Effectiveness of soil tillage and Arbuscular Mycorrhizal (AM) fungi inoculation on fruit development of the cocoa plant (*Theobroma cacao* L.)

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Abstract. The research was conducted in the form of an experiment, aimed to determine the effect of soil tillage and Arbuscular Mycorrhizal Fungi (AMF) inoculation on the development of cocoa fruits. This research was carried out in Barang Village, Liliriaja District, Soppeng Regency, from March to September 2017. The factorial experiment was set based on a randomized group design pattern. The first factor was soil tillage consisted of four levels, namely without soil tillage (control); application of organic mulch of cocoa leaves and from the remaining of pruning; soil tillage without organic mulch; and soil tillage with organic mulch. The second factor was AMF inoculation which consisted of four levels, namely without AMF, AMF 7.5 g plant⁻¹, AMF 15 g plant⁻¹, and AMF 22.5 g plant⁻¹. The results show that the interaction between soil tillage with organic mulch and AMF inoculation of 22.5 g plant⁻¹ produced the highest number of seeds per 100 grams of dried cocoa beans (8% moisture content). The treatment of soil tillage with the use of organic mulch can increase the number of fruits formed, the number of seeds of cocoa.

1. Introduction

One of the important agricultural commodities that thrive in Indonesia is cocoa [1], and until 2017 it still ranks third in the world. Recently, the area and production of Indonesian cocoa has decreased very dramatically. The main causes of the decline in these production and production area are mainly due to aging of plants, land degradation and conversion of cocoa plantations [2], in addition to high levels of pest and disease attacks [3,4] due to the low level of crop management.

South Sulawesi is one of the largest cocoa producing regions in Indonesia. The area of cocoa plantations in South Sulawesi in 2018 is around 232,710 ha with a total production of around 124,768 tons with a productivity of 729 kg/ha involving around 262,998 heads of farmer families [5]. The development of cocoa plantations is very important because the cocoa sector is a source of income for farmers, can provide employment opportunities, and as a source of foreign exchange. The development of cocoa plantations, especially on dry land with fertility levels that have decreased, requires a relatively high amount of fertilizer. This condition can hamper the development of cocoa plantations because large quantities of liming and fertilizer are needed. To achieve good growth and yield of cocoa, better soil management and bio-agent inoculation such as Arbuscular Mycorrhizal
(AM) fungi are useful for improving soil rhizosphere and increasing plant resistance to extreme environmental conditions and being able to increase nutrient availability for the specific crop plants, phosphorus [6]. Nasaruddin's research results [7] reported that cacao plants and AM fungi can build a symbiosis to contribute to the supply of nutrients and water for plants, and at the same time, as biotrophs, it can obtain symbiotic carbon to develop hyphae and to complete their life cycle [8].

The role of mycorrhizae in changing soil properties and their relevance to plant physiology and growth is expected to be different from soils without mycorrhiza, especially physical, chemical, soil volume, soil substrate [9], and accompanying climatic conditions [10]. Thus, an integrative approach that takes into account the climatic conditions and edaphic nature of the soil is needed to maximize and stabilize mycorrhizal growth. Therefore, the combination of soil management with mulch application and soil softening is expected to support better growth and production of cocoa.

2. Methodology

This research was carried out in the form of an experiment on a 12-year-old farmer's cocoa farm, in Barang Village, Liliriaja District, Soppeng Regency, from June to August 2017 after the peak of harvest. The experiment was carried out based on the randomized block design. Soil tillage was set as the first factor consisted of four levels, namely without soil tillage and organic mulch (control) (P0); no tillage with organic mulch (P1); soil tillage without organic mulch (P2); and soil tillage with organic mulch (P3). The organic mulch materials were the cocoa leaves from the pruning wastes. Mulching was conducted with a leaf thickness of about 3 layers, and added simultaneously with soil tillage at a depth of about 20 cm from the soil surface around the plant disk distanced about 0.25 to 1 m from the plant tree.

The second factor was AMF inoculation consisted of four levels, namely: without AMF (M0), AMF inoculation 7.5 g plant\(^{-1}\) (M1), AMF inoculation 15 g plant\(^{-1}\) (M2), and AMF inoculation 22.5 g plant\(^{-1}\) (M3). Mycorrhizae used were obtained from Bogor Research Institute for Plantations in the form of zeolite culture. AMF inoculation was carried out by applying the fungi that previously mixed with compost 250 g per treatment circularly around the plant disk area about 0.75 m from the tree. Before sowing, each treatment dose is mixed evenly with compost around 250 g per treatment.

Observation data were tabulated then analysed using analysis of variance (ANOVA) sand continued with Tuke's test and regression test to determine the best dose of mycorrhiza.

3. Results

Analysis of variance show that the interaction of soil treatment and AMF inoculation had a highly significant effect (p<0.01) on the number of dry seeds per 100 g (water content (WC) of 8%). The tillage treatment had a very significant effect (p<0.01) on the number of cherells formed, the number of survived fruits, the number of harvested fruit, but does not significantly affect the number of cherells wilts. Mycorrhizal inoculation has a significant effect on all parameters observed except the number of cherells wilts, like in Gmelina stands [11].

The treatment of soil tillage by applying organic mulch (P3) showed higher number of cherells formed, the number of survived fruits, and the number of harvested fruits (Figure 1A) and significantly different than the other treatments. AMF inoculation was significantly different compared without control (p<0.05) and positively correlated linearly with the number of cherells formed which assumed to survive and the number of fruits harvested (Figure 1 B). The higher the AMF inoculation dose, the more fruits were formed, the more fruits are assumed to survive and the more fruits harvested by following equation of \( y = 0.7039x + 33,435; r = 0.99 \) **; \( y = 0.6806x + 12,188; r = 0.98 \) **; and \( y = 0.8x + 15,042; r = 0.99 \) **, respectively.

The interaction of soil tillage treatment with AMF inoculation had a very significant effect (p<0.01) on the number of dried seeds of per 100 g of seeds (WC 8%) and negatively correlated linearly with the parameter (Figure 2A). The higher AM fungi dose, the lower the number of seeds per 100 grams by following the respective equation of \( y = -0.5781x + 111.51; r = 0.67 \) *(control)*, \( y = -0.2732x + 107.64; r = 0.57 \) *(no tillage with organic mulch)* and \( y = -0.3565x + 105.76; r = 0.84 \) **
(soil tillage without organic mulch), but it is not significant in the treatment of soil tillage with organic compost. The AMF inoculation treatment of 22.5 grams per plant showed the lowest number of seeds per 100 grams. The Tukey's test in figure 2 B shows that AMF inoculation treatment of 22.5 g per plant resulted in higher number of seeds per fruit and highest pod index and was significantly different than without AMF inoculation ($\alpha = 0.05$).

![Figure 1](image1.png)

**Figure 1.** Histogram of number of cherells, cherells wilt, surviving young fruit, and harvested fruit (A); Regression for number of cherells, cherells wilt, surviving young fruit, and harvested fruit (B).
Figure 2. Regression of inoculation of AM fungi on different soil tillage (A) and histogram of inoculation of AM fungi on number of seeds per fruit and pod index (B).

4. Discussion
The results showed that the treatment of soil tillage and AMF inoculation significantly affected the number of cherells formed, the number of survived fruits, the number of harvested fruits, the number of seeds per fruit, number of dried seeds per 100 g (WC 8%) and pod index.

Soil tillage with organic mulch showed better generative plant growth parameter components than without tillage and other treatments. Improvement of root rhizosphere conditions can increase the
development of effective fine roots and root metabolism activities. Previous study conducted by Nasaruddin (2013 - unpublished) showed that intensive fine roots development was found in the condition of loose soil following soil tillage and mulching treatment using cocoa leaves around the plant disk about 1 m from the tree compared to control treatment. This is due to the effect of soil tillage performed that resulted in cutting of old roots causing loss of apical dominance of the roots that when supported by soil moisture and good soil aeration will induce fine root formation and root metabolic activity [12].

The ability of cocoa plants to absorb water and nutrients depends on the dynamics of fine roots (mortality and regrowth of fine roots) and their variation in time. Development of fine root in turn can contribute to a significant proportion of nutrient recycling in water and nutrient utilization depending on the temporal pattern of fine root growth [13]. Increased supply of water and nutrients from plant roots allows the development of above-ground plant organs and their metabolic activities which will contribute to the supply of assimilates and intermediate compounds needed for the development of the body which ultimately contributes to the observed generative parameters.

AMF inoculation is positively correlated linearly with the number of cherells formed, the number of survived fruit, and the number of fruits harvested and has a significant effect on the number of dried seeds per 100 grams seeds with a moisture content of 8% and pod index. The results of the research by Nasaruddin and Ridwan [14] reported that AM fungi inoculation could increase the chlorophyll content of cocoa leaves and the level of sunlight energy absorption. This will result in increased in photosynthesis rate of leaves which can further increase the number of fruits formed until harvest, the number of dried seeds per 100 g of cocoa beans (WC 8%) and pod index. At the time of anthesis of the cocoa flower, auxin concentration and flower retention are low. Fertilization and the possibility of pod development, depending on sources or sufficient external signals for pollination or pollen growth [15].

AM fungi are the main functional group of beneficial soil microbes that can build mutualistic symbiosis with roots of 80% of plant species [8]. This includes the most important plants for human consumption including cocoa [16]. AM fungi produce hyphae that grow intercellular in roots that form in root cells, where there is a nutrient exchange between the two symbionts. Up to 20% of total plant photosynthates are transferred to AM fungi, chemoheterotrophically used as carbon sources [17]. Such transfer allows this fungus to grow and form new spores. On the other hand, extensive extra radic mycelium explores the surrounding soil beyond roots and increases the surface of the root absorber (up to 40 times) [17]. Extracellular hyphae from AM fungi are able to absorb and translocate soil mineral nutrients, such as phosphorus (P), nitrogen (N), sulfur, potassium, calcium, iron, copper, and zinc, thereby increasing plant growth and biomass production [18]. In addition, AM fungi provide a variety of ecosystem services, increase water absorption, and increase plant tolerance to biotic and abiotic pressures [19]. Several studies report that AMF can modulate the synthesis of secondary metabolites in host plants, which leads to higher antioxidant enzyme activity and increases phytochemical levels that are diverse with a variety of plant metabolism activities [20–22] which ultimately allows improvement in production and productivity of the host plants.

5. Conclusions
Based on the results of the discussion above, it can be concluded that:

a) Interaction between soil tillage with organic mulch treatment and AMF inoculation of 22.5 g plant$^{-1}$ resulted in the best in number of dried seeds per 100 grams cocoa beans (WC 8%).

b) Soil tillage with organic mulch can increase the number of cherells formed, the number of survived fruit until harvest, the number of seeds per fruit, the number of dried seeds per 100 g seeds (8% water content) and pod index.

c) AMF inoculation correlates linearly to the number of cherells formed, the number of fruits harvested and can increase the weight of dried seeds (8% water content).
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