Effect Of Carrier Media for Biofertilizer of Phosphate Solubilizing Bacteria Bacillus sp to Peanut (Arachis hypogea) Growth

Tutik Nurhidayati1, Wirdhatul Muslihatin1, N.Firdausi1, E.P. Setyaningsih2, A.P.D Nurhayati1 and EkoPrasetyoKuncoro3

1Department of Biology, Faculty of Sciences, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia
2Department of Chemistry, Faculty of Sciences, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia
3Department of Biology, Faculty of Sciences and Technology, Universitas Airlangga, Surabaya, Indonesia

*Correspondence: wirdhabioits@gmail.com Accepted: 05july.2018 Published online: 13Aug. 2018

Phosphorus (P) is an essential macronutrients for plant growth and development. The content of phosphorus in the soil is very low and it is bound by soil colloids which made phosphorus can not be absorbed by crops. Biological fertilizer production requires carrier media to improve the viability of the bacteria. This study aimed to determine the combination of phosphate solubilizing bacteria carrier on plant growth of Arachis hypogea. The method used in the study were the cultivation of Bacillus sp, production of biofertilizer to phosphate solubilizing bacteria, the calculation of TPC (Total Plate Count), cultivation of Arachis hypogea and measurement of plant growth parameters. Data analyzed using ANOVA, if there was a significant difference then continued to Duncan test at 95% significance level. The result of this research showed there was no significance influence of carrier media to growth parameter of crops length, leaves number, leaves width, roots length, stem diameters, and dry weight. Therefore, there was significance influence of carrier media to number of flower.

Keywords: Bacillus sp, Biofertilizer, Carrier media, Phosphorus.

INTRODUCTION

Phosphorus is one of the most important elements in crop production. It has a defined role in plant development and growth such as cell division, development, photosynthesis, breakdown of sugar and regulation of metabolic pathways (Muraledharan, 2010). The concentration of soluble P in soil is usually very low because this macronutrient is bounded to soil colloids so it can not be absorbed by crops (Prasad, 2014).

Plants utilize fewer amounts of phosphatic fertilizers that are applied and the rest is rapidly converted into insoluble complexes in the soil (Vassilev and Vassileva, 2003). This leads to the need of frequent application of phosphate fertilizers, but its use on a regular basis has become a costly affair and also environmentally undesirable (Reddy et al., 2002).

Biofertilizer advised to be used as a solution for the problem. Biofertilizers accelerate certain microbial processes in the soil or rhizosphere which increase the extent of availability of nutrients such as nitrogen and phosphorus in a form easily assimilated by plants. Biofertilizers are low cost, renewable sources of plant nutrients, supplement chemical fertilizers and their use for agricultural improvement has been a topic of research for a number of years (Glick, 1995).

Phosphate solubilizing bacteria is one of the soil microorganism having capability to dissolve P ion bounded to soil cations such as Al, Fe, Ca, and Mg. They convert P ion into a form ready to
be absorbed by crops (Keneni et al., 2010). Phosphate solubilizing bacteria causes increase of available phosphorus for plants and promote plant growth. *Bacillus* have been identified as phosphate solubilizing bacteria and widely investigated on crop plants to improve the crop yield (Kang et al., 2014).

Biofertilizer production requires carrier media. The function of carrier media is for growing bacteria, packaging, and extending shelf life of biological agents (Shariati, 2013). Carrier media must contain organic nutrient such as nitrogen, organic carbon, phosphorus, potassium, and other nutrients. Organic nutrients can be processed into inorganic material for plant nutrients. Various carrier media has been tested, such as peat, vermicompost, and compost.

Therefore, the objective of this study is to determine the effects of combination of carrier media for phosphate solubilizing bacteria such as manure, sand, and soil to the peanut growth. Manure is a valuable source of nutrients for crops and can improve soil productivity. Manure components can be characterized as organic and inorganic nutrient content, primarily nitrogen, phosphorus, and potassium (Kessel et al., 1999). Soil plays role in the biological activity especially microorganism. The energy input into the soil ecosystems is derived from the microbial decomposition of dead plant and animal organic matter (Nielsen, 2002). Sand has good aeration.

**MATERIALS AND METHODS**

**Procedures**

1. **Subculture of bacteria isolates**  
   *Bacillus* sp cultivated at NA medium and incubated at 37°C for 24 hours. *Bacillus* sp as phosphate solubilizing bacteria was inoculated in pikovskaya medium and incubated at room temperature for 7 days. Colonies of bacteria had clear zone that were capable to solubilize phosphate.

2. **Biofertilizer**  
   Production of biofertilizer required different carrier media. Comparison of carrier media for biofertilizer consists 6 treatments (Manure: Sand: Soil); B1 (1: 1: 0), B2 (1: 1: 1), B3 (1: 0: 1), B4 (0: 1: 1), B5 (0: 0: 1), and B6 (0: 0: 1). Each carrier media homogenized with the same composition. It watered and humidified by molasses and distilled water (Nurhidayati, 2015). *Bacillus* sp which aged 24 hours inoculated at carrier media. Biofertilizer of phosphate solubilizing bacteria can be applied in crops when the concentration of bacteria has reached 10⁸ CFU/g (Muraleedharan, 2010).

3. **Cultivation and observation parameter**  
   Biofertilizer cultivated at 1 cm depth in 1 kg of soil within plastic. Vegetative growth (crops length, leaves’ number, leaves’ width, root’s length, stem’s diameters, net weight, and dry weight) and generative phase (flowers number) were the observation object.

**Research design and data analysis**  
Random sampling with one factorial (six biofertilizer composition) applied as research design. Data observation tested by general linear model at 95% (α = 0.05%) confidence level. It was conducted to observe any influences biofertilizer composition for peanut growth. Duncan test applied when P values not more then 0.05.

**RESULTS AND DISCUSSION**

**Crops length**  
Based on one-way ANOVA test, a combination of carrier media for phosphate solubilizing bacteria is indicated by the p-value (P> 0.05). Based on Table 1, which show that the lowest average on this parameter is B1 treatment. B1 contains manure and sand. The usage of sand as a carrier media is preferred because it has good aeration and it reduces drainage but sand has low water holding capability so that media will be easy to dry (Mumtaz et al., 2006). It becomes difficult to support bacterial growth.

The highest average on crops length is B3 treatments. B3 contains soil and manure. Manure components can be characterized as organic and inorganic nitrogen, phosphorus, and potassium that are the major nutrients of manure. So, bacteria can function properly (Lorimor, 2004). Soil plays role in the biological activity especially microorganism.
Table 1. Treatment variation on the average growth of the crops length, stem diameter, and root length for peanut plants (A. hypogaea L.)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Crops length (cm)</th>
<th>Stem diameter (cm)</th>
<th>Root lenght (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>28.54</td>
<td>0.59</td>
<td>19.34</td>
</tr>
<tr>
<td>B2</td>
<td>29.12</td>
<td>0.65</td>
<td>23.48</td>
</tr>
<tr>
<td>B3</td>
<td>30.02</td>
<td>0.60</td>
<td>20.08</td>
</tr>
<tr>
<td>B4</td>
<td>29.70</td>
<td>0.63</td>
<td>21.62</td>
</tr>
<tr>
<td>B5</td>
<td>29.80</td>
<td>0.63</td>
<td>21.38</td>
</tr>
<tr>
<td>B6</td>
<td>29.62</td>
<td>0.62</td>
<td>20.16</td>
</tr>
<tr>
<td>B7</td>
<td>26.28</td>
<td>0.53</td>
<td>17.92</td>
</tr>
</tbody>
</table>

B1 = manure : sand  
B2 = manure : soil : sand  
B3 = manure : soil  
B4 = soil : sand  
B5 = manure  
B6 = sand  
B7 = without treatment  
(control plants)

Organic material is energy source of carbon and nitrogen for soil microbes (Nielsen, 2002). Carrier media must maintain and improve numbers of microbes in long term so that it can be used as source of microbial nutrients (Ambak and Melling, 2000). Enhancement of crops length can be assumed that phosphate solubilizing bacteria has capable of producing IAA (Indol Acid Acetat). IAA can promote plant growth and stimulate cell elongation (Compant et al., 2010).

**Stem diameter**

Based on one-way ANOVA test, a combination of carrier media for phosphate solubilizing bacteria is indicated by the p-value (P>0.05). Table 1 shows the lowest average. B7 is control treatment without giving phosphate solubilizing bacteria. All treatments has the higher values than control treatment because of influence of giving carrier media of phosphate solubilizing bacteria. Phosphate solubilizing bacteria dissolved phosphate through various microbial processes including acid production so that it can be absorbed by crops (Keneni et al., 2010).

B2 treatment has the highest average on stem’s diameter parameter. B2 consists of manure, sand, and soil. That combination ensures availability of nutrients and aeration. Soil has good water holding capacity and nutrients. Water holding capacity of carrier proves that the carrier media has capacity to have bacteria (Arora et al., 2014). The characteristic of this carrier media is favorable condition for bacteria. Phosphorus plays a role to plant growth, such as stems, roots, and leaves (Anbuselvi, 2015).

**Root length**

Based on one-way ANOVA test, a combination of carrier media for phosphate solubilizing bacteria is indicated by the p-value (P>0.05). The results showed all treatments of carrier media for biofertilizer of phosphate solubilizing bacteria have longer roots than control treatment. B7 treatments is a control plant, it has the lowest values because there is no influence from phosphate solubilizing bacteria. Phosphate solubilizing bacteria Bacillus sp. has capable of improving phosphate in soil and absorption of phosphate (Chen et al., 2006).

B2 treatment provided the highest values on this parameter. B2 treatment is appropriate carrier media because it has the highest values on each parameter. Roots development very rapid because there is increase availability of phosphorus. The availability of soil P for plants is related to several plant characters, including morphological traits such as length and surface area of roots, root architecture, and root hairs (Williamson et al., 2001). The roots are not well developed, it can’t absorb more phosphate.

**Leaves number**

Based on one-way ANOVA test, a combination of carrier media for phosphate solubilizing bacteria is indicated by the p-value (P>0.05). Table 2 shows that B6 has the lowest average on leaves number.
Table 2. Treatment variation on the average growth of the leaves number and leaves width for peanut plants (*A. hypogaea* L.)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Leaves number</th>
<th>Leaves width (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>52.60</td>
<td>4.08</td>
</tr>
<tr>
<td>B2</td>
<td>78.20</td>
<td>4.93</td>
</tr>
<tr>
<td>B3</td>
<td>57.80</td>
<td>4.42</td>
</tr>
<tr>
<td>B4</td>
<td>56.00</td>
<td>4.42</td>
</tr>
<tr>
<td>B5</td>
<td>55.00</td>
<td>4.42</td>
</tr>
<tr>
<td>B6</td>
<td>51.80</td>
<td>4.59</td>
</tr>
<tr>
<td>B7</td>
<td>48.80</td>
<td>3.57</td>
</tr>
</tbody>
</table>

B6 treatment consists of soil which has good holding capacity of water and nutrients, but it has poor aeration. Without addition of manure, that media is relatively poor nutrients for bacteria growth (Buckley, 2001). B2 treatments provides the most number of leaves and others parameter, it assumed combination of manure, soil, and sand as carrier media can promote plant growth and bacteria growth.

When P is limiting, the most striking effects are reduction in leaf expansion and leaf surface area, as well as the number of leaves. The more leaves number, photosynthesis more higher, then result of photosynthesis is also increase. Phosphorus (P) is vital to plant growth and is found in every living plant cell. It is involved in several key plant functions, including photosynthesis, transformation of sugars, and energy transfer (Alberta, 1999).

**Leaves width**

Based on one-way ANOVA test, a combination of carrier media for phosphate solubilizing bacteria is indicated by the p-value (P> 0.05). The results show all treatments provide more leaves width than control treatment. Control treatment has the lowest values because of no influence from phosphate solubilizing bacteria. Inoculation of phosphate solubilizing bacteria can promote growth of leaves width. *Bacillus sp.* can dissolve phosphorus in the soil and turn it into inorganic phosphate so that can promote plant growth (Canbolat et al., 2004).

B2 provides the highest average on each parameters. B2 is a good composition for bacteria growth because it has the highest average each parameters. When availability of phosphate is enough, it can speed up photosynthesis process (Alberta, 1999). Phosphorus is known to play a significant role in plant photosynthetic activity thereby resulting in increase in leaf length and width and number of leaves (Egbuchua, 2015).

**Flowers number**

Based on one-way ANOVA test, a combination of carrier media for phosphate solubilizing bacteria is indicated by the p-value (P=0.00). This results show the differences of each treatment on flowers number parameters (Table 3). Phosphorus plays a role on generative phase. Role of nitrogen and phosphorus is balanced for vegetative phase, when entering generative phase, the role of phosphorus is more dominant than nitrogen because phosphorus is used to formation of flowers, fruits, and seeds (Sharma et al., 2013).

B2 treatment provides the most flowers number. It can be attributed to the most optimal carrier media for biofertilizer of phosphate solubilizing bacteria. Phosphate solubilizing bacteria produce low molecular weight organic, such as formic acid, acetic acid, propionic acid and fumaric acid. Organic acids will react with ions Ca²⁺, Fe³⁺ and Al³⁺ to bind phosphorus into a stable form (chelate) so that the phosphorus being free and available form to plants (Krishnaveni, 2010).

**Dry weight**

Based on one-way ANOVA test, a combination of carrier media for phosphate solubilizing bacteria is indicated by the p-value (P> 0.05). This results show that low dry weight is found in control plants (B7), it provides not optimal plant growth. It can be seen from low crops length and leaves number.
Table 3. Treatment variation on the average growth of the flowers number for peanut plants (A. hypogaea L.)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Flowers number</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>6.00 a</td>
</tr>
<tr>
<td>B2</td>
<td>11.60 b</td>
</tr>
<tr>
<td>B3</td>
<td>5.60 a</td>
</tr>
<tr>
<td>B4</td>
<td>5.80 a</td>
</tr>
<tr>
<td>B5</td>
<td>5.60 a</td>
</tr>
<tr>
<td>B6</td>
<td>6.60 a</td>
</tr>
<tr>
<td>B7</td>
<td>4.80 a</td>
</tr>
</tbody>
</table>

Description: Number which followed by the same letter at of the same column figured no significant differ based on Duncan test at 95% (α=0.05%) of confidence level.

Table 4. Treatment variation on the average growth of the dry weight for peanut plants (A. hypogaea L.)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>1.81</td>
</tr>
<tr>
<td>B2</td>
<td>2.49</td>
</tr>
<tr>
<td>B3</td>
<td>1.90</td>
</tr>
<tr>
<td>B4</td>
<td>2.04</td>
</tr>
<tr>
<td>B5</td>
<td>1.96</td>
</tr>
<tr>
<td>B6</td>
<td>1.99</td>
</tr>
<tr>
<td>B7</td>
<td>1.61</td>
</tr>
</tbody>
</table>

Addition of biofertilizer of phosphate solubilizing bacteria can improve plant growth (Gholami, 2009). Dry weight reflects to plant growth and amount of nutrient absorbed. The higher value of dry weight, then the more nutrient absorption and the better plant growth (Lynch, 1991). Addition of phosphate solubilizing bacteria can increase availability of phosphorus. It caused nutrient uptake and photosynthesis process so well. Result of photosynthesis also impact on dry weight.

CONCLUSION
The result of this research showed that there was no significance influence of carrier media to growth parameter of crops length, leaves number, leaves width, roots length, stem diameters, and dry weight. Therefore, there was significance influence of carrier media to number of flower.

CONFLICT OF INTEREST
The authors declare that they have no competing interests.

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AUTHOR CONTRIBUTIONS
This work carried out in collaboration between all authors. TutikNurhidayati designed the study, managed literature, wrote the protocol, and wrote the first draft of the manuscript. N. Firdausi, E.P. Setyaningsih, A.P.D. Nurhayati performed statistical analysis. WirdhatulMuslihatin and EkoPrasetyoKuncoro managed the analysis of the study and finalized draft of the manuscript.

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