The responses of soil enzyme and microbial activities of shallot plantation under treatments of Liquid Organic Biofertilizer and sprout extract and its effect on the yield

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Abstract. Soil enzyme activities can be used to measures of microbial activity, soil productivity, soil ecology and fertility. Soil enzyme and microbial activities act as the indicators of soil health. The objectives of this study were to examine the influence of liquid organic biofertilizer and sprout extract on soil enzyme and microbial activities and their effect in shallot yield. The experimental design was completely randomized block design with three different treatments as following liquid organic biofertilizer (LOB), sprout extract (SE), and mixed of liquid organic fertilizer and sprout extract. The result showed that treatment of LOB gave the highest activity of phosphomonoesterase and urease measured, and followed by SE, LOB+SE and control. The LOB treatment showed the highest microbial respiration as also indicated in the highest population number of total bacteria. However, LOB + SE treatment gave the highest number of IAA producing bacteria as well as the yield of shallot. Therefore, it is confirmed from this work that application of liquid organic biofertilizer and sprout extract potentially could increase soil enzyme activities, microbial population number and the yield of shallot.

1. Introduction

Soil enzyme activities play an important role on soil health, physical and chemical properties as well as soil ecology. Soil enzymes activities can be used to measures of microbial activity, soil productivity, and inhibiting effects of pollutants. Soil enzyme closely related to soil organic matters, and microbial activities [1]. Microorganisms act as the indicators of soil health, as they have active effects on nutritional cycling, also affecting the physical and chemical properties of soil. Microorganisms could change their population and activities and this respond be used for soil health assessment. In other hand, soil quality is an important indicator of good crop yield [2]. Phosphomonoesterase and urease enzyme involved in nutrient mineralization. Soil phosphomonoesterase and urease play a role in controlling phosphorus (P) and nitrogen (N) cycling. Phosphomonoesterase is considered as the pre-dominant phosphatases in most soils [3]. Phosphatase activity is a reliable soil quality indicator and rapidly detect management changes [4]. The activity of urease increases with organic fertilization such as compost, sewage sludge and straw mulch [5]. Urea plays a role in the regulation of N supply to plants after urea fertilization [6].

Shallot is one of the important commodities in our country, but shallot national production still low and not in the sustainable agriculture approach. Shallot productivity depends on agronomical technique and soil quality. One of method to increase shallot production is applying plant growth promoting
rhizobacteria. Biofertilizer contain beneficial microorganisms include plant growth promoting rhizobacteria. Biofertilizer has been identified as an alternative for increasing soil fertility and crop production in sustainable farming [7]. Liquid organic bio-fertilizers, can compete in the global market because of organic crop production, improved soil and seeds survival, their dosages are ten times less than carrier-based powder bio-fertilizers, and cuts the chemical fertilizer use by 15–40% [8]. The use of bio-fertilizers is one of the important components of integrated nutrient management, as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture [9]. The liquid organic biofertilizer application increases soil enzyme activity such as urease and phosphomonoesterase, also increases crop production [10,11]. Beneficial microorganisms in liquid organic biofertilizers protect the plants from pest and diseases and promoted to harvest the naturally available, biological system of nutrient mobilization. Liquid organic biofertilizers containing living microorganism of efficient strain for nitrogen fixation, solubilization and mobilization of P and K, plant growth hormone production useful for agriculture [12,13]. Plant growth-promoting rhizobacteria (PGPR) in bio-fertilizers are crop specific bio-fertilizers. PGPR produce metabolites and hormones thus improve root growth. Indole-3-acetic (IAA) hormone has important role for plant growth through L-tryptophan-dependent mechanisms [14]. Tryptophan is used by plants and bacteria as physiological precursors to produce IAA. Sprout extracts contain high concentration of proteinand amino acid including tryptophan which acts as precursor of IAA produced by Acinetobacter sp. [15]. Previous study showed that sprout extract added in culture medium increased the ability of bacteria to produce IAA hormone. IAA is very important to enhance plant growth and cell elongation. Although biofertilizers has been widely practiced to improve crop production, liquid organic biofertilizer and sprout extract have not been widely reported. The objectives of this study were to examine the influence of liquid organic biofertilizer and sprout extract on soil enzyme and total population of microbial activities and their effect in shallot yield. This research was expected to give an alternative to develop environmentally friendly fertilizer.

2. Materials and methods

2.1. Materials

 Shallots bulbs (Bima Brebes variety) were grown in raised beds in the field with the size of bed was 4m x 0.8m, with distance of 20 x 20cm in a row. Every bed contained 60 shallot bulbs. The experimental design was randomized block. We used three different treatments which were liquid organic biofertilizer (LOB), sprout extract (SE), and mixed of liquid organic fertilizer and sprout extract (SE) and control (C) with three replicates. Liquid Organic Biofertilizer used in this study was from our previous research (Beyonic StarTmik) [11]. LOB and SE were applied at 25 mL/plant for three times.

2.2. Soil Sampling

 Soil and root were collected from the rhizosphere of shallot plant at a depth of 5-10cm. One hundred grams of soil were taken from each treatment in three replicates using composite method. The soil and root samples obtained was mixed and collected into the polybag and stored in cooler box. Soil samples were taken in 7th day at 0 and 4 weeks after planting (WAP).

2.3. Total population of plant growth promoting rhizobacteria

 One gram of sample (mixed soil and root) was stored in Erlenmeyer and homogenized with distilled water (9 mL) by shaking them for 30 min and decimally diluted (10^3, 10^4, 10^5, 10^6) in distilled water. The last dilutions were taken with micropipette and spread on different specific cultural media with three replicates, namely skim milk agar medium for enzyme protease activity, tryptic soy agar medium for IAA producing bacteria activity and Pikovskaya agar medium [16] for phosphate solubilizing bacteria. Total population of bacteria was observed on nutrient agar medium. Phosphate solubilizing and protease enzyme producing bacteria activity were observed with the indication of clear zone on the
bacterial colonies. Total population bacteria was observed by Total Plate Count Method according to Cappucino & Sherman [17], Moa et al [18], and SNI 2897 : 2008e [19].

2.4. Phosphomonoesterase enzyme activity
Phosphomonoesterase activity was analyzed according to the standard method by Margesin [20]. One gram of soil sample was put into test tubes and added with 1 mL p-nitrophenylphosphate substrate and 4 mL buffer (pH 6.5) then homogenized by vortex and incubated at 38°C for 2h in a water bath. After incubated for 2 h, sample was added 1 mL of CaCl2 0.5 M and 4 mL of 0.5 M NaOH then diluted with 4.5 mL of distilled water. The absorbance of samples and controls were measured by spectrophotometer at a wavelength of 400 nm.

2.5. Urease enzyme activity
The standard method used to analyze urease activity was according to Kendeler [21]. Five grams of soil sample were put in Erlenmeyer added with 2.5 mL of urea substrates and incubated at 37°C for 2 h. Soil samples were added with 50 mL of 1M KCl solution, shaking for 30 min then the solution was pipetted into microtubes and centrifuged for 6 min at 12000 rpm. 0.5 ml of supernatant was pipetted and added with 4.5 mL of distilled water. Solution was added with Nessler reagent then mixed and let stand for 10 min. The absorbance was measured by spectrophotometer with a wavelength of 420 nm.

2.6. Respiration activity
Respiration activity was measured according to the standard method [22]. Twenty grams of soil samples were put in the glass bottles containing 20 mL 0.05 N KOH solution and incubated for 24 hours at room temperature. Soil sample was removed, and the solution was added with Phenolphthalein (PP) and titrated with 0.1 N HCl until colorless. The colorless solution was added with Methyl Orange (MO) and titrated again with 0.1 N HCl until orange color was formed. Every 1 ml of HCl is equivalent to 2.2 mg of CO2 and is equivalent to 2.6 mg of C-biomass produced every 100 g of soil.

2.7. Analysis of data
Collected data of enzyme, respiration and yield of shallot were analyzed using analysis of variance (ANOVA) followed by Test and Tukey (HSD) comparison at 5% level of significance using R [23,24].

3. Results and discussions

3.1. Total Population of plant growth promoting rhizobacteria
Total population of bacteria were observed under general and specific medium. The result showed that the soil samples contains bacteria that have some activity including phosphat solubilizing bacteria, IAA hormone producing bacteria, protease producing bacteria. The number of total population of bacteria in the soil of shallot rhizosphere were affected by each treatment (table 1). Application of liquid organic biofertilizer, sprout extract and mixed of liquid organic biofertilizer and sprout extract increased the total population of bacteria yet not significant different each other. This result related to the condition of soil. Application of biofertilizer for the previous research affected the soil microorganisms. Advantages of biofertilizer are to improve the physical, chemical and biological properties of the soil [7]. The effectiveness of biofertilizer depends on selective microorganism that may be useful for the soil [25]. Biofertilization is to accelerate the microbial processes availability of nutrients that can be easily assimilated by plant and to increase the number of useful microorganisms in soil [26]. Sprout extract contains high concentration of protein and be utilized for microbes.
Table 1. Total population of bacteria and phosphate solubilizing bacteria.

| Treatment   | Total Bacteria (×10^7 CFU/g) 0 WAP | Total Bacteria (×10^7 CFU/g) 4 WAP | Phosphate Solubilizing Bacteria (×10^5 CFU/g) 0 WAP | Phosphate Solubilizing Bacteria (×10^5 CFU/g) 4 WAP |
|-------------|----------------------------------|----------------------------------|-----------------------------------------------|-----------------------------------------------|
| Control     | 20.97 ± 8.98                     | 43.13 ± 15.54                   | 46.67 ± 11.55 ab                              | 96.67 ± 15.28 b                              |
| LOB         | 24.13 ± 5.37                     | 131.00 ± 53.56                  | 53.33 ± 11.55 ab                              | 133.33 ± 11.55 ab                            |
| SE          | 24.00 ± 10.39                    | 108.00 ± 11.31                  | 20.00 ± 0.00 c                                | 153.33 ± 30.55 a                             |
| LOB+SE      | 13.33 ± 1.15                     | 77.33 ± 32.39                   | 26.67 ± 11.55 bc                              | 140.00 ± 20.00 ab                            |

*LOB : Liquid Organic Biofertilizer
*SE : Sprout Extract
*LOB +SE :Liquid Organic Biofertilizer + Sprout Extract
*The numbers followed by different letters indicate significantly different at the level of 5%

Table 1 showed phosphate solubilizing bacteria in the soil sample. The data showed that application of LOB, SE and mixed of LOB+SE increased the number of phosphate solubilizing bacteria significantly compared to control. LOB contains phosphate solubilizing bacteria that solubilize the fixed phosphate and make it bioavailable. Soil bacteria have the competency to transform insoluble phosphates into soluble forms by the excretion of organic acids in the rhizosphere. These organic acids decline the soil pH and cause the dissolution of phosphate complexes and make them available to plants [27]. Mung bean sprout extract contain protein, carbohydrate and little amount of sugar. These nutrients were good for growth medium of microorganisms. According to Ilmi et al [28] mung bean sprout was a good alternative medium for growth of fungi.

Table 2. Total population of IAA producing bacteria and protease enzyme producing bacteria.

| Treatment   | IAA Producing Bacteria (×10^6 CFU/g) 0 WAP | IAA Producing Bacteria (×10^6 CFU/g) 4 WAP | Protease Enzyme Producing Bacteria (×10^6 CFU/g) 0 WAP | Protease Enzyme Producing Bacteria (×10^6 CFU/g) 4 WAP |
|-------------|---------------------------------------------|---------------------------------------------|-------------------------------------------------------|-------------------------------------------------------|
| Control     | 63.33 ± 32.33                               | 78.00 ± 10.39                              | 16.00 ± 2.00                                          | 48.67 ± 10.26 a                                      |
| LOB         | 55.33 ± 7.02                                | 142.67 ± 25.01                             | 16.67 ± 6.11                                          | 166.67 ± 90.18 a                                     |
| SE          | 74.00 ± 20.78                               | 152.67 ± 44.06                             | 17.33 ± 1.15                                          | 594.67 ± 400.81 a                                    |
| LOB+SE      | 51.33 ± 9.24                               | 119.33 ± 27.74                             | 21.33 ± 2.31                                          | 536.67 ± 193.99 a                                    |

*LOB : Liquid Organic Biofertilizer
*SE : Sprout Extract
*LOB +SE :Liquid Organic Biofertilizer + Sprout Extract
*The numbers followed by different letters indicate significantly different at the level of 5%

Total population of IAA producing bacteria and protease enzyme producing bacteria were showed in Table 2. The table show mean value with standard deviation. Increasing the number of IAA and protease producing bacteria in this picture indicated clearly related with each treatment where LOB containing PGPR that promoted IAA producing bacteria and SE containing protein could support the increasing proteolytic bacteria. Based on the data, total IAA and protease enzyme producing bacteria increased significantly from 0 WAP to 4 WAP but not significant different in each treatment.

3.2. Phosphomonoesterase enzyme activity
Phosphomonoesterase activity was monitored at 0 WAP and 4 WAP. The amount of phosphatase present in the soil varies with the microbial count and the extent of organic materials, mineral and organic fertilizers, tillage and other agricultural practices [29].
Figure 1 showed Phosphomonoesterase enzyme activity in the rhizosphere of shallot plantation. Application of liquid organic biofertilizer, sprout extract and mixed of liquid organic biofertilizer and sprout extract after 4 WAP increased phosphomonoesterase enzyme activity about 30%. Increasing enzyme activity was good indicator of increasing the soil fertility. This data was significantly different compare to control, where the phosphomonoesterase activity even reduce after 4 WAP. The increasing phosphomonoesterase activity might be associated with every treatment, which containing PGPR or organic substances and nutrition that promoting microbial growth and phosphomonoesterase. This enzyme activity is one of the parameters of soil fertility. Phosphatase activity can be a good indicator of the quality and quantity of organic matter in soils [4].

3.3. Urease Enzyme activity
Urease enzyme activity as good indicator of soil quality, because of the role of urease in the regulation of N supply to plants after urea fertilization [6]. Table 2 showed that at 0 WAP, urease enzyme activity in all treatments was almost similar. At 0 WAP, the treatments were not difference between each other. Due to this, no post-hoc test were done at 0 WAP. Application of liquid organic biofertilizer and mixed of liquid organic biofertilizer and sprout extract at 4 WAP increased the enzyme activity significantly different compare to control. Enzyme activity increased fourfold from 0 WAP to 4 WAP. Application of sprout extract increased urease enzyme activity not significant different compare to control. Application of LOB could boost soil enzyme activity. Urease activity increased in a soil management system such as organic fertilization [30]. Liquid organic biofertilizer contain beneficial microorganisms. The increase in the enzymatic activities may be related to changes in rhizosphere bacteria activity.
3.4. Respiration activity
Microbial respiration is the indicator of microbial activity in the soil. Microbial respiration at 4 WAP increased significantly compared to control for all treatments. At 0 WAP, there are no post-hoc test due to the treatments were not difference between each other. Soil respiration can be used to estimate soil microbial biomass. Application of liquid organic biofertilizer and sprout extract increased population of microorganisms and their activities. This soil biological activity consists of numerous individual activities and the formation of CO$_2$ is the last step of carbon mineralization [31].

3.5 Effect on yield
Biofertilizers play key role in the productivity and sustainability of soil and protecting environment, being cost effective, eco-friendly and recognized as a competitive option to chemical fertilizer to

![Figure 2. Urease enzyme activity. The numbers followed by different letters indicate significantly different at the level of 5%. LOB is Liquid Organic Biofertilizer, SE is Sprout Extract.](image2)

![Figure 3. Respiration activity. The numbers followed by different letters indicate significantly different at the level of 5%. LOB is Liquid Organic Biofertilizer, SE is Sprout Extract.](image3)
increase soil fertility and crop production in sustainable farming [32]. Microorganism containing in the biofertilizer produce metabolite and hormone that can improve plant growth, enhance plant nutrition intake and increase soil fertility and improve yield.

Figure 4. Fresh weight of bulbs. The numbers followed by different letters indicate significantly different at the level of 5%. LOB is Liquid Organic Biofertilizer, SE is Sprout Extract.

Figure 5. Number of bulbs in each clump on block. The numbers followed by different letters indicate significantly different at the level of 5%. LOB is Liquid Organic Biofertilizer, SE is Sprout Extract.

Figure 4 showed fresh weight of the tuber from the application of liquid organic biofertilizer and sprout extract were significantly different from the control. The increasing of shallot production generally relies on the synthetic fertilizer to obtain a high yield, but tends to cause an environmental pollution and the use of biofertilizer which produces growth regulators that can increase the growth and yield of the crop [33]. It has been reported that combination application of PGPR of bamboo and ammonium sulphate fertilizer were able to increase to agronomic parameter (fresh weight of bulbs/ha) of Allium ascalonicum L. [34]. Fresh weight parameter is the weight of bulbs shortly after harvest. Fresh weight of each treatment was not significant different. Application of sprout extract might be more
economic than other treatment, however sprout extract was natural product that might contains nutrients unstable in different time and source of sprout. Further study must be taken to investigated it. Increasing the level of organic nitrogen could enhance onion bulb weight and the height of the bulb. This study has been reported before [35,36]. Figure 5 showed the number of bulbs was confirmed that all treatment resulted significantly different from the control. Similar result was also reported before that application of organic biofertilizer could improve the bulbs weight [37].

4. Conclusion
Application of liquid organic biofertilizer and sprout extract enhanced soil biochemical properties including the population of general rhizosphere bacteria and PGPR that also reflected in increasing of the phosphomonoesterase and urease enzyme activity. All treatments also improved the yield of the shallot. Further research must be taken to investigate the appropriate optimum concentration of sprout extract to be applied. Natural raw material mung bean sprout extract gives the new preference as liquid organic fertilizer.

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