Growth and development of *Theobroma cacao* seedlings as a response to different dosages of vermicompost and arbuscular mycorrhizal fungi

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**Abstract.** This study aims to determine the response of cocoa (*Theobroma cacao* L.) seedlings to the application of vermicompost and arbuscular mycorrhizal fungi (AMF). The research was conducted at the Teaching Farm, Faculty of Agriculture, Universitas Hasanuddin from February to July 2018. The trial was set using a randomized block design (RBD). Three vermicompost dosages set as the first factor were 0, 25, and 50 g polybag\(^{-1}\), and three dosages of AMF used as the second factor were 0, 6, and 12 g polybag\(^{-1}\). The results showed that the application of 50 g polybag\(^{-1}\) of vermicompost and 6 g polybag\(^{-1}\) of AMF resulted in better seedling growth indicated by a higher number of leaves and stem diameter. The use of 25 g polybag\(^{-1}\) of vermicompost and 6 g polybag\(^{-1}\) of AMF increased the shoot and root fresh weight. Shoot:root ratio of the cocoa seedlings as the response of the plant to the application of vermicompost and AMF ranged between 0.38 and 0.53 compared to controls for both treatments ranged between 0.31 and 0.51.

1. **Introduction**

The development of cocoa continues to increase from year to the year indicated by the average of cocoa growth rate in Indonesia during 2015-2017 showing an increase in productivity of 772, 778, and 779 kg/ha in the three consecutive years [1]. The efforts in improving cocoa production to meet the demand in the future are facing problem in providing good quality seedlings. During plant growth in the nursery, a high-quality seedling should have a straight and strong taproot. Strong rooting system at the stage of seedlings will help the mature plants in absorbing nutrients from the deeper layer of the soil.

Cocoa seedlings can grow well when planted in soil containing organic matter such as manure and vermicompost. Use of vermicompost as much as 25 g per polybag to cocoa seedling as an organic fertilizer had been shown to increase seedling height, leaf numbers, stem diameter, root volume, root shoot ratio, and plant dry weight [2]. In addition to the use of inorganic and organic fertilizers, utilization of biological agents also receives much attention since it can increase soil fertility. One of the fungi that can be used and is effective in providing nutrient requirements for plants is mycorrhiza. Mycorrhizae have an important role in increasing plant growth by increasing nutrient uptake by expanding the surface of the absorption area.

Plants with endomycorrhiza can absorb more of the P element (10-27%) compared to the control plant (0.4-13%) [3]. Higher P uptake is due to the ability of the fungal hyphae to release P from
specific bound in the soil, making it available to plant through the synthesis of phosphatase enzymes [4]. Increased absorption of P is followed by increased absorption of other elements, resulting in better plant growth [3]. Organic matter is an important ingredient in improving soil fertility and stabilizing soil aggregates. Musfal [5] stated that organic matter could improve soil structure, as well as the movement of water in the soil, which is beneficial for plants and microorganisms. Given the importance of the organic matter, soil organic matter needs to be maintained. Farida [6] stated that to maintain soil organic matter and improve soil fertility, fertilization with organic fertilizers such as manure that serves to increase nutrients, maintain soil structure, increase the ability of soil to hold water and improve soil biological activities is necessary.

One of the organic material known to improve soil fertility is vermicompost. Vermicompost is a microbiologically active organic material formed from the interactions between earthworms and microorganisms during the decomposition of organic material [7]. The substance is rich in nutrients and has better quality than other types of organic fertilizers. Vermicompost of *Eisenia fetida* contains nitrogen 0.63%, phosphorus 0.35%, potassium 0.20%, calcium 0.23%, magnesium 0.26%, sodium 0.07%, copper 17.58%, zinc 0.007%, manganese 0.003%, iron 0.790%, boron 0.2221% [8]. Vermicompost contains humic acid substances which, together with clay, play a role in several complex reactions both directly and indirectly. According to Ibrahim et al. [9], vermicomposting process produce various plant growth-regulating compounds, particularly PGRs (Plant Growth Regulators) such as auxins, kinetins, and gibberellins that could be absorbed by humates and fulvates in vermicomposts. The plant regulators then released gradually on a time scale synchronized closely with plant growth. Also, humic substances can increase soil fertility by changing the physical, chemical, and biological conditions of the soil.

Vermicompost can be given to vegetable plants such as tomatoes, eggplant and mustard greens at a dose of 450-500 g/m² and given before planting or at planting with an array system or around the root area. The vermicompost has a crumbly texture that is dominated by the size of sand (grain diameter of 0.05-2 mm) and also can hold large water, which is around 14.45-1.68% [10]. Based on its physical properties, vermicompost has high porosity, aeration, and drainage and good water-holding capacities [11]. According to Mulat [10], vermicompost also contains growth-stimulating hormones for plants, such as gibberellin 2.75%, cytokinin 1.05%, and auxin 3.80%. The application of vermicompost is considered to increase some of the content of nutrients in the soil such as N, P₂O₅, K₂O, CaO, MgO, and Mn compared to other fertilizers.

2. Methodology

This research was conducted at the Teaching Farm, Faculty of Agriculture, Universitas of Hasanuddin University, Makassar, South Sulawesi from February to July 2018. The trial employed a Randomized Block Design with two factors, namely the application of vermicompost (K) as the first factor and AMF (M) as the second factor. The dosage of vermicompost applied was 0, 25, and 50 g polybag⁻¹ while AMF dosage applied were 0, 6, and 12 g polybag⁻¹. Thus, there were nine combinations of treatments, and each combination was repeated three times, which resulted in 81 experimental units.

AMF used was *Acaulaspora tuberculata* from the genus of *Acaulasporaceae* inoculated in a mixture of corn and sorghum plants mixed in Zeolite. One-month-old cocoa seedlings of Sulawesi clone originated from Timco Village, Cita Subdistrict, Soppeng Regency, South Sulawesi province, were transplanted into 30x40 cm sized polybags previously filled with planting media of 2:1 ratio of soil and manure. The vermicompost and AMF were applied according to the treatment at transplanting by mixing the vermicompost in the media. The AMF was applied by adding it in the rhizosphere of the seedling.

Parameters of observed were stem diameter, number of leaves, root volume, fresh shoot weight, fresh root weight, and shoot and root ratio. The data were analyzed using analysis of variance and followed by Tukey's difference test when there is a significant effect of the treatments.
3. Results and discussion

3.1. Effect of vermicompost and AMF on the growth of cocoa seedlings

Application of different dosages of vermicompost and AMF significantly affected the growth of cocoa seedlings. Although there is no consistent effect of vermicompost on leaf numbers, the stem diameter of cocoa seedling tends to increase with the dosage of vermicompost used. Table 1 shows that the seedlings applied with 50 g polybag$^{-1}$ of vermicompost and 6 g polybag$^{-1}$ of AMF resulted in the highest number of leaves and stem diameter with an average of 13.89 leaves and 16.87 mm, respectively.

The use of vermicompost in the planting media can have a direct and indirect effect on the growth and development of the plant. The direct effect of vermicompost on growth component of the seedling is due to the chemical properties of the substance, which is rich in nutrition such as macronutrients of N, P, and K [10]. The vermicompost also contains growth stimulating hormones for plants, such as gibberellin 2.75%, cytokinin 1.05%, and auxin 3.80% [10]. Availability of important nutrients in the soil to be absorbed by the seedling and the presence of plant growth regulator simultaneously resulted in better growth of the cocoa seedling, as shown in this recent study. The indirect effect of vermicompost is more attributed to the physical properties of high porosity, aeration, and drainage and good water-holding capacities [11]. This condition is favourable to the development of the rooting system of the seedlings.

Beside the vermicompost, mycorrhizal fungi inoculated to the seedling can also improve the P absorption by the plant, one of the macro element needed for plant growth [3]. Higher P uptake is attributed to the synthesis of phosphatase enzymes by the fungi hyphae turning P element in the soil into an available state for the plant [4]. Therefore, the use of vermicompost and AMF would benefit the growth of cocoa seedlings.

Table 1. Effect of different dosages of vermicompost and arbuscular mycorrhizal fungi (AMF) on the growth of cocoa seedlings.

| Dosage of Vermicompost | Dosage of AMF       | Tukey’s 0.05 k |
|------------------------|---------------------|----------------|
|                        | m0 0 g polybag$^{-1}$ | m1 6 g polybag$^{-1}$ | m2 12 g polybag$^{-1}$ |
|                        | Number of Leaves    | Stem Diameter (mm) |
| k0 (0 g polybag$^{-1}$)| 10.67$^v_b$         | 12.44$^x_b$       | 10.44$^y_b$         |
| k1 (25 g polybag$^{-1}$)| 12.33$^x_a$        | 10.00$^y_c$       | 12.45$^x_a$        |
| k2 (50 g polybag$^{-1}$)| 11.00$^y_b$        | 13.89$^x_b$       | 10.00$^y_b$        |
| Tukey’s 0.05 m         | 1.25                |

Numbers followed by the same letter in a row (xyz) and column (abc) means do not significantly differ based on Tukey’s p ≤ 0.05.
3.2. Effect of vermicompost and AMF on the development of cocoa seedlings

Application of vermicompost and AMF in various dosages on cocoa seedlings significantly interacted in affecting the development of the seedlings. Seedlings applied with 25 g polybag\(^{-1}\) and 6 g polybag\(^{-1}\) resulted in the highest fresh shoot weight (1.35 g) while for the parameter of fresh root weight, the best result obtained with the application of 25 g polybag\(^{-1}\) of vermicompost without AMF inoculation (11.83 g). Application of vermicompost and AMF to the growing media improve the availability of some important nutrients such as N, P and K which are the component needed for the synthesis of the main compound to establish plant body [10].

Table 2. Effect of different dosages of vermicompost and arbuscular mycorrhizal fungi (AMF) on the development of cocoa seedlings.

| Dosage of Vermicompost | Dosage of AMF | Tukey’s \(0.05\) \(k\) |
|------------------------|--------------|------------------|
|                        | m0 0 g polybag\(^{-1}\) | m1 6 g polybag\(^{-1}\) | m2 12 g polybag\(^{-1}\) |
| **Shoot FW (g)**       |              |                  |                  |
| k0 (0 g polybag\(^{-1}\)) | 1.28\(^a\)   | 1.07\(^b\)       | 1.00\(^a\)       |
| k1 (25 g polybag\(^{-1}\)) | 1.04\(^b\)   | 1.35\(^a\)       | 1.09\(^a\)       |
| k2 (50 g polybag\(^{-1}\)) | 0.90\(^b\)   | 1.07\(^x\)       | 1.06\(^x\)       |
| Tukey’s \(0.05\) \(m\) |              | 0.15             |                  |
| **Root FW (g)**        |              |                  |                  |
| k0 (0 g polybag\(^{-1}\)) | 9.33\(^x\)   | 7.33\(^c\)       | 4.83\(^z\)       |
| k1 (25 g polybag\(^{-1}\)) | 11.83\(^a\)  | 11.00\(^a\)      | 10.33\(^a\)      |
| k2 (50 g polybag\(^{-1}\)) | 6.67\(^c\)   | 9.17\(^c\)       | 5.50\(^c\)       |
| Tukey’s \(0.05\) \(m\) |              | 1.09             |                  |

Numbers followed by the same letter in a row \(^{(xyz)}\) and column \(^{(abc)}\) means do not significantly differ based on Tukey’s \(p \leq 0.05\). FW=fresh weight.

Table 3. Effect of different dosages of vermicompost and arbuscular mycorrhizal fungi (AMF) on shoot and root ratio of cocoa seedlings.

| Dosage of Vermicompost | Dosage of AMF | Tukey’s \(0.05\) \(k\) |
|------------------------|--------------|------------------|
|                        | m0 0 g polybag\(^{-1}\) | m1 6 g polybag\(^{-1}\) | m2 12 g polybag\(^{-1}\) |
| k0 (0 g polybag\(^{-1}\)) | 0.36\(^b\)   | 0.36\(^b\)       | 0.49\(^x\)       |
| k1 (25 g polybag\(^{-1}\)) | 0.51\(^a\)   | 0.53\(^a\)       | 0.45\(^b\)       |
| k2 (50 g polybag\(^{-1}\)) | 0.31\(^c\)   | 0.38\(^c\)       | 0.44\(^c\)       |
| Tukey’s \(0.05\) \(m\) |              | 0.04             |                  |

Numbers followed by the same letter in a row \(^{(xyz)}\) and column \(^{(abc)}\) means do not significantly differ based on Tukey’s \(p \leq 0.05\)

Similar to results on the parameter of the shoot and root fresh weight of the cocoa seedlings, the vermicompost and AMF dosage treatments interacted significantly in affecting the shoot and root ratio. Treatments resulted in the highest shoot, and root ratio of 0.53 was the application of 25 g polybag\(^{-1}\) vermicompost and 6 g polybag\(^{-1}\) AMF. Shoot:root ratio is an indicator whether growth is
directed more to above or below ground. Although optimal shoot:root ratio depends on the variety of the plant, the extent of changes in this ratio is also affected by the environment [12]. Normal planting condition would result in normal shoot and root growth while abiotic stress can lead to an imbalanced proportion of growth. In this recent study, it is shown that the use of vermicompost and AMF resulted in higher ratios (0.53) compared to control (0.36). This ratio indicates a normal shoot growth as a result of favorable condition either for nutrients absorption or light condition to support the growth and development of cocoa seedlings.

4. Conclusion
Based on the results of the study, it can be concluded that application of 50 g polybag\(^{-1}\) vermicompost with 6 g polybag\(^{-1}\) of AMF on cocoa seedlings resulted in better seedling growth indicated by a higher number of leaves and stem diameter. The highest fresh weight of shoot and shoot and root ratio obtained from the treatment of vermicompost dosage of 25 g polybag\(^{-1}\) and AMF dosage of 6 g polybag\(^{-1}\). The treatment of 25 g polybag\(^{-1}\) of vermicompost on the cocoa seedling resulted in highest root fresh weight.

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