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Enhancing frass fertilizer efficiency with rice husk biochar and chicken manure via black soldier fly (*Hermetia illucens*) larvae composting

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ABSTRACT

In recent years, the bioconversion of organic waste using *Hermetia illucens* larvae, commonly known as black soldier fly (BSF), has emerged as an innovative and environmentally sustainable solution. This study aimed to assess the efficiency of the frass fertilizer produced by BSF larvae (BSFL) reared on a composite substrate consisting of rice husk biochar (RHB) and chicken manure (CM). A factorial randomized block design with two factors and replications was employed. The first factor was the proportion of mixed substrates, consisting of RHB and CM at different ratios (v/v): 10% biochar (B1), 30% biochar (B2), and 50% biochar (B3). The second factor was the feeding rate for BSFL: 200 mg per larva per day (M1) and 100 mg per larva per day (M2). Feeding was carried out for 16 days, after which the frass was sieved through a 2 mm mesh. The resulting frass was designated as Kasgotchar. The parameters observed included BSFL growth performance, waste reduction efficiency (WRE), and frass quality. The results revealed that the formulation of the RHB and CM substrate significantly influenced larval growth, frass weight, WRE, residual substrate percentage (%SR), and nutrient composition (pH, P, K, C/N ratio, and Ca content). Different substrate ratios significantly affected larval growth performance, WRE, and frass quality. The highest larval growth was observed in the B2 (30:70 RHB/CM) treatment combined with M1 (200 mg/day). The highest WRE was recorded in B3 (50:50), M2 (100 mg/day), and B2M2 (30:70 and 100 mg/day). The best frass quality was obtained from B1 (10:90), M2 (100 mg/day), and B1M1 (10:90 and 100 mg/day).

Keywords: rice husk biochar, chicken manure, BSFL, waste utilization.

INTRODUCTION

In recent years, the management of organic waste has become a critical concern for ensuring food security and promoting environmental sustainability across various sectors, including agriculture and animal husbandry (Bhatia and Sindhu, 2024). An innovative approach that has shown considerable promise is the use of *Hermetia illucens* (black soldier fly larvae/BSFL) for producing organic fertilizers, particularly frass fertilizer (Liu et al., 2022; Siddiqui et al., 2024). BSFL not only convert organic waste into valuable products but also substantially reduce waste volume (Bruno et al., 2025; Nallakumar and Fernando, 2023).

Various types of substrates, including agricultural and animal waste, can be used for rearing BSFL (Msangi et al., 2022). Among them, rice husk biochar (RHB) and chicken manure (CM) have shown significant potential (Zhang et al., 2023; Qin et al., 2023). Biochar enhances the quality of soil by increasing carbon content and provides a favorable medium for BSFL growth due to its physical and chemical properties. In contrast, CM is rich in essential nutrients required for larval development.

BSFL are highly effective in improving waste reduction efficiency (WRE), achieving rates between 65% and 79% (Diener et al., 2011). They may also reduce the mass and concentration of elements such as heavy metals by 50–60% (Attiogbe et al., 2019; Newton et al., 2005) while supporting a diverse and abundant microbial community (Vogel et al., 2018). During the bioconversion process, heavy metals tend to accumulate in BSFL biomass, resulting in compost with lower heavy metal concentrations (Biancarosa et al., 2018). Harmful bacteria are often present in waste streams and can proliferate in compost (Der Fels-Klerx et al., 2018). However, pathogens are typically eradicated during the thermophilic phase within the first three days of composting, while BSFL composting predominantly occurs under mesophilic conditions (Piceno et al., 2017). Several studies have also demonstrated the significant reduction of intestinal bacteria in insect larvae, producing the frass that is sterile or contains minimal bacterial load (Mumcuoglu et al., 2001).

Numerous research studies have been carried out on the BSFL composting technology (Beesigamukama et al., 2021; Lalander et al., 2015), including evaluations of compost quality and comparisons with the compost produced using alternative methods (Alattar, 2012). However, the application of BSFL compost in horticulture has yielded inconsistent results. The studies conducted by Choi and Hassanzadeh (2019) and Quilliam et al. (2020) demonstrated that the plants cultivated in the soil enriched with BSFL compost displayed better growth, greater yields, increased nutrient uptake, and improved resistance to diseases. In contrast, Alattar (2012) found that BSFL compost performs poorly in maize cultivation. The study showed that maize grown in the soil amended with micro-aerobic fermentation (MF) residues exhibited a 109% increase in height and a 14% increase in leaf number compared to those grown with conventional aerated compost. Conversely, the maize grown in BSFL compost-amended soil was 39% shorter and had 19% fewer leaves. This result may be attributed to the larvae's limited ability to decompose certain nutrients into plant-available forms (Urra et al., 2019) or to nutrient assimilation by BSFL for biomass production (Shumo et al., 2019; Suantika et al., 2017).

Certain studies indicate that the BSFL-derived compost may have an imbalanced nutrient composition. Previous research has shown that BSFL assimilate a considerable amount of nutrients, including proteins from organic feed, to generate biomass, which reduces the nutritional quality in the resulting compost (Rehman et al., 2017). Suantika et al. (2017) reported decreased nitrogen levels alongside increased phosphorus and potassium concentrations in BSFL compost. This occurs because nitrogen is primarily used by BSFL for biomass synthesis, while phosphorus and potassium are excreted (Oonincx et al., 2015).

Therefore, this study aimed to assess the efficiency of frass fertilizer produced by BSFL (*Hermetia illucens*) reared on a composite substrate of RHB and CM. Performance indicators for BSFL include growth rates and WRE, while frass fertilizer evaluation focused on quality and maturity.

MATERIALS AND METHODS

This study was conducted in the community of Kuala Simeme, Namorambe District, Deli Serdang Regency, North Sumatra, Indonesia (N 3°29'44"; E 98° 40'08", 61 m above sea level) between September and October 2023.

Materials

Rice husks were collected from a rice milling facility in Sei Rampah Village, Sei Rampah District, Serdang Bedagai Regency, North Sumatra, Indonesia (N 3°28'53"; E 99°08'49", 1 m above sea level). Biochar was produced from rice husks through pyrolysis at 350 °C. Chicken manure was obtained from the broiler farm in Binjai Bakung Village, Pantai Labu District, Deli Serdang Regency, North Sumatra, Indonesia (N 3°38'53"; E 98°55'57", 2 m above sea level).

Rice husk biochar and chicken manure were analyzed at PT Socfindo Indonesia (Socfindo) Laboratory, which holds KAN accreditation, ISO

17025 certification, and consistently scores very high results in the Wageningen Evaluating Programs for Analytical Laboratories (WEPAL). Detailed analyses of pH, the nutrient levels (carbon organic (C), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg)), and the heavy metal levels (boron (B), iron (Fe), copper (Cu)) of rice husk biochar and chicken manure were conducted using recommended laboratory methods (BPSI, 2023). The results are presented in Table 1.

Analytical method used for these tested were C organic by using gravimetry with furnace, pH by H_2O (1: 5) electrometry, N by using Kjeldahl with spectrophotometer, P by using dry ashing-HNO with spectrophotometer, B by using dry ashing-HNO_3 with spectrophotometer and K, Mg, Ca, Fe, Cu by using dry ashing-HCl with AAS (atomic absorption spectrometry) methods.

Methods

BSF breeding colony

The BSF stock colony was obtained from the Maggot Rearing House in Namorambe, Kuala Simeme village. The management of BSF colonies can be categorized into three components: (1) Management of puparium, which involves the emergence of flies from pupae; (2) Net Management, where mating occurs and the flies lay eggs; and (3) Nursery management, where eggs hatch into newly emerged larvae (neonates) that subsequently develop into seed larvae (Anderberg, 2023).

The prepupal stage represents the final developmental phase of BSFL and requires a drier environment. This stage typically takes between two and six days to transform into pupae (Dzepe et al., 2020; Purkayastha and Sarkar, 2022). Pupae are more sensitive to high temperatures than to lower temperatures, with an optimal range for the prepupal stage of 25 to 35 °C (Chia et al., 2018). The optimal relative humidity for the prepupal stage is approximately 70% (Holmes et al., 2012). During the pupal stage, metamorphosis occurs, resulting in the emergence of flies within 14 to 20 days (Purkayastha and Sarkar, 2022). Besides temperature and humidity, other key factors influencing fly emergence include pupal material (Dzepe et al., 2020) and the presence of parasites (Barrett et al., 2022).

After emergence, mating occurs within two to eight days, with peak mating activity between 9:00 AM and 2:00 PM. Egg-laying begins two days after mating, with the highest level of egg-laying occurring between 1:00 and 2:00 PM (Purkayastha and Sarkar, 2022; Julita et al., 2020). Factors influencing BSF mating and egg-laying include temperature and relative humidity (Chia et al., 2018; Holmes et al., 2012), nutritional solution (Macavei et al., 2020), and sunlight exposure (Purkayastha and Sarkar, 2022; Macavei et al., 2020). Following harvesting, eggs are transferred to the nursery, where they incubate and hatch within two to four days. The newly hatched larvae (neonates) are fed a high-quality starter substrate and undergo a growth phase of at least five days before being used in experimental treatment. The optimal temperature for larval development is 25–35 °C (Chia et al., 2018), with a relative humidity is 70 percent (Holmes et al., 2012).

Production of Frass fertilizer

Initially, 60 g of BSF eggs were incubated to produce larvae. After 8 days, the neonates were collected and transferred into plastic trays (43 × 62 × 20 cm), which had been prepared with 1 kg of mixed substrates in accordance with the designated treatments. The feeding procedure was conducted over a span of 16 days, with a daily allocation of 1 kg. At the end of the feeding period, the frass was separated by sieving the combination of larvae and frass through a 2 mm sieve, and then the BSFL that successfully went through the sieve were manually extracted. The frass is called Kasgotchar. Then, the initial substrate quantity, the quantity of frass, and the weight of larvae were

Table 1. Selected chemical characteristics of rice husk biochar and chicken manure used in the experiments

Substrate	pH H_2O (1:5)	Nutrient levels						Heavy metal levels		
		C	N	P	Ca	Mg	C/N	B	Fe	Cu
		%						mg/kg		
RHB	7.31	17.1	1.17	0.21	0.197	0.12	14.61	23.13	1,180.7100	2.6551
CM	9.71	18.7	3.59	0.90	1.835	2.00	5.21	29.77	949.0232	9.7100

Note: RHB – rice husk biochar; CM – chicken manure; C – carbon organic; N – nitrogen; P – phosphorus; K – potassium; Ca – calcium; Mg – magnesium; B – boron; Fe – iron; Cu – copper.

Table 2. Formulations and compositions of treatments

No	Treatments	Mixed composition of substrates (v/v)		BSFL Fed (mg/day)
		RHB (%)	CM (%)	
1	B1M1	10	90	200
2	B1M2	10	90	100
3	B2M1	30	70	200
4	B2M2	30	70	100
5	B3M1	50	50	200
6	B3M2	50	50	100

Note: RHB – rice husk biochar; CM – chicken manure; BSFL – black soldier fly larvae.

recorded. The frass samples were subsequently preserved for further analysis.

The substrate for BSFL bioconversion consists of a mixture of RHB and CM in three different formulations. The initial factor involved the varied composition of substrates, comprising RHB and CM (v/v) with proportions of 10% biochar (B1), 30% biochar (B2), and 50% biochar (B3). The second Factor was BSFL Fed (200 mg/larva/day (M_1); 100 mg/larva/day (M_2). The analysis was carried out under 6 combinations of treatment with a total of 12 experimental units (see Table 2).

The experimental data were analyzed using analysis of variance (ANOVA) at a 95% confidence level to determine significant differences between the treatments. When significant effects were detected, means comparisons were performed using Duncan's multiple range test (DMRT) at the same confidence level. All statistical analyses were conducted using R software version 3.6.0.

Evaluation of growth and yield in black soldier fly larvae

The metrics evaluated for the growth performance of BSFL included the weight of final larvae (WFL), bioconversion rate (BCR), and growth rate (GR). The larvae were isolated through the process of sieving the frass, and the final larvae were subsequently weighed as WFL. BCR of BSFL was determined using the formula Equation 1 (Rehman et al. 2017). GR of BSFL was determined using the formula presented in Equation 2 (Meneguz et al. 2018).

$$BCR = \frac{TLB}{FA} \times 100\% \quad (1)$$

where: *BCR* – bioconversion rate, *TLB* – total larval biomass, *FA* – feed added.

$$GR = \frac{LABF(g) - LIBW(g)}{D} \times 100\% \quad (2)$$

where: *GR* – growth rate, *LABF*(g) – larva average body final (g), *LIBW*(g) – larva initial body weight (g), *D* – days of trial.

The metrics evaluated for WRE included the percentage of substrate reduction (% SR), the waste reduction index (WRI), and the efficiency of conversion of digested food (ECD). The amount of feed consumed by BSFL during treatment is referred to as feed consumption or substrate reduction. Feed consumption is quantified as a percentage using the formula provided by Meneguz et al. (2018) and Pliantiangtam et al. (2021) (Equation 3).

$$\%SR = \frac{DS(g) - RS(g)}{DS(g)} \times 100\% \quad (3)$$

where: *%SR* – substrate reduction, *DS*(g) – distributed substrate (g), *RS*(g) – residual substrate (g).

WRI is the ability of the larvae to reduce waste, considering the larvae's feeding time. The WRI value can be measured by using the equation formula (Meneguz et al. 2018, Pliantiangtam et al., 2021) (Equation 4).

$$WRI = \left(\frac{DS(g) - RS(g)}{DS(g)} \times 100\%\right)/DT \quad (4)$$

where: *WRI* – waste reduction index, *DS*(g) – distributed substrate (g), RS(g) – residual substrate, RS – residual substrate (g), DT – days of trial (day).

ECD refers to how effectively larvae transform the waste they consume during their rearing period. The ECD value is determined through the equation formula (Meneguz et al., 2018, Pliantiangtam et al., 2021) (Equation 5).

$$ECD = \frac{TFB(g)}{TBS(g) - RS(g)} \quad (5)$$

where: ECD – efficiency of conversion of digesteed food, TBF(g) – total final biomass, TBS(g) – total distributed substrate, RS(g) – residual substrat.

The distributed substrate refers to the initial amount of feed at the beginning of the treatment. The total final biomass includes both larvae and prepupae while the residual substrate represents the remaining feed after treatment, including undigested material and larva excreta. The growth performance of BSFL was measured using final larval weight, growth rate, and how well the biomass is converted. Also, WRE was determined based on substrate reduction, WRI, and the environmental conversion efficiency. All measurements were performed on a fresh matter basis.

Performance of frass fertilizer using black soldier fly larvae composting

The quality of frass was evaluated based on its macro- and micro-nutrient content, which are essential for optimal plant growth. Compost analysis was conducted twice, at 15 and 30 days after composting, in the Socfindo laboratory. The parameters assessed at day 15 included moisture, pH, C organic, N, P, and K. At day 30, Ca was additionally analyzed. Moisture content was determined gravimetrically, and pH (H_2O 1:5) was measured by using electrometry. Organic carbon and nitrogen were measured using the Walkley-Black and Kjeldahl methods with a spectrophotometer. Phosphorus assessment was performed using the dry ash-HNO method with a spectrophotometer, and potassium analysis was conducted using the dry ash-HCl method with AAS. Daily temperatures of the compost were recorded using a digital thermometer.

The quality and maturity of compost are determined through chemical indicators like pH, ammonia levels, and the C/N ratio, along with assessments of plant growth, germination tests, and microbial analysis (Basri et al., 2022). The frass maturity was determined by a germination test at *Capsicum annuum* L (chilli) and *Solanum lycopersicum* L (tomato). Frass extract was made by dissolving 50 grams of frass in 1000 milliliters of distilled water, which gives a 5% concentration. The seed germination and root elongation were checked to calculate the germination index (Bera et al., 2013).

$$GI = \frac{SG(\%) \times RE(\%)}{100} \quad (6)$$

where: *GI* – germination index, *SG*(%) – seed germination, *RE* – root elongation (%).

RESULTS AND DISCUSSION

Effect of mixed substrate composition on the growth performance and yield of BSFL

The growth performances of the BSF raised on a mixed substrate composition of RHB with CM, along with the quantity of BSFL fed, are described in Table 3. The mixed substrate composition of RHB with CM had a significant effect on WFL of BSFL (F = 8.84, df = 2, 5, p = 0.0228), BCR of BSFL (F = 8.84, df = 2, 5, p = 0.0033), and GR of BSFL (F = 8.84, df = 2, 5, p = 0.0228). The analysis revealed a notable impact of the BSFL that were fed on WFL (F = 12.72, df = 1,5, p = 0.0161) and BCR (F = 12.72, df = 2, 5, p = 0.0033). However, there were no significant differences observed in GR (F = 0.46, df = 1, 5, p = 0.5263). The interaction involving the mixed substrate composition of RHB with CM and BSFL did not demonstrate a significant effect on the growth performances of BSFL (WFL: F = 0.83, df = 2, 5, p = 0.4866; BCR: F = 0.83, df = 2, 5, p < 0.001; GR: F = 0.83, df = 2, 5, p = 0.4866).

It was noted that the weight and BCR of BSFL rose with higher daily feeding rates; however, the GR of BSFL diminished as the daily feeding rate increased. The BSFL fed 100 mg per larva daily exhibited the highest WFL and biomass conversion ratio (BCR) of 884.85 g and 5.33%, respectively. In contrast, the BSFL that received 200 mg per larva daily demonstrated the lowest WFL and BCR, recording values of 674.82 g and 4.22%, respectively. In the case of GR, the BSFL that received a daily feed of 100 mg per larva exhibited the highest WFL at 14.16 g/day, whereas BSFL fed 200 mg per larva per day recorded the lowest WFL at 12.83 g/day. M2 exhibited notable differences from M1 regarding the growth performance parameters of BSFL. The highest growth performances of BSFL were observed in B2, with B3 and B1 following in that order. B2 showed a notable difference from B1 regarding the weight of BSFL, while no significant difference was seen between B2 and B3. In the context of bioconversion parameters of BSF larvae, the B2 exhibited statistically different values from both B3 and B1, but B1 and B3 were similar to each other. The B2M2 group exhibited the highest growth performance of BSFL, while the B1M1 group showed the lowest growth performance of BSFL.

The findings indicate that the growth performance of BSFL was influenced by the substrate, particularly the mixture of RHB and CM. The reduced larval growth and lower fertilizer quality observed at 50% biochar addition are mainly because biochar is a carbon-rich and highly stable material that contains very limited readily available nutrients. When the proportion of biochar increases to 50%, the substrate becomes predominantly inert, leading to a relative deficiency of digestible organic matter for BSFL. This nutritional imbalance restricts larval biomass accumulation and slows substrate degradation. Previous studies have shown that BSFL performance is strongly dependent on the nutrient quality and digestibility of the feeding substrate (Qin et al., 2022; Meneguz et al., 2018). In addition, RHB specifically has a rigid, silica-rich structure that is physically hard and difficult for larvae to manipulate. As the RHB content increases, the substrate becomes coarser, and the proportion of edible organic material accessible to BSFL decreases. Consequently, larvae experience a form of "nutritional dilution," resulting in slower growth. This phenomenon is consistent with the reports that BSFL exhibit lower conversion efficiency when reared on substrates with high lignocellulosic or lignin content due to their poor digestibility.

The weight of BSFL varied between 509.95 g and 1073.50 g in this study. Sari et al. (2023) said that the type of food given to BSFL during their growing time influences how much they weigh. Pliantiangtam et al. (2021) discovered that the final weight of fresh larvae, when raised on a mix of coconut endosperm and soybean curd residue in a 20:80 ratio with added supplements, was 27.7 grams. This was notably different from the 15.0 grams seen when the same mix was used in an 80:20 ratio with supplements. The substrate weighed 2182 kg, and when 858 kg of poultry manure was added, it produced 540 kg (62.9%) of BSFL frass and 54.9 kg (6.4%) of BSFL (Basri et al., 2022). The substrates mixed with 20% biochar produced much higher amounts of larvae in both wet and dry conditions than the control and gypsum treatments (Beesigamukama et al., 2020).

The BCR observed in this study varied between 3.19% and 5.89%. The percentage of BCR found here was lower than the results from Mulu et al. (2023). They reported that a mix made up of fruit waste, water hyacinth, and manure in the ratio of 55:35:10 gave a maximum BCR of 30.71%. Conversely, the lowest BCR was noted for the proportion 65:25:10, which recorded a value of 22.96%. The study conducted by Rohmanna and Maharani (2022) reported the highest BCR for BSFL reared on domestic waste, ranging from 14.4% to 14.6%. In contrast, the lower BCR was observed for BSFL on the solid decanter, with values between 4.5% and 5.3%.

The findings indicate that the GR of BSFL varied between 8.67 g/day and 19.12 g/day. According to Vodounnou et al. (2024), the amount of weight gained was between 2.19 ± 0.09 g/day and 4.53 ± 0.02 g/day when using composting materials like soy-bran, yam peel, cassava peel, and sweet potato peel. The correlation analysis indicated a robust relationship between daily weight gain and both organic matter and phosphorus content, as well as a significant correlation with the C/N ratio.

Table 3. Growth performances and waste reduction efficiency of BSFL at the end of frass composting

Treatments	30 days					
	WFL (g)	BCR (%)	GR (g/day)	% SR	WRI	ECD
B_1	642.60±187.60 ^{b}	4.02±1.17^{b}	8.92±0.36	54.69±0.44^{b}	1.82±0.01^{b}	7.34±2.18
B_2	942.45±185.33 ^{a}	5.89±1.16^{a}	18.92±0.29	62.50±3.09^{a}	2.08±0.10^{a}	9.39±1.39
B_3	754.60±72.41^{ab}	4.72±0.45^{b}	12.65±3.48	54.84±3.31^{b}	1.83±0.11^{b}	8.59±0.31
M_1	674.92±152.73 ^{b}	4.22±0.95^{b}	14.16±5.09^{a}	55.94±3.99	1.86±0.13	7.52±1.50^{b}
M_2	884.85±164.09 ^{a}	5.33±1.03^{a}	12.83±5.47^{b}	58.75±5.33	1.96±0.18	9.35±0.89^{a}
B_1M_1	509.95±36.84	3.19±0.23	8.67±1.23	55.00±3.54	1.83±0.12	5.79±0.05
B_1M_2	775.25±199.05	4.85±1.24	9.18±6.64	54.38±1.77	1.81±0.06	8.88±2.00
B_2M_1	811.40±0.97	5.07±0.19	18.71±1.03	60.31±0.44	2.01±0.01	8.41±0.38
B_2M_2	1073.50±37.48	6.71±0.23	19.12±1.25	64.69±1.33	2.16±0.04	10.38±0.57
B_3M_1	703.40±110.87	4.40±0.69	15.11±3.70	52.50±0.88	1.75±0.03	8.36±1.18
B_3M_2	805.80±34.22	5.04±0.21	10.19±1.14	57.19±0.44	1.91±0.01	8.81±0.44

Values represent mean±SEM. Means followed by the different letters at each column are significantly different at P < 0.05 (DMRT) Key: B1 = substrate with 10% RHB and 90% CM, B2 = substrate with 30% RHB and 70% CM, B3 = substrate with 50% RHB and 50% CM, M1= BSF larvae fed 100 mg/day, M2= BSF larvae fed 100 mg/day, B1M1 = substrate with 10% RHB and 90% CM and BSF larvae fed 100 mg/day, B1M2 = substrate with 10% RHB and 90% CM and BSF larvae fed 200 mg/day, B2M1 = substrate with 30% RHB and 70% CM and BSF larvae fed 100 mg/day, B2M2 = substrate with 30% RHB and 70% CM and BSF larvae fed 200 mg/day, B3M1 = substrate with 50% RHB and 50% CM and BSF larvae fed 100 mg/day, B3M2 = substrate with 50% RHB and 50% CM and BSF larvae fed 200 mg/day.

The parameters measured for WRE included the percentage of SR, WRI, and ECD. The efficiency of waste reduction by the BSF raised on a mixed substrate of RHB and CM, along with the amount of BSFL fed, is detailed in Table 3. The percentage of SR and WRI was notably influenced by the mixed substrate composition of RHB with CM (% SR: F = 21.84, df = 2, 5, p = 0.00338; WRI: F = 21.84, df = 2, 5, p = 0.00338). However, ECD did not exhibit a significant difference (F = 8.84, df = 2, 5, p = 0.0966). The ECD showed a significant impact from the BSFL feeding (F = 0.46, df = 1, 5, p=0.0298), whereas % SR and WRI did not exhibit significant differences (%SR: F = 6.50, df = 1, 5, p = 0.05133; WRI: F = 6.50, df = 1, 5, p = 0.05133). The interaction of mixed substrate composition of RHB with CM and BSFL fed was also not significant (% SRI: F = 2.43, df = 2, 5, p = 0.18283; WRI: F = 2.43, df = 2, 5, p = 0.18283; ECD: F = 0.83, df = 2, 5, p = 0.2940).

The most effective WRE was noted in B2 (65.5%; 2.08; 9.39) and B2M2 (64.69%; 2.16; 10.38). The minimum values were observed in B1 (54.69%; 1.82; 7.34), B2M2 (52.50%; 1.75), and B1M1 for ECD (5.79). In terms of % SR and WRI, B2 exhibited a statistical difference from both B1 and B3, while no statistical difference was seen between B3 and B1.

The data indicated that WRE improved as the daily feeding rate was increased. The WRE of BSFL fed 200 mg/larva/daily demonstrated the highest percentages of SR, WRI, and ECD at 58.75%, 1.96, and 9.35, respectively. In contrast, the BSFL fed 100 mg/larva/daily exhibited the lowest values of 55.94%, 1.86, and 7.52, respectively. M2 exhibited notable differences from the M1 at ECD. According to Nyakeri et al. (2017), the rates of substrate conversion varied between 44.7% and 81.8%. Furthermore, Diener et al. (2011) indicated that BSFL demonstrates a significant WRE ranging from 65–79%. Ahmad et al. (2023) demonstrated that the WRI value for food waste (5.31 g/day), chicken feed (5.40 g/day), rice bran (5.01 g/day), and garden waste (4.40 g/day). Rohmanna and Maharani (2022) found that the efficiency of BSFL on solid decanter waste was significantly lower than on domestic waste, with rates of 8.3% and 59.3% respectively. Liu et al. (2018) showed that the effectiveness of BSFL in reducing waste was influenced by the type of substrate used for feeding.

Effect of mixed substrate composition and BSF larvae fed on the quality of frass

The changes in temperature throughout the BSFL composting stages are shown in Figure 1. Initial temperature ranged from 28–34 °C, with the lowest temperature being recorded for substrates mixed with 50% rice husk biochar (28.7 °C for BSFL fed 200 mg/day and 29.9 °C for BSFL fed 100 mg/day). A temperature rise was observed for all experimental treatments from the initial until the second week. The temperature peaked between 36–37.5 °C on the second week of BSFL composting, except for substrates mixed with 30% biochar (fed 100 mg/day), which reached the temperature peak in the third week. The highest temperatures were measured in the substrates that had 10% biochar added (37.5 °C for fed 200 mg per day), and 50% biochar (37.3 °C for fed 200 mg/day and 37 °C for fed 100 mg/day) in the second week of experiments, respectively. In week 4, a sharp temperature decline was observed (30–33 °C). Subsequently, temperature variations remained minimal until the conclusion of the experiments. Lalremruati and Devi (2021) described the composting process as comprising two primary phases: the initial phase involves microbial activity during the mesophilic stage, which facilitates the decomposition of organic material, followed by the maturation phase. During the mesophilic stage, the temperature gradually rises to the average level of approximately 40 °C. In the maturation phase, the finished compost stabilizes within an optimal temperature range of 32 to 60 °C. Keeping BSFL at the right temperature helps them better reduce *Escherichia coli* (Liu et al., 2008).

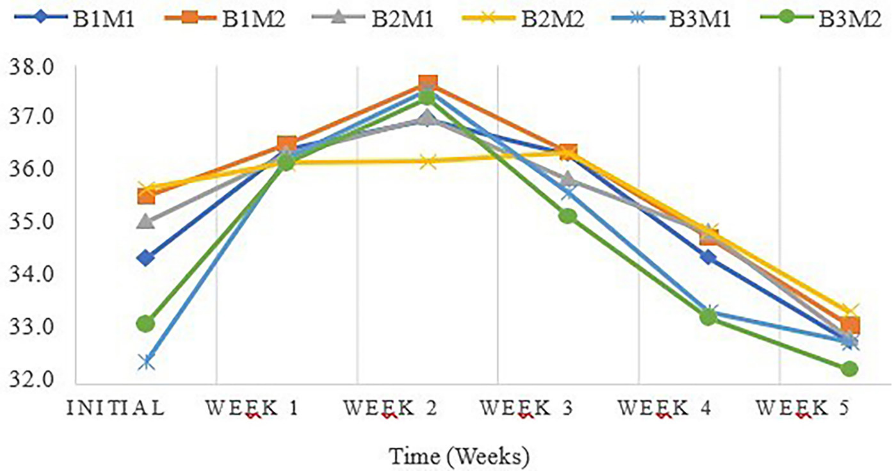

Figure 1. Fluctuations in temperature throughout BSFL composting process for each of the treatments

The pH across all treatments varied between 8.5 and 8.9 (Figure 2). The pH exhibited a notable influence from the mixed substrate composition of RHB with CM (F = 15.817, df = 2, 5, p = 0.00688), whereas the impact of BSFL feeding was not statistically significant (F = 2.354, df = 1, 5, p = 0.18554). The interplay of the mixed substrate composition of RHB with CM and BSFL feeding was likewise found to be insignificant (F = 0.136, df = 2, 5, p = 0.87581). The highest pH levels were found at the end of the composting process for the materials mixed with 10% biochar, reaching 8.68 for those given 200 mg per day. The materials with 50% biochar had the lowest pH, measuring 8.59 for those given 100 mg per day. All the pH levels seen in the different treatments were within the recommended range for solid organic fertilizer, as stated in the Indonesian National Standards SNI 7763-2018 (BSN, 2018). Beesigamukama et al. (2020) observed that the pH of frass was influenced by the amendments applied to the substrate and the duration of composting. Notably, the substrate treated with gypsum exhibited the lowest pH values, whereas the substrate containing 15% biochar recorded the highest pH value. During the initial week of composting, there was a notable increase in pH levels, which peaked between 7.9 and 9.1 during the first and third weeks. The pH level of BSFL frass is usually between 7.0 and 8.0, which is good for helping plants grow (Surendra et al., 2020) and supports the good bacteria that live in BSFL frass (Choi and Hassanzadeh, 2019).

Fluctuations in moisture levels throughout the BSFL composting treatments are illustrated in Figure 2. The moisture content exhibited a notable influence from the mixed substrate composition of RHB with CM (F = 3.793, df = 2, 5, p = 0.0995). However, the treatment involving BSF larvae showed no significant difference (F = 0.068, df

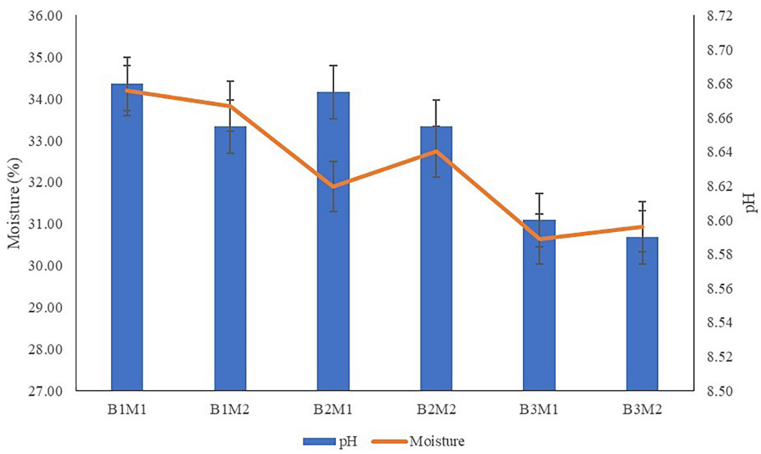

Figure 2. pH and moisture analysis of compost bioconversion by BSF Larvae

= 1, 5, p = 0.8047). Additionally, the interaction between the mixed substrate composition of RHB with CM and the feeding of BSF larvae was also found to be non-significant (F = 0.138, df = 2, 5, p = 0.8742). The moisture content across all treatments exhibited a variation ranging from 30 to 34%. The substrates incorporating 10% biochar exhibited the highest moisture content, measuring 34.20% for those receiving 200 mg/day, whereas the substrates with 50% biochar displayed the lowest moisture content at 30.64% for a 200 mg/day feeding rate. According to Basri et al. (2022), the moisture in BSFL frass can be between 30% and 72%. Higher moisture levels make it harder to harvest because dry frass is seen as not fully mature compost, which might contain harmful substances that can damage plants. The best moisture level is between 45% and 50%, as found by Razmjoo et al. (2015). Moisture levels below 30% will restrict bacterial activities, while the porosity of the compost diminishes when moisture exceeds 65% (Lalremruati and Devi, 2021).

Nutrient composition of BSFL frass fertilizer is delineated in Table 4. The mixed substrate composition of RHB with CM exhibited a notable impact on C-organic, with statistical results indicating F = 7.348, df = 2, 5, and p = 0.0325. The feeding of BSF larvae and the interaction involving the mixed substrate composition of RHB with CM and BSF larvae did not yield significant results for C-organic. The B1 was clearly different from B3, but there was no statistical difference between B1 and B2. The frass produced by the BSFL had the highest organic carbon content of 22.23% when they were fed 200 mg of feed each day, and the lowest was 21.41% when they were given 100 mg daily. According to Lomonaco et al. (2024), the composition of frass macronutrients was determined by the type of feed the larvae eat. Liu et al. (2019) found that when BSFL were fed chicken manure, the organic carbon in their waste was 23.6%, with pig manure giving 26.8%, and cow manure giving the highest at 27.7%.

The mixed substrate composition of RHB with CM exhibited a notable impact on calcium levels (F = 2.344, df = 2, 5, p = 0.000797). The feeding of BSF larvae and the interaction involving the mixed substrate composition of RHB with CM and BSF larvae were found to be insignificant concerning calcium levels. The calcium content in B1 exhibited a significant divergence from both B2 and B3, while a notable distinction was also observed between B2 and B3. The frass generated by BSFL, nourished with 200 mg per larva per day, exhibited the highest calcium content at 0.85%. In contrast, the frass from BSFL fed 100 mg per larva per day displayed the lowest calcium content, recorded at 0.80%. Liu et al. (2019) documented that the organic carbon content of BSFL frass derived from chicken manure was 23.6%, from pig manure was 26.8%, and from cow manure was 27.7%.

The analysis revealed no notable impact on C/N (F = 1.563, df = 2, 5, p = 0.297) concerning the treatment involving a mixed substrate composition of RHB with CM treatment, the BSF larvae fed treatment, and the interaction of both treatments. Research shows that the C/N of the frass generated by BSFL decreases with increasing daily feeding rate. The frass made by BSFL that received 100 mg of feed each day had the highest C: N ratio of 8.19, while the frass from BSFL that

Table 4. Macronutrient contents in BSFL frass at selected periods during frass composting

Treatments	30 days					
	C (%)	C/N	Ca (%)	N (%)	P (%)	K (%)
B_1	23.79±1.35 ^{a}	8.75±1.25	0.95±0.05 ^{a}	2.75±0.35	1.38±0.02 ^{a}	5.21±0.33 ^{a}
B_2	22.00±1.36 ^{ab}	8.13±0.61	0.85±0.08 ^{b}	2.72±0.23	1.35±0.01 ^{a}	4.20±0.20 ^{b}
B_3	19.67±1.31 ^{b}	7.31±0.80	0.66±0.03 ^{c}	2.71±0.25	1.31±0.03 ^{b}	3.19±0.09 ^{c}
M_1	21.41±1.85	8.19±0.89	0.80±0.13	2.64±0.26	1.34±0.04	4.21±1.04
M_2	22.23±2.50	7.96±1.25	0.85±0.16	2.81±0.24	1.36±0.03	4.19±0.80
B_1M_1	22.75±0.10	8.56±1.26	0.93±0.08	2.69±0.41	1.37±0.02	5.39±0.43
B_1M_2	24.83±0.18	8.94±1.73	0.98±0.01	2.82±0.42	1.39±0.02	5.04±0.08
B_2M_1	21.77±2.29	8.60±0.48	0.79±0.04	2.53±0.13	1.34±0.01	4.13±0.31
B_2M_2	22.22±0.37	7.66±0.05	0.92±0.02	2.90±0.03	1.36±0.01	4.27±0.02
B_3M_1	19.71±1.49	7.34±0.36	0.67±0.02	2.70±0.33	1.29±0.01	3.12±0.00
B_3M_2	19.63±1.71	7.28±1.34	0.65±0.04	2.72±0.27	1.33±0.03	3.26±0.04

received 200 mg of feed each day had the lowest ratio of 7.96. The C: N ratio helps show how stable and active organic fertilizers are in the soil. The C/N ratio in the BSFL frass derived from different food sources can fluctuate between 8:1 and 27:1 (Basri et al., 2022).

Values represent mean±SEM. Means followed by the different letters at each column are significantly different at P < 0.05 (DMRT) Key: B1 = substrate with 10% RHB and 90% CM, B2 = substrate with 30% RHB and 70% CM, B3 = substrate with 50% RHB and 50% CM, M1 = BSF larvae fed 100 mg/day, M2 = BSF larvae fed 100 mg/day, B1M1 = substrate with 10% RHB and 90% CM and BSF larvae fed 100 mg/day, B1M2 = substrate with 10% RHB and 90% CM and BSF larvae fed 200 mg/day, B2M1 = substrate with 30% RHB and 70% CM and BSF larvae fed 100 mg/day, B2M2 = substrate with 30% RHB and 70% CM and BSF larvae fed 200 mg/day, B3M1 = substrate with 50% RHB and 50% CM and BSF larvae fed 100 mg/day, B3M2 = substrate with 50% RHB and 50% CM and BSF larvae fed 200 mg/day.

The mixed substrate composition of RHB with CM exhibited no significant effect on nitrogen levels (F = 0.021, df = 2, 5, p = 0.979). Furthermore, the interaction between the mixed substrate composition of RHB with CM and the feeding of BSF larvae also did not yield significant results for nitrogen. The optimal nutritional composition of frass was noted in B1 (2.75%), M2 (2.81%), and B2M2 (2.90%). The performance metrics revealed the least favorable outcomes in B3 at 2.71%, M1 at 2.64%, and B2M1 at 2.53%. In the case of N, a notable distinction was observed between B1 and both B2 and B3, with B2 and B3 also exhibiting a significant disparity. Liu et al. (2019) measured the nitrogen level in BSFL frass that came from CM, which had 2.3% nitrogen, pig manure with 2.4%, and cow manure with 1.9%. The nutritional composition of the frass mainly depends on the nutrients present in the material that the larvae were fed (Surendra et al., 2020). For agronomic applications, a total nitrogen content exceeding 0.6 is deemed acceptable (Basri et al., 2022).

The mixed substrate composition of RHB with CM exhibited a notable influence on P, as indicated by the statistical analysis (F = 10.892, df = 2, 5, p = 0.0151). The feeding of BSF larvae and the interaction involving the mixed substrate composition of RHB with CM and BSF larvae were found to be insignificant for P. The most elevated nutritional composition of frass was noted in B1 (1.38%), M2 (1.36%), and B1M2 (1.39%). The performance metrics indicated that the lowest values were recorded in B3 at 1.31%, M1 at 1.34%, and B3M1 at 1.29%. For P, the B1 exhibited a notable distinction from B3, but there was no statistical difference between B1 and B2. Liu et al. (2019) found that the BSFL frass from CM had 1.1% phosphorus, from pig manure had 2.1%, and from cow manure had 1.0%. Commercial organic fertilizer has 2.3% total nitrogen, 2.3% total phosphorus, and 2.3% total potassium. For agronomic applications, a total phosphorus concentration exceeding 0.22 is deemed acceptable (Basri et al., 2022).

The mixed substrate composition of RHB with CM exhibited a notable impact on K, as indicated by the statistical analysis (F = 112.634, df = 2, 5, p < 0.0001). The feeding of BSFL and the interaction involving the mixed substrate composition of RHB with CM and BSF larvae were found to be statistically insignificant for K. The superior nutritional composition of frass was noted in B1 (5.21%), M1 (4.21%), and B1M1 (5.39%). The performance metrics revealed the least favorable outcomes in B3 at 3.19%, M2 at 4.19%, and B3M1 at 3.12%. In the case of K, a notable distinction was observed between B1 and both B2 and B3, while B2 and B3 also exhibited a significant disparity. Liu et al. (2019) documented the potassium content in the BSFL frass composted from CM (1.8%), pig manure (1.0%), and cow manure (0.2%). Lopes et al. (2022) observed variations stemming from the differing concentrations of carbohydrates, proteins, and fiber present in the feed substrates. The frass produced by BSFL, obtained from food waste, indicates a total potassium concentration ranging from 0.2 to 2.1. For agronomic applications, it is deemed acceptable for total potassium levels to exceed 0.25 (Basri et al., 2022).

The improved nutrient profile and frass quality observed in the B1 treatment (10% biochar + 90% chicken manure) can be attributed to a combination of biological and chemical mechanisms. Biologically, the moderate addition of rice husk biochar enhances the habitat for decomposer microorganisms, such as *Bacillus*, *Pseudomonas*, and *Actinomycetes*, which accelerate organic matter mineralization and nitrogen cycling (Chen et al., 2024; Li et al., 2022). The micro-pores of biochar provide a refuge for microbes against predation and moisture fluctuation, promoting stable enzyme activities. Chemically, the alkaline

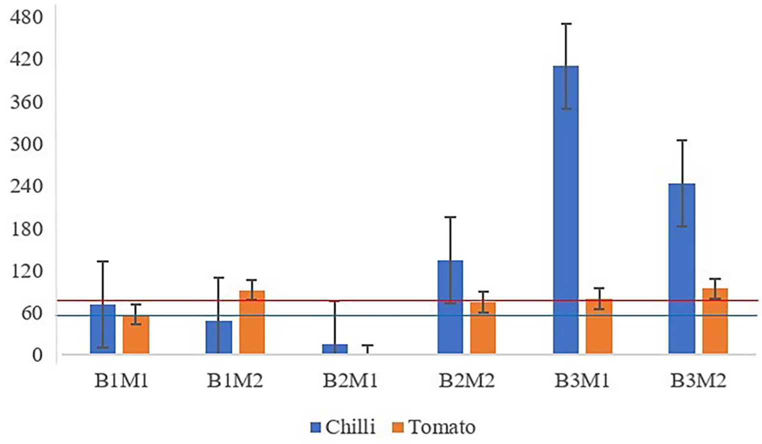

Figure 3. GI of compost bioconversion by BSFL for each of the treatments for chili and tomato. The blue line represents the germination index 60% and the red line represents the germination index 80%

and cation-exchange properties of biochar facilitate ammonium adsorption, reduce NH_3 volatilization, and improve nutrient retention in the frass matrix (Beesigamukama et al., 2020). Therefore, the 10% biochar ratio provides an optimal balance between microbial activity, nutrient stabilization, and organic matter transformation, resulting in superior frass quality compared to higher biochar dosages.

The peak germination index (GI) for tomatoes and chilies was observed in B3M2 and B3M1, respectively. The minimum GI for both tomato and chili was observed in B2M1. The GI for chili was higher than that of tomato. Immature compost may contain phytotoxic elements or substances detrimental to plants, thereby hindering seed germination. The GI value serves as a phytotoxicity level indicator and compost maturity (Lončarić et al., 2024). A GI value of 80% indicates the absence of phytotoxic substances in the compost (Zucconi et al., 1981). If the GI is 80%, it means there are no harmful substances in the compost that could hurt plants (Zucconi et al., 1981). If the GI is over 60%, it means the compost has finished maturing (Soriano-Disla et al., 2010). Mature compost that does not have harmful effects includes types B2M2, B3M1, and B3M2 (Figure 3). When compost is mature, it has less of the harmful stuff that can stop plants from growing. Immature compost has more of these harmful substances and can make it harder for plants to grow compared to mature compost (Diaz et al., 2007). Harmful chemicals like heavy metals, certain phenolic compounds, acids, and salt accumulation are often found in compost that has not undergone decomposition (Luo et al., 2018).

CONCLUSIONS

The different proportions of mixed substrate from RHB and CM significantly affected larval growth performance of larvae, WRE, and frass quality. The highest larval growth performances were observed in treatment B2 (30:70 RHB/CM), M1 (BSFL Fed 200 mg/day). The highest WRE was recorded in B3 (50:50), M2 (100 mg larvae/day), and the B2M2 combination (30:70 and 100 mg larvae/day). of the best frass quality was obtained in B1(10: 90), M2 (100 mg larvae/day), and the B1M1combination (10:90 and 100 mg larvae/day).

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