Growth and Yield of Soybeans in Various Growing Media Composition and Inoculation of Rhizobacteria on Marginal Soils

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ABSTRACT
Soybean is one of the leading commodities that is being developed in Indonesia. Today, the increase of soybean needs is not followed by its production capacity. Meanwhile, the area of productive land for soybean farming is decreasing due to land conversion for non-agricultural needs. One effort that can be an alternative is using marginal land by applying appropriate technology such as manure and biofertilizer. So that, it was necessary to know the composition of manure and biofertilizer, which was appropriate to increase the growth and yield of soybean plants on marginal soils. Complete Randomized Design was used in the experiment with two factors. The first factor was the ratio of manure: soil (v:v) there are 0:1, 1:1, 1:2, and 2:1. The second factor was rhizobacteria inoculum, which included without rhizobacteria, exogenous rhizobacteria, and indigenous rhizobacteria. Results showed that the composition of the best planting medium for growth and yield of soybean is manure: soil 1: 1 and 1: 2. The source of the rhizobacteria inoculum is not a significant difference to the soybean’s growth and yield. Manure and soil 1: 2 with indigenous inoculum tended to produce the best total number of nodules and effective root nodules. Manure and soil 1: 1 with indigenous inoculum produce the best of seeds number. Manure and soil 1: 2 or 2:1 with exogenous inoculum tended to produce the best seed index weight (g per 100 seeds).

Keywords: biofertilizer; Glycine max; manure; marginal soil; media composition

INTRODUCTION
Soybean (Glycine max (L) Merril.) is a food crop widely known by the community because it is a source of vegetable protein at an affordable price by most people. The demand for soybeans in Indonesia as food is very high at 2.18 million tons in 2014 and 2.32 million tons in 2015. Meanwhile, soybean production in 2015 was 963,183 tons, so the shortage of these needs must be met from imports. The increase in soybean production was sustained by adding the harvested area of around 24,670 hectares or 4.01% (BPS 2015). Marginal land can be interpreted as low-quality land because it has several limiting factors if used for certain purposes (latosol soil). The limiting factor can be overcome by input or costs that must be spent. Common problems in latosol soil are acid soil reactions, low organic matter content, low nutrient availability and reserves, and high Al saturation (Maas et al. 2020; Ikhwani et al. 2021; Wardani et al. 2021; Wei et al. 2021). The typical reaction of this soil will impact increasing Al content, which is toxic to plants, besides affecting P availability because P is fixed in the form of Al-P (Widawati dan Suliasih 2020; Fernandes et al. 2021; Minardi et al. 2021). Therefore, a necessary effort needs to be found as an alternative to improve soil fertility and optimize the management of soil organic matter (Diptaninggsari dan Rivaie 2021; Thapa et al. 2021).

Organic fertilizers are the best natural soil enhancers rather than artificial or synthetic enhancers. In general, organic fertilizers contain not only low macronutrients but also contain sufficient amounts of micronutrients which are very necessary for plant growth. One livestock that has the potential as a source of organic fertilizer is goats. In previous studies, Luo et al. (2021) found that the addition of goat manure at the ratio of 1: 2 and 1: 1 (v:v) showed a significant effect on the addition of fresh leaves weight the kailan plant. Tayyab et al. (2018) reported that applying goat manure and urea to alfisols could increase the yield of red chilies, a combination of 25% N goat manure and 75% N urea produced red chilies of 10.92 t/ha with an increase of 20.35%.

In agriculture, microbes as biological fertilizers are essential because they play a role in food security and sustainable crop production (Gerrewey et al. 2020). Added by (Jabborova et al. 2021), biological nitrogen fixation (BNF) is a nitrogen fixation process in an agricultural system that is inexpensive and environmentally friendly. Many research results show the
positive effect of inoculation in increasing BNF (Budiastuti et al. 2021).

If the necessary rhizobia strain is not present in the soil, then inoculation in legumes with the appropriate rhizobia is essential (Meng et al. 2015). The research results by Sánchez-Chino et al. (2015) showed that Legume fix on soybean plants in all research locations increased the yield by 50% against control and, when combined with Teprosyn Mo, could increase the yield by 205.62%.

Previous experiments have shown that inoculation of legumes provides a considerable opportunity to increase the production of legumes both in quality and quantity, also reduces the use of artificial fertilizers (Wu et al. 2005; Konvalinková et al. 2017; Ferrarezi et al. 2022). Inoculum originating from ex-soybean farming soil still has many bacteria and is cheap, so it is crucial to investigate its effect on soybean growth and yield. The objective of this study was to know the composition of manure and biofertilizer, which was appropriate to increase the growth and yield of soybean plants on marginal soils.

MATERIALS AND METHODS
Experimental site
The research has been carried out from February 2018 to May 2018 in an Education and Research Gardens at Sliper Agricultural Institute in Sleman, Yogyakarta, Indonesia.

Materials
The materials used were Anjasmoro cultivar, goat manure as organic fertilizer, latosol soil as growing media, polybags, plastic, commercial rhizobacteria (Rhizobium sp, Pseudomonas sp, and Bacillus sp) as exogenous rhizobacteria.

Growing media preparation and rhizobacteria inoculation
Latosol soil (pH 5.5) from Patuk, Gunung Kidul, Yogyakarta was used as growing media in polybags 30x30 cm. The ratio of manure: the soil is described below. Inoculating rhizobacteria is done by mixing the seeds in rhizoka (1 g per 200 g of seeds); previously, the seeds are moistened with water. Seeds then were planted in growing media. Meanwhile, indigenous rhizobacteria source was obtained from ex-soybean farming soil. Inorganic fertilizer was only used for treatments without manure.

Harvest and data collection
Root nodule and chlorophylls total were measured 30 days after planting (DAP). Measurement of chlorophylls totals used Minolta SPAD. Harvest was performed 90 DAP and measurement of plant height, fresh plant weight, plant dry weight, fresh root weight, root dry weight, seed weight total per plant, seed weight per 100 seeds, pod number, and pod weight.

Experimental design
The study was conducted using a Factorial design arranged in a Completely Randomized Design (CRD) consisting of 2 factors. The first factor was the ratio of manure: soil (v:v) including 0:1, 1:1, 1:2, and 2:1. The second factor was rhizobacteria inoculum, which included without rhizobacteria, exogenous rhizobacteria, and indigenous rhizobacteria. So, there are 12 combinations treatments; each combination was replicated seven times.

Statistical analysis
The data were analyzed by ANOVA (Analysis of Variance) using the SPSS program. Duncan's Multiple Range Test (DMRT) determined the differences among treatment means at P < 0.05.

RESULTS AND DISCUSSION
Soybean’s growth on marginal soil
The results showed that the composition of the growing medium had a significant effect on plant height, total chlorophyll, plant fresh weight, plant dry weight, fresh root weight, root dry weight, and nodule weight (Table 1). Meanwhile, variation of rhizobacteria inoculum source had no significant effect on soybean growth. So that, the composition of manure and soil 1:1 and 1:2 generally indicated appropriate for soybean’s growth. Nevertheless, the interaction between manure and soil ratio (v:v) and rhizobacteria inoculation was shown in nodule number and adequate nodule number (Table 2).

The composition of the growing medium had a significant effect on plant height, total chlorophyll, plant fresh weight, plant dry weight, fresh root weight, root dry weight, and nodule weight (Table 1). The composition of manure: soil ratio 1:1 and 1:2 generally indicated appropriate for soybean’s growth. The provision of manure on latosol soil has excellent benefits, such as the availability of more P elements. This is because the results of the decomposition of organic matter in the form of humic acids that form chelate bonds with Al and Fe ions can reduce the solubility of Al and Fe ions and also release P, which is fixed by Al and Fe so that P availability increases (Tonks et al. 2017; Grzyb et al. 2021; Sukmasari et al. 2021). Organic matter would improve cation exchange capacity (CEC) and form a chelated cation (Sudadi et al. 2018; Agus et al. 2019; Dai et al. 2020). Therefore, the addition of organic matter to marginal land is needed.

The variation of rhizobacteria inoculum source had no significant effect on soybean growth and yield (data not shown). This means that the treatment without the bacteria gave the same soybean growth and yield as that given the inoculum. Treatment of manure: soil ratio 1:2 with indigenous inoculum tended to produce the best total number of nodules and effective root nodules (Table 2). This shows that the inoculum originating from the soil previously planted with soybeans still contains a lot of effective Rhizobium bacteria. Indigenous Rhizobium sp inoculated can form nodules, but not all inoculants are effective for soybean plants (Abd-Alia et al. 2014; Banerjee et al. 2016; Goyal and Goel 2018; Franzini et al. 2019; Ilangumaran et al. 2021). Rhizobium bacteria are soil microbes capable of binding free nitrogen in the air into ammonia (NH3), which will be converted into amino acids become nitrogen compounds needed by plants to grow and develop (Tilak et al. 2006; Ahmed et al. 2016; Ren et al. 2019).
Yield production of Soybean

The result (Table 3) shows that the composition of manure: soil ratio (v:v) 1:1, 1:2, and 2:1 generally gives better results than 0:1 + NPK for soybean’s production yield. Besides these benefits, manure can also improve the physical properties of the soil. Organic matter makes the soil loose and crumb so that aeration and thinning are better and easier to penetrate the roots of plants. Previous research shows that providing solid compost or manure can increase plant height, number of flowers, number of pods, number of nodules, dry weight of stover, root dry weight, protein content, and seed fat soybean plants (Endriani et al. 2021; Latawiec et al. 2021; Zumpf et al. 2021)

Kinds of rhizobacteria inoculation resulted in the same seed weight, pods number, and pods weight of soybean on marginal soil. The number of pods is not followed by seed formation or filling because of many empty pods, as seen from the low seed weight. Inoculants do not always increase soybean yield (De Gregorio et al. 2017; Widawati dan Suliasih 2020; Sahile et al. 2021). Several researchers had reported that the effectiveness of inoculants was reduced when soil conditions after planting were very dry or too wet or when soil pH was below 6.0. In this study, the pH of the latosol soil used was 5.5.

The result (Table 4) shows that manure and soil (0:1) + NPK, without inoculum, are low in 100 seed index weight because of the low number of effective nodules formation. According to Khan et al. (2020), the number of nodules is an indicator of successful Rhizobium inoculation, which is often used to assess its effect on the growth and yield of soybean plants. Treatment of Manure: Soil (0:1) + NPK + without inoculum shows that pod and seed formation is inhibited due to the low number of effective root nodules. This indicates that many pods are hollow, as indicated by the low seed index weight.

### Table 1. Soybean’s growth on marginal soil with variation of manure

| Variables          | 0:1 + NPK | 1:1       | 1:2      | 2:1      |
|--------------------|-----------|-----------|----------|----------|
| Plant height (cm)  | 36.00 ± 1.21 c | 43.50 ± 1.24 b | 53.17 ± 1.67 a | 37.83 ± 1.23 c |
| Chlorophylls (U)   | 36.31 ± 0.55 a | 34.22 ± 0.81 a | 34.67 ± 0.70 a | 29.72 ± 1.62 b |
| Plant fresh weight (g) | 23.77 ± 0.92 c | 37.18 ± 1.92 ab | 38.58 ± 2.35 a | 32.21 ± 2.75 b |
| Plant dry weight (g) | 19.50 ± 0.71 c | 28.51 ± 1.20 a | 29.38 ± 1.63 a | 23.69 ± 1.96 b |
| Root Fresh weight (g) | 6.16 ± 0.29 b | 6.98 ± 0.29 ab | 7.92 ± 0.30 a | 6.47 ± 0.44 b |
| Root dry weight (g)  | 5.55 ± 0.25 b | 6.22 ± 0.25 b | 7.06 ± 0.25 a | 5.85 ± 0.37 b |
| Nodules weight (g)  | 0.90 ± 0.15 c | 2.26 ± 0.23 b | 3.91 ± 0.09 a | 0.97 ± 0.28 c |

**Remarks:** Means followed by the same letter on the line indicate no significant differences based on Duncan’s Multiple Range Test at p<0.05.

### Table 2. Soybean’s nodules on variation of manure: soil ratio (v:v) and rhizobacteria inoculation

| Treatments                              | Nodule numbers | Effective nodules |
|-----------------------------------------|----------------|------------------|
| Manure : Soil (0:1) + without inoculum  | 25.00 ± 6.03 ef | 20.33 ± 5.69 ef  |
| Manure : Soil (0:1) + exogenous rhizobacteria | 50.00 ± 12.34 cd | 48.67 ± 11.46 cd |
| Manure : Soil (0:1) + indigenous rhizobacteria | 36.00 ± 5.85 de | 32.67 ± 5.46 de  |
| Manure : Soil (1:1) + without inoculum  | 61.67 ± 14.24 bcd | 59.67 ± 13.25 bcd |
| Manure : Soil (1:1) + exogenous rhizobacteria | 49.67 ± 2.96 cd | 48.33 ± 2.73 cd  |
| Manure : Soil (1:1) + indigenous rhizobacteria | 86.67 ± 28.04 abc | 83.33 ± 27.72 abc |
| Manure : Soil (1:2) + without inoculum  | 88.67 ± 3.28 abc | 84.33 ± 2.03 abc |
| Manure : Soil (1:2) + exogenous rhizobacteria | 105.33 ± 4.37 ab | 103.00 ± 3.06 ab |
| Manure : Soil (1:2) + indigenous rhizobacteria | 121.00 ± 14.18 a | 117.33 ± 13.37 a |
| Manure : Soil (2:1) + without inoculum  | 18.33 ± 3.33 f | 14.00 ± 3.51 f  |
| Manure : Soil (2:1) + exogenous rhizobacteria | 14.00 ± 1.53 f | 11.67 ± 2.19 f  |
| Manure : Soil (2:1) + indigenous rhizobacteria | 57.00 ± 16.50 cd | 57.00 ± 13.65 bcd |

**Remarks:** Means followed by the same letter on the line indicate no significant differences based on Duncan’s Multiple Range Test at p<0.05.
CONCLUSIONS
Results showed that the composition of the best planting medium for growth and yield of soybean is manure and soil ratio 1:1 and 1:2. The source of the rhizobacteria inoculum was no significance difference to the soybean’s growth and yield. Treatment of manure and soil ratio 1:2 with indigenous inoculum tended to achieve the highest total number of nodules and effective root nodules of soybean. Manure and soil ratio 1:1 with indigenous inoculum produce the best seeds number. Manure and soil ratio 1:2 or 2:1 with exogenous inoculum tended to produce the best seed index weight (g per 100 seeds).

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