THE ROLES OF ARBUSCULAR MYCORRHIZAE IN SUPPORTING THE MANGROVE GROWTH

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ABSTRACT

The current study aims to determinate the potential of Arbuscular mycorrhizae in supporting the mangrove growth, the role of Arbuscular mycorrhizae on the improvement of mangrove growth in terms of its colonization level with the mangrove roots, the mangrove ability of Arbuscular mycorrhizae in the way of supporting the rehabilitation of mangroves, and giving the recommendation to the local government related to the potential of mycorrhizal fungi in rehabilitating the degraded magrove areas living in the coastal areas of Kwandang, North Gorontalo regency.

Moreover, this study used a descriptive qualitative analysis method using ex-post-facto approach. To collect the data, it used a pruposive sampling on rhizosfer and mangrove roots in order to observe the spore density, the diversity and the mycorrhizal colonization.

The results of this study show that the ability of Arbuscular mycorrhizae in supporting the mangrove growth is categorized in the high level. It can be seen from the spora density. On average in research location, the number of the spores that were successfully isolated is 42 spores/ 100 grams of soil. Arbuscular mycorrhizae has a role in the way of increasing the mangrove growth. This can be seen from the high percentage of colonization with mangrove roots whereas 57,87%.

Keywords: mangrove, arbuscular mycorrhizae

INTRODUCTION

The mangrove forest is considered as one of the important ecosystems in coastal areas, both ecologically and economically. This forest has high productivity, a primary producer in estuary water, and in turn as well as supporting fisheries. Mangrove forests are very useful, especially as an ideal habitat for the survival of living things around the coast. Mangrove forests also play a role in tourism, conservation, education, and research area [17].

According to the data from the Directorate General of Land Rehabilitation and Social Forestry, Indonesia's mangrove forest area is 8.6 million ha, consisting of 3.8 million ha in forest areas and 4.8 million ha outside forest areas. The mangrove ecosystem covering an area of 8.6 million hectares shows that 44.73% in forest areas and 87.50% in non-forest areas have been damaged or degraded [7]. The damage is generally caused by excessive mangrove conversion, conversion to ponds, rice fields, plantations, industry, settlements, pollution, sedimentation, pests and diseases, and other influences. The rapid loss of mangrove forests has triggered increased coastal erosion, which has caused damage to the natural habitat of fish and shrimp, the increased intrusion of seawater into the land, and affected the livelihoods of coastal fishermen.

In fact, one of the mangrove areas in Indonesia is located in the coastal area of

Kwandang, North Gorontalo District, Gorontalo Province. This mangrove area has advantages in terms of biodiversity and is unique from a variety of species that live not based on zoning patterns in general [11]. One of the rare mangrove species in the world is found on the coast of Kwandang, namely Ceriops decandra. Ecologically, this area is faced with the problem of local ecosystem damage, particularly mangrove forest damage.

Most of the mangrove forest area in this area has experienced shrinkage due to human activities logging and harvesting mangrove wood for fuelwood fulfillment and building construction. This is proven by the results of the study conducted by [3]. That study stated that mangrove damage in 2010 reached an increase of 41% comparing to 21% in 2000, therefore, the total damage to mangroves in 2010 had reached 62%. Importantly, the mangrove areas that have been damaged have reached 687.3 acres.

The efforts of rehabilitations carried out in the mangrove area include replanting, but the success rate is very low [2]. The low success of reforestation is thought to be due to poor mangrove growth, or mangroves are attacked by pests and diseases.

To improve the success of rehabilitation of mangrove forests and coastal forests, it is necessary to know the condition of the soil in the presence of microorganisms in these locations. One of the microorganisms that play a role in mangrove growth is Arbuscular mycorrhizae. An Arbuscular mycorrhiza is a form of a symbiotic relationship between fungi and higher plant roots.

The role of arbuscular mycorrhizae is very important, especially in terms of conservation of the nutrient cycle, helping to improve soil structure, transferring of carbon in the root system, overcoming degradation of soil fertility, and protecting plants from disease. The presence of mycorrhizae is very important for the resilience of an ecosystem, plant stability, and maintenance of biological diversity.

RESEARCH METHODOLOGY

The current study was conducted in the coastal areas of mangroves located in Kwandang, north Gorontalo regency of Gorontalo province. It was also conducted in the biology laboratory of the science faculty of Gorontalo State University.

The study used a descriptive-qualitative analysis using Ex-post-facto approach.

Tools used for the present study: Laminar airflow, oven, water bath, beaker, Erlenmeyer, centrifuge, centrifuge tubes, Compound and binocular microscopes, analytical scales, spore filters (425 µm, 212 µm, 106 and 45 µm), test tubes, Petridis, pH-meters, Salinometers, lux meters, hygrometers, thermometers, microscopes, glass objects, Bunsen lights, and cameras. Materials used: mangrove rhizosphere sediment (100g soil/sample point), mangrove roots, 60% sucrose, Trypan blue, 1% HCL, 10% KOH, glycerol, and Aquadest.

1. Soil Sampling and Mangrove Roots, one sample was taken by obtaining ± 3 points of soil around the tree and on all different tree species with a depth of 10-30 cm, then the soil sample was composited by stirring, and then 1 kg was taken as a sample.

   Along with soil sampling, root samples were also taken to see the AMF colonization contained in it. The roots taken are good in tree roots with a depth of 10-30 cm.

2. Isolation of Mycorrhizal Spores, In relation to research procedure, the technique used in isolation was the pour-filter method [1] followed by the centrifugation method [4].

   The working step of the filter pouring technique is to weigh the soil sample as much as 100 grams then mix it with 300 ml of water and stir evenly, then filtered in a filter set with sizes 425 µm, 212 µm, 106, and 63 µm respectively from top to bottom. At the top of the filter, it is sprayed with tap water to make it easier for the filter material to pass.

The material passing through the bottom filter and the second from the bottom is then transferred to the centrifuge tube. The filter results are added with 60% of sucrose. The centrifuge tube is tightly closed and centrifuged at 3000 rpm for 5 minutes. Furthermore, the supernatant solution was poured into 0.5 mm filter paper, rinsed with flowing distilled water to remove sucrose.

The remaining sediment is put into a petri dish and then spore observation was carried out using a compound microscope to calculate the number of spore populations per sample.

3. Arbuscular Mycorrhizal Colonization in Plant Roots, The AMF Observation of root colonization in mangrove plants used coloring techniques, namely, the roots were washed thoroughly with distilled water.

   Then the roots were soaked in 10% KOH and put in a water bath, temperature 80ºC for 10 minutes. Then the roots were washed with running water 3-5 times, the roots then were soaked in 1% HCL solution for 1 day and washed with distilled water, then given 0.05% Trypan blue dye and left for 1 day.

   The next step The roots are cut along 2 cm and then placed in a row on the object-glass. Every 5 pieces of roots were covered with a cover glass and then observed each piece of roots under a microscope. In the observation book, a minus sign (-) is given for each field of view where there is no Mycorrhizal structure and a sign (+) for each field of view where mycorrhizae are found (hyphae, Arbuscular, vesicles, or intra-radical spores).

RESULTS RESEARCH AND DISCUSSIONS

1. Arbuscular mycorrhizae Support Capacity in Supporting the Mangrove Growth in terms of Spore Density.
   a. Its results based on the identification results obtained 2 genes namely Glomus and Gigaspora.

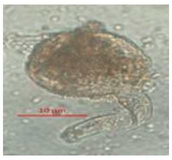
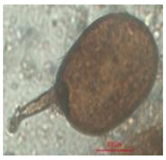
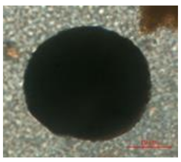
      The Glomus classification is as follows:
      Class : Zygomycetes
      Order : Glomeromycota
      Sub order : Glomineae
      Family : Glomeraceae
      Genus : Glomus
      Species : Glomus versiformes, Glomus ambisporum, Glomus bareale, Glomus canadense, glomus etunicatum, Glomus constrictum.
      Source: [4][9]] [10]

      The Gigaspora classification is as follows:
      Class : Zygomycetes
      Order : Glomeromycota
      Sub order : Glomineae
      Family : Glomeraceae
      Genus : Gigaspora
      Species : Gigaspora sp
      Source: [4] and [10]

The identification results of Arbuscular Mycorrhizal spores isolated from rhizosphere of mangroves are illustrated in table 1.

Tabel 1. The identification results of Arbuscular Mycorrhizal Spores Isolated from Rhizosphere of Mangrove Plants.

No.	Arbuscular Mycorrhizal	Description	Spore areas
1	 Glomus versiformes	The spore is round. Its color is brown. The surface of the spore is smooth and its hyphae is released	2902,673 µm2

2	 Gigaspora sp	The spore is round. Its color is blackish brown. Its surface is smooth and the hyphae of the spore is released	491,194 µm2
3	 Glomus sp	The spore is round. Its color is clear yellow. Its surface is smooth and its hyphae is still attached without bulbous suspensors	273,906 µm2
4	 Glomus ambisporum	The spore is round. Its color is black. Its surface is smooth and there are supporting hyphae	477,242 µm2
	 Glomus bareale	The spore is oval. Its color is brown. Its surface is smooth and the hyphae are still attached without bulbous suspensors	482,993 µm2
6	 Glomus canadense	The spore is oval. Its color is yellow. The surface of the spore is smooth and the hyphae are still attached without the bulbous suspensor	77,898 µm2
7	 Glomus constrictum	The spore is round. Its color is black. Its surface is smooth and the hyphae is released.	617,285 µm2

Source: Primary data

Tabel 2. Types of Arbuscular Mycorrhizal Spores Isolated from Rhizophere of Mangrove Plants.

No	Arbuscular Mycorrhizal species	Host Plants							
		R. mucronata	R. apiculata	C. decandra	B. gymnorrhiza	S. alba	X. granatum	C. tagal	A. marina
1	Glomus constrictum	√	√	√		√	√	√	√
2	Glomus versiforme	√	√	√		√	√	√	√
3	Glomus boreale				√			√	
4	Gigaspora								√
5	Glomus ambisporum			√	√	√	√	√	√
6	Glomus canadense							√	
7	Glomus sp							√	

2. Spore Density and Diversity, the results show that the arbuscular mycorrhizal population contained in the rhizosphere of mangrove plants at the research site has a high-density value. The density of mycorrhizal spores in mangrove plants is presented in Figure 1.

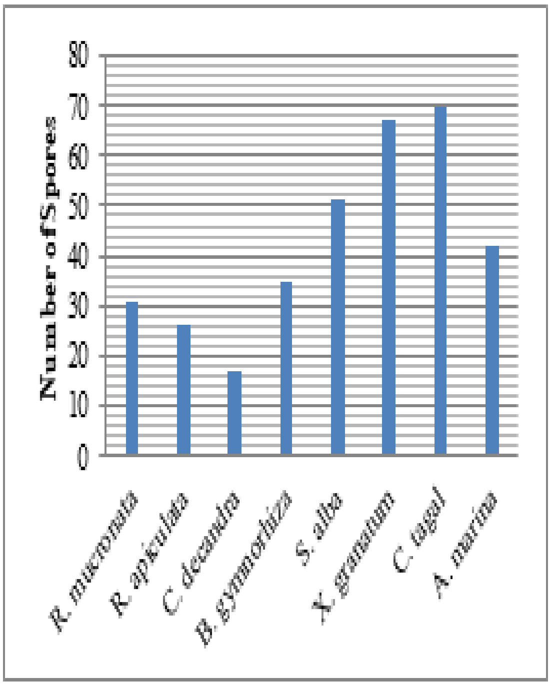


Figure 1. The spore Density of Mangroves in Kwandang

Table 3: Density (K), Frequency(F), Relative abundance (KR), Important value (NP), Diversity (H'), Uniformity (E), and Dominance (D) of Arbuscular Mycorrhizae of Mangroves in Kwandang.

Kinds of host plants	INP Mangrove (%)	K/100g	F	KR	INP	H'	E	D
R. mucronata	8.05	31	0.23	79	39.61	0.06	0.86	0.56
R. apiculata	22.8	26	0.04	15	7.52	1.173	0.84	0.31
C. decandra	13.1	17	0.23	80	40.11	1.085	0.98	0.31
B. gymnorhiza	25.2	35	0.34	118	59.17	0.692	0.99	0.48
S. alba	54.2	51	0.08	28	14.04	1.09	0.78	0.32
X. granatum	4.95	67	0.02	10	5.01	1.059	0.96	0.35
C. tagal	7.75	70	0.002	1	0.50	1.773	0.98	0.16
A. marina	36.5	42	0.02	8	4.01	1.352	0.97	0.24
Average		42	0.12	42.3	21.24	1.03	0.92	0.34

3. The Role of Arbuscular Mycorrhizae in Increasing Mangrove Growth in terms of the level of colonization with mangrove roots. In 8 species of mang-rove plants studied are known to be colonized with Arbuscular mycorrhi-zae. The presence of mycorrhizae is characterized by the presence of inter-nal hyphae, external hyphae, vesicles, and Arbuscular in the root system of plants

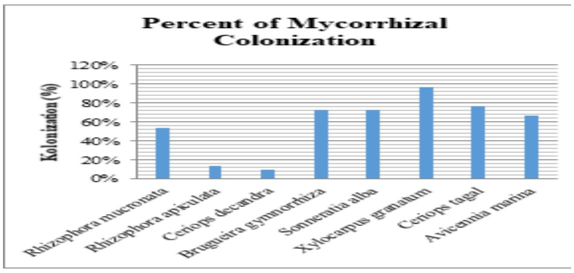
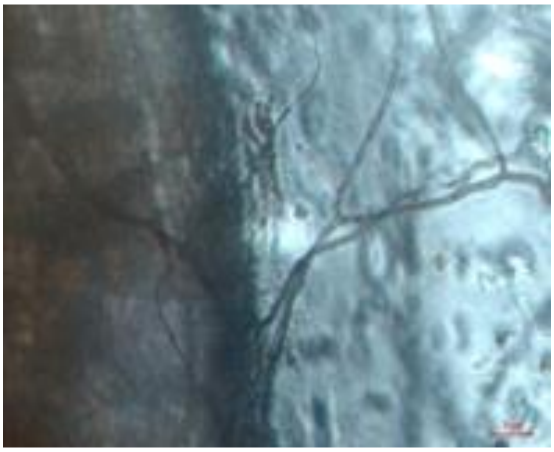


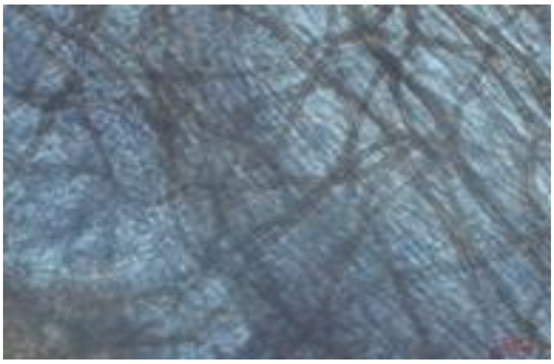
Figure 2. Percent of Mycorrhizal Root Colonization in The Area of Mangroves in Kwandang

Table 4. The structures of Arbuscular Mycorrhizal and of Mycorrhizal roots in the mangrove

Host plants	Arbuscular Mycorrhizal structures				Colonizatio (%)
	HI	HE	V	A	
Rhizophora mucronata	4	2	10	0	53,3%
Rhizophora apiculata	3	1	0	0	13,3 %
Ceriops decandra	1	2	0	0	10%
Brugueira gymnorrhiza	18	1	3	0	73,3%
Sonneratia alba	16	0	5	1	73,3 %
Xylocarpus granatum	11	2	15	1	96,6 %
Ceriops tagal	17	1	5	0	76,6 %
Avicennia marina	14	4	1	1	66,6 %

Note: HI: internal hyphae, HE: External hyphae. V: vesicle, A: Arbuscular





Figture 3. Colonization of Arbuscular Mycorrhizal with mangroves, HI; Internal Hyphae, HE; External Hyphae, V; Vesicles, A; Arbuscular and S; Internal spores.

DISCUSSION

1. The carrying capacity of Arbuscular Mycorrhizae in supporting mangrove growth in terms of spore density, The Arbuscular Mycorrhizae status in the soil can be seen from the density of spores per gram of soil. In this case, per 100 grams of soil is taken from the rhizosphere of the sample plants. Referring to Walker (1992) in [18], the population of Arbuscular mycorrhizae spores is categorized as high if the number is 14-161 per 100 grams of soil. Based on the results of research, the Arbuscular Mycorrhizae spores that were isolated from the research location are in the high category, on average, 42 spores per 100gr of soil samples. The high population of Arbuscular Mycorrhizae spores in saline soil is because of the types of Arbuscular Mycorrhizae at the study location. It has an osmoregulation mechanism such as the osmotic adjustment mechanism of halophytes in saline soil by storing sodium and chloride ions. Thus, the osmotic potential in the cells is lower than the soil solution [6].

   The high density of Arbuscular mycorrhizae spores gives great support for the growth of the host, in this case, mangroves. According to Rohimat (2002) in [14], the highest number of spores in a plant shows the best results on all observation variables. This is supported by the results of research by [14] that a high number of spores can increase the growth and yield of chili plants. The more spores found, the greater the chance that symbiosis will occur between mycorrhizae and mangroves. This symbiotic relationship provides enormous benefits for mangroves for their survival because mycorrhizae play an important role, especially in terms of conservation of the nutrient cycle, helping to improve soil structure, transporting carbon in the root system, overcoming degradation of soil fertility and protecting plants from disease.

   Related to the results of the calculation of Mycorrhizal spore diversity in the research site, it has a moderate Mycorrhizal diversity. A diversity index is used to determine the level of diversity of species in a community. A community is said to have high diversity if the community is composed of many species with the same and almost the same species abundance. Conversely, if a community is composed of a few species and if only a few species are dominant, the species diversity is low. The results of the calculation show that the average Mycorrhizal diversity in the Kwandang mangrove area is at the value of H '= 1.03, which means that Mycorrhizal diversity is in the medium category. This shows that the Mycorrhizal consortium has moderate diversity, sufficient productivity, fairly balanced ecosystem conditions and moderate ecological pressure [5]. This condition can be seen from the results of research that only found 7 species with different abundances. The lowest abundance of 1 was found in the host plant of Ceriops tagal and the highest abundance was 118 in the host plant of Brugueira gymnorrhiza. The difference in the number of high abundance influences the diversity of a community. The role of the host tree on the abundance of Mycorrhizal spores in the rhizosphere is related to the resulting root exudate, where the root exudate which is a source of energy will affect the germination of Arbuscular Mycorrhizae spores.

2. The Role of Arbuscular Mycorrhizae in Increasing Mangrove Growth in terms of the level of colonization with mangrove roots. Based on the results of the study, the average percent of colonization obtained by the research location, namely the mangrove area, is in the high category, namely 57.87%. This is following the statement [12] emphasized that the percent colonization of more than 30% is included in the high category. These data indicate that all mangrove species sampled were able to colonize with arbuscular mycorrhizae. Mycorrhizal colonization with roots can be seen from the formation of arbuscular mycorrhizal structures in the mangrove roots consisting of hyphae (external and internal), vesicles, arbuscules and internal spores.

   Gaining the data from the calculation of the percent of colonization illustrates that there is great potential for mycorrhizae to be used as biological agents for mangroves so that they can help in the growth process. This is expected to compensate for the degradation that occurs continuously due to human activities. Large-scale deforestation without reforestation will harm the mangrove ecosystem. The impact of mangrove damage has a major impact on the biotic life around it. Various types of biota such as fish, shrimp, crab, shellfish and birds have lost their habitat to find food or make nests to live in, this can cause these biotas to lack food and eventually die. Besides, the loss of mangrove forests is very dangerous for the lives of people on the coast.

3. Tsunamis and strong winds from sea to land can at any time threaten the people. Importantly, the role of arbuscular mycorrhizae in mangrove growth is its ability to absorb phosphorus nutrients. Besides, roots that have mycorrhizae can absorb nutrients in bound form and those that are not available to plants. Mycorrhizal roots can increase nutrient uptake capacity because the life time of the colonized roots is extended and the degree of branching and root diameter is enlarged, so that the surface area of root absorption is expanded. External hyphae on mycorrhizae can absorb elemental phosphate from the soil, and are immediately converted into polyphosphate compounds. The polyphosphate compound is then transferred into the hyphae and broken down into organic phosphate which can be absorbed by plant cells.

   The increase in plant nutrient absorption facilitated by mycorrhizal fungi will increase plant response in the form of vegetative growth and increase in the growth of the generative phase which in turn increases plant production [15] Another factor that affects plant growth is faster, namely the association between arbuscular mycorrhizae and plants which can stimulate the production of hormones such as IAA (indole acetic acid), cytokinins, auxins and gibberellins [8].

The results of [16] research show that Arbuscular Mycorrhizae can increase plant growth and increase resistance to biotic and abiotic stresses in many agricultural plants.

Inoculation of Mycorrhizal fungi increased potato yield, namely the number of tubers was greater and the size of potato tubers was larger. Arbuscular Mycorrhizae can increase plant productivity by around 25%-50% which includes plant health, yield quality, tolerance to water stress, fertilization efficiency and can suppress the development of pathogenic microbes in the soil [1]. Several previous studies explained that arbuscular mycorrhizae can increase plant growth. This of course also applies to mangrove plants, the greater the mycorrhizal infection, the higher the growth of the host.

This is very important to support the restoration of degraded mangrove ecosystems. Another function of soil function in general is to play a role in determining the carbon cycle in forest areas. Meanwhile, mangrove forests play an important role in the global carbon cycle, which is considered an important and sustainable carbon sink [16]. Seeing this connection, arbuscular mycorrhizae can indirectly reduce global warming which is currently an environmental problem. Damage to the mangrove ecosystem is not only caused by humans but also caused by pests and diseases.

Arbuscular mycorrhizae have an important role in preventing pathogenic microbes that can cause disease in mangroves. Mycorrhizae will use up all the excess carbohydrates and root exudates, creating an environment unsuitable for pathogenic growth. Besides, there are mycorrhizal fungi that can produce antibiotics that can inhibit pathogens [8]. This is supported by the results of research [13] which states that mycorrhizae can reduce the development of root rot caused by Rigidoporus microporus.

CONCLUSIONS

As the conclusions, the current study presents some concluding important points which are follows:

1) The carrying capacity of arbuscular mycorrhizae in supporting mangrove growth in the coastal area of Kwandang, North Gorontalo Regency is determined in the high level. This can be seen from the density of spores. The average number of spores isolated was 42 spores/100 grams of soil,
2) Arbuscular mycorrhizae play a role in increasing mangrove growth. This can be seen from the high percentage of colonization with mangrove roots, showing 57.87%.

Arbuscular mycorrhizae have the potential to rehabilitate degraded mangrove areas, therefore, they can be used as biological agents in helping mangrove growth.

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