rice bran and chicken feed were commercial products. The chicken feed used was Hi-Pro-Vite®-511-BRAVO.

Experiment of oviposition attractant

Each test container (10 × 15 × 3 cm) held 500 g of the fermented attractant covered with mesh to prevent BSF from depositing their eggs directly in the container. Egg traps comprise five units of four wooden sheets (30 × 3 × 0.5 cm), separated by the push pin for each and fastened together by rubber bands at each end. Each container was put in a BSF cage (1 × 1 × 1.25 m) at a distance and egg traps were arranged on every container (Fig. 1). The experiment was carried out every two days for 28 days (14 times). The weight of the eggs was ranked from lowest to highest and scored on a scale of 1–3, with three being the highest. During each observation period, each container was replaced at random.

Experiment of hatching substrate for early larvae

The experiment was performed by a completely randomized design with three treatments, *i.e*., rice bran, chicken feed, and a mixture of rice bran and chicken feed (1:1), each in triplicate. For each hatching substrate (200 g), approximately 300 mL of water was added and was mixed thoroughly until soft and placed in the respective container (13 cm diameter x 9 cm height). For each container, a wire mesh (4 x 4 cm) that bent at the edges was placed on the substrate as the “egg stand” and was covered by a tissue sheet on the top so the egg did not directly expose to the substrate (Fig. 2). After placing 1 g of BSF eggs on the wire mesh, the container was screened with gauze to prevent the infestation from pests or other insects. After seven days, several parameters, including fresh weight, dry weight, and moisture content of BSF larvae, were measured. This whole experiment was conducted in duplicate or two units simultaneously to cover the need for parameter measurement of hatching substrates and the subsequent trial.

Experiment of growing substrate

This experiment was combined with the hatching substrate resulting in six combinations, *i.e*., A1B1 (rice bran-banana peel), A1B2 (rice bran-pineapple peel), A2B1 (chicken feed-banana peel), A2B2 (chicken feed-pineapple peel), A3B1 (rice bran+chicken feed-banana peel), A3B2 (rice bran + chicken feed-pineapple peel), each in triplicate.

For pre-treatment, two growing substrates were chopped to 0.5 cm in size. Each treatment had nine test containers separated into three units for each observation period (7, 13 and 19 days). Each container contained 20 g of the substrate and 200 7-day-old BSF larvae derived from the hatching substrate experiment (Fig. 3) and labeled for identification. Each container received 20 g of additional substrate every three days until the study was completed. The weight of larvae and substrate was measured at seven days, 13 and 19 days of experiments.

Parameters measured were larval biomass (dry weight) (1), substrate consumption (2), waste reduction index (3), and proximate analysis (crude protein, crude fat, moisture content, ash and crude fiber), with the formula as following:

$$\text{Larval weight (mg)} = \frac{\text{total larval weight (g)}}{\text{the amount of larva}} \quad (1)$$

$$\text{Substrate consumption (\%)} = \frac{W\text{-}R}{W} \times 100 \quad (2)$$

$$\text{Waste reduction index (WRI)} = \frac{D}{t} \times 100,\ D = \frac{W\text{-}R}{W} \quad (3)$$

Where W = total amount of feed provided (g), R = remaining substrate (g), D = substrate consumption, t = days of trial (day).

Harvesting larvae and proximate analysis

The 21-day-old BSF larvae were separated from the residue by sieving, and the larvae were washed off to eliminate the dirt. Larvae were dipped in hot water for about 1 min and sun-dried subsequently to reach constant weight. Dry larvae were grounded to powder for proximate analysis. The proximate analysis was done according to the Association of Official Analytical Chemists (AOAC 2006) and was performed on three representative treatments, selected by their highest performance.

Statistical analysis

Data collected from all treatments were compared by ONE-WAY ANOVA ($P < 0.05$) using SPSS 19.0. The normal distribution and homogeneity of variance of the data were confirmed by Shapiro–Wilk and Levene’s test, respectively. Duncan’s new multiple range test was used for a post hoc test at 5%.

Results

Experiment of oviposition attractant

All treatment of fermented fruit waste could attract BSF for oviposition (Table 1). The fermented rotten orange and pineapple peel had an equal score and was significantly different from the fermented overripe jackfruit ($P < 0.05$). In comparison to the other two attractants, jackfruit performs poorly. In another study, fermented pineapple fruit attractant produced the highest egg weight compared to other fermented substrates, such as banana, bran, and cassava (Lamin *et al.* 2020). On the contrary, Sripontan *et al*. (2017) found no BSF eggs in fresh pineapple attractant.

Experiment of hatching substrate

The chicken feed treatment produced the highest value in all parameters with statistically significant differences in fresh weight of BSFL as compared to rice bran and rice bran+chicken feed (Table 2). Rice bran and rice bran+chicken feed substrate did not reveal statistically different values in all measured parameters, but rice bran+chicken feed consistently outperformed rice bran.

Substrate consumption

The chicken feed-pineapple peel treatment had the highest substrate consumption for all periods (Fig. 4), with the highest value reaching 55.07% for the 13-day experiment. The substrate consumption of the chicken feed-pineapple peel in the 7-day experiment indicated a statistically significant difference compared to all treatments ($P > 0.05$). The percentage of substrate consumption of all banana peel (A1B1, A2B1, A3B1) and pineapple peel (A1B2, A2B2, A3B2) treatments ranged from 26.77–29.54 and 26.25–43.24%, respectively. All treatments had a similar tendency of substrate consumption, which was maximum in the 13-day and decreased in the 19-day experiment.

Waste reduction index

The maximum substrate reduction occurred in the 13-day and declined in the 19-day for all treatments (Table 3). The highest WRI was found in the chicken feed-pineapple peel treatment but was statistically insignificant ($P < 0.05$). WRI for banana peel and pineapple peel substrates ranged from 1.58–1.83 and 1.46–1.98%, respectively (Fig. 5). The previous study revealed comparable results to this current study, with the WRI of banana peel being 1.50% (Putra *et al.* 2020).

Larval biomass

Larval weight increased over time until 19 days, but there were no significant differences among all treatments ($P > 0.05$) (Fig. 6). The highest dry weight at the end of the experiment was chicken feed-pineapple peel (12.6 ± 1.15 mg), while the lowest was in the rice bran-pineapple peel (9 ± 1.67 mg). Another trial showed that fermented pineapple peel substrate could produce a higher dry weight of up to 16 mg (unpublished data).

Proximate analysis

A1B1 (rice bran-banana peel), A2B2 (chicken feed-pineapple peel), and A3B1 (rice bran + chicken feed-banana peel) were the selected treatments for proximate analysis. The highest percentage of protein, fat, and carbohydrate in

Table 1: Average scoring value (mean ± SE) based on the level of BSF egg weight obtained from different fruit waste attractant substrates during the experiment

Attractant substrate	Average scoring value
Fermented rotten oranges	2.36 ± 0.19^{b}
Fermented pineapple peel	2.36 ± 0.19^{b}
Fermented overripe jackfruit	1.29 ± 0.12^{a}

Note: Different letters within the column indicate significantly different results ($P < 0.05$)

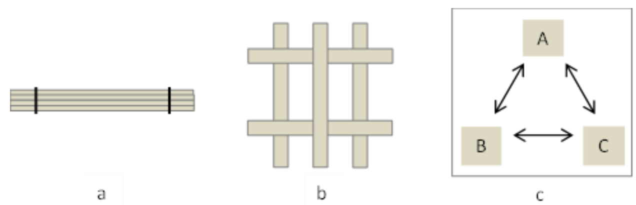

Fig. 1: Experimental illustration of oviposition test, (**a**) single wooden-sheet egg trap (side view), (**b**) egg traps order on each container (top view), (**c**) layout of test container in BSF cage replaced randomly every 2 days (capital letters represent each attractant container)

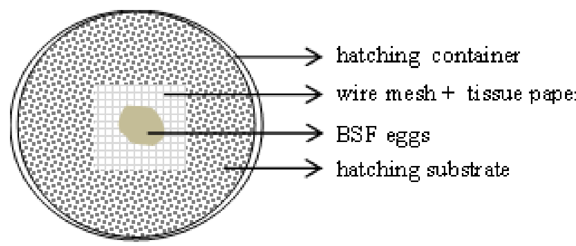

Fig. 2: Illustration of the hatching substrate container (top view)

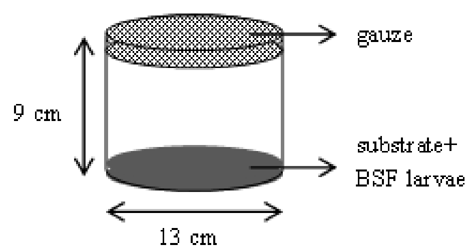

Fig. 3: Illustration of growing substrate container

Table 2: Effect of different hatching substrates on growth performance of 1 g of Black Soldier Fly eggs for seven days experiment (mean ± SE)

Hatching substrates	Fresh weight (g)	Dry weight (g)	Moisture content (%)
Rice bran	153.29 ± 3.67[a]	41.82 ± 0.87[a]	72.71 ± 0.46[a]
Chicken feed	244.05 ± 34.03[b]	53.42 ± 10.39[a]	78.38 ± 1.61[b]
Rice bran + chicken feed	159.49 ± 11.56[a]	42.96 ± 1.18[a]	72.89 ± 1.24[a]

Note: Different letters within the column indicate significantly different results (DNMRT 5%)

Table 3: Comparison of reduced substrate weight (dry weight) and WRI of BSF larvae at different treatments during observation (n = 3)

Treatment	Days	Total substrate (g)	Substrate residue (g)	Substrate reduction (g)	WRI (%)
A1B1	7	8.61	7.97	0.64	1.06
	13	14.35	8.2	6.15	3.3
	19	20.09	14.05	6.04	1.58
A1B2	7	10.8	9.03	1.77	2.34
	13	18	11.77	6.23	2.66
	19	25.2	18.2	5.3	1.46
A2B1	7	8.61	7.52	1.09	1.81
	13	14.35	9.34	5.01	2.68
	19	20.09	13.11	6.98	1.83
A2B2	7	10.8	6.79	4.01	5.3
	13	18	8.09	9.91	4.24
	19	25.2	15.73	9.47	1.98
A3B1	7	8.61	7.27	1.34	2.22
	13	14.35	8.55	5.8	2.82
	19	20.09	13.53	6.56	1.72
A3B2	7	10.8	9.32	1.48	1.95
	13	18	11.49	6.51	2.78
	19	25.2	17.55	7.65	1.6

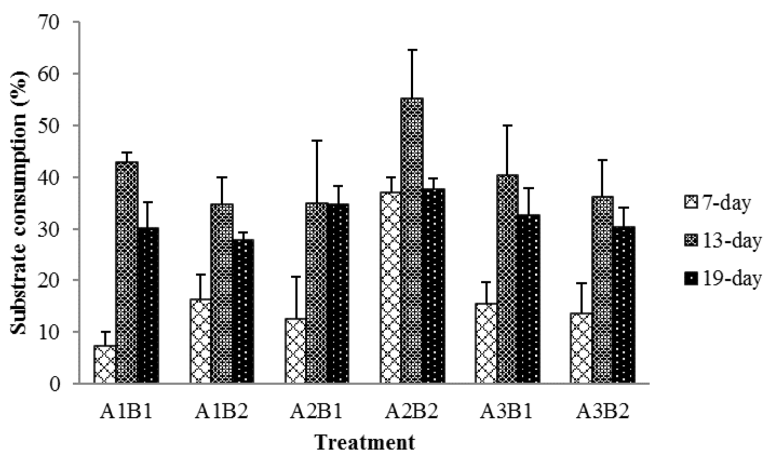

Fig. 4: Effect of different fruit waste substrates on substrate consumption (mean ± SE) of Black Soldier Fly larvae for 19 days experiment. [Details: A1B1 (rice bran-banana peel), A1B2 (rice bran-pineapple peel), A2B1 (chicken feed-banana peel), A2B2 (chicken feed-pineapple peel), A3B1 (rice bran+chicken feed-banana peel), A3B2 (rice bran+chicken feed-pineapple peel)]

larvae was shown in A3B1, A2B2 and A1B1, respectively. However, we found that all selected treatments are high in protein. The fat and ash content were similar among treatments, yet the differences were high in carbohydrates and crude fiber levels.

Discussion

The current study found that the effectiveness of the three fruit wastes varied in attracting BSF to lay eggs. The insignificant of fermented jackfruit might be due to the absence of jackfruit as a feeding substrate in MinaGot House, while rotten orange and pineapple peel are occasionally used. Prior exposure to a substrate as a food source could affect insect behavior in oviposition, especially for dipteran (Jaenike 1983). Several studies have shown that BSF preferences among various attractants are not consistently referring to the same substrate despite testing the identical one (Nyakeri *et al.* 2017; Sripontan *et al.* 2017; Boafo *et al.* 2023). BSF tend to choose organic matter to which they have ever been exposed (Sripontan *et al.* 2017; Ewusie *et al.* 2019; Boafo *et al.* 2023). Nonetheless, fermented jackfruit still attracts female BSF in oviposition, probably due to the scent of fermentation. Specific volatile

Table 4: The nutritional content of 21-day-old BSF larvae reared on different substrates

No.	Parameter % (g/100g)	Treatment		
		A1B1	A2B2	A3B1
1.	Moisture content	4.25	6.6	5.68
2.	Ash	8.3	8.13	7.55
3.	Crude protein	58.02	59.19	61
4.	Crude fat	23.79	24.74	23.04
5.	Carbohydrate	5.5	1.31	2.61
6.	Crude fibre	0.9	0.24	0.18

Note: A1B1 (rice bran-banana peel), A2B2 (chicken feed-pineapple peel), A3B1 (rice bran+chicken feed-banana peel)

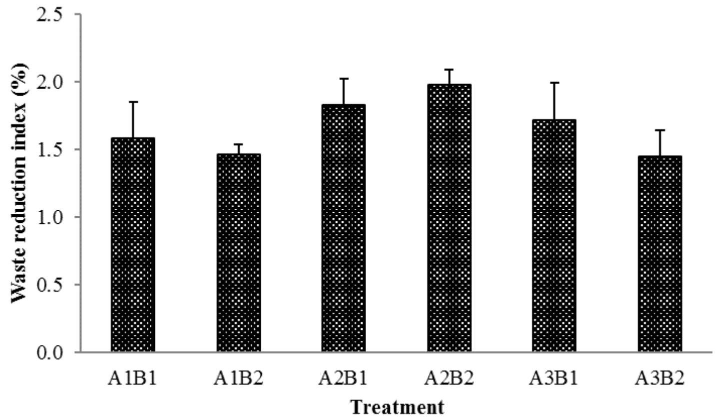

Fig. 5: Effect of different fruit waste substrates on waste reduction index (mean ± SE) of Black Soldier Fly larvae for 19 days experiment. [Details: A1B1 (rice bran-banana peel), A1B2 (rice bran-pineapple peel), A2B1 (chicken feed-banana peel), A2B2 (chicken feed-pineapple peel), A3B1 (rice bran+chicken feed-banana peel), A3B2 (rice bran+chicken feed-pineapple peel)]

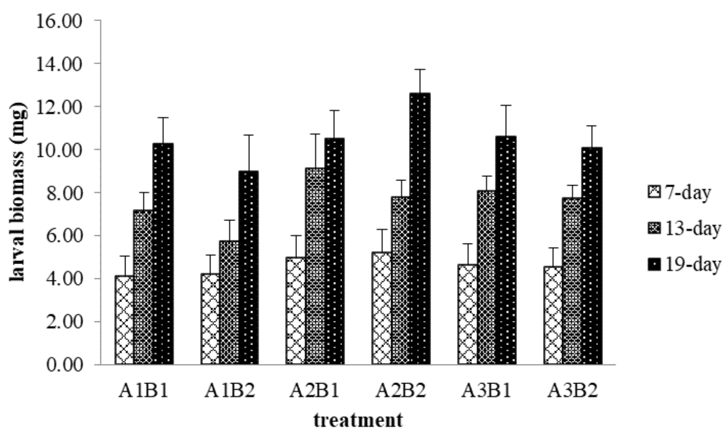

Fig. 6: Effect of different fruit waste substrates on dry weight (mean ± SE) of Black Soldier Fly larvae for 19 days experiment. [Details: A1B1 (rice bran-banana peel), A1B2 (rice bran-pineapple peel), A2B1 (chicken feed-banana peel), A2B2 (chicken feed-pineapple peel), A3B1 (rice bran+chicken feed-banana peel), A3B2 (rice bran+chicken feed-pineapple peel)]

compounds from decaying materials could also influence BSF oviposition (Scieuzo *et al.* 2021).

Chicken feed outperformed the other two treatments as hatching substrates (Table 2). Although both are nutritious, rice bran is higher in crude fiber than chicken feed. The chicken feed contains crude protein min. 21.0–23.0%, crude fat 5.0%, crude fiber 5.0%, while rice bran contains: 12–16% protein, 12–23% fat, and 23–30% fiber (Chen *et al.* 2023). Higher protein content and lower fiber result in better performance in chicken feed than rice bran. The high-nutrient substrate promotes larval growth resulting in high larval biomass (Hem *et al.* 2008). Besides, the chicken feed also has optimal moisture content compared to rice bran. Moisture content is crucial in controlling larval movement and determining substrate texture, substrate consumption, growth, and mortality of larvae (Makkar *et al*. 2014; Palma *et al.* 2018). While ideal water content can also increase larval weight (Lalander *et al.* 2020).

Substrate consumption data showed that banana peel and pineapple peel are feasible substrates for BSF larvae. The difference in substrate reduction in each observation period indicated that the highest rate of substrate reduction occurred in the second week, and the lowest substrate reduction occurred in the third week (Table 3). These data revealed that the level of feeding activity of BSF larvae varies from time to time. The decrease in substrate consumption in the 19-day experiment also take place, due to some larvae having reached the pre-pupae, which was no longer consuming.

The WRI was less than 2% in all treatments. Considering the two growing substrates given are fibrous, the WRI was insignificant. Based on the highest WRI, pineapple peel outperformed banana peel. These data imply that the palatability of pineapple peels inclines higher than banana peels for BSF.

In terms of biomass yield, there was no significant difference between banana peel and pineapple peel. The result is in line with the macronutrient content of the two substrates, which are comparable except for fiber content. According to Khan and Perveen (2010), banana peel contains crude protein (5.5–7.87%), crude fat (2.24–11.6%), ash (9–11%), carbohydrates (59.51–76.58%), moisture content (85%), and fiber (47–53%), whereas pineapple peel contains crude protein (5–9%), crude fat (2–3%), ash (4–6%), carbohydrates (50–80%), moisture content (82–88%), and fiber (1–6%) (Baidhe *et al.* 2021).

Larval weight increased periodically until the 19-day experiment, while substrate consumption declined in the 19-day experiment (Fig. 4, 6). We propose that the substrate consumed is not directly converted into biomass. So, the peak of consumption occurs in the second week, and the increase in biomass occurs afterward. As known, the larval continues to grow until it reaches "critical weight" in which hormone levels fluctuate, causing a shift to the next life stage (Edgar 2006). While some of the larvae had reached the prepupae stage, some others were still actively consuming the substrate given, so their biomass continued to increase during the third week of the experiment.

Overall, we found that the yield of larval biomass on growing substrates tended to reflect the yield of larval biomass on hatching substrate, where the highest was in the treatment of chicken feed (A2B1, A2B2) followed by rice bran + chicken feed mixtures (A3B1, A3B2) and rice bran (A1B1, A1B2). This point was also clearly illustrated by the conflicting results of the rice bran-pineapple peel and chicken feed-pineapple peel treatments (Fig. 6). Although both were fed with pineapple peel substrate, rice bran-pineapple peel produced the least biomass while chicken feed-pineapple peel produced the highest biomass in all experimental periods. These findings revealed that the hatching substrate has a greater impact on the yield of larval biomass in this study.

Analysis of proximate showed that all selected treatments (A1B1, A2B2, A3B1) had high protein content (Table 4) even though the substrate protein given was low. This finding is in line with previous studies, which found a protein content of BSF larvae above 30% even though the protein content in the substrate was lower (Spranghers *et al.* 2016; Shumo *et al.* 2019). Prior studies have also found that the protein of larvae did not reflect the protein of substrate (Beniers and Graham 2019; Danieli *et al.* 2019; Eggink *et al.* 2022; Kawasaki *et al.* 2022).

Generally, there were no significant differences between larval nutrients except for fiber. We found that the fiber content of the larvae inclines to represent the fiber content of the substrate. The highest fiber in larvae was found in the rice bran-banana peel, where the two types of substrates were also high in fiber. However, larval composition is highly dependent on harvest age, rearing medium, and processing technology (Apri and Komalasari 2020).

This research denoted the feasibility of several tropical fruit wastes in supporting BSF rearing. The study of other fruit waste and specific volatile compounds that meet the efficiency for BSF attractant is interesting to explore further. The high fiber of fruit waste substrate could be assisted by pre-treatment represented by the study of Isibika *et al.* (2019).

Conclusion

All three fermented fruit wastes, rotten orange, pineapple peel, and overripe jackfruit, could serve as oviposition attractants for BSF, yet their efficiency varies. Performance of chicken feed as the hatching substrate outperformed rice bran or a combination of both. Pineapple peel was slightly excelled banana peel in terms of yield and larval growth as growing substrates. The larvae of three selected treatments, *i.e.*, A1B1 (rice bran-banana peel), A2B2 (chicken feed-pineapple peel), and A3B1 (rice bran + chicken feed-banana peel), possess high protein levels (58–61%).

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Author Contributions

RR, MM and REP designed the study, RR and RRw performed the experiments, RR, RRw, MM and DD analyzed the data, RR and RRw wrote the paper with input from all authors.

Conflicts of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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