The effectiveness of liquid biofertilizer from waste bioconversion using black soldier fly larvae on the growth of arabica coffee seedlings

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Abstract. The organic waste treatment process using Black Soldier Larvae (BSF) produces liquid biofertilizer (LB) that contains functional microbes and macro and micronutrients that potentially can stimulate plant growth. The study aimed to determine the effectiveness of liquid biofertilizer from waste bioconversion by BSF on the growth of arabica coffee seedlings. The experiment used a randomized block design with 7 treatments, which consisted of: no treatment (control), 1.5% LB spraying, 3% LB spraying, 6% LB spraying, 3% LB watering, 6% LB watering, and 12% LB watering, with 4 replications and each replication containing 10 plants. All treatments were given once a month, to the 1-month-old seedlings. The test results showed that the application of LB significantly increased stem diameter and the number of leaves of Arabica coffee seedlings 4 months after application (MAA). In seedlings aged 4 MAA, spraying 6% LB resulted in the highest increase in stem diameter, while the highest number of leaves was obtained from watering 3% LB. Additionally, in 12 months old seedlings, Arabica coffee's highest fresh shoot weight was obtained by spraying 1.5% LB compared to other treatments.

1 Introduction

Plant nutrient management is one of the critical factors to producing optimal crop production. The use of inorganic fertilizers in agriculture today is generally excessive, resulting in wastage and soil pollution. The use of organic fertilizers and biofertilizers is an effort to restore soil fertility (physical, chemical, and biological) through the input of environmentally friendly materials. Organic fertilizers and biofertilizers can be produced from organic waste processing, one of which is through the bioconversion process using Black Soldier Fly (BSF).

BSF larvae are the immature stage of the Hermetia illucens L. fly (Diptera: Stratiomyidae), which can grow well on various organic wastes such as food waste such coconut pulp, tofu waste [1, 2], vegetables, and fruits [3–5], manure and liquid organic

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waste (compost leachate) [6]. BSF larvae can decompose organic waste containing 60–90% water [7]. Decomposition products by BSF can be used as organic fertilizers and biofertilizers that have to potential to improve the soil quality and stimulate plant growth, and the larvae produced can be used as animal feed (chickens, fish, and another animal) because they contain around 40–50% protein and 29–32% fat [3, 7, 8] and the decomposition of organic waste by BSF can reduce or even stop the spread of bacteria that cause disease to humans (Escherichia coli, Salmonella spp.) [9,10]. Waste treatment using BSF larvae can also prevent and reduce greenhouse gas emissions to avoid climate change disasters [11].

Organic waste treatment through the bioconversion process by BSF larvae can produce liquid biofertilizers that contain N-fixing microbes and, phosphate solubilizing, decomposer, and produce growth-promoting hormones (auxin, gibberellins, cytokinins) as well as macro and micronutrients. The quality of a biofertilizer depends on the effectiveness of the microbes it contains, the living microbial population, its suitability for the host plant, and the environmental conditions in which the microbes live. A biofertilizer product must meet quality requirements and its effectiveness on various plant commodities is tested before being marketed to the consumers. Research on bioconversion products using BSF larvae have been carried out on pakchoi [12], maize [13], sugarcane [14], and cocoa plant [15], but no studies on coffee plants were found. Arabica coffee plant is a perennial crop that is cultivated in the highlands. Therefore, it is necessary to study the effect of the dose and method of biofertilizer application of organic waste bioconversion using BSF larvae on the growth of Arabica coffee plant. This study aimed to determine the effectiveness of liquid biofertilizer from waste bioconversion by BSF on the growth of arabica coffee seedlings.

2 Materials and methods

The research was conducted in the greenhouse of the Indonesian Industrial and Beverage Crops Research Institute, located in Sukabumi Regency, Indonesia, from April 2019 to June 2020. The materials used were liquid biofertilizer (LB) produced by the Bioconversion industry, Arabica coffee seeds of the Sigararatang variety, soil media collected from Pakuwon, Sukabumi Regency, chicken manure, polybag 15 cm x 25 cm, and 60% paranet.

The biofertilizer used is derived from the bioconversion of vegetable, fruit, dairy industry waste, grated coconut waste, and palm oil cake. BSF larvae feed on organic waste to produce a liquid that is placed in a temporary shelter, followed by the process of mixing liquid and BSF frass and the incubation process for microbial propagation in a closed bioreactor [16]. The biofertilizer used has the characteristics of pH 8.92, 0.11% total N, 0.17% organic C, 0.08% total P, 0.31% total K, 8.066 ppm Indole-3-acetic acid (IAA), 13.12 ppm Gibberellic acid (GA-3), 3.101 ppm Trans-zeatin, and 1.786 ppm Kinetin. This biofertilizer also contains microbes i.e. *Azotobacter* sp., *Azospirillum* sp., *Pseudomonas* sp., *Trichoderma* sp. [15].
2.1 Design of experiment

The experiment was arranged in a randomized complete block design (RCBD) with 7 treatments and 4 blocks, each block containing 10 plants. The treatments consist of: no treatment (control), 1.5% LB spraying, 3% LB spraying, 6% LB spraying, 3% LB watering, 6% LB watering, and 12% LB watering. All treatments were carried out once a month. The watering treatment volume was 25 ml per polybag of block, while spraying application volume was 10 sprays per polybag of block.

The soil media was sieved through a 5 mm sieve and then mixed with 2:1 (v/v) manure and put into polybags. Arabica coffee seedling starts from the nursery stage until a pair of leaves appear then the seedlings are ready to be transferred to polybags. LB treatment was carried out on Arabica coffee seedlings aged 1-month-old after planting/transferring from the nursery in polybags. The watering was done every 2 days until the field capacity conditions. Pest and disease control was carried out when an attack occurred. Weeds are controlled manually.

2.2 Observed variables

The growth of coffee seedlings was observed at 4 MAA (months after application) and 11 MAA, on the following parameters: plant height, stem diameter, and several leaves. In the 3rd and 4th leaf areas, fresh weight and dry weight of shoots and roots were weighed after the seedlings were harvested at the age of 12 months after planting.

2.3 Statistical analysis

The results of the observations were then analyzed using variance (ANOVA) followed by the DMRT test with a significance level of 5%. The data was analyzed using the STAR program.

3 Result and discussion

This liquid biofertilizer experiment was carried out on coffee seedlings with planting media that had been given organic fertilizer without the addition of inorganic fertilizer. The experiment was carried out on seedlings aged 1 month after planting/transferred from the nursery to the age of 12 months in polybags. Based on the quality standard of generatively propagated coffee seedlings, the age of ready-to-distribute seedlings is 4-12 months.

The results of observations on Arabica coffee seedlings until the age of 4 MAP (months after application) showed that 6% LB spraying treatment had a significant effect on stem diameter and 3% watering had a significant effect on leaf number (Figure 1). However, LB treatment did not significantly affect the height of Arabica coffee seedlings. These results indicate that spraying 6% LB or 3% LB watering can best support the growth of coffee seedlings aged 4 MAP.

Spraying at 6% LB concentration produced the largest stem diameter. In general, the development of stem diameter will support the growth of organs at the top, such as the height of the stem and the number of leaves [17]. The development of stem diameter is
influenced by the availability of P and K nutrients. P elements play a role in plant metabolic processes so that they can increase the growth of plant stem diameters, while K nutrients play a role in strengthening plant stems [18]. Liquid biofertilizer spraying will increase the abundance of beneficial microbes in promoting plant growth. The liquid biofertilizer used in this study contains *Azotobacter* spp., *Azospirillum* spp., *Pseudomonas* spp., and *Trichoderma*, which has been reported by several studies that the microbes belonging to this genus can bind N, and dissolve P and K nutrients [15]. Liquid biofertilizer also contains phytohormones (IAA, GA3, and Zeatin) which are produced by microbes. In addition, the dissolved nutrients in LB will also have a direct effect on the development of coffee seedling stems. Although the amount of phosphorus contained in organic fertilizers is not as much as the number of inorganic fertilizers, it still contributes to growth [19].

![Graph](image)

*Fig. 1.* In seedlings aged 4 MAP, spraying 6% LB resulted in the highest increase in stem diameter, while the highest number of leaves was obtained from 3% LB watering.

Watering of LB on planting media has the potential to increase the abundance and activity of microbes in the root area, which is beneficial for plant growth. Watering LB with a concentration of 3% provided the most optimal root area conditions in meeting the available nutrient needs and the supply of growth-promoting hormones at the age of 4 MAP coffee seedlings. In addition, the dissolved nutrient content in biofertilizers can also directly increase the nutrient content available in the planting medium. According to Ji *et al.*[20], the nutrients in liquid organic fertilizer play a vital role in the early stage of chrysanthemum growth, especially in the growth of plant leaves. Meanwhile, excess concentrations of biofertilizer may encourage nutrient immobilization, due to microbes and the levels of available nutrients in relatively high concentrations stimulating in the planting medium [21].

The optimal LB concentration for the growth of Arabica coffee seedlings in this study was lower than the optimal concentration for the growth of 5-month-old cocoa seedlings as reported by Sasmita *et al.*[15]. The research of Sasmita *et al.*[15] found that the 6% LB watering treatment resulted in the highest growth of 5-month-old cocoa seedlings. This is probably because the growing media used in the Arabica coffee study used more organic fertilizers than in the cocoa seedlings experiment as reported by Sasmita *et al.*[15]. The results of other studies on chili plants with soil media and organic fertilizer showed that the application of liquid fertilizer from sewage treatment using BSF by 10% can increase plant vegetative growth which is comparable to the treatment of optimum NPK dose [22]
Meanwhile, Uliyah et al.[23] reported that the application of liquid organic fertilizer from BSF bioconversion gave the same response to plant height, plant length, stem diameter and several tillers of Panicum maximum cv Mombasa grass.

**Table 1.** Effect of application of spraying and watering of liquid biofertilizer on the increase in height, stem diameter, number of leaves, and leaf area of Arabica coffee seedlings aged 11 MAP.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant height (cm)</th>
<th>Stem diameter (mm)</th>
<th>Number of leaves</th>
<th>The 3rd leaf area (cm²)</th>
<th>The 4th leaf area (cm²)</th>
<th>The average leaf area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.80</td>
<td>2.77 c</td>
<td>15.66</td>
<td>48.60</td>
<td>45.27</td>
<td>46.94</td>
</tr>
<tr>
<td>1.5% LB spraying</td>
<td>22.55</td>
<td>2.91 c</td>
<td>17.89</td>
<td>48.58</td>
<td>50.06</td>
<td>51.88</td>
</tr>
<tr>
<td>3% LB spraying</td>
<td>21.16</td>
<td>2.73 c</td>
<td>15.97</td>
<td>49.87</td>
<td>45.6</td>
<td>47.74</td>
</tr>
<tr>
<td>6% LB spraying</td>
<td>20.83</td>
<td>3.14 bc</td>
<td>15.11</td>
<td>49.46</td>
<td>49.28</td>
<td>49.32</td>
</tr>
<tr>
<td>3% LB watering</td>
<td>22.91</td>
<td>3.55 ab</td>
<td>16.99</td>
<td>53.82</td>
<td>51.62</td>
<td>52.72</td>
</tr>
<tr>
<td>6% LB watering</td>
<td>22.71</td>
<td>3.08 c</td>
<td>15.78</td>
<td>49.64</td>
<td>49.28</td>
<td>47.96</td>
</tr>
<tr>
<td>12% LB watering</td>
<td>23.52</td>
<td>3.88 a</td>
<td>16.00</td>
<td>48.58</td>
<td>52.04</td>
<td>50.31</td>
</tr>
</tbody>
</table>

Note: The numbers followed by the same letter for each treatment are not significantly different based on the DMRT test at the 5% level.

The observations on the growth of coffee seedlings aged 12 months or 11 MAP (months after application) showed that LB watering had a significant effect on the increase in stem diameter, with 12% LB watering treatment producing the highest stem diameter (Table 1).
Table 2. Effect of application of spraying and watering of liquid biofertilizer on the fresh and dry weight of shoot and root of Arabica coffee seedlings aged 11 MAP (months after application).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>Control</td>
<td>15.92 b</td>
<td>5.09</td>
</tr>
<tr>
<td>1.5% LB spraying</td>
<td>20.59 a</td>
<td>6.52</td>
</tr>
<tr>
<td>3% LB spraying</td>
<td>17.57 ab</td>
<td>6.08</td>
</tr>
<tr>
<td>6% LB spraying</td>
<td>16.05 b</td>
<td>4.46</td>
</tr>
<tr>
<td>3% LB watering</td>
<td>16.28 b</td>
<td>6.3</td>
</tr>
<tr>
<td>6% LB watering</td>
<td>15.89 b</td>
<td>5.93</td>
</tr>
<tr>
<td>12% LB watering</td>
<td>18.33 ab</td>
<td>6.95</td>
</tr>
</tbody>
</table>

Note: The numbers followed by the same letter for each treatment are not significantly different based on the DMRT test at the 5% level.

Table 2 shows that 1.5% LB spraying significantly increased the fresh weight of shoots at 11 MAP, with an increase of 22.68% from the control. Tables 2 show that, spraying LB 1.5% resulted in the highest number of leaves and fresh weight of shoot while watering LB 12% resulted in the greatest growth in stem diameter. This is presumably because the application through foliar spraying allows the supply of N with the presence of N₂-fixing microbes and the production of growth-promoting hormones, especially auxin directly to the leaves, making it more effective in increasing leaf growth. The content in LB from waste bioconversion using BSF contains *Azotobacter* spp. and *Azospirillum* spp. which is based on other studies that both microbes are N₂-fixing microbes [24]. Meanwhile, the application of LB watering can increase stem diameter through a direct mechanism, namely the production of phytohormones by microbes that can stimulate plants and indirectly through the mobilization of nutrients such as P and K in the root area. Bioconversion of coffee husks by BSF larvae can supply P and K needs in plants that lack these two elements in the soil [25].

The level of concentration of liquid fertilizer can affect the permeability of leaf cells and determine the number of nutrients that can be absorbed in the fertilization process [26]. This study found that the best LB spraying concentration for coffee seedlings aged 11 MAP was lower than the optimum LB concentration for coffee seedlings aged 4 MAP. This is presumably because spraying on the leaves that are routinely carried out every month will cause the accumulation of nutrients and phytohormones in plants, so low concentrations can increase plant growth, especially leaf organs. Fertilizer concentrations that are too high can inhibit the absorption of other nutrients, causing elemental deficiency [27].
Spraying LB and watering LB with a certain concentration can increase plant fresh weight, indicating that this treatment enables the plants to absorb water and nutrients more optimally, which in turn increases photosynthetic activity and affects the increase in wet weight and plant weight [27].

4 Conclusions

The test results showed that the application of LB significantly increased the stem diameter and number of leaves of Arabica coffee seedlings aged 4 MPA (months after application). In seedlings aged 4 MPA, spraying 6% LB resulted in the highest increase in stem diameter, while the highest number of leaves was obtained from 3% LB watering. In 12 months old seedlings, the heaviest fresh crown weight of Arabica coffee was obtained from spraying 1.5% LB compared to other treatments.

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References