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THE EFFECTIVENESS OF ADDING MYCORRHIZES AND FERTILIZER COMPOSITION ON THE GROWTH OF SPINACH (*Amaranthus* sp)

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ABSTRACT

The production of organic spinach commodities has not been able to keep up with market needs; an effort is needed to produce quantity and organic spinach. The research was carried out in the laboratory and Greenhouse of the Faculty of Agriculture, University of KH.A. Wahab Hasbullah February – May 2021. The aim was to determine the effectiveness of giving mycorrhizae to spinach plants given various compositions of organic fertilizers with Trichoderma sp. decomposers, to improve the quality and quantity of harvested spinach plants. The research design used was a completely randomized design, with five treatments; M0 (8 g mycorrhizae with 0% organic fertilizer as a negative control), M1 (8 g mycorrhizal administration with 25% organic fertilizer), M2 (8 g mycorrhizae with 50% organic fertilizer), M3 (8 g mycorrhizae with organic fertilizer) organic 75%), M4 (8 grams of mycorrhizae with NPK Mutiara 16:16:16 fertilizer 5 grams as a positive control), with five replications. The results of the M2 treatment showed good growth in plant height (18.26 cm), the number of leaves (8.40 strands), plant wet weight (3.65 g), and root length (13.69 cm) compared to other treatments. Mycorrhizal spinach plants were effective in absorbing available nutrients in organic fertilizer 50% of the composition of the growing media but less effective on 25% organic fertilizer, 75% of the composition of the growing media, and NPK Mutiara 16:16:16 fertilizer. Organic fertilizers with local Trichoderma sp isolate decomposers could supply the nutrients needed by spinach plants for their growth. Organic fertilizers with a composition of 50% on plants that were given mycorrhizae might be able to suppress the use of inorganic fertilizers (NPK).

Keywords: Spinach, Mycorrhizae, Organic Fertilizer, Trichoderma sp

INTRODUCTION

Spinach (Amaranthus tricolor L.) is one of the most widely grown and developed vegetable commodities by horticultural farmers. This species can grow in the highlands to lowlands, with an environment that supports its growth as well as on critical land. Spinach cultivation has a short growing period, so farmers are able to harvest in a short time, and economically it can help farmers get quick sales results. In addition, the plant has a fairly good nutritional content, USDA

(Setiawati, et al, 2018) stated that it contained protein,ascorbic acid, and mineral nutrients (Ca, Fe, Mg, K, and Na) which are considered vegetables nutritional value. Moreover, every 100 grams of spinach contains 39.9 g of protein, 358 mg of calcium, 2.4 mg of iron, 0.8 mg of zinc, 18 mg of vitamin A, and 62 mg of vitamin C. The health benefits of spinach can restore the ability of the kidneys to work and help the digestive system. become smooth (Setiawati, et al. 2018). Therefore, many people like the vegetable commodity.

However, the production of spinach commodities has not been able to keep up with market needs, because it is generally cultivated by farmers with small land areas. Moreover, consumers in cities generally tend to have an awareness to improve health quality through consumption of vegetables, so that it affects the high demand for vegetable commodities. Based on the National Vegetable production data in Indonesia for 2015 – 2019, the spinach commodity in 2019 produced 160,306 tons, a decrease compared to 2018 which was 162,263 tons (BPS, 2019). Meanwhile, East Java Province was only able to contribute 9.1 percent of spinach commodity (14,601 tons) of the total production of spinach commodity (160,306 tons) in Indonesia (BPS, 2019).

On the other hand, public awareness of the quality of organic vegetable commodities for consumption and not easily damaged (perishable) is getting higher. This encourages efforts to produce spinach which is high both in quantity and organic quality.

The addition of nutrients through the application of organic fertilizers to the land is part of the effort to obtain such spinach commodities. Organic fertilizers are able to restore soil properties biologically, chemically, and physically. The decomposed organic matter contained in the fertilizer acts to bind loose particles, a source of plant nutrients and food for soil microorganisms. Giving organic fertilizer can increase the ability to dissolve elements, such as P, K, Ca, and Mg. Besides, it also increases C-organic, cation exchange capacity, and the ability of the soil to hold water.

Organic trash must undergo a decomposition process to become organic fertilizer. The manufacture of organic fertilizers cannot be separated from the role of decomposers which help speed up the process into mature organic fertilizers, and contain elements to help plant growth.

Trichoderma sp is one of the fungi that is useful in the process of decomposition of materials for organic fertilizers. Another benefit of this fungus is that it can play a role in encouraging plant growth and as a biological agent (Rizal and Susanti, 2018). *Trichoderma sp can decompose nutrients bound in the soil, and produce glycotoxin and viridian antibiotics which are used to protect plant seeds from disease, and produce* β-1,3- glucanase and chitinase enzymes that act as solvents for pathogenic cell walls (Rizal and Susanti, 2018).

Trichoderma sp has the ability to easily reproduce, is easy to monitor so it can maintain its presence in the environment, and does not contain chemical elements that cause harmful effects to the environment, animals, and humans (Febrianti, *et al*, 2019).

Research on the potential of Trichoderma sp as a decomposer in compost and biological agents has been recently carried out. Setyadi,et al (2017) melaporkan aplikasi kompos reported the application of Trichoderma sp. when the seedbed in chili plants showed insignificant different results from the Simantri compost treatment made from livestock waste. The time of shoot emergence, plant height, and number of leaves showed no significant difference. The study did not provide additional mycorrhizae that could help the absorption of nutrients in the Trichoderma compost used, so there was no difference with other compost applications.

Miftakhurrohmat and Fauzyah, (2018) in their research reported that the application of Trichoderma sp and manure had a positive effect on red spinach production compared to inorganic fertilizers. The organic materials used are limited to cow and chicken manure, without the composition of raw materials and the addition of other agricultural biomass.

Lehar, Salli, and Sine, (2018) concluded that the application of chicken waste organic fertilizer could interact with Trichoderma sp for the control of Phytophthora infestans and the yellow cyst nematode. Trichoderma sp was given two weeks after planting by watering the soil that had been given organic fertilizer, without knowing whether the maturity of the organic fertilizer was decomposed by Trichoderma sp or other decomposing microorganisms in the soil that was given material for organic fertilizer.

the suboptimal plant growth was the result of the unwise application of inorganic fertilizers/NPK; excessive and in a long time (Satria, et al. 2015). This can affect the unbalanced availability of nutrients. As a result, plants could not absorb nutrients optimally, and it interfered with the plant physiological processes and plant stem diameter growth. Therefore, it is necessary to add organic fertilizers other than inorganic for plant needs. Ananto (2020) stated that giving chicken manure significantly affected the growth of soursop seedlings, compared to cow and goat manure, so it is necessary to have a further study on cow manure as raw material for compost.

The use of mycorrhizal biological agents as the main ingredient of organic fertilizer, being a supporter to reduce the use of chemical synthetic fertilizers among farmers, and the success of plant cultivation on marginal land (Susanti,et al. 2020). The benefits of mycorrhizae in biological agent fertilizers are as a soil enhancer, not the same as synthetic chemical fertilizers in general, but as plant's assistants to take nutrients around them that cannot be absorbed by plants through their roots. Moreover, plants whose roots are infected with mycorrhizae will help to find and absorb water in the soil, making it suitable for use on dry/marginal land in helping plant growth and development (Susanti,et al. 2020).

Research on the use of mycorrhizae as fertilizer for biological agents has also been carried out on vegetable crops. Hadianur, et al. (2016) showed that Gigaspora sp was a type of mycorrhizal that could provide optimal growth and production of tomato (Lycopersicum esculentum Mill) using inorganic basic fertilizers, without the addition of compost. Suhardjadinata, (2020) studied the interaction between CMA inoculation with NPK fertilizer on the growth and production of tomato plants. The results showed a positive interaction between CMA inoculation with NPK fertilizer which could save the recommended use of NPK fertilizer between 25% to 50% and increase yield by 7.09% to 7.82%, without any organic fertilizer as a comparison. Nainggolan, et al. (2020) found that there was better vegetative and generative growth in long bean plants, with the application of mycorrhizal biofertilizer 5g/plant, than other doses. The manure used was limited to chicken manure, which in this study at a dose of 15 tons/ha could not significantly increase the growth and yield of long beans.

Based on this, an assessment was carried out which aimed to determine the effectiveness of giving mycorrhizae to spinach plants that were given various compositions of organic fertilizers with the decomposers of Trichoderma sp. , to obtain the quantity and organic spinach production.

MATERIALS AND METHODS

1 Research Implementation

The research was carried out in the laboratory and Green house of the

Faculty of Agriculture, KH.A. University. Wahab Hasbullah (Unwaha) Jombang, East Java, in February – May 2021.

2 Research Design

Completely Randomized Design (CRD) was used as the research design, consisting of five treatments and each repeated five times. The treatments were; M0 (8 g mycorrhizae with 0% organic fertilizer as a negative control), M1 (8 g mycorrhizal administration with 25% organic fertilizer), M2 (8 g mycorrhizae with 50% organic fertilizer), M3 (8 g mycorrhizae with organic fertilizer) organic 75%), M4 (8 grams of mycorrhizae with 5 grams of NPK fertilizer as a positive control).

2.3 Planting

The materials needed were seeds of pickled spinach (Amaranthus tricolor L.) Bangkok variety, yard soil, sand, mycorrhizal biofertilizer, and organic fertilizer/compost of cow dung and straw with a Trichoderma sp local isolate of culture decomposer developed by the Faculty of Agriculture Unwaha, NPK Pearl fertilizer. 16-16-16 and label paper. The equipment used was poly tray, polybag 16x25 cm, hoe, trowel, scales, camera, ruler, and raffia rope.

Preparing for planting, spinach seeds were soaked in warm water for approximately two hours so that the dormancy breaks quickly (Sidemen, et al. 2017). After that, the spinach seeds were planted in a polytray together with mycorrhizal biological agent fertilizer in the hole, then closed again (Susanti *et al.*, 2018). Mycorrhiza is derived from the mycorrhizal biological agent fertilizer which was propagated by the Faculty of Agriculture, Unwaha. Furthermore, after the plant was 7 days old, the plant and the soil were transferred to the planting media in polybags.

The composition of the planting media was a mixture of yard soil and sand with a balanced composition, and the addition of organic fertilizer based on treatment. The organic fertilizer/compost used was composed of cow dung (75%) and straw (25%), which had been decomposed with the help of Trichoderma sp. local isolates (Figure 1.) for 2 months.

(A) (B)

Figure 1. (A) Culture of Trichoderma sp, (B) Germinated Trichoderma Spores showing viability (400x magnification) (Personal Document, 2020)

The planting of spinach was carried based on the treatment when the seeds transferred to the polybag media. Organic fertilizer with the appropriate dose of treatment was mixed homogeneously with the planting media in polybagsThe dosage for the composition of the planting media was a full polybag and was left 5 cm of space measured from the top edge of the polybag. Furthermore, maintenance and care of the spinach plant were carried out, until it was 28 days old after planting, harvesting was carried out.

4 Root Coloring

Staining or painting the roots aimed at knowing the infection (colonization) of mycorrhizae in the root tissue. The equipment used was a microscope, scissors, object-glass, Petri dish, filter, needle preparations, slide glass, vial tube, and electric stove. The materials used were spinach plant roots which were suspected to have been infected with mycorrhizae, water, Aquadesh, 10% KOH (Potassium Hydroxide) solution, 1% HCL solution, alkaline H2O2, and lactophenol trypan blue. The staining method (Susanti, et al. 2018) was the boiled roots in a 10% KOH solution. The roots were then cleaned with water until no brown color appeared on the roots, then soaked in an alkaline H2)2 solution for 10 minutes. After 10 minutes, the roots were taken and dipped in 1% HCl for 3-4 minutes. The roots were cleaned again with lactophenol Trypan Blue, then left for a night, for the roots to absorb such solution. After that, the roots were placed on a preparat glass and covered with a coverslip and observed under a microscope.

5 Calculating Microbial Spore Density

The density of Trichoderma sp and mycorrhizal spores contained in each of the solid media was carried out to determine the quality control of each fungal spore contained in the tested material. The materials tested were compost and mycorrhizal biological agents the multiplication from the Faculty of Agriculture, Unwaha. The needed materials were: mushroom inoculum Trichoderma sp on the PDA media in culture test tubes, 100 ml aquades , aluminum foil, 70% alcohol. The tools needed were: analytical balance, magnetic stirrer, Neubauer Improve type Haemocytometer, Erlenmeyer 100 ml, volume syringe 1.0 ml, binocular microscope, hand counter, scissors, sampling spoon.

The hemocytometer type Neubauer Improve was prepared and placed on the microscope object table, covered with a cover glass. Furthermore, the counting area on the hemocytometer was observed with a magnification of 40x. A sampling of the test material on solid media was carried out using aluminum foil and put into 100 ml of Erlenmeyer. The test material used was mycorrhizal biological agent fertilizer. Aquadest was added until it reached 100ml. Then the solution was shaken until homogeneous using a magnetic stirrer for 15 minutes. The spore suspension from the results was taken as much as 0.2 ml using a syringe. Furthermore, the spore suspension was dripped slowly on the counting plane using a syringe through two sides until the canal was full, Let it stand for one minute to stabilize the position. Observations were repeated to obtain a focus on the spore count field. The number of spores contained in the counting box (a + b + c + d + e) was calculated using a 40x magnification using a hand counter. checking the counting was carried out for each count box. Spores located on the boundary line of the counting box were only counted on the left and top of the counting box. After knowing the number of spores in the calculation box, the number of spores/ml was calculated by the following formula:

$$S=\frac{X \times 10^3}{L\ (mm^2) \times t(mm) \times d}$$

Description:
S: number of spores/ml
X: number of spores counted
L : area of the counting box (0.04 x 5 = 0.2 mm2)
t : depth of field count (0.1mm)
d : dilution factor
103: calculated suspension volume
(1 ml = 103 mm3)

6 Observation Parameter

The observation Parameters were; 1) plant height measured until harvest time. The measurement of the boundary between the roots and stems, to the highest part of the plant, 2) the number of leaves, calculated every week during the vegetative phase until the beginning of the generative which marked by the appearance of flowers. The leaves counted were those that were completely open (Juhriah,et al. 2018), 3) wet weight of the plant, all parts of the plant were weighed at the harvest time or at the age of 30 days after planting. The soil close to the plant roots was cleaned with water so that all roots can be weighed, and 4) using a ruler, root length was measured after harvest, it was calculated from the base of the rootstock to the tip of the longest root.

Observational data were analyzed using the F test at the 5% level. The BNT test was carried out if there were significantly different results, with an error rate of 5%.

The root observations were carried out to determine whether the roots of spinach plants were infected with mycorrhizae. The observations were made seven days after seeding. Root samples of each treatment were taken and the level of infection was checked by staining the roots. Roots were declared infected if one or more types of propagules, i.e. vesicles, arbuscular, or internal hyphae, were present in the root cortex tissue.

The percentage of infected roots is calculated by the formula:

$$\%\ \text{infected roots} = \frac{number\ of\ infected\ roots}{number\ of\ observed\ roots(10)} X100\%$$

Note: number 10 indicates the number of observed root samples

The temperature and humidity at the time of testing were observed, starting from the time of planting the seedlings to the planting medium until harvesting (28 days).

RESULTS AND DISCUSSION

1 Roots of Spinach Plants Tested

Based on the observations of spinach roots which fed by mycorrhizal biological agent fertilizer, it was found that at the age of 7 days after planting, On average, mycorrhizal infected 10 to 27 percent of the roots. This showed that the mycorrhizae contained in the carrier media had good virulence, and there was compatibility between infected spinach plants and mycorrhizae.

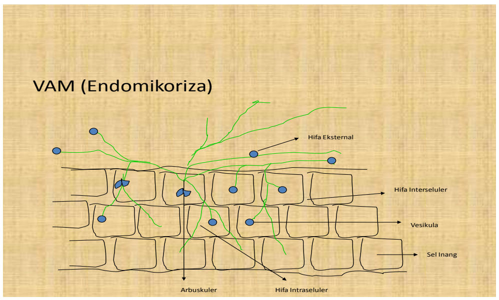
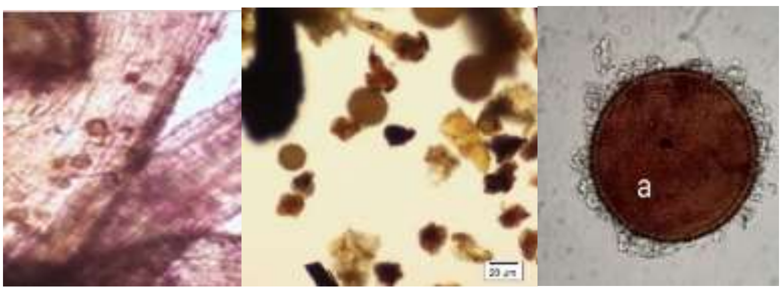


Figure 2. How Mycorrhizae infect the roots of host plants

The mechanism of mycorrhizae infected plant roots began with mycorrhizal spores germinating around plant roots, then through hyphae infected plant roots. The hyphae entered the root cortex, grew and developed in it (Figure 2).

Within the root tissue, mycorrhizal hyphae could form arbuscular, and/or versicular. Furthermore, the arbuscular produced hyphae that grew into internal and external hyphae.

Figure 3 (A) showed a cross-section of the roots of spinach plants in which there were arbuscular, and external and internal hyphae of mycorrhizae. The occurrence of infection or symbiotic colonization between plant roots and mycorrhizae could be characterized by the presence of round structures called vesicles and arbuscules (Sukmawaty, et al. 2016).



(A) (B) (C)

Figure 3. (A) Roots of spinach plants that which were infected by mycorrhizae (20µm), (B) Mycorrhizal spores in mycorrhizal carrier media produced by the Faculty of Agriculture, Unwaha (Personal document, 2020), (C) Mycorrhizal spores (400x magnification) (Afifah Document, 2020)

Based on the observation on the roots of spinach plants, it was clear that the type of mycorrhizal used was endomycorrhiza. This type of mycorrhizal was included in the group of mycorrhizae vesicular-arbuscular (MVA) the obligate symbionts character, which are widely found in agricultural plants.

2 Spinach Plant Height Tested

According to the results of observations on the effectiveness of mycorrhizae with the addition of organic fertilizer made from the decomposer Trichoderma sp. local isolates on spinach (Amaranthus tricolor L.), the plant height growth chart found and shown in the following figure

Figure 4. The graph of tested spinach plant growth

Description: M0 = 8 gr of mycorrhizae with 0% of organic fertilizer; M1 = 8 gr of mycorrhizae with 25% of organic fertilizer; M2 = 8 gr of mycorrhizae 50% of organic fertilizer; M3 = 8 gr of mycorrhizae with 75% of organic fertilizer, M4 = 8 gr of mycorrhizae with 5 gr of NPK fertilizer

It is known that each treatment experienced plant height growth. Up to the seventh day, on average, the growth in the five treatments was the same. It is suspected that mycorrhizal-infected roots still had not worked effectively to absorb nutrients and water in the planting media. The ability of plants to absorb nutrients was only known on the results of measurements on the 14th, 21st, and 28th days, which indicated that there were differences in plant height from each treatment. The M2 treatment had a significant plant height compared to the other four treatments.

Salisbury and Ross (*in* Satria, et al. 2015) stated that the increase in the size of the plant organs as a whole was the result of the increase in the size of the plant organs as a result of the increase in cell tissue produced by the increase in cell size. The highest average value of spinach plants was in treatment M2 (16.26 cm) compared to other treatments. The lowest value was in control M0 (9.79cm) without fertilizer application. While the M1, M3, and M4 treatments had no significantly different values, respectively 10.25 cm, 10.31cm, and 11.06cm (Table 1). The addition of organic fertilizer/compost to the growing media was important for the availability of nutrients because it had good potential for the growth of spinach plants. Sembiring et al. (2020) also stated that available nutrients were absorbed by plant roots, which were then used in the metabolic process, to encourage plant height increase. Organic materials that undergo decomposition process by decomposing microbes, produced elements of N-total in compost. (Yurmiati and Hidayati, 2008, in Yuniarti, et al. 2019). Furthermore, Sarief (1983, in Yuniarti, et al. 2019) claimed that the protein contained in organic matter was decomposed by microbial decomposers, undergoing a mineralization process into organic nitrogen in the form of NO^-.

This caused the availability of nutrients in the planting medium. Plants infected by mycorrhizae had a superior ability to absorb nutrients from the soil. Mycorrhizae that infected plant roots would form and produce both internal and external hyphae tissues that were symbiotic with the roots of the host plant. Mycorrhizal external hyphae helped roots in finding and absorbing nutrients and water. These hyphae had a longer reach than the roots of the host plant. So that the availability of nutrients in the form of compost, plant roots assisted by fine mycorrhizal hyphae, absorbed these nutrients and water became more optimal.

3 Number of leaves and plant weight of Spinach Tested

The data gathered for the analysis on the number of spinach leaves tested shown in Table 1. Significant results were also found in the number of spinach leaves treated with M2. It was known that the highest number of leaves in the range of 8-9 strands appeared in the M2 treatment, compared to the M0, M1, M3, and M4 treatments, which on average ranged less than 8 strands. The number of nutrients absorbed by plants for the process of photosynthesis was influenced by the given fertilizer. The content of N elements in compost played an important role in the vegetative phase of plants, especially leaf formation. The formed leaves became a place for the photosynthesis process then it affected the vegetative and generative growth of plants.

In correlation to the presence of mycorrhizae, the content of organic matter (BO) in the compost affected the work of external hyphae to infect the roots of the host plant. A fairly good BO could increase root infection consequently, the nutrients absorbed by plants increased as well, this supported the vegetative growth of plants. Plants that had adequate nutrients could help the growth of leaf area to be wide, and a lot of chlorophyll could be produced (Sari, et al. 2016).

Tabel 1. Average Result of the Observations of Spinach Plants (Amaranthus tricolor L) tested.

Treatment	Observation Parameters							
	height (cm)		Number of leaves (strands)		Weight (gr)		Root length (cm)	
M0	9.79	a	5.40	a	1.37	a	8.46	a
M1	14.65	b	7.20	bc	2.24	b	12.42	d
M2	18.26	e	8.40	cd	3.65	cd	13.69	de
M3	15.71	c	8.00	c	2.66	bc	10.45	b
M4	16.06	cd	8.00	c	2.68	c	10.68	c

Description: the numbers in the same column followed by the same letter show no significant difference in the 5% BNT test. M0 = 8 gr of mycorrhizal with 0% organic fertilizer; M1 = 8 gr mycorrhizae with 25% organic fertilizer; M2 = 8 gr mycorrhizae with 50% organic fertilizer; M3 = 8 gr mycorrhizae with 75% organic fertilizer, M4 = 8 gr mycorrhizae with 5 gr of NPK fertilizer

The number of leaves formed, affected the wet weight of the plant, as seen in Table 1. Polii (2009, *in* Sari, et al. 2016) argued in his research that an increase in the number of plant leaves would automatically increase the fresh weight of the plant. The observation on the wet weight of the tested spinach plants showed that the M2 treatment weight about 3.65 grams, significantly different from M1, M3, M4, 2.24gr, respectively 2.24gr, 2.66gr, and 2.68gr, and quite significantly different from M0, which was 1.37gr.

This case was influenced by the number of nutrients in compost, with the amount of N contained in it absorbed by plants fed by mycorrhizae. The amount of N absorbed by plants will increase the fresh weight of the spinach plants tested. The content of compost in the soil can have a positive effect on stimulating plant growth (Sari, Maghfoer and Koesriharti, 2016) Adanya kerja simbiosis dengan mikoriza, dapat membantu tanaman dalam penyerapan hara dari tanah secara optimal.

Stems influenced the wet weight of spinach plants, also influenced by stems. Based on the results of observations of plant stems for each treatment, the green M2 treatment looked darker and had an upright structure, followed by M1, M3, M4, and M0, which tended to be lighter green.

Probably, it was because plants grew less optimal due to the excessive doses of fertilizer. The inhibition of the osmosis process resulted from the condition of the concentrated soil solution, which was caused by excessive use of fertilizer, et al. 2015). This would interfere with the physiological processes of the plant and caused the plant not to grow optimally.

4 The Tested Spinach Plant Root Length

The average root length of the tested spinach plants was shown in Table 1. The M2 treatment had longer roots than the other treatments, up to 13.69 cm, significantly different from M1, M3, and M4.

Figure 5. The display of root length of spinach plants for each treatment 30 days after planting

Description: M0 = 8 gr of mycorrhizal with 0% organic fertilizer; M1 = 8 gr of mycorrhizal with 25% organic fertilizer; M2 = 8 gr of mycorrhizal with 50% organic fertilizer; M3 = 8 gr of mycorrhizal with 75% organic fertilizer, M4 = 8 gr of mycorrhizal with 5 grams of NPK fertilizer

The addition of mycorrhizae in each treatment helped the growth of plant roots, the hyphae searched for and absorbed nutrients and water that were less able to be reached by its host plant roots (Hartoyo, et al. 2015). Hyphae tissue that infected the host plant, in its growth directed nutrients to the rhizosphere area of the plant, could increase the vegetative and generative growth of the plant. External hyphae had a more effective surface for absorbing nutrients, which then transferred to the host plant. (Muis *et al.*, 2016). This showed a positive correlation between the application of mycorrhizae with compost and the tested inorganic fertilizers, on the growth of height, the number of stalks, and the leaflets of plants. The condition of nutrients in the soil also determined root morphology. Salisbury and Ross (1997, *in* Satria,et al. 2015) stated that the plant would be in a safe condition if sufficient nutrients are available, by forming a shallow root system. Giving cow manure would provide nutrients to the soil, so the plants would expand their roots.

The propagation of Trichoderma sp. which was applied to plantations and became a bio-decomposer, breaking down organic waste such as litters of twigs and leaves into the qualified compost (Setyadi,et al. 2017). Trichoderma as a decomposer of the tested organic was assumed to work more if it was homogeneously mixed with the planting media used for planting 7 days old spinach seedlings after nursery.

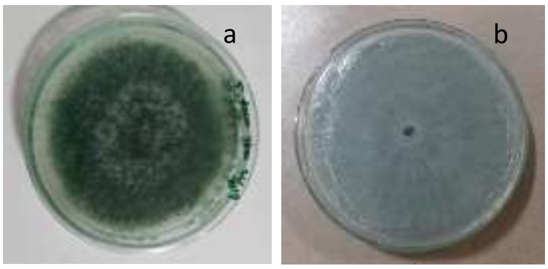


Figure 6. The cross-section of the surface of Trichoderma sp. isolate local used in the treatment. Fungus was grown on PDA media, 7 days after inoculation (a) top, (b) bottom (Susanti, et al. 2021)

In addition, the viability of the Trichoderma sp fungus used also affected the ability of the fungus as a decomposer and helped the availability of nutrients in the soil. It is known that the viability of the fungus was 100 percent, which indicated that the fungus was in good condition.

Trichoderma sp which was given to the 7 days old melon plants after seeding, could not work optimally so that it had no significant effect on the growth of the height and number of leaves (Valentine, et al. 2017). Mycorrhizae were given at the time of nursery to colonize and immediately infect plant roots when the roots were still young. This made it easy for mycorrhizae to penetrate and infect plant roots, therefore, when transferred to the planting medium, the roots were already infected with mycorrhizae and could immediately help absorbed nutrients in the spinach plants roots.

.4 The correlation between Mycorrhizae and Compost Fertilizer with Trichoderma sp decomposer and Inorganic Fertilizer on the tested Spinach Plants

Trichoderma sp contained in organic fertilizers might also affect the results of observations of the tested plants. Susanti *et al.*, (2018) concluded that the fungus was able to increase the quantity and width of leaves. The composition of the compost tested by giving mycorrhizae did not have a negative impact on the growth of spinach plants. It could be seen from the results of the observation, through this treatment, the plants increased until the observation ended (table 1). Valentine, et al, (2017) reported that the application of mycorrhizae and Trichoderma sp. affected the roots of melon plants. The collaboration of these two types of microbes stimulated the root formation that could increase the speed of plant growth and development. In this case, especially produced healthy plant roots.

In the M3 and M4 treatments (Table 1), generally, the average height, number of leaves, and wet weight of the spinach plants tested showed insignificantly different results. Spinach plants infected with mycorrhizae to the addition of organic and inorganic fertilizers (NPK) had less effect on these parameters.

The composition of organic fertilizers of about 75% of the total planting medium, providing abundant nutrient content for plants so that the roots did not need to work hard to find and absorb available nutrients in the rhizosphere area of the plant. This condition affected the activity of mycorrhizae, where mycorrhizae would form more spores than hyphae. This was because the environment around the rhizosphere was in fertile conditions. While the inorganic fertilizers (NPK) were known able to quickly supply these elements available for plants, making it easier for plants to reach and absorb elements contained in the inorganic fertilizers. It also affected the characteristics of mycorrhizae in the rhizosphere, which would form more spores than hyphae. Elpawati, et al. (2016) Stated that compost contained nitrogen and phosphate in the form of complex compounds which were very difficult for plants to absorb. Because of the presence of mycorrhizae, plants were able to take advantage of these elements. (Suryatmana *et al*. 2009 *in* Krisdayani, et al. 2020). However, the weakness was that the plants could not use the supplied inorganic fertilizer optimally. Inorganic fertilizers in the soil would undergo leaching so that some were lost, or could not be absorbed by plants and left in the soil. Based on Cassmar *et al.* (1996 *in* Yuniarti,et al. 2019) the range of the number of elements absorbed by plants was N, and K reached 30-50 percent, and P ranged from 15-20 percent. Additionally, the synthetic chemical compounds contained in NPK fertilizers affected on mycorrhizal life, which was assumed to reduce the mycorrhizal population in the rhizosphere.

This proved that the application of mycorrhizal biological fertilizers on the planting media that was given 25% of organic and 75% of inorganic fertilizers did not significantly impact the growth of spinach. Meanwhile, the application of 50% organic fertilizers, gave a positive correlation to the growth of spinach plants, then the application of 50% organic fertilizer to plants treated with mycorrhizae suppressed the use of inorganic fertilizers (NPK).

According to Sutedjo (2008, *in* Yuniarti, Damayani and Nur, 2019) Plants obtained nutrients in the soil quickly with the application of inorganic fertilizers. The combination of organic and inorganic fertilizers increased nitrogen absorption by cultivated plants. (Kubat et al. 2003, *in* Yuniarti, Damayani and Nur, 2019).

Rokhminarsi,et al. (2019) found that mycorrhizae and Trichoderma played a role in helping the supply of nutrients to reduce synthetic fertilizers and affect plant growth which ultimately affects chili fruit yields.

The use of Trichoderma sp helped to accelerate the process of matter organic weathering. Stated that good compost for cucumber plant growth was made from cow dung which was decomposed by Trichoderma harzianum. This could reduce the waiting period for compost maturation, from 2 to 3 months to less than a month (Okalia et al. 2017). The acceleration of composting process period showed that microbes could help accelerate the process of decomposition of organic matter into mature compost.

The livestock waste process that applied to crop cultivation was useful for soil structure and fertility. The source of livestock waste as raw material for fertilizer contained nutrients. Large livestock manure is rich in potassium, magnesium, nitrogen, and calcium. However, the main function of manure was to maintain the physical structure of the soil so that roots grew well. Soil capacity to hold water and cations increased, due to the application of manure, therefore, by giving manure and inorganic, it could inhibit leaching by rainwater and soil erosion (Melsasail, et al, 2019).

5 The Effect of Temperature and Humidity on Mycorrhizal Development in Spinach Plants

Light intensity was the main element in the photosynthesis process in spinach plants which produced carbohydrates, including roots. This condition caused the sensitivity of the plants to mycorrhizal fungal infections. The intensity of light directly affected the temperature. Based on observations, it was known that the average temperature at the time of testing was 33oC for the highest, and the lowest was 27oC with humidity was 60 RH - 70 RH. This was in accordance with the optimal conditions required for mycorrhizae in roots and increase the spore population in the rhizosphere. Mycorrhizae would actively produce spores and form hyphae in the process of infection to the roots at relatively high temperatures, depending on their type (Harran,et al. 1992 *in* Kamid,et al. 2020). Gigaspora mycorrhizae from Florida soil experienced the best germination at 34^{o}C (Harran,et al. *in* Kamid,et al. 2020). Furthermore, ita was found that the development of mycorrhizal physiology was not inhibited at a temperature of around 35^{o}C then decreased its activity at temperatures above 40^{o}C. (Harran,et al. *in* Kamid,et al. 2020).

CONCLUSION

Mycorrhiza amaranth (Amaranthus sp) was effective in absorbing available nutrients in organic decomposer fertilizer local isolate Trichoderma sp 50% of the composition of the planting media but less effective in applying 25%, 75% of organic fertilizer, and NPK Mutiara 16:16: 16 as much as 5 grams.

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