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Evaluation of Fertilizer Value of Residues Obtained after Processing Household Organic Waste with Black Soldier Fly Larvae (*Hermetia illucens*)

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Received: 30 May 2020; Accepted: 12 June 2020; Published: 17 June 2020

Abstract: The residue generated by the black soldier fly (*Hermetia illucens*, BSF) during the processing of organic waste is considered a suitable crop fertilizer. However, no detailed studies have investigated the fertilizer value of the residue obtained from processing household organic waste. In this study, experimental household organic waste (EHOW) was processed by BSF at 200 mg of EHOW per head for 15 days at 27 °C. To evaluate the fertilizer value of the obtained BSF larvae production residue (BSFR), the chemical composition and microbiota were analyzed, and Komatsuna (*Brassica rapa* var. *perviridis)* cultivation tests were conducted. BSFR results demonstrated higher ammonium nitrogen and lower nitrate nitrogen, and the highest above-ground dry matter weight of Komatsuna. Although the relative abundance of *Escherichia* was low, the relative abundance of Xanthomonadaceae, which contains a genus that causes disease in plants, was high. Therefore, the presence of plant pathogens in the BSFR microbiota should be considered. Finally, the effects of BSFR on the external environment requires more detailed investigation.

Keywords: organic fertilizer; *Hermetia illucens*; compost; household organic waste; microbiota profile; principal component analysis

1. Introduction

The concept of using fly larvae for processing organic waste was initially proposed almost 100 years ago [1]. More recently, the black soldier fly (BSF), which is raised on animal manure or household organic waste (HOW) as feed for livestock, is being considered as an efficient way to recycle unutilized resources in a sustainable manner [2]. However, due to restrictions from sanitary laws and a lack of public acceptance for processing HOW for this purpose [3], several companies currently raise BSF larvae on cereal byproducts. Subsequently, the BSF larvae meal is sold as feed for animals [4] and BSF larvae production residue (BSFR) is sold for fertilizer.

Although using only cereal byproducts for BSF larvae production will maintain the safety of feed for livestock, this regulation may inhibit the possibility of sustainable resource recycling technology. The BSFR could be used as an organic fertilizer because BSF larvae can use livestock manure and HOW as a food source [5,6], which is typically disposed of as organic waste [6]. However, most previous studies on BSF larvae production have been conducted to develop efficient organic waste treatment conditions for BSF larvae production.

A previous study on the density of BSF larvae reported that a larval density of 1.2 larvae/cm^2 and a feeding rate of 163 mg/larva/day (dry base) were optimal for ideal organic waste disposal [7]. Although, in the case of one feeding, the individual larvae weight was more affected by the nutrient concentration of the feed than the density of larvae [8].

Efficient BSF larvae production methods are currently being investigated; however, very few studies currently exist on the fertilizer value of BSFR made from HOW. In a recent study, a mixture of municipal solid organic waste from factories and households was treated with BSF larvae [6]. This study reported that the heavy metal content in municipal solid organic waste was reduced and the heavy metal content in the residue was below the threshold for fertilizer use [6]. However, the study did not investigate microorganisms in the residue and evaluate its effects on plants. Therefore, a detailed evaluation of the HOW-derived BSFR has still not been conducted. Although some microbial benefits of BSF larvae production have been reported, in particular a reduction in *Escherichia coli* in livestock manure [9,10], it appears that only one study has investigated the microbiota of BSFR [11]. Furthermore, to date no detailed studies that analyzed the fertilizer value of BSFR exist. Thus, this present study aimed to better explain the nutrient composition and suitability of BSFR as a fertilizer in comparison with commercial compost.

2. Materials and Methods

2.1. Preparation of BSF Larvae and Experimental Household Organic Waste

BSF eggs were obtained from an adult breeding department in a laboratory (Research Institute of Environment, Agriculture and Fisheries, Osaka Prefecture) and the larvae were reared for seven days following the methods described by Nakamura et al. [12]. Experimental household organic waste (EHOW) was created according to a previous study [13]. Specifically, the composition of EHOW was 17% cabbage, 17% carrot, 16% potato, 10% horse mackerel, 8% ground pork, 5% apple pomace, 5% banana peel, 4% grapefruit pomace, 4% orange pomace, 3% rice, 3% bread, 3% wheat noodle, 3% Chinese noodle, and 2% eggshell. These ingredients were finely chopped using a food processor and uniformly mixed to create EHOW. EHOW was frozen and stored at −20 °C until required for the experiments.

2.2. Chemical Composition of BSFR and Commercial Compost

The seven-day-old BSF larvae were placed on EHOW (200 mg/larvae, dry base) at 27 °C for 15 days to create BSFR. A composition analysis was undertaken to compare BSFR to cow, horse, and poultry waste composts in order to clarify the value of BSFR as fertilizer and commercial compost.

Concentration of total organic carbon (TOC), total nitrogen (TN), carbon to nitrogen ratio (C/N), ammonium nitrogen (NH_4^+-N), nitrate (NO_3-N), phosphorus (P), potassium (K), sodium (Na), copper (Cu), calcium (Ca), iron (Fe), magnesium (Mg), zinc (Zn), manganese (Mn), crude ash, pH, and electrical conductivity (EC) were measured. The concentration of carbon in each sample was analyzed using a CHN analyzer (MT-6, YANACO, Tokyo, Japan) according to the manufacturer's instructions. Water content (950.01), total nitrogen (955.04), ammonium nitrogen (920.03), nitrate nitrogen (930.01), phosphorus (958.01), potassium (983.02), sodium (965.09), copper (965.09), calcium (965.09), iron (965.09), magnesium (965.09), zinc (965.09), and ash (955.03) within the samples were analyzed according to the Official Methods of Analysis of the Association of Official Agricultural Chemists (AOAC) [14]. The value of pH and EC were measured using a pH meter (pH-22B, HORIBA, Tokyo, Japan) and an EC meter (EC-33B, HORIBA, Tokyo, Japan) according to the manufacturer's instructions, respectively.

2.3. Analysis of Microbiota in BSFR and Commercial Compost by Amplicon Sequencing

DNA was extracted from EHOW, BSFR, and three commercial composts (cow, horse, and poultry composts) using a Fast DNA SPIN kit for soil (MP Biomedicals, California, CA, USA), according to the manufacturer's instructions. The variable region V3-V4 of bacterial 16S rRNA

genes were amplified using universal primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) [15]. The polymerase chain reaction (PCR) mixture was composed of 10 µM forward primer, 10 µM reverse primer, 2 × KAPA HiFi HotStart ReadyMix (KAPA BIOSYSTEMS, MA, USA), and the extracted fecal DNA template. The first set of PCR conditions were as follows: initial denaturation at 94 °C for 3 min, followed by 25 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension step at 72 °C for 10 min. The second set of PCR conditions for index attachment were initial denaturation at 98 °C for 30 s, followed by 8 cycles of 98 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, and a final extension step at 72 °C for 5 min. The amplicons were purified using AMPure XP beads (Beckman Coulter, Brea, CA, USA). Paired-end sequencing of all libraries was performed on an Illumina MiSeq sequencer (Illumina, San Diego, CA, USA) using a MiSeq Reagent kit v3 (600 cycles; Illumina) according to the manufacturer's instructions.

2.4. Plant Cultivation Test 1

This experiment was conducted according to the methods issued by the Food and Agricultural Materials Inspection Center [16]. For the experiment, pots (113φ × 65 mm) without drainage holes were used. Each compost was used as a fertilizer and 250 g of soil sod (moisture 33.09%, TN 0.42%, NH_4^+-N 0.00%, NO_3-N 0.09%, K 0.05%, P 0.00%, ash 56.94%, pH 7.0, and EC 0.5 mS/cm) was sterilized by an autoclave, placed in a plastic bag and stirred for 1 min. The TN content of each pot was adjusted to 100 mg and 200 mg in each pot for two treatment groups—A group (n = 3) and B group (n = 3), respectively. Thirty Komatsuna (*Brassica rapa* var. *perviridis*) seeds were sown in each pot. To prevent the premature death of the Komatsuna, a 25 mg equivalent of chemical fertilizer (i.e., ammonium sulfate, phosphorus pentoxide, and potassium oxide) was added to all groups. Table 1 shows the fertilization amount in each treatment group and the contents of total nitrogen, available phosphoric acid, and potassium in each group. After sowing the Komatsuna seeds, the plants were cultivated in a constant temperature room at 25 °C and 70% humidity for a 14-h light period and a 10-h dark period for 21 days. To prevent the soil drying out, watering was undertaken once per day after germination. The germination rate on day 5 and the fresh and dry weight of the above-ground portion on day 21 were measured.

Table 1. Fertilization amount for the cultivation test 1.

	Group	EHOW	BSFR	Cow	Horse	Poultry
A	Amount Applied (g)	10.25	4.72	6.44	14.72	3.09
	Nitrogen [a] (mg)	100.00	100.00	100.00	100.00	100.00
	Phosphorus [a] (mg)	6.54	2.34	5.99	3.62	3.41
	Potassium [a] (mg)	267.28	89.68	90.14	1020.77	20.22
B	Amount Applied (g)	20.50	9.43	12.87	29.43	6.19
	Nitrogen [a] (mg)	200.00	200.00	200.00	200.00	200.00
	Phosphorus [a] (mg)	13.08	4.69	11.99	7.24	6.83
	Potassium [a] (mg)	534.56	179.37	180.28	2041.55	40.43

[a] Calculated amount. EHOW—experimental household organic waste, BSFR—black soldier fly residue.

2.5. Plant Cultivation Test 2

This experiment was conducted according to the methods issued by the National Agriculture and Food Research Organization [17]. The amounts of compost and sod applied from each group are displayed in Table 2. The amount of poultry compost was decreased to half of the level of other compost types to avoid excessive application of nitrogen. Unsterilized compost and 250 g of the soil sod was placed in a plastic bag and stirred for 1 min. Then, mixed soil was placed into pots (113φ × 65 mm) without drainage holes. Sixteen Komatsuna seeds were sown in each pot (n = 5). After sowing, the plants were cultivated in a constant temperature room at 25 °C and 70% humidity for a 14-h light period and a 10-h dark period for 21 days. To prevent the soil drying out, watering was

undertaken once per day after germination. The germination rate on day 5 and the fresh weight of the above-ground portion and total leaf number on day 21 were measured.

Table 2. Fertilization amount for the cultivation test 2.

Group	Control	BSFR1	BSFR2	BSFR3	Cow	Horse	Poultry
Amount applied (g)	0.00	25.18	12.60	8.33	25.20	25.52	12.49

2.6. Statistical Analysis

The chemical composition and the results of the cultivation tests were statistically analyzed by one-way analysis of variance (ANOVA), Games– Howell nonparametric post-hoc tests, Spearman rank-order correlation coefficients (Spearman's rho), and principal component analysis (PCA) on the correlation matrix of the chemical composition using Statistical Product and Service Solutions (SPSS) software (SPSS Statistics 25, International Business Machines Corporation, Armonk, NY, USA). PCA was also performed using the statistical software PAST4.0 [18] and a biplot diagram was created. The analysis of the microbiota data was performed according to the method described in Kawasaki et al. [13].

3. Results

3.1. Chemical Composition of BSFR and Commercial Compost

The chemical composition of each sample is shown in Table 2. The chemical composition of the samples was significantly different between groups, excluding the value of K (Table 3). BSFR had the highest concentration of NH_4^+-N and highest EC value among the samples. The moisture content of BSFR was lower than that of EHOW but higher than found in livestock manure. The C/N was lower than EHOW and similar to the cattle manure. The nitrogen content of BSFR was similar to the poultry manure. The nitrate nitrogen content of BSFR was the next highest, and the nitrate nitrogen content of BSFR was the lowest among the composts. The P content of BSFR was almost equal to the EHOW. BSFR had the highest value of Ca, but when compared to the livestock manure, the mineral content of BSFR had lower values of all minerals. The ash content of BSFR was higher than EHOW but not livestock. The pH of BSFR was neutral.

Table 3. Chemical composition of EHOW and fertilizers (dry matter basis).

Items	EHOW	BSFR	Cow	Horse	Poultry	SEM	*p*-Value
Moisture (%)	78.01^{a}	55.60^{b}	39.98^{c}	27.80^{d}	14.21^{e}	5.93	<0.01
C (%)	45.43^{a}	35.84^{b}	25.34^{c}	35.12^{d}	27.40^{e}	1.90	<0.01
N (%)	0.98^{ad}	2.16^{b}	1.55^{ac}	0.68^{d}	3.23^{bc}	0.25	<0.01
C/N	48.09	16.61^{a}	16.31^{a}	53.88	8.58^{b}	5.22	<0.01
NH_4^+-N (%)	0.07^{a}	0.88^{b}	0.08^{a}	0.25^{c}	0.48^{d}	0.08	<0.01
NO_3-N (%)	0.04^{a}	0.10^{b}	0.57^{c}	0.15^{ab}	1.25^{d}	0.12	<0.01
P (%)	0.06^{a}	0.05^{b}	0.09^{c}	0.02^{d}	0.11^{e}	0.01	<0.01
K (%)	0.12^{a}	0.07	0.18	0.16^{b}	0.19	0.02	0.12
Na (%)	0.04^{a}	0.08	0.10^{b}	0.11^{b}	0.09^{b}	0.01	<0.01
Cu (%)	0.00^{a}	0.01^{b}	0.03^{c}	0.14^{d}	0.06^{e}	0.01	<0.01
Ca (%)	0.09^{a}	1.00^{b}	0.13^{c}	1.25^{b}	2.14^{b}	0.21	<0.01
Fe (%)	0.03^{a}	0.24^{b}	1.15^{c}	7.15^{d}	1.04^{c}	0.71	<0.01
Mg (%)	0.02^{a}	0.09^{b}	0.13^{c}	0.20^{abc}	0.21^{bc}	0.02	<0.01
Zn (%)	0.00^{a}	0.01^{a}	0.17^{b}	0.44^{c}	0.36^{d}	0.05	<0.01
Mn (%)	0.00^{a}	0.01^{a}	0.23^{b}	0.14^{c}	0.29^{b}	0.03	<0.01
Ash (%)	1.04^{a}	12.65^{b}	18.00^{c}	33.74^{d}	38.74^{d}	3.70	<0.01
pH	6.20^{a}	7.40^{a}	9.10^{a}	8.17^{b}	8.30^{ab}	0.26	<0.01
EC (mS/cm)	3.67^{a}	9.67^{b}	5.53^{c}	2.30^{d}	7.27^{e}	0.70	<0.01

Different alphabet characters indicate significant difference (Games–Howell nonparametric post-hoc test, $p < 0.05$). SEM—standard error of the mean. *p*-values were computed by one-way ANOVAs and represent the significant differences in the results.

3.2. PCA of the Chemical Composition

The PCA plot is shown in Figure 1. The correlation coefficients between each chemical component are shown in Supplementary Table S1. The PCA on the correlation matrix of the chemical composition showed four components with eigenvalues higher than 1 (Supplementary Table S2). The contributing rate of each principal component (PC) was PC 1, 47.55%; PC 2, 28.90%; PC 3, 13.02%; PC 4, 6.10%; and the total contributing rate of the three components was 95.57%. BSFR plotted between EHOW and cow in PC 1, plotted close to poultry in PC 2, plotted close to horse in PC 3, and plotted close to cow and horse in PC 4.

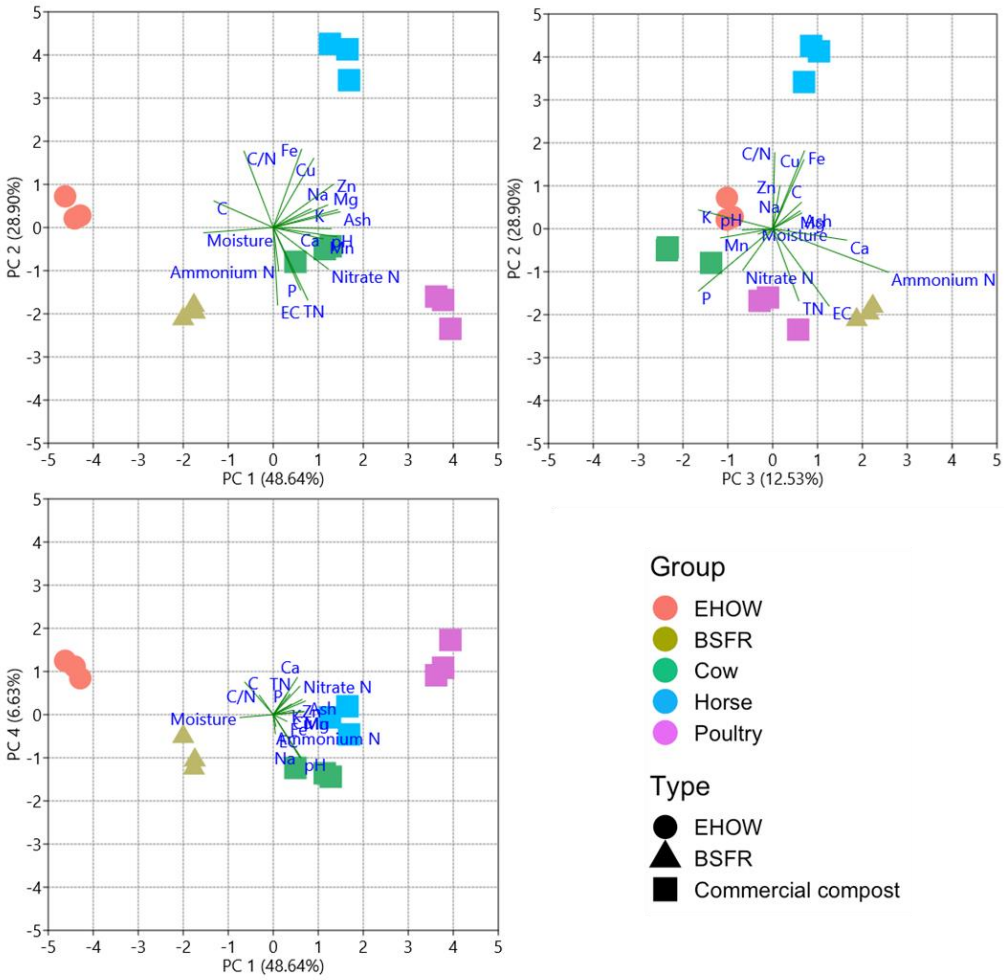

Figure 1. Principal component analysis of the groups.

3.3. Relative Abundance in Microbiota of BSFR and Commercial Compost

Up to 10 highly abundant bacteria were identified for each study group (Table 4). The most abundant bacteria for BSFR was Bacillaceae, similar to poultry. However, *Sporosarcina* and Xanthomonadaceae, which recorded the second and third highest abundance in the microbiota of BSFR, did not show high abundance of microbiota in any other group. Lactobacillales, *Carnobacterium*, and *Escherichia*, were observed in high abundance within the microbiota of EHOW, but were not found in high abundance in BSFR. *Corynebacterium*, *Bacillus*, *Virgibacillus*, and Aerococcaceae were detected in BSFR and also in horse, poultry, or both. *Trichococcus*, *Natronobacillus* and Erysipelotrichaceae were detected only in BSFR.

Table 4. The 10 most abundant microbial taxonomic groups (relative abundance, %) in the samples.

No.	EHOW		BSFR		Cow		Horse		Poultry	
	Taxonomy	**(%)**	**Taxonomy**	**(%)**	**Taxonomy**	**(%)**	**Taxonomy**	**(%)**	**Taxonomy**	**(%)**
1	Lactobacillales	31.33	Bacillaceae	22.91	*Halomonas*	15.27	Bacillales	16.52	Bacillaceae	51.31
2	*Carnobacterium*	21.55	*Sporosarcina*	13.21	*Georgenia*	10.27	[Weeksellaceae]	9.61	Bacillales	21.69
3	*Escherichia*	16.68	Xanthomonadaceae	9.82	Bacillaceae	7.75	*Pseudomonas*	6.64	*Corynebacterium*	5.79
4	*Enterococcus*	11.16	*Corynebacterium*	9.49	Sphingobacteriaceae	7.20	Jonesiaceae	4.41	*Yaniella*	4.99
5	*Lactococcus*	5.06	*Bacillus*	8.05	Flavobacteriaceae	6.68	*Bacillus*	3.45	Aerococcaceae	2.55
6	*Vagococcus*	3.63	*Virgibacillus*	6.22	Promicromonosporaceae	4.73	*Corynebacterium*	3.34	*Virgibacillus*	2.15
7	*Lactobacillus*	2.54	*Trichococcus*	4.54	*Marinimicrobium*	3.42	Porphyromonadaceae	3.32	*Bacillus*	1.93
8	*Proteus*	1.47	Aerococcaceae	4.51	*KSA1*	2.83	Alcaligenaceae	3.04	*Lactobacillus*	0.94
9	*Pseudomonas*	1.18	*Natronobacillus*	3.39	Clostridia	2.83	*Georgenia*	2.49	*Salinicoccus*	0.88
10	Enterococcaceae	1.06	Erysipelotrichaceae	2.92	Bacillales	2.39	Bacillaceae	2.17	*Lentibacillus*	0.65
	Total	95.66		85.05		63.36		54.99		92.88

Microbial classification at the lowest possible taxonomic level and their relative abundance in the microbiota of the samples (n = 4).

3.4. Analysis of Microbial Diversity for between Groups

When α-diversity (Chao 1 index: richness, Shannon index: evenness) was compared among the groups, no significant difference among groups was observed for any α-diversity index (Figure 2).

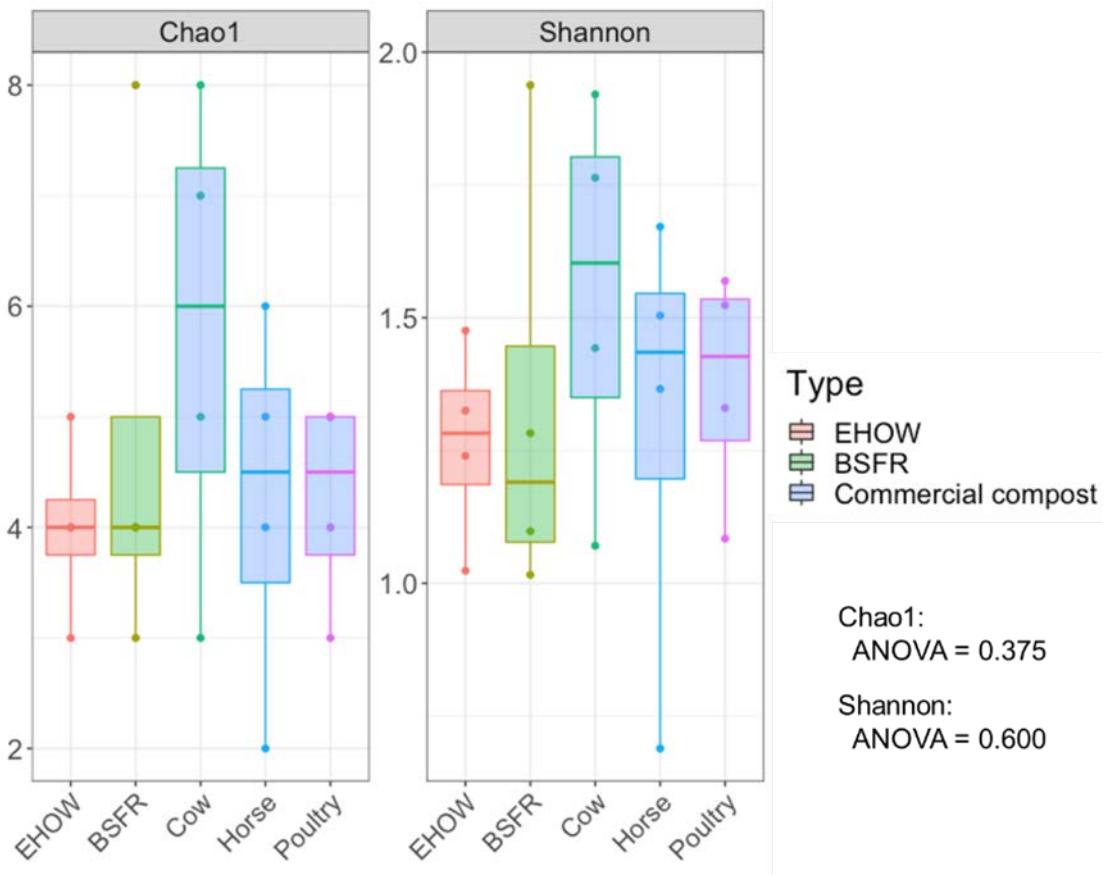

Figure 2. Alpha diversity indices (Chao1 and Shannon) of microbial communities in the groups (n = 4). ANOVA—analysis of variance.

Regarding β-diversity based on unweighted and weighted UniFrac distance, BSFR and poultry were closely clustered in the principal coordinate analysis (PCoA) plots of the first three axes (axes 1, 2, and 3; Figure 3). BSFR data were located away from those of EHOW and Cow. Permutational multivariate analysis of variance (PERMANOVA) indicated that the β-diversity in the microbiota of the groups had differences among groups (PERMANOVA < 0.05; Figure 3).

3.5. Plant Cultivation Test 1 (Same Amount of Nitrogen)

On day five of the experiment, the germination rate for the Komatsuna seeds was lower in all groups when starting N was 200 mg, compared to 100 mg (Table 5). BSFR showed the highest value when starting total nitrogen was 100 mg, while horse showed the highest value for the above-ground fresh weight of Komatsuna when starting total nitrogen was 200 mg on day 21. BSFR showed the highest above-ground dry weight of Komatsuna regardless of the starting N amount ($p < 0.05$). There was no difference in the physical appearance of Komatsuna on day 21 in A group, but EHOW revealed smaller leaves and one pot with a low germination rate was recorded for horse (Figure 4).

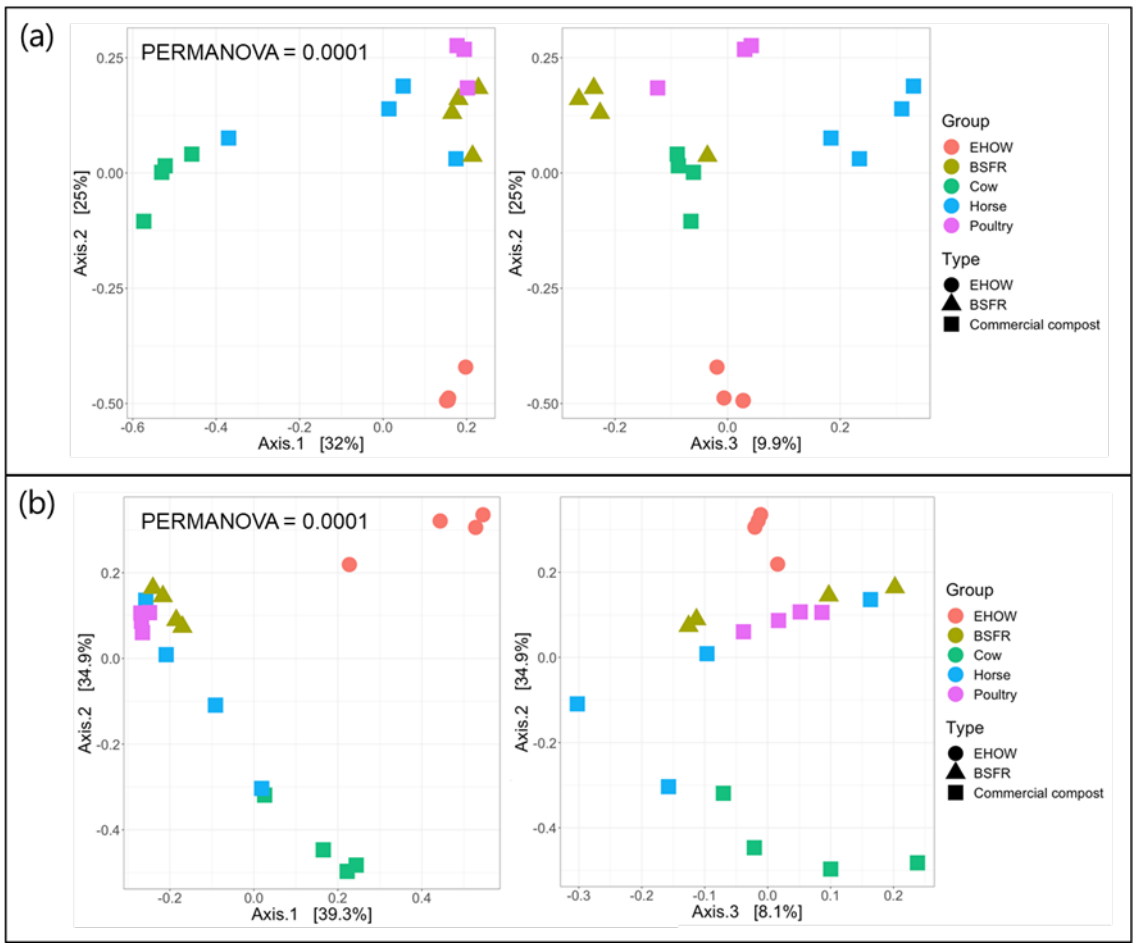

Figure 3. Beta diversity of microbial communities in the samples (n = 4). (**a**) Unweighted and (**b**) weighted UniFrac distance principal coordinate analysis plots of β-diversity measures of the microbiota communities in the samples (n = 4). PERMANOVA—permutational multivariate ANOVA.

Table 5. Results of the cultivation test 1 showing germination rate, fresh weight, and dry weight of Komatsuna.

	Group	EHOW	BSFR	Cow	Horse	Poultry	SEM	*p*-value
A	Germination rate (%)	72.22	70.00	67.78	72.22	70.00	3.01	0.99
	Fresh weight (mg/strain)	141.36	172.10	157.49	171.49	129.60	7.41	0.29
	Dry weight (mg/strain)	9.35	11.45	10.96	8.67	8.71	0.40	0.04
B	Germination rate (%)	42.22	42.22	51.11	32.22	44.44	2.59	0.25
	Fresh weight (mg/strain)	68.71^{a}	149.98^{bc}	124.41^{bc}	156.41^{c}	99.48^{ab}	9.05	<0.01
	Dry weight (mg/strain)	5.09^{a}	11.45^{b}	9.16	10.30	7.97	0.65	<0.01

Groups A, B: Starting total nitrogen in each group was (A) 100 mg and (B) 200 mg. Different alphabet characters indicate significant difference (Games–Howell nonparametric post-hoc test, $p < 0.05$). SEM—standard error of the mean. *p*-values were computed by one-way ANOVAs and represent the significant differences in the results.

3.6. Plant Cultivation Test 2

On day five of the experiment, the germination rate for the Komatsuna seeds was lower in BSFR1 and poultry ($p < 0.05$). The total leaf number of Komatsuna on day 21 revealed the highest value for BSFR2, while BSFR1 recorded the lowest value. On day 21, the fresh weight of the above-ground Komatsuna plant material was highest for BSFR2, while BSFR1 and poultry recorded the lowest values for fresh weight above-ground (Table 6). BSFR2 was the most frequently observed in Komatsuna material on day 21 (Figure 5).

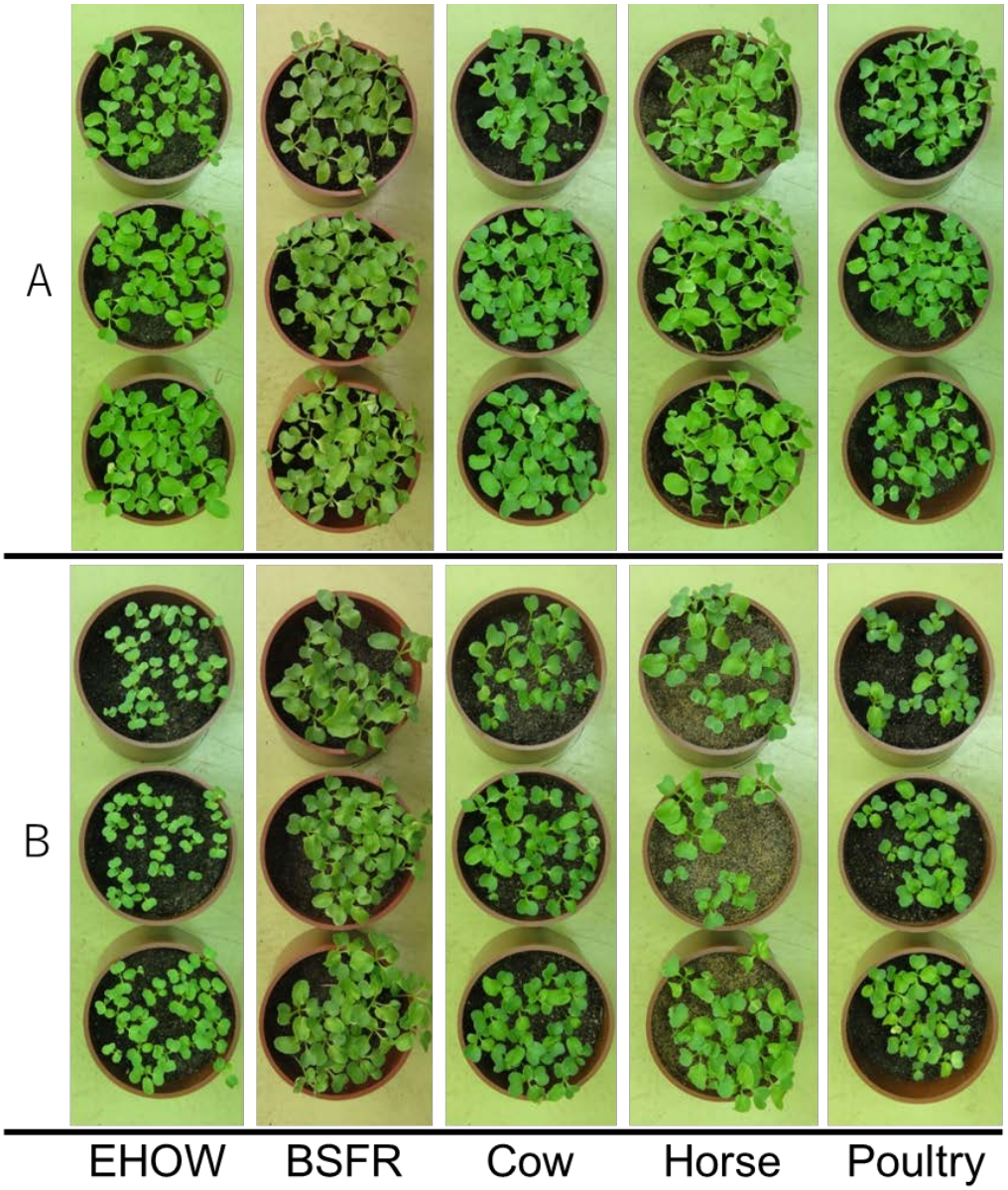

Figure 4. Plants on day 21 of the cultivation subjected to the two N levels ((**A**) = 100 mg, (**B**) = 200 mg) and the tested organic matrices (EHOW, BSFR, cow manure compost, horse manure compost, poultry manure compost) (plant cultivation test 1).

Table 6. Results of the cultivation test 2 showing germination rate, total leaf number, and fresh weight of Komatsuna.

Group	Control	BSFR1	BSFR2	BSFR3	Cow	Horse	Poultry	SEM	*p*-value
Germination rate (%)	85.00	53.75 ^{a}	73.75	75.00	78.75	86.25 ^{b}	57.50 ^{ac}	2.57	<0.01
Total leaf number	30.40 ^{a}	17.50 ^{ab}	49.20 ^{c}	47.60 ^{c}	41.80 ^{cd}	35.60 ^{ad}	10.20 ^{b}	2.50	<0.01
Fresh weight (g/plant)	1.05 ^{a}	0.60 ^{ac}	4.53 ^{b}	3.51 ^{b}	2.77 ^{b}	1.54 ^{ac}	0.39 ^{c}	0.27	<0.01

Different alphabet characters indicate significant difference (Games-Howell nonparametric post-hoc test, $p < 0.05$). SEM—standard error of the mean. *p*-values were computed by one-way ANOVAs and represent the significant differences in the results.

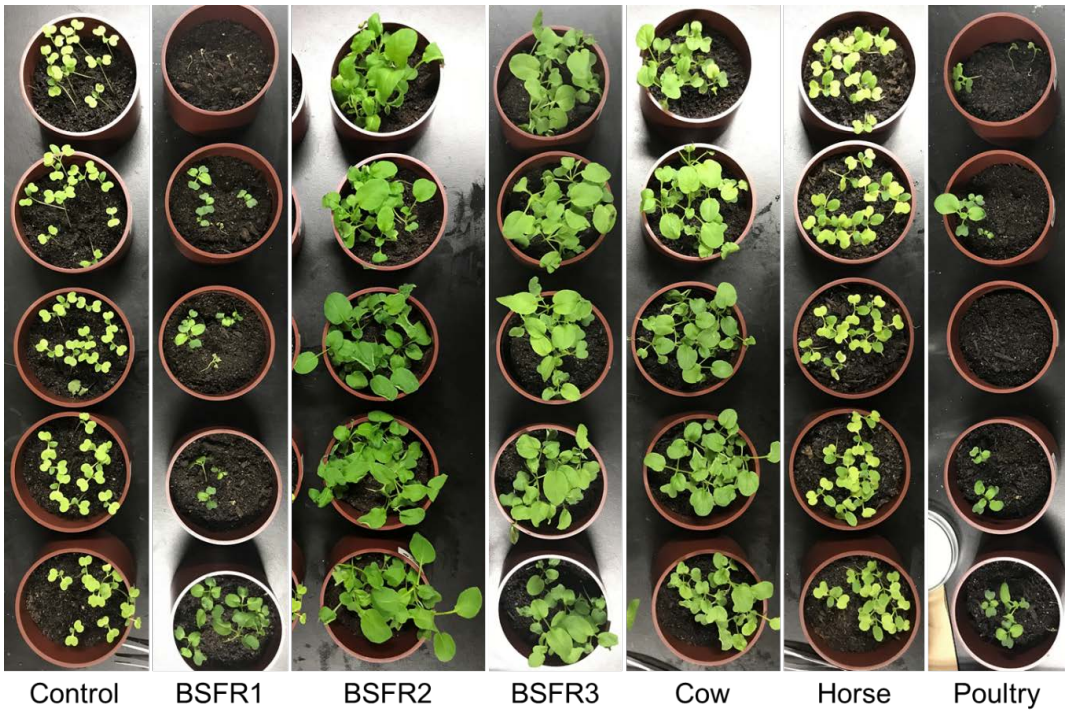

Figure 5. Physical appearance of Komatsuna on the 21st day of cultivation test 2.

4. Discussion

The final chemical composition of BSFR differed from EHOW and other commercial composts, and was distanced from the other groups in the PCA plots. BSFR had a higher N and ash in its chemical composition compared to EHOW because BSFR includes larval feces and molted residues. Moreover, P and K were slightly lower which is possibly due to their use for larval growth. Since BSFR was produced only by larval processing and air-drying, its final composition was similar to poultry manure composts. However, the concentration of NO_3-N in the poultry manure was higher than NH_4^+-N because it had been processed through the animal, deposited, and subsequently dried. Conversely, BSFR showed a higher concentration of NH_4^+-N than NO_3-N, which is likely as BSFR was only treated by larvae and did not experience the microbial fermentation process associated with animal digestion and deposition. Previous reports indicate that the application of fertilizers and manures derived from livestock excrement containing high levels of NO_3-N have led to the accumulation of NO_3-N in vegetables and drinking water, resulting in health risks [19]. However, BSFR, unlike conventional livestock manures, may be a new fertilizer with less concern of potential NO_3-N accumulation. Moreover, since higher cation exchange capacity (CEC) soil can retain ammonium ions in the soil colloids [20,21], BSFR may be suitable in higher CEC soil. However, NH_4^+-N can volatilize as ammonia when it comes into contact with the alkaline soil, and volatilize as nitrite gas when it comes into contact with acidic soil [20–22]. This gas can cause injury to plants (e.g., tomato, green pepper, or eggplant) [23,24]. Hence, the application method for BSFR would be a future concern.

In the PCA, a positive correlation was observed among the analyzed properties excluding moisture, C, C/N ratio, and EC in PC 1. Thus, PC 1 could be considered in the evaluation of the main fertilizer nutrients for plants, such as N, P, and K. Moreover, a positive correlation was also recorded for pH, which is acidic in the early stages of composting due to organic acids, but gradually mineralizes and becomes alkaline [25,26]. This might indicate that the concentration of fertilizer nutrient compositions in the samples increased as the PC value increased positively, and the pH tended to be alkaline. Whereas, the negative values were larger in the samples with a higher C/N ratio due to the abundance of organic matter. In general, organic wastes have high moisture and high organic matter, and if they are applied as fertilizer without fermentation by composting, the germination of plants will be

inhibited and their growth will be damaged [27]. Thus, higher negative PC values would indicate that the sample is close to the unfermented condition and does not sufficiently work as a fertilizer. In PC 2, negative correlations were found for TN, P, and EC, while positive correlations were found for C/N ratio, Cu, and Fe. These results indicate that for PC 2, when the value of the PC increased negatively, the sample was rich in TN and P, and the EC value was also high; when the value of the PC increased positively, the C/N ratio was high and the Cu and Fe content were high. In PC 3, there was a positive correlation with ammonium nitrogen, which suggests that the concentration of NH_4^+-N was higher when the PC value was positively increased. In PC 4, each sample plotted in approximately the same position, a situation that also makes it difficult to characterize the other PC. PC 1 represents the compost characteristics of each sample, and PCs 2 and 3 represent the detailed compost characteristics that support PC 1.

The livestock manures are plotted at different locations in the plots of PC 1 and 2 due to their corresponding characteristics. Despite this, they are considered ready for use as fertilizer. The plots demonstrated that EHOW was plotted at a farther distance from the livestock manures and is not ready for use as a fertilizer in its original condition. Although BSFR is closer to livestock manures than EHOW, it had high moisture and a high EC value for the conditions used in this study. Therefore, BSFR can be better processed for fertilizer use by increasing the period of larval processing, drying, and reducing the organic matter.

The top 10 microbiota for BSFR in terms of relative abundance were similar to those in poultry, but the structure of BSFR demonstrated a distinct composition. Unlike poultry manure, BSFR is not subject to composting for a long period of time, which was evident from its chemical composition. This may occur because BSFR is a dry product of animal feces. *Sporosarcina*, which exhibited a high abundance of BSFR microbiota was also detected in the BSF larvae and the feed residue [11]. Hence, this suggests that *Sporosarcina* originated from larvae. In the α diversity, BSFR did not show any significant differences with other commercial composts, indicating that the diversity of constituent bacteria was similar to commercial composts. In the β diversity, BSFR plotted at far distances from EHOW, which suggests that changes in the constituent bacteria were caused by larval processing. Moreover, although BSFR plotted near horse, it was plotted closest to poultry, indicating that the microbiota of BSFR was closest to that of poultry manure, similar to the results of the top 10 bacterial composition.

It is important to determine whether BSFR is safe for plant and human health when considering its use as a fertilizer. The abundance of *Escherichia* in EHOW was reduced by larval processing, which confirms that BSFR can be used as a fertilizer, in addition to a commercial compost. However, caution should be taken in the application of BSFR as a fertilizer for vegetables of Brassicaceae, vineyards, or citrus orchards, as Xanthomonadaceae was recorded in high abundance in the microbiota of BSFR. This family includes two genera *Xanthomonas* and *Xylella* which can cause disease in plants [28–31].

In the Komatsuna cultivation test, there was no difference in the germination rate for standard condition A when fertilizer was applied to a starting total of 100 mg of total nitrogen per pot. Although BSFR contains Xanthomonadaceae, which includes a potentially disease-causing bacteria, no pathogens were observed in the Komatsuna during the cultivation test and they appeared to grow normally. Moreover, under condition A, the highest values for the fresh and dry weights of the Komatsuna were from BSFR. Hence, BSFR can be applied as a commercial fertilizer if the amount of nitrogen is adjusted to standard application levels. The conversion of organic waste into BSFR by BSF larvae is better than using organic waste as fertilizer directly as the germination rates of EHOW and the BSFR Komatsuna were almost the same under condition B. However, the fresh and dry weights for the day 21 EHOW and BSFR Komatsuna were more than twice as high in the initial BSFR group. The common fertilizer values (nitrogen, phosphorus, and potassium) and the values of other chemical compositions that were altered by larval processing may have affected Komatsuna growth. This is strongly suggested by the weakly acidic pH that is suitable for growing plants but was only recorded in EHOW [16], as well as the fresh weight which was the lowest of all groups. Therefore, rather than directly using the organic waste as a fertilizer it is beneficial in plant production to use organic waste as feed for BSF larvae and

then use the residue as fertilizer. Under the conditions of the present study, it is evident that if BSFR is applied at 1/20 of the amount of soil, then there is no yellowing of leaves due to nitrogen deficiency, which was observed in the horse group. This result was also apparent when BSFR was applied at 1/30 of the amount of soil. The germination of Komatsuna was inhibited when 1/10 of the amount of BSFR was applied to the soil. These results indicate that applying such a large amount of BSFR to the soil is not recommended. This recommendation is further reinforced as BSFR recorded the highest EC value.

5. Conclusions

In summary, BSFR derived from EHOW could be an incomplete compost based on its chemical composition for short term larval processing. This process mainly focuses on larvae production, but it contains a significant amount of ammonium nitrogen which could be an effective N source for plant nutrition. This may reduce environmental pollution of nitrates in the soil, which is typical of several composts that are not properly stabilized. However, the presence of plant pathogens in the BSFR microbiota needs to be considered. The effects of BSFR on the environment, soil microbial community, and plant productivity require more detailed investigation in a mid-term experiment.

Supplementary Materials: The following are available online at http://www.mdpi.com/2071-1050/12/12/4920/s1. Table S1: Spearman rank-order correlation coefficients of the chemical composition of the samples. Table S2: Component Matrixa of the variables.

Author Contributions: Writing—original draft preparation, K.K.; writing—review and editing, T.K., H.H., Y.M., and Y.F.; insect preparation, H.H.; examination, K.K. and T.K.; data analysis, K.K. and T.K.; project administration, K.K., Y.M., and Y.F.; and funding acquisition, K.K., Y.M., and Y.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Environment Research and Technology Development Fund (1-1604) of the Ministry of the Environment, Japan.

Acknowledgments: We thank Shun-ichiro Iwase, Tomohiro Seyama, Akihisa Izumo, Koji Kasai, and the staff of the Research Institute of Environment, Agriculture and Fisheries, Osaka Prefecture for their assistance in taking care of the insects.

Conflicts of Interest: The authors declare no conflict of interest.

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