

Effects of Bradyrhizobia and Phosphate-solubilizing bacteria on soybean (*Glycine max* L. Merrill) cultivated on Ferralsols of Cujut district, DakNong province, Vietnam

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Abstract— A field experiment was conducted in Summer-Spring cropping season 2016 at Cujut district, DakNong province, Vietnam to study the effects of rhizobia and phosphate-solubilizing bacteria (PSB) on soybean (cv. Cujut) cultivated on ferralsols. The experiment consisted of six treatments as follows: control (no fertilizer, no inoculant), 240 kg/ha NPK 15-15-15, rhizobial inoculant [with liquid cover seeds] + 20 kg N/ha applied at 10 days after sowing [DAS], PSB inoculant [with liquid cover seeds] + 20 kg N/ha at 10 DAS, rhizobial and PSB inoculant [with liquid cover seeds] + 400 kg fertilizer/ha + 20 kg N/ha at 10 DAS and endophytic bacteria inoculant [with liquid cover seeds] + 400 kg fertilizer/ha + 20 kg N/ha at 10 DAS from June to August, 2016. The results showed that application of rhizobial inoculant and/or PSB inoculant produced significantly higher yield component, grain yield than control and did not differ from 240 kg/ha NPK 15-15-15. Consequently, application of rhizobia and PSB improved soil fertility after harvesting however using mixture of rhizobia and PSB inoculation plus 400 kg biofertilizer/ha + 20 kg N/ha for soybean cultivation supported yield component, grain yield and oil, protein in seed than control and equivalent with treatment of chemical fertilizer (240 kg/ha NPK 15-15-15). This technique not only increased grain yield, incomes for farmers but also improved soil fertility.

Keywords - Endophytes, Ferralsols, Phosphate-solubilizing bacteria, Rhizobia, Soybean.

I. INTRODUCTION

Symbiotic nitrogen fixation, a key component in biological nitrogen fixation, has not been as successful in substituting for chemical fertilizer as initially expected. Rhizobial inoculants seem to be an attractive and cost effective source of N for soybean cultivation in the Mekong Delta, Vietnam [1]. Phosphorus plays an important role in the plant's energy transfer system since phosphorus deficiency retards growth and tillering [2]. In soil, phosphorus is quite abundant but it reacts readily with iron, aluminum and calcium to form insolubly compounds. These reactions results in very low phosphorus availability and low efficiency phosphorus fertilizer used by plants [3].

Soybean (*Glycine max*) is one of the most important oil seed crop in the world. It contains 18 to 22% oil, highly desirable in diet and have 40 to 42% of good quality protein [2]; Soybean protein is rich in valuable amino acid lysine (5%) in which most of the cereals are deficient [4]. Soybean, like other legumes, fixes atmospheric nitrogen in association with gram-negative soil bacteria of the genera *Bradyrhizobium* and *Sinorhizobium* [5][6]. Many rhizobial inoculant products have been applied for soybean cultivation for along time [7]. However there were many researches showed that many PGPR as PSB supported good nodulation and rhizobia-legume symbiosis [8][9], the results led to high grain yield and protein content in seeds [10]. The biofertilizer (consisted of rhizobia and PSB) was not only as well as soybean grain yield applying with 100 kg N and 60 kg P₂O₅/ha but also quality soybean seed [protein and lipid content in seed] was higher than soybean seed using of chemical fertilizers at Dong Thap province, Mekong Delta, Vietnam [11].

DakNong province is situated in the highland of Vietnam, it locates from 107°42'03" to 107°44'44" E and from 11°59'01" to 12°40'56" N and CuJut is a district of DakNong province, it locates the north of province (Figure 1) [DakNong province locates in Central High Land of Vietnam]. The soils are mainly red latosols (from origin of volcanic mountain) or ferralsols (FAO classification) with a pH range of 4.61 - 4.91. They are considered a good nutrient, with an average organic matter of 2.75 - 4.06%, a total nitrogen range of 0.11 - 0.13%, but it has concentrations of low available phosphorus, cation exchange



FIGURE 2. BIOFERTILIZER (GRANULE)

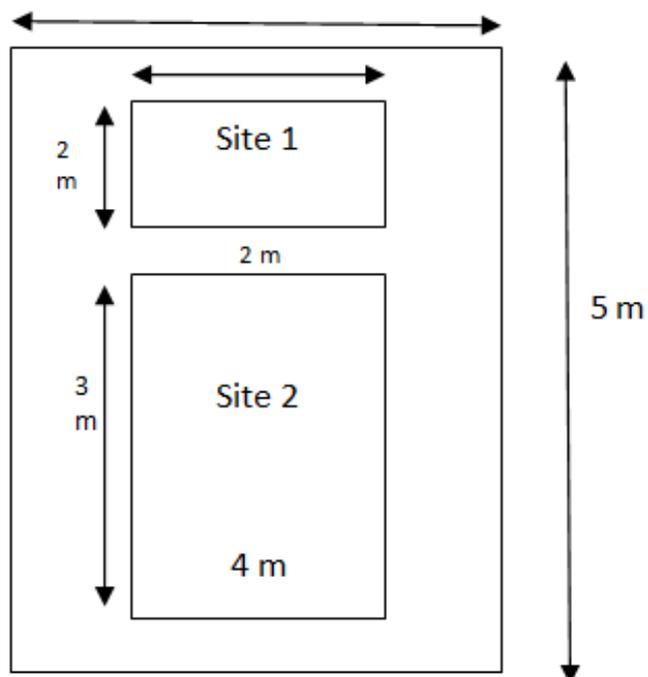


FIGURE 3. LAYOUT OF 1 PLOT WITH SITE 1 AND SITE 2

2.4 Yield component and Grain yield

Plant height and yield component were recorded at the time of maturity. Five randomly plants were taken at site 1 (Figure 3) to measure plant height, number of pods/plant, hundred seed weight. All plants in site 2 (Figure 3) were harvested to calculate grain yield after soybean seeds were oven dried at 70°C for constant weight. Soybean seed was also chosen to ground for determination of total nitrogen and oil contents.

2.5 Estimation of protein and oil

Total nitrogen content of soybean seed was determined by Micro-Kjeldahl method as recommended by AOAC, 1975 [16]. Nitrogen contents were multiplied by dry matter-based factor 5.71 to determined total protein content [17] and Oil content of soybean seeds was estimated by adopting Soxhlet Ether Extraction method [17].

2.6 Soil analysis

After harvesting, soil samples of each treatment were collected to analyse as soil pH with pH meter, N total by Micro-Kjeldahl method, Available P₂O₅ by Colorimetric method [18], Organic matter with Walkley-Black method.

2.7 Statistical Analysis

All the data pertaining to the present investigation were statistically analyzed as per the method described by Gomez and Gomez [19]. The statistically significance of various effects was tested at 5 per cent level of probability.

III. RESULTS AND DISCUSSION

3.1 Effect of Rhizobia and PSB on Nodule number and Shoot Dry Weight (DW)

Inoculated rhizobia on soybean seeds enhanced nodule number/plant however nodule number/plant of control and PSB together with endophytic bacteria treatments also appeared (Table 1), this showed that high native rhizobia population in soil and they infected into young soybean roots and formed the first nodules at main root in Treat 1 and Treat 2. (Figure 4).

TABLE 1

EFFECTS OF RHIZOBIA, PSB, ENDOPHYTIC BACTERIA AND MINERAL FERTILIZERS ON NODULATION AND DRW WEIGHT (DW) OF SHOOT OF SOYBEAN (CV. CUJUT) CULTIVATED ON FERRALSOLS OF CUJUT DISTRICT, DAKNONG PROVINCE IN SUMMER-SPRING 2016

Treatment	Nodule number / plant *	DW of nodule /plant (mg) *	DW of shoot/plant (gr) *
Treat. 1	11.35 d	44.35 d	3.429 b
Treat. 2	13.43 c	44.54 d	3.430 b
Treat. 3	15.30 b	52.35 c	4.240 a
Treat. 4	18.33 a	91.81 a	4.007 a
Treat. 5	18.25 a	64.42 b	4.230 a
Treat. 6	17.20 a	68.63 b	3.260 b
F calculated	**	**	**
C.V (%)	8.04	9.28	6.19

Treat.1: control (no fertilizer and without inoculation), **Treat. 2:** Application 240 kg NPK (15-15-15)/ha, **Treat 3:** Rhizobial Inoculation + 20 kg N/ha, **Treat. 4:** PSB Inoculation + 20 kg N/ha, **Treat. 5:** Rhizobial + PSB Inoculation + 400 kg biofertilizer (granule)/ha + 20 kg N/ha; **Treat. 6:** Endophytic bacteria inoculation + 400 kg biofertilizer (granule)/ha + 20 kg N/ha.

*The numbers followed by the same letter do not differ at 1% level significantly



FIGURE 4. EFFECTS OF RHIZOBIAL, PSB AND ENDOPHYTIC BACTERIA ON SOYBEAN NODULATION OF SOYBEAN CULTIVATED ON FERRALSOLS

PSB inoculated on soybean seeds before sowing had the highest DW of nodule/plant in comparison to rhizobial and mixture of rhizobia and PSB treatments, this result showed that phosphorus requirement in nodulation and development of nodule was important in this stage however no difference about the growth of soybean plant (DW of shoot) between these three treatments while endophytic bacteria did not affect on DW of shoot of soybean (Table 1)

Interestingly, DW of nodule root had correlation with DW of shoot (Figure 5) at 5% level, this showed that the development of nodule or the effectiveness of rhizobia affected to the growth of soybean plant.

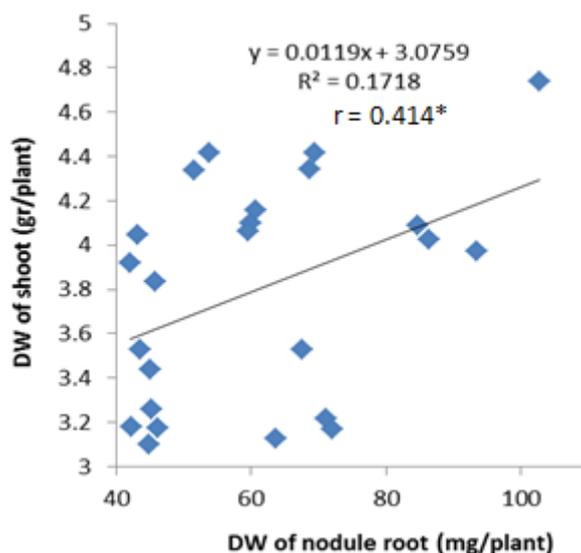


FIGURE 5. THE CORRELATION BETWEEN DW OF NODULE ROOT (mg/plant) AND DW OF SHOOT (gr/plant)

The result from Table 2 showed that inoculation of rhizobia, PSB, Endophytic bacteria and chemical fertilizers did not affect to plant height, effective branche number/plant and 100-seed weigh of soybean however these factors influenced to effective pod number/plant and total of pod/plant especially in the treatments of chemical fertilizers (Treat. 2) and rhizobial inoculant (Treat. 3).

TABLE 2

EFFECTS OF RHIZOBIA, PSB, ENDOPHYTIC BACTERIA AND MINERAL FERTILIZERS ON YIELD COMPONENT OF SOYBEAN (CV. CUJUT) CULTIVATED ON FERRALSOLS OF CUJUT DISTRICT, DAKNONG PROVINCE IN SUMMER-SPRING 2016.

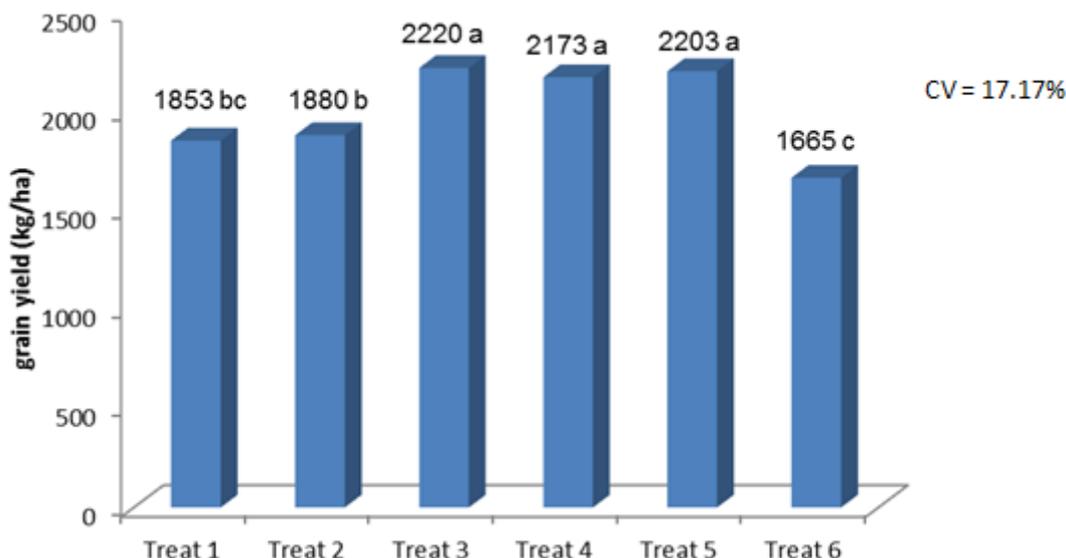
Treatment	Plant height (cm)	Effective branche number/plant	Effective Pod number/plant	Total of pod / plant	100-seed weigh (gr)
Treat. 1	75.75	1.60	8.10 a	20.35 b	16.58
Treat. 2	77.15	1.85	7.75 ab	22.68 ab	17.96
Treat. 3	77.75	2.10	7.95 a	24.55 a	17.02
Treat. 4	76.45	1.80	8.05 a	21.93 b	16.34
Treat. 5	74.70	1.50	7.45 b	21.95 b	16.86
Treat. 6	68.90	1.50	7.05 bc	17.87 c	15.89
F calculated	n.s	n.s	*	**	n.s
C.V (%)	8.76	26.43	5.51	7.22	7.07

n.s : not significant

Treat.1: control (no fertilizer and without inoculation), **Treat. 2:** Application 240 kg NPK (15-15-15)/ha, **Treat 3:** Rhizobial Inoculation + 20 kg N/ha, **Treat. 4:** PSB Inoculation + 20 kg N/ha, **Treat. 5:** Rhizobial + PSB Inoculation + 400 kg biofertilizer (granule)/ha + 20 kg N/ha; **Treat. 6:** Endophytic bacteria inoculation + 400 kg biofertilizer (granule)/ha + 20 kg N/ha.

*The numbers followed by the same letter do not differ at 1% level significantly

These yield components affected to grain yield (Figure 6) and these treatments as rhizobial inoculation (Treat. 3), PSB inoculation (Treat. 4) and mixture of rhizobial and PSB inoculation (Treat. 5) had the highest grain yield and they differed from other treatments significantly (Figure 6).



Means within a column followed the same letter/s are not significantly different at $p < 0.05$

Treat. 1: control (no fertilizer and without inoculation), **Treat. 2:** Application 240 kg NPK (15-15-15)/ha, **Treat 3:** Rhizobial Inoculation + 20 kg N/ha, **Treat. 4:** PSB Inoculation + 20 kg N/ha, **Treat. 5:** Rhizobial + PSB Inoculation + 400 kg biofertilizer (granule)/ha + 20 kg N/ha; **Treat. 6:** Endophytic bacteria inoculation + 400 kg biofertilizer (granule)/ha + 20 kg N/ha.

FIGURE 6. EFFECTS OF RHIZOBIA, PSB, ENDOPHYTIC BACTERIA AND CHEMICAL FERTILIZERS ON GRAIN YIELD (KG/HA) OF SOYBEAN (CV. CUJUT) CULTIVATED ON FERRASOLS OF CUJUT DISTRICT, DAKNONG PROVINCE, VIETNAM IN SUMMER-SPRING CROPPING-SEASON 2016

Grain yield of chemical fertilizer treatment (Treat. 2) did not differ significantly with grain yield of control treatment (Treat. 1) therefore application chemical fertilizers for soybean cultivation in this ferrasols (Cujut district) had no efficiency while application of endophytic bacteria had the lowest grain yield. DW of soybean shoot had close correlation very significantly with pod number/plant (Figure 7) and grain yield (Figure 8) while DW of nodule root had correlation with DW of shoot at different < 0.05 , therefore DW of nodule root had correlation with pod number/plant and grain yield indirectly.

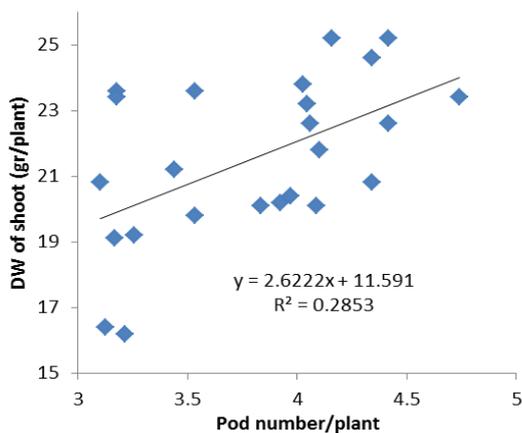


FIGURE 7. CORRELATION BETWEEN DW OF SHOOT (gr/plant) WITH POD NUMBER/PLANT

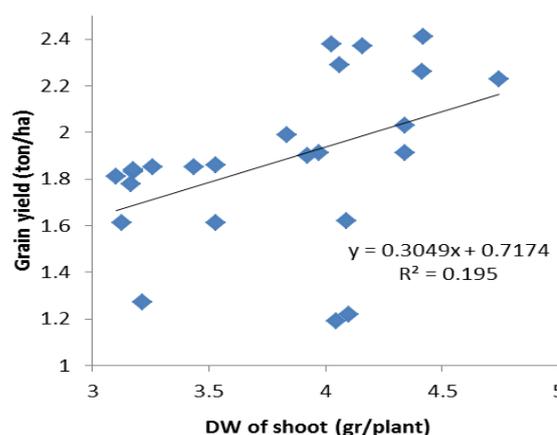


FIGURE 8. CORRELATION BETWEEN DW OF SHOOT (gr/plant) WITH GRAIN YIELD (ton/ha)

Application of chemical fertilizer (Treat. 2) and rhizobial and PSB inoculation (Treat. 5) for soybean cultivation increased protein content in seed but total of protein in seed/ha of treat. 2 was lower than total of protein of treat. 5 because grain yield of this treatment was low, this led to low total of protein of treat. 2 (Table 3); Protein content in seed of treatments of rhizobial inoculation (Treat. 3) and PSB inoculation (Treat. 4) were lower than protein content in seed of treat. 2 and treat. 5 and total of protein/ha of two treatments were also lower than treat. 5 however lipid content in seed of these two treatments were higher than and this led to total of lipid in seed/ha of two treatments were the highest while endophytic bacteria

inoculation for soybean seed before sowing did not enhance yield component, grain yield, protein content in seed but increasing lipid content in seed (Table 3).

TABLE 3
EFFECTS OF RHIZOBIA, PSB, ENDOPHYTIC BACTERIA AND MINERAL FERTILIZERS ON PROTEIN CONTENT, LIPID CONTENT IN SOYBEAN SEED AND TOTAL OF PROTEIN AND TOTAL OF LIPID IN SOYBEAN SEED/HA CULTIVATED FERRALSOLS OF CUJUT DISTRICT, DAKNONG PROVINCE IN SUMMER-SPRING CROPPING-SEASON 2016

Treatment	Protein content in seed (%)	Total of protein in seed /ha (kg/ha) *	Lipid content in seed (%)	Total of lipid in seed/ ha (kg/ha)
Control (no fertilizer, without inoculation)	29.16 c	53.995 d	20.841 c	38.607 b
240 kg NPK (15-15-15)/ha without inoculation	32.34 a	60.841 c	20.512 c	38.554 b
Rhizobial inoculation, 20 kg N/ha	30.88 b	68.424 b	21.600 b	47.917 a
PSB inoculation, 20 kg N/ha	30.12 b	65.469 b	21.408 b	46.495 a
Rhizobial + PSB inoculation + 400 kg biofertilizer + 20 kg N/ha	33.13 a	72.964 a	20.686 c	45.560 a
Endophytic bacteria inoculation + 400 kg biofertilizer + 20 kg N/ha	28.32 d	47.205 e	22.144 a	36.888 b
F calculated	**	**	**	**
C.V (%)	3.29	7.03	2.25	6.60

*Total protein in seed/ha = protein content in content (%) x grain yield

**Total Lipid in seed/ha = lipid content in content (%) x grain yield

+The numbers followed by the same letter do not differ at 1% level significantly

Soybean cultivation on ferrasols improves soil pH and organic matter in soil but decreased N total in soil perhaps requirement of soybean plant use a big amount of nitrogen and available phosphorus for the development of shoot, root and pod especially soybean seed (contains more than 34% protein) (Table 4). Application of chemical fertilizer in soybean cultivation supported soybean plants and enhanced soil pH, chemical parameters as N total, P available and organic matter in soil however rhizobial inoculation (Treat. 3), PSB inoculation (Treat. 4) and mixture of rhizobia and PSB inoculation (Treat. 5) also had good effectiveness as chemical fertilizer treatment (Treat. 2) while endophytic bacteria inoculation (Treat. 6) only improper soil pH and N total.

TABLE 4
EFFECTS OF RHIZOBIA, PSB, ENDOPHYTIC BACTERIA AND MINERAL FERTILIZERS ON pH AND CHARACTERISTICS OF FERRALSOLS BEFORE AND AFTER CULTIVATED SOYBEAN.

Treatment	pH	N total (%)	P available (mg P ₂ O ₅ /soil kg)	Organic Matter (%)
Initial	4.91 c	0.141 bc	30.98 cd	2.36 c
Control (no fertilizer, without inoculation)	5.87 ab	0.135 c	28.33 d	2.53 b
240 kg NPK (15-15-15)/ha without inoculation	5.96 a	0.160 ab	38.67 ab	2.71 ab
Rhizobial inoculation, 20 kg N/ha	5.89 ab	0.178 a	37.45 ab	2.69 ab
PSB inoculation, 20 kg N/ha	5.87 ab	0.155 b	36.40 b	2.83 a
Rhizobial + PSB inoculation + 400 kg biofertilizer + 20 kg N/ha	5.93 a	0.160 ab	40.19 a	2.62 b
Endophytic bacteria inoculation + 400 kg biofertilizer + 20 kg N/ha	5.89 ab	0.165 ab	32.79 c	2.39 c
F calculated	**	**	**	**
C.V (%)	1.38	0.079	13.62	5.24

*The numbers followed by the same letter do not differ at 1% level significantly

3.2 Economical efficiency

Based on grain yield, application of NPK (240 kg 15-15-15) in soybean cultivation did not differ from control (no fertilizer, without inoculation) while rhizobia inoculation plus 20 kg N/ha or PSB inoculation plus 20 kg N/ha and mixture of rhizobia and PSB inoculation plus 400 kg biofertilizer and 20 kg N/ha enhanced grain yield from 367 kg, 320 kg and 350 kg soybean seed/ha, respectively in comparison to control (Table 5) and farmers earned income 12,845,000; 11,200,000 and 12,250,000 VND, respectively while they must pay to cost of fertilizer as 367,000; 347,000 and 762,000 VND, respectively.

TABLE 5
ECONOMICAL EFFICIENCY IN SOYBEAN CULTIVATION WITH CHEMICAL FERTILIZERS, RHIZOBIA, PSB, ENDOPHYTIC BACTERIA INOCULATION

Treatment	Grain yield (kg/ha)	Enhanced seed weigh* (kg/ha)	Soybean seed price (35,000 VND/kg)	Cost of fertilizer** (for 1 ha)	Benefit income (VND/ha)
Control (no fertilizer, without inoculation)	1853	0	0	0	
240 kg NPK (15-15-15)/ha without inoculation	1880	27	945,000	1,680,000	- 735,000
Rhizobial inoculation ¹ , 20 kg N/ha	2220	367	12,845,000	367,390	12,478,000
PSB inoculation ¹ , 20 kg N/ha	2173	320	11,200,000	347,390	10,852,610
Rhizobial ² + PSB inoculation ² + 400 kg biofertilizer + 20 kg N/ha	2203	350	12,250,000	762,390	11,487,610
Endophytic bacteria inoculation ³ + 400 kg biofertilizer + 20 kg N/ha	1665	-188	0	767,390	0

*enhanced seed weigh = grain yield (treat) – grain yield (control)

** 1 kg NPK = 7,000 VND/kg, 1 litre rhizobia liquid, endophytic bacteria = 50,000 VND, 1 litre PSB liquid = 25,000 VND
20 N = 43,3 kg urea x 7,300 VND/kg

15 litres of rhizobial inoculant/ha, 22.5 litres rhizobial liquid and 2.5 PSB liquid, 35 litres of endophytic bacteria inoculant/ha,
1 kg biofertilizer = 1,000 VND

Low soil pH and N total are characteristics of ferralsols [20]; while concentration of available P₂O₅ and organic matter in ferralsols depended on cultural practices. Besides N requirement for soybean growth through nitrogen biological fixation, soybean plants require many other nutrients as phosphorus and P had quite prominent effects on nodulation, growth and yield parameters [21]. Many soils throughout the world are P-deficient because the free phosphorus concentration (the form available to plants) even the fertile soil is generally not higher than 10 µM even at pH 6.5 where it is most soluble [22]. To circumvent the problem of P deficiency, chemical fertilizers are added to the soils but cost of chemical phosphatic fertilizers is high [23] and low efficiency (<0.1%) [24]. Phosphorus biofertilizers in the form of microorganisms, especially phosphate-solubilizing bacteria in rhizosphere, can help in increasing the availability of accumulated phosphates for plant growth by solubilization [25][26].

Application of phosphorus along with PSB improved phosphorus uptake by plants and yields indicating that the PSB are able to solubilize phosphates and to mobilize phosphorus in crop plants [27]. PSB enhanced the phosphorus availability to plants by mineralizing organic P in soil and by solubilizing precipitated phosphate [28][29][30]. Dubey et al. [31] have also reported significant increased in grain yield of soybean due to co-inoculation of phosphorus solubilizers. Our results showed that application biofertilizer (mixture of thermophosphate and PSB) for soybean cultivation had high grain yield, protein yield and oil yield as applying NPK or inorganic fertilizer without inoculation [14]. PSB also are capable of transforming soil phosphorus to the forms available to plant and oil and protein yield were also maximum with PSB inoculant or biofertilizer. It is reported that soybean inoculated by *Bradyrhizobium* bacteria and phosphate solubilizing bacteria increased the seed yield [32][33]. Rana et al. [4] calculated that the highest B:C ratio (1:39) were obtained from the crop sown with 45 kg P₂O₅/ha, *Rhizobium* and phosphorus solubilizing bacteria followed by the crop sown with 60 kg P₂O₅/ha, *Rhizobium* and phosphorus solubilizing bacteria and therefore saving of 15 kg P₂O₅/ha; our results also recognized mixture rhizobia and PSB inoculants + 400 kg biofertilizer and 20 kg N/ha had grain yield, oil, protein in seed was equivalent with treatment of 100 kg/ha thermophosphate (15% P₂O₅) + 25 kg/ha NPK 16-16-ha for soybean cultivation on ferralsols of DakLak province [14].

In the Mekong Delta, Son et al. [34] reported that application of *Bradyrhizobium japonicum* and PSB *Pseudomonas* spp. can enhance the number of nodules, dry weight of nodules, yield components, grain yield, soil nutrient availability and uptake of soybean crop. This result showed that application of rhizobia or/and PSB inoculation in soybean cultivation on ferralsols of Cujut district, DakNong province not also enhanced yield component, grain yield, incomes but only improved soil fertility.

IV. CONCLUSION

Rhizobial inoculation or/and Phosphate-solubilizing bacteria inoculation are good, cheap, effective techniques in soybean cultivation on ferralsols of Cujut district, DakNong province. These techniques not only increased grain yield, incomes for farmers but also improved soil fertility.

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