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The Colonization Vesicular Arbuscular Mycorrhiza

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The Colonization Vesicular Arbuscular Mycorrhiza in Different of Dosage Form in the Root Tissue of C3 and C4 Plants

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A B S T R A C T

A study aimed to identify and determine the percentage of Vesicular Arbuscular Mycorrhiza (VAM) structure colonies in C3 and C4 Plant Root Tissues as Biological Agents in Different Dosage Forms was conducted from May 2022 to August 2023. At coordinates 3°59'30" S and 119°38'43" E, an altitude of 37.0 m above sea level. This study used a factorial design. Factor 1 is the form of VAM dosage consisting of powder/control, sachet, and tablet dosage forms. In contrast, the other factors are the types of plants, namely *Pueraria javanica*, *Vigna radiata*, *Amaranthus tricolour* from the C3 plant group and *Saccharum officinarum*, *Zea mays*, *Sorghum bicolour* from the C4 plant group. The parameters observed were the level of colonization of VAM structures on plant roots in the form of hyphae, arbuscules, vesicular, and spores.The results of the study showed that the VAM dosage form did not inhibit colonization of the roots of C3 and C4 plant groups with different levels of colonization percentage by VAM structures; the level of VAM colonization in the Sachet dosage form had a higher colonization level, and a more complete VAM structure was found compared to other VAM dosage forms. The novelty found in this study is that the mycorrhizal sachet dosage form has a better colonization rate than the powder and tablet dosage forms in C3 and C4 plants.

Introduction

The most widely known types of mycorrhiza are endomycorrhiza, ectomycorrhiza and ectendomycorrhiza (Dyshko *et al.,* 2024; Perotto and Balestrini, 2024). Mycorrhiza (biotrophic fungi) are included in the group of fungi that can cooperate with plant roots and are not pathogenic (Enebe and Erasmus, 2023; Priyashantha *et al.,* 2023). Roots infected by ectomycorrhizae generally have short, blunt root tips covered by a coat of fungal tissue and no or few root hairs. The fungus takes over the role of the root hairs in absorbing nutrients. The fungus

grows between the root cortex cells within the coat to form a heartig net. Infected roots usually enlarge and branch (Priyashantha *et al.,* 2023; Chauhan *et al.,* 2022).

Mycorrhiza is widely used in agriculture and forestry because it can be associated with plant roots by colonization of the root cortex tissue (Chauhan *et al.,* 2022; Martin and Heijden, 2024) and until now, the use of mycorrhiza is also applied in the livestock sector to increase the production of green fodder. Mycorrhiza with a mutualistic symbiosis with plant root tissue is called Vesicular Arbuscular Mycorrhiza (VAM) (Huey *et al.,* 2020; Khaliq *et al.,* 2022). VAM can colonize plant roots using propagules (Kowal *et al.,* 2020; Zhou *et al.,* 2020). These propagules consist of spores, mycelium, and hyphae from the mycorrhiza, infected roots, and planting media infested by mycorrhiza (Eve and Alessandro, 2023; Huey *et al.,* 2020).

Vesicular Arbuscular Mycorrhiza are obligate facultative organisms (Diagne *et al.,* 2020; Jaitieng *et al.,* 2021). Therefore, they need a host of plant roots that can associate with VAM (Gough *et al.,* 2020; Paredes-Jacome *et al.,* 2022). The host plant provides carbohydrates from photosynthesis to VAM (Fall *et al.,* 2022; Wang and Wu, 2023) and in return, VAM helps absorb nutrients such as potassium, phosphorus, and nitrogen from the soil (Fall *et al.,* 2022; Qi *et al.,* 2022; Wahab *et al.,* 2023).

In plants that are symbiotic with VAM, the root absorption area is expanded by VAM mycelium so that the absorption of nutrients, especially P, becomes more remarkable. The rate of P entry into VAM hyphae can reach six times faster than the rate of P entry through root hairs. Plants symbiotic with VAM can generally survive environmental stress, such as drought, salinity, and heavy metal contamination (Muhammad *et al.,* 2024; Wahab *et al.,* 2023).

The occurrence of symbiotics between VAM and plants can be known by the level of colonization that occurs in the roots. The presence of VAM can reveal the colonization of structures produced by VAM, including hyphae, mycelia, vesicles, arbuscular, and spores. With the presence of one or more VAM structures, it can be said that there is a symbiotic by VAM on its host plant (Muhammad *et al.,* 2024; Wahab *et al.,* 2023; Wang and Wu, 2023). In plants that are symbiotic with VAM, the root absorption area is expanded by VAM mycelium so that the absorption of nutrients, especially P, becomes more remarkable. The rate of P entry into VAM hyphae can reach six times faster than the rate of P entry through root hairs. Mycorrhizae can also increase plant growth by protecting plants from root pathogens and toxic elements. The structure of mycorrhizae can function as a biological shield against root pathogens. Mycorrhizal fungi can release antibiotics that can kill pathogens.

The mechanism of protection can be explained as follows: (1) The presence of a hyphal membrane (coat) can function as a barrier to the entry of pathogens; (2) Mycorrhiza utilizes almost all excess carbohydrates and other exudates, thus creating an environment that is unsuitable for pathogens, (3) Mycorrhizal fungi can secrete antibiotics that can kill pathogens; (4) Plant roots that mycorrhizal fungi have infected cannot be infected by pathogenic fungi, indicating the presence of competition (Islam *et al.,* 2022; Lyu and Smith, 2022; Liu *et al.,* 2024).

The association between VAM and host plants in forming colonization is shown, where the higher the percentage of colonization, the higher the level of compatibility between host plant root exudates and mycorrhiza (Brundrett and Tedersoo, 2020; Lyu and Smith, 2022; Yusnizar *et al.,* 2024). The process of root colonization by VAM is divided into four stages.

The first stage is the germination of spores and the growth of VAM hyphae. The second stage is the penetration of hyphae into the root cell tissue of the plant. The third stage is the development and growth of hyphae in the root cell tissue. The fourth stage is further developing the internal system and its duties to increase plant absorption of water and nutrients (Warman *et al.,* 2022; Yin *et al.,* 2024).

The traditional use of VAM as a biological agent has long been carried out in powder dosage form. However, this dosage form has several disadvantages, including inconsistent application doses because it only uses relative doses and the potential for spore loss due to wind pressure and rainwater. In addition, the powder dosage form has a large mass, making it difficult to move in the field. Therefore, an innovative VAM dosage form with more spores and a higher colonization rate is needed.

The plant organ that is directly related to mycorrhiza is the plant root. There are two known architectures of plant root systems: taproot system architecture, which is generally found in legumes or C3 plants, and fibrous root

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system, which is usually found in cereal plants (C4) (Ceritoglu *et al.,* 2020; Sharma *et al.,* 2024; Zhiyong *et al.,* 2022). According to Islam *et al.,* (2022) and Liu *et al.,* (2024), the large surface area of plant roots can increase the occurrence of VAM colonization as long as other factors do not act as inhibitors.

Information and explanations regarding the Percentage of VAM Structure Colonies in C3 and C4 Plant Root Tissues as Biological Agents in Different Dosage Forms need to be found. Therefore, a study aimed to identify and determine the percentage of VAM structure colonies in C3 and C4 Plant Root Tissues as Biological Agents in Different Dosage Forms. This study provides initial information on the VAM dosage form suitable for C3 and C4 plant roots.

Materials and Methods

Study Area

The research was conducted at the Screen House, Faculty of Agriculture, Animal Husbandry and Fisheries, Muhammadiyah University of Parepare, and the Agroplastid Mini Laboratory from May 2022 to August 2023. At coordinates 3°59'30" S and 119°38'43" E, the altitude was 37.0 m above sea level, the average daily air temperature was 28OC, the average air humidity was 75%, and the average rainfall was 319.78 mm.

Procedures

Research Design

This study used a factorial design. Factor one is the VAM dosage form, which consists of powder/control, sachet, and tablet dosage forms. In contrast, the other factors are the types of plants, namely *Pueraria javanica*, *Vigna radiata*, and *Amaranthus tricolour* from the C3 plant group and *Saccharum officinarum*, *Zea mays*, and *Sorghum bicolour* from the C4 plant group.

Preparation of mycorrhizal dosage form

VAM in powder dosage form is a propagule consisting of spores, vesicular, arbuscular, and hyphae of VAM, pieces of colonized roots, and propagation media containing VAM. This dosage form is needed to manufacture VAM in sachet form using readily biodegradable packaging. Each sachet contains 5 g of propagule, and for the manufacture of VAM in tablet form, VAM propagules and clay minerals are used as binders with a ratio of 5:5.

Planting and treatment application

VAM in powder dosage form is a propagule consisting of spores, vesicular, arbuscular, and hyphae of VAM, pieces of colonized roots, and propagation media containing VAM. This dosage form is needed to manufacture VAM in sachet dosage form using readily biodegradable packaging. Each sachet contains 5 g of propagule, and for the manufacture of VAM in tablet dosage form, VAM propagules and clay minerals are used as binders with a ratio of 5:5.

Identification and determination of the percentage of VAM structure colonization

Identification and determination of the percentage of VAM structure colonization on each plant root was carried out after the plant was 60 DAP. Observation of root colonization began with root staining, referring to the Clapp *et al.,* (1996) method, and it had the following stages: 1). The roots were washed thoroughly with distilled water. 2). The roots were soaked in 20% KOH for 48 hours. 3). The roots were washed with water until clean using a filter, then soaked in 0.1 M HCl. 4).

The roots were soaked in a trypan blue solution for 48 hours without washing. 5). The roots were soaked in a destaining solution for 24 hours. 6). The roots were cut to 1 cm in size, then arranged parallel to the object glass and covered with a cover glass. The characteristics of colonized roots are the discovery of propagules in the form of hyphae, vesicular, arbuscular, and spores in the root cortex cell tissue.

Root staining was carried out to prove the presence of VAM in the roots of cocoa plants. Before staining, the host plant roots were washed until cleaned from any soil/sand still attached. Then, the lateral roots were selected, cut, and collected. As much as 5-10% of the lateral root collection was randomly taken and then put in a plastic bottle (volume 8 mL) containing 50% alcohol.

The roots are placed in a 10% KOH solution soaked for 24 hours, then rinsed with water for 5 minutes. If the roots still look blackish, soak them for 1–2 minutes in a 10% H2O2 solution. After that, the roots are soaked in a 2% HCl solution for 24 hours. Furthermore, the roots are

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placed in a staining solution consisting of 100 ml of lactic acid, 100 ml of glycerin, 50 ml of distilled water, and 0.13 g of acid fuchsin, then soaked for 24 hours. Finally, the roots are soaked again in a destaining solution consisting of 100 ml of lactic acid, 100 ml of glycerin, and 50 ml of distilled water, soaked for 24 hours, and the roots are ready to be observed.

Before the observation, the root was prepared with five pieces in one replication. The roots were cut to a length of 1 cm, arranged on a glass object, covered with a covered glass, and labeled according to the treatment. Furthermore, the preparation was observed under a microscope with a magnification of 10-40 times.

Data Analysis

The percentage of VAM colonies was calculated using the Phillips and Hayman (1970) formula, which can be seen in equation 1:

$$\text{Percent of colonization (\%)} = \frac{\text{Number of colonized roots}}{\text{Number of roots observed}} \times 100$$

The colonization level criteria are determined O'Connor *et al.,* (2001) criteria, which are determined based on the percentage of calculated percentage of colonization.

Results and Discussion

Percentage of VAM colonization

The results of observations of the percentage of VAM colonization in different dosage forms on various roots of C3 and C4 plants (Table 2).

Percentage colony of VAM structures

Figure 1 shows that the VAM structure has different levels of colonization percentage at various VAM dosage forms. Colonization in the roots was found in hyphae, arbuscular, vesicular, and spore structures.

Cross-section of roots colonized by VAM structures in different dosage form

The VAM structure in the root tissue of each type of plant and the found structures hyphal, arbuscular, vesicle, and spore (Figure 2)

The percentage of VAM structures that colonize roots indicates the capacity of the plant root system to interact with VAM spores. In general, colonization is formed by spores compatible with the physiological processes in the roots of plants.

This type of colonization is generally mutualistic symbiosis, meaning plants and VAM benefit each other. The percentage value of colonization formed indicates the level of ability for mutualistic symbiosis between VAM and host plants. The ability of VAM to reproduce in the rhizosphere is related to the percentage of colonization. The root structure of a plant also influences VAM colonization. Plants with broad and deep root systems have a high chance of being colonized by VAM.

The results of observations of the percentage of VAM colonization in different dosage forms on roots of C3 and C4 plants showed low (2.50%) to high (96.25%) colony status (Table 1). Host sensitivity to the colonization process, climate, and soil factors generally greatly influence VAM colonization. According to Hazzoumi *et al.,* (2022) and Madouh and Quoreshi (2023), the type of plant that functions as a host in the growth and development of VAM must be able to adapt and not be susceptible to pathogens that can interfere with the colonization process. In addition, fine roots can increase mycorrhizae's effectiveness on the host, affecting the optimal response of mycorrhizal symbiosis in colonizing. This form of symbiosis is mainly mutualistic symbiosis, although it can sometimes be a weak parasitic symbiosis.

High colonization levels were detected in the roots of C3 plants (Table 2). This is likely because C3 plants are legumes that generally form root nodules and can form a symbiosis with rhizobia. The positive effects of VAM application on nodule growth, nitrogenase activity, and leghemoglobin content increased nitrogen uptake in some plants (Fall *et al.,* 2022; Kamau *et al.,* 2021).

The increase in nodule dry weight was due to the positive effect of VAM on increasing P status, which allowed the host plant to form nodules and promoted nitrogen fixation by rhizobia. Increasing the availability of plant phosphorus through VAM will increase the quantity and quality of legume root nodules (Calderon and Dangi, 2024; Ishaq *et al.,* 2023). In addition, inoculating plants with VAM and rhizobia increased stomatal conductance and the efficiency of photosystems I and II in the photosynthesis process (Hu *et al.,* 2020; Kamau *et al.,* 2021).

Figure 1 shows that the structure of VAM has different levels of colonization percentage. Internal hyphae can colonize up to 82.89%, followed by Arbuscular (44.44%), vesicular (50.61%), and spores (25.56%).

In the infection process, according to Boyno *et al.,* (2023) and Khaliq *et al.,* (2022), the beneficial relationship between VAM and plants occurs due to the exchange of signals between the two symbionts, where strigolactones derived from plants are perceived by VAM (Korek and Marzec, 2023; Mashiguchi *et al.,* 2021), which in turn produces a mixture of chitoligosaccharides and lipochitooligosaccharides that appear to function as VAM signaling molecules to plants, both chitoligosaccharides, and lipochitooligosaccharides can activate the symbiotic signaling pathway (Rush *et al.,* 2020; Solis *et al.,* 2022), followed by the growth of VAM hyphae towards the roots, the formation of hypopodium on the root surface, and entering through the epidermal cell layer into the root cortex (Chen *et al.,* 2021; Russo and Genre, 2021).

After penetration, hyphae grow intracellularly or extracellularly in the cortex; hyphae form hyphal coils outside the cortex (Bharathy and Muthukumar, 2023; Chandwani *et al.,* 2023). A plant signaling process is required for the establishment of VAM symbiosis in arbuscular, which are the source of symbiosis (Shi *et al.,* 2023; Wang *et al.,* 2023).

Once inside the root, there are two distinct types of VAM colonization strategies, although intermediate forms of these strategies are common (Boeraeve *et al.,* 2019; Ceulemans *et al.,* 2019).

Arum-type colonization involves hyphae spreading between cortical cells before they penetrate the inner cortical cells to form highly branched, terminally differentiated structures called arbuscules; and Paris-type colonization, hyphae spreading through intracellular pathways of cortical cells, where hyphal coils or arbuscules are formed. Arbuscules are the site of nutrient transfer between the plant and VAM and are, therefore, essential for VAM symbiosis (Huo *et al.,* 2021; Tominaga *et al.,* 2022).

The VAM colonization is also influenced by the root system of a type of vegetation (Thind *et al.,* 2022; Zhou *et al.,* 2020); plants with a broad and deep root system have a high chance of forming colonies with VAM (Madouh and Quoreshi, 2023; Salim *et al.,* 2020). In addition, the presence of hair roots can also increase the effectiveness of VAM on the host, which affects the symbiotic response of VAM in colonizing optimally (Beslemes *et al.,* 2023; Chauhan *et al.,* 2022).

The unique characteristics of VAM are that it is located inside the host root cells, the hyphae are not septate, and they are vesicular and arbuscular. The hyphae that are in the host root cells are the starting point of penetration and are directly connected to the hyphae that are outside the roots; arbuscular function as a means of transferring nutrients between VAM and its host, while vesicles are formed at the tips of the hyphae in the host tissue and function as a place for food reserves (Corcoz *et al.,* 2022; Khaliq *et al.,* 2022; Yuwati *et al.,* 2020). VAM infection in the roots can be seen through chemical staining (Figure 2). The infected root cells will be more giant and expand but do not damage the root cells; even if viewed from the outside, it looks like there is no change.

Observation of the VAM structure in the root tissue of each type of plant found hypha, arbuscular, vesicle, and spore (Figure 2). VAM spores form this structure and play a vital role in the association process. The hyphae produced from the germination of VAM spores absorb nutrients and water from the outer surface into the roots (Kalamulla *et al.,* 2022; Yusnizar *et al.,* 2024).

The arbuscular structure is a hyphal structure that branches, is shaped like a tree, and is located between the cell wall and the cell membrane. It is a temporary storage place for minerals, nutrients, and sugars and for exchanging nutrients and carbon between VAM and the host plant (Rini *et al.,* 2021; Yuwati *et al.,* 2020). Vesicles are thin-walled structures formed from swelling at the tips of hyphae, round, oval, or irregular in shape. Vesicles act as organs for storing food reserves such as lipids and, at certain times, act as spores, which defend VAM's life (Giovannini *et al.,* 2020; Natawijaya *et al.,* 2022).

Meanwhile, spores are the reproductive organs of VAM and are formed from single or colonial extraradical hyphae (sporocarps) (Sugiura *et al.,* 2020; Yamato *et al.,* 2022). Spores are composed of polysaccharides, lipids, proteins, and chitin. Spores germinate during reproduction and produce hyphae that infect the host plant's roots (Kalamulla *et al.,* 2022; Silwer, 2020). During reproduction, spores first germinate and produce hyphae that infect the host plant's roots (Satria *et al.,* 2023; Warman *et al.,* 2022).

Table.1 Colonization level criteria

S.No	Percentage	Criteria
1	0	Not colonized
2	<10	Low
3	10-30	Medium
4	> 30	High

Table.2 Percentage and status of AMF colony structures in different dosage forms in various C3 and C4 plants.

VAM dosage form	Species	Family	% VAM Structure Colony				% colony	Colony status*
			HI	A	V	S		
Powder	*Puraria javanica* (C3)	Fabaceae	100	85	90	60	83.75	High
	Vigna radiata (C3)	Fabaceae	100	100	100	85	96.25	High
	Amarantus gangenticus (C3)	Amaranthaceae	100	100	100	80	95.00	High
	Saccharum officinarum(C4)	Poaceae	85	0	10	0	23.75	Medium
	Zea mays (C4)	Poaceae	90	0	35	10	33.75	High
	Shorgum bicolor (C4)	Poaceae	95	5	60	20	45.00	High
Sachet	*Puraria javanica* (C3)	Fabaceae	100	100	70	20	72.50	High
	Vigna radiata (C3)	Fabaceae	100	100	80	80	90.00	High
	Amarantus gangenticus (C3)	Amaranthaceae	100	100	100	30	82.50	High
	Saccharum officinarum(C4)	Poaceae	50	5	15	0	23.33	Medium
	Zea mays (C4)	Poaceae	95	25	55	5	45.00	High
	Shorgum bicolor (C4)	Poaceae	90	30	75	15	52.50	High
Tablet	*Puraria javanica* (C3)	Fabaceae	90	70	30	20	52.50	High
	Vigna radiata (C3)	Fabaceae	80	70	10	10	42.50	High
	Amarantus gangenticus (C3)	Amaranthaceae	0	0	10	0	2.50	Low
	Saccharum officinarum(C4)	Poaceae	55	0	0	0	13.75	Medium
	Zea mays (C4)	Poaceae	95	0	50	15	40.00	High
	Shorgum bicolor (C4)	Poaceae	85	10	35	10	34.00	High

Note: HI, Internal Hyphae; A, Arbuscules; V, Vesicles; S, Spores;*, colony status based on O'Connor *et al.,* (2001).

Figure.1 Percentage colony of VAM structures in various dosage forms.

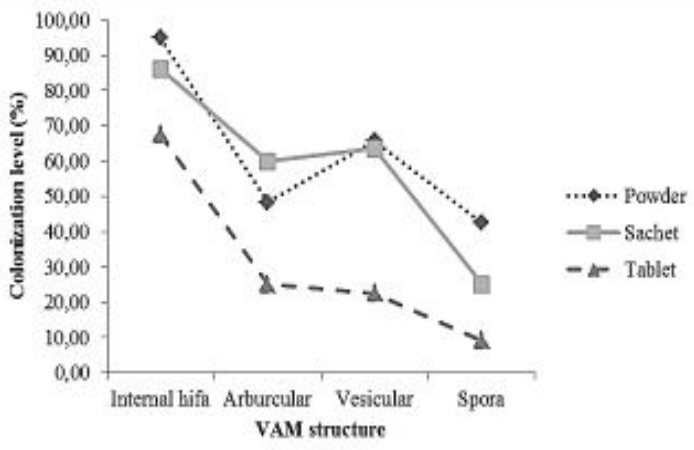
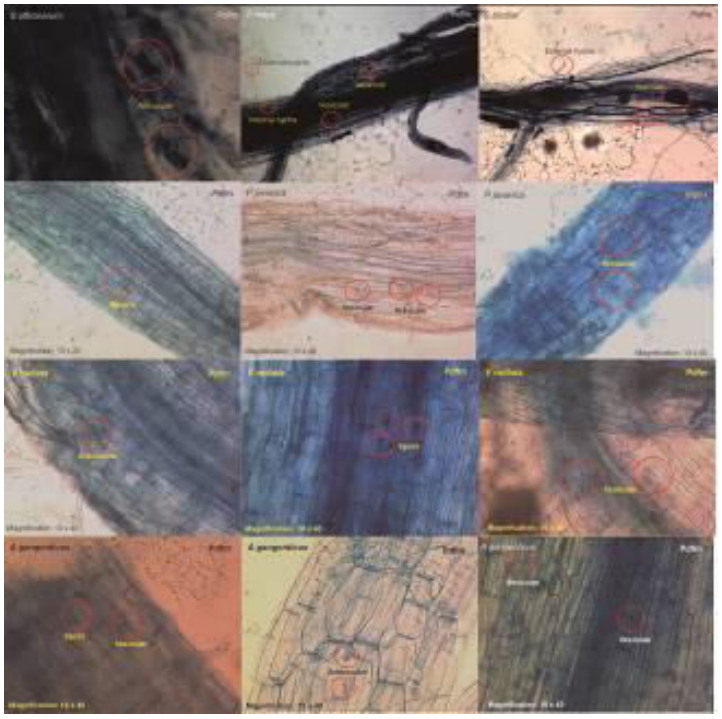
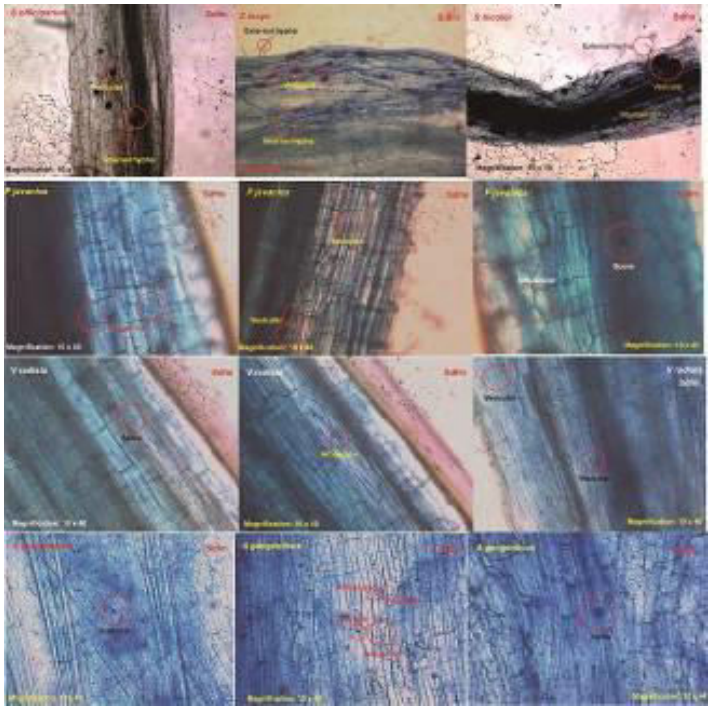


Figure.2 Cross-section of roots colonized by VAM structures in different dosage forms in various C3 and C4 plants. (Pdfm, Powder dosage form; Sdfm, sachet dosage form; Tdfm, tablet dosage form)





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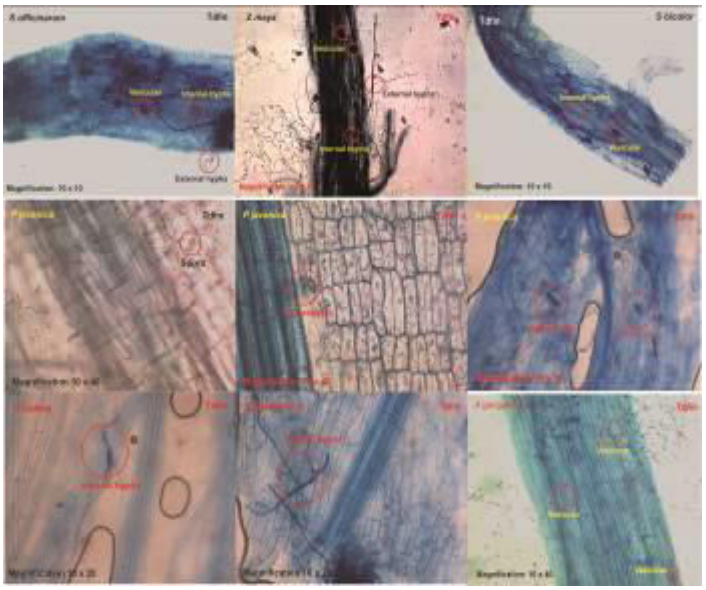

The research that has been conducted provides information that VAM dosage forms show different colonization levels of C3 and C4 plant root tissue. VAM in the sachet, tablets, and powder dosage forms is more capable of colonizing C3 plant root tissue than other plants. The hyphae structure of VAM is more dominant in colonizing the root tissue of C3 and C4 plants compared to other VAM structures. VAM in sachet dosage form colonizes deep plant root tissue (taproots), while tablet and powder dosage forms colonize short plant root tissue (fibrous roots), which can be concluded in this study.

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Author Contributions

1 Muh. Akhsan Akib: Investigation, formal analysis, writing—original draft. Andi Nuddin: Validation, methodology, writing—reviewing. Gusmiaty:—Formal analysis, writing—review and editing. Retno Prayudyaningsih: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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