The Effect of Inoculation Phosphate Solubilizing Bacteria and *Rhizobium sp.* on Plant Growth and Production of Cowpea (*Vigna unguiculata*)

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Abstract— *The constraints of Alfisol or Mediterranean soils pose unique management challenges for maintaining soil fertility and agricultural productivity. The aim of the research was to study the effect of couple inoculation Rhizobium and Phosphat Solubilizing Bacteria (PSB) on plant growth and production of Cowpea in Alfisol or Mediterranean soils. The experiment used a Randomized Block Design with treatment as combination concentration of PSB and Rhizobium sp. The treatment consist of : control; PSB of 10 ml/L and Rhizobium sp 10 ml/kg seed; PSB of 10 ml/L and Rhizobium sp 20 ml/kg seed; PSB of 10 ml/L and Rhizobium sp 30 ml/kg seed; PSB of 20 ml/L and Rhizobium sp 10 ml/kg seed; PSB of 20 ml/L and Rhizobium sp 20 ml/kg seed; PSB of 20 ml/L and Rhizobium sp 30 ml/kg seed; PSB of 30 ml/L and Rhizobium sp 10 ml/kg seed; PSB of 30 ml/L and Rhizobium sp 20 ml/kg seed; PSB of 30 ml/L and Rhizobium sp 30 ml/kg of seed. Each treatment was repeated with three repetitions. Data analysis used analysis of variance at the 5% level, followed by LSD test at the 5% level. The results showed that couple inoculation PSB and Rhizobium sp. increase significantly N and P soil, plant growth of Cowpea i.e the plant height, number of root nodules, fresh weight, shoot dry weight and total dry weight, the average 100 seed weight, population of PSB in soils and Rhizobium in root cowpea compared by control.*

Keywords— Alfisol, Mediterranean soil, Phosphat Solubilizing Bacteria, Rhizobium sp., Vigna unguiculata.

I. INTRODUCTION

In Malang Regency, Mediterranean soil develops from limestone, calcareous sandstone, marl and basalt andesite. The Mediterranean land is found in Karst plains, tectonic plains, tectonic hills, lower volcanic slopes, old volcanic plains and old volcanic hills. Soil cross section is medium to very deep, good drainage, slightly fine to fine texture, slightly acidic to alkaline pH, medium to very high CEC and high to very high base saturation (BS), low to medium in C-organic and total N content., P_2O_5 (medium to high) and total K_2O low to moderate (Yatno *et al.*, 2017). Mediterranean soil has problems with being too alkaline and low in organic matter, macro and micro nutrient levels as well as low water retention capacity and poor drainage (Tri Pamungkas, 2021). Red Mediterranean soil derived from limestone has a higher soil pH value than soil derived from sandstone. The main problem with Mediterranean soil is the high soil pH which will give rise to direct problems, namely suitability for plants and availability of nutrients, namely low availability of microelements and phosphate because they are bound by Ca^{2+} (Supriyadi, 2007). Soil quality and fertility in Mediterranean calcareous agricultural soils are very important to be managed so that soil productivity can be sustainable. The development of Mediterranean land requires pioneer plants that can increase soil fertility, one of which is legumes. Legume crops can be a practical solution for improving calcareous soils because they contribute to nitrogen fixation, organic matter accumulation, and improved soil health. The results of Tri Pamungkas' research showed that the addition of organic fertilizer significantly increased the growth of plant height, number of leaves, and stem diameter of sugar cane seedlings planted in Mediterranean soil. Legume crops are useful as human and animal feed, wood energy, and as soil-improving components of agricultural and agro forestry systems through its association with bio-fertilizers (Havugimana *et al.*, 2016).

Cowpea is included in the minor legume group which has prospects for future development as a source of food, animal feed, green manure, industry, and health. It belongs to the Leguminosae family and as important legume crop growing across the world mainly intropical and subtropical regions (Papa et al., 2020; Kebede and Bekeko, 2020). It is classified as food crops, feed and industrial raw materials. According to Safitri et al. (2016) stated that cowpea seeds contain a lot of nutrition, every 100 g contains 24.4 g protein, 56.6 g carbohydrates, 1.9 g fat, 481 mg calcium, 399 mg phosphorus, and 2.68 g phytic acid. Cowpea also has a higher vitamin B1 content than green beans. The potential for cowpea seeds is quite high, which can reach 1.5 - 2 tons/ha depending on the variety, location, growing season and cultivation applied. Cowpea has the advantage of having a low fat content so it can minimize the negative effects of fatty food products (Saputro et al., 2015).

Cowpea is a leguminous plant that has also the ability to bind N_2 from the atmosphere. The ability of cowpea to fix nitrogen through biological nitrogen fixation is a cheap and sustainable alternative to inorganic fertilizers. This is because the performance of cowpea depends on the rhizospheric characteristics; hence, it is able to form a beneficial association with microorganisms present in the rhizosphere (Abdel-Fattah *et al.*, 2016). The potency of N_2 fixation by cowpea can be optimized by inoculation of *Rhizobium sp.* Morphologically, cowpea roots spread to a depth of soil between 30-60 cm and can be in symbiosis with *Rhizobium sp*. to bind free Nitrogen (N_2) from the air which then forms root nodules. Inoculation of Rhizobia isolates significantly increased the number of root nodules, compared to plants that were not inoculated (Kebede et al., 2020). Symbiosis between cowpea and *Rhizobium sp.* can increase the availability of N to support plant growth and production. *Rhizobium sp* is one of the bacteria that is very useful for agriculture which acts as a nitrogen fixer from the atmosphere. Nitrogen is one of the main elements in the production of food crops, especially legumes. By fixing nitrogen symbiotically, a cheap source is obtained and can help reduce production costs, especially on infertile soils (Suryantini, 2015). The development of cowpea plants in calcareous areas has several obstacles related to the level of soil fertility. In the calcareous soil, high levels of calcium can also cause problems with the presence of soil potassium. Clay colloids can adsorb and exchange base cations depending on their valence. Cations with three and two valences are more strongly adsorbed by colloids compared to cations with one valence (Putra and Hanum, 2018). As a result, potassium which has a valence of one, will be suppressed by high Ca in calcareous soils which has a valence of two. In calcareous or normal soils, P gets immobilized by cations such as Ca^{2+} to form a complex calcium phosphate ($Ca_3(PO_4)_2$). Nearly, 70 to 90% of phosphorus fertilizers applied to soils is fixed by cations and converted inorganic P (Walpola and Yoon, 2012).

Optimizing the role of cowpeas as a pioneer crop planted in Mediterranean soil requires agronomic practise, one of which is the application of biological fertilizer to help increase the availability of nutrients in the soil, especially elements of N and P to support growth and production. However, the availability of P in the soil is lacking, even though the total amount of P in the soil is high through repeated fertilization applications. This is due to the nature of P which is easily fixed by metals, colloids and calcium which makes it unavailable for plants. Agricultural soils in general also face constraints on the availability of P elements for plants. Even though fertilization has been done many times for each planting, because P is tightly bound by soil colloids, the availability of P element in the soil is low. Likewise with soils that have high Al-dd content, generally also have constraints on the availability of P elements. Low availability of phosphorus (P) in calcareous soils can effect sustainability of improvement in cereals crops yield. A higher amount of calcium in calcareous soils precipitates the P, thus making it immobile in soil. Inoculation of phosphate solubilizing bacteria (PSB) could be helpful in the sustainable management of immobile P in soil (Wahid *et al.*, 2020).

An alternative to increase the efficiency of fertilization in overcoming P fixation by Al, Fe and Ca is by utilizing phosphate solubilizing microorganisms. Phosphate solubilizing bacterial inoculants are microbes given to plants for the process of dissolving P which is bound to become a form available to plants. It play an important role in the biogeochemical cycle of soil P, including dissolution, mineralization, and immobilization of P, thereby increasing P bioavailability and plant nutrition. Root-associated PSB, can modulate plant root systems and stimulate root absorptive capacity and nutrient uptake, including P acquisition (Bargaz *et al.*, 2021). PSB facilitates the conversion of stable phosphorus to active phosphorus and increases the residual phosphorus fraction in compost (Sun *et al.*, 2023). *Bacillus subtilis* is one of PSB which is able to decompose P bonds in the soil and can enhance plant P uptake through remobilization of recalcitrant forms of P in soil. The results of research conducted by Batool and Iqbal (2019) on *Triticum aestivum* L., recommend the use of PSB as biofertilizer, as an alternative to chemical fertilizer.

Providing N and P nutrients in calcareous soil requires technology in the form of the use of biofertilizers which can support the availability of nutrients needed by Cowpea plants to support their growth and production. This study aims to determine the optimal concentration of application of Phosphate solubilizing bacteria and *Rhizobium sp.* to increase biology soil fertility and the growth of cowpea cultivated in Alfisol or Mediterranean soils.

II. MATERIALS AND METHODS

2.1 Study area and soil characteristic

The research was conducted at the Screenhouse of the Faculty of Agriculture, Wisnuwardhana University, Madyopuro Village, Kedungkandang District, Malang City, which is located at an altitude about 500 m above sea level. The type of soil used is the Alfisol or Mediterranean taken from the Bantur area of Malang Regency. The soil used has the following characteristics (primary data from laboratory analysis): pH (H_2O)=7.6 (slightly alkaline), C-Org= 0.73% (very low), N-tot= 0.08% (very low); P-Olsen= 34 ppm (medium); K-dd= 0.24 cmol(+)/kg (low); Ca-dd= 10.20 cmol(+)/kg (high).

2.2 Data collection

Growth parameters observed included plant height, number of root nodules, plant fresh weight, shoot and root dry weight, total dry weight, and weight of 100 seeds, soil PSB population and *Rhizobium sp.* population. Plant height was measured from the base of stem to the top of plant shoot using ruler. The number of root nodules was measured by removing roots at the end of the vegetative phase. The number of effective root nodules is calculated from the pink nodules in color. At the age of 70 days after planting, three samples cowpea plants were taken in each treatment in each replication, then were measured the total fresh weight, dry weight of roots and shoots, and total dry weight of plants. The plant samples were washed then rinsed with distilled water to remove dust and soil on the surface. After the total fresh weight was calculated, then the roots and shoots plant were dried in the oven at 70 ºC for 48 hours and weighed to measure the root dry weight and shoot dry weight (Rahayu et al., 2022). The weight of 100 seeds was measured by weighing 100 cowpea seeds taken from each sample plant on each experimental unit after harvest. Calculation of microbial populations of Rhizobium used the Selective Medium Total Plate Count (TPC) method. A total of 10 grams of sample was suspended in 90 ml of solution sterile physiological saline, followed by serial dilution using 9 ml of sterile physiological saline to level dilution 10-5. A total of 100 l of the suspension then spread into Yeast Mannitol (YM) Agar selection medium added with Congo Red, then incubated 3-10 days at room temperature room (28-30 ºC). Its growth is observed and its population calculated based on the Most Probable method Number (MPN). Calculation of the population of Phosphate Solubilizing Bacteria using the Selective Medium Total Plate Count (TPC) method. A total of ten grams of sample was suspended in 90 ml of solution sterile physiological saline, then serial dilutions were carried out using 9 ml of sterile physiological saline to a 10-5 dilution. A total of 100 l of the suspension was then spread over the selection medium Pikovskaya Agar, and incubated for 7 days at room temperature (28 + 2 ºC). Growth was observed and the number of colonies counted has a clear zone around the colony based on the MPN method (Peraturan Menteri Pertanian, 2011). Soil chemical properties were analyzed consist of pH (H2O 1:1), C organic (Walkley and Balck), Nitrogen (Kjeldahl), P (Olsen), K dan Ca (NH4OAC pH 7.0) (Agricultural Research ad Development Agency, 2012).

2.3 Data analysis

This experiment used a randomized block design, consisting of 9 treatments, namely: control (without PSB and *Rhizobium sp.* innoculants); PSB inoculant concentration of 10 ml/L and *Rhizobium sp.* 10 ml/kg seed; PSB innoculant concentration of 10 ml/L and *Rhizobium sp.* 20 ml/kg seed; PSB innoculant concentration of 10 ml/L and *Rhizobium sp.* 30 ml/kg seed; PSB inoculant concentration of 20 ml/L and *Rhizobium sp.* 10 ml/kg seed; PSB innoculant concentration of 20 ml/L and *Rhizobium sp.* 20 ml/kg seed; PSB inoculant concentration of 20 ml/L and *Rhizobium sp.* 30 ml/kg seed; PSB inoculant concentration of 30 ml/L and *Rhizobium sp.* 10 ml/kg seed; PSB inoculant concentration of 30 ml/L and *Rhizobium sp.* 20 ml/kg seed; PSB inoculant concentration of 30 ml/L and *Rhizobium sp.* 30 ml/kg seed. Each treatment combination was repeated three times. Observational data were analyzed at the 5% level of variance, followed by the 5% BNT test if there was a significant effect of the treatment.

III. RESULTS AND DISCUSSION

3.1 Plant Growth and Production of Cowpea

The results of the varians analysis showed that there was a significant effect on the concentration of Phosphate Solubilizing Bacteria (PSB) and *Rhizobium sp.* on cowpea plant height. Table 1. shows that the height of cowpea plants was significantly different between those treated with phosphate solubilizing bacteria (PSB) and Rhizobium bacteria and those without treatment (control). From the first to the ninth week of observation, the average height of cowpea plants in the control showed significantly different and lower than the cowpea applied with PSB and Rhizobium. Until the end of the observation, which was 63 days after planting (DAP), the cowpea plants grown on media with the inoculation of PSB 20 ml/l and Rhizobium 30 ml/kg, produced the highest growth in plant height (155.33 cm) compared to the other treatments. In fast growth, especially in the vegetative phase, plants need N elements which play an important role in cell division, the formation of amino acids and proteins, chlorophyll and many potential enzymes that are important for photosynthesis and plant growth (Fitriatin et al., 2017), as well as important for stimulating root growth and increasing the uptake of other nutrients for plants (Nget *et al.*, 2022). So that with higher Rhizobium inoculation it helps the availability of N in the soil.

TABLE 1
PLANT HEIGHT (CM) OF COWPEA (*VIGNA UNGUICULATA*) AS INOCULATION OF PHOSPHATE SOLUBILIZING BACTERIA AND *RHIZOBIUM SP.*

<table>
<tr><th rowspan="2">PSB and Rhizobium Concentration</th><th colspan="18">Plant height (cm)</th></tr>
<tr><th colspan="2">7 dap</th><th colspan="2">14 dap</th><th colspan="2">21 dap</th><th colspan="2">28 dap</th><th colspan="2">35 dap</th><th colspan="2">42 dap</th><th colspan="2">49 dap</th><th colspan="2">56 dap</th><th colspan="2">63 dap</th></tr>
<tr><td>0 ml/L and 0 ml/kg</td><td>10,17</td><td>a</td><td>13,17</td><td>a</td><td>19,17</td><td>a</td><td>26,50</td><td>a</td><td>30,83</td><td>a</td><td>40,83</td><td>a</td><td>56,67</td><td>a</td><td>62,67</td><td>a</td><td>69,33</td><td>a</td></tr>
<tr><td>10 ml/L and 10 ml/kg</td><td>17,67</td><td>b</td><td>26,17</td><td>b</td><td>59,50</td><td>c</td><td>74,50</td><td>b</td><td>91,00</td><td>b</td><td>112,33</td><td>bc</td><td>106,17</td><td>b</td><td>110,50</td><td>b</td><td>116,00</td><td>b</td></tr>
<tr><td>10 ml/L and 20 ml/kg</td><td>18,17</td><td>b</td><td>25,33</td><td>b</td><td>61,50</td><td>c</td><td>68,17</td><td>b</td><td>94,50</td><td>b</td><td>111,83</td><td>bc</td><td>127,17</td><td>c</td><td>132,83</td><td>bc</td><td>136,83</td><td>bc</td></tr>
<tr><td>10 ml/L and 30 ml/kg</td><td>18,83</td><td>b</td><td>28,33</td><td>b</td><td>63,33</td><td>c</td><td>75,83</td><td>b</td><td>96,00</td><td>b</td><td>113,17</td><td>bc</td><td>120,67</td><td>bc</td><td>126,83</td><td>bc</td><td>134,67</td><td>bc</td></tr>
<tr><td>20 ml/L and10 ml/kg</td><td>16,67</td><td>b</td><td>25,33</td><td>b</td><td>49,83</td><td>bc</td><td>76,83</td><td>b</td><td>91,33</td><td>b</td><td>111,83</td><td>bc</td><td>133,50</td><td>c</td><td>136,83</td><td>bc</td><td>146,17</td><td>c</td></tr>
<tr><td>20 ml/L and 20 ml/kg</td><td>17,50</td><td>b</td><td>27,33</td><td>b</td><td>57,00</td><td>bc</td><td>79,33</td><td>b</td><td>96,17</td><td>b</td><td>92,17</td><td>b</td><td>131,17</td><td>c</td><td>136,17</td><td>bc</td><td>149,50</td><td>c</td></tr>
<tr><td>20 ml/L and 30 ml/kg</td><td>18,00</td><td>b</td><td>27,67</td><td>b</td><td>61,50</td><td>c</td><td>81,17</td><td>b</td><td>101,17</td><td>b</td><td>117,83</td><td>c</td><td>136,17</td><td>c</td><td>141,17</td><td>c</td><td>155,33</td><td>c</td></tr>
<tr><td>30 ml/L and 10 ml/kg</td><td>18,00</td><td>b</td><td>24,67</td><td>b</td><td>39,00</td><td>b</td><td>75,50</td><td>b</td><td>101,83</td><td>b</td><td>126,33</td><td>c</td><td>134,67</td><td>c</td><td>138,33</td><td>c</td><td>144,67</td><td>c</td></tr>
<tr><td>30 ml/L and 20 ml/kg</td><td>18,50</td><td>b</td><td>24,83</td><td>b</td><td>52,67</td><td>bc</td><td>75,33</td><td>b</td><td>94,17</td><td>b</td><td>117,67</td><td>c</td><td>125,50</td><td>c</td><td>128,67</td><td>bc</td><td>142,33</td><td>c</td></tr>
<tr><td>30 ml/L and 30 ml/kg</td><td>16,50</td><td>b</td><td>29,00</td><td>b</td><td>56,83</td><td>bc</td><td>77,67</td><td>b</td><td>98,33</td><td>b</td><td>119,50</td><td>c</td><td>127,83</td><td>c</td><td>133,17</td><td>bc</td><td>143,83</td><td>c</td></tr>
<tr><td>BNT 5%</td><td colspan="2">3,005</td><td colspan="2">6,68</td><td colspan="2">18,41</td><td colspan="2">19,13</td><td colspan="2">20,44</td><td colspan="2">22,52</td><td colspan="2">16,45</td><td colspan="2">27,24</td><td colspan="2">25,28</td></tr>
</table>

Note: the numbers followed by the same letter in the same column are not significantly different in the Least Significant Difference Test (LSD) at the 5% level. PSB = Phosphate Solubilizing Bacteria; PSB = Phosphate Solubilizing Bacteria; hst = days after planting; dap = day after planting

Table 2 shows that the control treatment and lower concentration of PSB and Rhizobium, namely 10 ml/l and Rhizobium 10 ml/kg, tended to produce an average weight of 100 cowpea seeds lower than cowpea plants which were inoculated with the higher concentration of PSB (20 ml/l and 30 ml/l) and Rhizobium concentration (20 ml/kg and 30 ml/kg). The results of (Nyaga and Njeru, 2020) research showed that rhizobia inoculation significantly increased cowpea yields where inoculation with native isolates recorded 22.7% increase in yield when compared to uninoculated control in the first season and 28.6% increase in yield in the second season.

TABLE 2
WEIGHT OF 100 SEEDS, NUMBER OF ROOT NODULES, ROOT LENGTH OF COWPEA (*VIGNA UNGUICULATA*) AS INOCULATION OF PHOSPHATE SOLUBILIZING BACTERIA AND *RHIZOBIUM SP.*

PSB and Rhizobium Concentration	100 seed weight (g)		The Number of root nodule		Root length (cm)	
0 ml/L and 0 ml/kg	7,03	A	1,83	A	9,00	a
10 ml/L and 10 ml/kg	7,17	A	2,17	A	9,33	ab
10 ml/L and 20 ml/kg	7,75	abc	4,00	Ab	12,00	bcde
10 ml/L and 30 ml/kg	8,32	bc	5,00	Bc	12,83	cde
20 ml/L and 10 ml/kg	7,20	Ab	7,00	Cd	10,00	abc
20 ml/L and 20 ml/kg	10,45	D	10,00	E	11,00	abcd
20 ml/L and 30 ml/kg	12,14	E	11,00	Ef	11,00	abcd
30 ml/L and 10 ml/kg	8,65	C	9,00	De	10,83	abcd
30 ml/L and 20 ml/kg	11,77	E	10,00	E	13,00	de
30 ml/L and 30 ml/kg	12,27	E	13,33	F	14,00	e
BNT 5%	1,13		2,34		2,88	

Note: The numbers followed by the same letter in the same column are not significantly different in the Least Significant Difference Test (LSD) at the 5% level. PSB = Phosphate Solubilizing Bacteria.

Observation of the root nodules number showed that the control treatment and the low concentration of PSB and Rhizobium respectively 10 ml/l and 10 ml/kg, tended to produce number of cowpea nodules as 1-2 root nodules. This result showed lower than the higher concentration of PSB (20 ml/l and 30 ml/l) and Rhizobium (20 ml/kg and 30 ml/kg). The highest number of root nodules was produced in the inoculation of PSB 30 ml/l and Rhizobium 30 ml/kg and the application of PSB 20 ml/l by soaking the seeds in Rhizobium 30 ml/kg. The results of this study are in line with the results of Habete and Buraka (2016) research, that showed the presence of rhizobium bacteria inoculation increased the number of nodules per plant which was significantly different for the two varieties of beans. Furthermore, both N fertilizer application and rhizobium inoculation increased 100-seeds weight and seed yield per ha. For better soil health and reduced production costs, it is advisable to use bacteria treated seeds. The root length parameter showed that the cowpea plants that were inoculated with PSB 30 ml/l and Rhzobium sp. 30 ml/kg produced an average root length longer than the other treatments, while the control cowpea plants produced short root lengths. The result of (Chandra and Kumar, 2008) research showed that combined inoculation treatment of *Rhizobium sp.*+ Phosphate Solubilizing Bacteria, PSB (Bacillus megaterium) + PGPRs LK-786 (Kurthia sp.) produced the highest and significantly more number and dry weight of nodules and plant dry weight of Lentil (Lens culinaris L.) than *Rhizobium sp.* alone at different intervals. Combined inoculation of chickpea with Rhizobia and Phosphate Solubilizing Bacteria (PSB), Bacillus sp., and Enterobacter aerogenes showed a significant enhancement of chickpea nodulation, biomass production, yields and N, P, and protein content in grains as compared to single inoculation or single application of N or P. Formulation of biofertilizers based on tasted strains could be used for chickpea co-inoculation in P-deficient soils (Benjelloun *et al.*, 2021). Rhizobia in association with a legume host have the exceptional ability to form root nodules. PGPR with rhizobial inoculation enhanced nodulation, growth and yield of lentil (Lens culinaris) (19.8 nodules/plant, 70.6 mg nodule dry weight/plant, 1 605kg/ ha) as compared to Rhizobium alone (17.8 nodules/plant, 64.3mg/plant and 1 546kg/ha resp.) and non-inoculated control (13.9nodules/plant, 47.7mg/plant and 1 401kg/ha resp.) (Khanna and Sharma, 2011).

TABLE 3
PLANT FRESH WEIGHT, SHOOT DRY WEIGHT, ROOT DRY WEIGHT, AND TOTAL DRY WEIGHT OF COWPEA (*VIGNA UNGUICULATA*) AS INOCULATION OF PHOSPHATE SOLUBILIZING BACTERIA AND *RHIZOBIUM SP.*

PSB and Rhizobium Concentration	Fresh Weight (g)		Root Dry Weight (g)		Shoot Dry Weight (g)		Total Dry Weight (g)	
0 ml/L and 0 ml/kg	65,17	a	0,61	a	18,79	a	19,39	a
10 ml/L and 10 ml/kg	72,00	ab	0,65	a	24,56	b	25,22	b
10 ml/L and 20 ml/kg	73,67	ab	0,47	a	25,31	b	25,78	b
10 ml/L and 30 ml/kg	75,50	ab	0,60	a	25,84	b	26,43	b
20 ml/L and 10 ml/kg	78,67	bc	0,79	a	26,73	b	27,53	b
20 ml/L and 20 ml/kg	80,67	bc	0,53	a	26,78	b	27,30	b
20 ml/L and 30 ml/kg	91,33	c	0,63	a	31,34	c	31,97	c
30 ml/L and 10 ml/kg	71,33	ab	0,44	a	24,54	b	24,97	b
30 ml/L and 20 ml/kg	74,33	ab	0,58	a	25,34	b	26,02	b
30 ml/L and 30 ml/kg	79,83	bc	0,53	a	27,26	bc	27,94	bc
BNT 5%	12,84		0,35		4,37		4,11	

Note: the numbers followed by the same letter in the same column are not significantly different in the Least Significant Difference Test (LSD) at the 5% level. PSB = Phosphate Solubilizing Bacteria.

Table 3 shows that the fresh weight of cowpea was significantly different between those treated with phosphate solubilizing bacteria and Rhizobium bacteria and those without treatment (control). The control showed plant fresh weight (65 g) lower than cowpea inoculated with PSB and Rhizobium. In cowpea plants inoculated with 20 ml/l PSB and *Rhizobium sp* 10 ml/kg, 20 ml/l PSB and 20 ml/kg *Rhizobium sp.*, 20 ml/l PSB and 30 ml/kg Rhizobium resulted in a higher average fresh weight than those inoculated with 10 ml/l PSB at all of Rhizobium concentrations level.

The result showed that there were no significant difference on root dry weight between the plants inoculated with PSB and Rhizobium and the control. Meanwhile, there were significant differences on shoot dry weight and total plant dry weight between control and those applied with PSB and *Rhizobium sp*. The highest shoot dry weight and total dry weight were shown in plants that were inoculated with PSB 20 ml/l and Rhizobium 30 ml/kg. The plant growth needs Nitrogen and Phosphorus as a mineral nutrient. Although Phosporus is abundance in soil, both in organic and inorganic forms, its availability is limited because P mostly occurs in insoluble forms. The average soil P content is about 0.05% (w/w) but only 0.1% of the total P is available to plants due to its low solubility and soil fixation. An adequate supply of phosphorus during the early phases of plant development is important for supporting the development of the reproductive parts of the plant. This is because P plays an important role in increasing root branching and increasing sturdy plants and resistance to disease. Phosphorus accounts for about 0.2 - 0.8% of the plant's dry weight. With the application of phosphate solubilizing microorganisms (PSM) it is the best environmentally friendly means to help the availability of plant P nutrients (Sharma *et al.*, 2013). The combined inoculation of Arbuscular Mycorrhizal Fungi (AMF) and Phosphate Solubilizing Bacteria (PSB) with ground rock phosphate RP had more potential to improve maize-wheat yields and P uptake comparable to those obtained by using expensive phosphatic fertilizers in P deficient calcareous pH soils (Wahid *et al.*, 2020). The research results of Hashem et al. (2019) showed that inoculation of plant roots with rhizobia may result in a small number of nodules depending on the root nodulation process which is based on the exchange of signals between the host and bacteria leading to the formation of rhizobia in the host tissue, nodulation and plant growth through increased day element uptake from the soil.

Rhizobium and phosphate solubilizing bacteria are important for plant nutrition. These microbes also have an important role as plant growth-promoting rhizobacteria (PGPR) in plant biofertilizers. Inoculation of Rhizobium and BPF either singly or both with the use of phosphate fertilizer significantly increased root and shoot weight, plant height, seed yield, seed P content, leaf protein content and leaf sugar content. Inoculation of Rhizobium and BPF either singly or both with the addition of phosphate fertilizer improves seed production 30% - 40% better than using only phosphate fertilizer . The synergistic relationship between rhizobium inoculation and BPF which produces growth regulators such as IAA and GA can improve root growth both in length and root weight which in turn can increase water and nutrient uptake which helps improve plant canopy growth (Afzal and Bano, 2008). The role of Bacillus subtilis which plays a direct role in P solubilization, it can help the availability of P nutrients in the soil, and can increase plant growth. Among microbial inoculants, the Rhizobium + PSB was found most effective in terms of nodule number (27.66 nodules plant^{-1}), nodule fresh weight (144.90 mg plant^{-1}), nodule dry weight (74.30mg plant^{-1}), shoot dry weight (11.76 g plant^{-1}), and leghemoglobin content (2.29mg g^{-1} of fresh nodule) and also showed its positive effect in enhancing all the yield attributing parameters, grain and straw yields of Chickpea (Tagore *et al.*, 2013).

3.2 Population of Soils Phosphate Solubilizing Bacteria and Population of Cowpea Roots *Rhizobium sp.*

The results of laboratory analysis showed that inoculation of Phosphate Solubilizing Bacteria (PSB) *Bacillus subtilis* increases the soil PSB population (Table 4). The highest soil PSB population of 2.31 x 10^7 CFU/g was obtained from the PSB treatment at concentration of 20 ml/L compared to the control i.e population of 1.98 x 10^6 CFU/g (Table 4). This is in line with the results of Fitriatin et al. (2017) that the PSB population in the Rhizosphere of corn plants can be increased by application of PSM biofertilizer (Phosphate Solubilizing Microorganisms) to 2.0 x 10^{11} CFU/g compared to control (without PSM biofertilizer) as PSB population of 1 .2 x 10^{11} CFU/g. The use of PSB could significantly increase pH, available phosphorus and several kinds of trace elements both in the rhizosphere and non-rhizosphere soil. The PSB secreted small molecular organic acids to dissolve inorganic phosphorus and changed the soil properties, which changed the rhizosphere microbial community indirectly. PSB can produce various organic acids with low molecular weight, which alter the soil pH, solubilizing the phosphate from acid or alkaline soils (Liu *et al.*, 2020).

Alfisols or Mediterranean soils are a group of red soils caused by high iron content and low humus content (Wijanarko et al., 2007). Organic matter is a source of energy for soil macro and micro fauna. Soils with low organic matter content have an effect on low soil microbiological activity and population. Biological fertility (microbiological population) of soil can be increased by addition of organic matter as well as the addition of biological fertilizers. The results showed that increasing the concentration of Rhizobium to 30 ml/kg of seed could increase the soil Rhizobium population up to 1.29 x 10^7 CFU/g compared to no application of Rhizobium (control) as a population of 2.87 x 10^6 CFU/g. The increase in microbial population in the soil can be due to the application of bioinoculants which induce better root proliferation and are responsible for high soil enzyme activity resulting in higher biomass production and extended root exudates (Prathima *et al.*, 2022).

TABLE 4
POPULATION OF SOIL PSB AND POPULATION OF COWPEA ROOT *RHIZOBIUM SP.*

Treatment	Type of Bacteria	Population of Bacteria (CFU/g)
R0 (Control)	*Rhizobium sp.*	2,87 x 10^6
R1 (10 ml/kg seeds)	*Rhizobium sp.*	5,60 x 10^6
R2 (20 ml/kg seeds)	*Rhizobium sp.*	1,01 x 10^7
R3 (30 ml/kg seeds)	*Rhizobium sp.*	1,29x 10^7
B0 (Control)	PSB *Bacillus subtilis*	1,98 x 10^6
B1 (10 ml/L)	PSB *Bacillus subtilis*	3,14 x 10^6
B2 (20 ml/L)	PSB *Bacillus subtilis*	2,31 x 10^7
B3 (30 ml/L)	PSB *Bacillus subtilis*	9,61 x 10^6

3.3 Nitrogen and Phosporus of Soils

Table 5 shows that the Nitrogen total in soils was significantly different between those treated with phosphate solubilizing bacteria and Rhizobium bacteria and those without treatment (control). Planting media and plants inoculated with PSB 10 ml/L and 30 ml/kg *Rhizobium sp.*, PSB 20 ml/L and 30 ml/kg *Rhizobium sp.*, PSB 30 ml/L and 30 ml/kg *Rhizobium sp.*, resulted N total in the soils was higher compared to other treatments. The research result of Wei et al. (2023) showed that long-term co-application of rhizobium and PK (phosphorus and potassium fertilization) promoted soybean nodule dry weight by 33.94% compared with PK + N (nitrogen and PK fertilization), and increased soybean yield by average of 32.25%, 5.90%, and 5.00% compared with CK (non-fertilization control), PK, and PK + N, respectively. The main effect of two rhizobium strain (Faba bean, Fb17 and Fb18) positively improved soil porosity, but soil bulk density was negatively infuenced. Soil chemical parameters such as organic carbon, total N, available P, available sulfur, Ca, Mg, and K were positively influenced (Chimdi et al., 2022). Inoculation Rhizobium can increase the availability of N in the soils through nitrogen fixation capability. The research of Oktaviani et al. (2017) showed that combination of Rhizobium and compot 200 g resulted highest value on the total soil N. The highest N soil is produced from combination treatment of 0.245 g, 200 g and Rhziobium compost as much as 25g.

Table 5 also shows that application of 30 ml/L PSB resulted in significantly higher P-total compared to controls and lower PSB concentrations. Total soil P levels in the media and plants applied were 30 ml/L PSB and 20 ml/kg *Rhizobium sp.* showed higher results than the control (without PSB and Rhizobium application) and other treatments. This is very possible because in calcareous soil Ca fixation of soil P becomes a form that is insoluble in the soil solution, and as a consequence is not available to plants. It could be mobilized, converted into soluble P forms using of PSM (Kalayu, 2019). Soil phosphate solubilizing bacteria (PSB) inoculation with mineral P can improved postharvest soil fertility relative to pre-harvest by improving soil organic matter from 0.61% to 0.70%, lowering pH from 7.74 to 7.68, and improving soil total N from 0.04 to 0.09%, ABDTPA-extractable P from 2.07 to 3.44 $mg.kg^{-1}$, and potassium (K) concentrations from 100.27 to 129.45 $mg.kg^{-1}$ (Khan *et al.*, 2022).

TABLE 5
THE CONTENT OF NITROGEN AND PHOSPORUS SOILS AS INOCULATION OF PHOSPHATE SOLUBILIZING BACTERIA AND *RHIZOBIUM SP.*

PSB Bacillus subtilis* and *Rhizobium sp. concentration	**N-Tot (%)**		**P-Tot (mg.kg-1)**	
0 ml/L and 0 ml/kg	0,07	a	208	a
10 ml/L and 10 ml/kg	0,21	b	421	b
10 ml/L and 20 ml/kg	0,28	b	454,67	bc
10 ml/L and 30 ml/kg	0,42	c	611,67	bcd
20 ml/L and 10 ml/kg	0,21	b	470,33	bc
20 ml/L and 20 ml/kg	0,28	b	623,33	cd
20 ml/L and 30 ml/kg	0,42	c	616	cd
30 ml/L and 10 ml/kg	0,21	b	604	bcd
30 ml/L and 20 ml/kg	0,28	b	943	e
30 ml/L and 30 ml/kg	0,42	c	723	d
BNT 5%	0,07		192,85	

Note: the numbers followed by the same letter in the same column are not significantly different in the Least Significant Difference Test (LSD) at the 5% level. PSB = Phosphate Solubilizing Bacteria.

IV. CONCLUSION

Inoculation of Phosphate Solubilizing Bacteria (PSB) and *Rhizobium sp.* to cowpea (*Vigna unguiculata*) grown on Alfisol or Mediterranean soils, has an influence on the growth and yield of cowpea plants, namely cowpea plant height, number of root nodules, fresh weight per plant, shoot dry weight and total plant dry weight and average weight of 100 seeds. It's also influence on increasing of soils PSB population and cowpea roots Rhizoobium. Total of N and P soils increased by inoculation of PSB and Rhizobium.

The inoculation of PSB 20 ml/l and 30 ml/l and Rhizobium 10 ml/kg, 20 ml/kg and 30 ml/kg resulted in higher plant height, plant fresh weight, shoot dry weight, total dry weight, and weight of 100 seeds of cowpea higher than the other treatments. The highest number of root nodules was produced in the treatment of PSB 30 ml/l and Rhizobium 30 ml/kg. The highest soil PSB population was resulted in the treatment of 20 ml/L and the highest population of cowpea roots *Rhizobium sp.* were produced in the 30 ml/kg treatment. The inoculation of Rhizobium 30 ml/kg produced the highest N-total soil, meanwhile inoculation of PSB 30 ml/L produced the highest P-total soils.

REFERENCES

[1] Abdel-Fattah, G. M. *et al.* (2016) 'The Impact of the Arbuscular Mycorrhizal Fungi on Growth and Physiological Parameters of Cowpea Plants Grown under Salt Stress Conditions', *International Journal of Applied Sciences and Biotechnology*, 4(3), pp. 372–379. doi: 10.3126/ijasbt.v4i3.15775.

[2] Afzal, A. and Bano, A. (2008) 'Rhizobium and phosphate solubilizing bacteria improve the yield and phosphorus uptake in wheat (Triticum aestivum)', *International Journal of Agriculture and Biology*, 10(1), pp. 85–88.

[3] Agency, A. R. and D. (2012) *Technical Guidelines for Chemical Analysis of Soil, Plant, Water and Fertilizer. Ministry of Agriculture of Republic of Indonesia (in Indonesian).*

[4] Bargaz, A. *et al.* (2021) 'Benefits of phosphate solubilizing bacteria on belowground crop performance for improved crop acquisition of phosphorus', *Microbiological Research*. Elsevier GmbH, 252(May), pp. 1–21. doi: 10.1016/j.micres.2021.126842.

[5] Batool, S. and Iqbal, A. (2019) 'Phosphate solubilizing rhizobacteria as alternative of chemical fertilizer for growth and yield of Triticum aestivum (Var. Galaxy 2013)', *Saudi Journal of Biological Sciences*. King Saud University, 26(7), pp. 1400–1410. doi: 10.1016/j.sjbs.2018.05.024.

[6] Benjelloun, I. *et al.* (2021) 'Co-inoculation of mesorhizobium ciceri with either bacillus sp. Or enterobacter aerogenes on chickpea improves growth and productivity in phosphate-deficient soils in dry areas of a mediterranean region', *Plants*, 10(3), pp. 1–15. doi: 10.3390/plants10030571.

[7] Chandra, R. and Kumar, R. (2008) 'Influence of PGPR and PSB on Rhizobium leguminosarum Bv. viciae Strain Competition and Symbiotic Performance in Lentil', *World Journal of Agricultural Sciences*, 4(3), pp. 297–301.

[8] Chimdi, A., Negasa, D. and Chala, G. (2022) 'Effects of Rhizobium Inoculation and P Fertilizer Levels on Selected Soil Properties, Yield, and Yield Components of Faba Bean (Vicia faba L.): The Case of Abuna Gindeberat, West Shewa Zone, Ethiopia', *Applied and Environmental Soil Science*, 2022, pp. 1–10. doi: 10.1155/2022/3635989.

[9] Fitriatin, B. N., Agustina, M. and Hindersah, R. (2017) 'Populasi Bakteri Pelarut Fosfat , P-Potensial Dan Hasil Jagung Yang Total Phosphate Solubilizing Bacteria (Psb), Soil Potential P And Yield Of Maize (Zea mays . L) Affected By The MPF Application Grown On Jatinangor Ultisols', *Jurnal Agrologia*, 6(2), pp. 75–83.

[10] Habete, A. and Buraka, T. (2016) 'Effect of Rhizobium Inoculation and Nitrogen Fertilization on Nodulation and Yield Response of Common Bean (Phaseolus vulgaries L.) at Boloso Sore, Southern Ethiopia', *Journal of Biology, Agriculture and Healthcare*, 6(13), pp. 72–75. Available at: www.iiste.org.

[11] Hashem, A., Tabassum, B. and Fathi Abd_Allah, E. (2019) 'Bacillus subtilis: A plant-growth promoting rhizobacterium that also impacts biotic stress', *Saudi Journal of Biological Sciences*. King Saud University, 26(6), pp. 1291–1297. doi: 10.1016/j.sjbs.2019.05.004.

[12] Havugimana, E. *et al.* (2016) 'Role of dual inoculation of Rhizobium and arbuscular mycorrhizal (AM) fungi on pulse crops production', *Walailak Journal of Science and Technology*, 13(1), pp. 1–7.

[13] Kalayu, G. (2019) 'Phosphate Solubilizing Microorganisms : Promising Approach as Biofertilizers', *International Journal of Agronomy*, 2019, pp. 1–7.

[14] Kebede, E. *et al.* (2020) 'Symbiotic effectiveness of cowpea (*Vigna unguiculata* (L.) Walp.) nodulating rhizobia isolated from soils of major cowpea producing areas in Ethiopia', *Cogent Food and Agriculture*. Cogent, 6(1), pp. 1–16. doi: 10.1080/23311932.2020.1763648.

[15] Kebede, E. and Bekeko, Z. (2020) 'Expounding the production and importance of cowpea (*Vigna unguiculata* (L.) Walp.) in Ethiopia', *Cogent Food and Agriculture*. Cogent, 6(1). doi: 10.1080/23311932.2020.1769805.

[16] Khan, H. *et al.* (2022) 'Coupling phosphate-solubilizing bacteria (PSB) with inorganic phosphorus fertilizer improves mungbean (Vigna radiata) phosphorus acquisition, nitrogen fixation, and yield in alkaline-calcareous soil', *Heliyon*. The Author(s), 8(3), pp. 1–10. doi: 10.1016/j.heliyon.2022.e09081.

[17] Khanna, V. and Sharma, P. (2011) 'Potential for enhancing lentil (Lens culinaris) productivity by co-inoculation with PSB, plant growth-promoting rhizobacteria and Rhizobium', *Indian Journal of Agricultural Sciences*, 81(10), pp. 932–934.

[18] Liu, J. *et al.* (2020) 'Exogenous phosphorus-solubilizing bacteria changed the rhizosphere microbial community indirectly', *3 Biotech*. Springer International Publishing, 10(4), pp. 1–11. doi: 10.1007/s13205-020-2099-4.

[19] Nget, R. *et al.* (2022) 'Responses of Soybean Genotypes to Different Nitrogen and Phosphorus Sources: Impacts on Yield Components, Seed Yield, and Seed Protein', *Plants*, 11(3). doi: 10.3390/plants11030298.

[20] Nyaga, J. W. and Njeru, E. M. (2020) 'Potential of Native Rhizobia to Improve Cowpea Growth and Production in Semiarid Regions of Kenya', *Frontiers in Agronomy*, 2(December), pp. 1–13. doi: 10.3389/fagro.2020.606293.

[21] Oktaviani, E. and Susanto, D. (2017) 'Effect Of Inoculant Rhizobium And Compost As Amelioration On Growth Of Peanut (Arachis Hypogaea) In Soil Post-Coal Mining', *International Journal of Scientific & Technology Research*, 6(02), pp. 4–8.

[22] Papa, M., Hetharie, H. and Polnaya, F. (2020) 'Keragaan Pertumbuhan dan Produksi Aksesi-Aksesi Kacang Tunggak Lokal Asal Maluku', *Jurnal Budidaya Pertanian*, 16(1), pp. 88–94. doi: 10.30598/jbdp.2020.16.1.88.

[23] Peraturan Menteri Pertanian (2011) 'Pupuk Organik, Pupuk Hayati Dan Pembenah Tanah', *Peraturan Menteri Pertanian Nomor 70/Permentan/SR.140/10/2011*, pp. 1–109.

[24] Prathima, M. N. *et al.* (2022) 'Influence of Bioinoculants on Growth and Yield of Cowpea (*Vigna unguiculata* L.) under Field Condition', *Agricultural Research Communication Centre (ARCC) Journals: Legume Research: An International Journal*, I, pp. 1–6. doi: 10.9734/ijecc/2022/v12i1030773.

[25] Putra, I. A. and Hanum, H. (2018) 'Kajian Antagonisme Hara K , Ca Dan Mg pada Tanah Inceptisol yang Diaplikasi Pupuk Kandang , Dolomit dan Pupuk KCl terhadap Pertumbuhan Jagung Manis (Z ea mays saccharata L .) Antagonism Study K , Ca And Mg Nutriens On The Inceptisol Soil that Applied Man', *Elkawnie: Journal of Islamic Science and Technology*, 4(1), pp. 23–44.

[26] Rahayu, Y. S., Maghfoer, M. D. and Wardiyati, T. (2022) 'The potential of intercropping of Crotalaria juncea on the reduction of Pb accumulation in Brassica rapa and Phaseolus vulgaris grown on Pb-contaminated soil', *Journal of Degraded and Mining Lands Management*, 9(4), pp. 3745–3752. doi: 10.15243/jdmlm.2022.094.3745.

[27] Safitri, F. M. *et al.* (2016) 'Pengembangan getuk kacang tolo sebagai makanan selingan alternatif kaya serat', *Jurnal Gizi dan Dietetik Indonesia (Indonesian Journal of Nutrition and Dietetics)*, 4(2), p. 71. doi: 10.21927/ijnd.2016.4(2).71-80.

[28] Saputro, D. H., Andriani, M. and Siswanti (2015) 'Karakteristik Sifat Fisik Dan Kimia Formulasi Tepung Kecambah Kacang-Kacangan Sebagai Bahan Minuman', *Jurnal Teknosains Pangan*, 4(1), p. 11. Available at: www.ilmupangan.fp.uns.ac.id.

[29] Sharma, S. B. *et al.* (2013) 'Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils', *Springer Plus*, 2(587), pp. 1–14. doi: 10.1128/jcm.35.12.3305-3307.1997.

[30] Sun, L. *et al.* (2023) 'Effects of phosphate-solubilizing bacteria on phosphorus components, humus and bacterial community metabolism during spent mushroom substrate composting', *Environmental Technology and Innovation*. Elsevier B.V., 32, pp. 1–13. doi: 10.1016/j.eti.2023.103341.

[31] Supriyadi, S. (2007) 'Kesuburan Tanah Di Lahan Kering Madura', *Embryo*, 4(2), pp. 124–131.

[32] Suryantini (2015) 'Pembintilan dan Penambatan Nitrogen', *Monograf Balitkabi*, pp. 234–250.

[33] Tagore, G. S. *et al.* (2013) ' Effect of Rhizobium and Phosphate Solubilizing Bacterial Inoculants on Symbiotic Traits, Nodule Leghemoglobin, and Yield of Chickpea Genotypes ', *International Journal of Agronomy*, 2013, pp. 1–8. doi: 10.1155/2013/581627.

[34] Tri Pamungkas, S. S. (2021) 'Pemanfaatan Tanah Mediteranian Sebagai Media Pembibitan Budset Tebu (Saccharum officinarum L.) Varietas Bululawang dengan Penambahan Pupuk Kandang pada Dosis yang Berbeda', *Mediagro*, 17(2), pp. 107–119. doi: 10.31942/md.v17i2.4918.

[35] Wahid, F. *et al.* (2020) 'Sustainable management with mycorrhizae and phosphate solubilizing bacteria for enhanced phosphorus uptake in calcareous soils', *Agriculture (Switzerland)*, 10(8), pp. 1–14. doi: 10.3390/agriculture10080334.

[36] Walpola, B. C. and Yoon, M. (2012) 'Prospectus of phosphate solubilizing microorganisms and phosphorus availability in agricultural soils : A review', *African Journal of Microbiology Research*, 6(37), pp. 6600–6605. doi: 10.5897/AJMR12.889.

[37] Wei, W. *et al.* (2023) 'Long-term fertilization coupled with rhizobium inoculation promotes soybean yield and alters soil bacterial community composition', *Frontiers in Microbiology*, 14(May), pp. 1–16. doi: 10.3389/fmicb.2023.1161983.

[38] Wijanarko, A., Sudaryono and Sutarno (2007) 'Characteristics of Chemical and Physical Soil Alfisols in East Java and Central Java', *Iptek Tanaman Pangan*, 2(2), pp. 214–226.

[39] Yatno, E. *et al.* (2017) *Atlas Peta Tanah Semi Detail Skala 1 : 50.000 Kabupaten Malang Provinsi Jawa Timur*. 2017th edn. Edited by Sukarman and M. Anda. Malang: Balai Besar Penelitian dan Pengembangan Sumberdaya Lahan Pertanian- Badan Penelitian dan Pengembangan Pertanian- Kementerian Pertanian.