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Harvesting of Residual Soil Phosphorus on Intensive Shallot Farming in Brebes, Indonesia

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ABSTRACT

Accumulated residual soil phosphorus (P) on shallots farming in Brebes can be harvested through the application of ameliorants or bio-fertilizers. The information on the effect of ameliorants and bio-fertilizers on soil P fractions is limited. The study objective was to evaluate the transformation of accumulated P to available forms by adding humic substance (CHS), bio-fertilizers (CBF), phosphate solubilizing bacteria (PSB), or phosphate solubilizing fungi (PSF) on soil from Brebes. The experiment was conducted in rhizobox that has two compartments, namely inner compartment (rooting area) and outside compartment (non-rooting area). Shallots were planted for 26 days, observed for their growth, and analyzed for their P absorption. Soil samples in rooting and non-rooting area were analyzed for their P fractions after planting. The results indicated that the addition of CHS, CBF, PSB or PSF increased the harvesting of residual soil P through its transformation to a more labile P as high as 0.67% in rooting area. The dynamic of transformation in rooting area gave better information of harvesting P. The capability of harvesting accumulated P was in the order of CBF, CHS, PSF and PSB. For harvesting residual P, addition of humic substance or bio-fertilizers should be made in the rooting area.

INTRODUCTION

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 ($H_2PO_4^-$ and 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

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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 $P_2O_5ha^{-1}$, 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 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; Tiecher, 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, Syekhfani, & 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 subtance) 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 × 5 × 12 cm) as seedbed of shallots planting (rooting area) and outside compartment (10 × 10 × 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 *Azotobakter vinelandii*, *Azospirillum lipoferum*, *Bradyrhizobium japonikum*, *Bacillus thuringiensis*, *Lactobacillus* sp, *Samlharomyces cerevisae*, *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% P_2O_5), and KCl (61% K_2O). The supply of P_2O_5 from CHS, DM, IF, and DM+IF treatments was 0.0014, 13.7, 25.7, and 26.6 mg P_2O_5 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

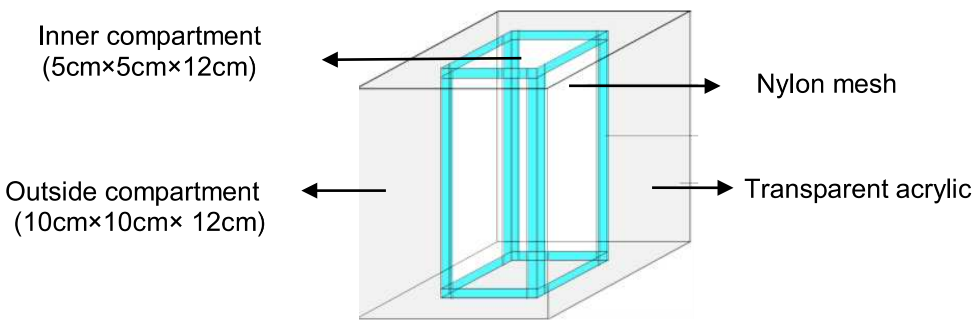

Fig. 1. Root area box (rhizobox) made of transparent acrylic

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 $CaCl_2$-Pi. Phosphorus fractions include (1) 0.01 mol L^{-1} $CaCl_2$ (inorganic $CaCl_2$-P (Pi)) (P is available for plants, and in this study it is included in labile P), (2) 0.5 mol L^{-1} $NaHCO_3$ extraction of inorganic P (Pi) and organic P (Po) (labile inorganic P weakly adsorbed on the surface of crysltalline 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} 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} 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 crystobalite. This soil had a heavy clay texture, with clay content of 80%. Soil was slightly acidic with pH H_2O 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_2O_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_2O_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	Parameter	Content
Mineral type: quartz, monmorillonite and crystabalite			
Soil texture: heavy clay		P fractions (mg $P_2O_5 kg^{-1}$):	
Sand (%)	1.10	$CaCl_2$-Pi	1.06
Silt (%)	18.7	$NaHCO_3$-Pi	32.9
Clay (%)	80.2	NaHCO3-Po	3.86
pH H_2O	5.75	NaOH-Po	15.5
Organic-C (%)	1.40	HCl-Pi	46.8
CEC (cmol kg-1)	32.7	Total P	2,183

Table 3. Treatments for harvesting residual P in soil of intensive farming, Brebes

Treatments	Plant height (cm)	Number of leaves	Number of tillers	Plant dry weight (g)	P content (%)	P uptake (mg)
Control	38.4	20.1	4.44	2.76	0.19 bc	5.32 b
CHS	37.1	19.9	4.78	2.65	0.18 cd	4.77 b
CBF	41.3	21.2	4.44	2.75	0.19 bc	5.22 b
PSB	39.2	21.2	4.33	3.19	0.17 cd	5.45 b
PSF	37.2	24.8	5.33	2.84	0.16 d	4.60 b
DM	40.1	23.7	4.78	3.04	0.22 a	6.71 a
IF	38.4	24.0	4.78	3.27	0.17 cd	5.74 ab
DM+IF	40.5	25.8	5.33	3.27	0.21 ab	6.89 a

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

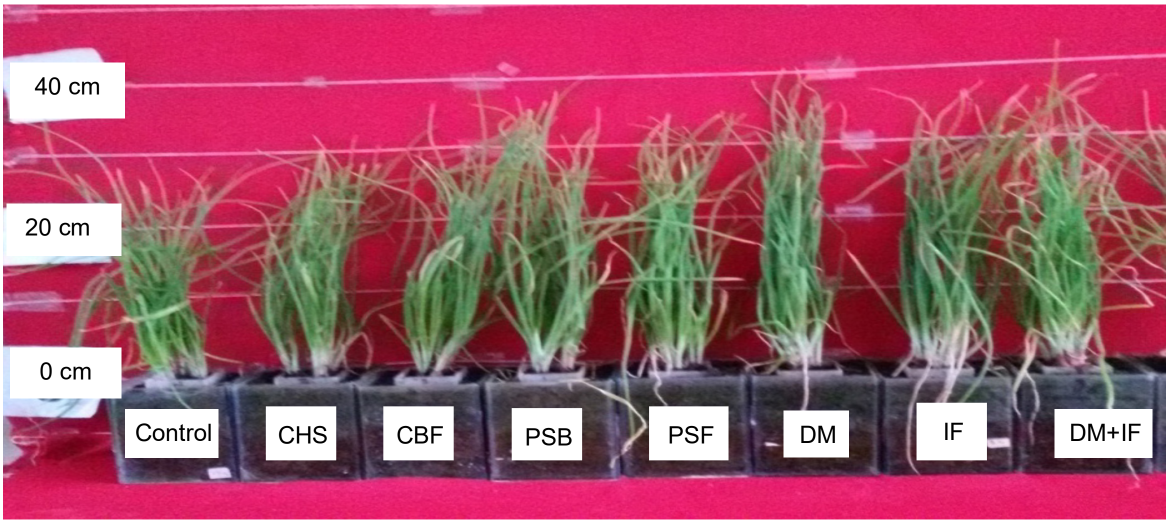

Fig. 2. Shallot 26 days after planting

Plant Growth, Biomass, P Content and P Uptake

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) which was not significantly different in all the treatments. However, the best plant growth and P uptake were mostly found in manure, inorganic fertilizers, and combination of manure and inorganic fertilizers (DM, IF, and DM+IF). This phenomenon may occur because the organic fertilizers of duck manure provided organic C and other macro and micro elements, and thus improve physical and biological properties of soil. The application of manure or compost in combination with inorganic fertilizers has a better effect compared to the application of only one of the fertilizers on soil quality and crop production (Agbede, 2010; Lee et al., 2009). Organic matter also became the adhesive of the free-loose granules and the main source of nitrogen, phosphorus, and sulfur, and increased available water for plants and the energy source for microorganisms.

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 CaCl2-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 > PSB> 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_2O_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. 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, CBF, PSB, and PSF 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 = control > CHS > CBF = PSF > PSB. All treatments had no significant effect on the NaOH-Po fraction. The average of NaOH-Po fraction was lower in the rooting area than in the non-rooting area.

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_2O_5													
	$CaCl_2$-Pi		$NaHCO_3$¬-Pi		$NaHCO_3$-Po		NaOH-Pi		NaOH-Po		HCl-Pi		Residual P	
	R	NR	R	NR	R	NR	R	NR	R	NR	R	NR	R	NR
	(mg kg-1)													
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
	(%)													
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 > NaHCO3-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, 2005; Plante, 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 (Mehrvarz, 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 fractionsin 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		Moderately Labile P		Sum of Labile and Moderately Labile P		Residual P	
	R	NR	R	NR	R	NR	R	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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