The effect of application of compost and frass from Black Soldier Fly Larvae (*Hermetia illucens* L.) on growth of Pakchoi (*Brassica rapa* L.)

D Agustiyani*1, R Agandi2, Arinafril2, A A Nugroho1 and S Antonius1

1 Research Center for Biology, Indonesian Institute of Sciences (LIPI), Bogor, Indonesia
2 Faculty of Agriculture, Sriwijaya University, Kampus Indralaya, Ogan Ilir, Indonesia

DA and SA contributed equally as main authors

*Email: titinagustin@yahoo.com

Abstract. Pot experiment was conducted to observe the effect of frass (insect feces) from Black Soldier Fly larvae (BSFL) as soil amendments at different application rates in comparison with other organic fertilizers on Pakchoi (*Brassica rapa* L). The experimental design was completely random with seven treatments, namely compost, compost+LOB (liquid organic biofertilizer), 5%, 10%, and 15% BSF frass, chemical fertilizer (NPK, equivalent to 10 kg/ha), and soil as a control. After 35 days planting, the result showed that Pakchoi cultivated with compost+LOB, compost, and BSF frass produced the highest biomass compared to control and NPK treatment significantly. The highest total plant weight was achieved in the treatment of Compost+LOB (24.4 gr), followed by 10% BSF frass (23.3 gr), compost (21.8 gr), 15% BSF frass (20.7 gr), 5% BSF frass (19.8 gr), NPK (1.3 gr) and control soil (0.9 gr). While the chlorophyll content was not significantly different between all treatments, the application of BSF frass tended to be higher but with curly leaf symptoms than other treatments. The population of *P* solubilizing bacteria and the activity of PME-ase enzyme in soil were also higher in the treatment of compost, compost+LOB, and BSF frass compared to control and NPK treatment.

1. Introduction

Agricultural development in Indonesia is constrained by problems with soil quality. One of the major issues is the low organic matter content. Other common problems inherent to tropical soils are soil acidity, excessive aluminum, and calcium deficiency [1]. Low soil qualities are also caused by poor land management [2]. Application of composted material as fertilizer does not only provide essential nutrients to plants, it also improves soil quality. Composted organic material contains essential nutrients for plant growth, especially N and P [3]. Therefore, increasing soil organic matter has the added benefit of improving soil quality and enhancing the long-term sustainability of agriculture [4]. It is well known that compost can improve not only the physical and chemical properties of the soil but also improve the soil microbial community. The primary role of organic amendments is to rejuvenate the soil by creating a favorable soil-plant–microbial environment and to make the live-live soil system for healthy plant growth [5].

Various organic materials can be used as a basis for making compost and solid fertilizers, including agricultural waste, sludge from wastewater treatment, animal manure, fish feed, shredded paper, mushroom growth media, and other organic wastes. Recently, frass, which is a by-product of Black Soldier Fly Larvae (BSFL) rearing, has been used as an alternative organic fertilizer. The frass contains substantial amounts of nutrients [6] that could be useful in crop production if converted into organic...
fertilizer. It has been reported that frass application positively impacts plant growth of *Helianthus annulus* by increasing stem width as well as fat content in seeds. However, since frass quality may be affected by the type of insect substrate [7], the effect of frass on plant growth may not be possible to be generally concluded.

Soils which are rich in reduced organic carbon will support extensive soil microbial communities. Microbes of the rhizomicrobiome play key roles in nutrient acquisition and assimilation, improve soil texture, secreting, and modulating extracellular molecules such as hormones, secondary metabolites, antibiotics, and various signal compounds, all leading to enhancement of plant growth [8]. Research has demonstrated that inoculating plants with Plant Growth-Promoting Rhizobacteria (PGPR) can be an effective strategy to stimulate crop growth. Furthermore, these strategies can improve crop tolerance for the abiotic stresses (e.g., drought, heat, and salinity) and also biotic stresses (pests and diseases) [9].

The use of PGPR in agriculture does not only reduce agrochemicals usage, but it is also more environmentally-friendly [10,11]. This discovery has resulted in multifunctional PGPR based formulations for commercial agriculture. LIPI has been developing an innovative PGPR-based organic biofertilizer, LOB-Beyonic StarTmik-LIPI (LOB-BS). It is a Liquid Organic Biofertilizer (LOB) inoculant that contains microorganisms with abilities of nitrogen-fixing, phosphate solubilizing, organic acid secretion, and growth regulator secretion, which aims to minimize the use of synthetic fertilizers and agrochemicals [12].

At present, organic fertilizers are not widely used in intensive arable agriculture and less known about the effects of frass of BSFL on the crop. High input agricultural systems that supply only major nutrients to the crop may suffer from a lack of secondary nutrients (e.g., Ca, Mg, and S) and micronutrients (e.g., Fe, Cu, and Zn), which can impact yield and nutritional quality of harvested products [13]. Organic fertilizers are generally needed in a relatively large amount; which is often difficult to be practiced by farmers. The use of insect BSF frass as organic fertilizer is a relatively new concept. Adoption of a new concept or product as fertilizer in any farming system requires information on its performance in terms of how it influences crop growth, yield, nutrient uptake, and efficiency usage in comparison to existing fertilizers.

Pakchoi is a vegetable crop that has economic value. Pakchoi plant production depends on environmental conditions and nutrient content in the soil. In order to explore the utilization of BSF frass as a novel organic fertilizer, growth experiments involving Pakchoi (*Brassica rapa* L) were conducted in pot experiments to determine the effects of this fertilizer on plant growth and soil fertility (soil biochemical properties).

### 2. Material and Methods

#### 2.1. Plant Litter Compost

Compost was originated from bioconversion of mix plant litter waste and cow dung added with *Fusarium equiseti*, *Aspergillus fumigatus*, and *Trichoderma harzianum* fungal decomposer that shows cellulose, lignin peroxidase, and protease enzyme activities.

#### 2.2. BSF Larvae Feces (BSF frass)

The BSF frass fertilizer was a product obtained from a local farmer of an integrated garbage disposal site of *Perumahan Mutiara Bogor Raya*.

#### 2.3. Liquid Organic Biofertilizer (LOB)

LOB-BS contains 11 PGPR isolates that have activities of P solubilizing, N-fixing, produce growth and biocontrol agent. All isolates were fermented with the addition of organic matter [12].
2.4. Application of several formulas of soil conditioner/organic fertilizers on Pakchoi grown under greenhouse

Application of several formulas of soil conditioner on Pakchoi plants in ultisol soil was carried out in the greenhouse. Pot experiments were conducted by completely randomized design consisting of 7 treatments with the basic component of the BSF frass and five replications in each treatment. The seed was sowing in germination material for 7 days then transferred to a polybag (20 x 30cm) filled with a specific growth medium designed for this study with a medium total weight of 2 kg. There were 7 types of growth medium as treatments:

A. Soil (control)
B. Soil and chemical fertilizer NPK (equal to 10 kg/Ha)
C. Soil and compost (2:1)
D. Soil and BSF frass 5% (100 gr)
E. Soil and BSF frass 10% (200 gr)
F. Soil and BSF frass 15% (300 gr)
G. Soil and compost (2:1) + LOB (Liquid Organic Biofertilizer, 25 mL/L)

Plant growth was observed with parameters that included plant height and number of leaves. Pakchoi plants were harvested 35 days after planting, followed by measurement of weight of canopy, roots, total plants, and chlorophyll content. Soon after harvesting, the soil samples were taken for measurement of microbial population and enzyme activity in the soil.

2.5. Soil biochemical properties and bacterial population

To evaluate the effect of organic fertilizer application on soil fertility, some soil biochemical properties were measured, including soil enzymes (urease and phosphomonoesterase/PMEase) and the number of the bacterial population (phosphate solubilizing bacteria and proteolytic bacteria) after 35 days of planting.

2.5.1. Measurement of phosphomonoesterase activity in the soil. Soil samples were weighed as much as 1 g and put into test tubes and added with 1 mL of p-nitrophenylphosphate (p-NPP) substrate and 4 mL of universal phosphate buffer (pH 6.5) and then homogenized with vortex and incubated at 38°C for 2 hours in the water bath. After incubation, 1 mL of 0.5 M CaCl₂ was added and homogenized, then added with 4 mL of 0.5 M NaOH and diluted into 4.5 mL of distilled water. The control solution does not use p-NPP substrate. The absorbances of samples and controls were measured by a spectrophotometer at a wavelength of 400 nm [14].

2.5.2. Measurement of urease activity in the soil. Soil samples were weighed as much as 5 g and put in an erlenmeyer flask. Soil samples were added with 2.5 urea substrates, covered, and then incubated at 37°C for 2 hours. Soil samples were added with 50 mL of 1M KCl solution, shaken for 30 minutes, and centrifuged at 12000 rpm. As much as 0.5 ml of supernatant were added with 4.5 ml of distilled water and Nessler reagent and allowed to stand for 10 minutes. Absorbance was measured at a wavelength of 420 nm [15].

2.5.3. Numeration of P solubilizing bacteria. The medium used to calculate the population phosphate solubilizing bacteria was Pikovskaya media, containing 5.0 g Ca₃(PO₄)₂, 0.2 g NaCl, 0.2 g KCl, 0.1 g MgSO₄.7H₂O, 0.00025 g MnSO₄.7H₂O, 0.00025 g FeSO₄.7H₂O, 0.5 g (NH₄)₂SO₄, 0.5 g yeast extract, 10.0 g glucose, and 20.0 g agar (per L medium). The serial dilutions of soil samples used were 10⁻³ and 10⁻⁴. 20-50 µl of each dilution samples were inoculated on Pikovskaya medium, then incubated at 30°C for two days. The development of a clear zone around the bacterial colony indicated P solubilization activity. The colonies with clear zone were then counted and calculated (CFU/g soil) [16].
2.5.4. Numeration of proteolytic bacteria. The medium used to calculate the population of proteolytic bacteria was Skim Milk Agar (SMA) medium containing 1.0 g glucose, 2.5 g yeast extract, 1.0 g casein, 10.0 g skim milk, and 22.0 g agar (per L medium). The serial dilutions of soil samples used were $10^{-5}$ and $10^{-6}$. 20-50 µl of each dilution samples were inoculated on SMA agar medium, then incubated at 30 °C for two days. The development of a clear zone around the bacterial colony indicated protease enzyme activity. The colonies with clear zone were then counted and calculated (CFU/g soil) [17].

2.6. Analysis of phosphorus (P) and potassium (K) content in leaves and soil
Phosphor and potassium content in leaves and soil were measured after harvest. The K and P elements were extracted by wet ashing using a mixture of concentrated acids HNO$_3$ and HClO$_4$. Macro levels of K in the extract were measured using AAS (Shimadzu), while the level of P was measured using spectrophotometer UV-Vis, Uvmini-1240 Shimadzu [18].

2.7. Statistical analysis
Experimental data were analyzed by Analysis of Variance (ANOVA). If F-values showed significance, Post-hoc comparisons of means were tested using Duncan test at the 0.05 probability level.

3. Results and discussions
The effect of various soil conditioner formulas on the growth of Pakchoi plants was observed for 35 days. Pakchoi plant growth parameters observed were plant height, number of leaves, wet weight of the upper plant (canopy), and root weight. Plant height of Pakchoi showed a maximum increase at 3 weeks after planting, the treatment of compost, BSF frass 5%, 10%, 15%, and compost + LOB showed significant higher growth compared to control and NPK treatment (Figure 1).

![Figure 1](image_url). Increase in Pakchoi height for 5 weeks for each treatments.
Figure 2. Increase in number of Pakchoi leaves for 5 weeks for each treatments.

The number of leaves increased until the age of 5 weeks after planting. The number of leave in treatment of compost, BSF frass 5%, 10%, 15%, and compost+LOB were significantly higher compared to control and NPK treatment (Figure 2).
Application of compost and BSF frass greatly affected the wet weight of the upper plants (canopy) and roots. The highest wet weight of the plant was in the compost+LOB treatment, followed by 10% BSF frass, compost, 15% BSF frass, and 5% BSF frass, respectively. Statistical analyses showed significant different from control and NPK treatment (Figure 3A). The highest root weight was also in the compost+LOB treatment, followed by compost, 15% BSF frass, 10% BSF frass, and 5% BSF frass respectively. Those five treatments were significantly different from control and NPK treatment (Figure 3B). Pakchoi plant performance (Figure 3D) showed curling leaves on BSF frass treatment. It was still not clear why these BSF frass treatments caused curling leaves. It might be caused by the unbalanced plant hormones in this treatment. These plant hormones imbalance may be occurred due to the joint action of BSFL and microorganisms during composting. Compost and vermicompost product contained three important phytohormones (Indole 3-acetic acid, Gibberellic acid, Kinetin) [19]. Something similar might happened in BSF frass. However, chemical compounds and the use of BSF frass as organic fertilizer is still needed to be studied in more detail. It is necessary to combine BSF frass with other organic materials or enriched it with PGPR-based biofertilizer.

Leaves chlorophyll content between treatments was not so significantly different, but there was a tendency of higher content in BSF frass treatment than compost and control. The highest chlorophyll
content was in plants treated with 15% BSF frass, followed 10% BSF frass, 5% BSF frass, compost + LOB, NPK, compost, and control respectively (Figure 4).

![Leaf chlorophyll content two and for weeks after planting for each treatment.](image)

**Figure 4.** Leaf chlorophyll content two and for weeks after planting for each treatment.

Compost and BSF frass applications were not only had a positive effect on plant growth but also on soil biological properties. The population of P-solubilizing and proteolytic bacteria were higher in the BSF frass and compost + LOB treatments compared to control NPK, and compost treatment (Figure 5A and 5B).

![Number of P solubilizing bacteria (A) and proteolytic bacteria (B) on each soil treatment after harvesting.](image)

**Figure 5.** The number of P solubilizing bacteria (A) and proteolytic bacteria (B) on each soil treatment after harvesting.

The biological nature of soil is also indicated by the enzymatic activity. PME-ase and urease enzymes were observed in this study. PME-ase activity tend to be higher in the BSF frass, compost + LOB and compost compared to controls and NPK treatments (Figure 6A). The same trend also occurred in activity of urease enzyme, but the activity of urease enzyme in 15% BSF frass treatment is relatively low compared to the control (Figure 6B).
Figure 6. PME-ase (A) and urease enzyme (B) activities in each soil treatment after harvesting.

Table 1. Phosphorus (P) and Potassium (K) content at leaf and soil on each treatment after harvesting.

| Sample           | P (%) | K (%) | P (%) | K (%) |
|------------------|-------|-------|-------|-------|
| Control          | 0.210 | 0.037 | 1.209 | 0.066 |
| Compost          | 0.900 | 0.086 | 2.031 | 0.172 |
| Compost + LOB    | 0.885 | 0.070 | 1.652 | 0.154 |
| NPK              | 0.215 | 0.039 | 1.315 | 0.083 |
| BSF frass (5%)   | 0.635 | 0.066 | 1.534 | 0.153 |
| BSF frass (10%)  | 0.835 | 0.079 | 1.477 | 0.255 |
| BSF frass (15%)  | 0.855 | 0.089 | 2.572 | 0.264 |

Leaf P contents in treatments of compost, compost+LOB and BSF frass ranged from 0.635% to 0.900%. They were relatively higher than control (0.210%) and NPK treatment (0.215%). Leaf K content also showed the same tendency as P content. Leaf contents in the treatments of compost, compost+LOB, BSF frass were higher (1.477-2.572%) compared to control (1.209%) and NPK treatment (1.315%). Phosphorus (P) and potassium (K) content in the potting soil after harvesting also showed the same tendency as in the leaf (Table 1).

The effect of various organic fertilizers on Pakchoi growth was significantly different compared to control and NPK treatments (Figure 1, 2, and 3). These results indicated that the application effect of compost and BSF frass were effective in supporting plants growth. It is possible that compost and BSF frass as organic fertilizer have acted several things to benefit the soil, it could add organic matter, which improved soil physical properties like the way water interacts with the soil and the soil with vast numbers of beneficial microbes. The composted organic material contains essential nutrients for plant growth, especially N and P [3]. Solid fertilizers produced by BSFL (BSF frass) may allow plants to fulfill their requirements of phosphorus (P) and potassium (K), which is considered quite lacking in common soil [20]. Data on Table 1 showed that P and K content in leaf and soil in treatment of BSF frass, compost+LOB, and compost were relatively higher than 1 control and NPK treatments. However, the highest and the best performance of plant growth was in treatment of compost + LOB (Figures 3). The Pakchoi leaf performance in BSF frass treatment was curling, although the chlorophyll content was relatively high (Figure 3C and 4). This study showed that compost enriched with LOB Beyonic Startmik containing PGPR with functions on nitrogen-fixing, phosphate solubilization, organic acid secretion, and growth regulator secretion have the best effect on productivity and quality of plant crop.

The basic concept of applying organic fertilizer is to create healthy soil. There are many parameters used as indicators in soil health, such as microbial diversity and soil enzyme activities. Soil enzyme have been suggested as suitable indicators of soil quality because of their closed relationship with soil biology,
easy to measure, and their rapid response to change in soil management and environment [21-23]. Soil enzymes can promote the transformation of matter and energy in soil, and activity of soil enzymes has a close relationship with soil nutrients and their availability. The result showed that application of organic fertilizer (compost and BSF frass) significantly affected soil biological properties indicated by higher populations of soil bacteria (P solubilizing and proteolytic bacteria) and soil enzyme activities (PME-ase and urease) compared to NPK and control treatment (Figure 4 and 5). Phosphomonoesterase/PME-ase are important enzymes involved in the P cycle of soil. These enzymes are great agronomic significance because they hydrolyze compounds of organic P and transform them into different forms of inorganic P, which can be assimilated by plants [24]. The high PME-ase activity in soil might indicate the high population and activity of P solubilizing bacteria in the soil. Data in Figure 5A showed that the populations of P solubilizing bacteria were higher in treatments of compost+LOB and BSF frass.

Phosphate solubilization is a very important plant growth-promoting activity due to its ability to change insoluble phosphate which are unavailable to plants to soluble forms. Activities of urease in the soil treated by compost and BSF frass were significantly different from NPK and control treatment. However, the urease activity in treatment of 15% BSF frass was relatively low. Urease is a nickel dependent enzyme that catalyzes the hydrolysis of urea to two moles of ammonia (NH₃) and one mole of carbon dioxide (CO₂). A variety of substances have been reported to slow down urease catalytic activity, in which several of them are urea analogs that compete with the natural substrate for the urease active site. A wide variety of organic compounds and metal cations (e.g., Hg²⁺, Cd²⁺, Ag⁺, among others) are also potentially to inhibit ureases [25,26]. Urease activity in soil may originate from plant residues, animal waste, or soil microbes. Therefore, the addition of plant materials (compost) and animal wastes, including BSF frass may supply urease to the soil. The optimum pH of soil urease activity has been reported to be 6.5 to 7.0 [27].

4. Conclusions
The present study in the applications of several formulas of organic fertilizers with the basic biomass component of compost and BSF frass on Pakchoi (Brassica rapa L) were confirmed to improve plant growth and soil biochemical properties. The supporting effect of BSF frass on the soil biochemical properties as well as agronomical properties were comparable with compost, however compost enrichment with LOB (Compost + LOB) performed the highest yield. This study has demonstrated the potential of BSF frass as a biofertilizer, however the use of BSF frass as biofertilizer still needs to be studied in more detail, by combining it with other organic materials or enriched it with FGPR based-biofertilizer.

Acknowledgments
We are very grateful to TPST Mutiara Bogor Raya (integrated landfills) for providing BSF frass. This work was supported by BRIN PRN-LPDP Mandatory project 2020.

References
[1] Hue N V 1992 Tropical and Subtropical Agricultural Research, Progress and Achievements (Honolulu: The Pacific Basin Administrative Group University of Hawaii and University of Guam)
[2] Verheye W H 2007 CONTOUR Newsletter of The Asia Soil Conservation Network ASOCON 19
[3] Beltran E M, Miralles de Imperial R, Porcel M A, Delgado M M, Beringola M L, Martin J V and Bigeriego M 2002 Proc. 12th ISCO Conf. Effect of sewage sludge compost application on ammonium nitrogen and nitrate-nitrogen content of an Olive Grove soils
[4] Laird D A, Martens D A and Kingery W L 2001 Soil Sci. Soc. Am. J. 65 1413–8
[5] Mahanty T, Bhattacharjee S, Goswami M, Bhattacharyya P, Das B, Ghosh A and Tribedi P 2017 Environ. Sci. Pollut. Res. 24 3315–35
[6] Lander C H, Fidjeland J, Diener S, Eriksson S and Vinnerås B 2014 Agronomy for Sustainable Development 35 261–71
[7] Kagata H and T. Ohgushi 2012 Population Ecology 54 75–82
[8] Ahemad M and Kibret M 2014 J. King Saud Univ. Sci. 26 1–20
[9] Matthew C E and Olubukola O B 2018 Appl. Microbiol. Biotechnol. 102 7821–35
[10] Backer R, Rokem J S, Ilangumaran G, Lamont J, Praslickova D, Ricci E, Subramanian S and Smith D L 2018 Front. Plant Sci. 9 1473
[11] Lugtenberg B and Kamilova F 2009 Ann. Rev. of Mic. 63 541–56
[12] Antonius S, Agustiayani D, Laili N, Imamudin H and Sutisna E 2019 Pupuk organik hayati cair dan proses pembuatannya Patent No. 000064813 Jakarta: Ministry of Law and Human Rights Republic of Indonesia
[13] Davis D R 2009 Int. Conf. Declining fruit and vegetable nutrient composition: What is the evidence? HortScience (Beijing, China) p 15–19
[14] Margesin R 1996 Enzymes involved in phosphorus metabolisms: Acid and alkaline phosphomonoesterase activity with the substrate p-nitrophenyl phosphate. In: Schinner F, R. Ohlinger, E. Kandeler, & R Margesin (eds), Methodds in Soil Biology (Berlin Heidelberg: Springer-Verlag) pp. 213–7
[15] Kandeler E. 1996. Enzymes involved in nitrogen metabolisms: urease activity by colorimetric technique. In: Schinner F, R. Ohlinger, E. Kandeler, & R Margesin (eds.), Methodds in Soil Biology. Springer-Verlag, Berlin Heidelberg. pp. 171–4.
[16] Subba Rao N S 1999 Soil microbiology (4th edition of soil microorganisms and plant growth) (USA: Science publishers, Inc.)
[17] Uyar F, Porsuk I, Kızil G and Yilmaz E I 2011 Eurasian J. BioSci. 5 1–9
[18] Burt R 2004 Soil Survey Laboratory Methods Manual Soil Survey Investigations Report (Vers 4) Natural Resources Conservation Service United States Departement of Agriculture
[19] Ravindran B, Wong J W, Selvam A, Sekaran G 2016 Bioresour Technol. 217 200–4
[20] Ramadhani E P, Rachmisanti H, Gede S and Mia R. 2017 Proc. Int. Conf. on Green Technology vol 8
[21] García-Ruiz R, Ochoa V, Viñegla B, Hinojosa, M B, Peña-Santiago R and Liébanas G 2009 Appl. Soil Ecol. 41 305–14
[22] Burke D J, Weintraub M N, Hewins C R and Kalisz S 2011 Soil Biol. Biochem. 43 795–803
[23] Henry H A L 2012 Soil Biol. Biochem. 47 53–9
[24] Rashid M I, Mujawar L H, Shahzad T, Almeelbi T, Ismail I M I and Oves M 2016 J. Mic. Res 41 33–44
[25] Abalos D, Jeffery S, Sanz-Cobena A, Guardia G, Vallejo A 2014 Agric Ecosyst Environ 189 1–13
[26] Silva A G B, Sequeira C H, Sermarini R A and Rafael O R 2017 Agron J 109 1–13
[27] Paulson K N and Kurtz L T 1970 Proc. Soil Science Society of America 37 707–10