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Root colonization by vesicular arbuscular mycorrhizal in different dosage forms and effect on C3 plants root morphology

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Abstract. The application of vesicular arbuscular mycorrhizal (VAM) in powder dosage form has been used for a long time. However, this dosage form has a non-uniform application dose and allows VAM spores to be lost through wind and rainwater, so innovation is needed in the VAM dosage form. A study aimed at knowing dosage forms of VAM as a biological agent that affects colonization level and C3 plant's root morphology was carried out at Screen House Laboratory, Faculty of Agriculture, Animal Husbandry and Fisheries, University of Muhammadiyah Parepare using a factorial design based on completely randomized design (CRD). Treatment of VAM dosage forms (factor 1) used was powder (A1), sachet (A2), and tablet (A3). While the C3 plant species (factor 2) used were *Puraria japonica* (B1), *Vigna radiata* (B2), and *Amaranthus gangeticus* (B3). Observations were conducted on the percentage of colonized roots and the C3 plants' root morphology. The results showed that the combined treatment of VAM dosage forms (powder, sachet, and tablets) and C3 plant species (*Puraria javanica, Vigna radiata, Amaranthus gangeticus*) showed significant effects on colonized roots percentage and C3 plant root morphology. So it can be recommended that VAM dosage forms (sachet and tablet) are efficient and effective for the mobilization and field application of VAM.

Keywords: endomycorrhiza, hifa, infection, tablet.

1. Introduction

Vesicular arbuscular mycorrhizal (VAM) in its role as a biological agent greatly assists plant growth through external hyphae in increasing water and nutrient absorption, tolerance, and plant resistance to biotic and abiotic stresses [1]. The pre-conducted research reveals that population density of

indigenous mycorrhizal spores in post-mining areas of Sorowako nickel mining, Kebung Raya Jompie, and pepper plantations found *Glomus* sp., *Acaulospora* sp., and *Gigaspora* sp., in various quantities. Indigenous mycorrhiza originating from the Sorowako nickel mining post-mining planting area has been identified as having a degree of adaptation and tolerance to several heavy metals with high concentrations and has been cultured using various combinations of planting media and host plants

Conventional application of VAM has long been carried out in powder dosage forms. Still, this dosage form has several drawbacks including having non-uniform application doses because it only uses relative doses and allows the loss of spores due to windblown and exposure to rainwater [2]. In addition, conventional preparations have a large mass making them challenging to mobilize to the field. Therefore an innovative VAM dosage form is needed which has a high number of spores with a more significant infection rate of 50% [3].

Plant organs that are directly related to mycorrhizae are plant roots. The commonly known plant root system is the taproot system which is generally found in plants of the C3 group and the fibrous root system is generally owned by plants of the C4 group. According to [4] the wider the root surface, the greater the chance of infection. So The suitability of the VAM dosage form is needed with the plant root system so that an effective infection process occurs.

The VAM dosage form technology that is packaged in a simple, practical, and applicable form is still very limited. Commercially, VAM dosage forms and storage are needed to be more attractive, easy to apply, and quantity and quality controlled. So engineering studies on technological innovation of VAM dosage forms are needed to present dosage forms that are practical and have a high infection rate in the root system of C3 plant groups.

Utilization of biological natural resources, especially VAM which has been adapted and compatible with heavy metal-polluted environments is the object of this research. VAM is a biological asset that can be developed optimally so that it can be used as an efficient and effective biological agent by modifying the dosage form of VAM. Research that aims to determine the level of VAM colonization in different dosage forms and its effect on the morphology of C3 plant roots is very important to be carried out to find and recommend VAM dosage forms for application in the field.

2. Materials and methods

This research was conducted at the Agrotechnology Laboratory and Screen House, Faculty of Agriculture, Animal Husbandry and Fisheries, Muhammadiyah University of Parepare. This study used a factorial design based on a completely randomized design (CRD). The treatment of the VAM dosage form factors (factor 1) used was powder (A1)/control, sachets (A2), and tablets (A3). While the C3 plant species factor (factor 2) used was *Puraria japonica* (B1)/control, *Vigna radiata* (B2), and *Amaranthus gangeticus* (B3).

The powder dosage form is a standard propagule consisting of zeolite, sand, husk charcoal, pieces of host plant roots, and VAM spores. This standard propagule will be used to make sachets dosage forms by using sachets packaging and to make tablet dosage forms by adding organic adhesives. The planting medium used consisted of soil and compost in a ratio of 1:1.

Planting begins by making a planting hole in a planter bag filled with medium. Each planting hole is filled with a VAM dosage form (for VAM powder, use 5 g of propagules per planter bag; for VAM sachets, use one sachet per planter bag; and for VAM tablet, use one tablet per planter bag). Furthermore, each planting hole that contained 1 type of VAM dosage form, was planted with one of the C3 plants (3 seeds per planter bag). The planting material used is composite seeds. Finally, the planting hole is covered with medium and watered with use sprayer. Thinning is done 7 days after planting (DAP) by cutting and leaving 1 plant per planting bag.

The percentage of colonized roots by VAM was calculated based on the formula from Phillips & Hayman [5]. While for the root morphology variables included root length, number of secondary roots per 1 cm of the primary root, and diameter of secondary root, root volume [6]. Observational data were analyzed using Factorial Variance Analysis Based on a Completely Randomized Design, and continued with Duncan's Test it had a significant effect.

The calculate the percentage of colonized roots, semi-permanent root preparations were first made. Percent colonization was calculated from the number of colonized roots from 10 observed root sections. Colonized roots are characterized by the presence of hyphae, vesicles, arbuscles, or spores in the root tissue. VAM colonization percentage was calculated based on the formula of Phillips & Hayman, [5].

$$Colonized\ Root\ Percentage\ (\%) = \frac{Total\ colonized\ root}{Total\ examined\ root} \times 100$$

The percentage of colonized roots was determined based on the modified Rajapakse & Miller, [7] categories as follows:

Table 1. Categories of the percentage of roots colonized with VAM

No	Colonized roots (%)	Categories
1	<5	Very low
2	6-25	Low
3	26-50	Medium
4	51-75	High
5	>75	Veri high

3. Results and discussion

Observing the percentage of root colonization by VAM showed that the combined treatment of VAM dosage forms and C3 plant species showed various phenomena. However, in general, all VAM dosage forms could colonize the root tissue of C3 plants, although they were still in the medium to veri high category (Figure 1). Root colonization is a symbiotic process between host plant roots and VAM.

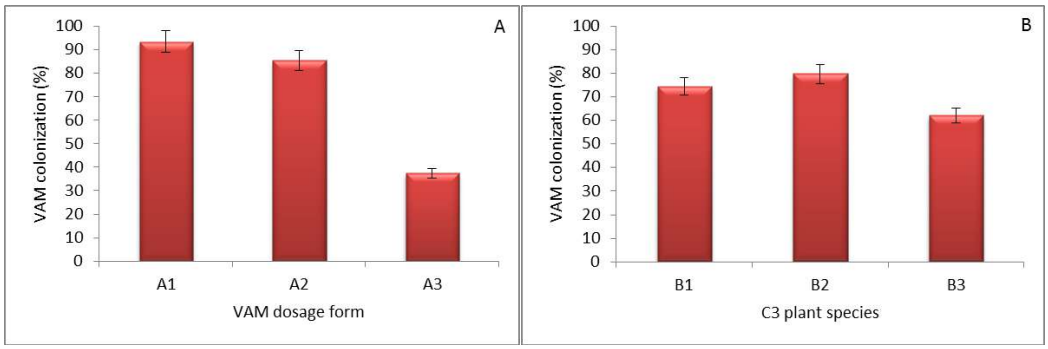

Figure 1. Percentage of root colonization by VAM in the VAM dosage forms (A) and C3 plant species (B) at 30 DAP. Values were the mean ± se of 10 root sections examined. Vertical bars indicated standard error values (se) with 5% (A1, powder dosage forms; A2 sached dosage forms; A3 tablet dosage forms; B1, *Puraria javanica*; B2, *Vigna radiata*; B3, *Amarantus gangeticus*).

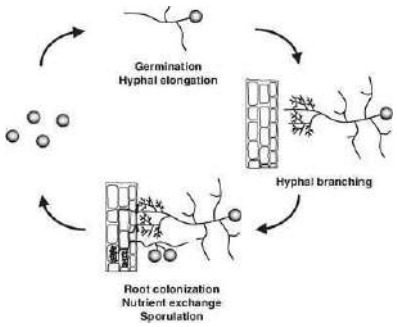

Figure 2. Development of a Functional Arbuscular-Mycorrhizal Symbiosis [12]

The form of symbiosis that occurs between VAM and plant roots is a form of mutualistic symbiosis, namely that VAM obtains nutrients as an energy source from plants, and plants obtain additional uptake of water and nutrients from VAM's living activities. Figure 1A shows that VAM in the form of shazet and tablet dosage forms has spore performance that is more active in colonizing plant root tissue, with the colonization level being in the very high category. Meanwhile, the level of VAM colonization in the root tissue of C3 plants was more dominant in the root cortex tissue of *Vigna radiata* (*V. radiata*) compared to *Puraria japonica* (*P. javanica*) and *Amarantus gangeticus* (*A. gangeticus*). Figure 2 shows that the VAM life cycle begins when propagules in the form of spores and hyphae come into contact with a suitable host. Hyphae will penetrate by forming appressoria to enter the root cells of the host plant. VAM hyphae will develop widely in the intercellular spaces in the cortex, forming intraradical hyphae, and then forming arbusculars and vesicles. The last VAM cycle will create defense organs in the form of spores [8][9][10][11]. VAM organelles found in the root cortex tissue of C3 plants were found in the form of hyphae, arbuscular, and spore structures (Figure 3)

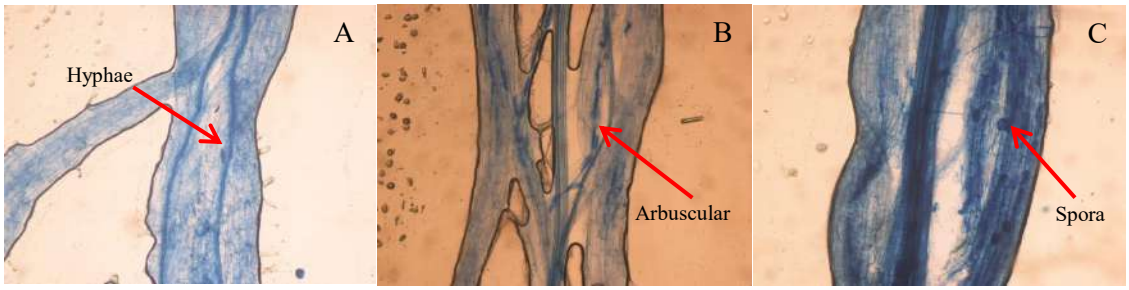

Figure 3. Cross-section of root colonization by hyphae (A), arbuscular (B), and spores (C) of arbuscular mycorrhizae in the combined VAM dosage form and C3 plant species.

Hyphae are one of the VAM structures in the form of fine threads that function as absorbers of nutrients from the outside, and arbuscular are colonization units that have reached deeper cortical cells and penetrated the cell walls, and formed a complex branching system of hyphae, which looks like a small tree with branches. The arbuscular structure acts as a site for exchange of nutrients and carbon [13] while the vesicle is a bloated structure formed on the main hyphae, functioning as a storage organ. This structure also functions as a resting spore. In the presence of one or more of the VAM structures, it can be said that colonization by VAM has occurred.

This phenomenon is suspected *V. radiata* can create a good rhizosphere environment for the development of VAM spores so that all VAM dosage forms (powder, sachets, and tablets) can colonize root tissue. According to Bangkele et al [14] and Vetterlein et al [15], the rhizosphere is a microenvironmental zone that is around plant roots. It is also often interpreted or limited as micro-sized materials or materials and microorganisms that are still attached to plant roots [16]. The rhizosphere region will always be an environment in which metabolic activity is always more active, rapidly changing, and more competitive than the surrounding soil. As an illustration, in the rhizosphere, there are around 106-109 bacterial population cells, and about 105 to 106 fungi per gram of rhizosphere soil [17], [18]. The activity of rhizosphere microorganisms is affected by the exudate produced by plant roots, but according to Weng et al [19] and Sanana et al [20] in general, spores are formed if there is a decrease in the number of nutrients from the roots during VAM symbiosis and the plant will die.

In theory, the area of the rhizosphere is affected by the area covered by the activity of plant roots and the microorganisms associated with them. The results of the analysis of variance for root morphology variables showed that the VAM dosage form and C3 plant type had a significant to a very significant effect on root length (A), number of secondary roots in primary roots (B), the diameter of secondary roots, and root volume. Meanwhile, the results of Duncan's test showed that the application of VAM dosage forms to various types of C3 plants for each root morphological variable showed various phenomena (Figure 4).

The phenomenon that occurs is C3 plants that have long roots tend to produce fewer secondary roots with larger diameters so as to increase root volume, and this phenomenon is found in *V. radiata* in various VAM dosage forms. Meanwhile, another phenomenon is that plants with short roots tend to have more secondary roots with relatively small diameters so that they can reduce root volume, as shown by *P. Javanica* and *A. Gangeticus* as illustrated in Figure 5. This is possibly caused by the concentration of growth hormone contained in the root area produced by VAM according to the grade of infection that occurs. According to Palupi et al [21] that VAM can stimulate plant growth including the roots. Meanwhile, according to Mandasari et al [6], plants that have good roots, if the plant has long roots, a broad surface, large volume, and small diameter.

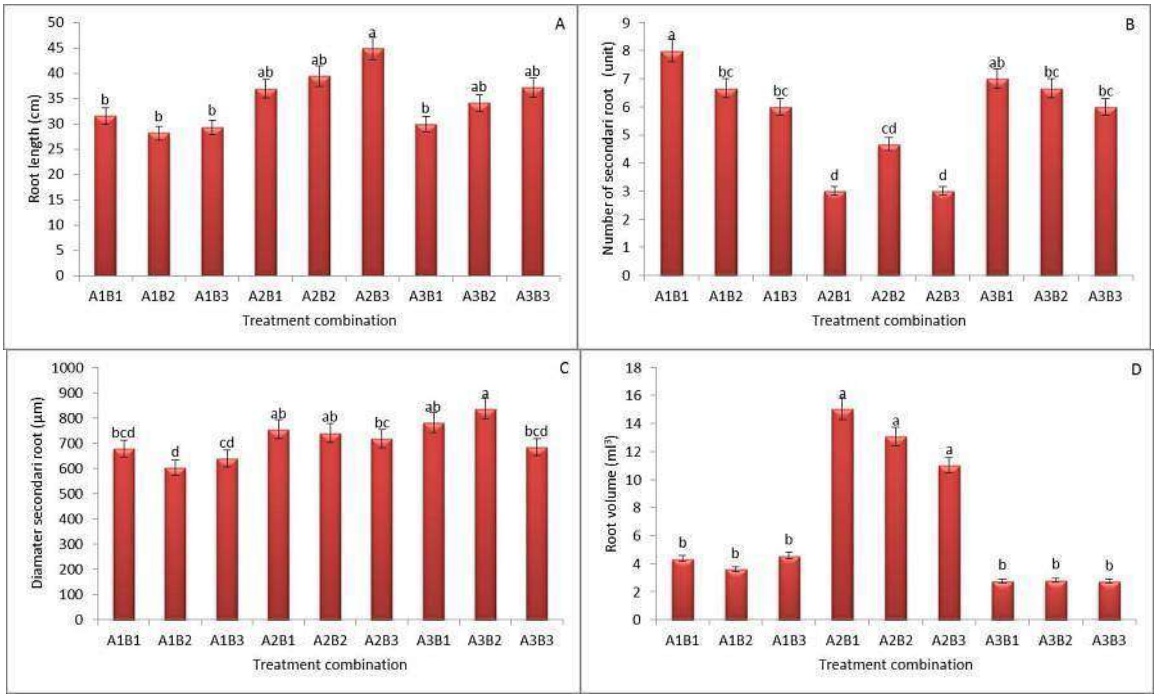

Figure 4. Root morphology in the treatment combination of VAM dosage form and C3 plant species at harvest age (70 DAP for *P javanica* and *V radiata*; 30 DAP for *A gangeticus*). Values were the mean ± se of 15 plant. Vertical bars indicated standard error values (se) with 5%. (A1, powder dosage forms; A2, Sached dosage forms; A3, tablet dosage forms; B1*, P javanica*; B2, *V radiata*; B3, *A gangeticus*).

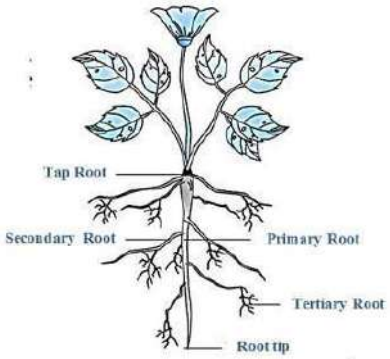

Figure 5. Illustration of C3 legume root architecture

In addition to internal factors, external factors of plants also greatly affect the development of plant roots. The addition of root length is a root response to the availability of water and nutrients, and the observation of root length aims to provide information on the ability of a plant's roots to absorb water and nutrients. The low availability of nutrients gives a response to more root development so it becomes heavier because the roots are trying to get enough nutrients and water for plant development. Mastur [22] saying that plants that grow in conditions of lack of water will form a greater number of roots but with lower growth results than plants in sufficient water.

4. Conclusion

The combined treatment of VAM dosage forms (powder, sachet, and tablets) and C3 plant species (*P javanica, V radiata, A gangeticus*) showed various effects on the percentage of colonized roots and root morphology, so that, can be recommended for applications of VAM in the field should to use sachet or tablet dosage forms.

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