

Effect of AMF propagule dosage forms on the growth and production of *Amaranthus tricolor* L.

M. A. Akib^{1*}, Syatrawati Syatrawati², R. Prayudyaningsih³, S. Antonius³, and T. Kuswinanti⁴

¹Universitas Muhammadiyah Parepare, Department of Agrotechnology, Faculty of Agriculture, Animal Husbandry and Fisheries, Parepare, Indonesia

²Politeknik Pertanian Negeri, Department of Food Crop Production Technology, Pangkajene dan Kepulauan, Indonesia.

³National Research and Innovation Agency (Badan Riset dan Inovasi Nasional-BRIN), Research Center for Applied Microbiology, Cibinong, Jawa barat. Indonesia.

⁴Hasanuddin University, Department of Plant Pest and Disease Science, Faculty of Agriculture, Makassar, Indonesia

Abstract. Red spinach (*Amaranthus tricolor* L) contains vitamins (A, B2, B6, K, and folate), proteins, carbohydrates, fats, minerals, fiber, iron, magnesium, manganese, potassium, and calcium. Red spinach also contain anthocyanins (red pigments) that function as antioxidants to prevent free radical oxidation. The use of biofertilizers can give benefits to the soil and plants. Mycorrhizal fungi can be used as a type of biological fertilizer. The application of arbuscular mycorrhizal fungi (AMF) in powder dosage forms can help the growth of plants. However, this dosage form has disadvantages, so a study is needed to determine the effectiveness of AMF in various dosage forms. This study applied the Latin Square Design (LSD), with three treatments of AMF propagule dosage forms, namely: tablets, sachets, and powders, without AMF application as a comparison/control. The results showed that the effectiveness of AMF propagule in dosage form sachets provided a reasonable infection rate with fertile plant growth and harvest index value was not significantly different from other dosage forms, due to the attack of plant-disturbing organisms before harvest. So, further research is needed to determine the effect of the AMF propagule dosage form in physiological review without invasion of plant-disturbing organisms.

1 Introduction

Spinach (*Amaranthus* sp.) is a vegetable plant originating from the Americas and now this plant is spread throughout the world. Spinach is generally used as a culinary dish, such as vegetables and chips [1, 2]. Red spinach (*Amaranthus tricolor* L) contains many vitamins (A, B2, B6, K, and folate), protein, carbohydrates, fat, minerals, fiber, iron, magnesium, manganese, potassium, and calcium [3, 4]. Red spinach contains anthocyanins (red pigments) which act as antioxidants to prevent free radical oxidation [5, 6].

* Corresponding author: akhsanbagus@umpar.ac.id

The use of biofertilizers can provide benefits to the soil and plants. Biofertilizers are live microorganisms that are added to the soil as inoculants to facilitate or provide nutrients for plants [7, 8]. One type of biofertilizer that can be used is mycorrhizal [9, 10]. The use of mycorrhizae as biological fertilizer can be used as an alternative to avoid soil damage due to the use of inorganic fertilizers [11, 12] and increase the efficiency of nutrient absorption [13, 14].

Mycorrhizae are organisms from a group of fungi that form a symbiotic relationship of mutualism between fungi and plant roots [15, 16]. Mycorrhizae have great potential as biological fertilizers because mycorrhizae are microorganisms that have a very important role for plants, namely facilitating the absorption of nutrients in the soil to increase plant growth [13, 14], as a biological barrier against root pathogen infection [17, 18], increasing water availability for plants [19], and increasing production growth hormones such as auxins, cytokinins, and gibberellins for their host plants [20, 21]. In addition, mycorrhizae are also able to adapt to extreme environments, especially in marginal soils such as dry areas, low pH, acid soils, and soil contaminated with heavy metals [22, 23].

The application of AMF propagule in powder dosage form has long been used by farmers, but this dosage form has several drawbacks, including having inconsistent application doses and allowing loss of spores due to wind and rain. Therefore, it is necessary to innovate the AMF propagule dosage form which has an infection rate greater than 50%.

The technology for the AMF propagule dosage form which is packaged in a simple, practical, and applicable form is still very limited. Commercially, AMF dosage forms and storage are more attractive, easy to do, and controlled in quantity and quality, so research is needed to study the effect of AMF propagule in dosage forms different on red spinach, which is also the purpose of this study.

2 Research methods

The research was conducted in the village of Bukit Harapan, the city of Parepare, Indonesia, at the coordinates of 3°59'30,264" S; 119°38'42,996" E. This experimental form is based on Latin square design (LSD) with 4 treatments, namely MA in tablet, sachet, powder, and without mycorrhizal application (control). Each dosage form contains 5 g of AMF propagule. An organic adhesive is needed to make tablet dosage form, while organic bags that are readily biodegradable is needed for the sachet's dosage form.

Parameters observed were plant height, leaves number, plant dry weight, harvest index, and level of AMF colonization on roots of red spinach. To estimate the harvest index, the used of formula comparison of the economic weight/leave weight (BE) with the biological weight/root, stem, and leave (BB), or calculated using the formula: BE/BB [24, 25].

The preparation of roots of red spinach infected with MA was carried out at the Biotechnology Laboratory of the Makassar Environmental and Forestry Research and Development Center (BP2LHK Makassar). The percentage of root infection was calculated based on the number of all observed roots and followed the formula in Equation.1 [26]. Observational data were analyzed using the F test and the mean value and shown in the bar chart.

$$\% \text{ AM Fungi colonization} = \frac{\sum \text{infected root area}}{\sum \text{total area of the roots observed}} \times 100\% \quad (1)$$

3 Results and discussion

Results of variance analysis showed that AMF propagule dosage form gave the effect of insignificant to significant on each age of plant vegetative growth. Figures 1a and 1b, show

that the AMF propagule dosage form significantly affected the height and leaves a number of red spinaches at the beginning of growth (14 to 21 days after planting/DAP) and had no significant effect at the age of 28 to 42 DAP, it was suspected that the mutualistic symbiosis between mycorrhizae and plant roots had been well established at the age of 14 DAP so that the plants experienced fertile growth. According to Molinari et al. [27] and Oliveira et al. [28] mycorrhizal infection can occur after 7 days after being inoculated, so a mutualistic symbiotic relationship can occur.

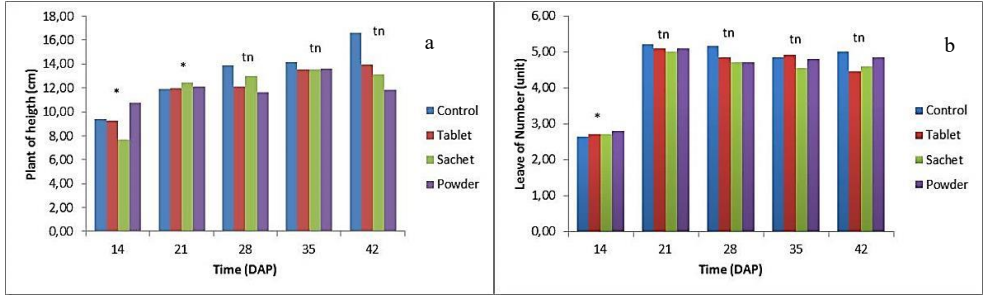


Fig. 1. Average values of the height (a) and leaves number (b) of red spinach given different MA propagule dosage forms.

The effect caused by plants with growth that is too fertile is the incident of caterpillar attacks on plant shoots and leaves so that plants experience abnormal growth at the age of 21 DAP, especially in plants that receive AMF propagule treatment in different dosage forms, so that plants experience growth slow and tends to stagnate which causes plant growth to tend to be uniform so that the results of the analysis of variance for ages 21 to 42 DAP have no significant effect. According to Biswas et al. [29] and Sedo et al. [30] plants that are too fertile can have soft leaf tissue easily attacked by pests and plant diseases.

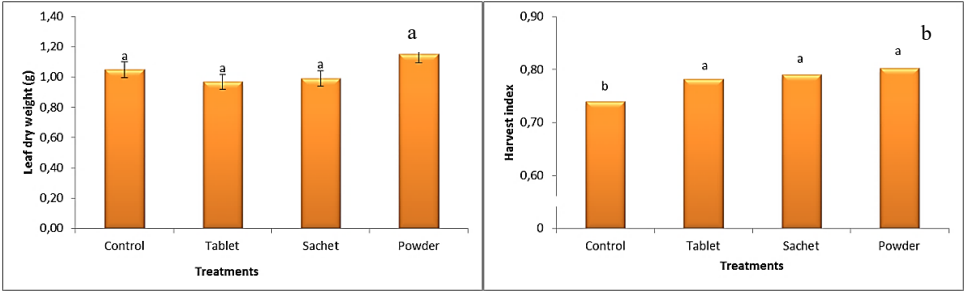


Fig. 2. Average values of leaf dry weight (a) and harvest index (b) of red spinach given different AMF propagule dosage forms.

Result of variance for leaf dry weight showed that the treatment of AMF propagule in different dosage forms had no significant effect on leaf dry weight of red spinach (Figure 2a), this was because after the plant experienced fewer leaves due to pest attack, so the plant had leaf dry weight lighter and uniform. However, in Figure 2 it is shown that the harvest index value is influenced by the propagule AMF in different dosage forms. However, the average value indicates that red spinach receiving AMF propagule treatment tends to be higher and significantly different if compared to the control plants.

The dry weight of plants was not sufficient to show the effectiveness of the AMF propagule dosage form treatment at shaping plant growth, so the use of the harvest index (HI) as an indicator of the ability to spread assimilate by plants to their economic parts needed to be done. The average of HI is generally below 1 which indicates that the harvested plant parts

(leaves) tend to be less than the unharvested plant parts (stems and roots), this is because some parts of the plant leaves have been attacked by caterpillars and insects thus reducing the ability plants to produce assimilate. According to Porker et al. [31] and Suminarti and Susanto [32] harvest index (HI) is the ability of plants to transmit assimilate, if the harvest index is high (>1), then the distribution of assimilation results is greater to the harvested economic organs, contrary if the harvest index is low (<1), the assimilation to the economic organs is smaller. Furthermore Adijaya et al. [33] and Alemu [34] stated that the harvest index describes the ratio of photosynthate translocated to the harvested plant part (leaves, tubers, or seeds). According to Rubatzky and Yamaguchi [35] and [36] that the longer the harvest age of a plant, the lower the harvest index, this is because increasingly plant organs are succulent, hardened, and fibrous. However, the parts harvested in the form of stems and leaves have a heavyweight. So, the younger the harvest age of a plant, the higher the harvest index, and the older the harvest age of a plant, the lower the harvest index.

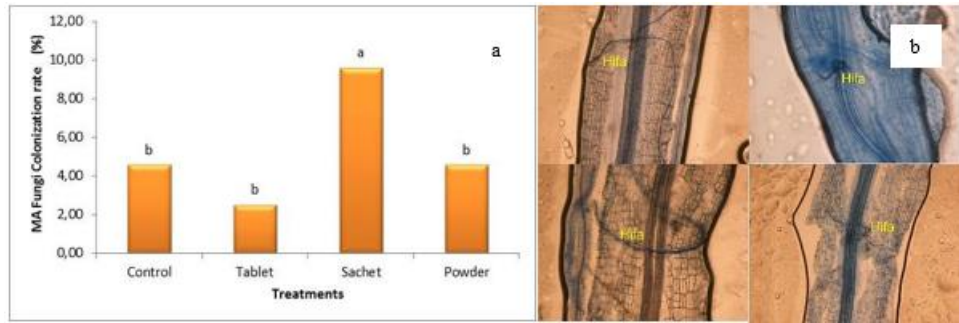


Figure 3. Average value of colonization rate (a) and cross-section of roots infected with hyphae (b) AMF in different dosage forms.

The AMF colonization rate on red spinach roots showed a significant difference with the use of AMF propagule in different dosage forms (Figure 3a), AMF propagule in sachet dosage form gave better infection results than in other dosage forms. This was possible because the spores contained in the sachets dosage form are not reduced by water splash or wind blow, so it is more effective in carrying out infections on the red spinach roots. The AMF part which carried out infections was dominantly by the AMF internal hyphae (Figure 3b). This is possible because the assimilate supply from the host plant as an energy source is reduced due to biotic attack so that the development of spores in plant root tissues is hampered.

4 Conclusion

The performance of AMF propagule in sachets dosage form showed a better infection rate with the growth of red spinach which was more fertile at the beginning of planting, but the harvest index value obtained was more uniform due to the attack of pest organisms before harvesting age.

The author would like to thank The Ministry of Education, Culture, Research, and Technology. Directorate General of Higher Education, Research, and Technology which has provided support through the research competition (Penelitian Dasar Unggulan Perguruan Tinggi-PDUPT) in 2022-2024.

References

1. A. Rahmi and W. Khazanah, *JAND J. Appl. Nutr. Diet.* **1**, 28 (2022)
2. H. Soetjpto, M. Krisdayanti, and N. R. Aminu, *Makara J. Sci.* **25**, 85 (2021)
3. S. M. El-Sayed, *Heliyon* **6**, e03278 (2020)
4. A. Prasetyo, A. Baihaqi, L. Indreswari, L. Fisiologi, F. Kedokteran, and U. Jember, *J. Agromedicine Med. Sci.* **6**, 87 (2020)
5. B. Eppang, Nurhaeni, Khairuddin, A. Ridhay, and Jusman, *KOVALEN J. Ris. Kim.* **6**, 53 (2020)
6. G. F. C. Mejica, R. Ramaraj, and Y. Unpaprom, in *Conf. 13 Th Semin. Sci. Technol. 6 – 7 Oct. 2020*, (Universiti Malaysia Sabah, Malaysia, Malaysia, 2020), pp. 253–255
7. A. I. Daniel, A. O. Fadaka, A. Gokul, O. O. Bakare, O. Aina, S. Fisher, A. F. Burt, V. Mavumengwana, M. Keyster, and A. Klein, *Microorganisms* **10**, 1220 (2022)
8. E. K. Mitter, M. Tosi, D. Obregón, K. E. Dunfield, and J. J. Germida, *Front. Sustain. Food Syst.* **5**, 1 (2021)
9. F. Hazra and R. P. Novtiar, *J. Ilmu Tanah Dan Lingkungan.* **22**, 35 (2020)
10. W. Wangiyana, I. G. P. M. Aryana, and N. W. D. Dulur, *IOP Conf. Ser. Earth Environ. Sci.* **637**, (2021)
11. D. Kuila and S. Ghosh, *Curr. Res. Microb. Sci.* **3**, 100107 (2022)
12. D. Trejo, W. Sangabriel-Conde, M. E. Gavito-Pardo, and J. Banuelos, *Agriculture* **11**, 934 (2021)
13. B. R. W. Giono, M. S. Solle, M. I. Idrus, and S. Sofyan, *J. Trop. Soils* **26**, 75 (2021)
14. M. D. Sukmasari, U. Dani, and A. A. Wijaya, *IOP Conf. Ser. Earth Environ. Sci.* **748**, (2021)
15. W. A. Elkhateeb, T. Somasekhar, P. W. Thomas, T. C. Wen, and G. M. Daba, *J. Microbiol. Biotechnol. Food Sci.* **11**, 1 (2021)
16. N. C. Johnson and K. S. Gibson, *Front. Plant Sci.* **11**, (2021)
17. N. Goicoechea, *Plants* **9**, 1668 (2020)
18. W. Weng, J. Yan, M. Zhou, X. Yao, A. Gao, C. Ma, J. Cheng, and J. Ruan, *Microorganisms* **10**, 1266 (2022)
19. S. Wu, Z. Shi, X. Chen, J. Gao, and X. Wang, *PeerJ* **10**, 1 (2022)
20. S. J. Barker and D. Tagu, *J. Plant Growth Regul.* **19**, 144 (2000)
21. S. Djauhari, J. D. Elriyono, and B. T. Rahardjo, *J. Trop. Plant Prot.* **2**, 19 (2021)
22. S. Branco, A. Schauster, H. Liao, and J. Ruytinx, *New Phytol.* (2022)
23. M. Usman, T. Ho-Plágaro, H. E. R. Frank, M. Calvo-Polanco, I. Gaillard, K. Garcia, and S. D. Zimmermann, *Front. For. Glob. Chang.* **4**, (2021)
24. W. Liu, P. Hou, G. Liu, Y. Yang, X. Guo, B. Ming, R. Xie, K. Wang, Y. Liu, and S. Li, *Food Energy Secur.* **9**, 1 (2020)
25. J. H. Ramírez-Silva, M. G. Lozano-Contreras, and G. Ramírez-Jaramillo, *OALib* **09**, 1 (2022)
26. M. Brundrett, N. Bougher, B. Dell, T. Grove, and N. Malajczuk, *Working with Mycorrhizas in Forestry and Agriculture Mycorrhizas of Australian Plants View Project Banksia Woodland Restoration Project View Project* (The Australian Centre for International Agricultural Research (ACIAR), 1996)
27. S. Molinari, M. Akbarimotlagh, and P. Leonetti, 1 (2022)

28. T. C. Oliveira, J. S. R. Cabral, L. R. Santana, G. G. Tavares, L. D. S. Santos, T. P. Paim, C. Müller, F. G. Silva, A. C. Costa, E. L. Souchie, and G. C. Mendes, *Sci. Rep.* **12**, 1 (2022)
29. S. Biswas, B. Mahato, P. Panda, and S. Guha, *J. Entomol. Res.* **33**, 219 (2009)
30. R. Sedo, P. Mudjirahardjo, and E. Yudaningtyas, *J. EECCIS* **13**, 31 (2019)
31. K. Porker, M. Straight, and J. R. Hunt, *Front. Plant Sci.* **11**, 1 (2020)
32. N. E. Suminarti and S. Susanto, *J. Agro* **2**, 15 (2015)
33. I. N. Adijaya, N. L. G. Budiari, I. M. R. Yasa, A. R. K. Sari, and T. F. Silitonga, *IOP Conf. Ser. Earth Environ. Sci.* **911**, (2021)
34. G. A. Alemu, *J. Nat. Sci. Res.* (2019)
35. V. E. Rubatzky and M. Yamaguchi., *World Vegetables: Principles, Production, and Nutritive Values.*, 2nd ed. (Springer, New York, 1997)
36. A. F. Tauk, M. T. Darini, and Z. Zamroni, *J. Ilm. Agroteknologi* **4**, (2020)