Phosphorus (P) is one of essential nutrients in the growth and development of plants. Soil P is found in the organic and inorganic forms and ranges from ionic forms in solution to highly stable forms associated with organic matter and minerals (Nash et al., 2014; Rodrigues, Pavinato, Withers, Teles, & Herrera, 2016; Shen et al., 2011). Plants obtain P from the soil solution as reactive anion forms, absorbed in orthophosphoric ions \( \text{H}_2\text{PO}_4^- \) and \( \text{HPO}_4^{2-} \). P can also be absorbed in other forms, such as pyrophosphate, metaphosphate, and possibly also absorbed in the form of water-soluble organic substances. Phosphates are needed for plants in energy transfer, protein activation, and regulation of chemical metabolism processes (IPNI, 2013). P deficiency can affect vegetative growth of a plant.

Most farmers have fertilized the soil with inorganic or chemical fertilizers for nutrient needs of crops and to ensure production. Brebes Regency is one of the most important production centers of shallot in Indonesia that practiced intensive agriculture including fertilization. Farmers in this area annually planted four times of shallot or interspersed with other crops (e.g. chili, eggplant, soybeans, corn) or fallowed, and one time of rice (Muliana, Anwar, Hartono, Susila, & Sabiham, 2018). In each planting, inorganic fertilizers are applied on a regular basis rate regardless the soil
nutrient status and actual plant requirements. This prolong intensive inorganic fertilization is not accompanied by the addition of organic matter has resulted in the accumulation of nutrients, in particular phosphorus since it is easily fixed by soil minerals. Another study indicated that the rate of P fertilization varied considerably among farmers, ranging from 22 to 171 kg of P$_{\text{O}}$ ha$^{-1}$, but there was no significant correlation with shallot yield (Muliana, Anwar, Hartono, Susila, & Sabiham, 2018). IAARD (2006) showed that 99.95% of the 54,421 ha area of intensive shallot farming in Brebes has high to very high status of soil P. In addition, this high P mostly in the unavailable form (Hartono et al., 2015). The accumulated residual P as a result of prolong intensive inorganic P fertilization is known as legacy P (Chen et al., 2017; Ringeval, Nowak, Nesme, Delmas, & Pellerin, 2014; Rodrigues, Pavinato, Withers, Teles, & Herrera, 2016; Rowe et al., 2016). If it is not absorbed by plants, P from inorganic fertilizer can be immobilized by many reactions in soil, such as adsorption onto Al/Fe oxyhydroxides and clay minerals, immobilization by Al, Fe, and Ca ions, and complex reactions with soil organic substances (Chen et al., 2017; Powers et al., 2016; Rodrigues, Pavinato, Withers, Teles, & Herrera, 2016; Tischer, Santos, Kaminski, & Calegari, 2012; Yan, Wang, Zhang, Zhang, & Wei, 2013; Yan, Wei, Hong, Lu, & Wu, 2017). Depending on the source of P fertilizers, as much as 60 to 85% added P in the fertilizers is accumulated in the soils as residual P (Chen et al., 2017; Haygarth et al., 2014; Powers et al., 2016; Sattari, Bouwman, Giller, & van Ittersum, 2012). The accumulation of residual P is commonly occurred in agricultural soils that managed by smallholders. Smallholders usually have lack information and guidance for the right fertilization according to the soil conditions and plant needs. Smallholders tend to apply excessive amounts of fertilizers to secure plant production without considering the amount of nutrients in the fertilizers as well as the present nutrient status of the soil. In the future, the nutrient management to fulfill plant needs should also consider residual nutrients in the soil, in particular residual P.

Principally, the residual P in soil can be utilized by plant if the immobilized P can be converted to available form by organic materials, microorganisms or other chemical substances. The materials that have proven to increase the availability of the residual P including manure (Andrians, Syekhifi, & Nuraini, 2015), organic matter (Wahyudi & Handayanto, 2015), and phosphate solubilizing microorganisms (Alori, Glick, & Babalola, 2017; Khan, Jilani, Akhtar, Saqlan, & Rasheed, 2009). Biological fertilizers (microorganisms) such as phosphate solubilizing bacteria (PSB) and phosphate solubilizing fungi (PSF) can dissolve the adsorbed soil P (Diep & Hieu, 2013). The use of other materials such as humic substance is reported to decrease P sorption in high Al and Fe oxides soils (Hanudin, Sukmawati, Radjagukguk, & Yuwono, 2014; Hartono, Indriyati, & Selvi, 2013). Most of these studies were more concentrated on the effect of ameliorants and bio-fertilizers on plant growth, while information on its effects on soil P fractions is limited.

Besides being easily adsorbed by the soil, P is relatively immobile in the soil. Therefore, to effectively increase the availability of P, reactions in the rhizosphere should be considered. One way of studying plants related to the rhizosphere is to use a rhizobox. Rhizobox generally consists of two compartments, namely the inner compartment for root growth and the outer compartment is not for root growth, facilitating the comparison between rhizosphere and non-rhizosphere.

In relation to the ability of biological fertilizers (microorganisms such as PSB and PSF) as well as organic fertilizers (humic substance) to mobilize P, decrease soil P sorption, increase availability and absorption of P by plants, it is interesting to study the dynamics of soil P fractions in rhizosphere and non-rhizosphere. The objective of this study was to evaluate harvesting of residual P by the plant through its transformation to available forms by addition of humic substance (CHS) and bio-fertilizers (CBF, PSB and PSF) to the soil of intensive shallot farming from Brebes.

**MATERIALS AND METHODS**

The study was conducted from May 2015 to December 2016. The soil sample was collected from Siasem and Kersana Villages, Brebes District. The shallot planting experiments were conducted in a green house in Balumbang Jaya, Darmaga, West Bogor. Plant and soil analysis was conducted at the Laboratory of Soil Chemistry and Soil Fertility, Department of Soil Science and Land Resource, Faculty of Agriculture, Bogor Agricultural University. The study consisted of one factor comprising eight treatments in a completely randomized design with three replications, resulting in 24 experimental
units (Table 1). The growth and P uptake of shallots, and P fractions of soils after shallots planting were tested by analysis of variance at 5% significance level, while the difference between treatments was tested by Tukey's test at 5% level using SAS software version 9.2.

### Planting Preparations and Treatments

Soil sampling was conducted at a depth of 0–20 cm, comprised of 16 sites from Siasem Village and 24 sites from Kersana Village. All soil samples were combined, air dried and grinded to pass 2 mm sieve. This research used a rhizobox as container for planting shallots. Each rhizobox prepared had two compartments separated with nylon mesh, namely inner compartment (5 x 5 x 12 cm) as seedbed of shallots planting (rooting area) and outside compartment (10 x 10 x 12 cm) (non-rooting area) (Fig. 1). Air-dried soil sample was equivalent to one kg of absolute dry weight and was treated according to Table 1, proportionally placed into each rhizobox, and then watered with distilled water to the field capacity. Three shallot bulbs were planted on the inner compartment and grown for 26 days.

Four of the eight treatments were the focus of this study for increasing the availability of residual P, i.e. solid CHS (commercial humic substance), liquid CBF (commercial bio-fertilizers), PSB (phosphate solubilizing bacteria), and PSF (phosphate solubilizing fungi). The commercial humic substance contains 0.04% P. Commercial bio-fertilizers contain several microorganisms, namely Azotobacter vinelandii, Azospirillum lipoferum, Bradyrhizobium japonikum, Bacillus thuringiensis, Lactobacillus sp, Samiharomyces cerevisiae, Microbacterium lactium, Phanerochaete sp, and Paenibacillus macerans. Phosphate solubilizing bacteria (BPF-9/ not yet identified) and phosphate solubilizing fungi (FPF-4/ Aspergillus niger) were the collection of the Laboratory of Soil Biotechnology, Department of Soil Science and Land Resource, Faculty of Agriculture, Bogor Agricultural University. The other three treatments, i.e. duck manure (DM), inorganic fertilizers (IF) and their combination (DM+IF), in addition of control treatment, were used as comparison. The duck manure contains a total of 0.12% of P was collected from a duck farm in Brebes. Inorganic fertilizers comprised of urea (44% N), ZA (21% N), SP-36 (34% P₂O₅), and KCl (61% K₂O). The supply of P₂O₅ from CHS, DM, IF, and DM+IF treatments was 0.0014, 13.7, 25.7, and 26.6 mg P₂O₅ kg⁻¹, respectively. The contribution of these P was added to total P for percentage calculation of P fractions to the total P.

### Table 1. Treatments for harvesting residual P in soil of intensive farming, Brebes

| Code | Treatments |
|------|------------|
| Control | Without treatment |
| CHS | Commercial humic substance (3 kg ha⁻¹) |
| CBF | Commercial bio-fertilizers (7 L ha⁻¹) |
| PSB | Phosphate solubilizing bacteria (5 ml kg⁻¹) |
| PSF | Phosphate solubilizing fungi (5 ml kg⁻¹) |
| DM | Duck manure (10 t ha⁻¹) |
| IF | Inorganic fertilizers (Urea 250; ZA 180; SP-36 150; KCl 150 kg ha⁻¹) |
| DM+IF | DM (10 t ha⁻¹) + IF (Urea 125; ZA 60; SP-36 75; KCl 75 kg ha⁻¹) |

Remarks: CHS = commercial humic substance, CBF = commercial bio-fertilizers, PSB = phosphate solubilizing bacteria, PSF = phosphate solubilizing fungi, DM = duck manure, IF = inorganic fertilizers

![Fig. 1. Root area box (rhizobox) made of transparent acrylic](image)
The shallots used for this research was Bima Brebes variety. Seeds were derived from a shallot farm in Brebes, which have been stored for two months. The shallot bulbs used for planting were firstly selected for normal size and not deformed ones.

**Growth Observations and Plant Analysis**

Growth observations and measurement included plant height, number of leaves, and number of tillers. Plant biomass (without root) was harvested after 26 days of planting, oven dried at 60°C for 48 hours, and weighed. Plant analysis for P uptake was conducted by dry digestion followed by digestion with nitric acid. The concentration of P in the filtrates were measured with the method of Murphy & Riley (1962) and by UV-Vis spectrophotometer (UV-1280, Shimadzu, Japan).

**Analysis of Soil P Fractions**

Soil samples for analysis of P fractions were taken separately from the rooting area and the non-rooting area. The soil samples were analyzed without prior drying. Fractionation of P was based on the modified Tiessen & Moir (1993), in which the resin Pi was replaced with CaCl
_\text{2}\_\text{Pi}_. Phosphorus fractions include (1) 0.01 mol L
^-1\text{CaCl}_2 (inorganic CaCl
_\text{2}\_\text{P} (Pi)) (P is available for plants, and in this study it is included in labile P), (2) 0.5 mol L
^-1\text{NaHCO}_3 extraction of inorganic P (Pi) and organic P (Po) (labile inorganic P weakly adsorbed on the surface of crystalline compounds and labile organic P compounds with low recalcitrance like ribonucleic acid and glycerophosphate, highly related to the absorption by plants and microorganisms), (3) 0.1 mol L
^-1\text{NaOH} extraction of Pi and Po (moderately labile inorganic P strongly adsorbed onto Fe and/or Al oxidesand clay minerals, and moderately labile organic P mainly associated with fulvic and humic acids adsorbed onto the minerals and/or soil organic matter surfaces), and (4) 1.0 mol L
^-1\text{HCl} extraction of Ca-bound Pi (moderately labile inorganic P associated with apatite, adsorbed by negatively charged oxide surfaces, or other sparingly-soluble Ca-P compounds). A separate untreated sub sample was determined for its total P by digestion with concentrated sulfuric and fluoric acids. Each of the filtrates measured for its P concentration using colorimetric ascorbic acid method (Murphy & Riley, 1962) and by UV-Vis spectrophotometer (UV-1280, Shimadzu, Japan). The residual P (more recalcitrant and non-labile organic and inorganic P fractions) was calculated as subtraction of the sum of labile and moderately labile P fractions from total P.

**RESULTS AND DISCUSSION**

**Initial Soil Characteristics**

The initial soil characteristics are shown in Table 2. The soil of intensive farming in Brebes contained quartz, montmorillonite and crystalbalite. This soil had a heavy clay texture, with clay content of 80%. Soil was slightly acidic with pH H
_2\text{O} of 5.75. Organic-C content was low (1.40%), and cation exchange capacity (CEC) was high (32.7 cmol kg
^-1\). The total P was 2,183 mg P
_2\text{O}_5 kg
^-1\ and categorized as very high in the criterion of Eviati & Sulaeman (2012). The distribution of the P fractions in the soil was in the order of residual P (94.4%) >> HCl-Pi (2.14%) > NaHCO
_3-Pi (1.51%) > NaOH-Pi (1.03%) > NaOH-Po (0.71%) > NaHCO
_3-Po (0.18%) > CaCl
_2-Pi (0.05%). The available P (CaCl
_2-Pi) was very low, only 1.06 mg P
_2\text{O}_5 kg
^-1\ (in this study it was included in labile P). The labile P (CaCl
_2-Pi + NaHCO
_3-P) and the moderately labile P (NaOH-P + HCl-Pi) were 1.7 and 3.9% to the total P, respectively. The residual P was very high, almost 95% of the total P, because of a prolong P fertilization of the soil.

**Table 2. Soil properties before treatments**

| Parameter          | Content | P fractions (mg P
_2\text{O}_5 kg
^-1\) |
|--------------------|---------|-----------------|
| Sand (%)           | 1.10    | CaCl
_2-Pi           | 1.06 |
| Silt (%)           | 18.7    | NaHCO
_3-Pi            | 32.9  |
| Clay (%)           | 80.2    | NaHCO
_3-Po            | 3.86  |
| pH H
_2\text{O}       | 5.75    | NaOH-Po          | 15.5  |
| Organic-C (%)      | 1.40    | HCl-Pi           | 46.8  |
| CEC (cmol kg
^-1\) | 32.7    | Total P          | 2,183 |
The observation results of the plant height, number of leaves, number of tillers, plant dry weight, content and uptake of P by shallot after 26 days of planting are shown in Table 3. The growth conditions at 26 days after planting are shown in Fig. 2. The treatments had no significant effect on plant height, number of tillers, and plant dry weight. However, there was a significant different effects of the treatments on P content and P uptake. The overall results of plant growth and P absorption indicated that the comparative conventional treatments (DM, IF and DM+IF) had better effects than that of the alternative treatments (CHS, CBF, PSB and PSF). The addition of duck manure, inorganic fertilizers, and their combination resulted higher P content and P uptake by plants compared to other treatments. These results, however, did not followed by significant different effects of the treatments on plant height, number of leaves, number of tillers, and plant dry weight. This is probably because the soil had enough supply of the necessary nutrients for plant growth, such that the increasing supply of nutrients in particular P from duck manure and inorganic fertilizers or their combination resulted in the inefficient use of P by plant. From four alternative treatments, PSB and PSF relatively had better effects on the plant growth compared to the control. PSB tended to have higher plant height, number of leaves, and plant dry weight while PSF tended to have higher number of leaves, number of tillers, and plant dry weight compared to the control.

These data indicated that the application of P fertilizer in IF and DM+IF treatments did not significantly affect the plant growth. The unsignificant effect of the application of P fertilizer in IF and DM+IF treatments supported by plant growth (plant height, number of leaves, number of tillers, and plant dry weight).
Variation of the population density of fruit flies (Anastrepha spp.) considering the MTD during the study period. The CaCl$_2$ both in the rooting and non-rooting areas compared to the initial soil (32.9 mg kg$^{-1}$ P$_2$O$_5$).

The NaHCO$_3$-Pi fraction was lower in the rooting area than non-rooting areas. The order of NaHCO$_3$-Pi fraction in the rooting area from the highest was: DM > DM+IF > IF > CHS > PSF > CBF > control; while that in the non-rooting area was: DM > DM+IF > control > IF > CBF > CHS > PSF > PSB. The NaHCO$_3$-Po decreased significantly by the application of DM in the rooting area; and increased and decreased significantly by the application of CHS and DM+IF, respectively, in the non-rooting area. The highest NaHCO$_3$-Po fraction was achieved by CBF for the rooting area and by CHS for the non-rooting area. In the contrary, NaHCO$_3$-Pi and NaHCO$_3$-Po were higher in the rooting area than non-rooting area. When P availability is low, organic matter, microbes, and plant roots produced organic acids could release the bound P through several reactions such dissolution of mineral (Shen et al., 2011; Hinsinger, 2001; Richardson, 2001), ligand exchange reaction (Hinsinger, 2001; Richardson, 2001) chelation and complex formation with P adsorbent agents (Plante, 2007; Richardson, 2001), and enzymatic hydrolysis of organic P (Richardson, 2001).

The average fractions of CaCl$_2$-Pi and NaHCO$_3$-Pi on the rooting area were lower than that of the non-rooting area. This illustrated that the P-nutrient depletion process occurred in the form of the CaCl$_2$-Pi and NaHCO$_3$-Pi fractions in the rooting area due to P uptake by the plant. Nutrient depletion by plants can be recompensed either by the diffusion from non-rooting area, or the release of the unavailable nutrient (in this case probably from the moderately labile NaOH-P and HCl-P fractions) in the rooting area.

The NaOH-Pi fraction was significantly increased by the applications of DM and DM+IF both in the rooting and non-rooting areas, increased significantly by the applications of CHS and IF in the rooting area, and decreased significantly by the applications of CHS and IF in the non-rooting area compared to the control. Overall, the average NaOH-Pi fraction was higher in the rooting area than non-rooting area. The order of NaOH-Pi fraction in rooting area from the highest was: DM > DM+IF > IF > CHS > CBF > PSF > PSB > control; while that in the non-rooting area was: DM > DM+IF > IF > CHS > PSF > CBF > control. The NaHCO$_3$-Po fraction was lower in the rooting area than in the non-rooting area.

P Fractionations

Phosphorus in the soil is in inorganic (Pi) and organic (Po) forms, with varying degrees of solubility or availability. The results showed that all forms of labile P in the rooting and non-rooting areas were significantly affected by the treatments (Table 4). Only NaOH-Pi in the rooting and non-rooting areas and HCl-Pi in the rooting area were significantly affected by the treatments for moderately labile and recalcitrant P pools.

All treatments resulted in a higher CaCl$_2$-Pi both in the rooting and non-rooting areas compared to the control. The CaCl$_2$-Pi fraction was increased significantly by the treatments with the exceptions of DM+IF in the rooting area and CBF in the non-rooting area compared to the control. The average CaCl$_2$-Pi fraction was lower in the rooting area than non-rooting area. The order of CaCl$_2$-Pi fraction in the rooting area from the highest was: DM > CBF > IF > PSF > CHS > PSF > DM+IF > control; while that in the non-rooting area was: IF > DM+IF > DM > PSF > CHS > PSB > CBF > control. These suggested that all given inputs can increase available P (CaCl$_2$-Pi) for plants.

The NaHCO$_3$-Pi fraction increased significantly by the applications of DM and DM+IF both in the rooting and non-rooting areas, whereas the application of IF significantly increased this fraction only in the rooting area (Table 4). The NaHCO$_3$-Pi fraction in the rooting and non-rooting areas also increased in all the treatments including the control compared to the initial soil (32.9 mg kg$^{-1}$ P$_2$O$_5$). This indicated that the root of shallot has the ability of extracting unavailable P. The addition of humic substance and bio-fertilizers, however, can increase this ability. The average NaHCO$_3$-Pi fraction was lower in the rooting area than non-rooting areas. The order of NaHCO$_3$-Pi fraction in the rooting area from the highest was: DM > DM+IF > IF > CHS > PSF > CBF > PSB > control; while that in the non-rooting area was: DM > DM+IF > control > IF > CBF > CHS > PSF > PSB.

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Table 4. Effect of treatments on P fractions on rooting area and non-rooting area of shallot 26 days after planting

| Treatments | Fraction of P<sub>2O</sub><sub>5</sub> | CaCl<sub>2</sub>-Pi | NaHCO<sub>3</sub>-Pi | NaHCO<sub>3</sub>-Po | NaOH-Pi | NaOH-Po | HCl-Pi | Residual P |
|------------|----------------------------------|--------------------|---------------------|---------------------|---------|---------|--------|-----------|
|            |                                  | R                  | NR                  | R                   | NR                  | R                   | NR       | R         |
| Control    | 0.94 d                           | 1.04 d             | 34.3 d              | 43.8 b              | 6.69 bc             | 3.22 bc             | 27.8 de | 27.0 b    | 11.5     | 21.0     | 39.3 c   | 44.0     | 2.050               | 2.031               |
| CHS        | 1.26 bc                          | 1.39 c             | 37.0 cd             | 40.8 b              | 8.93 abc            | 13.4 a              | 30.9 bc | 25.2 c    | 11.8     | 19.0     | 47.4 bc | 43.2     | 2.035               | 2.029               |
| CBF        | 1.45 ab                          | 1.09 d             | 36.3 cd             | 41.3 b              | 10.4 ab             | 2.09 c              | 30.2 bcd | 24.9 c    | 13.2     | 21.0     | 47.3 bc | 41.2     | 2.032               | 2.040               |
| PSB        | 1.27 bc                          | 1.37 c             | 34.9 d              | 36.7 c              | 7.99 bc             | 7.02 b              | 26.8 e  | 22.6 d    | 12.5     | 24.0     | 45.5 bc | 42.1     | 2.073               | 2.037               |
| PSF        | 1.16 cd                          | 1.43 c             | 37.0 cd             | 40.4 b              | 8.45 abc            | 7.70 b              | 29.8 cd | 24.9 c    | 13.0     | 18.6     | 45.7 bc | 46.9     | 2.037               | 2.032               |
| DM         | 1.63 a                           | 1.62 b             | 54.6 a              | 60.3 a              | 2.49 d              | 2.94 c              | 35.2 a  | 37.2 a    | 13.2     | 11.8     | 66.6 a  | 59.6     | 2.008               | 2.008               |
| IF         | 1.44 ab                          | 2.07 a             | 40.7 bc             | 43.7 b              | 6.44 bc             | 7.25 b              | 32.4 abc | 27.0 b    | 11.4     | 20.8     | 48.5 bc | 42.8     | 2.055               | 2.052               |
| DM+IF      | 1.07 cd                          | 1.63 b             | 43.7 b              | 59.2 a              | 5.55 c              | 1.85 d              | 32.9 ab | 36.5 a    | 14.5     | 8.3      | 53.3 b  | 52.5     | 2.043               | 2.034               |
| Average    | 1.28                             | 1.46               | 39.8                | 45.8                | 7.12                | 5.68                | 30.8    | 28.2      | 12.6     | 18.1     | 49.2    | 46.5     | 2.042               | 2.033               |

| Treatments | Fraction of P<sub>2O</sub><sub>5</sub> | CaCl<sub>2</sub>-Pi | NaHCO<sub>3</sub>-Pi | NaHCO<sub>3</sub>-Po | NaOH-Pi | NaOH-Po | HCl-Pi | Residual P |
|------------|----------------------------------|--------------------|---------------------|---------------------|---------|---------|--------|-----------|
|            |                                  | (%)                | (%)                 | (%)                 | (%)     | (%)     | (%)    | (%)       |
| Control    | 0.04                             | 0.05               | 1.58                | 2.02                | 0.31    | 0.15    | 1.28   | 1.25      | 0.53     | 0.97    | 1.81    | 2.03     | 94.5                | 93.5                |
| CHS        | 0.06                             | 0.06               | 1.71                | 1.88                | 0.41    | 0.61    | 1.42   | 1.16      | 0.54     | 0.88    | 2.18    | 1.99     | 93.7                | 93.4                |
| CBF        | 0.07                             | 0.05               | 1.67                | 1.90                | 0.48    | 0.10    | 1.39   | 1.15      | 0.61     | 0.97    | 2.18    | 1.90     | 93.6                | 93.9                |
| PSB        | 0.06                             | 0.06               | 1.61                | 1.69                | 0.37    | 0.32    | 1.24   | 1.04      | 0.58     | 1.11    | 2.10    | 1.94     | 94.1                | 93.8                |
| PSF        | 0.05                             | 0.07               | 1.70                | 1.86                | 0.39    | 0.35    | 1.37   | 1.15      | 0.60     | 0.86    | 2.10    | 2.16     | 93.8                | 93.6                |
| DM         | 0.08                             | 0.07               | 2.51                | 2.78                | 0.11    | 0.14    | 1.64   | 1.71      | 0.61     | 0.54    | 3.07    | 2.74     | 92.0                | 92.0                |
| IF         | 0.07                             | 0.10               | 1.88                | 2.01                | 0.30    | 0.33    | 1.49   | 1.24      | 0.53     | 0.96    | 2.23    | 1.97     | 93.6                | 93.5                |
| DM+IF      | 0.05                             | 0.08               | 2.02                | 2.73                | 0.26    | 0.09    | 1.51   | 1.68      | 0.67     | 0.38    | 2.45    | 2.42     | 93.1                | 92.7                |
| Average    | 0.06                             | 0.07               | 1.83                | 2.11                | 0.33    | 0.26    | 1.42   | 1.30      | 0.58     | 0.83    | 2.27    | 2.14     | 93.5                | 93.3                |

Remarks: The number in the same column followed by the same letter is not significantly different from the Tukey’s test (p > 0.05); CHS = commercial humic substance, CBF = commercial bio-fertilizers, PSB = phosphate solubilizing bacteria, PSF = phosphate solubilizing fungi, DM = duck manure, IF = inorganic fertilizers.
The HCl-Pi fraction was higher after all treatments although only significantly increased by the application DM and DM+IF in the rooting area compared to the control. The HCl-Pi in the non-rooting area was not significantly affected by the treatments. The average of HCl-Pi fraction was higher in the rooting area than non-rooting area. The increasing of HCl-Pi fraction in the rooting area from the highest was: DM > DM+IF > IF > CHS > CBF > PSF > PSB > control.

The mean percentage of each P fraction to the total P both in the rooting and non-rooting areas from the highest was in the order of HCl-Pi > NaHCO$_3$-Pi > NaOH-Pi > NaOH-Po > NaHCO$_3$-Po > CaCl$_2$-Pi (Table 4). This order was same as the order of the initial soil. The values, however, were all higher compared to that of the initial soil, which suggested that the treatments resulted in the transformation of non-labile residual P into a more labile P pools. Only small proportion of the residual P transformed into a more labile P. The initial soil contained 1.74 and 3.88 mg P$_{2O_5}$ kg$^{-1}$, respectively for labile and moderately labile P. The average labile and moderately labile P after treatments were 2.22 and 4.27 mg P$_{2O_5}$ kg$^{-1}$ respectively in the rooting area, and 2.24 and 4.27 mg P$_{2O_5}$ kg$^{-1}$ respectively in the non-rooting area (Table 4). The uptake of P in this study which was 5.59 mg P$_{2O_5}$ kg$^{-1}$ in average (Table 3) should be considered, previously being the labile P in the rooting area. Most of the P was in the form that cannot be available to the plant (residual-P). According to Ludwick (1998), P in soil at pH < 4 is mainly bound by Fe, at pH 5.0–5.5 by Al, and at alkaline pH by Ca. Organic acids added or produced by microbes or plant roots react with Ca, Al and Fe through the chelating, increasing the accessibility of soil P to enzymatic hydrolysis thereby releasing P in the soil (Bhattacharyya, Chakrabarti, Chakraborty, & Nayak, 2005; Kovar & Claassen, 2007; Richardson, 2001).

The higher levels of moderate labile fractions (NaOH-P and HCl-P) in the soil of this study indicated that the soil was saturated with P and was difficult to dissolve. Thus, the application of P-releasing materials is required in this soil. The application of organic (duck manure) and inorganic fertilizers increased the available P fraction (CaCl$_2$-P and NaHCO$_3$-P) as well as less available P (NaOH-P and HCl-P) in both rooting and non-rooting areas. This indicated that both could contribute to phosphorus in all the fractions of both available and adsorbed forms. Organic fertilizers released P in the various fractions and release P as an available form, whereas inorganic fertilizers release dissolved P which can be available to plants, and some can be adsorbed as the less available forms (NaOH-P and HCl-P).

The other inputs, that is humic substance, bio-fertilizers, phosphate solubilizing bacteria, and phosphate solubilizing fungi increased the available CaCl$_2$-Pi fraction in the rooting and non-rooting areas, while the NaHCO$_3$-Pi fraction was also increased but not significant and only in the rooting area. The input released P, compensating P absorption by the plants in the rooting area. The diffusion of P from the non-rooting area to the rooting area was proven by the lower levels of NaHCO$_3$-Pi fraction in non-rooting areas of four alternative treatments compared to the control. The dynamics of P fraction in the rooting area gave better information about the phenomenon of harvesting P, because the transformation from the unavailable to the available form that is directly absorbed by the plant. Phosphate solubilizing microbes would produce organic acids such as citrate, malate, oxalate, and acetate as chelating agents (Arcand & Schneider, 2006). P-solubilizing bacteria also excrete phosphatase and phytase that can mineralize organic P and produce phosphate (Mehrzarv, Chaichi, & Alikhani, 2008).

**Dynamics Transformation of Residual P to Labile P in Rooting and Non-Rooting Areas**

The dynamics transformation of residual P to labile P in rooting area and non-rooting area is presented in Table 5. The P fractions in the rooting area gave better information about the harvest of P since it showed higher transformation of residual P to more labile forms, compared to that of the non-rooting area. Table 5 also presents the difference to the control in percentage (treatment – control), the labile P increased from 0.11 (PSB-control) to 0.29% (CBF-control) with average of 0.21% in the rooting area, and decreased/increased from -0.14 (PSB-control) to 0.34% (CHS-control) with average of 0.03% in the non-rooting area (lower part of Table 5). The moderately labile P increased from 0.29 (PSB-control) to 0.56% (CBF-control) with average of 0.46% in the rooting area, and decreased from -0.07 (PSF-control) to -0.23% (CBF-control) with average of -0.17% in the non-rooting area. Overall, the dynamics transformation of residual P was indicated by the total change of the more labile P (sum of the
difference of the labile and the moderately labile P to the control). The more labile P increased from 0.40 (PSB-control) to 0.85% (CBF-control) with average of 0.67% in the rooting area, and decreased/ increased from -0.01 (PSF-control) to 0.13% (CHS-control) with average of -0.14% in the non-rooting area. These results suggested that in addition to the transformation of residual P to the more labile P, there was also a transformation of the moderately labile P to labile P, and the transformations were more pronounce in the rooting area. Later, it is also suggested that the alternative treatment is better to be applied in the rooting area. Among the alternative treatments CBF is the best in increasing the labile P, followed by CHS, PSF, and PSB. From the total P, the labile P fractions amounted to about 1.9–2.6%, moderately labile P amounted to about 3.6–4.2% while the remaining was residual P amounted to about 93.4–94.5%, both in the rooting and non-rooting areas. The overall dynamic transformations of P indicated that addition of bio-fertilizers and humic substance can be utilized to harvest residual P. Further studies are still needed to utilize the high legacy P in the agricultural soil.

**CONCLUSION AND SUGGESTION**

The addition of humic substance and bio-fertilizers can improve the harvesting of residual P in the intensive shallots farming soil in Brebes. The improvement of harvesting residual P was indicated by the increasing of the more labile P in the rooting area. In this research, however, the harvesting of residual P by addition of humic substance or bio-fertilizers was not followed by significant effects on the shallot growth up to 26 days after planting. The average increase of the more labile P, were only as high as 0.67% in rooting area. The capability of harvesting residual P was in the order of commercial bio-fertilizers > commercial humic substance > phosphate solubilizing fungi > phosphate solubilizing bacteria. To harvest residual P, the addition of humic substance, bio-fertilizers, and other similar materials should be in the rooting area. Further studies are still necessary for utilizing the high legacy P in the agricultural soil.

**Table 5. The harvesting of residual P and its comparison with the control**

| Treatment | Labile P R | Labile P NR | Moderately Labile P R | Moderately Labile P NR | Sum of Labile and Moderately Labile P R | Sum of Labile and Moderately Labile P NR | Residual P R | Residual P NR |
|-----------|------------|-------------|-----------------------|------------------------|----------------------------------------|----------------------------------------|-------------|-------------|
| Control   | 1.93       | 2.21        | 3.63                  | 4.24                   | 5.55                                  | 6.45                                  | 94.5        | 93.5        |
| CHS       | 2.17       | 2.56        | 4.15                  | 4.03                   | 6.32                                  | 6.58                                  | 93.7        | 93.4        |
| CBF       | 2.22       | 2.05        | 4.18                  | 4.01                   | 6.40                                  | 6.06                                  | 93.6        | 93.9        |
| PSB       | 2.03       | 2.08        | 3.91                  | 4.09                   | 5.94                                  | 6.16                                  | 94.1        | 93.8        |
| PSF       | 2.15       | 2.28        | 4.07                  | 4.16                   | 6.22                                  | 6.45                                  | 93.8        | 93.6        |
| Average   | 2.10       | 2.24        | 3.99                  | 4.10                   | 6.09                                  | 6.34                                  | 93.9        | 93.7        |
| CHS-Control | 0.24     | 0.34        | 0.53                  | -0.21                  | 0.77                                  | 0.13                                  | -0.77       | -0.13       |
| CBF-Control | 0.29     | -0.17       | 0.56                  | -0.23                  | 0.85                                  | -0.40                                 | -0.85       | 0.40        |
| PSB-Control | 0.11     | -0.14       | 0.29                  | -0.15                  | 0.40                                  | -0.29                                 | -0.40       | 0.29        |
| PSF-Control | 0.22     | 0.07        | 0.46                  | -0.07                  | 0.67                                  | -0.01                                 | -0.67       | 0.01        |
| Average   | 0.21       | 0.03        | 0.46                  | -0.17                  | 0.67                                  | -0.14                                 | -0.67       | 0.14        |

Remarks: CHS = commercial humic substance, CBF = commercial bio-fertilizers, PSB = phosphate solubilizing bacteria, PSF = phosphate solubilizing fungi
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