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Plant growth analysis and arbuscular mycorrhizal colonization in *Amaranthus tricolor* L.

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Abstract

Red spinach (*Amaranthus tricolor* L) is a horticultural vegetable that contains anthocyanins, protein, calcium, phosphorous, iron, vitamin A, vitamin C, and B vitamin. However, this plant has not become a major commodity, so it is generally grown in lands with a low level of soil fertility. Application of arbuscular mycorrhizal (AM) fungi in powder dosage form can help plant growth with a positive effect. However, this dosage form has several weaknesses, so a study aimed at knowing the effectiveness of AM fungi in different dosage forms is needed in physiological studies to analyze plant growth and the level of colonization of red spinach roots in the vegetative stage. This research was carried out using the model of the Latin square design (LSD) with three treatments for AM dosage forms of fungi: tablets, sachets, and powders, and without mycorrhizal application (control). The results showed that AM fungi in sachet dosage form could colonize the roots of spinach plants with the highest percentage, although physiological studies have not given consistent results. The sachet dosage form of AM fungi is the novelty of this study. AM fungi in a sachet dosage form can are recommended as the dosage form the most attractive, efficient, and effective to be applied and mobilized to farmers' fields.

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Introduction

Spinach is a horticultural plant that has many types and discoveredin 60 families that can grow on various types of land and in different ecosystems (Ribera et al., 2021; Ulianych et al., 2020). One type of spinach is red spinach (*Amaranthus tricolor* L.) originating from tropical America(Sumarmi et al., 2020). Red spinach contains high anthocyanins and functions to prevent the formation of free radicals (Shafura et al., 2022). The results showed that red spinach contained 6350 ppm anthocyanins in the leaves and 2480 ppm in the stems (Pebriantiet al., 2015).

In addition to antioxidants, red spinach also contains protein, calcium, phosphorus, iron, vitamin A, vitamin

B, and vitamin C. In addition to the benefits of the leaves (Febriyani et al., 2019; Fevriaet al., 2021), spinach root can be used as an antipyretic, diuretic, anti-toxic, diarrhea medicine, and to clean the blood (Ganjare and Raut, 2019; Peter and Gandhi, 2017).

Cultivation of red spinach is still limited due to the condition of agricultural land with low nutrient content. According to Fajeriana et al., (2022) and Nawaz et al., (2017), red spinach productivity can be increased if it is planted in land conditions with high organic matter content and sufficient nutrient availability, pH 6-7, and the use of biological agents.

The application of mycorrhizae as a biological agent technology has been widely studied and has given positive results for plant growth. However, the application of mycorrhizae is still in the powder dosage form. This dosage form has several disadvantages, including having a non-uniform application dose and allowing spore loss due to wind and being washed away by rainwater. The unavailability of technology for MA fungi dosage forms packaged in sachets and tablets is important in this study, as a solution to improve the performance of MA fungi which is efficient, effective, and environmentally friendly. so a study is needed that objectives to determine the effectiveness of AM fungi in various dosage forms in physiological studies. (analysis of spinach plant growth in the vegetative phase). Plant growth analysis is a way to follow the dynamics of photosynthesis by measuring dry matter production.

The accumulation of dry matter reflects the ability of plants to bind sunlight energy through the process of photosynthesis, as well as its interaction with other environmental factors. The components of growth analysis include Leaf Area (LA), Leaf Area Index (LAI), Relative Growth Rate (RGR), and Net Assimilation Rate (NAR).

Research methods

The research was carried out at the Agroplastid farm in Parepare city, Indonesia, at the coordinates of 3˚59'30,204" S; 119˚38'42,936" E in an experimental form based on the Latin Square Design with three treatments of dosage form AM fungi, namely: tablets, sachets, powders and without mycorrhizal application (control).

Plant growth analysis was implemented by observing the Leaf Area (LA), Leaf Area Index (LAI), Relative Growth Rate (RGR) variables, and net assimilation rate (NAR). The formula for each growth analysis variable is shown in table 1.

Preparation of red spinach roots infected with AM Fungi was carried out at the Biotechnology Laboratory of the Makassar Environmental and Forestry Research and Development Center (BP2LHK Makassar). The colonization of AM Fungi in roots could be calculated based on the number of all roots observed with using the formula from Brundrett et al., (1996).

$$\%\ \text{AM Fungi colonization} = \frac{\sum \text{infected root area}}{\sum \text{total area of the roots observed}} \times 100\%$$

The Institute of Mycorrhizal Research and Development, USDA Forest Service, Athens, Georgia has classified the number of root infections into 5 classes: grade 1, very low (if infection was 0% - 5%), grade 2, low (if infection was 6% - 25%), grade 3, moderate (if infection was 26% - 50%), grade 4, high (if infection is 51% - 75%), grade 5, very high (if infection was 76%-100%) (Rai et al., 2019).Data of plant growth analysis and the percentage of infected roots, were analyzed with the F test and Middle-value test which are shown in graphical.

Results and discussion

Analysis of variance showed that the dosage form of AM fungi an effect was not significant until significant effects on the observed components of the red spinach.Variance analysis for variables of LA and LAI showed that the application of biological agents in different dosage forms had a non-significant effect, but average values obtained were very diverse. Plant growth spinach at 30-40 DAP, LA and LAI phenomena appear the same in all treatments. However, when the plants reached the age of 50 to 60 DAP, a high LA value was also followed by a high LAI value. The phenomenon provided information that the plant leaves had shaded each other (Figures 1A and 1B). Campbell, (2022) stated that an LAI >1 indicates the occurrence of shading between leaves, the shaded leaves will cause the rate of photosynthesis to be not optimal because the shaded leaves will be parasitic (Pignon et al., 2017; Wang et al., 2021; Wijeratne et al., 2008).

The treatment of MA fungi in tablet dosage forms stimulated the growth of red spinach with very high LA and LAI values at the age of 60 DAP. Allegedly, AM fungi in tablet dosage form have AM fungi spores that have adapted and symbiosis with plant roots to help absorb water and nutrients to meet plant needs.

The absorption of water and nutrients can be increased by the formation of internal and external hyphae (Irmawati and Gofar, 2020; Prameswari et al., 2021). External hyphae are an important part of AM fungi that are outside the roots and function to absorb phosphorus in polyphosphate, these compounds are then transferred into the internal and arbuscular hyphae where they are broken down into organic phosphates which are released into the host plant cells (Madrid-Delgado et al., 2021; Smithet al., 2003). AM fungi can help plants absorb water and nutrients needed for photosynthesis, while plants provide assimilation for the survival of AM fungi (Bahram and Netherway, 2022; Elliott et al., 2021).

A high LAI value does not always provide benefits for plants, depending on the morphology and leaf sitting position (Khairunisa, 2015; Parker, 2020). Red spinach plants given AM fungi in different dosage forms had a negative impact, namely a decrease in the net assimilation rate (NAR) and relative growth rate (RGR) (Figure 2A and 2B). The ANOVA results showed that NAR and RGR were not affected by the treatment of AM fungi in different dosage forms. The net assimilation rate (NAR) is produced from the net-assimilation process per unit leaf area and time (Díaz-López et al., 2020; Hilty et al., 2021). The rate of assimilation is not constant with time but decreases with increases in plant age and is linearly related to leaf area and plant dry weight (Aslani et al., 2020; Hilty et al., 2021). The inhibition of leaf development will impact to leaves' reduced capacity to absorb light (Bielczynski et al., 2017; Cioć and Pawłowska, 2020; Yavari et al., 2021). The NAR has an opposite relationship with the increase in LAI, where the higher the LAI value as at the age of 50-60, the lower the NAR value because the plant leaves shade each other so that it affects the production of assimilation. Net assimilation rate according to Aslani et al.,(2020) and Li et al., (2016) is strongly influenced by the spread of sunlight on the plant canopy, the existence of leaves that shade each other will reduce the NAR value. This opinion is in accordance with the statement of Orzech et al., (2022) and Samidjo, (2021)that the net assimilation rate depends on the level of sunlight to plants. The spread of solar radiation on the canopy determines the dry matter production rate per unit leaf area during vegetative growth. Likewise, Raffo et al., (2020) and Yang et al., (2021) states that the production of plant dry matter depends on the acceptance of sunlight on plants and the availability of water. Relative growth rate (RGR) is rate of increase in plant dry weight per unit of dry weight (Sun et al., 2021; Zulkarnaini et al., 2019). The decrease in NAR affects reduced in RGR of plant. As explained by Francescangeli et al., (2006) and Osone et al., (2008) that decrease in RGR was caused by increase in LA which caused LAI to increase, so that reducing photosynthesis rate and increasing respiration rate, which caused that use more assimilate.

According toVillar et al., (2005) and Almodares et al., (2007), the older age of plant, so the lower of RGR. The RGR is also closely related to the use efficiency of light by leaves, in this case, LA and NAR will affect RGR. An increase in LA followed by a high NAR can increase RGR.

Table 1. Formulation of plant growth analysis.

Variable	Unit	Formula	Source
LA	cm^2	Wr/Wt x LK	(He et al., 2020; Musa et al., 2020; Schrader et al., 2021)
LAI	No Dimension	$(LA_2 + LA_1/2).1/G$	(Aschonitiset al., 2014; Chenet al., 1997; R. Yang et al., 2022)
RGR	$g.g^{-1}.day^{-1}$	$\ln W_2 - \ln W_1 / T_2 - T_1$	(Kumar & Kaliyaperumal, 2015; Pommerening & Muszta, 2015)
NAR	$g.cm^{-2}.day^{-1}$	$(W_2\text{-}W_1/T_2\text{-}T_1)$. $(\ln LA_2\text{-}\ln LA_1/LA_2\text{-}LA_1)$	(Díaz-López et al., 2020; Li et al., 2016)

Note : LA = Leaf area (cm^2), Wr = Leaf replica paper weight (g), Wt = total paper weight (g), LK = Total paper area (cm^2), G = Ground area, W=dry weight, T=time.

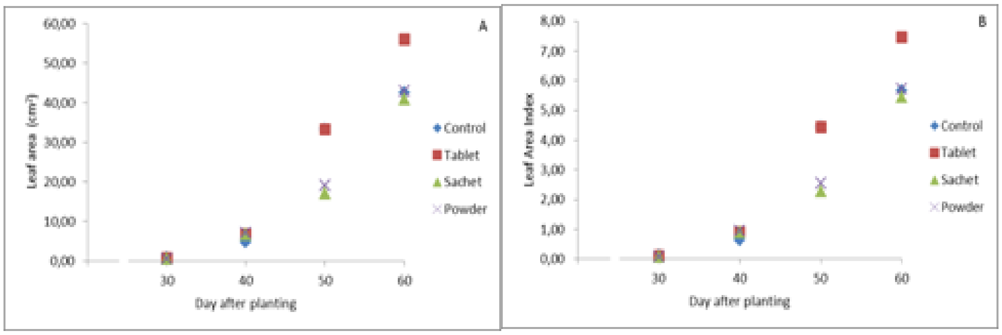

Fig. 1: The average LA and LAI of red spinach at treating different AM fungi dosage forms.

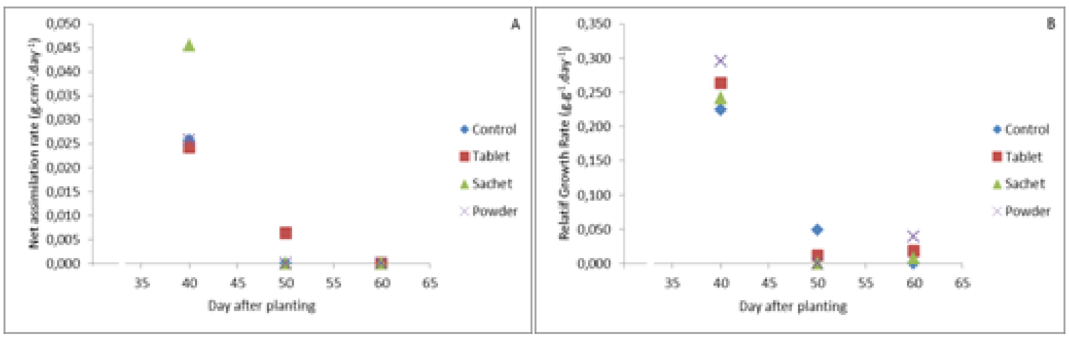

Fig. 2: The average NAR and RGR of red spinach at treating different AM fungi dosage forms.

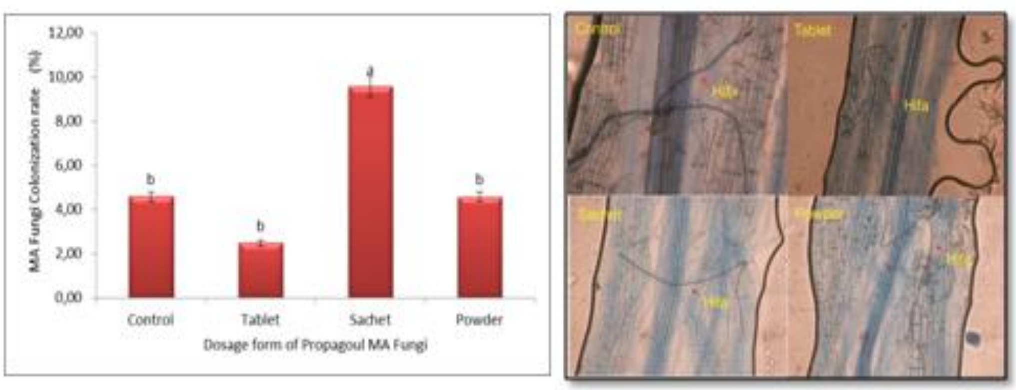

Fig. 3: The average root colonization level of red spinach at treating different AM fungi dosage forms.

AM fungi propagule dosage form had a positive effect on the colonization rate of AM fungi on plant roots, the highest colonization rate was obtained in the sachet dosage form compared to other dosage forms, but based on the classification of number of root infections by Institute of Mycorrhizal Research and Development, USDA Forest Service, Athens, Georgia, then the percentage of colonization rates in all propagule dosage forms is still classified as very low grade (0% - 5% infection) to low grade (6% - 25% infection), this is presumably because apart from the MA fungus, it is still in the process adaptation to the host plant is also possible that the supply of assimilating for MA fungi is very low so the performance of AM fungal infection in plant roots classified as low, as can be show in Fig. 3.

The AM fungi in a sachets dosage form can colonize the roots of red spinach with the highest percentage, so that can be recommended as a dosage form of AM fungi that is more attractive, efficient, and effective to be applied and mobilized to farmers' land. Although physiological studies it has not given consistent results.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

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