Effectiveness of plant growth promoting microrganisms as biofertilizer for soybeans under oil palm plantations on tidal land

Suryantini and A.A. Rahmiana
Indonesian Legumes and Tuber Crop Research Institute (ILETRI/Balitkabi). Jl. Raya Kendalpayak Km 8, PO Box 66 Malang 65101, East Java, Indonesia
tinibalitkabi@gmail.com

Abstract. A research to study the effectiveness of biofertilizers on nodulation, growth and productivity of soybean was carried out at two locations on type D tidal lands in South Kalimantan, using a randomized block design with four replications. The treatments tested were N-fixing bacteria *Rhizobium japonicum*, P solubilizing bacteria (PSB) *Pseudomonas sp* and mycorrhiza biofertilizers, combined with inorganic nitrogen (N) and phosphorus (P) fertilizers in the form of Urea and SP36 with low (25:25), moderate (50:50) and high (100:100) kg/ha doses. At the first location, the highest seed yield (2.04 t ha) was obtained in the rhizobium + PSB treatment combined with moderate dose of Urea and SP36 (50:50). This yield was 34% increase compared to seed yield at the same fertilizer rate without any biofertilizer. The same response was also obtained in second location where the soil was more acidic. The highest seed yield (1.9 t/ha) was obtained in the rhizobium + PSB treatment with a moderate dose of N and P fertilizer (50:50). This treatment resulted in 70% yield increase compared to that obtained by the treatment of the same fertilizer rate without bio fertilizer. In treatment of rhizobium + PSB + mycorrhiza, the seeds yield obtained were not different from the rhizobium + PSB treatment. This yield increase was supported by increasing the number and weight of root nodules, leaf chlorophyll index, weight of 100 seeds, and the number of filled pods per plant. This shows that the tested biofertilizers was effective on tidal land, and was able to increase soybean productivity.

Keywords: Bio fertilizer, inorganic fertilizer, soybean, tidal land, oil palm trees.

1. Introduction
The problems faced in soybean production basically include two main things namely, less extensive of harvest area and low productivity. The harvest area of soybean in Indonesia is around 700 thousand hectares with an average productivity of 1.4 t/ha (BPS 2015). Meanwhile, to achieve self-sufficiency, at least 1,700 ha of harvest area is needed, with an average productivity of 1.54 t/ha [1]. Soybeans can be developed in a variety of ecosystems, ranging from optimal to sub-optimal lands. The optimal land is actually quite potential to support an increase of national soybean production. This land, however, are continue to decrease from year to year as a result of switching to the non-agricultural purposes. Besides that, the development of soybeans on optimal land must also hardly compete to other crops especially maize which more profitable [2]. For the future, sub-optimal land uses as a soybean development area will have good prospects
Suboptimal land which has enough potential for growing soybeans *i.e.* tidal land has been widely used for oil palm plantation. In Indonesia, tidal land area reaches around 23.1 million hectares spread in the coastal plains of Sumatra, Kalimantan, Irian Jaya and Sulawesi. The land is now widely used for oil palm plantations, and has great potential for developing soybean plants, especially under oil palm plantations that are less than five years old. According to Gunawan (2013) the area of oil palm plantations in Indonesia has now reached 13.5 million hectares [3], and continues to grow of around 450 thousand hectares each year [4]. Seeing the vast area of tidal land, and the increasing amount of productive land that has been converted to the non-agricultural sector, the use of tidal land in the future will be very important to support increased soybean production to strengthen food security. The main problem currently faced is high soil acidity due to pyrite oxidation, this condition is generally followed by decreasing nutrient availability [5, 6]. Acidic soils contain high Al, Fe, and Mn that can be toxic to the crops, and lack of essential macro nutrients such as N and P.

In acid soils, P binds with aluminium, iron and manganese to form insoluble compounds, or do not available for plant uptake. Phosphate solubilising microorganisms can produce several organic acids and enzymes that are able to convert phosphate which is difficult to dissolve into a substance that can be easily assimilated by plants and therefore improve the supply of phosphorus in the soil [7]. In acid soils, N level tends to be low because the rate of N mineralization is positively correlated with pH (P < 0.05), i.e. N mineralization increases significantly with an increases of soil pH [8]. The opposite is certainly the case with low pH or acidic soils. The use of chemical fertilizers can temporarily alleviate soil fertility problems, but in the long run it will not only increase the cost of agricultural production but also damage the soil and accumulate some chemical elements that are harmful to the environment. One inexpensive and long-term technical solution is the use of biological fertilizers such as P-solubilizer and symbiotic N-fixers. Bio fertilizers will be effective in tidal land if the microbes are tolerant to acidity constraints. With the acquisition of rhizobium and phosphate solubilizing bacteria inoculums tolerant to acidic conditions, N and P requirements of soybean plant can largely be supplied through N2 fixation by rhizobium bacteria and P solubilization by phosphate solubilizing bacteria [9,10].

Phosphate solubilisation by microbes is an important process in natural ecosystems, especially agricultural land. Several types of microbes such as bacteria, fungi and actinomycetes are reported to be active in conversion of insoluble phosphate to soluble phosphate. The use of P-solubilizing bacteria as a biological fertilizer can increase crop yields because of its ability to dissolve insoluble inorganic phosphate compounds such as tricalcium phosphate, hydroxyapatite, and rock phosphate [11, 12] Microbial inoculation of P solubilizer was able to increase the availability of P from phosphate rock which was applied to the soil within twenty days, from 0.67 ppm to 17.78 ppm [13]. The use of P-solubilizing along with rhizobium inoculants produces a synergistic effect that is benefit to plants has been reported by many researchers. Inoculation of a combination of Bradyrhizobium and Pseudomonas strains increased soybean yields by 38% and 12% in pot and field experiments, respectively, compared to results obtained in the treatment of P-solubilizing alone [14]. Inoculation of N-fixing bacteria combined with P-solubilizing bacteria positively influences root expansion, plant performance, water assimilation and nutrient elements [15]. The same thing was reported by Ahsan et al. (2012) that the combination of P solubilizing bacteria and rhizobium significantly increased plant height, number and weight of nodules, number of pods per plant and soybean seed yield and saved 40% of P fertilizer use [16].

Tran Thi Ngoc Son et al. (2007) reported that the application of *Bradyrhizobium japonicum* and P solubilizing bacteria (*Pseudomonas* spp.) in soybeans + 20 kg N/ha fertilization increased the number and weight of nodules, yield components and yield of seeds compared to conventional fertilizer treatment by farmers [17]. Furthermore it was reported that the treatment not only saved 40-60 kg N and 60 kg P2O5 kg / ha but also gained higher economic efficiency in the form of Marginal benefit Cost Ratio (MBCR) of up to 43.98%. In alluvials, a combination of rhizobium and PSB can also increase soybean yields [18]. This study, therefore, aims to examine the effectiveness of rhizobium and P solubilizing bacteria as well as mycorrhiza bio fertilizers in various doses of N and P inorganic
fertilizers, on nodulation, growth and yield of soybean grown between young oil palm trees on tidal land. In addition to N-fixing and P solubilizing bio fertilizers, another bio fertilizer namely mycorrhizae is also reported to be effective in increasing nutrient uptake by plants. This is because mycorrhizal colonization at plant roots can expand the field of root absorption in the presence of external hyphae that grow and develop in roots. In lentil, combined application of Rhizobium + VAM increased seed yield by 45% over the control, as well as 24% and 28% over a single inoculation of VAM and Rhizobium, respectively [19].

The purpose of this study was to examine the effectiveness of P-solubilizing (Pseudomonas sp.), N-fixing (Rhizobium japonicum) and mycorrhiza biofertilizers, at several doses of urea and SP36 fertilizer (as sources of N and P elements) on nodulation, growth and productivity of soybeans in tidal land.

2. Material and methods

The experiment was conducted at two locations of tidal land types D in Barito Kuala Regency of South Kalimantan Province (2 ° 29'50" S; 114 ° 20'50" E; 1-3 meters above sea level), in the growing season of 2016. The experimental plots were laid out in a randomized complete block design (RCBD), with four replications. The tested treatments are presented in the following table.

| Treatment number | Dose of fertilizer (kg / ha) |
|------------------|------------------------------|
|                  | Urea(*)| SP36(*)| KCl(*)| Rhizobium| PSB| Mycorrhiza |
| 1                | 100    | 100    | 100   | 0       | 0  | 0         |
| 2                | 50     | 50     | 100   | 0       | 0  | 0         |
| 3                | 25     | 25     | 100   | 0       | 0  | 0         |
| 4                | 50     | 100    | 100   | 0.3     | 0  | 0         |
| 5                | 50     | 50     | 100   | 0.3     | 0  | 0         |
| 6                | 50     | 50     | 100   | 0.3     | 0  | 0         |
| 7                | 25     | 25     | 100   | 0.3     | 0  | 0         |
| 8                | 100    | 100    | 100   | 0.3     | 0  | 0         |

*) as a source of N, P, and K nutrients respectively. PSB: P solubilizing bacteria

Each treatment combination was plant in a 4 m x 5 m plot size, with plant spacing of 40 cm x 15 cm, two plants / clump. The Anjasomo variety was used as plant materials. All plots received basal fertilizer of 500 kg / ha Dolomite as the soils had Al saturation below 20%. In soils with Al saturation higher than 20%, more Dolomite was given until Al saturation reduced to about 20%. Inoculant of N-fixing (Rhizobium japonicum) and P-solubilizing (Pseudomonas sp) is a peat-based biofertilizer product from the Balitkabi Soil Microbiology Laboratory, which has been proven effective in soybeans in acid dry lands, whereas mycorrhizae is a commercial biofertilizer.

Dolomite and manure 1 t/ha were applied during tillage. Biofertilizer of Rhizobium and PSB were applied before planting, by mixing it with seeds that have been moistened with water, while mycorrhiza was applied as a cover for planting holes. Inorganic fertilizers of Urea, SP36 and KCl were applied in furrows next to the plant rows, a week after planting.

Data collected includes:
1. Chemical properties of soil before planting: soil pH, organic C, nutrients N, P, K, Ca, Mg, Al, Mn, and Fe.
2. Plant height at 40, 60 and 80 days after planting (DAP), which were measured at 10 sampled plants per plot.
3. Leaf chlorophyll index at 40 and 60 DAP, number and weight of nodules at 40 DAP, and plant dry matter at 60 DAP (10 samples/plot)
4. Number of filled pods and seed weight per plants (10 samples/plot), 100 seed weight and seed weight obtained from 2 m x 5 m harvest plot.

Weed, pest and disease controls were carried out intensively. Harvesting was done when the leaves are yellow and the pods are brown. An analysis of variance was done on data obtained from each parameter. The Duncan Multiple range test was used to compare treatment means.

3. Results and Discussion.

Tidal land under oil palm trees in two locations, namely Sidomulyo and Kolam Makmur Village showed relatively similar soil nutrient status with very low to moderate status, in average. Both sites are acid soils, where Kolam Makmur soils are more acid than that of Sidomulyo soils (Table 1).

| Location / Village | C-org % | pH | N % | P2O5 ppm | K | Ca | Mg | Al-dd | H-dd | KTK Me/100 g |
|-------------------|--------|----|-----|----------|---|----|-----|------|------|-------------|
| Sidomulyo         | 7.86   | 5.0| 0.33| 17.5     | 0.21| 0.47| 0.41| 6.22 | 7.33 | 133         |
| Kolam Makmur      | 9.15   | 4.4| 0.41| 28.5     | 0.41| 0.45| 0.48| 4.10 | 9.80 | 141         |

3.1 Number and weight of nodules

On tidal land of Sidomulyo and Kolam Makmur villages, which had never been planted with soybeans before, there were nodules on the roots of Anjasamoro variety despite it was not inoculated with rhizobium. This showed that the soil has an infective (capable of forming nodules) natural rhizobium population and they are compatible with the soybean variety. The number of nodules in Sidomulyo ranged from 9.4-57.1 nodules/plant, and it was higher than that in Kolam Makmur that ranged from 3.5-33.5 nodules/plant. This later site was more acidic than that the first. Bio fertilizer rhizobium, in fact, was able to compete with natural rhizobium at these locations, as seen from the formation of significantly higher numbers and weight of root nodules in inoculated plants. In Sidomulyo, the plants with rhizobium treatment produced 50.6 nodules/plant or it produced three times higher number of nodules than that produced by plants with no biological fertilizer with only 13 nodules/plant (Table 2). Likewise in Kolam Makmur, there were a doubled number of nodules produced by the rhizobium treatment (27.7 nodules/plant) than the number of nodules produced by the plant with no rhizobium treatment which produced 8.5 nodules per plant only (Table 3). In Sidomulyo the combination of rhizobium bio fertilizer with PSB and mycorrhiza did not beneficial in increasing the number of nodules compared to rhizobium treatment alone. Whereas in Kolam Makmur, the highest number of nodules was obtained in the treatment of rhizobium + PSB + mycorrhiza + high dose of Urea and SP36 fertilizers (100:100).

An increase in number of nodules was followed by an increase in dry weight of nodules with the similar pattern/trend. In Sidomulyo, the highest nodules weight was obtained in rhizobium + PSB treatment, but it was not significantly different from rhizobium treatment alone (Table 2). Whereas in Kolam Makmur, low dose fertilizer treatment (25:25) without any rhizobium application showed high nodules weight (0.24 g/plant) compared to other fertilizer doses. The addition of rhizobium applied together with low dose fertilizer treatment resulted 0.31 g/plant of nodules although this is non-significantly different result. Significant increase in nodular weight was obtained in the combination treatment of rhizobium + PSB + mycorrhiza + high dose of Urea and SP36 fertilizer (100:100) with nodule weight of 0.35 g/plant (Table 3). In both locations, inorganic and biological fertilizers significantly affected the number of root nodules. High doses of Urea and SP36 fertilizer tend to decrease the number of nodules, whereas rhizobium bio fertilizer increased the number of nodules (Tables 2 and 3). It seems that the dose of N fertilizer has a significant effect on the number and weight of root nodules. In treatment without any biological fertilizer, application of Urea and SP36 in moderate (50:50) and high (100:100) quantities resulted in lower numbers and weights of nodules.
compared to those of low dose fertilization (25:25) (Tables 2 and 3). This result is in accordance with Argaw and Tsige (2015) who reported that increasing the dose of N fertilizer to 20 kg N/ha (equal to 40 kg Urea/ha) significantly decreased the number and weight of nodules compared to the lower dose of N fertilizer. High concentrations of nitrate and ammonium in the soil inhibit the formation of nodules, number of rhizobium infection points in roots, nodules development, N fixation, and nitrogenase activity [20,21].

Besides nitrogen, soil P levels also affect nodulation. P deficiency inhibited N₂ nodulation and fixation, whereas the availability of sufficient P in the soil increased N₂ nodulation and fixation [22,23]. However, in this study without any rhizobium inoculation, increasing the P fertilizer dosage to 100 kg SP36/ha tended to reduce the number and weight of root nodules. Conversely, with rhizobium inoculation there was no decrease in nodulation although the P fertilizer dosage was gradually increased from 25 to 50 and 100 kg SP36/ha, instead it tended to increase in number and weight, especially in Kolam Makmur, where the number of nodules increased significantly at the dose of 100 kg SP36/ha. This seems to be related to the number of indigenous rhizobium populations in the rhizosphere, although in this study that population was not observed. Without rhizobium inoculation, it is suspected that rhizobium populations in the soil are low so that a high P supply tended to suppress the formation of root nodules. Conversely, rhizobium inoculation increased the number of rhizobium populations in the soil so that a high P supply can be applied and used for nodule formation. As reported by Miao et al. (2007), that at the level of inoculation of 102 cells/mL, P supply did not affect the number of nodules. At the level of inoculation of 105 cells/mL, an increase in P supply had been increased both the number and size of nodules [24]. However, a more definite mechanism regarding the relationship between rhizobium population and P level with nodulation, needs to be searched to any further.

3.2 Dry matter of plant and leaf chlorophyll index
In Sidomulyo a significant increase in plant dry matter was obtained in combination treatment of rhizobium + PSB + moderate dose (50:50) of Urea and SP36 fertilization. The dry matter was increased by 90% with the application of bio fertilizers than that at the same dose of fertilizers (50:50) without any bio fertilizer. While the highest plant dry matter was obtained in the combination treatment of rhizobium + PSB + mycorrhizae + high inorganic fertilizers dose (100:100). The application bio fertilizers increased (by 51.9%) the dry matter over those at the same dose of fertilizer (100:100) without bio fertilizer (Table 2). Similarly in Kolam Makmur, the highest plant dry matter was obtained in rhizobium treatment with Urea and SP36 fertilizer (50:100) but not significantly different from that obtained in rhizobium + PSB combination treatment with lower fertilizer dosage (50:50). In both locations the use of mycorrhizal bio fertilizer was fail to produce a higher increase, likewise the addition of Urea and SP36 fertilizers to a high dose (100:100). This shows that the combination of rhizobium and PSB bio fertilizers increased the efficiency of fertilizer use by plants.

Leaf chlorophyll index is one measure of plant N levels. In Sidomulyo, increase in the amount of inorganic fertilizer tended to increase the chlorophyll index. The significant effect of treatment on leaf chlorophyll index was only seen at 40 DAP, presumably because the plant has just recovered from N deficiency conditions at 60 DAP. The definite reasons need further research. The highest leaf chlorophyll index in Sidomulyo at 40 DAP was obtained in rhizobium + PSB combination treatment + Urea and SP36 fertilization (50:50), which was significantly higher than the same and higher fertilizer dosage, of (50:50) and (100:100) respectively. In Kolam Makmur at 40 DAP, rhizobium + PSB treatment + Urea and SP36 fertilization (50:50) produced a significantly higher chlorophyll index compared to the same fertilizer dosage but without biological fertilizer. The effectiveness of rhizobium + PSB combination remained consistent until 60 DAP observation, with chlorophyll index equivalent to those of Urea and SP36 at high doses (100:100). This shows the important role of PSB, might be in the supply of P, to support the N fixation process by rhizobium.
Table 3. Effect of inorganic and bio fertilizers on number and dry weight of root nodules, dry matter of plants, and leaf chlorophyll index of soybean in the tidal land of Sidomulyo Village.

| Urea Kg/ha | SP36 Kg/ha | Biological fertilizer | Nodule number/Plant | Nodule dry weight/plant (g) | Plant dry matter (g) | Chlorophyll index 40 DAP | Chlorophyll index 60 DAP |
|------------|------------|-----------------------|---------------------|----------------------------|---------------------|--------------------------|--------------------------|
| 100        | 100        | 0                     | 9.4 c               | 0.31 d                     | 7.43 c             | 33.8 bc                  | 42.0 ab                  |
| 50         | 50         | 0                     | 10.1 c              | 0.24 e                     | 4.76 e             | 33.7 bc                  | 39.1 b                   |
| 25         | 25         | 0                     | 19.6 bc             | 0.44 c                     | 6.11 d             | 32.1 c                   | 39.2 b                   |
| 50         | 100        | Rhizobium (Rhi)       | 50.6 a              | 0.51 ab                    | 9.58 ab            | 37.7 ab                  | 43.3 ab                  |
| 50         | 50         | Rhi+PSB               | 57.1 a              | 0.54 a                     | 10.21 ab           | 39.5 a                   | 41.2 ab                  |
| 50         | 50         | Rhi+PSB+Mycorrhiza    | 39.6 ab             | 0.45 bc                    | 8.78 b             | 38.5 ab                  | 42.8 ab                  |
| 25         | 25         | Rhi+PSB+Mycorrhiza    | 34.5 ab             | 0.45 bc                    | 6.98 cd            | 37.6 ab                  | 40.4 ab                  |
| 100        | 100        | Rhi+PSB+Mycorrhiza    | 39.3 ab             | 0.48 abc                   | 10.29 a            | 40.5 a                   | 45.4 a                   |

The number followed by the same letter is not significantly different at the LSD level of 5%

Table 4. Effect of inorganic and bio fertilizers on number and dryweight of root nodules, drymatter of plants and chlorophyll index of leaves of soybean in the tidal land of Kolam Makmur Village.

| Urea Kg/ha | SP36 Kg/ha | Bio fertilizer | Nodule number/Plant | Nodule dry weight/plant (g) | Plant dry matter (g) | Chlorophyll index 40 DAP | Chlorophyll index 65 DAP |
|------------|------------|----------------|---------------------|----------------------------|---------------------|--------------------------|--------------------------|
| 100        | 100        | 0              | 3.5 e               | 0.04 c                     | 2.79 d             | 33.6 bc                  | 39.3 a                   |
| 50         | 50         | 0              | 18.6 d              | 0.24 b                     | 4.13 cd            | 28.8 d                   | 35.9 b                   |
| 50         | 100        | Rhizobium (Rhi) | 23.4 c              | 0.31 ab                    | 6.57 a             | 36.4 ab                  | 38.1 ab                  |
| 50         | 50         | Rhi+PSB        | 27.7 b              | 0.28 ab                    | 6.20 ab            | 36.1 ab                  | 40.1 a                   |
| 50         | 50         | Rhi+PSB+Mycorrhiza | 21.0 cd         | 0.32 ab                    | 6.30 a             | 35.9 ab                  | 39.7 a                   |
| 25         | 25         | Rhi+PSB+Mycorrhiza | 22.4 c              | 0.25 ab                    | 4.91 bc            | 36.4 ab                  | 40.6 a                   |
| 100        | 100        | Rhi+PSB+Mycorrhiza | 33.5 a             | 0.35 a                     | 5.68 ab            | 37.0 a                   | 39.6 a                   |

The number followed by the same letter is not significantly different at the LSD level of 5%

3.3 Plant height

In Sidomulyo, the plants grew 48.3 - 67.5 cm tall and it was relatively higher than those at Kolam Makmur which was between 39.3 -55.5 cm. In both locations the treatment of inorganic and bio fertilizers significantly affected plant height. In Sidomulyo, plant height at with rhizobium + PSB + Mycorrhiza + high dose (100:100) Urea and SP36 was the highest compared to other treatments consistent at 40, 60 and 80 DAP. However, this plant height was not significantly different from those treated with rhizobium + PSB + moderate (50:50) Urea and SP 36 treatment (Table 4). In Kolam Makmur, the similar trend to that in Sidomulyo was also presence. The highest plant height was obtained in the combination of rhizobium + PSB bio fertilizer treatment + Urea and SP36 at medium dose (50:50) both at 40, 60 and 80 DAP. Adding mycorrhizal bio fertilizer did not find any useful for further increase of plant height (Table 5). These results indicate that the combination of rhizobium and
PSB biological fertilizer could increase the efficiency of the use of nitrogen and phosphorus fertilizer in soybean plants.

Table 5. Effect of inorganic and bio fertilizers on soybean height in tidal land of Sidomulyo Village.

| Urea Kg/ha | SP36 Kg/ha | Bio fertilizer | Plant height (cm) |
|------------|------------|----------------|------------------|
| 100        | 100        | 0              | 40 DAP 60 DAP 80 DAP |
| 50         | 50         | 0              | 49.4 b 54. cd 55.8 cd |
| 25         | 25         | 0              | 48.3 b 51.8 d 53.8 d |
| 50         | 100        | Rhizobium (Rhi)| 56.1 ab 63.4 ab 63.7 ab |
| 50         | 50         | Rhi+PSB        | 54.7 ab 63.9 ab 65.2 ab |
| 50         | 50         | Rhi+PSB+Mycorrhiza | 59.8 a 65.3 a 67.2 ab |
| 50         | 25         | Rhi+PSB+Mycorrhiza | 51.2 ab 58.6 bc 60.0 bc |
| 100        | 100        | Rhi+PSB+Mycorrhiza | 59.6 a 67.0 a 67.5 a |
| LSD 5%     |            |                | 5.37 6.69 8.49 |
| CV (%)     |            |                | 6.63 7.37 9.69 |

The numbers followed by the same letter is not significantly different at the LSD level of 5%.

Tabel 6. Effect of inorganic and bio fertilizers on soybean height in tidal land of Kolam Makmur Village.

| Urea Kg/ha | SP36 Kg/ha | Bio fertilizer | Plant height (cm) |
|------------|------------|----------------|------------------|
| 100        | 100        | 0              | 40 DAP 60 DAP 80 DAP |
| 50         | 50         | 0              | 39.3 c 46.1 b 46.0 ab |
| 25         | 25         | 0              | 40.8 bc 44.5 b 44.2 b |
| 50         | 100        | Rhizobium (Rhi)| 46.5 ab 49.4 ab 52.2 ab |
| 50         | 50         | Rhi+PSB        | 48.7 a 53.0 a 55.5 a |
| 50         | 50         | Rhi+PSB+Mycorrhiza | 46.7 ab 51.4 a 52.9 ab |
| 25         | 25         | Rhi+PSB+Mycorrhiza | 44.5 ab 48.8 ab 51.3 ab |
| 100        | 100        | Rhi+PSB+Mycorrhiza | 49.6 a 52.1 a 55.0 a |
| LSD 5%     |            |                | 6.34 4.68 9.28 |
| CV (%)     |            |                | 9.58 7.75 9.02 |

The number followed by the same letter is not significantly different at the LSD level of 5%.

3.4 100 seeds weight

The 100 seeds weight in Sidomulyo and Kolam Makmur were relatively the same, which ranged from 14-17 g. In Sidomulyo the use of Urea and SP36 fertilizer at a high dose (100:100) increased the weight of 100 seeds compared to the lower doses of fertilizers. However, the highest of 100 seeds weight (17.79 g) was obtained by treatment rhizobium + PSB with medium dose fertilizer (50:50) which increased significantly compared to those of the same dose of fertilizers (50:50) or even higher (100:100) without biofertilizers (Table 6). Unlike in Sidomulyo, in Kolam Makmur the 100 seeds weight among treatments was not significantly different. The treatment of inorganic and biofertilizers had no effect on the weight of 100 seeds (Table 7).

3.5 Number of filled pods per plant

In Sidomulyo the application of high dose of Urea and SP36 fertilizers (100:100) increased the number of filled pods per plant by 25% compared to those in moderate and low fertilizers doses. The
highest number of filled pods was obtained in rhizobium + PSB biofertilizer treatment + moderate (50:50) dose fertilizer, with an increase of 68.8% from that obtained by inorganic fertilizers with the same dose (50:50). This result was similar to other biological fertilizer treatments but requires inorganic fertilizer with a higher dose so it is less efficient than the combination of Rhizobium + PSB biological fertilizer.

In Kolam Makmur, the use of inorganic fertilizers Urea and SP36 from low to high dosages did not increase the number of filled pods per plant. While the combination of Rhizobium + PSB biological fertilizer + medium dose fertilizer (50:50) had been increased the number of filled pods by 67.9% compared to the number of pods of that obtained by similar dose of inorganic fertilizers only. However, the highest number of filled pods was obtained in treatment of rhizobium + PSB + mycorrhizae + high dose fertilizer (100:100). This was 65.8% and 92.4% higher than those of fertilized by inorganic alone with the same (100:100) and moderate dose (50:50), respectively.

3.6 Seed weight per plant
In Sidomulyo, the application of Urea and SP36 at low dose (25:25) without biofertilizer produced the lowest seed weight per plant compared to other treatments. Adding the fertilizer to moderate amounts (50:50) did not increase the seeds weight. When the moderate dose fertilizers was combined with rhizobium + PSB, the 26% increase in seed weight per plant from the same fertilizer dose (50:50) without bio fertilizer, occurred. The increase in weight of the seeds was similar to that obtained in high dose (100:100) fertilizers. This shows that the combination of rhizobium + PSB biofertilizer increased the efficiency of the use of Urea and SP36 fertilizer in soybeans. The highest seed weight per plant was obtained in treatment of rhizobium + PSB + mycorrhizae + high dose fertilizer (100:100) which increased by 21.5% compared to seed weight per plant at the same fertilizer dose (100:100) without biological fertilizer (Table 6).

In Kolam Makmur, fertilizing in moderate (50:50) to high (100:100) doses did not increase the seeds weight per plant compared to that fertilized with the lowest dose (25:25). While low-dose fertilizer (25:25) + combination of rhizobium + PSB + mycorrhizae bio fertilizer produced 60% increase compared to that low-dose fertilizers (25:25) without biological fertilizer. Higher seed weight was obtained in combination treatment of rhizobium + PSB + moderate dose fertilizer (50:50) but it was not significantly different from that treated in rhizobium + PSB + Mycorrhiza with low dose fertilizer (25:25). This shows that in addition to being efficient, the use rhizobium + PSB and rhizobium + PSB + mycorrhiza was also able to increase the effectiveness of Urea and SP36 fertilizers on soybeans in both locations in tidal land.

Table 7. Effect of inorganic and bio fertilizers on 100 seeds weight, filled pods number and seed weight per plant, and seed yield of soybean in the tidal land of Sidomulyo Village

| Urea | SP36 | Bio fertilizer | 100 seeds weight(g) | filled pods number/ plant | Seed weight/plan t (g) | Seed yield (t/ha) |
|------|------|----------------|---------------------|-------------------------|----------------------|------------------|
| 100  | 100  | 0              | 16.70 bc            | 23.0 c                  | 17.56 bc             | 1.50 d           |
| 50   | 50   | 0              | 15.79 d             | 18.9 d                  | 14.56 d              | 1.52 d           |
| 25   | 25   | 0              | 14.78 e             | 18.4 d                  | 15.00 d              | 1.61 cd          |
| 50   | 100  | Rhizobium (Rhi)| 17.27 ab            | 31.3 a                  | 18.04 b              | 1.91 ab          |
| 50   | 50   | Rhi+PSB        | 17.79 a             | 31.9a                   | 18.35 b              | 2.04 a           |
| 25   | 25   | Rhi+PSB+Mycorrhiza | 16.74 bc           | 26.5 b                  | 15.57 cd             | 2.06 a           |
| 100  | 100  | Rhi+PSB+Mycorrhiza | 15.61 d            | 23.4 c                  | 15.63 cd             | 1.79 bc          |
| 16.72 bc | 33.5 a | 21.34 a | 2,13 a         |                         |                      |                  |
| LSD 5% | 0.90 | 2.5          | 2.09                | 0.21                    |                      |                  |
| CV (%) | 5.75 | 7.12      | 8.37                | 8.21                    |                      |                  |

The numbers followed by the same letter is not significantly different at the LSD level of 5%
Table 8. Effect of inorganic and bio fertilizers on 100 seeds weight, filled pods number and seed weight per plant, and seed yield of soybean in the tidal land of Kolam Makmur Village

| Urea Kg/ha | SP36 Kg/ha | Bio fertilizer | 100 seed weight (g) | filled pod number/plant | Seed weight/plant (g) | Seed yield (t/ha) |
|------------|------------|----------------|---------------------|------------------------|----------------------|------------------|
| 100        | 100        | 0              | 16.53 a             | 12.3 c                 | 11.04 c              | 0.83 cd          |
| 50         | 50         | 0              | 17.15 a             | 10.6 c                 | 10.78 c              | 0.70 d           |
| 25         | 25         | 0              | 16.98 a             | 11.0 c                 | 11.00 c              | 0.83 cd          |
| 50         | 100        | Rhizobium (Rhi)| 17.17 a             | 17.9 b                 | 18.45 a              | 1.03 b           |
| 50         | 50         | Rhi+PSB        | 17.40 a             | 17.8 b                 | 18.86 a              | 1.19 a           |
| 50         | 50         | Rhi+PSB+Mycorrhiza | 15.88 a           | 16.5 b                 | 13.52 b              | 0.96 bc          |
| 25         | 25         | Rhi+PSB+Mycorrhiza | 15.99 a           | 17.2 b                 | 17.72 a              | 0.96 bc          |
| 100        | 100        | Rhi+PSB+Mycorrhiza | 17.53 a           | 20.4 a                 | 18.99 a              | 1.24 a           |
| LSD 5%     |            | Ns             | 2.2                 | 1.67                   | 0.14                |
| CV (%)     |            | 6.32           | 7.18                | 5.55                   | 7.34                |

The numbers followed by the same letter is not significantly different at the LSD level of 5%; ns: non significant

3.7 Seed yield
In Sidomulyo and Kolam Makmur, inorganic fertilizers in the form of Urea and SP36 from low to high doses were unable to increase soybean yield. Conversely, the use of a combination of rhizobium + PSB biological fertilizer + moderate dose fertilization was able to increase soybean yield. In Sidomulyo, rhizobium + PSB + 50 kg Urea + 50 kg SP36 increased yield by 34% and 36% compared to that of (50:50) and (100:100) fertilizers without biofertilizers. The same thing in Kolam Makmur, a combination of rhizobium + PSB + 50 kg Urea + 50 kg SP36 fertilization increased yields by 70% and 43% compared to that of the same (50:50) and high dose (100:100) fertilizer dosages without biofertilizer. The addition of mycorrhiza bio fertilizer to the combination of rhizobium + PSB had no benefit for higher yield improvement. This shows that the use of rhizobium and PSB bio fertilizers, in addition to, saving the use of Urea and SP36 fertilizers, it also gave a noticeable increase in yield compared to inorganic fertilizers alone. The increase in soybean yields in both locations was supported by the increase in parameters of nodulation, plant growth, number of filled pods, seed weight per plant and weight of 100 seeds, although in Kolam Makmur Village the weight of 100 seeds was not significantly different.

4. Conclusion
At two locations of oil palm plantations on tidal lands, the combination of inorganic fertilizers and bio fertilizers was more effective than inorganic fertilizer alone in increasing nodulation, plant growth, leaf chlorophyll index, components of yield and seed yield of soybean. Inorganic fertilizers in the form of Urea and SP36 in low, moderate to high doses of 25:25, 50:50 and 100:100, were unable to increase soybean yield. Whereas rhizobium + PSB bio fertilizer + 50 kg Urea + 50 kg SP36 fertilization successfully increased yield by 34% and 36% in Sidomulyo, and 70% and 43% in Kolam Makmur compared to yields of soybean applied by similar dose of fertilizer (50:50) and higher dose (100:100) without bio fertilizers.

5. Acknowledgement
The authors would like to acknowledge Indonesian Agency for Agricultural Research and Development (IAARD) for the research funding, and also to Mr. Urip Sembodo and Prof, Arief Harsono for the support during the research.
References

[1] Harsono A, Indiati S, Suryantini, Sucahyono D, Subandi, Taufiq A 2015 Laporan Hasil Penelitian Tahun 2015 (Balitkabi: Jakarta) pp 65.

[2] Harsono A, Sriwahyuni, Suryantini D, Sucahyono, Subandi and Runik DP 201. Laporan Hasil Penelitian Tahun 2016. Badan Litbang Pertanian. Puslitbang Tanaman Pangan. (Balitkabi: Jakarta) pp 68.

[3] Gunawan I 2013 Luas Kebun Sawit Mencapai 13.5 Juta Hektare (Tempo: Jakarta).

[4] Medan Bnis 2014 Lahan sawit di Indonesia tumbuh 450.000 hektare per tahun access http://mdn. Biz.id/n/85971.

[5] Shamsuddin J, Sarwani M 2002 Symposium 17th WCSS 14-21 August 2002(Thailand).

[6] Suriadikarta DA J. Litbang Pertanian 2436-45.

[7] Babana AH, Dicko AH, Maïga K, Traoré D 2013 J. Microbiology & Microbial Res. 1(1) 1-6.

[8] Zhao W, Zhang J, Müller C, Cai Z 2017 Soil Research 56(3) 275-283

[9] Suryantini. 2011 Populasi Bakteri Pelarut Fosfat Pada Lahan masam Lampung Timur dan Banjarnegara Jawa tengah. Winarto A. Sari KP (Eds.) Prosiding Seminar Nasional. Pusat Penelitian dan Pengembangan Tanaman Pangan 189-196

[10] Gull I, Hafeez FY, Saleem M, Malik KA 2004 Aust. J. Exp. Agric. 44 623-628

[11] Panhwar QA, Radziah O, Zaharah AR, Sariah M, Razi IM 2011 J Environ Biol 32(5) 607-12

[12] Khalil S, Sultan T 2000. International Soil Sci. 1 79-87.

[13] Aftab A, Asghari B, Fatima M. 2010. Agron. Sustain. Dev. 30 (2): 487-495.

[14] German MA, Burdman S, Okon Y, Kigel J 2000 Biol. Fertil. Soils. 3 259-264.

[15] Ahsan MR, Akter M, Alam MS, Haque MMA 2012 J. Agrofor. Environ 6(1) 33-37

[16] Tran-Thi NS, Cao ND, Truong TMG and Tran TAT 2007 Omonrice 15 135-143.

[17] Yashima H, Fujikake H, Yamazaki A, Ito S, Sato T, Tewari K 2005 Soil Sci. Plant Nutr. 7 981–990.

[18] Xia X, Ma C, Dong S, Xu Y, Gong Z 2017 Soil Science and Plant Nutrition 63(5) 470–482.

[19] Nguyen VD and Cao ND 2004 Can Tho University Journal 1 98-104

[20] Yaseen T, Ali K, Munsif F, Rab A, Ahmad M, Israr M and Baraich AK 2016 Pak. J. Bot. 48(5) 2101-2107

[21] Rotaru V, Sinclair TR 2009 Environ Exp Bot 66 94-99.

[22] Yakubu H, Kwari J D, Sandabe MK 2010 Nigerian Journal of Basic and Applied Science 18(1) 19-26

[23] Argaw A, Tsigie A 2015 Chem. Biol. Technol. Agric. 2 1-13

[24] Miao SJ, Qiao YF, Han XZ, An M 2007 Pedosphere 17 36–43.