The Effect of Local Micro Organism and Mycorrhizal Fungi on Anatomical and Morphological Responses of Red Chili (Capsicum annuum L.) at Different Soil Water Level

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Abstract. Red chilli as a vegetable commodity plays an important role for Indonesian people and its cultivation depends on the level of water availability, which is still a problem in certain area in Indonesia. This study aimed to describe the effect of local microorganism (LMO) concentration and different soil water levels on the anatomical and morphological responses of red chilli by adding the mycorrhizal fungi in all treatments during the cultivation. This experimental study used Randomized Block Design (RBD) with two factors, namely the concentration of LMO (0mL/L, 25 mL/L, 50 mL/L, 75 mL/L) and different soil water levels based on field capacity measurement (100%, 75%, 50%, and 25%) with 3 replications. The parameters obtained included anatomical parameters (stomatal density and trichome density) and morphological parameters (plant height, leaf number, wet biomass, root length, mycorrhizal infection and relative water content of leaves). Data obtained from this study was in the form of morphological response of red chili and anatomic parameters. Data obtained in this study were analyzed by two-ways ANAVA and continued by the Duncan test. The results showed that there was an effect of LMO concentration and different soil water levels by the addition of mycorrhizal fungi on plant height, wet biomass, relative water content of leaves with the highest effect of LMO concentration at 75 ml/L with water level of 25%. In the stomatal density, the LMO concentration and water levels that significantly effect was 25 ml/L with water level of 25%. Although there was no significant effect on leaf number, root length, mycorrhizal infection and trichome density parameters due to the treatments. These findings recommended that red chilli could be cultivated better in the interaction of 75 mL/L of LMO concentration and soil water level of 25%.

1. Introduction
The use of red chilli in Indonesia based on the calculation of the Food Balance in 2002 until 2013 increased from 654 to 1.72 thousand tons, which shows an increase of 11.14% [1]. Red chilli plant widely cultivated on agricultural land both on a large and small scale and its cultivation depends on the water availability [2]. Water has an important role in plant growth because it plays a role in composing protoplasm, mineral nutrient solvents, medium for all chemical reactions, plays a vital role in photosynthetic reactions, maintains cell turgidity, and cell growth [3]. Mycorrhizae fungi play an important role to alleviate plant growth in drought conditions due to its role in regulating various host aquaporins through transporting water and facilitating membrane diffusion processes for other molecules such as CO₂, silicon, boron, urea, or ammonia [4]. In addition to being affected by water, plant growth was also influenced by the advantages of fertilizers such as local microorganism (LMO) from golden snail because it contains N-available, higher C/N ratio and P nutrient content compared to LMO from banana weed and LMO from rabbit urine and also contains nutrient content such as K, Ca,
Mg, Cu, Zn, Fe, and Mg [5]. Therefore, it was necessary to know more about the application of interaction between the LMO concentration and different soil water levels with the addition of mycorrhizal fungi in all treatments to the anatomic and morphological responses of red chilli (Capsicum annuum L.).

There is a connection between LMO, water availability, and mycorrhizae fungi. Water availability has a great impact on microbial community structure [6]. Where water availability have an effect on increasing host-microbe interactions [7]. In this case, mycorrhizal fungi have an ability to improving water availability. Mycorrhizal fungi can stimulate water lifting from roots to shoots via higher water apoplastic flow [8]. Increasing the availability of water will have an impact on increasing interaction between LMO with plants.

2. Methods
2.1. LMO Preparation
Golden snail (Pomacea canaliculata) in 1.25 kg has been ground than added rice-washing water as much as 2.5 L, 0.25 kg brown sugar, 2.5 L of coconut water and 2 L of water [5], mix for all of this and incubating for 21 days.

2.2. Water Level Determination
Determination of water level based on field capacity measurement as gravimetrically and simple lysimeter method by taking 5 kg soil from depth on 0-20 cm [9], and was added the water to the soil until saturated and the water stopped to drip out of the polybag. Then the soil was weighted after giving water and 100% field capacity was determined by reducing the final weight of the soil with the weight of the soil at the beginning.

2.3. Planting of Red Chili Seeds
Good quality of red chilli seeds was chosen and then soaked in warm water for about one hour. After that, the seed was stored for 24 hours. Basic fertilizer was added and mixed with 20 grams of mycorrhizae fungi-contain soil. This soil will be as a medium for planting the red chilli seeds.

2.4. Treatment
LMO was applied at the age of 10-15 days after seedling. After the red chilli plants reached the age of 21 days, the level of water supply could be applied and the LMO has applied again at the age of 30-35 days after seedling, 40-50 days after the seedlings are approximately 250 mL/plant.

2.5. Measurement of Stomata and Trichome Density
The abaxial leaves from the third to fifth sample leaves were coated from shoots [10] with colourless nail polish then dry at the air ± 40 minutes, coated polish again uses 1.5 cm tape to be lifted/peeled. The taped sticking parts were affixed to the object-glass and observed using a 400 magnification with diameter of 0.52 mm². The calculated by equations:

\[
\text{Stomatal density} = \frac{\text{Number of stomata}}{\text{broad field of view} (\text{mm}^2)}
\]

\[
\text{Trichome density} = \frac{\text{Number of trichome}}{\text{broad field of view} (\text{mm}^2)}
\]

2.6. Measurement of Plant Morphology
Measurements on plant height from the base of the stem to the main stem tip which was calculated at the age of 40, 50, 60 and at 71 days after seedling because at this age the red chilli was in the generative stage starting at 30-115 days after seedling which is obtained in the afternoon. The number of leaves that counted in this study was the number of leaves of red chilli that opens perfectly with clear leaf reinforcement which was calculated at the age of 40 days after seedling, 50 days after seedling, 60 days after seedling and at 71 days after seedling because at this age the plants were in the generative stage. The number of leaves was counted in the afternoon at the same time. The root length was calculated
from the base to the end of the stem at 71 days after seedling. Plant biomass was measured at 71 days after seedlings in the form of fresh roots, stems, and leaves. Relative water content of the leaves carried out at 71 days after the seedling. Measurements were modified by weighing fresh leaves to determine fresh weight (FW), then put in a cup containing distilled water until all the leaves were submerged, covered with filter paper and stored at room temperature for 18-24 hours. If the remaining water was left, the remaining water was removed while the sample was drained with tissue, then weighed to obtain the weight of turgid (TW). Then it dried using oven in 60°C for 2x 24 hours to get dry weight (DW) [11]. The relative moisture content of the leaf was obtained from the formula:

\[ RWC = \frac{FW - DW}{TW - DW} \times 100\% \]

The percentage (%) of mycorrhizal infections was obtained at 71 days after seeding through calculations [12]:

\[ \% \text{ Infection} = \frac{\text{Total root infection}}{\text{Total root infection} + \text{Non infection}} \times 100\% \]

2.7. Statistical analysis
Statistical analysis was performed using Two-Ways ANOVA to determine the effect of LMO concentration and different levels of soil water with the addition of mycorrhiza fungi to the anatomical response and morphology of the red chilli plant. If there are significant statistical differences, then was proceed with the Duncan test with a significance of 5%.

3. Results and Discussions
The effect of LMO concentration and different soil water levels with the addition of mycorrhizal fungi to morphological responses of red chilli in a) height plant, b.) number of leaves, c.) wet biomass, d.) root length, e.) mycorrhizal infection and f.) relative water content of leaves, were shown in Figure 1.
There was a significant effect of the interaction between the LMO concentration and different water soil level with the addition of mycorrhizal fungi to the height of red chili plant (Figure 1a), wet biomass (Figure 1c), and relative water content of leaves (Figure 1f), with the highest effect of the LMO concentration of 75 ml/L with a water level of 25%. However, there was no significant effect of the interaction between the LMO concentration and the different water levels to the parameters of leaf number (Figure 1b), root length (Figure 1d), and mycorrhizal infection (1e).

Other data that obtained from this study was the effect of LMO concentration and different water level with the addition of mycorrhizal fungi on anatomic parameters a.) stomatal density and b.) trichome density that is shown in Figure 2.

There was a significant effect of the interaction between LMO concentration and different water level with the addition of mycorrhizal fungi to the stomatal density (Figure 2a) with the highest effect found in the 25 ml/L LMO concentration with a 25% water level. However, there was no significant effect of the interaction between the LMO concentration and different water level on the trichome density (Figure 2b).

Plant growth was defined as a series of events that can be identified through qualitative changes such as germination and flowering or quantitatively such as the number of leaves, number of flowers, etc. in plant structures [13]. Growth occurs because of the increase in the number of protoplasts followed by the increase in the size of the weight and the number of cells that will affect in the meristem tissues to produce new cells that divide, enlarge and differentiate [14]. The division in meristem tissues occur because of mitotic division at the root tip and the end of the stem that depending on the availability of carbohydrates, protein mainly water in the big capacity and the effect will be seen in vegetative growth of plants such as increasing plant height, leaf number, stem diameter and root growth [15]. Plant
growth besides being influenced by the level of water availability also influenced by hormones that found in plants such as the auxin, cytokinins, and gibberellins which play an important role in cell expansion [16].

LMO from golden snail used in this study was known to contain nutrients such as N and Fe which play an important role as a constituent of proteins, amino acids, nucleotides, and enzymes [16] for cell division related to protein synthesis. Mycorrhizal fungi play a role in increasing water and nutrient uptakes such as N, P and K [17]. If the availability of N nutrients in plants increases, cell division in plants will also increase. The symbiosis between mycorrhizal fungi and root will increase root growth, architecture and functional hydraulic roots for water and nutrient uptake, absorbs and transportation [18] which has an impact on increasing the relative water content in plant tissues (Figure 1f.). The higher relative moisture content of the leaves indicates that the plant is more tolerant. The increase in the water supply will increase the potential of turgor so that the process of plant cell division will increase too. In addition, mycorrhizal fungi will increase also the diffusion of ions for cell elongation related to the action of auxin hormones in cell formation [16]. This event can be observed with the increasing of plant height (Figure 1a) and the increasing of the stomatal density (Figure 2a.). The density of the stomata was related to the number of stomata that influence the photosynthesis and transpiration. The increase of the density of stomata will increase the transpiration so that there was an increase of CO₂ uptake for dark reactions (Calvin cycle) in photosynthesis by fixation of CO₂ which produces carbohydrates [16].

The results of this study showed that the increase of stomatal density was not followed by an increase in leaf trichome density (Fig. 2b) caused by plant responses further minimized cell division for stomata formation compared to trichomes. Although with high stomatal density, plants will not lose much water through transpiration due to the absorption process which compensates for the rate of water loss through transpiration due to the presence of root symbiosis with mycorrhizal fungi [19].

LMO also contain nutrients Mg, Fe, Zn and Cu which play a role in arranging leaf chlorophyll for photosynthetic materials mainly in bright reactions. If the chlorophyll content of leaves was high, the photosynthesis process will increase so that the photosynthetic compounds produced such as carbohydrates will also increase. These photosynthetic compounds were used for cell division and composting plant biomass (Figure 1c). If the biomass value of a plant was greater, it indicates that the nutrient content absorbed by plants was also getting bigger [20] and more water content in plants because the weight of the plant tissue about 90-95% consisting of water [16,18].

The application of LMO and mycorrhizal fungi with the different water level availability did not appear to have an effect on increasing the number of leaves (Figure 1b). It was because more photosynthetic assimilates are distributed to the roots than to the leaves for the expansion of the root system. Another factor that affects the decrease in the number of leaves is the presence of pest attacks Aphis gossypii causes leaf malformations to become curved, perming, shrinking, thickening, chlorosis and declining before adulthood. Then Thrips which attack the main leaf on young leaves with symptoms of colour change from light green to brown copper, thickened surface, leaves become wrinkled and when touched will fall/fall out [21, 22].

LMO and mycorrhizae fungi with the different water availability also had no effect on root length (Figure 1d) and mycorrhizal infections (Figure 1e) but these two parameters had other parameters due to their important role in transporting water and nutrients from the soil. Microscopic observations showed that most of the roots were infected by mycorrhizae fungi by finding internal hyphae in the roots. Mycorrhizae fungi are able to aggregate soil grains so that the ability of the soil to store water increases [23] to be absorbed by plants and the effects of various biochemical reactions on plants can be carried out.

4. Conclusion
Based on the data analysis can be concluded that there was an effect between the interaction of the LMO concentration and different water levels by the addition of mycorrhizal fungi on plant height, wet biomass, and relative water content of leaves with the highest effect of LMO concentration at 75 ml /L and water level of 25%. There was no significant effect of the interaction between the LMO concentration and different water level with the addition of mycorrhizal fungi on the response of red chilli (Capsicum annuum L.) at parameters leaf number, root length, mycorrhizal infection and leaf
trichome density. These findings recommended that red chili can be cultivated better in the interaction of 75 mL/L of LMO concentration and soil water level of 25%.

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