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The improvement of coastal soil fertility using soil conditioner from biocompost inoculated with phosphate-solubilizing microbes, *Bradyrhizobium* and arbuscular mycorrhizal fungi to increase soybean production

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Abstract The results showed that the application of biocompost inoculated with phosphate-solubilizing microbes (PSM), arbuscular mycorrhizal fungi (AMF) and *Bradyrhizobium* improved the soil quality which resulted to increased growth, yield and quality of soybeans. The optimum doses of biocompost in Beringin Raya Village was : 7.08 ton ha^{-1} which resulted in a 89.99% root infection, (2) 12.41 ton ha^{-1} which produced a total of 290 g of grains per plant, 14.51 ton ha^{-1} which produced 32.47 g of grains per plant, and 13.15 ton ha^{-1} which produced 1.94 kg of grains per plot. The optimum doses of biocompost in Lempuing Village were 10.5 tons ha^{-1} which resulted to 91.8% root infection, 11.74 ton ha^{-1} which produced 29.94 g of grains per plant, and 11.86 ton ha^{-1} which produced 1.80 kg of grains per plot.

Keywords: Biocompost, Coastal land, Mycorrhiza,Phosphate-solubilizing microbes, Soybean

Introduction

Indonesia has 81,000 km of beach, which is the world's second longest beach, after Canada's beach. Meanwhile, Bengkulu Province has ± 525 km of beach (Pemprov, 2015). Coastal soil is composed mainly of primary minerals, especially quartz (SiO_2), which is difficult to decompose and contain little nutrients, so this soil does not provide sufficient nutrients for plants. Its low water holding capacity also leads to nutrient leaching. In general, coastal soil has the following properties: low water content, high evapotranspiration rate, low organic matter content and low levels of nitrogen and phosphorus (Finkl, 2005).

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The utilization of coastal land in Bengkulu is limited due to restricted availability of technology and plant varieties suitable for this land. It is therefore necessary to invent a cultivation technology and create new varieties which are resistant to salt water. Some researchers have managed to cultivate plants in coastal soil such as chilli pepper (Istiyanti *et al*., 2015), peanut (Holbrook *et al*., 2008), shallot (Iriani, 2013), soybean (Bertham *et al*., 2018), and maize (Ekowati and Nasir, 2011). The results of those studies showed that coastal land is suitable for plant cultivation if there is an appropriate technology. The other technology for improving coastal soil properties is the utilization of microorganisms such as arbuscular mycorrhizal fungi, phosphate-solubilizing microbes, rhizobia and bradyrhizobia. Previous studies have shown the effectiveness of new varieties, arbuscular mycorrhizal fungi (AMF), phosphate-solubilizing fungi (PSF), and rhizobia for increasing the number and weight of soybean grains (Saptiningsih, 2007; Oktaviani *et al.*, 2014; Bertham *et al*., 2018).

Arbuscular mycorrhiza fungi (AMF) are endomycorrhizae that help increase available P for plants (Joner and Johansen, 2000). van Aarle *et al*. (2002) added that the extraradical mycelium might be involved in hyphal P acquisition. The external hyphae of mycorrhizae could release organic acids to bind with some metal cations, so the soil pH increases (Kumar *et al*., 2015). According to Keneni *et al.* (2010) phosphate-solubilizing bacteria (PSB) can dissolve phosphate ion bound to cations such as Al, Fe, Ca, and Mg and convert them into available P, which can be taken up by plants. The results of a study by Astuti *et al.* (2013) showed that phosphate-solubilizing bacteria could increase the fresh weight, height, N, and P in tomatoes. Bertham (2002) reported that the residue of *Aspergillus niger* PSF used in peanut cultivation was able to increase the yield of soybeans. Bertham (2006) successfully isolated 55 strains of bacteria from the rhizosphere of soybeans growing in Ultisol soil from three locations in Bengkulu. The 55 strains consisted of 40 strains of *Bradyrhizobium* and 15 strains of *Rhizobium.* Out of the 40 strains of *Bradyrhizobium,* two were effective in increasing the productivity of soybean varieties: Ceneng, Pangrango and Wilis under agroforest ecosystem of *Scorodocarpus borneensis* (Burm. F) and soybeans. Out of the 15 strains of *Rhizobium,* two were effective in increasing the productivity of three varieties of soybeans: Ceneng, Pangrango and Wilis in the field (Bertham and Inoriah, 2009).

In addition, the application of organic matter is absolutely necessary to improve the quality of coastal soil. The positive role of organic matter in increasing chili pepper production in coastal soil was reported by Bertham *et al.* (2018). Organic matter functions as a substrate for microbes having symbiotic relationships with plants, so the presence of organic matter attracts soil microbes and promote plant growth (Bot and Benites, 2005). One type of organic matter that can be used for improving coastal soil is biocompost which is made from coffee husk. Coffee husk is abundant in Bengkulu, especially in the Rejang Lebong District. Data from the Rejang Lebong Bureau of Statistics in 2011 showed that the coffee production was 18,480.6 tons, which presumably produced 6,468.21 tons of compost from coffee husk that was not utilized (Adnan, 2014). Afrizon (2015) reported that the nutrient content in compost made from coffee husks met the national standards (SNI 19-7030-2004) on the quality of compost. The addition of 80% coffee husk, 10% manure, 10% rice bran without sugar and bioactivator (EM4) resulted to a compost which has 2.443% N, 0.286% phosphorus (P_2O_5), 2.9% potassium (K_2O) and C:N ratio of 9.75:15.99%. The dry coffee beans has 51.2% coffee husk, which amounts to 30.222 tons per year and when composted, may substitute 339.628 tons urea, 31.116 tons SP-36 and 525.826 ton KCl. Melawati (2002) reported that a mixture of 25-50% coffee husk and manure from cattle resulted in an organic compost having good structure. Furthermore, Baon *et al.* (2005) stated that coffee husk could decompose quicker than other materials. Bertham *et al.* (2018) showed that soybeans grew well in media with compost made from coffee husks.

Soybean (*Glycine max* (L.) Merrill) is a commodity potentially grown in coastal soils. It is an important commodity in Indonesia as a source of plant protein, industrial raw material and animal feeds. The soybean productions in Indonesia from 2014-2018 were 955 to 983 metric ton with productivities of 1.551-1.444 tons ha^{-1} (Kementerian Pertanian, 2019). The need for soybeans is increasing with the increasing population and this need cannot be met by domestic production although in the last five years, there has been an increase in its production. Two of the constraints in meeting soybean self-sufficiency are the decreasing area for soybean cultivation and low productivity.

The objective were to determine the optimum doses of biocompost mixed with phosphate-solubilizing microbes (PSM), *Bradyrhizobium* and arbuscular mycorrhizal fungi (AMF) for improving the soil propertie and the effect of compost on the growth and yield of soybean at two coastal land sites.

Materials and Methods

This study was conducted from March-July 2018 in Beringin Raya Village and Lempuing Village, Bengkulu Province, Indonesia. The sites were located approximately 100 m from the shoreline and the soil was an Entisol, the texture was silty sand. The experiment was laid out in a split-plot design, the study sites were the main plots (Beringin Raya and Lempuing) and biocompost doses were the sub-plots (0 ton ha^{-1},5 tons ha^{-1},10 tons ha^{-1}, and 15 tons ha^{-1}). Each treatment was replicated four times.

The land was cleared of weeds and tilled using hoes. Plots measuring 1.5 x 3.3 meters were made at 50 centimetres apart from each other. Biocompost was then added according to the treatment allocations. The prepared soil was allowed to rest for seven days before planting. In all treatments, basic fertilizers (200 kg ha^{-1} dolomite with an active material $CaMg(CO_3)_2$, 50 kg ha^{-1} Urea, 50 kg ha^{-1} SP-36 (phosphate fertilizer) and 50 kg ha^{-1} KCl) were used. Urea was given twice: half at the time of planting and the rest when the plants were one month old. SP-36 (phosphate fertilizer) and KCl were given at the time of planting.

The soybean seeds were first inoculated with PSM, *Bradyrhizobium* and AMF. The seeds were inoculated with *Bradyrhizobium* and PSM by mixing them using 20% gum powder, while inoculation with AMF was done by adding 2.5g of inoculant into each planting hole. Before soil tillage soil sample was made from soil samples were taken and analyzed in the Soil Science Laboratory at the University of Bengkulu. After soil tillage, planting holes with a depth of 5 cm were made using a wooden digger, with a planting distance of 30 x 30 cm, so there were 60 plants in each experimental unit.

The care for the plants were: watering, dead seedling replacement, thinning, weeding and pest and disease control. Harvesting was done twice, the first one was during the vegetative phase, and the last one was during the generative phase. In the first harvest, five plants in the middle of the plot were sampled randomly and a composite soil sample was taken from each plot. The first harvesting was done on the 40^{th} day after planting or when 10% of the soybean plants were blooming. The second harvesting was when the pods had become dry and turned brown.

The variables measured were pH, KCl, organic C(%), tissue N (%), tissue P(%), the population of PSM, root inoculation (%) by AMF, dry weight of plant (grams), number of nodules, weight of nodules, number of pods per plant, number of grains per plant, weight of grains per plant, weight of 100 grains, weight of grains per plot, production, fat content of grains (%), carbohydrate content of grains (%), and protein content of grains (%). The data were analyzed using ANOVA at 5% significance level. The significantly different variables in the main plots were analyzed further using DMRT at 5% significance level, while the significantly affecting variables in subplots and interaction between plots and subplots were analyzed using orthogonal polynomials.

Results

Soil properties and effect of treatments

The analyses of variance showed that site had a highly significant effect on tissue P, total dry weight of nodules, root infection, the number of pods per plant, the weight of grains per plot, carbohydrate content, and protein content. Site also had significant effects on pH (KCl), organic C, tissue N, the dry weights of plants, the numbers of root nodules per plant, the numbers of grains per plant, and the weight of grains per plant, but it had no significant effects on the PSM population, the weight of 100 grains, or fat content. The dose of biocompost had a highly significant effect on organic C, tissue P, the number of root nodules per plant, the weight of nodules per plot, root infection, number of all pods per plant, weight of grains per plant, weight of grains per plot, protein content, and tissue N. However, had no significant effect on pH (KCl), the dry weight of grains per plant, the population of PSM, or weight of 100 grains. The interaction between site and biocompost dose had highly significant effect on root infection, and it had a significant effect on the number of grains per plant, the weight of grains per plant and the weight of grains per plot. However, the interaction between site and biocompost dose had no significant effect on pH (KCl), organic C, tissue N, tissue P, the dry weight of plants, the number of root nodules, the dry weight of nodules per plant, the population of PSM, the number of pods per plant, the weight of 100 grains, fat content, carbohydrate content or protein content of grains (Table 1).

The effects of sites on the variables observed

In comparing the sites, the results showed that the optimum doses of biocompost at the two sites were different the for variables: AMF root colonization, the number of grains per plant, the weight of grains per plant, and the weight of grains per plot. This difference was due to the differences in soil characteristics between the two sites. As a result, the need for organic matter input in each site was different.

The effect of the combination of site and biocompost dose

The orthogonal polynomials showed that biocompost dose and root colonization of AMF in Beringin Raya had a quadratic correlation with a regression equation as follows:$y = -0.125x^2 + 3.325x + 72.875$, and R^2=0.9517. In Lempuing these two variables had a quadratic correlation with the regression equation: y= $-0.2x^2 + 4.2x + 69.75$, and R^2=0.9956. These mean that the increase of biocompost dose led to the increase of root colonization until an optimum point, above which the opposite correlation occurred. The orthogonal polynomial equation showed that the optimum dose of biocompost in Beringin Raya was 7.08 ton ha^{-1}, resulting in a root colonization of 89.99%, while in Lempuing it was 10.5 ton ha^{-1}, resulting in root colonization of 91.8 % (Figure 1.a).

The biocompost dose and number of grains per plant in Beringin Raya had a quadratic correlation with regression equation as follows: y= $-0.375x^2 + 9.305x + 232.2878$, and R^2 = 0.9717. This means that the increase of biocompost dose led to the increase number of grains per plant until the optimum point, above which the opposite correlation occurred. The optimum dose of biocompost in Beringin Raya was 12.41 ton ha^{-1}, resulting in 290 grains per plant. In Lempuing, biocompost dose and number of grains per plant had linear correlation with the following equation:y = 1.35x + 234.63, and R^2 = 0.4438. This means that the dose given in Lempuing did not reach the optimum level, so the increase of biocompost dose led to the increase of number of grains per plant (Figure 1.b).

Table 1. The summary of ANOVA results

Variables	Calculated F			Coefficient of variation (%)
	Site	Biocompost dose	Interaction	
pH KCl	15.73*	2.59 ^{ns}	1.53 ^{ns}	2.12
Organic C (%)	33.29*	12.55**	1.07 ^{ns}	6.02
Tissue N (%)	10.57*	6.16*	0.48 ^{ns}	10.46
Tissue P (%)	41.28**	12.99**	0.52^{ns}	6.49
Dry weight of plants (g)	25.58*	2.74 ^{ns}	0.07 ^{ns}	12.03
Number of all nodules per plant	12.04*	6.01**	0.17^{ns}	16.88
Total dry weight of nodules (g)	55.02**	7.29**	0.72^{ns}	1.29
Population of PSM(10^4 cfu g^{-1})	1.18 ^{ns}	2.38 ^{ns}	0.09 ^{ns}	18.35
Root inoculation (%)	75.00**	55.36**	5.18**	4.49
Number of pods per plant	201.84**	13.93**	0.35 ^{ns}	4.76
Number of grains per plant (g)	10.30*	11.49**	3.68*	7.21
Weight of grains per plant (g)	26.34*	38.72**	3.23*	3.05
Weight of 100 grains (g)	5.44 ^{ns}	0.25 ^{ns}	0.16 ^{ns}	4.18
Weight of grains per plot (kg)	89.75**	45.45**	3,78*	2.84
Fat content (%)	0.04 ^{ns}	1.74 ^{ns}	2,19 ^{ns}	9.97
Carbohydrate content (%)	411.16**	0.78 ^{ns}	1.79 ^{ns}	9.46
Protein content (%)	295.85**	7.72**	2.49 ^{ns}	6.26

Notes : **=highly significant *=significant, ^{ns}=not significant

Table 2. The mean value of variables observed in two sites of study

Variables	Sites	
	Beringin Raya	Lempuing
pH (KCl)	5.84 a	5.67 b
Organic C (%)	2.45 a	2.32 b
Tissue N (%)	3.97 a	3.53 b
Tissue P (%)	0.98 a	0.64 b
Dry weight of plants (g)	10.50 a	9.08 b
Weight of nodules (g)	0.09 a	0.07 b
Number of nodules (g)	17.44a	13.00a
Population of PSM (10^4 cfu g^{-1})	158.39a	140.02a
Root infection (%)	90.00 a	83.75 b
Number of pods	167.53 a	160.31 b
Number of grains per plant (g)	261.75 a	244.75 b
Weight of grains per plant (%)	30.26 a	28.82 b
Weight of 100 grains (%)	15.84a	15.59a
Weight of grains per plot (kg)	1.83 a	1.73 b
Fat content (%)	16.38a	16.51a
Carbohydrate content (%)	22.88 b	30.50 a
Protein content (%)	36.55 a	25.81 b

Note: the numbers in the same row followed by different letters are significantly different according to DMRT at 5% significant level.

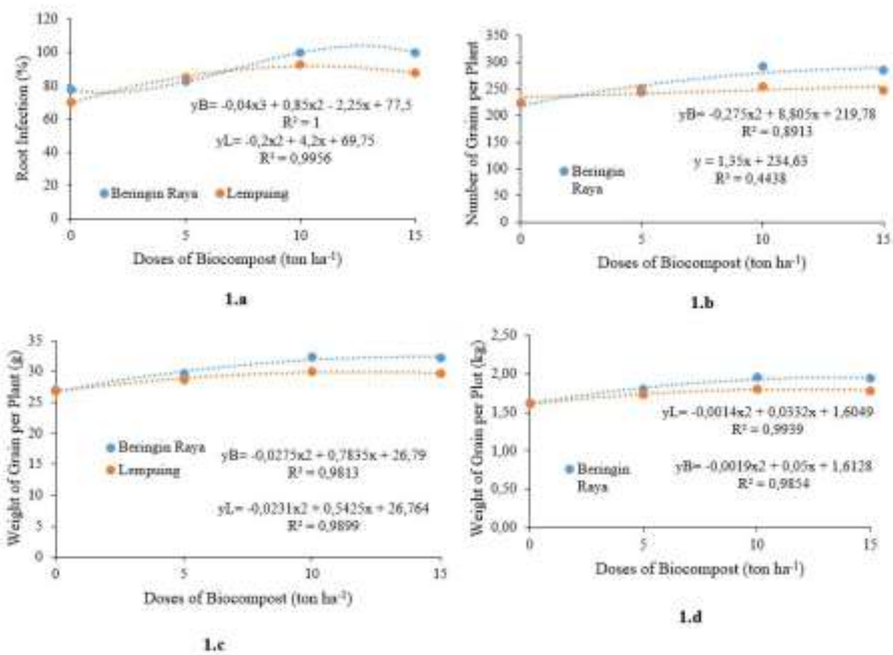

Figure 1. The correlation between treatment combinations (site and dose of biocompost) for root inoculation (1.a), number of grains per plant (1.b), weight of grains per plant (1.c) and weight of grains per plot (1.d)

The orthogonal polynomials showed that biocompost dose and the weight of grains per plant in Beringin Raya and Lempuing had quadratic correlation. The regression equation for Beringin Raya was $y = -0.0275x^2 + 0.7835x + 26.79$and $R^2 = 9813$, while in Lempuing it was $y = -0.0231x^2 + 0.5425x + 26.764$and $R^2 = 9899$. This means that the increase of biocompost dose caused an increase in weight of grains per plant until the optimum point, above which the opposite correlation occurred. The optimum dose in Beringin Raya was 14.51 ton ha^{-1} and produced 32.47 g of grains per plant, while in Lempuing it was 11.74 ton ha^{-1} and resulted in 29.94 g of grains per plant (Figure 1.c).

The orthogonal polynomials showed that biocompost dose and weight of grains per plot in Beringin Raya and Lempuing had quadratic correlation. The regression equation for Beringin Raya was $y = -0.0019x^2 + 0.05x + 1.6128$ and $R^2 = 9854$, while in Lempuing , it was $y = -0.0014x^2 + 0.0332x + 1.6049$ and $R^2 = 9854$. This means that the increase of biocompost dose caused an increase in the weight of grains per plot until the optimum point, above which the opposite correlation occurred. The optimum dose in Beringin Raya was 13.15 ton ha^{-1}, which produced 1.94 kg of grains per plot, while in Lempuing it was 11.86 ton ha^{-1}, which resulted 1.80 kg of grains per plot (Figure 1.d).

The effects of biocompost dose on variables observed

The orthogonal polynomials showed that biocompost dose had linear correlation with organic C ($y = 0.0263x + 2.1916$ and $R^2 = 0.8878$), tissue N ($y = 0.0436x + 3.4222$ and $R^2 = 0.6668$), number of nodules ($y = 0.6225x + 10.55$and $R^2 = 0.7077$), number of grains per plant ($y = 3.015x + 230.64$ and $R^2 = 0.7901$), and protein content ($y = 0.2962x + 28.956$ and $R^2 = 0.9917$) (Figure 2).

The orthogonal polynomials in Figure 3 showed that biocompost dose had quadratic correlation with tissue P ($y = -0.0047x^2 + 0.1088x + 0.4057$and $R^2 = 0.9217$), the dry weight of plants ($y = -0.4971x^2 + 10.829x + 43.318$ and $R^2 = 0.9548$), the number of pods ($y = -0.1381x^2 + 3.4856x + 149.87$ and $R^2 = 0.9283$), root infection ($y = -0.125x^2 + 3.325x + 72.875$ $R^2 = 0.9517$), weight of grains per plant ($y = -0.0253x^2 + 0.663x + 26.777$ and $R^2 = 0.9846$) and weight of grains per plot ($y = -0.0016x^2 + 0.0416x + 1.6088$ and $R^2 = 0.9888$). Based on the regression equation, the optimum dose of biocompost for tissue-P was13.6 ton ha^{-1}, which produced a tissue-P of 1.14%. The optimum dose of biocompost for the for weight of nodules per plant was 10.89 ton ha^{-1}, and than dose produced 10.29 mg of nodules per plant. The optimum dose of biocompost for number of pods was 12.61 ton ha^{-1}, which produced 171.86 pods, and for root infection, it was 13.3 ton ha^{-1}, which produced 94.99%

infection. The optimim dose of biocompost for the weight of grains per plant was 13.10 ton ha^{-1}, which resulted in 31.12 g of grains, and for the weight of grains per plot, it was 13 ton ha^{-1}, and produced 1.88 kg of grains.

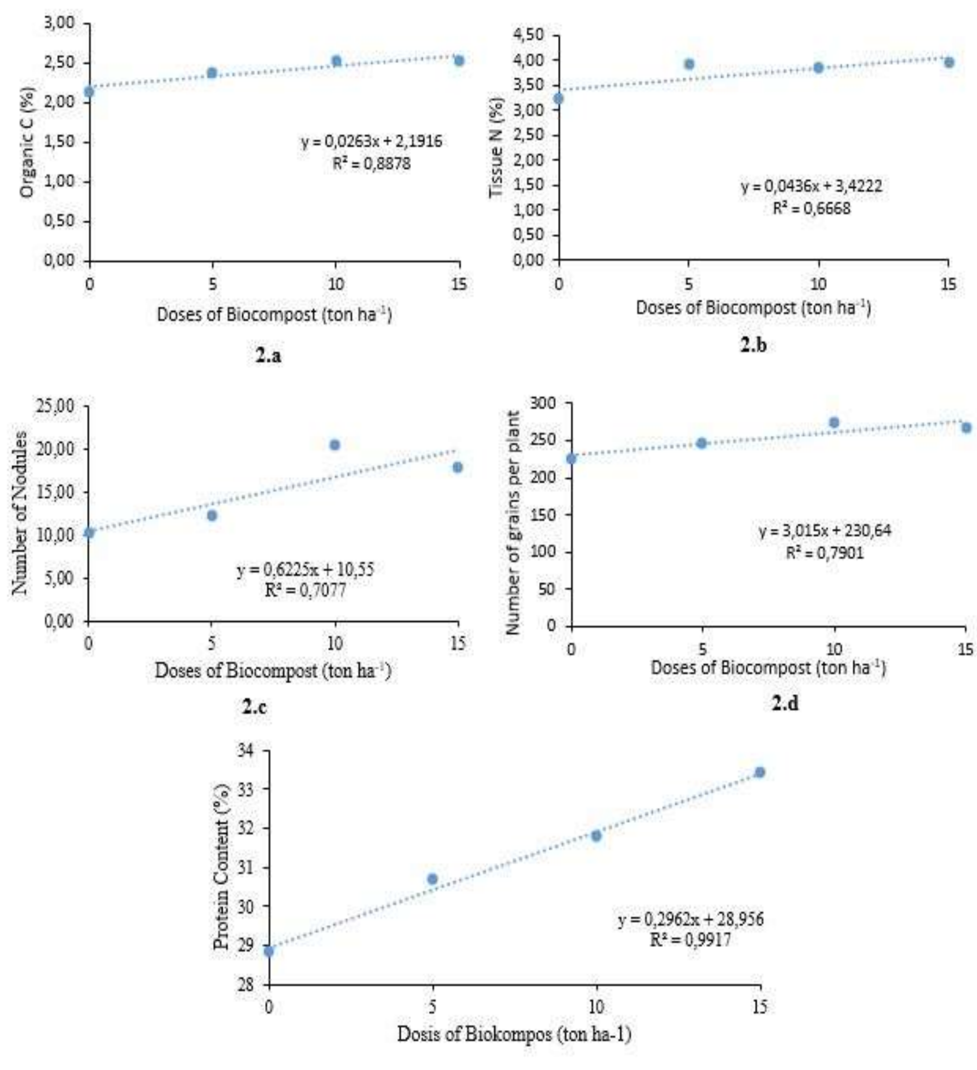

Figure 2. Correlation between biocompost dose and organic C (2.a), tissue N (2.b), number of nodules (2.c), number of grains per plant (2.d), and protein content (2.e)

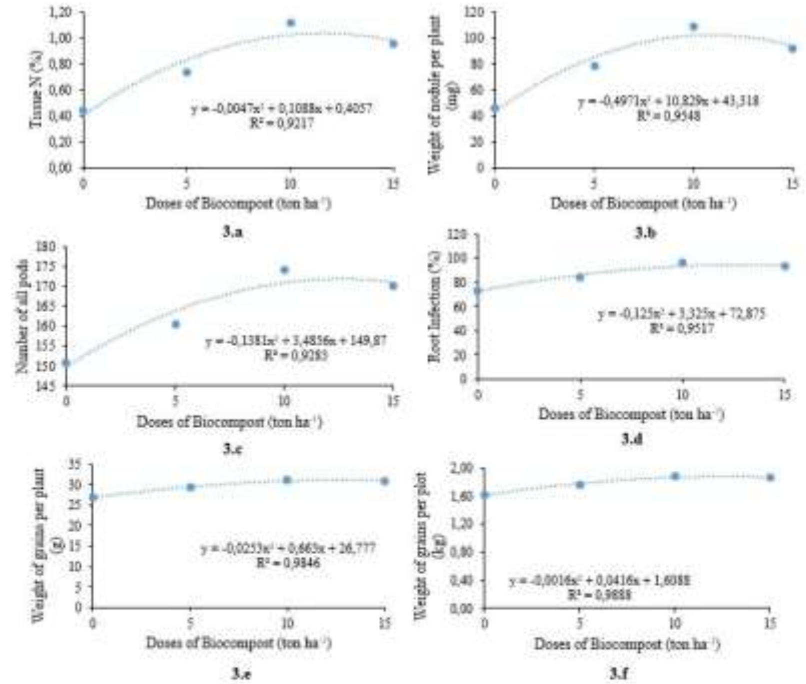

Figure 3. Regression between biocompost dose and tissue P (3.a), weight of nodules per plant (3.b), number of all pods (3.c), root (3.d), weight of grains per plant (3.e), weight of grains per plot (3.f)

Discussion

The coastal land for this study was Alluvial Marine (Entisol). The soil in Beringin Raya had the following properties: pH (H_2O) of 6.1 and pH (KCl) of 5.7, total N 0.13%, P_2O_5 of 4.31 ppm, available K of 0.10 me/100 g, and organic C of 2.03 %. The soil texture was silty sand and had 89.2% sand, 1.62% silt and 9.18% clay. The soil properties in Lempuing were as follows: pH (H_2O) of 6.0 and pH (KCl) of 5.5, total N of 0.25%, P_2O_5 of 4.31 ppm, available K of 0.10 me/100 g, and organic C of 2.03%. The soil texture was silty sand and had 93.23 % of sand, 2.52% silt and 4.25% clay.

One observed result in this research was the difference in soil characteristics and fertility in the two sites studied. These results were in line with the results of Barus *et al.* (2013) who showed that the interaction between the soil types and compost addition affected the growth of corns. According to Pinatih *et al.* (2015), the need for nutrients for plant growth and production is influenced by the ability of the soil to supply the nutrients for plants. The decline of soil fertility affects its productivity, so addition of nutrients through fertilization is necessary in order to get high agricultural production.

Soil characteristics, together with other factors, determine the success of plant cultivation. The soil analyses in the beginning of this study showed that Beringin Raya had better soil fertility than Lempuing. Therefore, the results showed that the same doses of biocompost in Beringin Raya resulted in higher pH (KCl), andorganic C than in Lempuing, which in turn led to the higher uptake of N and P by the plants. The study of Afandi *et al.* (2015) showed that the increase of organic content in an Entisol soil increased the uptake of N and P in sweet potatoes. In addition, Lubis *et al*. (2015) reported that with increased pH of soil significantly increased the N content in plants, in addition to the N and P uptake by varieties of soybeans in an Inseptisol soil in a screen house.

Soil pH and organic C also affect soil microbial activities. This was evident in this study, which showed that the weight and number of nodules, population of PSM, and root infection in Beringin Raya were higher than those in Lempuing were. Similar results have been reported by previous studies showing that higher pH led to a higher percentage of root colonization by mycorrhizae (Saputra *et al.*, 2015) and a higher number and weight of root nodules (Murni, 2009). The increased microbial activities also increased the N and P uptake by plants as shown in the study by Bertham and Inoriah (2009) in which *Rhizobium* and phosphate-solubilizing fungi affected the dry weight of plants, P uptake, and the number of pods and nodules of plants. The external hyphae of mycorrhizae help bring water and nutrients into the rhizosphere. Meanwhile, *Rhizobium* can increase the availability and uptake of N in soil and the plant growth hormone IAA gibberellin which can improve the growth of soybeans (Novriani, 2011). Additionally, according to Suliasih *et al.* (2010) the main role of PSM is its effect on the metal bonding with phosphates in soil. These microbes are able to release organic acids, such as formic acid, acetic acid, propanoic acid, lactic acid, glycolic acid, fumaric acid, succinic acid which form chelates with cations bounded with P so that $H_2PO_4^-$ becomes free and available for plant uptake.

The improvement of soil chemical and biological properties leads to the improvement of nutrient uptake, which in turn improves the growth of soybeans. This was evident in this study showing that soil in Beringin Raya produced better growth of soybeans than that in Lempuing.

The dose of biocompost had linear correlation with organic C, tissue-N, the number of nodules, the number of grains per plant, and protein content. This means that the biocompost doses in this study did not reach optimum level. Therefore, the increase in the dose led to the increase of organic C, N tissue, the number of nodules, the number of grains per plant, and protein content. These results were in agreement with those of Valentiah *et al.* (2015) who showed that the addition of coffee husk had a significant effect on soil properties, namely increasing organic C, total N, P and K. Pujiyanto (2007) stated that coffee husk can be used as a natural soil ameliorant to improve the soil carrying capacity and plant production.

The application of coffee husk increased the availability of organic matter in the soil which in turn increase *Rhizobium's* activities (Suryantini, 2012), as shown in the increase of N uptake by plants in this study. As a result, the number of grains per plant increased. According to Amir *et al.* (2015), translocation of N from root nodules to all plant tissues increases tissue N concentration and assimilatory nitrate reductase (ANR) which increases N uptake.

Nitrogen is the raw material for plant protein. The higher N uptake by plants, the higher their nitrogen content will be. It was evident in this study that protein content in soybean grains increased with the increasing nitrogen uptake. This was supported by the study of Indradewa *et al*. (2004) who showed that the weight of nitrogen in leaves and grains, which was 0.93% and 0.70% respectively, had correlated with the activity of nitrate reductase.

The application of biocompost at different doses had a quadratic correlation with tissue P, the dry weight of plants, the number of pods, root infection, the weight of grains per plant, and the weight per of grains per plot. These data showed that the optimum dose of biocompost had been passed, meaning that the tissue P, dry weight of plants, number of pods, root infection, weight of grains per plant, and weight of grains per plot increased with the increasing dose of biocompost until the optimum dose; then, the value of these variables declined with the increasing dose.

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