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The Effect of Water and BiofertilizerPhosphate Solubilizing Bacteria (PSB) Toward the Availability of P and Absorptions of Phosphor in Soybean Crop (*Glycine max*)

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Abstract:*Phosphate Solubilizing Bacteria acts as biological fertilizer by way of the ability to dissolve P inorganic and mineralization of P organic to become P which is available for plants through 3 mechanism, those are: (1) produce organic acids (2) acidification of medium pH which is caused by excretion of* H^+ *by bacteria, (3) phosphate enzyme which is produce by bacteria. Greenhouses research has been done to find out the effect of dosage of water and consortium of biological fertilizer Phosphate solvents Bacteria in entisols toward the availability of soils phosphate, growth and absorptions of phosphor by crops. The experiment is arranged by using factorial completely randomized design which consists of 2 factors those are dosage of water and biological fertilizer PSB. The research finds that there are interactions between dosage of water and consortium of biological fertilizer PSB toward the availability of P and absorptions of P crops. Distribution of consortium of 4types of biological fertilizer PSB can increase P available in soils and dry weight roots' Stover consecutively 39,07%; 79,39% and 22,86%. Dosage of water 100% of field capacity contribute the highest value to the absorption of P crops that is 4,00mg/crop, dry weight of roots' stover is 2,47 gram and dry weight canopy stover that is 19,46gram.*
Keyword:*Water, Phosphate Solubilizing Bacteria, Consortium Bacteria, P available, P absorption.*

I. Background

Phosphorus is second essential nutrient for crops after nutrient nitrogen. The content of P in plant tissues is in the range of 0.2% to 0.8% of the dry weight of plants (Sharma et al., 2013). Plants acquire P from the soil solution in the form of anions phosphate which largely absorbed by plants in the form of primary orthophosphate (H2PO4-) and secondary ortho phosphate (HPO4-2) and only a fraction is absorbed in the form PO4-3. In soil phosphate ions are highly reactive, reacts with kationCa^{2+}, Mg $^{2+}$ (at a neutral ground and calcareous), and Fe^{3+}, Al^{3+} (on acid soils) to form mineral deposits cation-phosphate (ThanandEgashira, 2008), so that P is not available to crops. Formation of deposits cation-phosphate compound causes only a small part of P fertilizer can be absorbed by plants. Hilda and Fraga (2000) reported that more than 80% of P is added into the soil become the P-insoluble in acid soil and unavailable to plants due to strong fixed inside the compound of Al and Fe-phosphates. Sofyandkk (2003) reported that the efficiency of phosphate fertilizers in paddy soil is very low, only about 10-20%. Total P in ground quite a lot, but the form of P that may soon lower the amount absorbed by plants only from 0.01 to 0,2 mg/kg soil (Handayanto and Hairiyah, 2007) .Therefore, maintaining the level of concentration of P in soil solution through increased P-fixed dissolving is an effective way to increase the availability of P for plants.

Lack of water in the soil can also inhibit the leaching of fertilizers and also the release of nutrients in both the mass flow mechanism and diffusion of nutrient solution to the root surface. Drought can concentrate the soil solution that can damage plant tissues due to plasmolysis. Rapid percolation will leach many soluble fertilizer materials. The leaching of fertilizer nutrients increased in coarse-textured soil as the ability to hold water and small nutrients. On the other hand water availability will affect the activity of soil microorganisms, including the process of humification and mineralization of organic matter. Therefore, the management of soil moisture becomes an important aspect in the management of soil fertility. Fertilization is one effort nutrient management, but it would not give the expected results, if it is not accompanied by soil moisture management (Notohadiprawiro, 2006). Less efficient use of P fertilizer can be overcome in various ways, including the use of Microorganisms Solvents Phosphate (MPF) one of which is a Phosphate Solubilizing Bacteria(PSB), which is capable of dissolving P inorganic and mineralization P organically into P available to plants (Rao, 1982; Kpomblekou and Tabatabai, 1994). The ability of the dissolution of phosphate by the PSB is closely linked to its ability to secrete organic acids (Banik and Dey, 1982; Rao 1994; Goenadi, 1996; Zhang et al, 1997). Phosphate Solubilizing Bacteriaplay a role in the transformation of P by: (1) the production of organic acids, (2) acidification pH of the medium caused by the excretion of H + by bacteria, (3) enzyme phosphate produced by bacteria (SubbaRao, 1982b; Illmer and Schinner, 1992; Illmer et al., 1995; De Freites et al., 1997).

Transformation of P by bacterial solvent P through three mechanisms those can increase the availability of phosphate in the soil. Dissolution generally caused by the production of phosphate enzymes and organic acids such as acetic acid, formic acid, lactic acid, oxalic acid, malic acid and citric acid produced by these microbes. besides the dissolution is strongly influenced by the availability of water in the soil. This study aims to determine the effect of the dosage of water and potential consortium PSB biological fertilizers affect the availability of phosphate in the soil, growth and absorption of phosphor by crops.

II. Materials and Methods

The research was conducted at Greenhouse Faculty of Agriculture, University of Mataram, the exploration of phase bacteria were performed in the Laboratory of Microbiology, Faculty of Agriculture, University of Mataram and soil and plant tissue analysis conducted at the Laboratory of Soil Science, Faculty of Agriculture, University of Mataram. This research was conducted in January 2016 - July 2016.

Experimental design

This study uses a completely randomized design (CRD) with factorial treatment consists of two factors. The first factor is the dosage of water that consists of 60% of field capacity (L1), 80% of field capacity (L2) and 100% (L3) at field capacity. The second factor is the distribution of a biofertilizer comprising PSB without giving PSB (P0), the distribution of two types of consortium PSB (P1), giving three types consortium PSB (P2) and the distribution of four types consortium PSB (P3). There are 12 combinations of treatments, each treatment was repeated 3 times so that there are 36 units of pot experiment.

Isolation of indigenous bacteria from soil samples

Isolation is done by dilution methods. Dilution of 10-1 obtained by ten grams of soil from the rhizosphere of plants *Tithoniadivesifolia* put into 90 ml of sterile distilled water, then shaken with shaker for 1 hour at 120 rpm. Furthermore taken 1 ml of the soil suspension then added to a test tube containing 9 mL of normal saline, and then shakes it until homogeneous. Further dilution by means 10-2 obtained by taken 1 ml from dilution tube 10-1 that transferred to the next tube, and so on until the last dilution series in this study were at 10-7 dilution series (Figure 3). A total of 0.1 of a serial dilution of 10-4, 10-5, 10-6, and 10-7 Pikovskaya is grown on solid media to see the population of bacteria growing phosphate solvent. The grown bacteria isPhosphate Solubilizing Bacteria (PSB) with an indication to form a clear zone (holozone) in the area around colony.

Purification Phosphate Solubilizing Bacteria

The results of the Phosphate Solubilizing Bacteriaisolation is purified by removing each isolated isolate on NA media using scratch method (Streak plate methods). Isolates those grown on a free basis (not forming colonies) were taken using a needle ent, ent needle then touched at one point area on the center of the solid pikovskaya media. Observations were made during 7x24 hours, if the isolate form a clear zone on the solid pikovskaya media isolates it was indicated as pure isolates of Phosphate Solubilizing Bacteria(PSB).

Selection of potential Phosphate solvents Bacteria of the purification results.

Bacteria from the isolationresult are obtained by 12 isolates to be selected. Selections are made against bacteria that could potentially high in dissolving phosphate characteristics: Colonies of bacteria thick, large diameter clear zone and has a phosphate Dilution Index> 1,5. From selection process is expected to obtain four potential isolates Phosphate Solubilizing Bacteria.

Test of Selected Antagonist Bacteria

Of the four selected bacteria is carried antagonist. Each test of the two different isolates Phosphate Solubilizing Bacteria were grown by side (within ± 1 cm) in a single petri pikovskaya solid media. If there are four isolates were elected, there are six tests of petri antagonist those repeated three times each. Each petri incubated for 3x24 hours at room temperature condition, if both bacteria in a petri form a clear zone then the bacteria are not antagonistic.

Bacteria proliferation for biological fertilizer consortium PSB

Breeding biofertilizer PSB.

1 ose of elected isolates PhosphateSolubilizing Bacteriadeclared as not antagonistic grown in 25 ml Erlenmeyer containing 10 ml of liquid pikovskaya media. Each erlenmeyer then incubated for 2x24 hours on shaker with a speed of 120 rpm.

Multiply of biological fertilizers PSB.

Suspension of Phosphate Solubilizing Bacteriain 25 ml Erlenmeyer taken 10 ml, and then inserted into a 200 ml Erlenmeyer containing 90 ml of liquid pikovskaya. Furthermore, the incubation conducted for 4x24 hours at shaker with speed of 120 rpm.

Calculation of the population of PSB biological fertilizer.
After an incubation period of Phosphate Solubilizing Bacteriaof each consortium calculated bacterial population by dilution methods to define the population that will be given into the soil (the bacterial population has reached ± 108-109cfu / ml).

Preparation of Plantation Media

Soil as a growing medium is Entisol from the village Nyerot, Jonggat sub-district, Central Lombok regency, West Nusa Tenggara, Indonesia, compositely taken at a depth of 0-20 cm, and then dried and filtered aired by 2 mm sieve eye strain. Ground weight on every plastic bag is 8 kg/polybag.

Preparation of seeds and Planting.

Organic fertilizer given into the soil at a dose equivalent to 5 tonnes/ha, equivalent to 20 g/polybag and inoculant PSB as a biological fertilizer is given at a dose of 20 ml/polybag with density 108-109cfu/ml, respectively provided at the time of planting. As an inorganic fertilizer in the form of 50 kg urea/ha, equivalent to 0.20 g of urea/polybag and 100 kgNPK Phonska/ha, equivalent to 0.40 grNPK/polybag. Urea and NPK fertilizers are given at the age of 14 HST. Watering is done in the morning/afternoon with daily watering with water supply volume based on treatment. Seeds of soybean seed varieties used are Anjasmoro. Seeds were planted in a way in drill as many as three seeds in each hole in polybag and thinning after 1 week old plants with two leaves of plants that grow health. After soybean plants reach the end of vegetating (6 weeks after planting), the plant is taken to determine the dry weight roots and crop top. The soil in the pot then wind dried, stirring evenly to analyze the availability of P (Olsen method).

Data analysis.
The observed data in this study were analyzed by analysis of variance *Unvariate* at 5% level ($P \leq 0.05$), in the event of a real interaction then continued with Duncan Multiple Range Test (DMRT) at 5% level.

III. Result

P Soils Availability Residue

Results of variance showed that there was a significant interaction between soil moisture and a consortium of bacteria to the availability residue of P in soil (P = 0,048) at 6 MST (Table 3). Effect of different doses of the availability of water does not significantly affect the availability residueof P in the soil. But the influence of the biological fertilizer consortium PSB showed a significant influence on the availability residueof P in the soil.

Table-1: Analysis of Variety dosage of water and a consortium PSB toward P availableresidue in the soil

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Dosage of water	,559	2	,279	,964	,396
Consortium PSB	6,936	3	2,312	7,979	,001*
Dosage of water *consortium PSB	4,415	6	,736	2,540	,048*

*Description: * = Significant*

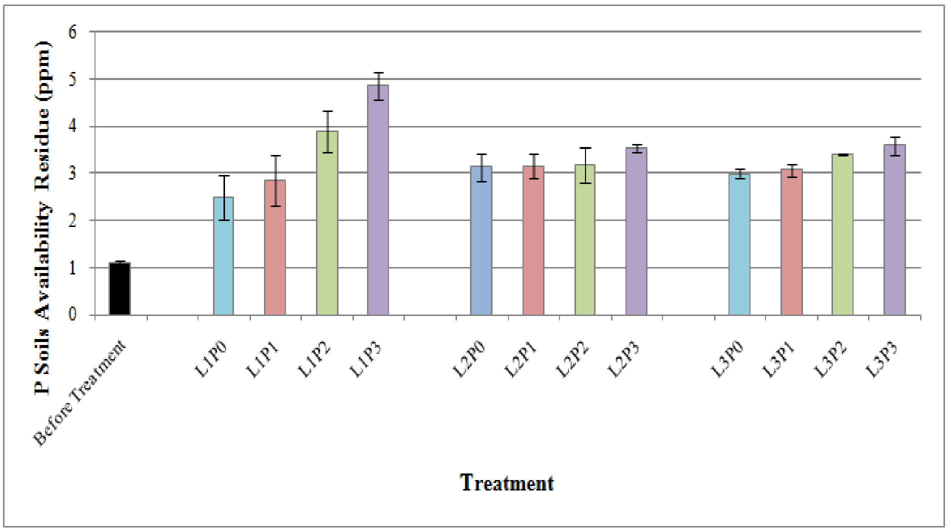
Distribution of consortium PSB biofertilizer with dose of consortium of 2 types of biofertilizers PSB (P1), consortium of dose of three types of biofertilizer PSB (P2), and consortium of dose of four types of biofertilizer PSB (P3) successively increasing availability P soil 5,15% ; 21,68% and 39,07% compared with no distribution of consortium PSB biofertilizer.

Table-2: Effect of the interaction between the dosage of water and a consortium PSB against P-available residue in soil.

P Soils Availability Residue (ppm)				
	Consortium of Phosphate Solvents Bacteria (PSB)			
Dosage of Water (ml)	Without PSB (P0)	Consortium 2 PSB (P1)	Consortium 3 PSB (P2)	Consortium 4 PSB (P3)
60% of field capacity (L1)	2,49 a	2,84 a	3,89 ab	4,85 b
	A	A	A	B
80% of field capacity (L2)	3,13 a	3,14 a	3,19 a	3,53 a
	A	A	A	A
100% of field capacity (L3)	2,99 a	3,06 a	3,40 ab	3,59 b
	A	A	A	A

Description: The numbers followed by the same letter are not significantly different according to Duncan's multiple range test further (DMRT) at the 5% significance level. Lowercase is read horizontally (row) and a capital letter is read vertically (column).

Figure-1: Effect of dosage of water and the amount Consortium Biofertilizer different PSB toward P-available residue in the soil.



Absorption of P

Significant interaction occurs (P = 0.015) between the dosage of water and the provision of a consortium of biofertilizers PSB against P absorption of soybean crop at age 6 WAP (Table 5). Distribution of consortium biofertilizer PSB with dose of 2 types of biofertilizers PSB (P1), dose 3 types of biofertilizers PSB (P2), and dose of 4 types of biofertilizers PSB (P3) consecutively increase P absorption of crops 35.16%, 58.01% and 79.39% compared with no distribution of consortium PSB biofertilizer. At the dosage of water 100% of field capacity value P absorption is high, in amount of 4,00mg / plant.

Table-3: Analysis of Variety dosage of water and consortium of PSB toward P absorption of crops.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Dosage of water	4,441	2	2,220	21,900	,000*
Consortium PSB	13,862	3	4,621	45,577	,000*
Dosage of water * consortium PSB	2,041	6	,340	3,355	,015*

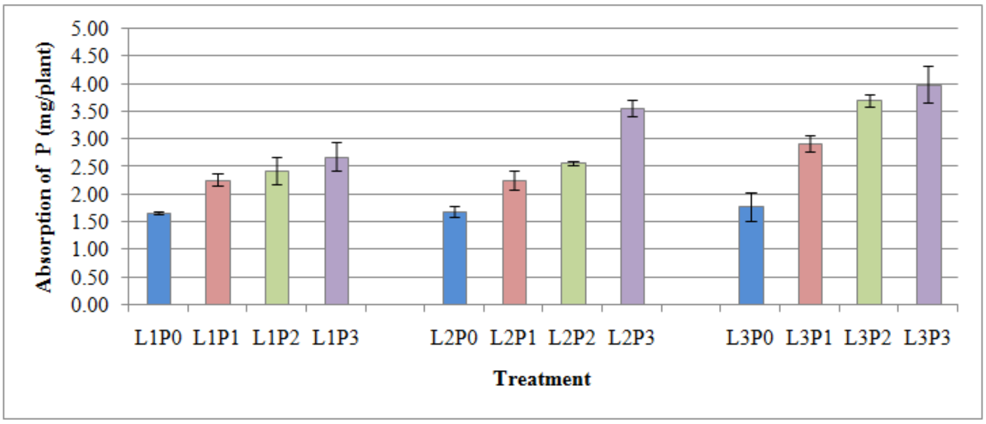
*Description: * = Significant*

Table-4: the effect of the interaction between the dosage of water and consortium of PSB toward P absorption of crops.

	Absorption of P (mg/plant)			
	Consortium of Phosphate Solvents Bacteria (PSB)			
Dosage of Water (ml)	Without PSB (P0)	Consortium 2 PSB (P1)	Consortium 3 PSB (P2)	Consortium 4 PSB (P3)
60% of field capacity (L1)	1,66 a	2,27 b	2,43 b	2,68 b
	A	A	A	A
80% of field capacity (L2)	1,70 a	2,26 b	2,57 b	3,56 c
	A	A	A	AB
100% of field capacity (L3)	1,79 a	2,92 b	3,70 c	4,00 c
	A	B	B	B

Description: The numbers followed by the same letter are not significantly different according to Duncan's multiple range test further (DMRT) at the 5% significance level. Lowercase is read horizontally (row) and a capital letter is read vertically (column).

Figure-2 : Effect of dosage of water and the number of different consortia PSB toward P absorption of crops.



Dry weight of Root stover

The dose of water with consortium of biological fertilizer PSB improve root stover dry weight at 6 MST, but the interaction was not significant (P = 0.807) (Table 7). Distribution of consortium biofertilizer PSB (P1-P3) were able to increase the weight of dry roots stover consecutively 9.09%, 16.54% and 22.86% compared to the roots of plants without biological fertilizers PSB (P0) (Table 8). At the dosage of water 100% of field capacity is highest value of dry weight root stover, in amount of 2.47 grams.

Table-5: Analysis of Variety dosage of water and consortium PSB toward dry weight of root stover.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Dosage of water	,907	2	,454	3,247	,056
Consortium PSB	2,771	3	,924	6,613	,002*
Dosage of water * consortium PSB	,414	6	,069	,493	,807

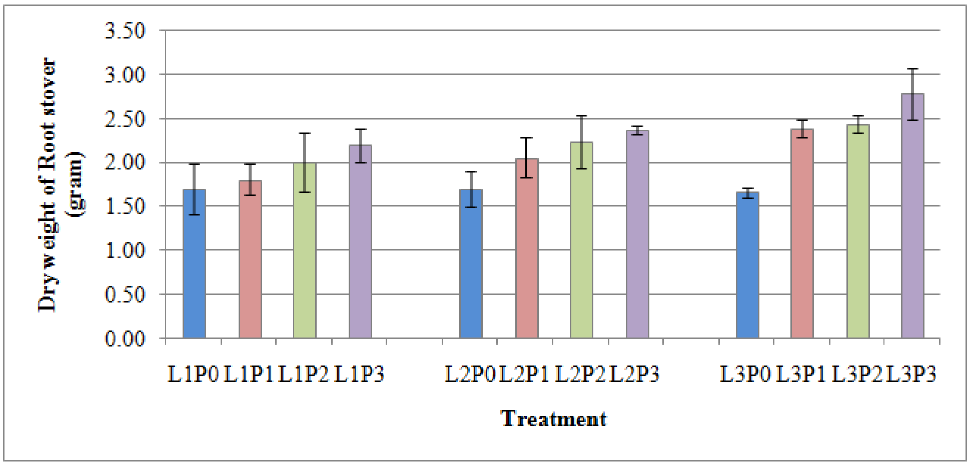
*Description: * = Significant*

Table 6: Effect of dosage of water and consortium of PSB toward dry weight of root stover.

Dosage of water (ml)	**Consortium bacteria**				**Average**
	Without PSB (P0)	**2 PSB (P1)**	**3 PSB (P2)**	**4 PSB (P3)**	
	--------------------------------- gram ---------------------------------				
60% of field capacity (L1)	1,70	1,81	2,00	2,19	1,93 a
80% of field capacity (L2)	1,70	2,06	2,24	2,37	2,09 ab
100% of field capacity (L3)	2,33	2,38	2,43	2,47	2,40 b
Average	1,91 a	2,08 b	2,22 b	2,34 b	

Description: The numbers followed by the same letter are not significantly different according to Duncan's multiple range test further (DMRT) at the 5% significance level.

Figure 3. Effect of dosage of water and distribution of the number of different consortia PSB toward dry weight of root Stover



Dry Weight of Stover Canopy

The interaction between the dosage of water and the amount of the distribution of consortium PSB biofertilizer does not have significant effect (P = 0.950) toward dry weight of stover canopy (Table 9). Whereas the influence of the dosage of water and consortium of biofertilizers PSB respectively very significant on the dry weight of stover canopy. Values of dry weight stover header at the dosage of water at 60% of field capacity (L1), 80% of field capacity (L2) and 100% of field capacity (L3) each shows highly significant difference (Table. 10) in a row is 13 , 04 grams; 16.07 grams and 19.46 grams. While the distribution four types of consortium of biofertilizer PSB (P3) were able to increase the weight of dry stover canopy of 24.47% compared to the crops canopy without being given a biofertilizer PSB (P0).

Table 7: Analysis of Variety dosage of water and consortium PSB to dry weight of stover canopy

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Dosage of water	247,861	2	123,930	85,637	,000*
Consortium bacteria	18,152	3	6,051	4,181	,016*
Dosage of water * consortium bacteria	2,253	6	,376	,259	,950

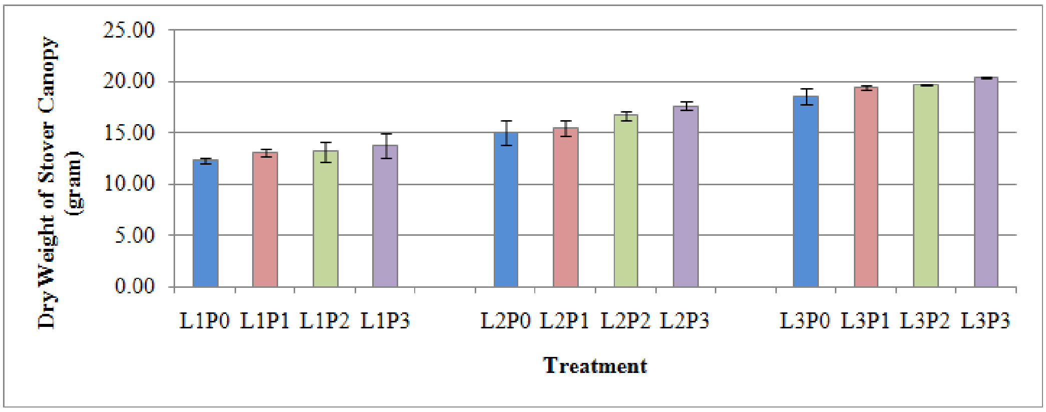
*Description: * = Significant*

Table 8: Effect of dosage of water and consortium of PSB toward dry weight of stover canopy

Dosage of water (ml)	**Konsorsium Bakteri**				**Average**
	Tanpa PSB (P0)	**2 PSB (P1)**	**3 PSB (P2)**	**4 PSB (P3)**	
	---------------------------------- gram ----------------------------------				
60% of field capacity (L1)	12,26	13,05	13,15	13,69	13,04 a
80% of field capacity (L2)	15,00	15,46	16,65	17,56	16,17 b
100% of field capacity (L3)	18,49	19,40	19,62	20,34	19,46 c
Averege	15,25 a	15,97 ab	16,47 ab	17,19 b	

Description: The numbers followed by the same letter are not significantly different according to Duncan's multiple range test further (DMRT) at the 5% significance level.

Figure 4. Effect of dosage of water and the number of distribution of different consortium PSB to dry weight of stover canopy



IV. Discussion

There an increase of the availability of P in the soil before and after the distribution of consortium PSB biological fertilizer. It shows that the distribution of consortium PSB able to increase the availability of P in the soil thus allowing the high absorption of P by crops. PSB able to secrete organic acids which can form complex compounds which poorly soluble. The formation of complex compounds will lead to the fixation of P decreased thereby increasing the available P (Whitelaw, 2000). It caused by organic acids especially humic acid and fulvic acid results of decomposition will form a complex compound (chelate) with Al, Fe and Ca it can help to release phosphate (P). In addition, according to Susilowati *et al*, 2015 to get biofertilizer effective in improving availability of P in the soil it needs to developed consortium Phosphate Solubilizing Bacteriathat consist of two or more groups species of PSB-indigenous selected. Consortium Phosphate Solubilizing Bacteriais a group of bacterial species that cooperate within a group to have a higher capability in dissolving phosphate compared to single bacteria. Results of previous studies proved that the mixture of cultures (*Bacillus*, *Streptomyces*, *Pseudomonas*.) Is more effective in mineralization of P-organic compared to each bacteria inoculation (Molla*et al*., 1984).

The distribution of amount of consortium biofertilizer PSB (P) at different dosage of water (L) is very significant on P absorption of crops. P absorption was lowest of the treatment without giving PSB and increase along with the the addition of consortium of biofertilizers on the third PSB different doses of water. Increased availability of P cause different concentrations of the soil increases so that the rate of diffusion to the root get higher (Indrayana, 1994). Nurhayati (2009) also states that the size of P absorption in soil depends on the availability of P in soil solution because many nutrients are absorbed through the roots.Increasing dry weight of stover of the crops' roots by 16.16% compared to the roots of plants without being given consortium of biofertilizers PSB. This is because the high availability P in the soil as a result of biofertilizer PSB distribution which stimulates growth of roots crops. Besides, distribution dose of water 100% of field capacity is the highest roots' dry weight. The highest percentage of groundwater levels provides the greatest response and decreases as the low percentage of soil water content, in accordance to the opinion of Gardner et al. (1985) that during vegetative development Lack of water can reduce the rate of widening leaves and stem extension. Declining groundwater levels, the declining growth of crops. Nurhayati stated (2009) soil water content desired for soybean growth is in the condition of field capacity (100% water is available).

Value of dry weight of stover canopy at the dosage of water at 60% of field capacity (L1), 80% of field capacity (L2) and 100% of field capacity (L3) each show high significant difference. It can be concluded that the dosage of water direct or indirect effect on crops. According Rosadi and Darmaputra (1998) states that the soybean crop is experiencing a shortage of water available up to (60-70%) in the vegetative phase can still be maintained if it is irrigated at flowering. It can directly lead to a decrease in plant turgor. Turgor pressure is crucial in determining the size of the plant, affect the enlargement and multiplication of plant cell, opening and closing of stomata, leaf development, the formation and the development of flowers (Islami and Utomo, 1995). It does not directly affect the physiological processes such as photosynthesis, nitrogen metabolism, nutrient absorption and translocation fotosintate (Salisbury and Ross, 1985). Water demand for soybeans is equivalent to the amount of water that evapotransiviration ranged between 300-350 mm during its growth (Kung, 1971; Doorenbos and Kassam, 1979). It can be seen in Entisols which is able to sustain plant growth, although in reeling condition.

V. Conclusion

1. There is a significant interaction between the dosage of water and distribution of consortium biofertilizers PSB toward the available P and absorption of P plants.
2. Distribution of consortium biological fertilizer can PSB increase the available P, P absorption, dry weight of stover root and dry weight of plant stover compared to absence of consortium biological fertilizers PSB.
3. At the dosage of water 100% field capacity provide value absorption of P plant, dry weight of stover root, dry weight of highest stover canopy.

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