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Phosphate Solubilizing Study on the Determination of Inoculant Dose and Composition for Biofossi Fertilizer

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A B S T R A C T

Phosphorus availability becomes a major problem on the productivity of soil and rice in Indonesia. Utilization of P source which has a slow release property is one of the solutions to these problems. Biofossi fertilizer is a natural phosphate organic fertilizer which is enriched with phosphate solubilizer bacteria (PSB), to improve the solubility of P and absorption by the plant so that P fertilization becomes more efficient. Experiment conducted on January 2015 at the experimental station of Agriculture Faculty-Padjadjaran University, Bandung, located in Ciparay, West Java. The aim of the experiment was to determine the PSB composition and its inoculation dose toward natural phosphate. Inoculum used was of *Bacillus* sp and *Pseudomonas* sp, which was injected to a carrier material. Through previous test results, the carrier used had a composition of 50 % peat + 28 % compost + 5 % humic acid + 2 % nutrition + 15 % rice husk. To test the dose of PSB inoculation, experimental design of factorial randomized complete block was applied. As the first factor was P_2O_5 content in the phosphate source, consisting of four levels as follows: (1) p_0 = without P_2O_5 (as control); (2) p_1 = 22 % P_2O_5 (163 kg ha^{-1} natural phosphate from Blora); (3) p_2 = 26 % P_2O_5 (138 kg ha^{-1} natural phosphate from Morocco); (4) p_3 = 36 % P_2O_5 (100 kg ha^{-1} SP-36 fertilizer). Second factor was the combination of dose and type of PSB inoculant, consisting of seven levels as follows: (1) b_0 = without inoculation of PSB (as control); (2) b_1 = *Bacillus* sp inoculant in the dose of 1 kg ha^{-1}; (3) b_2 = *Bacillus* sp inoculant in the dose of 2 kg ha^{-1}; (4) b_3 = *Pseudomonas* sp inoculant in the dose of 1 kg ha^{-1}; (5) b_4 = *Pseudomonas* sp inoculant in the dose of 2 kg ha^{-1}; (6) b_5 = *Bacillus* sp + *Pseudomonas* sp consortium in the dose of 1 kg ha^{-1}; (7) b_6 = *Bacillus* sp + *Pseudomonas* sp consortium in the dose of 2 kg ha^{-1}. By using the ^{32}P radioisotope techniques it was known that a consortium of bacteria *Bacillus* sp + *Pseudomonas* sp was capable of dissolving P on the treatment p_0, as well as in the treatments p_1, p_2 and p_3. In this treatment, some of the highest response was obtained, i.e. the grain dry weight, P uptake and P fertilizer efficiency.

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INTRODUCTION

Phosphorus availability becomes a limiting factor for rice crop and soil productivity. P concentration is usually less than 0.01 % of the total soil [1]. In Indonesia, most of the lowland soil has been P saturated, but this element cannot be utilized as much as possible [2]. The high P absorption by soil colloids makes P unavailable for plants. The addition of P by fertilizers does not meet an adequate level of efficiency. The use of resources that are slow in releasing P is one of the promising alternatives to maintain the availability of P, to avoid P absorption, and to improve P fertilizer efficiency. Some actions that are known to be able to release P absorption in the soil colloids are (1) the inoculation of phosphate solubilizer microbe and (2) the addition of organic material [3].

The need for phosphate source fertilizer was the driving force behind this research. Biofossi is the natural phosphate fertilizer enriched by several

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minerals to fulfill the nutrients needed by rice plant. The phosphate solubilizer microorganism is added to facilitate P solubilization and P absorption by plant so that P fertilization becomes more efficient. *Pseudomonas* sp and *Bacillus* sp are the most important species of phosphate solubilizing bacteria which are widely used as biofertilizer [4]. An inoculant is carried in a material containing nutrients as an energy source for microorganisms to maintain their viability within a prescribed period.

A formulation of the carrier material becomes the determining factor in maintaining microorganism’s viability. A carrier material, in addition to maintain the microorganism’s viability, should also be able to provide nutrients for the inoculant during its lifetime. A carrier material is chosen from the material with energy and nutrient source suitable for the inoculated microorganisms. The composition of carrier material formulation was also being studied in this experiment.

The ingredient used in the formulation of Biofossi fertilizer was the natural phosphate which was chosen either from Blora or from Morocco whose P_2O_5 was 22 % and 26 % respectively. Silica mineral and humic acid were also added in accordance with the functional nutrient need by rice plant and soil characteristics. The entire materials were combined in such way to be 100 %. The best composition was determined from previous experiments. Inoculation dose and phosphate solubilizing bacteria (PSB) composition toward natural phosphate were the targets to be studied in the experiment, in addition to the type of natural phosphate source to be selected. Radioisotope of ^{32}P was used to describe the contribution of each type of natural source, the inoculation dose, and the composition to the increase of P availability.

EXPERIMENTAL METHODS

The experiment was conducted in January 2015 in the experimental station of CIRA-BATAN, Pasar Jumat, Jakarta Selatan using wetland soil from Ciparay, Bandung Regency. Analysis of the soil characteristics of the experiment showed that the soil belonged to Ultisols type. Soil pH value of 5.2 indicated that the soil was acidic, with a low content of organic C, N, and C / N ratio (1.52 %; 0.19 %; 8). Potential K_2O content was high (32 mg 100 g^{-1}). The values of CEC and base saturation were very high (25.60 cmol kg-1 and 82 % respectively). This may indicate the plant's ability to store nutrients, but may also indicate the high probability of leaching and weathering in the soil [5].

PSB was inoculated to the sources of P fertilizer, i.e. SP-36 (containing 36 % P_2O5) and natural phosphate rocks from Blitar and Morocco (containing 22 % and 26 % P_2O_5 respectively). The types of PSB used were *Pseudomonas* sp and *Bacillus* sp. Inoculation technique used was of a single inoculum and a mixture of both, injected to the carrier material consisting mineral nutrients and organic matter.

The carrier material consisted peat moss, straw compost, humic acid, nutrient solution and rice husk. The rice husk which has a stable carbon content was combined with humic acid as a source of labile organic carbon. The addition of rice husk was based on its potential as a microhabitat for the microorganism. A test on the potential of husk as a carrier material had also been conducted in this experiment. Thus the contents of the four carrier materials to be tested were as follows: (1) m_0 = 50 % peat + 28 % compost + 7.5 % humic acid + 2 % nutrition + 12.5 % rice husk; (2) m1= 50 % peat + 28 % compost + 5 % humic acid + 2 % nutrition + 15 % rice husk; (3) m_2= 50 % peat + 28 % compost + 2.5 % humic acid + 2 % nutrition + 17.5 % rice husk; (4) m_3= 50 % peat + 28 % compost + 0 % humic acid + 2 % nutrition + 20 % rice husk.

The test was then followed by the determination of the dose and the composition of PSB inoculation toward natural phosphate as the ingredients of Biofossi fertilizer. Dose of microorganism’s inoculation in biological fertilizer was determined based on the application of techniques described in Singh and Purohit (2011) [4]. For an inoculation technique by seedling treatments, the standard dose of inoculation used was 1 kg - 2 kg to 10 kg - 15 kg of seeds.

Greenhouse experiment was conducted in this study by applying the experimental design of factorial randomized complete block. The first factor was P_2O_5 content in the phosphate source consisting of four levels as follows: (1) p_0 = without P_2O_5 (as control); (2) p_1 = 22 % P_2O_5 (163 kg ha^{-1} natural phosphate from Blora); (3) p_2 = 26 % P_2O_5 (138 kg ha^{-1} natural phosphate from Morocco); and (4) p_3 = 36 % P_2O_5 (100 kg ha^{-1} SP-36 fertilizer). The second factor was the dose and the composition of PSB inoculant consisting of seven levels as follows: (1) b_0 = without inoculation of PSB (as control); (2) b_1 = *Bacillus* sp inoculant in the dose of 1 kg ha^{-1}; (3) b_2 = *Bacillus* sp inoculant in the dose of 2 kg ha^{-1}; (4) b_3 = *Pseudomonas* sp inoculant in the dose of 1 kg ha^{-1}; (5) b_4 = *Pseudomonas* sp inoculant in the dose of 2 kg ha^{-1}; (6) b_5 = *Bacillus* sp + *Pseudomonas* sp consortium in the dose of 1 kg ha^{-1}; and (7) b_6 = *Bacillus* sp + *Pseudomonas* sp consortium in the dose of 2 kg ha^{-1}.

A ^{32}P radioisotope with an activity of 30 mCi in the form of carrier-free solution $KH_2{}^{32}PO_4$ was applied to the soil in the pot before planting. Each pot contained 10 kg of air-dried soil which was then processed into mud labeled with 50 cc of ^{32}P and incubated one day before planting. In the next day, a Sidenuk rice variety of 10 days-after-sowing (DAS) which had been inoculated with PSB was transplanted. Rice crop was maintained until fully ripe, and then an analysis was taken to collect the data. Parameters observed in this experiment were inoculant viability in the carrier material, ^{32}P counted in the plant sample, P absorption derived from each treatment, plant yield, and P uptake efficiency.

RESULT AND DISCUSSION

Viability testing of PSB inoculant in carrier material

A carrier material consisting of peat, compost and nutrients got an addition of humic acid and charcoal husk. Husk charcoal has been known to play a role as a habitat for microorganisms, so that the existence of microorganisms was protected. This condition was able to influence microorganism's ability to maintain the viability. The meaningful difference in the PSB viability was found as the effect of the addition of rice husk and humic acids in the carrier material.

Table 1. Phosphate solubilizer bacteria viability testing in carrier materials enriched by rice husk and humic acid.

Carrier Materials Formula	Number of PSB Colony ($\times 10^8$ cfu g^{-1})
m_0 = 50 % peat + 28 % compost + 7,5 % humic acid + 2 % nutrition + 12.5 % charcoal husk	7,78 a
m_1 = 50 % peat + 28 % compost + 5 % humic acid + 2 % nutrition + 15 % charcoal husk	8,07 b
m_2 = 50 % peat + 28 % compost + 2.5 % humic acid + 2 % nutrition + 17 % charcoal husk	8,01 b
m_3 = 50 % peat + 28 % compost + 0 % humic acid + 2 % nutrition + 20 % charcoal husk	7,78 a

Remark: the average value followed by the same letter in the same column, are not significantly different at 5 % level of testing according to Duncan's Multiple Range Test

The data in Table 1 indicated a different response when the husk was added to the carrier material formula. The m_1 formula (50 % peat + 28 % compost + 5 % humic acid + 2 % nutrition + 15 % charcoal husk) caused the highest number of PSB colony (8.07×10^8 CFU g^{-1}) and was not significantly different from m2 formula. In general, the whole treatments showed responses due to the rice husk addition. However, the data showed that the number of PSB colonies decreased as the percentage of rice husk increased and the percentage of humic acid lowered. In this case, rice husk did not provide enough nutrition for PSB, in spite of its abundance. The percentage of low humic acid was more decisive as a source of nutrition. That was why the number of colonies decreased when humic acid availability decreased. The best viability of inoculant PSB in the formulation carrier achieved when the rice husk was added in a percentage of 15 %, followed by 5 % humic acid of the total weight of the carrier material.

Count value of ^{32}p in the plant samples from each treatment

The interaction between phosphate source and dose inoculation of PSB caused significant differences on ^{32}P count values response in the plant.

Table 2. The effect of phosphate sources and PSB dose of inoculation against ^{32}P count values in plants.

Inoculation Dose	Phosphate Sources			
	p_0 = 0	p_1 = 163 kg ha^{-1} NPB (22 % P_2O_5)	p_1 = 138 kg ha^{-1} NPM (26 % P_2O_5)	p_2 = 100 kg ha^{-1} SP-36 (36 % P_2O_5)
	cpm ^{32}P			
b_0 = 0 inoculation	608.1 c C	579.5 bc C	572.2 b C	557.6 a B
b_1 = 1 kg ha^{-1} *Bacillus* sp	581.6 c B	553.2 ab B	549.7 a C	562.0 b C
b_2 = 2 kg ha^{-1} *Bacillus* sp	563.7 b B	554.2 b B	541.8 a B	566.8 bc C
b_3 = 1 kg ha^{-1} *Pseudomonas* sp	559.8 b B	529.3 a B	527.1 a B	563.1 b C
b_4 = 2 kg ha^{-1} *Pseudomonas* sp	531.6 c A	507.4 b B	481.1 a A	537.5 c B
b_5 = 1 kg ha^{-1} *Bacillus* sp + *Pseudomonas* sp	511.4 c A	462.2 a A	471,3 a A	497,4 b A
b_6 = 2 kg ha^{-1} *Bacillus* sp + *Pseudomonas* sp	501.0 b A	469.3 a A	467,8 a A	522,9 b B

Remark: the average value followed by the same letter in the same column, are not significantly different at 5 % level of testing according to Duncan's Multiple Range Test

Data in Table 2 indicated a decline in ^{32}P count values at the time of PSB inoculation into the soil given various P sources. The count values in the soil were reduced without any addition of PSB and fertilizer (p_0b_0). The decrease in ^{32}P count values occurred because of the presence of a P source other than that from the soil. It then "diluted" the concentration of P in the plant tissue [6,7]. Thus the proportion of P uptake by plants was then divided according to the number of available P sources in the soil. It could be argued that a low count value of ^{32}P indicated high P uptake of plants against various sources of P in the soil.

Some of PSB inoculation treatment caused a significant response of ^{32}P count value, in comparison with the treatment of no PSB inoculation at all. The interaction between PSB inoculation and P sources showed that the highest ^{32}P count values (581.6 cpm ^{32}P) was achieved as a response to the inoculation of 1 kg ha^{-1} *Bacillus* sp to the soil. In this case, the soil acted as the only source of P for the plants and other soil organisms that needed P for their metabolism. The highest ^{32}P count value on this treatment showed that a lot of P nutrient was not diluted since no nutrient P sources were added to the soil. Statistically, as a response to the dose of PSB inoculation treatment, ^{32}P count value of the inoculation of 2 kg ha^{-1} *Bacillus* sp was not significantly different from that of the inoculation of 1 kg ha^{-1} *Pseudomonas* sp. Thus, it can be said that the P dissolution ability of *Bacillus* sp was not different from *Pseudomonas* sp when the source of P was only available from the soil.

Another highest response of ^{32}P count value (579.5 cpm ^{32}P) was obtained in the no inoculation treatment to the P nutrient sources of Blora natural phosphate with 22 % P_2O_5. This condition indicated a low level of P availability due to the lack of technology to improve P dissolution from natural phosphate. Inoculation of PSB would be the one of the technologies that can improve the dissolution of P from natural phosphate. High rate of solubilized P was depicted in a very low ^{32}P count value response (462 cpm ^{32}P) in the inoculation treatment of 1 kg ha^{-1} *Bacillus* sp + *Pseudomonas* sp to the soil. Nevertheless, the statistical response of this treatment was not significantly different from the inoculation dose of 1 kg ha^{-1} *Bacillus* sp + *Pseudomonas* sp to Moroccan natural phosphate (26 % P_2O_5). Similarly, no significant difference occurred when the time of inoculation dose was increased to 2 kg ha^{-1} on both sources of P. From the response of ^{32}P count value, it could be argued that the inoculation of *Bacillus* sp + *Pseudomonas* sp at a dose of 1 kg ha^{-1} and 2 kg ha^{-1} were having the same ability in solubilizing P from natural phosphates from both Blora and Morocco.

Plant p-uptake derived from each treatment

The composition of plant P-uptake could be described by using the ^{32}P count values based on the P availability in the soil. Calculation of P-absorbed derived from various P sources in the soil was a result of ^{32}P count value conversion into %^{32}P, and then proportionally fractionated into %P derived from various P sources in the soil. The effect of the treatment in the variation of P sources and PSB inoculation doses caused significant differences in the P uptake from various P sources.

The data in Table 3 showed since there was no phosphate source given (p_0) then the highest P uptake came from the soil (1034.9 g plant P^{-1}). It showed that the plants were able to utilize P-soil when the only available source of P_2O_5 was only from soil. The value of P uptake derived from soil was higher in comparison with P uptake derived from P-source applied. Supposedly, the addition of phosphate did not necessarily improve the availability of P in the soil. This condition could be caused by a low P dissolution from P sources added to the soil and an adsorption of P by the soil colloid and the metal elements.

Soil microorganisms have an important role in nutrient cycle [8]. The existence of rhizosphere area is supporting the availability of energy sources for microorganisms. Rice roots have an aeration system, controlled by gene, which is able to modify the environment that supports rice to grow under anaerobic condition [9]. The aeration system is able to release O_2 out to the growing environment, resulting in pH changes, hence the slower release of P retained by soil colloid and metallic elements. This condition was able to help the plants to obtain P nutrient from the soil while there is no P sources added from outside.

Table 3. Single effect of various P sources and PSB dose of inoculation against P-uptake in plants.

Treatments	P-uptake from various P sources (µg P plant^{-1})		
	P-df soil	P-df P source	P-df PSB inoc.
P sources (p)			
p_0 = 0	1034.9 b	0.0 a	0.0 a
p_1 = 163 kg ha^{-1} NPB (22 % P_2O_5)	422.5 a	201.2 b	439.7 b
p_2 = 138 kg ha^{-1} NPM (26 % P_2O_5)	413.7 a	229.0 b	501.4 b
p_3 = 100 kg ha^{-1} SP-36 (36 % P_2O_5)	623.6 b	244.4 c	523.9 c
PSB dose of inoculation (b)			
b_0 = 0 inoculation	1034.6 a	1174.4 a	0.0 a
b_1 = 1 kg ha^{-1} *Bacillus* sp	1146.1 a	1122.3 a	423.3 b
b_2 = 2 kg ha^{-1} *Bacillus* sp	1215.5 b	1023.5 a	329.1 c
b_3 = 1 kg ha^{-1} *Pseudomonas* sp	1179.9 b	1120.4 a	464.7 d
b_4 = 2 kg ha^{-1} *Pseudomonas* sp	1220.8 b	1175.8 a	435.8 d
b_5 = 1 kg ha^{-1} *Bacillus* sp + *Pseudomonas* sp	1330.8 b	1034.9 a	348.6 cd
b_6 = 2 kg ha^{-1} *Bacillus* sp + *Pseudomonas* sp	1390.8 b	1080.7 a	389.7 cd

Remark: the average value followed by the same letter in the same column, are not significantly different at 5 % level of testing according to Duncan's Multiple Range Test

An addition of P source caused the diversity of P sources in the soil. It means that the plants gained a P element not only from soil but also from others. This was proven on the treatment of additional P sources of natural phosphate (p_1 and

p_2), where the absorbance of P from soil decreased because the plant also absorbed P elements from rock phosphate. Similarly, the addition of P fertilizer SP-36 (p_3) also lowered the P uptake from the soil. The comparison of P uptake in the treatments p_1, p_2 and p_3 showed the high uptake of P from the soil (623.6 g plant P^{-1}) in the treatment p_3. This condition indicated a low uptake of P from SP-36 fertilizer. P ions of fertilizer SP-36 had undergone the adsorption reaction before it could be used by the plants.

Inoculation of PSB at different doses caused differences in P uptake response derived from the soil. Phosphorus uptake derived from the soil showed the highest uptake rate (1390.8 g plant P^{-1}) in the treatment of *Bacillus* sp and *Pseudomonas* sp consortium at a dose of 2 kg ha^{-1}. In this case, both inoculants worked synergistically to increase the P availability in the soil and also to improve plants ability to absorb P nutrients which was in the limited quantity (P_2O_5 content in soil is 24 mg kg^{-1}). Widawati and Suliasih [10,11] observed that the *Bacillus* sp and *Pseudomonas* sp had a good ability to dissolve phosphates. These bacteria played a role in the transfer of energy, protein preparation, coenzymes, nucleic acids and other metabolic compounds which can increase the activity of P uptake in plants that grow in an environment with low P. The PSB inoculant effectiveness in the process of organic P mineralization occurred through the production of phosphates enzyme, phytase and nuclease.

The PSB activity was more effective in releasing P adsorbed in the soil so that the concentration of available P and P uptake by plants increased [11]. Phosphorus uptake derived from phosphate sources showed the highest response (244.44 g $plant^{-1}$ P) of P uptake in the treatment p_3 (100 kg ha^{-1} fertilizer SP-36). This condition was caused by the form of P_2O_5 in fertilizer SP-36 that was easily absorbed and readily available to plants. However, this P uptake response was not significantly different from the response of P uptake derived from natural phosphate. This showed that the plant was responding to the P concentration changes in the soil solution due to the addition of P sources. The plant's response was in accordance with the limited quantities of P in the soil (P_2O_5 content in soil is 24 mg kg^{-1}, classified as moderate).

The addition of PSB in these experiments demonstrated its ability to improve the P dissolution from all sources of organic P. Inoculation of *Pseudomonas* sp at a dose of 2 kg ha^{-1} was able to cause highest P uptake response derived from P sources (1175.8 g plant P^{-1}) in comparison with other treatments. The result from Puspitawati et al showed that *Pseudomonas* sp had the ability to dissolve tricalcium phosphate by 14 % in media solution and 9 % in the soil [12]. In this experiment, the non-significant response between each treatment inoculation showed that plants absorbed P from any source of P in the soil because the plants have the ability to adapt with the environmental conditions [9]. The rice plant is able to modify root hair according to its growth environment, such as the availability of nutrient supply, especially N and P [13].

Plant P uptake response derived from P sources showed no significant differences in the treatment of PSB dose of inoculation. In this case plants had been able to increase the availability of P in the rhizosphere surrounding area, thus it provided a response that was not statistically different from the response of plants that received PSB inoculation. Phosphorus uptake by the root hairs may able to achieve 90 % of total P uptake that was carried out by plants in the soils with low P availability [13].

Phosphorus availability condition in the plants with no PSB inoculation was supported by lowland characteristic that has a high CEC value. In the soil with high CEC value, lots of base cation exchange occurred in the soil colloid surface resulting in the release of H^+ from plant roots [14]. This condition resulted in a decrease of rhizosphere pH value which then increased the release of P element retained by soil colloids.

The impact of P sources and PSB dose of inoculation caused significant differences in the P uptake response derived from PSB dose of inoculation independently. Data in Table 3 showed that the highest P response was derived from PSB dose of inoculation in p_3 treatment (100 kg ha^{-1} SP-36 fertilizer). The easily available and ready to use form of P_2O_5 in SP-36 fertilizer had brought the highest P uptake (523.9 µg P $plant^{-1}$) in p_3 treatment.

Phosphorus uptake response derived from PSB inoculation was also shown in the treatment with no P sources (p_0). This suggested that the cycle of nutrient availability remained ongoing although no P solubilizing microorganisms inoculated into the soil. Nutrient cycle can be done biologically through the existence of indigenous microorganisms in the soil and through the balance of chemicals in the soil. Every gram of soil consisted of 10^5-10^8 CFU bacteria, 10^6-10^7 CFU actinomycetes, 10^5-10^6 CFU fungi, and 10^4 CFU algae [4, 8]. Interaction between microorganisms with the rhizosphere was associated with the cycle of nutrient availability in the soil. In addition, in the soil with high clay content there was also the ability to hold organic material in the clay colloids thus providing better energy source for microorganisms.

The inoculation dose of *Pseudomonas* sp at 1 kg ha^{-1} had achieved the highest P uptake (464.7 µg P $plant^{-1}$) in comparison with other treatments. Better characteristics of one inoculum compared to the other had become a trigger for higher P uptake response derived from PSB inoculant. *Pseudomonas* sp, a species of aerobic bacteria that uses H_2 or carbon as a source of energy, acted as a phosphate solubilizer, fixed nitrogen and produced plant growth regulator for plants [15]. The highest response of P uptake was in line with response of straw dry weight which was also the highest response due to single inoculation of *Pseudomonas* sp. It explains the utilization of P in the plants tissue to form plants biomass.

Plant production

The interaction between P sources and PSB dose of inoculation treatment caused a significant difference in grain dry weight response.

Table 4. Single effect of P sources and PSB dose of inoculation against grain dry weight.

Treatments	Grain Dry Weight (t ha^{-1})	Δy (%)
P sources (p)		
p_0 = 0	6.20 a	
p_1 = 163 kg ha^{-1} NPB (22 % P_2O_5)	6.74 b	8.71
p_2 = 138 kg ha^{-1} NPM (26 % P_2O_5)	6.88 b	10.96
p_3 = 100 kg ha^{-1} SP-36 (36 % P_2O_5)	6.94 b	11.94
PSB dose of inoculation (b)		
b_0 = 0 inoculation	6.29 a	
b_1 = 1 kg ha^{-1} *Bacillus* sp	6.30 a	0.16
b_2 = 2 kg ha^{-1} *Bacillus* sp	6.55 ab	4.13
b_3 = 1 kg ha^{-1} *Pseudomonas* sp	6.70 ab	6.51
b_4 = 2 kg ha^{-1} *Pseudomonas* sp	6.62 b	5.24
b_5 = 1 kg ha^{-1} *Bacillus* sp + *Pseudomonas* sp	6.85 a	8.90
b_6 = 2 kg ha^{-1} *Bacillus* sp + *Pseudomonas* sp	7.08 b	12.56

Remark: the average value followed by the same letter in the same column, are not significantly different at 5 % level of testing according to Duncan's Multiple Range Test
Δy = percentage of yield increase against 0 treatment (p_0 and b_0)

Data in Table 4 showed that the highest dry weight grain (7.08 t ha^{-1}) was achieved as a response to b_6 treatment (2 kg ha^{-1} inoculation dose of *Bacillus* sp and *Pseudomonas* sp). This condition was in line with the level of P uptake of plant whether P uptake from the different sources of phosphate or P uptake from PSB inoculation. The fulfillment of P nutrient in early stage of plant growth was to stimulate root formation, tiller number development, flowering, plant yield increase and to accelerate ripening [16]. In the consortia of *Bacillus* sp and *Pseudomonas* sp treatment, it could be said that both bacteria synergistically increased P availability for the plants, then P element distributed evenly inside the plant tissue [17]. The percentage of yield increase due to this treatment was 12.56 % in comparison with the treatment with no inoculation (b_0).

The PSB inoculation treatment, whether single or in consortia, showed an increase in dry weight of grain. Statistically, there were no differences among grain dry weight data as a response to no PSB inoculation or with PSB inoculation. This was presumably due to the low availability of N, P and C-organic in the soil which was needed as the energy for microorganism's activities. It was unlike the case of grain dry weight response to different source of P which showed a significant difference among the data. Different sources of phosphate caused different response of grain dry weight due to the different content of P_2O_5 of each source. Natural phosphate used in this experiment had P_2O_5 content of 26 % which was lower than P_2O_5 content of SP-36.

Phosphorus uptake efficiency

The single effect of phosphate sources and PSB dose of inoculation showed a significant difference of P uptake efficiency.

Table 5. Single effect of P sources and PSB dose of inoculation against phosphorus uptake efficiency.

Treatment	P Uptake Efficiency (%)
P Sources (p)	
p_0 = 0	10.68 a
p_1 = 163 kg ha^{-1} NPB (22 % P_2O_5)	15.36 b
p_2 = 138 kg ha^{-1} NPM (26 % P_2O_5)	16.21 b
p_3 = 100 kg ha^{-1} SP-36 (36 % P_2O_5)	19,61 b
PSB dose of inoculation (b)	
b_0 = 0 inoculation	15,17 a
b_1 = 1 kg ha^{-1} *Bacillus* sp	20,93 ab
b_2 = 2 kg ha^{-1} *Bacillus* sp	23,33 ab
b_3 = 1 kg ha^{-1} *Pseudomonas* sp	26,73 b
b_4 = 2 kg ha^{-1} *Pseudomonas* sp	28,58 b
b_5 = 1 kg ha^{-1} *Bacillus* sp + *Pseudomonas* sp	25,06 ab
b_6 = 2 kg ha^{-1} *Bacillus* sp + *Pseudomonas* sp	28,72 b

Remark: the average value followed by the same letter in the same column, are not significantly different at 5 % level of testing according to Duncan's Multiple Range Test

The data in Table 5 showed that the application of fertilizer SP-36 achieved highest P uptake efficiency (19.61 %). According to Widawati and Suliasih [10], high P plant uptake could be due to the high content of P_2O_5 in P source thus resulting in high P uptake efficiency. The soil used in this experiment had lower P availability, causing a plant response to P added in the form of readily available. Phosphorus uptake efficiency had increased into 80 % in the plants fertilized with SP-36 in comparison with plant with no additional P fertilizer. However, statistically the P uptake efficiency of SP-36 fertilizer was not different in comparison with P uptake efficiency of natural rock phosphate.

The plant had become more responsive to the addition of P from outside of the soil due to the lower P_2O_5 content of the soil.

Consortium of *Bacillus* sp and *Pseudomonas* sp at the dose of 2 kg ha^{-1} had the highest P uptake efficiency (28.72 %) in comparison with other treatments. In this case, it can be said that the consortium resulted in as much as 28.72 % of P nutrient were absorbed by plant. As stated by Syers *et al* [13] that rice plant is more responsive to the addition of P nutrient. However the inoculation of PSB led to increase the P availability from various P sources in soil, so that it became easily absorbed by plants. High P uptake efficiency from the inoculation treatment was able to reduce the dosage of chemical fertilizer given, thus potentially can be developed to improve soil productivity [13,17].

CONCLUSION

The ^{32}P radioisotope technique showed that consortia of *Bacillus* sp + *Pseudomonas* sp at the dose of 2 kg ha^{-1} had the best capability in the dissolution of P whether it is from soil or from natural phosphate. This treatment also caused the highest grain dry weight (7.08 t ha^{-1}) and plant P uptake with the nutrient P efficiency of 28.72 %. The consortia of *Bacillus* sp + *Pseudomonas* sp carried in a carrier material consisting of 50 % peat + 28 % compost + 5 % humic acid + 2 % nutrition + 15 % rice husk were able to maintain the highest number of colonies (8.07 x 10^8 CFU g^{-1}) within the prescribed period. The ability of those two inoculants in solubilizing P was an evidence that the types of natural phosphates from both Blora and Morocco could be used as P source for Biofossi fertilizer.

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