IIETA International Information and Engineering Technology Association
Advancing the World of Information and Engineering

International Journal of Design & Nature and Ecodynamics
Vol. 17, No. 6, December, 2022, pp. 921-927
Journal homepage: http://iieta.org/journals/ijdne

Inoculation by Mycorrhizal on Combinations of Planting Media and Host Plant Types and Their Effect on Plant Vegetative Growth

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https://doi.org/10.18280/ijdne.170613

Received: 16 July 2022
Accepted: 6 October 2022

Keywords:
mycorrhizae, medium for growth, upland rice, corn, shallot

ABSTRACT

This study intends to examine the types of media and host plants inoculated with mycorrhizae with the aim of obtaining suitable media and plants adapted to mycorrhiza. By knowing the types of media and suitable host plants, it is hoped that there will be findings on good and effective inoculant media for spore multiplication as mycorrhizal inoculant media materials. The rationale is that it is necessary to find alternative planting media and suitable types of host plants for mycorrhizal propagation as mycorrhizal inoculants that farmers can easily use. Utilization of mycorrhizal inoculants is the application of biofertilizers that are environmentally friendly and have good prospects to be the choice of farmers. The study used a factorial randomized block design (FRBD) which consisted of two treatment factors. The first factor was the planting medium (M) consisting of 4 levels, namely m1 = soil mixed with zeolite (50 : 50 ratio), m2 = soil mixed with organic fertilizer (50 : 50 ratio), m3 = soil mixed with tailings (50 : 50 ratio), m4 = soil mixed with fine sand (ratio 50: 50). The second factor was the type of host plant (I) with 3 levels, namely: i1 = corn plant, i2 = shallot plant, i3 = upland rice plant. All treatments were repeated 3 times. The study lasted for 8 months, carried out at the Laboratory of the Faculty of Agriculture, Universitas Siliwangi, Tasikmalaya, starting from April to November 2019. The results showed that there was an interaction between the growing media and the type of host plant inoculated with mycorrhiza on the parameters of plant height, number of leaves and number of root infections 35 days after planting. For the parameter number of leaves aged 21 and 28 days, the highest wet weight and dry weight of the upper plant was in upland rice, while the highest lower dry weight was in leeks. Media that has good potential to be used as a mycorrhizal inoculant is a mixture of soil with manure and a mixture of soil with zeolite, while the type of plant that is good as a host plant is shallots.

1. INTRODUCTION

The usage of biological fertilizers is expanding now that it is known that certain species of microorganisms have beneficial properties for supplying nutrients to plants and suppressing pest organisms. Natural fertilizer is any item that contains live organisms capable of providing nutrients to the soil, enhancing the performance of enzymes and metabolism, and perhaps promoting plant development. Cyanobacteria, for example, is a type of microbe that has been shown to improve total nitrogen in soil by 0.21 per cent and plant nitrogen intake by 0.91 mg plant-1 [1].

Mycorrhizae are fungi that live on the roots of plants. Some reside on the roots' surface (ectomycorrhizae) and those whose hyphae penetrate the root tissue of the plant (mycorrhizae/endo mycorrhizae). Endo mycorrhizae are more commonly referred to as MVA (vesicular-arbuscular mycorrhizae). MVA can generate specific organs termed arbuscules and vesicles, each of which works as a transporter of nutrients and a storage location for food reserves. MVA inoculation can boost agricultural crop yields since it aids in nutrient uptake expansion and converts unavailable nutrients to available nutrients for plants [2].

Mycorrhizal plants are typically more drought-resistant than non-mycorrhizal plants because mycorrhizae aid in the absorption of water when the plant roots cannot do so and can endure drought damage to the cortical tissue. Mycorrhizae contribute to environmental improvement and are capable of adapting to unacceptable environmental conditions [3]. Beneficial soil bacteria that aid in plant growth and production are biofertilizers or biological fertilizers. Bacteria, fungi, and actinomycetes can all produce natural fertilizers. Numerous mycorrhizae have been identified and isolated from the rhizosphere of plant roots, including Glomus, Acaulospora, Gigaspora, and Sclerocystis [4-6]. Arbuscular mycorrhizal fungi (AMF) are a subclass of endomycorrhizal fungi. Mycorrhizal association occurs naturally in practically all tropical and subtropical agricultural plants. Mycorrhizal fungi can boost plant yield in stressful environments. Purnomo [7] demonstrates that using Gigaspora margarita and Acaulospora sp can increase the production of chilli plants under Al (aluminium) stress. Similarly, Setiadi and Setiawan [8] research demonstrates that the mycorrhizal species Gigaspora margarita, Acaulospora sp., and Glomus sp. can thrive in postnickel mining circumstances. Arbuscular mycorrhizal fungi have a unique potential to enhance the absorption of insoluble phosphorus, both naturally occurring and produced from fertilizers, in marginal soils with low P content.

According to the research findings of Nurmasyitah et al. [9], mycorrhizae can significantly increase soil chemical fertility, such as pH value, accessible P, and CEC, compared to soils lacking arbuscular mycorrhizal fungi. The total nitrogen content of the earth is determined by its type and the number of mycorrhizae inoculated. In a stressful situation, adding mycorrhizae such as Gigaspora margarita, Acaulospora sp., and Glomus sp. to plant cultivation increased plant yield. One sign of mycorrhizae in plants is the presence of many spores in the root region (rhizosphere).

While mycorrhizae can thrive in various environments and on a variety of hosts, some plants are host specific. Mycorrhizal inoculants derived from Glomus moderate species increased the pH, accessible P, and CEC values [9]. Numerous species of vesicular-arbuscular mycorrhizae (MVA) have been discovered in the root zone of sorghum [4], teak [5], and citrus trees [6]. Mycorrhizal research has been conducted on a variety of agricultural and forest plants. Mycorrhizae is a symbiotic mutualism (non-pathogenic relationship) between natural fungus and the roots of higher plants that are frequently utilized as biological fertilizers.

Although many studies regarding the association of mycorrhizae and host plants have been carried out, research on the types of media which suitable for the propagation of mycorrhizae and their host plants has not been widely carried out. Therefore, research on the type of media and specific host plants needs to be conducted to get a suitable combination in mycorrhizal propagation that can support plant growth.

2. MATERIAL AND METHODS

The experiment was conducted using a factorial randomized block design (FRBD). The first factor is the planting medium (M), which has four levels: m1=soil mixed with zeolite (50:50 comparison), m2=soil mixed with organic fertilizer (50:50 comparison), m3=soil mixed with tailings (50:50 comparison), and m4=soil mixed with fine sand (50:50 comparison). In contrast, the second factor is the host plant type (I), with three levels: i1=corn plants, i2=onion plants. All treatments were repeated three times. Data were examined using ANOVA and Duncan's test to demonstrate that treatment influenced all observed response variables.

To investigate root colonization using a staining procedure similar to that described by Clapp et al. [10] with the staining stages described below: 1) thoroughly washed the roots with distilled water; 2) soaked the roots in 20% KOH for 48 hours; 3) the roots were washed with water until clean, then soaked in 0.1 M HCl; 4) soaked the roots in a solution of trypan blue for 48 hours without washing; 5) soaked the roots in a destaining solution for 24 hours; 6) After cutting the roots to a length of 1 cm, they were aligned parallel to the slide and covered with a coverslip. The proportion of root colonization was estimated using the methodology [11].

% Colonization = colonized field of view multiplied
by 100% of the entire field of view

Isolated spores from soil samples using the wet filter pour method [12] and centrifugation [11]. Used the following steps to isolate spores: 1) 20 g of soil was taken and mixed with water until 500 ml was obtained; 2) the soil suspension was poured into a graded filter with sizes of 125 and 63 m from top to bottom; 3) the soil suspension was poured into a test tube, and then added 1/3 of the 60 per cent sucrose solution; 4) centrifuged the test tube at a speed of 2,300 rpm for approximately 3 minutes. 5) A somewhat clear liquid in the tube's centre (float) is a transition between glucose solution and water, which is sucked up with a pipette and rinsed and filtered through a 63 m filter; 5) The results are placed in a petri dish and viewed under a microscope to determine the spore density. The formula for calculating spore density is as follows:

$$\text{Spore density} = \frac{\text{spore count}}{\text{weight of dirt analyzed}}$$

They measured numerous growth metrics to ascertain the host plants' response, including plant height, leaf count, wet weight, dry upper weight, and lower dry weight.

3. RESULTS AND DISCUSSION

The study of the soil employed as a growing medium revealed that it was slightly acidic (pH 6.4), had a moderate nitrogen content, and contained 25% P_2O_5 HCl, including the high category of 53 mg/100 g with a meagre C/N ratio of 3.94. The average temperature is 27 degrees Celsius, and the intermediate humidity level is 80%. The findings of a statistical investigation of the effect of mycorrhizae on the height of maize, shallots, and upland rice may be found in Table 1.

Table 1. The results of a statistical investigation of the effect of mycorrhizae on plant height (cm) at 35 days after planting in response to differences in the growing environment and host plant type

Treatment	Host Plant Type		
	Corn	Red Onion	Gogo Rice
Soil+ zeolit	26,87 b	56,45 b	22,05 a
	A	B	A
Soil+Organic fertilizer	17,72 a	58,61 b	23,83 a
	A	B	A
Soil+tailings	22,05 ab	39,34 a	18,64 a
	A	B	A
Soil+sand	36,93 c	53,44 b	21,25 a
	B	C	A

Numbers followed by the same lowercase letter (vertical direction) and the same capital letter (horizontal direction), indicating no statistically significant variation in the degree of inaccuracy of 5% as determined by Duncan's Test.

For corn, a mixture of soil plus sand media can accelerate plant height compared to other media mixtures, while for shallots and upland rice, there is relatively no difference. Among the three host plants, the most suitable as host that can stimulate plant height growth is soil plus sand, namely the onion plant.

At 35 days following planting, there was a favourable interaction between the planting media and the host plant on plant height characteristics. For each host plant species evaluated, varied growing conditions resulted in a range of plant heights. For maize, a mixture of soil and sand performed best; however, for shallots, three varieties of mixed media performed similarly, namely earth with sand, mud with manure, and dirt with zeolite, all of which served as well soil with tailings. While all forms of the medium produced similar findings in terms of plant height for upland rice plants, onion plants on various growing media appeared to be more suited than corn and upland rice plants.

The results of previous study Sufardi et al. [13] showed that the application of organic ameliorants and mycorrhizae improved soil pH, total P (25 per cent HCl extract), available P (Bray 1), and soil P availability index hence increasing maize growth and yield. The combination of treatments that had the most significant influence on P status, maize growth, and yield was manure, or Gamal leaves mixed with mycorrhizae. Gigaspora margarita is a mycorrhizal fungus that is excellent for use in garlic production. It can improve plant height, leaf number, stem diameter, root length, and stem dry weight [3].

The impact of mycorrhizal inoculation on the number of leaves 21 days after planting is depicted in Table 2. The three different plant types displayed considerable differences. Each plant has a different amount of leaves at the same age, with the rice plant having the most. When compared to maize and shallots, rice plants have a higher level of mycorrhizal inoculant compatibility. Our results appear to be in agreement with Fitriyah's study [14], which found that mycorrhizal inoculation of 100 g kg^{-1} of soil had an impact on rice plant height, root decay ratio, tiller count, degree of root infection, and dry grain production (*Oryza sativa* L.).

Table 2. Statistical examination of the effect of mycorrhizae on the number of leaves (strands) aged 21 days after planting

Treatment	Host Plant Type			Average
	Corn	Red Onion	Gogo Rice	
Soil+ zeolite	2,06	3,72	13,41	6,40 a
Soil + Organic fertilizer	1,91	4.39	14,16	6,82 a
Soil + tailings	1,91	2,50	12,00	5,47 a
Soil + sand	2,46	3,33	13,66	6,48 a
Average	2,08 A	3,48 B	13,31 C	

Numbers followed by the same lowercase letter (vertical direction) and the same capital letter (horizontal direction), indicating no statistically significant variation in the degree of inaccuracy of 5% as determined by Duncan's Test.

Rice plants are easier to grow from seed to sapling than shallots. Because there will be a rise in the number of leaves for each sapling, the rate of leaf growth will thus be quicker. Even corn plants do not create saplings. Hence there will be fewer leaves than in the case of shallot plants, where tillers develop more slowly. Based on observations of the parameter number of leaves aged 21 days, it was determined that these changes in media and host plant species were more a result of genetic than mycorrhizal influences.

The number of leaves at the age of 28 days after planting showed the same outcomes as the number of leaves at the age of 21 days after planting in response to mycorrhizal administration (Table 3). There was no interaction between the application of mycorrhizal inoculation to different types of growing media and plant types, and variations in the number of leaves only occurred due to variations in plant species. Variations in the types of planting media did not show any differences at 21 days or 28 days after planting.

According to the two tables above (Tables 2 and 3), it can be explained that different mycorrhizal species, host plant species, and environmental factors affect how compatible mycorrhizae are with particular host plants. Mycorrhizal infections coming from the same host plant's rhizosphere will probably tend to be better than sources of spores coming from various host plants. The findings of Nurhayati [15], which demonstrated that different host plant species and inoculum sources had a substantial impact on the level of mycorrhizal infection, lend credence to this opinion.

Table 4 represents the outcome of a statistical examination of the number of leaves 35 days after planting. Only upland rice differed between the three types of host plants, specifically in the soil media mixture with an organic fertilizer that had more leaves. Across all planting media, it turns out that upland rice has the most leaves, followed by maize and shallots. The criterion "number of leaves" is greatly influenced by genetic characteristics, with upland rice having more tillers than shallots. Even the corn plants lack saplings.

Table 3. Statistical examination of the effect of mycorrhizae on the number of leaves (strands) aged 28 days after planting

Treatment	Host Plant Type			Average
	Corn	Red Onion	Gogo Rice	
Soil+ zeolite	2,67	4,05	14,83	7,18 a
Soil + Organic fertilizer	1,86	4,27	15,75	7,29 a
Soil + tailings	2,77	2,95	13,96	6,56 a
Soil + sand	2,71	3,72	12,50	6,31 a
Average	2,5 A	3,75 B	14,26 C	

Numbers followed by the same lowercase letter (vertical direction) and the same capital letter (horizontal direction), indicating no statistically significant variation in the degree of inaccuracy of 5% as determined by Duncan's Test.

Table 4. Statistical examination of the effect of mycorrhizae on the number of leaves (strands) aged 35 days after planting

Treatment	Host Plant Type		
	Corn	Red Onion	Gogo Rice
Soil+ zeolit	3,84 a A	5,63 a B	14,50 a C
Soil + Organic fertilizer	3,07 a A	5,61 a B	20,33 c C
Soil + tailings	4,13 a A	4,33 a A	16,50 b B
Soil + sand	4,49 a A	4,67 a A	14,25 a B

Numbers followed by the same lowercase letter (vertical direction) and the same capital letter (horizontal direction), indicating no statistically significant variation in the degree of inaccuracy of 5% as determined by Duncan's Test.

Each type of plant has different leaf growth characteristics during the vegetative phase. This will be determined among others by fertile and whether or not the planting medium. Among several types of mixed media tested, it showed that a good medium was soil containing organic fertilizer. the soil mixed with zeolite still showed a good effect, but the soil added with sand or tailings had a bad effect on increasing the number of leaves at the age of 35 days after planting.

As shown in Table 5, there is no interaction effect between the planting media and the host plant type on the plant's wet weight parameters. All media mix treatments on individual plants did not affect the wet weight parameters. However, upland rice had the highest damp weight than corn and shallots in all media mixtures. Onion had the second biggest wet weight, while corn had the smallest. Wet weight is inversely proportional to the number of leaves so that as the number of leaves increases, the plant's damp weight increases as well. Upland rice has a more significant number of leaves, which results in a heavier wet weight.

Table 5. Results of statistical analysis of the effect of mycorrhizae on the variation of growing media and host plant species on wet weight (g) at 35 days after planting

Treatment	Host Plant Type			Average
	Corn	Red Onion	Gogo Rice	
Soil+zeolite	3,84 a	5,63 a	14,50 a	4,17
Soil+Organic fertilizer	3,07 a	5,61 a	20,33 c	2,33
Soil+tailings	4,13 a	4,33 a	16,50 b	4,17
Soil+sand	4,49 a	4,67 a	14,25 a	7,00
Average	4,42 A	9,67 B	15,92 C	

Numbers followed by the same lowercase letter (vertical direction) and the same capital letter (horizontal direction), indicating no statistically significant variation in the degree of inaccuracy of 5% as determined by Duncan's Test.

Table 6. Results of statistical analysis of the effect of mycorrhizae on variations in planting media and host plant species on the upper dry weight (g) 35 days after planting

Treatment	Host Plant Type			Average
	Corn	Red Onion	Gogo Rice	
Soil+ zeolite	1,0	1,0	2,0	1,3 a
Soil + Organic fertilizer	1,0	1,2	3,5	1,9 a
Soil + tailings	1,0	1,5	2,0	1,5 a
Soil + sand	1,0	1,5	1,2	1,4 a
Average	1,0 A	1,3 A	2,3 B	

Numbers followed by the same lowercase letter (vertical direction) and the same capital letter (horizontal direction), indicating no statistically significant variation in the degree of inaccuracy of 5% as determined by Duncan's Test.

As shown in Table 6, there was no interaction effect between planting media and plant type on the maximum dry weight. On all types of growing media, mycorrhizal inoculation had no discernible influence on the top dry weight of all types of plants. However, upland rice had the largest dry weight of any plant kind, followed by corn and shallots.

Table 7. Results of statistical analysis of the effect of mycorrhizae on variations in planting media and host plant species on root dry weight (g) at 35 days after planting

Treatment	Host Plant Type			Average
	Corn	Red Onion	Gogo Rice	
Soil+ zeolite	1,12	1,83	1,33	1,83 a
Soil + Organic fertilizer	1,00	1,17	1,00	1,16 a
Soil + tailings	1,00	1,67	1,00	1,66 ab
Soil + sand	1,00	1,17	1,00	1,16 a
Average	1,04 A	1,46 B	1,08 A	

Numbers followed by the same lowercase letter (vertical direction) and the same capital letter (horizontal direction), indicating no statistically significant variation in the degree of inaccuracy of 5% as determined by Duncan's Test.

In Table 7, all growing media mixes had the same root dry weight. However, shallots had a more extensive root dry weight than maize and upland rice. After dewatering, root dry weight represents the quantity of biomass accumulated due to photosynthesis. Additionally, this biomass represents the amount of photosynthate transported to plant roots. Concentrated for particular plants whose economic value at the base (seeds), the accumulation of photosynthetic results is critical since crop yields are directly tied. According to Setiawati et al. [16], inoculation of phosphate-solubilizing bacteria (*Pseudomonas cepaceae*) and vascular-arbuscular mycorrhizae increased the dry weight of corn plant roots. As We know that phosphorus (P) is indispensable for plant growth [17]. To sustain crop yields, P is supplied to the soil as fertilizer, for example, in mineral, water-soluble form such as triple superphosphate [17].

At the same time, Sukmawati [18] found that the highest population of phosphate-solubilizing microbes was found seven weeks after planting. It could be due to an accumulation of roots, particularly young hearts (root cape), which results in increased exudate. Exudates generated by plant roots, such as sugar, provide carbon, and amino acids offer nitrogen for soil microorganism growth. The previous research reported an increase in soil P availability negatively affects the colonization of plant roots as well as the mycelium development of arbuscular mycorrhizal fungi (AMF) [19].

Table 8. Results of statistical analysis of the effect of mycorrhizae on variations in growing media and host plant species on root infection (root infection unit)

Treatment	Host Plant Type		
	Corn	Red Onion	Gogo Rice
Soil+ zeolit	78,00 a A	315,67 b A	86,33 a A
Soil + Organic fertilizer	27,67 a A	374,33 b B	129,33 a A
Soil + tailings	32,33 a A	218,67 ab A	70,33 a A
Soil + sand	283,00 a B	27,33 a A	55,67 a AB

Numbers followed by the same lowercase letter (vertical direction) and the same capital letter (horizontal direction), indicating no statistically significant variation in the degree of inaccuracy of 5% as determined by Duncan's Test.

Based on Table 8 on the parameters of root infection, it can be observed that the planting media and the host plant interact favourably. The presence of root infection suggests that mycorrhizal fungi have formed a symbiotic relationship with the plant's roots. Figure 1 showed colonization of mycorrhizal on shallot's root and Figure 2 exhibited the colonization on corn root. In Figure 1, it can be seen that there are coils of mycorrhizal hyphae on the outer surface of the roots of the shallot plant. This proves that there is an association between plant roots and mycorrhizae. Likewise, the same thing happens to corn plants The establishment of a specific structure of mycorrhizal colonization on the roots identifies the relationship between mycorrhizae and their host plant. The symbiotic relationship between AM and the roots of higher plants contributes significantly to plant nutrition and growth [20], and has been shown to increase the productivity of a variety of agronomic crops including maize [21].

The structure of internal and external hyphae, vesicles, arbuscules, and spores was established as a consequence of root sample observations (Table 8). Mycorrhizal spores produce a structure that plays a crucial part in the attachment process. The germination of spores results in the formation of hyphae, which play a role in the absorption of nutrients and water from the environment into the roots, which are then utilized in the development and expansion of the host plant.

The combination of soil media and organic fertilizers is typically superior to corn and upland rice for onion plants. Possibly because the onion root supplies a lot of nutrients in the form of metabolic waste. In mixed media containing sand, maize and upland rice had the highest infection rates. Observations of plant root staining revealed the existence of spherical entities known as vesicles and arbuscules. Figures 3 and 4 showed the structure of mycorrhiza spores from corn (*Glomus sp.*) and a spore from shallot (*Acaulospora* sp.) respectively. Infection or symbiotic colonization between plant roots and mycorrhizal fungus is indicated by the presence of this structure. Infection by mycorrhizal fungi can be affected by host sensitivity, environmental conditions, and soil variables. Numerous variables, like as fertilizer, plant nutrition, pesticides, light intensity, season, etc., have a significant impact on the degree of mycorrhizal infection [22]. Typically, the greater the amount of fertilizer applied the less mycorrhizal infection of plants. Thus, it may be stated that mycorrhizae thrive on marginal soils.

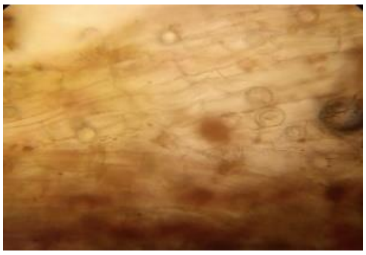

Figure 1. Colonization of shallot plant roots

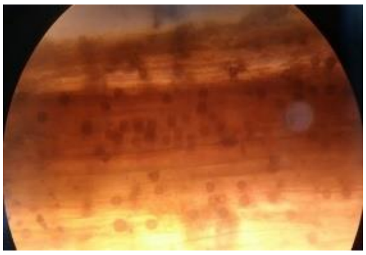

Figure 2. Colonization of corn plant roots

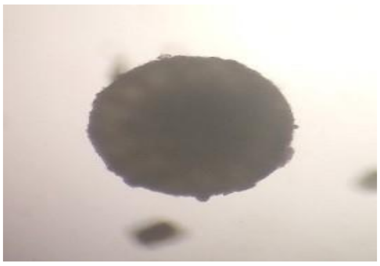

Figure 3. *Glomus* sp. spores from corn plants

Figure 4. Spores of *Acaulospora* sp. from shallot plants

Observations from Gazey et al. [23] experiment investigating the relationship between the development of mycorrhizas and increasing quantities of inoculum for three species of Acaulospora indicated that spores formed earlier in those treatments in which high quantities of inoculum were used. However, for each fungus there was not a close relationship between the length of external hyphae and the number of spores produced [23].

Arbuscular mycorrhiza naturally occurs in saline soils [24, 25]. Although salinity may affect the formation and function of mycorrhizas [24, 26, 27], several studies have shown that inoculation with arbuscular mycorrhiza fungi improves plant growth under salinity stress conditions [24, 28-30]. The arbuscular structure resembles a tree and is composed of intraradical hyphae between the cell wall and the cell membrane [31]. The arbuscular serves as a location for the exchange of nutrients and carbon between mycorrhizae and host plants, as well as a temporary storage location for minerals, nutrients, and carbohydrates. While vesicles are thin-walled structures created by swelling at the ends of hyphae, their shapes can be circular, oval, or irregular. Vesicles function as storage organs for food reserves such as lipids and, at times, as spores, which are mycorrhizal life's methods of defence. Moreover, spores, which are the self-replicating organs of mycorrhizal fungi, are produced by extraradical hyphae that take the form of a stump or colonies (sporocarps). Spores contain carbohydrates, lipids, proteins, and chitin as constituents. Spores contain mitochondria, endoplasmic reticulum, and vacuoles [32].

The application of arbuscular mycorrhiza aid in the implementation of organic farming systems for shallot growing [33]. Another research indicates the complex control mechanisms to regulate the levels of free and conjugated auxins, which are locally and systemically induced during the early stages of the formation of an arbuscular mycorrhizal symbiosis [34]. More research is needed to investigate the potential of indigenous arbuscular mycorrhiza and other microorganisms as biofertilizers or biopesticides for building sustainable farming systems on shallot and other crops.

4. CONCLUSIONS

Our results confirm that several growth parameters, such as plant height, number of leaves, and root infection, were influenced by the planting medium and the type of host plant. Among the three host plants, the most suitable host to stimulate plant height growth is the onion plant on soil and sand media. In all planting media, upland rice has the most leaves, followed by maize and shallots. Inoculation of mycorrhizae statistically had no noticeable influence on all plants' wet and dry weight in all types of growing media.

Meanwhile, mycorrhizal colonization on the roots identifies the relationship between mycorrhizae and their host plant. Our study found that combining soil media +organic fertilizers and onion plants provides the best interaction.

Based on the results of the various parameters observed, we conclude shallots planted in soil mixed with organic fertilizer are suitable as hosts for mycorrhizal propagation.

ACKNOWLEDGMENT

The authors would like to thank the management of LP2M-PMP of Universitas Siliwangi which has funded this research.

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