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Effect of biofertilizer on population of phosphate solubilizing bacteria under green onion cropping

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ABSTRACT

The plant cannot absorb phosphorus nutrient in the bound form but must be converted into an available form. Soil microbes have a role in the soil properties such as solubilized phosphor bound by acid secretion, and mineralization of organic phosphate components by converting into inorganic forms. Several types of microorganisms were identified. They can help the plants to provide the nutrients. Soil pH has a role in the availability of inorganic phosphorus. The aim of this study was to determine the effect of the addition of Bacillus aryabhattai to the population of phosphate solubilizing bacteria and soil pH. The research was conducted in the screen house and Integrated Laboratory of Indonesian Agricultural Environment Research Institute (IAERI) from December 2016 to March 2017. The experimental design was Completely Randomized Design with 4 treatment. The treatment involved Control (D1), Bacillus Aryabhattai (D2), Liquid Organic Fertilizer (D3), and Trichoderma (D4). The Pikovskaya medium was used to analyze the phosphate solubilizing bacteria. The results show that Bacillus Aryabhattai has a great influence on the increasing population of phosphate solubilizing bacteria. The population of phosphate solubilizing bacteria in the Bacillus Aryabhattai treatment was 3,1 x 107 cfu/ml, while in the control was 4,1 x 106 cfu/ml. There was found the similarities in trends between the number of phosphate solubilizing bacteria populations and soil pH. Bacillus Aryabhattai has a great potency to increase the plant nutrients especially phosphorus.
Keywords: *phosphate solubilizing bacteria, Bacillus aryabhattai, green onion*

INTRODUCTION

The Nitrogen, phosphorus, and kalium were the macro-essential component, which needed by the plant. There were not all components in the available form for the plant. Some of microorganism was identified can help the plant to provide the plant nutrients. Phosphorus is one of the major plant nutrients required in optimum amount for proper plant growth. Native soil phosphorus is organically bound and available in the form of phytin, or its derivatives has low solubility and mobility, and thus is not accessible to plants. This organic P must be hydrolyzed to inorganic P for plants utilization which

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can be mediated by phosphatases secreted by AM fungi or phosphate solubilizing bacteria (Gaur and Rana, 1990).

The green onion needs the phosphorus nutrients for growth and yield growth. The use of phosphorus fertilizer to increase agricultural production is not new to farmers. Phosphorus is needed by plants to promote the root growth and formation of a root system of seeds and young plants, as a constituent of cell nuclei, fat, and protein. The role of phosphorus nutrient for plants is for cell division, albumin formation, flower, fruit, and seed formation, accelerating plant maturity, digging roads, and keeping energy (Nahdudin et al, 2014).

Tricalcium phosphate was added as an insoluble source of inorganic phosphate which does not increase the P level in the rhizosphere soil. Various methods to solubilize insoluble phosphate to increase its availability by biologically mediated processes as mineralization and immobilization by phosphate solubilizing microorganisms have been discussed (Illmer and Schimmer, 1995; Zou et al., 1992). An increase in P availability to plants through the inoculation of phosphate solubilizing microorganisms has been reviewed several times in pot experiments (Kumar et al., 2001). Soil bacteria having the phosphate solubilizing capacity are called as Phosphate Solubilising Bacteria (PSB). They convert the insoluble phosphate into soluble form through the production of organic acids and make it available for plant uptake and nutrition. They are also useful as biofertilizers as they belong to the plant growth promoting Rhizobacteria (Satyaprakash et al., 2017).

Richardson (1994) discussed two strategies to improve the efficiency of P fertilizer utilization: 1) management of existing populations of soil microorganisms to optimize their capacity for P transformation to synchronize their nutrient mobilization activity with that of plant requirements, and 2) the introduction of a specific inoculant to enhance either the supply and availability of soil P or the uptake of P by plant roots.

There were some characteristics of phosphate solubilizing bacteria: 1. Phosphate solubilizing bacteria has the ability to dissolve organic P into dissolved phosphate forms available to plants; 2. Phosphate solubilizing bacteria secrete organic acids such as acetic acid, formic acid, lactic acid, oxalic acid, malic acid and citric acid produced by these microbes; 3. Phosphate solubilizing bacteria producing phosphatase enzymes such as acid and base phosphatase (Some phosphatase enzymes such as phosphomonoesterase, phosphodiesterase, triphosphomonoesterase, and phosphoamidase are generally present in the soil); 4. These enzymes are responsible for the process of hydrolysis of organic P into inorganic phosphate ($H_2PO_4^-$, HPO_4^{2-}) which is available to plant (Pang and Kolenk, 1986; Mearyard, 1999; Lal, 2002).

The aim of this study was to determine the effect of biofertilizer to the population of phosphate solubilizing bacteria green onion. Through this study

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will be expected to know which treatment that can increase the bacteria population and provide the plant nutrients.

MATERIALS AND METHODS

The research was conducted in the screen house and Integrated Laboratory, Indonesian Agricultural Environment Research Institute (IAERI) from December 2016 to March 2017. The andisols from Kaliangkrik, Magelang Regency was used in this experiment. The experimental Completely randomized design was used with 4 treatments. The treatment involved was Control (D1), Bacillus Aryabhattai (D2), Liquid Organic Fertilizer (D3), and Trichoderma (D4). The Pikovskaya medium was used to analyze the phosphate solubilizing bacteria. The composition of Pikovskaya medium for 100 ml medium was 1 g glucose, 0.5 g $Ca_3(PO_4)_2$, 0.05 g $(NH_4)_2SO_4$, 0.02 g KCl, 0.01 g $MgSO_4$, 0.05 g Yeast Extract, 0.005 g $MnSO_4$, 0.005 g $FeSO_4$, and 1.8 g agar.

Calculation of the number of bacteria is carried out using the total plate count method. The principle of this method is that if living microorganisms are grown on agar medium, then the cell will proliferate and form the colonies that can be seen directly and calculated with the eye without using a microscope .The Colony Forming Unit in solution can be calculated using the formula (Fardiaz, 1992):

cfu/ml = (no. of colonies x dilution factor)/volume culture

The phosphate solubilizing bacteria can be identified by grow it in the Pikovskaya medium which contains insoluble phosphorus $Ca_3(PO_4)_2$ after incubated in 30 ^{0}C for about 5 days. The bacteria which growth in the pikovskaya medium will dissolve phosphorus which signed by transparency zone (halo zone) in the surround of the bacteria colony (Suliasih and Rahmat, 2007).

RESULTS AND DISCUSSIONS

To measure the quantitative ability of phosphate dissolution from microorganisms, it is carried out by growing pure microorganisms in Pikovskaya liquid media (Ginting et al., 2005). In the medium which has turbid appearance, the colony of phosphate solubilizing bacteria will characterize by transparency zone in the circumference because of the phosphate dissolving. The bacteria which grow in the pikovskaya medium will dissolve phosphorus which be marked by transparency zone (halo zone) in the surround of the bacteria colony as shown in Figure 1. This is due to the dissolution of fine particles from $Ca_3(PO_4)_2$ (Suliasih and Rahmat, 2007).

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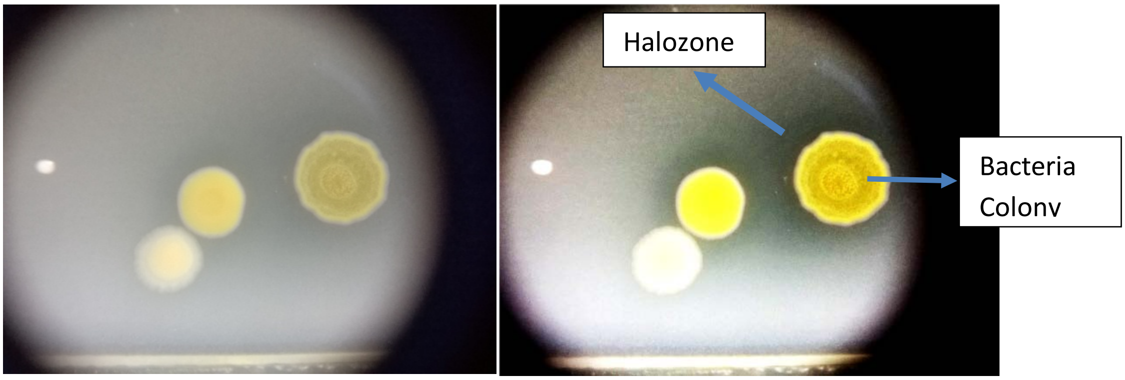

Figure 1. Transparency zone (halozone) in the surround of the bacteria colony

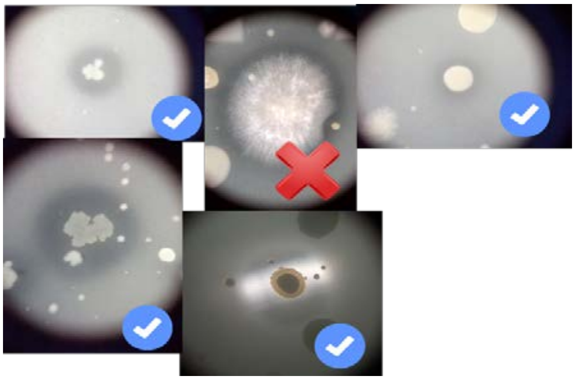

Figure 2. Isolation Result in Pikovskaya Medium

Figure 2 shows the isolation results in Pikovskaya medium. Some of the bacteria are categorized as phosphate solubilizing bacteria and a few numbers as fungi. The tranparency zone is the initial sign to determine the ability of a phosphate solubilizing microbia to dissolve phosphate. The wider the clear zone can qualitatively be considered as a sign of the ability to phosphate solubility in the growing medium (Nautiyal, 1999). The results of the total population of phosphate solubilizing bacteria showed in Table 1.

Table 1. Population of Phosphate Solubilizing Bacteria

No	Treatment	Phosphate Solubilizing Bacteria (cfu/ml)
1	Control	$4{,}1 \times 10^6$
2	*Bacillus Aryabhattai*	$3{,}1 \times 10^7$
3	Liquid Organic Fertilizer	$5{,}2 \times 10^5$
4	*Trichoderma*	$1{,}4 \times 10^5$

The result showed that the highest bacteria population is in treatment of Bacillus aryabatthai. The population of phosphate solubilizing bacteria in the

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Bacillus aryabhattai was 3.1 x 107 cfu/ml, while in the control was 4.1 x 106 cfu/ml. The population of phosphate solubilizing bacteria population in the liquid organic fertilizer and Trichoderma were 5.2 x 105 cfu/ml and 1.4 x 105 cfu/ml, respectively. The differences in the population of phosphate solubilizing bacteria from each treatment shows that these microbes have different abilities in solubilizing phosphate. The more population of phosphate solubilizing bacteria from each treatment accordingly, the ability of each type of microbial to phosphate solubility also getting higher. Ngawit (1999) states that the ability of each type of phosphate solubilizing bacteria in fixed phosphate solubility varies greatly, depending on the type and adaptability of the bacteria to the environment. Many bacteria produce enzymes which enhance releasing phosphate from organic P compounds. These bacteria also produce other biological materials such as auxin, gibberellic acid, vitamins and hormones that increase the dissolution of phosphate (He et al., 2002).

The highest population of phosphate solubilizing bacteria is stimulated by Bacillus aryabhattai, while the lowest one is affected by Trichoderma. Strains of Pseudomonas bacteria, Bacillus, Rhizobial, Enterobacter, Aspergillus and Penicillium are the most efficient in solubilizing phosphorus (Whitelaw, 2000). Rodriquezz and Fraga (1999) stated that in dissolving phosphate, Bacillus sp. also has the other PGPR (Plant growth promoting rhizobacteria) character because Bacillus sp. can synthesize IAA, promote the growth of germination significantly, and can produce anti-fungal compounds that can inhibit the growth of phytopathogenic fungi. Of several bacterial strains, it turns out that the genera Pseudomonas and Bacillus have a high ability to dissolve phosphate.

Liu et al., (2016) had isolated and identified phosphate solubilizing bacteria from the grapevine rhizosphere and revealed 5 species: Bacillus aryabhattai, B. megaterium, Klebsiella variicola, Stenotrophomonas rhizophila, and Enterobacter aerogenes. These phosphate solubilizing bacteria was used as biofertilizers which will increase the available P content in soils, minimize P-fertilizer application, reduce environmental pollution, and promote sustainable agriculture. Phosphate solubilizing fungi like Aspergillus, Penicillium and Trichoderma plays a role in dissolving compound difficult to dissolve phosporus, increasing available - P, improve plant growth and increase the efficiency of phosphate fertilization (Rao, 1994).

Figure 3 showed that there is a relation between total population of phosphate solubilizing bacteria and soil pH on 94 and 105 days after planting. Inoculation of Bacillus Aryabhattai in sgreen onion has soil pH 5.8 and 6.1 in 94 and 105 DAP (day after planting), respectively, which shown the highest soil pH. This is indicated that soil pH increase tend to increase population of phosphate solubilizing bacteria. Soil pH in 105 DAP was higher than in 94 DAP in all treatment. The range of soil pH in all treatment is 94 DAP was 5.3 - 5.8 (94

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DAP) and 5.4 - 6.1 (105 DAP). According to Taha et al.(1969), optimum bacterial growth at neutral pH and will increases with increasing soil pH. Soil pH values between 6 and 7.5 are best for P-availability, this is because at pH values below 5.5 and between 7.5 and 8.5 limits P from becoming fixed by aluminum, iron, or calcium, and hence, not being available for plant use (Azziz et al., 2012).

Phosphate solubility by phosphate solubilizing bacteria occurs chemically and biologically. The mechanism of chemical phosphate dissolution is the main mechanism carried out by bacteria. These bacteria excrete a number of low molecular weight organic acids such as oxalate, succinate, tartrate, citrate, lactate, ketoglutarate, acetate, formate, propionate, glycine, glutamate, glyoxylate, malate, fumarate (Illmer et al., 1992; Banik et al., 1982; Alexander, 1977; Beauchamp et al., 1997 in Ginting et al., 2005). The increase in organic acid is followed by a decrease in pH. The pH changes play an important role in increasing phosphate solubility. Furthermore, these organic acids will react with phosphate binders such as Al^{3+}, Fe^{3+}, Ca^{2+}, or Mg^{2+} to form stable organic chelate so that it is able to free bound phosphate ions and therefore can be absorbed by plants (Ginting et al., 2005).

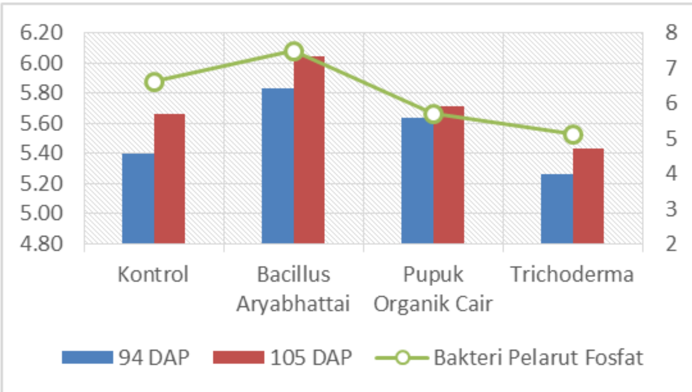

Figure 3. Graphic of comparison between soil pH with population of Phosphate solubilizing bacteria

The mineralization rate also increases with a pH value that is appropriate for the metabolism of microorganisms and the release of phosphate will increase as the pH value increases from acid to neutral. In addition, the mineralization rate turned out to be directly correlated with the number of the substrate. Soil-rich phosphate soils are the most active soil for the ongoing process of mineralization (Alexander, 1977). The availability of inorganic phosphorus is largely determined by soil pH, the amount and level of decomposition of organic matter and the activities of microorganisms in the soil (Lal, 2002).

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CONCLUSIONS

- Application of *Bacillus aryabhattai* under green onion culture increase the phosphate solubilizing bacteria.
- There is similarity trends between the population of phosphate solubilizing bacteria and soil pH of andisols.

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