14751_Utilization of Tithonia diversifolia bokashi and Mycorrhiza on cultivation of Allium ascolicum L. grown on post-mine sandpits soil

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Utilization of *Tithonia diversifolia* bokashi and Mycorrhiza on cultivation of *Allium ascolicum* L. grown on post-mine sandpits soil

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ABSTRACT

Post-mine sandpits C soil has a potential to be used for vegetable cultivation, nevertheless needs improvement on its physical, chemical, and biological properties through the input of microbial application technology and organic matter. The purpose of this study was to examine the effect of AMF and bokashi of *Tithonia diversifolia* in improving soil physical properties and yield of *Allium ascolicum*. The study used a two-factor Randomized Block Design. The first factor was AMF provision (control 0 g $plant^{-1}$, 4 g $plant^{-1}$, 6 g $plant^{-1}$, 8 g $plant^{-1}$, and 10 g $plant^{-1}$). The second factor was bokashi of *T. diversifolia* (control 0 t ha^{-1}, 3 t ha^{-1}, 6 t ha^{-1}, and 9 t ha^{-1}). The results showed that the application of AMF together with bokashi generated soil porosity and permeability that were suitable for the growth of shallot bulbs. The application of bokashi 9 t ha^{-1} increased bulbs diameter and bulbs fresh weight, although still below its potential due to unfavorable environmental factors. Thus, the successful application of AMF and organic materials need to pay attention on environmental factors in order to produce maximum effect.

Key words: AMF, bokashi, shallot, *T. diversifolia*.

INTRODUCTION

The land-use change of agricultural land to non-agricultural occurs in several regions of Indonesia. In the highlands, there are many changes in land use from vegetable cultivation to hotels, housing or tourist areas. This results in the narrowing of fertile agricultural land for the production of high economic value vegetable crops and requires to look for alternative soils even with not optimal carrying capacity, one of which is post-mine sandpits C soil.

Post-mine sandpits C soil is widely found in various regions in Indonesia as a source of gravel and sand for building purposes and a source of regional income. The further activity of these minerals mining causes damage to the ecosystem and is vulnerable to erosion, so the Government Regulation of the Republic of Indonesia no. 78 of 2010 requires that mining C land be reclaimed to function again according to its allotment. Thus, post-mine sandpits land reclamation using revegetation has a dual function, namely increasing the area of agricultural production land and remedying the ecology.

Post-mine sandpits C soil has several obstacles when it will be used for plant cultivation, namely the low C-organic content, sand-dominating texture (Hidayat et al., 2020), low water binding capacity (Ginting et al., 2018), and has not yet formed aggregates so that sensitive to erosion.

Agricultural cultivation activities on post-mine sandpits C soil must be initiated by eliminating the inhibiting factors. The addition of organic matter is a necessity considering that post-mine sandpits C soil has very low organic C (0.86%) (Hidayat et al., 2020). To support optimal plant growth requires a minimum of C-organic content > 2.5% (Patrick et al., 2013). Organic matter has been proven to improve soil density, soil porosity, and soil permeability (Hidayat et al., 2020), maintain water availability and improve soil aeration (King et al., 2020). In addition, organic matter is an energy source to support the development of soil decomposer microorganisms (Yin et al., 2019) and as the main ameliorant (Maftu'ah et al., 2014). One source of organic matter that can be utilized is Tithonia diversifolia which is a weed from the Asteraceae family. It can grow in extreme environments, its availability is abundant because the adaptability of the plant is high, moreover, it can grow at various altitudes (Obiakara & Fourcade, 2018). This plant can also increase soil nutrients, improve soil physical properties, and lead to increase crops productivity (Hafifah et al., 2016).

The problem in the application of organic matter is the high dose of organic matter, which ranges from 10 t ha^{-1} to 30 t ha^{-1} (Ginting et al., 2018). Therefore, in this study, we tried to reduce it based on the principle that organic matter is not positioned as a source of nutrients, but as a source of carbon and energy for beneficial soil microbes, namely Arbuscular Mycorrhizal Fungi (AMF).

AMF is a microbe that can be associated with almost all cultivated plants. This type of fungus has a special form of long external hyphae. According to Smith & Read (2008) the external hyphae of AMF can reach up to 30 meters per gram of soil, which is useful in post-mine sandpits C soil with high sand content to increase the bonding of the particles to make the soil more stable. Nurbaity et al. (2013) found that AMF increases the stability of andisol aggregates. Xiao et al. (2019) added that AMF can improve soil fertility in post-mine sandpits C soil.

The application of *T. diversifolia* organic matter together with AMF inoculation is expected to be synergistic. Organic matter improves soil physical properties and supplies carbon for AMF survival. Furthermore, AMF works in increasing aggregate stability, porosity, and soil permeability. AMF also plays a role in the decomposition of organic matter and releases high total P in the post-mine sandpits C soil so that the nutrients become available for the shallot plants that grow in porous conditions.

The purpose of this study was to determine the effect of *T. diversifolia* bokashi and AMF on the improvement of post-mine sandpits C soil properties and yield of shallot (*Allium ascolicum* L.) Batu Ijo variety.

MATERIALS AND METHODS

The research was carried out in Kutamandiri Tanjungsari Village, Bandung Regency with an altitude of 800 m above sea level from February to May 2019. The materials used in this study were post-mine sandpits C soil from sandstone mining in Giri Asih Village, Batujajar District, West Bandung Regency, mixed AMF inoculum (*Gigaspora* sp., *Glomus* sp., and *Aclauspora* sp.), 60% glucose solution, EM_4, alcohol, HCl (2%), KOH (10%), blue writing ink, onion bulbs of Batu Ijo variety, plant parts (leaves and young stems) of *T. diversifolia*, bran, urea fertilizer, TSP, KCl, and water. The tools used during the research were sample rings, soil sifter, polybag of 30 x 40 cm, 500 ml rinse bottle, soil sample weighing paper, oven, tissue paper, 250 ml Beaker glass, hoe, knife, microscope, digital scale, watering bucket, scissors, analytical balance, spore clamp, Petridish, spore net, shovel, caliper, thermometer, pH meter, permeability unit, label, plastic rope, stationery, pest trap, paranet, and camera.

In this study, two factorial randomized block design (RBD) was used with 20 treatment levels and 3 replications. The first factor was the addition of bokashi *T. diversifolia* and the second factor was the addition of FMA.

Factor 1, addition of *Tithonia diversifolia* bokashi (b) :

b0 : Control (without Bokashi)

b1 : Bokashi doses of 3 t ha^{-1} (9.37 g $polybag^{-1}$)

b2 : Bokashi doses of 6 t ha^{-1} (18.75 g $polybag^{-1}$)

b3 : Bokashi doses of 9 t ha^{-1} (28.12 g $polybag^{-1}$)

Factor 2, addition of Arbuscular Mycorrhizal Fungi (m)

m0 : control (without AMF)

m1 : AMF inoculum of 4 g $polybag^{-1}$

m2 : AMF inoculum of 6 g $polybag^{-1}$

m3 : AMF inoculum of 8 g $polybag^{-1}$

m4 : AMF inoculum of 10 g $polybag^{-1}$

Observation carried out in this research:

1. The degree of AMF infection in plant roots at harvest was calculated in units (%), using the grid line intersect method (Brundrett et al., 1998).

$$\text{colonization (\%)} = \frac{\text{the number of infected roots}}{\text{the total number of observed roots}} x100\%$$

2. Soil porosity was observed by calculating the total pore space of the soil with units of percent (%) and the formula (Kurnia et al., 2006):

$$\text{Total pore space (\%)} = 1 - \left[\frac{Bulk\,density}{Particel\,density}\right] x\ 100\%$$

3. Soil permeability in saturated solution in laboratory based on Darcy's law (Kurnia et al., 2006) in units (cm hour^{-1}):

$$\text{Permeability (K)} = \frac{QL}{AhL}\ \text{cm hour}^{-1}$$

Explanation:

Q : Water debit (cm^3 hour^{-1})

L : Thickness of soil sample (cm)

hL : Water surface height of soil sample and soil thickness (cm)

A : Surface area of the soil sample (cm^2)

4. Number of Bulbs Per Clump, observation was done after harvest on each plant.
5. Bulb diameter (cm), the measurement was carried out using a caliper. This observation was done after harvest on each plant.
6. Fresh weight of tubers per clump (g) observation was carried out at the end of the research by weighing the tubers harvested from each plant sample. Before weighing, the tubers must be cleaned of the soil attached.

Observation parameters were analyzed by Anova to determine the effect of treatment. If there was an effect of treatment, it was continued with Duncan's Multiple Range Test for further testing at the 5% level. The study started with making bokashi of *T. diversifolia* with 40 kg of plant material, 4 kg of bran, 25 g of glucose solution, and 100 ml of EM_4 in December 2018 at UIN Bandung campus. Soil from mining C was taken at a depth of 0-50 cm, and sieved with a 1x1 cm sieve diameter to separate the soil from the carried rock. The sifted soil was given additional bokashi according to the treatment. Preparation of planting media was carried out two weeks before planting. Moreover, the mixture of soil and bokashi was put into a polybag measuring 30 cm x 40 cm as much as 10 kg. Bokashi mixed with mining soil C one week before planting. The doses of bokashi used were 9.37 g polybag^{-1}, 18.75 g polybag^{-1}, and 28.12 g polybag^{-}

[1]. Meanwhile, AMF inoculation was carried out at the same time as planting shallot bulbs at doses of 4 g polybag^{-1}, 6 g polybag^{-1}, 8 g polybag^{-1}, and 10 g polybag^{-1} with mixed AMF types. The inoculum was applied with the carrier medium fine zeolite.

The onion bulbs used were Batu Ijo variety of medium size (10-15 g), healthy and fresh, not wrinkled, dense, and bright in color. Before planting, the dried outer skin of the tubers was cleaned. The bulb seedling was cut at ¼ part of the end of the bulb. Furthermore, 2/3 part of the tuber was immersed into the ground and covered with soil. Each polybag was planted one tuber.

The maintenance of shallot plants included watering, replanting, weeding, fertilizing and controlling of plant pests and diseases. Watering was done 2 times a day, if it rained then it was done according to the conditions. Replanting was done when the plant was 7 day after planting (DAP), weeding was done manually. Fertilization was carried out at the age of 21 DAP according to BALITSA (Vegetable Research Centre) recommendations i.e. 0.21 g polybag^{-1} urea, 0.14 g polybag^{-1} TSP, and 0.1125 g polybag^{-1} KCl.

RESULTS AND DISCUSSION

Degree of Root Infection

There was no interaction effect of AMF inoculants and *T. diversifolia* bokashi on the degree of root infection in late vegetative and generative observation times. At the end of vegetative observation, the value of the degree of infection was below 20% which was included in the low category. The value of the degree of infection increased to above 30% in the AMF and *T. diversifolia* bokashi treatments during the late generative phase (Table 1).

AMF inoculation increased the degree of root infection in the late vegetative, though not significantly. Likewise, the addition of *T. diversifolia* bokashi increased the degree of infection not significantly. In the treatment without inoculants, it was seen that there was a root infection. This was because in mining soil C there were indigenous AMF (Dodd, 2000). The results of wet screening analysis of soil samples taken from the rhizosphere of weeds growing in the mining area of excavation C found AMF sopra. Most of the spores were found in the rhizosphere of *T. diversifolia*, *Cynedrella nodiflora*, and *Impatiens balsamina*. The presence of AMF spores on *T. diversifolia* was the answer why under the independent influence of this weed bokashi generated a degree of infection. Suharno et al. (2014) also found indigenous AMF infecting several plants found on post-mine sandpits soil in Timika Papua, namely *Bracharia* sp. by 73.33%, *Setaria* sp. by 23.33% and *Bidens pilosa* by 63.33%. This informs that AMF has the potential to improve the remedy post-mine sandpits soils. The root infection by AMF can be identified in the presence of mycorrhizal organs such as internal hyphae, vesicles, and spores observed under a microscope (Figure 1).

The low value of infection degree in onion plants (Table 1) was influenced by environmental factors. The research conditions during the vegetative phase were often raining which resulted in wet soil and a relatively low average temperature of 24ºC. Hence, in this condition, the development of AMF was hampered since AMF will be able to infect host plants effectively and produce mycelia in relatively dry soil conditions with a temperature range of 28-35ºC. This is in line with the research of Hifnalisa et al. (2018) who found a low degree of mycorrhizal infection in coffee seedlings due to high rainfall and a temperature range of 22ºC. Wu & Ying-Ning (2017) stated that in dry soil conditions, AMF can maintain contact with roots, so that AMF will infect plant roots. High soil moisture inhibits spore germination in line with Brundrett & Tedersoo (2018) which states that spore germination and AMF workability are closely related to environmental conditions, especially temperature and soil moisture levels.

Another factor that caused a low degree of infection was the pH of the soil. In this study, the soil pH was relatively neutral. According to Sudheer et al. (2021), AMF has "Acidophilis" properties, which AMF actively develops in acidic conditions. Likewise, spores are found in a greater numbers under acidic conditions. Entering the generative phase, the environmental conditions were undergoing to change. Rainfall decreased and temperature increased, so that environmental conditions began to match the conditions desired by AMF, which led to an increase in the degree of root infection to a moderate category (Jerbi et al., 2020).

Soil Porosity

The application of AMF and *T.diversifolia* bokashi had an effect on soil porosity. The lowest average porosity percentage was 24.67% and the highest was 81.67% (Figure 1). The provision of *T. diversifolia* bokashi starting at a dose of 3 t ha^{-1} to 9 t ha^{-1} and AMF 6 g plant^{-1} was able to increase the average percentage of soil porosity compared to other treatments. This is in line with the research of Hidayat et al. (2017), namely the provision of AMF and various types of manure compost can improve soil porosity. According to Rahayu et al. (2018), the soil pore needed for shallots is around 60-75% until the end of the generative period. The results of the study were treated with AMF 6 g polybag^{-1} + *T. diversifolia* 6 t ha^{-1} (74.00%), AMF 8 g polybag^{-1} + without *T. diversifolia* (65.33%), and AMF 10 g polybag^{-1} + *T. diversifolia* 3 t ha^{-1} (62.00%) were sufficient for the pore needs of the shallot plant.

The addition of organic matter can determine the pore volume and size of the soil (Malik & Lu, 2015). The content of organic matter can improve the quality of soil's physical properties through the stimulation of soil microbes which makes the soil structure stable. Organic matter also helps the process of soil granulation, the more granulation of the soil formed, the more total of available soil pores.

The large amount of available organic C becomes a source of microbial food that makes the life of micro-fauna in the soil increase. According to Yang et al. (2017), the addition of AMF to the soil can help the formation of aggregates. AMF has hyphae that can release glomalin which is able to make the soil particles stick to one another. In sandy soil, glomalin from AMF hyphae acts as an adhesive (binder) of soil particles so that the soil structure becomes granular and many pores are formed. In soils that have low porosity, AMF hyphae are also able to penetrate the soil layer to find sources of water and soil nutrients. When AMF-infected roots grow lengthwise, these roots will break down the soil layer and new pores will be formed inside the soil.

Soil Permeability

The application of AMF together with *T. diversifolia* bokashi had an effect on soil permeability. The lowest average value of soil permeability was 0.52 cm $hour^{-1}$ and the highest was 2.57 cm $hour^{-1}$. The maximum point of soil permeability is found at *T. diversifolia* bokashi 3 t ha^{-1} and at AMF 10 g $polybag^{-1}$ (Figure 3). The application of *T. diversifolia* bokashi of 3 t ha^{-1} without AMF and with AMF 10 g $polybag^{-1}$ generated the highest permeability value and successfully entered the criteria for the medium permeability class (2.01-6.25 cm $hour^{-1}$) based on the permeability class criteria of Uhland and O'Neil (Kurnia et al., 2006). These data are consistent with the effect of AMF application and *T. diversifolia* bokashi on soil porosity (Figure 2) where AMF inoculation of 8 g $polybag^{-1}$ without organic matter and lower AMF inoculation (6 g $polybag^{-1}$) with *T. diversifolia* bokashi resulted in higher soil porosity which was sufficient for the soil pore needs of the onion.

The application of *T. diversifolia* bokashi affected the binding of soil particles into soil aggregates. As well as the formation of soil aggregates, it will produce pores that function as a way for water to enter the soil body (Nichols & Halvorson, 2013). According to King et al. (2020), organic matter that has undergone weathering has the ability to absorb water which is twice higher than the mass. In addition, organic matter also helps in binding water in the soil so that it can be utilized by plants. Besides organic matter, AMF is involved in the process of aggregate formation (Nurbaity et al., 2013) which will affect the pores formed. When the application of *T. diversifolia* bokashi and AMF simultaneously, organic matter from *T. diversifolia* will provide carbon for AMF living needs and activity in binding soil aggregates carried out by external hyphae (Curaqueo et al., 2010) thereby improving the percentage of porosity which ultimately increases soil permeability.

Number of Bulbs Per Clump

The application of AMF and *T. diversifolia* bokashi did not significantly affect the number of tubers (Table 1). The results showed that the average number of bulbs per clump was 3-5, appropriate to the potential for the number of bulbs per clump of 2-5 clumps.

The application of *T. diversifolia* increased the number of bulbs, though not significantly. Organic matter has a slow-release property which causes the availability of nutrients in the soil takes a long time to be absorbed by plants (El-Ramady et al., 2014), so tuber propagation did not increase significantly.

AMF inoculation also did not increase the number of bulbs significantly. This was related to unfavorable environmental conditions. According to Chandra (2018), AMF can develop at low soil moisture, which is 50-60%. This research took place in conditions of high rainfall which resulted in increased soil moisture so the AMF did not work optimally in increasing the uptake of P and K nutrients needed for bulbs formation. Yusriadi et al. (2017) added that AMF works well at acidic pH, while in this study the pH was neutral.

Bulb Diameter

The application of AMF and bokashi did not affect the diameter of shallot bulbs, but there was an independent effect from the provision of *T. diversifolia* bokashi. The increase of the doses increased the diameter of shallot bulbs and reached the highest value at a doses of 9 t ha^{-1}. Unlike the number of bulbs, *T. diversifolia* bokashi was able to provide the P and K elements needed for the formation of bulbs diameter, although it was not maximal, as seen the value was still below the plant description.

The formation of bulb diameter is also influenced by the planting medium used. Crumbly soil, lots of pores, and high nutrient content can help the tuber development process (Suprapto et al., 2018). The results of the research on the level of soil porosity, the combination of AMF inoculation and *T. diversifolia* bokashi resulted in the 60-75% porosity required for bulbs enlargement (Table 1).

Referring to the description of the potential of the shallot bulbs diameter namely 3-4.5 cm, while the results of the study only produced an average tuber diameter of 1.2-1.6 cm (Table 1), this indicates that the diameter of the onion bulbs produced in this study was low. The small diameter of the tubers was caused by the bokashi T. diversifolia which nutrients were not readily available for the plants. The slow-release nature of organic matter causes the supply of nutrients needed by plants during the generative period to be hampered (El-Ramady et al., 2014), hence the diameter of the tubers produced was not too large.

Based on the characteristics of the shallot plant, the bulbs will have a diameter of 3-4 cm if the climate at the time of planting is in optimal conditions, with a temperature of 25-32 ^{o}C and gets sunlight for more than 12 hours (Firmansyah & Bhermana, 2019). However, in the study, the average daily air temperature only reached 23.3^{o}C with high humidity (93.3%), and ± 8 hours of sun exposure, thus tuber growth was hampered.

Fresh Weight of Bulbs Per Clump

As in the observation of other tuber parameters, the application of AMF and *T. diversifolia* bokashi did not affect the fresh weight of shallots. The addition of bokashi *T. diversifolia* increased the fresh weight of shallot bulbs. The highest fresh weight was shown by giving 9 t ha^{-1} (Table 1). Bokashi *T.diversifolia* provides P and K that can be absorbed by plants (Isrun et al., 2018). The results of the bokashi *T.diversifolia* analysis showed that there was a P content of 1.89% and K of 3.50% which were able to support bulbs growth. Then the nutrients obtained by plants will be used for the formation of carbohydrates, proteins, and fats stored in the bulbs so that the fresh weight of the bulbs will increase (Jeptoo et al., 2013).

Referring to the description of the potential fresh weight of tubers per clump, it was ± 92 g clump^{-1}. While the results of the study only produced an average fresh weight of 20-25 g clump^{-1}, these results showed that the fresh weight of tubers per clump produced in this study was low (Table 1).

CONCLUSIONS

The application of AMF and *Tithonia diversifolia* bokashi generated appropriate soil porosity and permeability for the formation of shallot bulbs, but both were not able to produce shallot bulbs yield compared to the potential yield. The adding of *T. diversifolia* bokashi 9 t ha^{-1} increased the bulbs diameter and fresh weight of shallots, but still below its potency. Environmental factors that were less supportive contributed to the achievement of results which below the potential.

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Table 1 Effect of *Tithonia diversifolia* bokhasi and AMF on infection degree, growth and yield *Allium ascolicum* L.

Treatments	Average of infection degree (%)		Average number of bulbs per clump (pcs)	Average of Bulbs Diameter (cm)	Average of fresh weight of bulbs per clump (g)
	Late vegetative	Late generative			
T. diversifolia bokhasi					
b_0 (0 t ha^{-1})	13.97 a	27.75 a	3.93 a	1.02 a	11.63 a
b_1 (3 t ha^{-1})	15.43 a	30.41 a	4.67 a	1.67 b	26.43 b
b_2 (6 t ha^{-1})	16.83 a	31.41 a	5.13 a	1.41 ab	20.11 ab
b_3 (9 t ha^{-1})	14.64 a	31.60 a	5.40 a	1.89 b	32.01 b
AMF					
m_0 (0 g polybag^{-1})	10.09 a	16.05 a	4.83 a	1.37 a	21.13 a
m_1 (4 g polybag^{-1})	13.39 a	28.39 b	4.75 a	1.43 a	18.53 a
m_2 (6 g polybag^{-1})	18.35 a	34.39 c	4.67 a	1.52 a	23.11 a
m_3 (8 g polybag^{-1})	16.90 a	35.13 c	5.08 a	1.56 a	24.68 a
m_4 (10 g polybag^{-1})	17.36 a	37.51 c	4.58 a	1.60 a	25.28 a

Explanation: The average numbers in each column followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% level

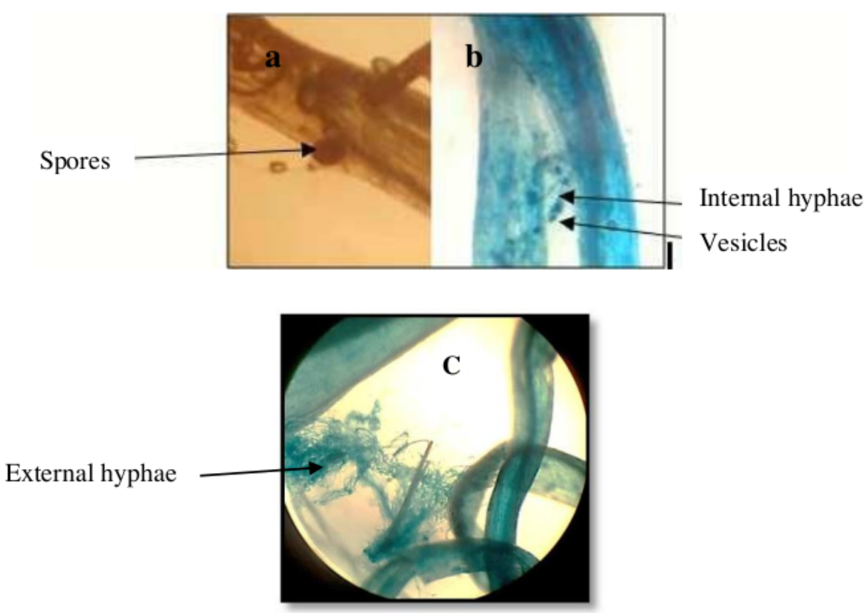

Figure 1. Shallot Root Infection Degree (late vegetative phase); a) Spores that develop in the root tissue; b) AMF organs that are inside and outside the root tissue; c) Degree of generative infection due to 40x microscope magnification

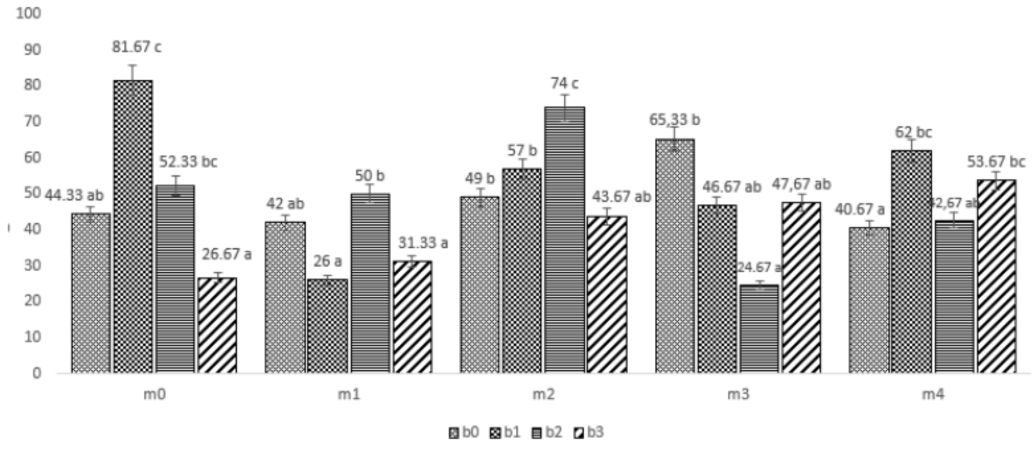

Figure 2. Effect of AMF and *T. diversifolia* bokashi on soil porosity (%)

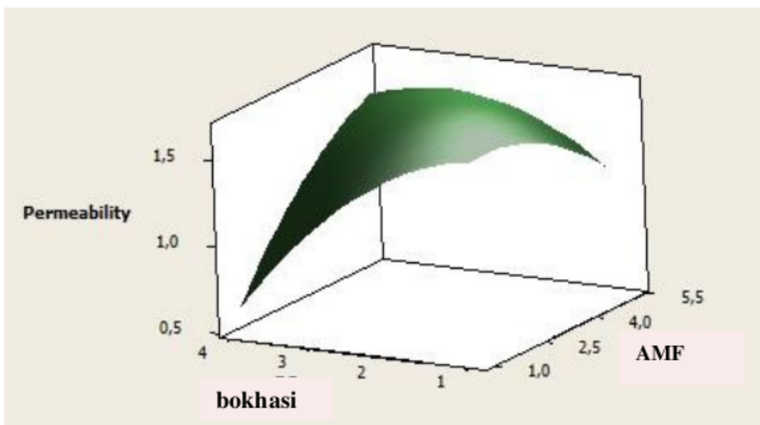

Figure 3. Maximm point AMF and *Tithonia diversifolia* bokashi on soil permeability (cm hour^{-1})

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