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# The Improvement of Yield and Quality of Soybeans in a Coastal Area Using Low Input Technology Based on *Biofertilizers*

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***Abstract*** **— Indonesia is an archipelagic country, having vast coastal areas which have not been utilized optimally for crop cultivation because their poor soil properties become a limiting factor for plant growth. It is, therefore, necessary to use cultivation technology, such as the use of *biofertilizers* to overcome this limiting factor. The objective of this study was to determine the appropriate *biofertilizers* for improving the quality of soybean seeds, the *rhizosphere* environment, and the soybean yield in a coastal area. The study was conducted in Bengkulu, Indonesia, using Randomized Complete Block Design, with a single factor, namely *biofertilizers*. Six treatments were applied, namely the addition of *Arbuscular Mycorrhizal Fungi* (AMF), phosphate–solubilizing fungi (PSF), *Rhizobium* (R), AMF+PSF, R+AMF, and R+PSF. The results of this study showed that, in general, the inoculation of *biofertilizers* could increase the yield of soybeans in the coastal area, as indicated by the higher yield of plants in this study than those as described in the literature. The dual inoculation of PSF+AMF resulted in the highest protein content, the largest populations of PSF and AMF, the highest percentage of root colonization of AMF, plant phosphorus concentration and absorption, the weight of seeds per plant and yield. Meanwhile, the highest fat content of seed was found in the plants inoculated with PSF. In coastal soil, the dual application of *biofertilizers*, namely PSF and AMF, should be done, instead of a single application. Further studies are needed to increase the protein content and to reduce the fat content of soybean seeds.**

***Keywords***— **coastal land; mycorrhiza; Rhizobium; phosphate–solubilizing fungi.**

## I. INTRODUCTION

Indonesia is an archipelagic country, having the second longest shoreline in the world after Canada according to various data from the internet. The length of Indonesia's shoreline is 106,000 km, and the area of its sea is 5.8 million km$^2$. Approximately 1,060,000 ha of land along the shoreline had the potential to be utilized for agriculture. Meanwhile, the length of shoreline in Bengkulu Province is ± 525 Km. So far, however, the coastal areas have not been utilized optimally. Several constraints hinder the utilization of coastal soils, namely unstable soil, high evapotranspiration, low soil moisture content, water retention capacity, and organic matter, very low Cation Exchange Capacity (CEC), organic C and calcium, and high soil salinity, and low nitrogen content.

Soybean (*Glycine max* (L.) Merrill) is an important agricultural commodity in Indonesia, as a source of protein, the raw material for industry and animal feed. Soybean is a source of protein, fat, and carbohydrate, which are important for improving the nutritional level of the community because they are safe for health. Therefore, the availability of soil nutrients, primarily N and P, is important to improve the nutritional level of soybean seeds. Nitrogen enhances plant growth and plays important roles in the formation of chlorophyll, fat, protein and other compounds. In addition to N., the availability of P in soil is also important because legumes are more responsive to P [1]. Phosphorus plays important roles in energy transfer within plant cells, and it can improve the effectiveness of the N function, and the efficiency of N uses [2].

The domestic production has not met the national demand for soybeans although in the last five years the domestic production of soybeans has increased. According to the Central Agency of Statistics of Indonesia (2016) [3], the productions of soybeans in Indonesia from 2010 to 2015 were 907,031 tons, 851,286 tons, 843,153 tons, 779,992 tons, 954,997 tons and 982,967 tons, with the productivity of 1.373–1.573 tons ha$^{-1}$. With the increasing demand for soybeans, it is important that the land along the shoreline be utilized for cultivation of soybeans. However, an appropriate cultivation technique is required to improve the quality of coastal soil, such as the utilization of local soil microbes and organic matter, which is environmentally friendly.

Soil microbes and organic matter play essential roles in the transformation which cause the changes in physical and chemical sandy soil properties [4], [5]. They are responsible for most (60–80%) of biological processes related to nutrient cycle and organic matter decomposition. Some soil microbes which potentially improve the productivity of coastal soil are *Arbuscular Mycorrhizal Fungi* (hereafter referred to as AMF), phosphate–solubilizing fungi (hereafter referred to as PSF) and *Rhizobium*. The AMF and PSF play essential roles in helping plants take nutrients, especially phosphates (P). AMF colonized the host roots and produced hyphae intensively so they can improve the plant nutrient and water absorption capacity [6]. PSF responsible for mineral phosphate solubilization is by lowering the soil pH by the microbial production of organic acids and mineralization of organic phosphorus by acid phosphates [7] so that increases the P concentration in the soil solution. An increase in P concentration in soil has been shown to increase P uptake and soybean yield [8]. *Rhizobium* is a genus of bacteria capable of providing nitrogen for plants. In symbiosis with legumes, such as peanut, Rhizobium infects the roots and forms root nodules, and inside the nodules of the host legumes, it fixes nitrogen from the atmosphere [9].

It has been evident that single and dual inoculation of *biofertilizers* can improve the productivity of soil and plants. Reference [10] have proved in their study that AMF from Bengkulu could develop the production of soybeans. The study of [8] showed that inoculation of PSF (*A. niger*) in soybeans fertilized with Tri-Calcium Phosphate (TCP) resulted in higher mean dry weight of plants (18.9 g) than that of plants without TCP addition and PSF inoculation (10.7 g), and that of plants added with TCP but without PSF inoculation (15.4 g). The study of [10] also concluded that dual inoculation of *Rhizobium* and AMF could increase the number of root nodules and plant biomass. Therefore, the objective of this study was to determine the appropriate input of soil microbes which could improve the quality of soybean seeds, the environment of *rhizosphere* and the yield of soybean in a coastal area.

## II. Material and Method

### A. Materials

The materials used in this study were soybean seeds (variety of *Anjasmoro*), inoculants of *Rhizobium*, AMF and PSF, synthetic fertilizers (Urea, SP36, and KCl), dolomite with the active material of $CaMg(CO_3)_2$, and compost made of coffee bean husks. The AMF and PSF were produced by the Laboratory of Soil Biology, the Faculty of Agriculture, University of Bengkulu. The AMF and PSF were taken from the *rhizosphere* of soybean plants from the previous study. The AMF had spores, hyphae, and *mycorrhizal*–infected roots, developed using trapping method in a mixed medium of sand and soil from soybean plant *rhizosphere*, with a ratio of 3:1 [11]. The PSF was cultured in the medium of *Pikovskaya*. The *Rhizobium* was taken from the root nodules of soybean growing on some soils of Bengkulu Province. Inoculants of PSF and *Rhizobium* were given in the media of peat, charcoal and calcium carbonate ($CaCO_3$) with the proportions of 9:0.75:0.25.

### B. Experimental Design

This study was conducted in Beringin Raya Village, Muara Bangkahulu Subdistrict, Bengkulu Province, Indonesia, from March to July 2017. This study used Randomized Complete Block Design, with a single factor, namely *biofertilizers* which we produced in the Soil Science Laboratory, the University of Bengkulu. Six treatments were applied, namely the addition of AMF, PSF, *Rhizobium* (R), AMF+PSF, R+AMF, and R+PSFF. Each treatment had three replications.

### C. Land Preparation

The land was cleared of weeds and then tilled with hoes. Three plots, each measuring 1.5 m x 3.3 m and 50 cm apart from each other, were made. The distance between replications was 100 cm. Then, the soil was added with 10 ton/ha of compost made of coffee bean husks, 200 kg $ha^{-1}$ of dolomite with the active compound of $CaMg(CO_3)_2$, 50 kg $ha^{-1}$ of Urea, 50 kg $ha^{-1}$ of SP36 and 50 kg $ha^{-1}$ of *KCl*. The Urea fertilizer was added twice, half dose during the planting and the rest when the plants were one–month old. The SP36 and *KCl* fertilizers were given at once, at planting time. Planting holes with a depth of ±5 cm were made.

### D. Planting, Caring and Harvesting of Plants

A total of 60 plants were planted with a planting distance of 30 cm x 30 cm. The caring of the plants consisted of watering, replacing the dead plants, thinning, weeding and pest and disease control. Harvesting was done in two stages, during the vegetative and generative stages. From each treatment plot, five plants in the middle of the plot were harvested. The harvesting at the vegetative phase was done when the plants were 40 days old or when 10% of the plants had started flowering, while the harvesting at generative phase was done when the pods had dried and turned dark brown.

### E. Data Collection

The variables recorded in this study were soil organic–C (%), population of AMF, population of PSF, root colonization (%), N and P content in tissues (%), plant height (cm), weight of seed per plants (g), weight of 100 seeds (g), production (ton $ha^{-1}$), and the content (%) of protein, fat and carbohydrate in seeds. Composite soil samples were analyzed to determine the organic–C(%), a population of PSF and AMF.

### F. Data Analyses

The data were analyzed using analysis of variance (ANOVA) at 5 % significant level [12]. If the mean of variables were significantly different among treatments, a further test was conducted using Duncan's multiple range test (DMRT) at 5% significant level.

## III. Results and Discussion

### A. Soil properties

The study site was located ± 100 meters from the shoreline, having Alluvial Marine soil (*Entisol).* The soil had a pH of 6.05 ($H_2O$ method) or 5.32 (*KCl* method), a total–N 0.31%, phosphorus ($P_2O_5$) 4.31 ppm, available K 24.4 me/100 g, Ca 13.32 me/100 g, Mg 6.69 me/100 g, and organic–C 1.93 %. The soil texture was silky sand, with 89.2 % of sand, 1.62 % silt, and 9.18 % clay.

### B. The Results of ANOVA

The results of ANOVA of all variables are presented in Table 1.

TABLE I
THE SUMMARY OF ANOVA

| Variables | F values | Coefficient of variance (%) |
|---|---|---|
| Organic–C | $0.24^{ns}$ | 6.42 |
| Population of PSF | 5.78* | 8.53 |
| Population of AMF | 6.85* | 19.74 |
| Root colonization | 5.38* | 5.04 |
| Tissue N | $0.53^{ns}$ | 7.82 |
| Tissue P | 4.52* | 9.94 |
| Plant height | 18.77** | 2.72 |
| Weight of seeds per plant | 16.81** | 2.22 |
| A weight of 100 seeds | $0.86^{ns}$ | 4.40 |
| Production | 8.10** | 4.01 |
| Protein content | 5.11* | 3.72 |
| Fat content | 5.19* | 5.68 |
| Carbohydrate content | $3.02^{ns}$ | 6.15 |

Note: $^{ns}$=not significantly different, *= significantly different, **=highly significantly different

The ANOVA shows that *biofertilizers* significantly affected plant height, weight of seeds per plant, plant production, populations of PSF and AMF, root colonization, tissue P, protein and fat content, but it did not significantly affect organic–C, tissue N, weight of 100 seeds, and carbohydrate content of soybean seeds.

### C. The Effect of Biofertilizers on the Rhizosphere Environment and Plant Nutrients

The results of this study showed that the dual application of *biofertilizers* using PSF and AMF inoculants resulted in the largest populations of PSF and AMF, and relatively higher organic C than the application of other *biofertilizer* inoculants, with the values of 68.33, 97.33, and 2.51% respectively (Table 2). This means that PSF and AMF can live together well so both fungi can grow optimally when applied together. Other researchers [13] also found that the growth of *arbuscular mycorrhizal* hyphae in the soil amended with beet waste fermented with *Aspergillus niger* was higher than that amended with beet waste without fermentation with *Aspergillus niger.* Also, soil organic matter plays important roles in the development of soil microbes. The more organic matter available in the soil is, the more optimal the development of soil microbes will be. The results of this study showed that the dual application of PSF+AMF resulted in relatively higher organic C than the application of other inoculants, namely 2.51%, so it also resulted in the largest populations of PSF and AMF. As we know soil microbes need organic matter as the energy input, so the increase of organic matter in the soil will increase the activities and growth of soil microbes, including PSF.

TABLE II
ORGANIC–C CONTENT, THE POPULATION OF PSF AND AMF AS AFFECTED BY THE ADDITION OF *BIOFERTILIZERS*

| *Biofertilizers* | Organic–C (%) | Population of PSF | Population of AMF |
|---|---|---|---|
| R | 2.21 | 38.67b | 19.00c |
| PSF | 2.35 | 61.33 a | 26.67bc |
| AMF | 2.24 | 39.33 b | 36.00 bc |
| R+PSF | 2.36 | 51.67 ab | 50.00 ab |
| R+AMF | 2.31 | 44.00 b | 79.00 a |
| PSF+AMF | 2.51 | 68.33 a | 97.33 a |

Note: the numbers in the same column followed by the same letters are not significantly different at 5 % level with DMRT.

The dual inoculation of PSF and AMF resulted in the highest percentage of root colonization, namely 96.67%, indicating the high quantity and effectiveness of *mycorrhizas*, so the absorption of P by the plants increased (Table 3). This was evident from the highest tissue P content in the plants treated with PSF+AMF inoculation, namely 1.18%. Root colonization by *mycorrhiza* is facilitated with the external hyphae which can absorb and move water and nutrients closer to roots [6]. Also, the role of PSF which fix unavailable phosphate and make it available to plants is another factor causing the high tissue P [7]. The role of PSF is more dominant in its effect on metal bound, which binds phosphates in the soil. The PSF can produced a variety of organic acids such as formic acid, acetic acid, propionic acid, lactic acid and succinic acid which can form chelates (stable complex) with cations that bind P, so that the phosphates ion becomes free from the bound and available for plant absorption [7], [8].

TABLE III.
THE MEANS OF ROOT COLONIZATION AND PLANT NUTRIENTS (N AND P) CONTENT AS AFFECTED BY THE ADDITION OF *BIOFERTILIZERS*

| Biofertilizers | Root colonization (%) | Tissue N (%) | Tissue P (%) |
|---|---|---|---|
| R | 66.67 b | 4.89 | 0.82 ab |
| PSF | 90.00 a | 4.88 | 0.34 b |
| AMF | 93.33 a | 3.98 | 0.85 a |
| R+PSF | 80.00 ab | 4.43 | 1.07 a |
| R+AMF | 93.33 ab | 4.34 | 1.13 a |
| PSF+AMF | 96.67 a | 4,54 | 1.18 a |

Note: the numbers in the same column followed by the same letters are not significantly different at 5 % level with DMRT.

### D. The Effect of Biofertilizers on the Growth and Yield of Soybeans

*Rhizobium* inoculant resulted in the higher plant height than the other *biofertilizers* because *Rhizobium* can increase N uptake by pants (Table 4). According to [14], *Rhizobium* can increase the N availability in the soil and N absorption by plants and produce *phytohormones* of IAA and gibberellins which can improve the growth of soybeans. The results of this study showed that the application of

*Rhizobium* resulted in tissue N of 4.89 %, higher than that of the other treatments. Although *Rhizobium* application resulted in the highest plant height, it did not produce the highest values of other growth variables or yield. It means that higher plants do not always have a higher yield.

Another researcher [15] also reviewed that the multi microbes containing *biofertilizer* significantly increased plant nutrient absorption and plant yield over the controls. The dual application of PSF and AMF resulted in the highest seed weight per plant (31.67 g) and production (3.05 tons/ha). Previously research [10] also found that the dual inoculation of A. *niger* and *Gigaspora* gave the highest production of soybeans. The dual application of PSF and AMF resulted in the highest seed weight and production because this treatment produced the largest populations of PSF and AMF and the highest root colonization, so the tissue P was also the highest. A high percentage of root colonization indicates that the *mycorrhizas* actively colonized the root and expand the area of water and nutrient absorption. Reference [6] explains that *mycorrhizas* colonized the root system of their host plants, producing intensive networks of hyphae, so the colonized roots are capable of increasing their capacity to absorb nutrients. The increased absorption of nutrients will increase the growth and yield of plants.

TABLE IV
THE GROWTH AND YIELD OF PLANTS AS AFFECTED BY THE ADDITION OF *BIOFERTILIZERS*

| *Biofertilizers* | Plant height (cm) | The weight of seeds per plant (g) | Weight of 100 seeds (g) | Production (ton/ha) |
|---|---|---|---|---|
| R | 66.49 a | 29.75 b | 15.20 | 2.80 b |
| PSF | 59.02 b | 28.45 cd | 16.53 | 2.83 b |
| AMF | 57.62 bc | 31.03 a | 16.56 | 2.86 ab |
| R+PSF | 56.42 bc | 27.55 d | 15.90 | 2.73 b |
| R+AMF | 56.72 bc | 29.09 bc | 16.33 | 2.50 c |
| PSF+AMF | 55.77 c | 31.67 a | 16.55 | 3.05 a |
| Plant description | 64–68 cm | – | 14.8–15.3 | 2.03–2.25 |

Note: the numbers in the same column followed by the same letters are not significantly different at 5 % level with DMRT.

Compared to soybean plants of the same variety as described in the literature, the soybean plants applied with *Rhizobium*, which had higher stems. However, the plants with other application had lower stems. The application of other *biofertilizers* resulted in a higher weight of 100 seeds than the weight of the same variety described in the literature. The plant production in this study was much higher than that of the same variety as described in the literature. Although the plant production was not measured directly, but through the conversion of plot area (1.5 m x 3.3 m), this information, at least, indicates that *biofertilizers* from local soil microbes have the potential for increasing the soybean yield, given that the study site is coastal soil which has properties and environmental factors which are not optimal for soybean plant growth. Inoculants of soil microbes increase soil biodiversity and enhance plant growth and yield as reported before [16].

*E. The effect of Biofertilizers on the Quality of Soybean Seeds*

The results of this study showed that the plants inoculated with PSF+AMF had the highest protein content in the seeds, namely 37.12% which was not significantly different from those treated with R+AMF inoculation (35.07%) but was significantly different from the plants treated with other inoculants (Table 5).

TABLE V
THE MEANS OF PROTEIN, FAT AND CARBOHYDRATE CONTENTS OF SOYBEAN SEEDS AFTER THE ADDITION OF BIOFERTILIZERS

| Biofertilizers | Protein content (%) | Fat content (%) | Carbohydrate content (%) |
|---|---|---|---|
| R | 32.77 b | 19.50 a | 24.28 |
| PSF | 33.04 b | 19.61 a | 23.49 |
| AMF | 33.11 b | 17.44 b | 26.21 |
| R+PSF | 34.24 b | 17.97 ab | 24.82 |
| R+AMF | 35.07 ab | 16.09 b | 21.71 |
| PSF+AMF | 37.12 a | 17.47 b | 24.51 |
| Plant description | 41.78–42.05 | 17.12–18.60 | – |

Note: the numbers in the same column followed by the same letters are not significantly different at 5 % level with DMRT.

The protein content in the other five treatments was not significantly different from one another. The higher protein content in seeds of plants inoculated with PSF+AMF was presumably due to the higher and more balanced nutrient availability. This study found that dual inoculation of PSF+AMF resulted in the largest populations of PSF and AMF and the highest percentage of root colonization and tissue P. The large population of PSF and AMF in *rhizosphere* of soybean plants increased the soil phosphorus dynamics which needed for activities of nitrogen-fixing bacteria, such as rhizobia, so the absorption of N also increased. Phosphorus, from the ATP hydrolysis, is required for the formation of nodules and the maximum activities of nodules [17]. The plants that get nitrogen through symbiosis also need larger amount of P than the plants fertilized with N, presumably because more P is required to form root nodules, signal transduction, and P–lipid which is needed for *bacteroid* activities within the nodules. In previous research [18] addition of 150 kg $ha^{-1}$ of P fertilizer to *Phaseolus* bean was reported to increase seed yield to 62% and also increase nitrogen fixation from an average of 8–60 kg $ha^{-1}$. Fertilizing at a dose of 30 kg $ha^{-1}$ $P_2O_5$ was reported to increase seed yield of pigeon pea up to 1300 kg $ha^{-1}$, but if combined with *Rhizobium* inoculation, the seeds yield reached 1800 kg $ha^{-1}$. The increase of tissue N increases the rate of photosynthesis, yield and protein content in the seed of soybean plants accordingly.

The highest fat content of seeds, 19.1%, was found in the plants inoculated with PSF, whereas the lowest one was found in the plants inoculated with R+AMF, namely 16.09%. These results showed that despite resulting in the highest protein content, the dual inoculation of PSF and AMF did not produce the highest fat content. It was the single inoculation of PSF which resulted in the highest fat content, 19.1%, although the protein content tended to be the lowest, second after the plants inoculated with *Rhizobium*. The protein content of seed was negatively correlated with the oil content [19].

In general, the types of *biofertilizer* inoculants did not significantly affect the carbohydrate content of the seeds. However, the single inoculation of AMF tended to give higher carbohydrate content than the other treatments, namely 26.21%, whereas the dual inoculants of R+AMF resulted in the lowest carbohydrate content, namely 21.71%. In general, the protein content of the seeds in this study was lower than that of the plants as described in the literature (41.78–42.05%), because the study site was the coastal area having marginal land which is not favorable for optimal growth of soybeans. Coastal soil is characteristically infertile. In alkaline soil, phosphate and calcium make insoluble complexes compounds Ca–P which are unavailable to plants. Also, in sandy soil, N content is low because it is easily lost through evaporation or leaching.

## IV. Conclusions

The results of this study showed that the inoculation of *biofertilizers* could increase the yield of soybeans in the coastal area, as indicated by the higher yield of plants in this study than those as described in the literature. The dual inoculation of PSF+AMF resulted in the highest protein content, the largest populations of PSF and AMF, the highest percentage of root colonization of AMF, tissue P, the weight of seeds per plant and yield. Meanwhile, the highest fat content of seed was found in the plants inoculated with PSF. In coastal soil, the dual application of *biofertilizers*, namely PSF and AMF, should be done, instead of single application. Further studies are needed to increase the protein content and to reduce the fat content of soybean seeds.

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