Bulgarian Journal of Agricultural Science, 23 (No 4) 2017, 596–603
Agricultural Academy

THE EFFECT OF ARBUSCULAR MYCORRHIZA AND ORGANIC MANURE ON SOYBEAN GROWTH AND NUTRIENT CONTENT IN INDONESIA

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Abstract

Samanhudi, S., A. Yunus, B. Pujiasmanto, V. R. Cahyani and D. S. Lestariana, 2017. The effect of arbuscular mycorrhiza and organic manure on soybean growth and nutrient content in Indonesia. *Bulg. J. Agric. Sci.*, 23 (4): 596–603

Soybean productivity can be increased by optimalizing marginal land (vertisols) to enlarge harvesting area. Application of biological fertilizer (*Rhizobium* and mycorrhizal inoculant) and organic fertilizer/manure (cow/goat/quail manure, and straw compost) are efforts to minimize external input to increase the use of marginal land to get high productivity. Application of organic manure that is enriched by straw compost and biological fertilizer (arbuscular mycorrhiza inoculation) could affect soybean growth and nutrient content on vertisols. Field experiment was done with two levels of mycorrhizal inoculation (without inoculation+10 g mycorrhizal inoculation per plant) and five levels of manure+straw compost (without manure; 10 t Ha^{-1} cow manure+5 t Ha^{-1} straw compost; 10 t Ha^{-1} cow manure+5 t Ha^{-1} straw compost; 10 t Ha^{-1} quail manure+5 t Ha^{-1} straw compost; 5 t Ha^{-1} straw compost). Application of mycorrhizal inoculation and manure treatment significantly affected on dry mass parameter, while the trends on root length, chlorophyll content, seed wet weight, protein content, total nodules, mycorrhizal-root colonization, nitrogen uptake and phosphorus uptake were higher when soybean inoculated with mycorrhizal+ manure+straw compost. We concluded that mycorrhizal inoculation incorporated manure enriched straw compost can be applied on soybean low input farming systems to support Low External Input Sustainable Agriculture Programme/LEISA.

Key words: soybean; arbuscular mycorrhiza; organic manure; vertisols
Abbreviations: AM – Arbuscular Mycorrhizal, SOC – Soil Organic Carbon, PGPR – Plant Growth Promoting Rhizobacteria

Introduction

Soybean productivity in Indonesia still need more improvement in order to meet the demand as import activities in soybean keeps increasing fulfill the domestic needs. Land resources is one of the factors that determine the success of agricultural activities. The conversion of agricultural land to residential and industrial interests will reduce fertile land for agricultural activities. So that the utilization of marginal land agricultural can be an alternative way to increase domestic soybean production.

Vertisols have the capacity to swell and shrink, induce cracks in the upper parts of the soil and distinctive soil structure throughout the soil. The formation of these specific features are caused by a heavy texture, a dominance of swelling clay in the fine fraction and marked changes in moisture content (Dengiz et al., 2012).

Application of organic amended soil recommended as a viable option to maintain good soil tilth compared with mineral fertilizers (Eusufzai and Fujii, 2012). Organic matter makes vertisols could accumulate more carbon than soil under con-

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tinous moist condition through the influence of damping and drainage on soil aggregation (Bravo-Garza and Bryan, 2005). Beside that low inputs of organic materials and excessive use of mineral fertilizers have contributed to a general reduction in soil organic carbon (SOC) (Zhang et al., 2015).

Soil can be improved using organic matter as known as organic fertilizer and biological fertilizer. Organic fertilizer can be cattle manure (cow manure and goat manure), poultry manure (quail manure), and farmyard manure (straw compost). Cattle manure can increase soil essential nutrient include Ca, Mg, K, and P (Wortman et al., 2011). The poultry manure is relatively a cheap source that cointain both macro nutrients (N, P, K, Ca, Mg, S) and micronutrients (Cu, Fe, Mn, B), also can increase soil carbon, N content, soil porosity, and soil microbial activity as well (Gosh et al., 2004). Straw incorporation is an effective practice for the improving of soil aggregate structure and stability (Zhang et al., 2014).

Rhizobium and arbuscular mycorrhiza both are kind of biological fertilizer. Soybean plants use atmospheric dinitrogen (N_2) by nitrogen fixation of root nodules associated with soil bacteria rhizobia (Ohyama et al., 2011), *Rhizobium japonicum* (Pierce and Bauer, 1983). Dinitrogen fixation by rhizobia provides nitrogen nutrition in a highly sustainable and economically competitive way thus contributing to environmentally agricultural production as well as high quality crop products (Vollmann, 2011). These bacteria can promote plant growth by regulating nutritional and hormonal balance, producing plant growth regulator, solubilizing nutrients and including resistance against plant pathogens (Nadeem et al., 2014).

Arbuscular mycorrhizal (AM) fungi are ubiquitous root symbionts that provide plant with nutrients and others benefits (Denison and Kiers, 2011) such as increase P uptake of soybean (Abdel-Fattah et al, 2014) by increasing enzim activities such as phosphatase (Mar Vazquez et al., 2000); enhance salinity tolerant of soybean plant (Younesi et al., 2013); increase soil aggregation (Bethlenfalvay and Schuepp, 1994; Borie et al, 2008; Leifheit et al, 2014); protect root from pathogen (Sikes, 2010); affect plant in water relation; and response to drought (Auge, 2004; Finlay, 2004). The combination application of fungi and Plant Growth Promoting Rhizobacteria (PGPR) could be meaningful for sustainable agriculture (Bashan, 1998; Ordookhani et al*.,* 2010; Zhuang et al., 2007).

Both organic and biological fertilizer are expected to increase land productivity that will directly impact on rising crop productivity and environmental sustainability to support agricultural programs LEISA. We now aim to study how the organic fertilizer (manure) enriched by straw compost and biological fertilizer (arbuscular mycorrhiza inoculation) could influence soybean growth and nutrient content grown in vertisols.

Materials and Methods

Plant Materials and Experimental Design

Materials used in this study were soybean of Grobogan cultivar from and Tuber Crops Research Institute Malang; *Rhizobium japonicum* from Microbiology Laboratory of Universitas Gadjah Mada; arbuscular mycorrhizal inoculum from Agency for the Assessment and Application of Technology Serpong; cow manure, goat manure, quail manure, and straw compost.

The experiment was conducted using a randomized block design arranged in a factorial. The first factor is mycorrhizal treatment which consists of two levels (without mycorrhiza inoculation (M0) and mycorrhizal inoculation 10 g/plant (M1). The second factor is various types of manure which are without manure (P0); cow manure 10 t Ha^{-1} enrichment straw compost 5 t Ha^{-1} (P1); goat manure 10 t Ha^{-1} enrichment straw compost 5 t Ha^{-1} (P2); quail manure 10 t Ha^{-1} enrichment straw compost 5 t Ha^{-1} (P3); and straw compost 5 t Ha^{-1} (P4). So we got 10 combinations of treatment and each treatment was repeated 3 times, thus there are 30 experimental plots.

Soil Tillage and Analysis

Soil tillage was done two weeks before planting, as the land was cleaned from the weeds and made plot with each plot is 200 cm x 200 cm. Soil sampling is analyzed before soil tillage and after harvesting period. Soil samples were taken at five points randomly sampled on area which is used. Soil samples were collected in a depth of 10 cm (top layer of soil). Five samples taken will then be composited. Approximately 200 g of soil were taken for analysis in the Chemistry and Fertility Soil Laboratory Faculty of Agriculture, Universitas Sebelas Maret on a parameters of total nitrogen, phosphorus available, potassium exchanged, organic carbon, organic matter, and C/N ratio.

The Organic Matter Analysis and Application

Organic matter which consist of manure and straw compost is analysed in the Chemistry and Fertility Soil Laboratory Faculty of Agriculture, Universitas Sebelas Maret on parameters of tested on the total nitrogen, phosphorus available, potassium exchanged, organic carbon, organic matter, and C/N ratio before applied to the land. The application of manure enrichment straw compost is given after soil tillage and two weeks before the seeds are planted. It is spreaded with appropriate doses of the each treatment on the plots treated with appropriate doses of the each treatment.

Seed Planting with Inoculation of Rhizobium Japonicum and Arbuscula Mycorrhiza

Rhizobium was inoculated in all soybean seeds. Seeds were moistened with water, drained, then added with the inoculant. Before application, the treatment of arbuscular mycorrhizal inoculation were tested first in the laboratory in parameters of spores density, kind, and viability. The mycorrhiza spores were applied at 30 spore each seed (approximately 300 spores/100 g media). 10 g of inoculant mycorrhiza arbuscular incorporated into the planting hole and the seeds that has been given with *Rhizobium* inoculants were placed into the planting hole, then covered the soil. The seeds which will be planted in each plot were treated with a spacing of 25 cm x 25 cm.

Measurement of Total Dry Mass

Total dry mass of the plant carried out at the time of maximum vegetative, with ovened all components on a fresh weight of the plant at a temperature of 80°C until it reaches a constant weight (48 hours).

Measurement of Root Length

Root length is measured from bottom stem until up root with ruler on centimeters of each plant (Figure 1).

Measurement Chlorophyll Content

The chlorophyll content in the leaves was analyzed from the fresh leaves by cutting trifoliate leaves 1 cm^2. The leaves were grinded in a mortar by adding 10 cc of methanol then transferred into Erlenmeyer and stored in the refrigerator for 24 h. 1 cc of the supernatant was taken then added with 10 ml of methanol. The observation was carried out using a spectrophotometer. The calculation of chlorophyll content:

chlorophyll a = $11{,}75A_{666}-2{,}35A_{653}$

chlorophyll b = $18{,}61A_{653}-3{,}96\ A_{666}$ (Lichtenthaler and Wellburn, 1983)

The content of chlorophyll is total chlorophyll (chlorophyll a + chlorophyll b) each cm^2 of leaf area.

Mycorrhizal Colonization (%)

Staining method was adopted from Phillip and Hyman (1970). Coloring phases samples were taken from root fibers on the left side and right side of the main stem that have been washed and soaked using 70% alcohol. In the laboratory the roots were soaked in 10% KOH until white or yellow translucent then washed with water. After that, the roots were soaked with 2% HCl for 24 hours then washed with water. Next step, the roots were soaked with 0.05% trypan blue staining until the roots color change into blue. The observation was carried out by cutting the roots. The percentage of mycorrhizal colonization is calculated with following formula:

$$\frac{\text{infection root}}{\text{total roots}} \times 100\%$$ (Younesi et al, 2013)

Measurement of Total Nodules

Total nodules were measured by calculated the number of root nodules each plant.

Measurement Protein Content in the Seeds, Nitrogen Uptake, and Phosphorus Uptake

Phosphorus uptake was analyzed by spectrophotometry method. While seed protein content and nitrogen uptake was analyzed using Kjeldhal method.

Statistical Analysis

Data were analyzed using statistical analysis two factor of variance (ANOVA). Means were separated by Duncan's Multiple Range Test.

Results

The soil was first analyzed before planting by taking five points of sample in 0-20 cm soil depth. The same thing was done to Cow manure, goat manure, quail manure, and straw compost to know the manure nutrient content. the primarily parameters were analyzed both soil sample and manure sample are macro nutrient content such as N, P, K, C-organic content, and C/N. Vertisols used as field trials has the characters of medium Nitrogen and Phosphorus, and low Potassium (Table 1). Manure quail has the highest N, P, K and the lowest C/N ratio content among others manure.

Plant dry mass is influenced by mycorrhizal inoculation and manure (Table 2). The highest dry mass and root length of the soybean were obtained by mycorrhizal soybean+10 t Ha^{-1} quail manure+5 t Ha^{-1} straw compost, which are 16.03 g and 26.73 cm respectively. Even though the highest chlorophyll content was obtained by mycorrhizal soybean+10 t Ha^{-1} cow manure+5 t Ha^{-1} straw compost which is 94.9/cm^2 leaf area but it is not significantly different especially with mycorrhizal soybean+10 t Ha^{-1} quail manure+5 t Ha^{-1} straw compost.

Mycorrhizal and manure treatment has no significant effect to the root length. It assumes that beside nutrient content, endogenous factors also take a part in affecting plant morphology. Soybean root length is growing better with mycorrhizal inoculation than non-mycorrhizal (see Table 2 and Figure 2). Mycorrhizal influences system of roots by increasing root branches so root area is wider.

Soybean plants were harvested at 85 days after plant

Table 1
Soil and manure characteristic under study

Number	Analysis	Soil pre-planting (0-20 cm)	Goat manure	Cow manure	Quail manure	Straw compost
1	N total	0.13 %	1.58%	1.02%	2.63%	1.26%
2	P_2O_5	9.41 ppm	1.50%	1.10%	2.50%	0.95%
3	K_2O	0.23me%	1.25%	1.08%	1.72%	1.56%
4	C-organic	1.50%	19.04%	13.51%	20.48%	23.80%
5	C/N ratio	11.54	12.05	13.25	7.79	18.89

Analyzed by: Chemistry and Fertility Soil Laboratory, Faculty of Agriculture, Universitas Sebelas Maret

Table 2
Effect of mycorrhizal inoculation incorporated with manure enriched by straw compost on soybean growth parameters (dry mass, length root, and chlorophyll content) grown in vertisols

Treatments	Dry mass (g)	Root length (cm)	Chlorophyll content (/cm^2 leaf area)
Mycorrhizal (M1)			
P0	10.02 ab	21.87 ab	81.95 a
P1	9.69 ab	23.17 ab	94.90 a
P2	10.96 ab	24.27 ab	78.01 a
P3	16.03 c	26.73 b	92.50 a
P4	11.27 ab	21.90 ab	83.79 a
Non-mycorrhizal (M0)			
P0	7.27 a	19.13 a	68.78 a
P1	8.01 ab	22.10 ab	58.59 a
P2	10.83 ab	23.30 ab	81.36 a
P3	11.95 b	25.87 ab	85.98 a
P4	8.27 a b	21.43 ab	88.11 a
Significance			
Mycorrhizal	*	ns	ns
Manure	*	ns	ns
Mycorrhizal x Manure	ns	ns	ns

Two-way analysis of variance (ANOVA) was done using Duncan's Multiple Range Test (DMRT) (95% significance level) to determine the main difference among treatments
* Significance by analysis of variance $P < 0.05$; ns: not significant
P0: without manure; P1: 10 t Ha^{-1} cow manure enriched by 5 t Ha^{-1} straw compost, P2: 10 t Ha^{-1} goat manure enriched by 5 t Ha^{-1} straw compost ; P3: 10 t Ha^{-1} quail manure enriched by 5 t Ha^{-1} straw compost ; P4: 5 tonHa^{-1} straw compost

when plants start having brown pods and falling yellow leaves (Figure 2). Mycorrhizal and manure did not give significant influenced on soybean yield. Table 3 shows that the average of soybean fresh weight and protein content on mycorrhizal soybean incorporated manure enriched straw compost is higher than non-mycorrhizal soybean incorporated manure enriched straw compost.

Mycorrhizal inoculation have significant effect on the root-

a)

b)

Fig. 1. Soyben root length (a) with mycorrhiza inoculation (M1); (b) non mycorrhizal inoculation (M0). Both incorporated manure enriched straw compost

mycorrhizal colonization. Nevertheless the average of total nodules, mycorrhizal colonization, nitrogen uptake, and phosphorus uptake on soybean with mycorrhizal incorporated with manure enriched by straw compost is higher than soybean non-mycorrhizal inoculation incorporated with manure enriched by straw compost (Table 4).

Discussion

In this study, the source of NPK nutrients were provided by the application of biological fertilizer (Rhizobium and mycorrhizal) and organic fertilizer (kind of manure which is enriched with straw compost). The application of mycorrhizal inoculation incorporated with kinds of manure enriched by straw compost showed a positive response to the content of nutrients available

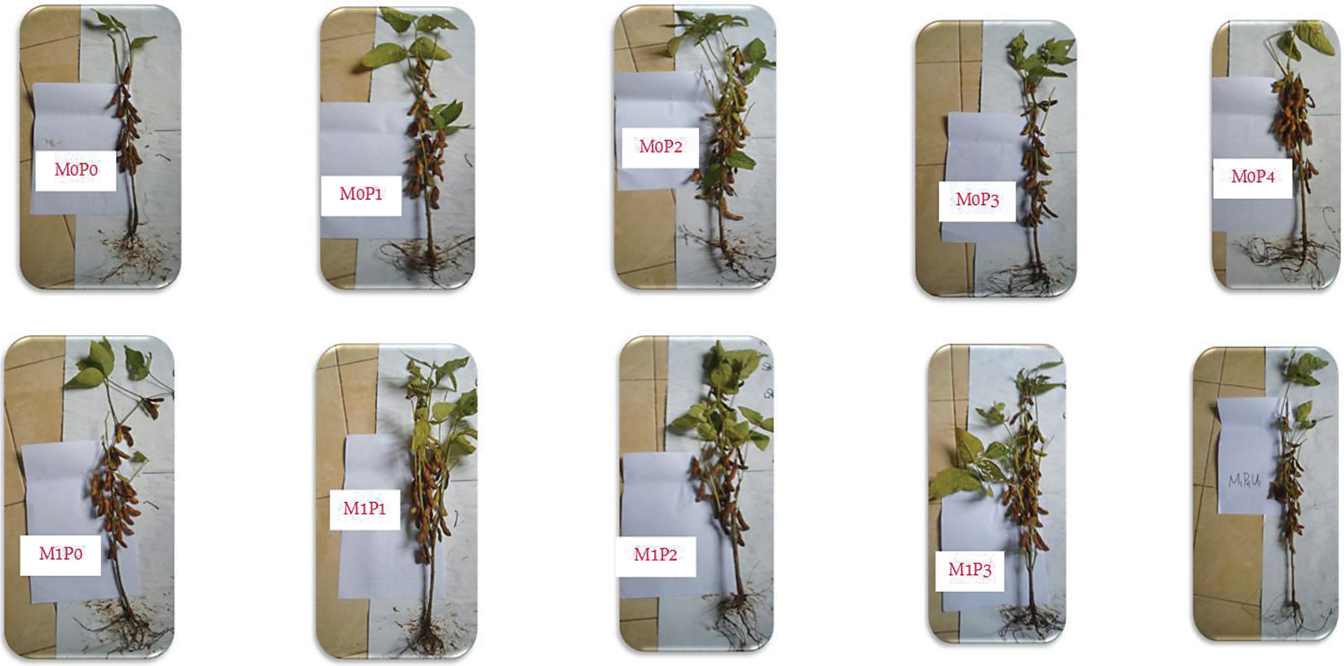

Fig. 2. Soybean growth performance in all treatments: M0 = non-mycorrhizal; M1 = with mycorrhizal; P0 = without manure; P1 = 10 t Ha^{-1} cow manure+5 t Ha^{-1} straw compost; P2 = 10 t Ha^{-1} goat manure+5 t Ha^{-1} straw compost; P3 = 10 t Ha^{-1} quail manure+5 t Ha^{-1} straw compost; P4 = 5 t Ha^{-1} straw compost

Table 3

Effect of mycorrhizal inoculation incorporated with manure enriched by straw compost on soybean yield parameters (seed wet weight, filled pods each plant, and protein content) grown in vertisols

Treatments	Seed wet weight per plant (g)	Percentage of filled pods per plant (%).	Seed protein content (%)
Mycorrhizal (M1)			
P0	23.2 b	69.54 a	43.76 a
P1	21.27 ab	81.63 a	43.44 a
P2	16.67 ab	66.58 a	45.37 a
P3	21.93 ab	68.93 a	42.96 a
P4	19.00 ab	73.19 a	46.29 a
Non-mycorrhizal (M0)			
P0	14.07 a	78.44 a	44.65 a
P1	17.60 ab	80.86 a	43.60 a
P2	18.60 ab	77.77 a	44.09 a
P3	21.73 ab	78.49 a	46.77 a
P4	18.33 ab	82.10 a	38.66 a
Significance			
Mycorrhizal	ns	ns	ns
Manure	ns	ns	ns
Mycorrhizal x Manure	ns	ns	ns

Two-way analysis of variance (ANOVA) was done using Duncan's Multiple Range Test (DMRT) (95% significance level) to determine the main difference among treatments. ns: not significant by analysis of variance $P<0.05$; P0: without manure; P1: 10 t Ha^{-1} cow manure enriched by 5 t Ha^{-1} straw compost, P2: 10 t Ha^{-1} goat manure enriched by 5 t Ha^{-1} straw compost, P3: 10 t Ha^{-1} quail manure enriched by 5 t Ha^{-1} straw compost ; P4: 5 t Ha^{-1} straw compost

Table 4
Effect of mycorrhizal inoculation incorporated with manure enriched by straw compost on soybean ineffectiveness and effectiveness nutrient uptake grown in vertisol

Treatments	Total nodules per plant	Mycorrhizal-root colonization (%)	Nitrogen uptake (%)	Phosphorus uptake (%)
Mycorrhizal (M1)				
P0	18.00 b	31.76 a	4.82 bc	0.49 a
P1	13.00 ab	56.33 a	3.52 a	0.46 a
P2	12.00 ab	39.00 a	4.03 ab	0.54 a
P3	12.00 ab	46.58 a	4.77 bc	0.52 a
P4	6.00 a	44.35 a	5.18 c	0.60 a
Non-mycorrhizal (M0)				
P0	7.00 a	22.67 a	3.94 ab	0.44 a
P1	10.00 ab	40.22 a	3.93 ab	0.56 a
P2	11.00 ab	22.38 a	4.17 abc	0.51 a
P3	8.00 a	25.33 a	4.42 abc	0.59 a
P4	6.00 a	27.67 a	4.19 abc	0.55 a
Significance				
Mycorrhizal	ns	*	ns	ns
Manure	ns	ns	ns	ns
Mycorrhizal x Manure	ns	ns	ns	ns

Values in each coloumn followed by the same letter(s) are not significantly different at $P < 0.05$ Duncan's multiple range test. ns: not significant by analysis of variance $P < 0.05$. P0: without manure; P1:10 t Ha^{-1} cow manure enriched by 5 t Ha^{-1} straw compost , P2:10 t Ha^{-1} goat manure enriched by 5 t Ha^{-1} straw compost ; P3: 10 t Ha^{-1} quail manure enriched by 5 t Ha^{-1} straw compost ; P4: 5 t Ha^{-1} straw compost

in the soil. Increased nutrient content of the soil is caused due to good cooperation given by mycorrhizal and variety of manure.

The AM fungi and manure play an important role in ecosystem through nutrient cycling (Jeffries et al., 2003; Shokri and Maadi, 2009; Yaseen et al., 2011). Plant growth response is indicated by plant dry mass and root length. Plant dry mass is the accumulation of photosynthate (dry matter) as a result of the photosynthesis process. The results showed that the highest plant dry mass is in the treatment of mycorrhizal inoculation incorporated of quail manure enriched straw compost, which is 16.03 g and significantly different among the treatments (Table 2). The quail manure has the highest nitrogen content than other manures (Table 1).

Nitrogen (N) plays a key role in the plant life cycle, as it is the main plant mineral nutrient needed for promote vegetative growth (El Debbay et al., 1994) and crop yield is affected by N content in plants as well (Muñoz-Huerta et al., 2013). The length of root indicate good vegetative growth that is promoted by N content (Figure 1). It is assumed that mycorrhizal has another function which is for modification of root architecture which results in better root growth due to numerous branched root (Berta et al., 2005).

Chlorophyll is a pigment in chloroplast which involve in photosynthesis process. Table 2 (chlorophyll content) and Table 4 (total nodules) show in the same treatments get the same trends that mycorrhizal soybean has higher total nodules and chlorophyll content compared to non- mycorrhizal soybean. Previous studies in Vollmann et al. (2010) and Zhao et al. (2005) stated that soybean nodulation affect chlorophyll content and protein content in plants. This indicates that there is fairly close relationship between the availability of nitrogen either from nitrogen fixation or N content in the soil (with application of manure and soybean-mycorrhizal symbiosis) and the net leaf photosynthetic.

The function of nitrogen not only improve quality and quantity of dry matter in leaves and protein content in grain crops (Casmann et al., 1980), but also chlorophyll production and other plant cell components (proteins, nucleic acids, amino acids), (Uchida, 2000).

Soybean seed is a commercial part and profitable value as soybean yield quality is indicated from soybean wet weight and seed protein content. Table 3 shows that mycorrhizal soybean has higher wet weight of seed and protein content than non-mycorrhizal soybean both incorporated manure enriched straw compost. But these were not showed on percentage of filled pods as we know that filled pods are influenced not only by nutrient but also light and water status. Protein content indicating quality of soybean and it is represented by nitrogen content in the seed. Table 3 shows protein content was not significantly different in all treatments due to soybean is derived from the same genetic source. Vollmann et al. (2000) stated that genetic variation affects the soy protein content.

The result of microbial infectivity and nutrient absorption effectiveness are of shown in Table 4. All treatments show there is no significant result in all parameters of total nodules, mycorrhizal colonization, nitrogen uptake and phosphorus uptake. Table 4 shows the trends of total nodules mycorrhizal-root colonization, nitrogen uptake, and phosphorus uptake are higher on mycorrhizal soybean than non-mycorrhizal soybean (both incorporated manure enriched straw compost). When all treatments were inoculated with *Rhizobium leguminosarum,* this could indicate that there is good cooperation among microbes. In mycorrhizal association, the plant-fungus interaction occur in mycorrhizosfer (Johansson et al., 2004). In this study, fungus also interact with other organisms like bacteria and synergistic interaction among them and not only promotes plant growth but also enhance the population of each other (Artursson et al., 2006). PGPR improves the development of the mycosimbionts and facilitates the colonization of plant root by mycorrhizal (Hildebrandt et al., 2002; Jaderlund et al., 2008). Nitrogen and phosphorus uptake were not significant due to mycorrhizal soybean and non-mycorrhizal soybean both incorporated manure enriched straw compost due to all treatments colonized with mycorrhizal. AM symbioses are bio trophic and also (usually) mutualistic, based on bidirectional transfers of organic carbon (C) from the plant and soil-derived nutrients (particularly phosphorus (P) but also nitrogen (N) and zinc (Zn) from the fungi (Jones and Smith, 2004; Govindarajalulu et al., 2005).

Conclusions

Mycorrhizal inoculation incorporated with manure enriched by straw compost gave a positive response to soybean growth. Even though quail manure has the highest nutrient content especially N, P, K among other manures, it does not mean it is the best manure to be applied in the farm. Statistical analysis showed that mycorrhizal inoculation and manure has significant effect on dry mass. Mycorrhizal inoculation incorporated with quail manure enriched by straw compost provides the highest dry mass and significantly different among the treatments. On the other parameters (root length, chlorophyll content, seed wet weight, percentage of filled pods each plant, protein content, total nodules, mycorrhizal-root colonization, nitrogen uptake, and phosphorus uptake) are not significantly different with interaction of mycorrhiza inoculation and kind of manure at the same doses. Nevertheless mycorrhizal inoculation incorporated with manure enriched by straw compost is the best combination to be applied for soybean cultivation in vertisols.

Acknowledgements

The authors are grateful to the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia that has funded this research through the scheme of Masterplan Percepatan dan Perluasan Pembangunan Ekonomi Indonesia (MP3EI) for fiscal year 2015.

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Received April, 4, 2017; accepted for printing June, 9, 2017