APPLICATIONS OF *Rhizoctonia Mycorrhiza* AND WITHOUT *Rhizoctonia Mycorrhiza* IN IMPROVING VEGETATIVE GROWTH OF *Dendrobium nindii* SEEDLINGS

APLIKASI *Rhizoctonia Mycorrhiza* DAN TANPA *Rhizoctonia Mycorrhiza* DALAM MENINGKATKAN PERTUMBUHAN VEGETATIF BIBIT *Dendrobium nindii*

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ABSTRACT

Dendrobium is a genus of orchid that has the most attraction among the public rather than other types, especially for orchid species such as *Dendrobium nindii* type. The obstacle in cultivating orchids species is the slow vegetative growth when compared to orchids resulting from crosses (hybrid orchids). This research aimed to determine the vegetative growth of *D. nindii* seedlings using *Rhizoctonia* sp. The research was conducted at the Greenhouse, Faculty of Agriculture, Tunas Pembangunan University from January to July 2023. The research used quantitative descriptive method consisting of one treatment with six replications and each replications contained ten plants. The factor was application of *Rhizoctonia* mycorrhiza and without application of *Rhizoctonia* mycorrhiza. The results showed that (1) the morphological characteristics of *Rhizoctonia* mycorrhiza were white colonies, with right-angled branches and two nuclei, (2) the application of mycorrhizal *Rhizoctonia* had a very significant effect on the vegetative growth of *D. nindii* as shown in the parameters of plant height, leaf length, number of leaves, root length, number of roots and fresh weight of seedlings, and (3) there was an association of *Rhizoctonia* mycorrhiza with the formation of the peloton structure.

Key words: *Dendrobium nindii*, *Rhizoctonia mycorrhiza*, Peloton structure, Vegetative growth.

ABSTRAK

Dendrobium merupakan salah satu genus anggrek yang memiliki daya tarik paling banyak di masyarakat diantara jenis anggrek lainnya terutama untuk anggrek spesies seperti *Dendrobium nindii*. Kendala dalam budidaya anggrek spesies adalah pertumbuhan vegetatif yang lambat bila dibandingkan dengan anggrek hasil persilangan (anggrek hibrida). Penelitian ini bertujuan untuk mengetahui pertumbuhan vegetatif bibit *D. nindii* dengan menggunakan *Rhizoctonia* sp. Penelitian dilakukan di Rumahkasa Fakultas Pertanian Unversitas Tunas Pembangunan dari Januari sampai Juli 2023. Penelitian menggunakan metode deskriptif kuantitatif dengan enam ulangan dan setiap ulangan terdiri enam tanaman. Faktor perlakuan adalah aplikasi *Rhizoctonia* mikoriza dan tanpa aplikasi *Rhizoctonia* mikoriza. Hasil penelitian menunjukkan (1) ciri morfologi *Rhizoctonia* mikoriza berupa koloni berwarna putih, dengan percabangan siku-

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siku dan berinti dua, (2) pemberian *Rhizoctonia* mikoriza berpengaruh sangat nyata pada pertumbuhan vegetatif *D. nindii* yang ditunjukan pada parameter tinggi tanaman, panjang daun, jumlah daun, panjang akar, jumlah akar dan berat segar bibit, dan (3) terjadi asosiasi *Rhizoctonia* mikoriza dengan terbentuknya struktur *peloton.*

Kata kunci : *Dendrobium nindii*, pertumbuhan vegetatif, *Rhizoctonia* Mikoriza, struktur *peloton*.

INTRODUCTION

The Dendrobium orchid is a genus of orchid that has the greatest attraction among the public among other types of orchids. This is because the orchids of this genus have high adaptation to their environment. Apart from its adaptability, other advantages of this genus are that it has a variety of types and colors, is long-lasting and does not fall off easily, and is easy to use in cut flower packaging (Latief et al., 2020). Therefore, this genus is very interesting and is in great demand by many consumers, making this type of orchid one of the most popular with consumers who love orchids. One of the favorite Dendrobium orchids is *Dendrobium nindii*.

The *Dendrobium nindii*, known as the blue horn orchid, is a type of epiphytic orchid that originates from the northern to eastern regions of Papua New Guinea. The flowers are purplish white with a purple labellum, strong false stems, usually dark in color, can reach 2 m in length and 4 cm in diameter, and oval-shaped leaves with a length of 5 to 15 cm (Puccio, 2018). The growth of the *D. nindii* orchid plant is very slow so special care is needed to increase its growth. Using appropriate planting media and utilizing soil microbes such as mycorrhiza are efforts that can be made to increase the growth and flowering of orchid plants (Herliana et al., 2018).

The *D. nindii*, like other orchid plants, is propagated using tissue culture techniques (in vitro). Even though using tissue culture techniques you will get more seeds because of the high success rate, this causes orchid embryos to be heterotrophic. Heterotrophic properties occur because orchid seeds are very dependent on the tissue culture media used and this will make it difficult when orchid seeds are grown in the field, because when cultivated orchids have to change their lifestyle from heterotrophic plants to autotrophic plants. Several methods that are widely used are providing growth regulators such as auxin and cytokinin to orchid seedlings to stimulate the vegetative growth process so that orchid seedlings can carry out photosynthetic reactions more optimally (Latief et al., 2020). Apart from using chemical growth regulators, organic growth regulators such as coconut water can also be used which are mixed into orchid tissue culture media (Tuhuteru et al., 2018).

The use of chemical and organic growth regulators is only temporary because it will be completely absorbed by the orchid seeds. However, there is another way, namely by applying mycorrhiza on plants (Siddiqui & Pichtel. 2008). Therefore, it is necessary to consider using other methods to increase vegetative growth, namely using microorganisms that are inoculated on orchid seeds, one of which is by using mycorrhizal fungi which can associate with orchids (orchid mycorrhiza). One group of mycorrhizal fungi that can be associated with orchids is *Rhizoctonia* mycorrhiza (Suryantini et al., 2015). Previous research

by Hossain et al. (2013) also proved that two endophytic fungi isolated from the roots of the epiphytic orchids *Rhynchostylis retusa* and *Aerides multiflorum* were used for biological hardening of *Cymbidium aloifolium* and *Cymbidium giganteum* seedlings raised asymbiotically. These fungal isolates showed similarities to *Rhizoctonia*-like fungi in cultural characteristics, microscopic features, and sequence of the internal transcribed spacer (ITS) region.

Rhizoctonia mycorrhiza belongs to the *Rhizoctonia* spp. and identified in orchids and a group known as anastomoses (Gondal et al., 2019). In nature, the association of *Rhizoctonia* mycorrhiza with orchid roots occurs when the seeds begin to germinate to form roots and shoots (protocorm) (Balestrini et al., 2014). *Rhizoctonia* mycorrhiza which is found on the roots of orchid species is a fungus that lives naturally and is in symbiosis with plant roots, thereby helping to meet the orchid's need for plant nutrition. Mycorrhizal fungi are beneficial for plants because they can increase the plant's ability to absorb water (Sari, 2018), improve the chemical, physical and biological properties of the soil, because the outer hyphae of mycorrhizal fungi are able to penetrate the pore spaces of the soil, both micro and micro. and macro. The external existence of hyphae and roots is very important because they are able to absorb and store soil moisture. The aim of this research was to determine the effect of *Rhizoctonia* mycorrhiza in increasing the vegetative growth of *D. nindii* seedlings. It is hoped that in the future it can be applied to other orchid species resulting from tissue culture (in vitro) propagation.

MATERIALS AND METHODS

This research was carried out from January to July 2023. *Rhizoctonia* mychorrizae was taken from the *Dendrobium lasianthera* collection using the Hossain method (2022) in the tissue culture laboratory of the Faculty of Agriculture, Tunas Pembangunan University (UTP) Surakarta. *D. nindii* seeds to be inoculated were obtained from the tissue culture laboratory with George et al. (2008) method and inoculation carried out at the UTP experimental garden.

Rhizoctonia mycorrhizal isolates were grown on Potato Dextrose Agar (PDA) media and incubated for 9 days and the colony shape and hypha structure were identified. After 9 days, five grams of *Rhizoctonia* mycorrhiza culture was mixed with 100 ml of sterile water. D. 8 month old *D. nindii* seedlings are placed in a pot filled with moss. Each *D. nindii* seedling was sprayed with 1 ml of *Rhizoctonia* mycorrhiza inoculum and first acclimatized in the greenhouse for 2 months. After 8 months of age, the roots of *D. nindii* seedlings were cut and examined under a microscope to see the association of *Rhizoctonia* mycorrhiza in the form of peloton structures. The growth of *D. nindii* seedlings was observed every week at the age of 8 to 10 months.

The research used quantitative descriptive method with namely : without application *Rhizoctonia* mycorrhiza (Mo), and with application *Rhizoctonia* mycorrhiza (M1). Each factor was repeated six times and each replication contained ten plants. The parameters observed were plant height, leaf length, number of leaves, root length, number of roots, plant fresh weight, and root peloton observations.

RESULTS AND DISCUSSION

Identification of *Rhizoctonia* in this research is very necessary, because the *Rhizoctonia* genus consists of 3 groups, namely mononucleate which is epiphytic in plants, binucleate which is mycorrhizal in orchids, and multinucleate which is pathogenic in plants such as *Rhizoctonia solani* (Oyetunde and Bradley, 2017). So the inoculated *Rhizoctonia* isolate really belongs to the binucleate group which is mycorrhiza in orchids (orchid mycorrhiza). After being subcultured into new PDA media, in the first week the results showed that the mycorrhizal *Rhizoctonia* isolated from the *D. lasiantera* orchid was white (Figure 1). However, in general, Rhizoctonia mycorrhizal isolates have different colors (Sneh et al., 2004). The color differences in *Rhizoctonia* are in accordance with Carling's opinion in Soelistijono et al. (2017), that of 26 isolates of *Rhizoctonia* spp. collected from *Pterostylis acuminata* orchid plants, 20 isolates were dark brown while the other 6 isolates were light brown.

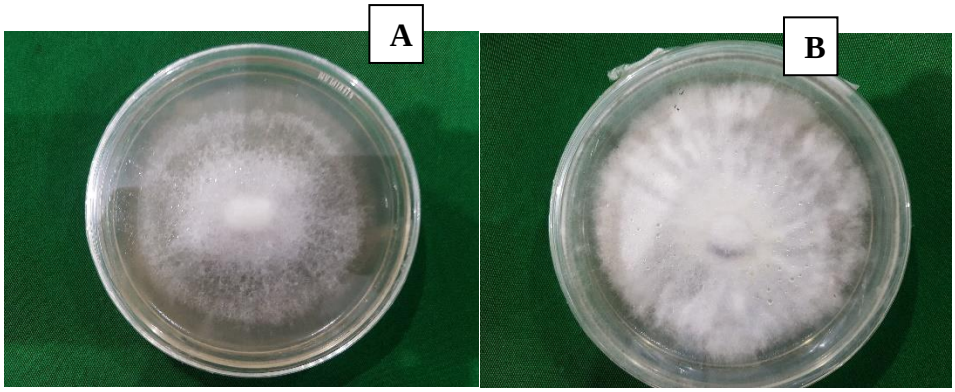

Figure 1. Growth and development of *Rhizoctonia* mycorrhiza colonies isolates from *Dendrobium lasianthera* on Potato Dextrose Agar media
Description : Development observation of *Rhizoctonia* mycorrhiza colonies on the 6rd day (A), and 9th day (B)

Previous research conducted by Agustini et al. (2009) stated that at the Cycloops Jayapura botanical garden, 10 orchid mycorrhiza were isolated from orchid plants with colony colors varying from white to black. This is also in accordance with the opinion of Dwiyanto et al. (2017) who stated that *Rhizoctonia* mycorrhiza colonies differ depending on each group. The rapid growth rate of *Rhizoctonia* mycorrhiza is expected to accelerate the formation of mycorrhizal associations with orchid seeds and the formation of peloton structures in the root cortex. To determine that *Rhizoctonia* sp. isolated from the roots of *D. lasianthera* is mycorrhizal, the core must be observed to prove that the isolate has two cell nuclei in its hyphae, which is a characteristic of mycorrhizal orchids (Sneh et al., 2004). Microscopic observation and identification of *Rhizoctonia* mycorrhiza to identify the branching form and number of cell nuclei can be seen in Figure 2.

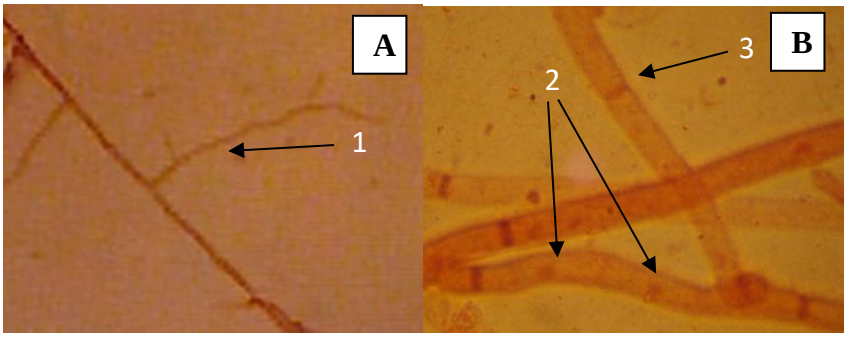

Figure 2. (A). *Rhizoctonia* mycorrhiza hyphae have angular branches at a magnification of 90 times (1), (B). Cell nucleus in *Rhizoctonia* mycorrhiza hyphae (2) and septa hyphae (3).

In Figure 2, it can be seen that the *Rhizoctonia* mycorrhiza hyphae appear reddish brown in color and had branches that form right angles when observed at 40 times magnification. Based on the results of observations carried out microscopically, it was obtained that the developing *Rhizoctonia* mycorrhiza colonies were clustered in the center and over time spread to the edges. In *Rhizoctonia* mycorrhiza isolates there were hyphae that were angular in shape with branches and are brownish in color. This is in accordance with what was stated by Soelistijono et al. (2020) that the branching hyphae of *Rhizoctonia* mycorrhiza are brownish in color and have a right-angled shape. In general, the characteristics of *Rhizoctonia* mycorrhiza are that it has two cell nuclei, brownish hyphae and there are perpendicular branches on the fungal hyphae. The same thing was also stated by Kasiamdari (2000), that hyphae with 2 cell nuclei are also another characteristic of *Rhizoctonia* sp., this can be seen in Figure 2B above. To determine the vegetative growth of *D. nindii* inoculated or not inoculated with *Rhizoctonia* mycorrhiza, it is necessary to observe morphology.

The results of observing the morphology of *D. nindii* seedlings that had been inoculated with *Rhizoctonia* mycorrhiza and those that had not yet can be seen in Figure 3 below.

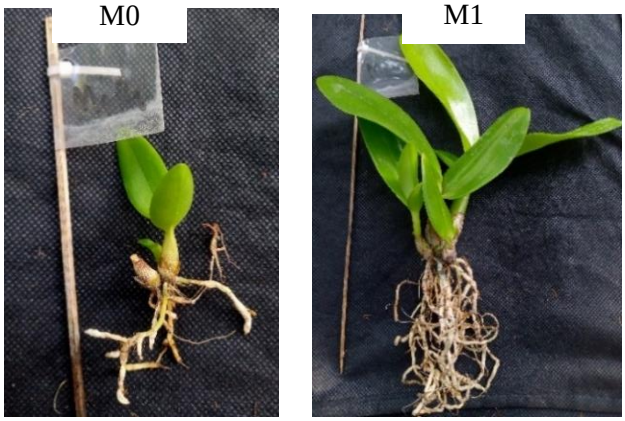

Figure 3. Comparison of the morphological appearance of plants without the application of *Rhizoctonia* mycorrhiza (Mo) and with the application of *Rhizoctonia* mycorrhiza (M1).

From Figure 3, it can be seen that D. *nindii* seedlings that had been inoculated with *Rhizoctonia* mycorrhiza (M1) had greener leaf color compared to those

treated without *Rhizoctonia* mycorrhiza (M0). This happened because inoculation of *Rhizoctonia* mycorrhiza on plants would cause better growth because they obtained nutrients that are assisted by mycorrhiza and could be utilized by plants so that the vegetative growth of *D. nindii* seedlings became better. Sufficient nutrients will influence photosynthesis in plants so that the photosynthesis obtained will increase and be used by plants in the formation of leaves and the number of leaves. According to Abdelghany (2016), the number of leaves is highly positively correlated with plant vegetative growth and development. Based on observations of root morphology, orchid seedlings treated with inoculation with *Rhizoctonia* mycorrhiza (M1) had longer roots and a large number of roots compared to plants without inoculation with *Rhizoctonia* mycorrhiza treatment (M0). This is in accordance with the opinion of Bierman and Linderman (1983), that roots treated with mycorrhiza have a higher auxin content than those not treated with mycorrhiza. From this opinion it can be concluded that *Rhizoctonia* mycorrhiza can produce hormones such as auxin which play a role in plant root growth. According to Bierman and Linderman (1983), mycorrhiza can play a role in plant rooting and can help produce the hormone auxin which also plays a role in elongating root cells. Observation of the roots shows that all the roots of the plant are white for mature roots, and for old roots they are light brown and young roots are greenish.

The use of mycorrhizal fungi is needed to change the heterotrophic pattern in tissue culture techniques to become autotrophic in the field. In several adult orchid species, an association was found between the orchid and its mycorrhiza (orchid mycorrhiza). Research by Calepo & Duffy (2023) proves that several orchid mycorrhizal taxa are often found associated with adult orchids, and this contributes to germination and early development of orchids.

Table 1. Effect of *Rhizoctonia* mycorrhiza application on the growth of *Dendrobium nindii* seedlings

Application	**Parameters**					
	Plant heigh (cm	Length leaf (cm)	Number of Leaf (sheet)	Number of root (sheet	Length root (cm)	Weight of plants (g)
Without aplication of *Rhizoctonia* michorrhizae (M0)	3,23	4,03	2,53	7,27	9,11	2,71
With aplication of *Rhizoctonia* michorrhizae (M1)	3,84	4,23	4,53	10,47	9,18	4,33

The results of applying *Rhizoctonia* mycorrhiza gave different responses, this can be seen in the parameters of plant morphology observations carried out in the final week of the study by looking at plant height, leaf length, number of leaves,

number of roots, root length and plant wet weight which can be seen in the table. 1.

From Table 1, it can be seen that the growth of *D. nindii* treated with *Rhizoctonia* mycorrhiza inoculation was better compared to plants that were not given *Rhizoctonia* mycorrhiza. This can be seen clearly in the differences in plant height, number of leaves, number of roots, and seed weight. This happened because *Rhizoctonia* mycorrhiza was able to help plants obtain the nutrients that orchids really need and could increase the vegetative growth of *D. nindii*. This is in accordance with research by Hossain et al. (2013) that mycorrhizal fungi isolated from the roots of the epiphytic orchids *Rhynchostylis retusa* and *Aerides multiflorum* were inoculated on *Cymbidium aloifolium* and *Cymbidium giganteum* seedlings grown in vitro. These two mycorrhizal fungi show similarities to the *Rhizoctonia* fungus. According to Senthilkumar et al. (2001), *Rhizoctonia* mycorrhiza are able to associate with orchids and form peloton structures. Therefore, observations of peloton formation need to be carried out to determine whether there is an association of *D. nindii* with *Rhizoctonia* mycorrhiza.

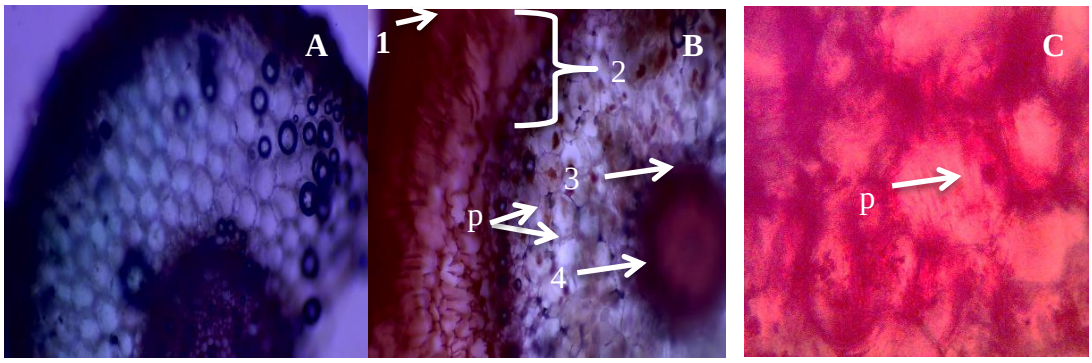

Figure 4. (A): Microscopic of cross-sectional roots with peloton in the treatment without application of *Rhizoctonia* mychorrhiza (with 10X magnification)
(B): Microscopic of cross-sectional roots with peloton in the application of *Rhizoctonia* mychorrhiza treatment (with 10X magnification)
(C) Microscopic of root cross section with peloton (with 40X magnification)
Description: 1) Root epiderm tissue, (2) Root cortex, (3) Central cylinder, (4) Root endoderm, (p) Peloton structure

From Figure 4 above, it can be seen that on the roots of plants treated with the application of *Rhizoctonia* michorrhizae there are coils of hyphae that are able to enter the root tissue called peloton. Smith & Read (2008) stated that the formation of peloton, namely hyphae of *Rhizoctonia* mycorrhiza which penetrate and infect plant roots and form dense clumps in the cortex of plant roots, shows that there is an association between *Rhizoctonia* mycorrhiza and orchids. The same thing was expressed by Muthukumar et al. (2013) that the root cortical cells of *Disperis neilgherrensi* contain fungal structures typical of orchid mycorrhiza (OM) and arbuscular mycorrhiza types. In contrast, the root tip only contains the structure of the orchid mycorrhiza fungus. Colonization of orchid mycorrhiza is characterized by a peloton of light and dark coloration with

regularly septated hyphae with varying diameters.

According to George et al. (2008), there are several stages in the formation of fungal associations with orchid plants, the first stage is the process of introducing the *Rhizoctonia* mycorrhiza to plant roots, stems or protochromes. After that, adhesion or attachment will occur, the hyphae attach themselves to the roots, forming a special structure on the root surface called a peloton. Then an infection process occurs in the plant cortex. *Rhizoctonia* mycorrhiza infects the roots and forms a network of hyphae in the root cortex tissue first to increase nutrient absorption in orchid plants (Iskandar 2001 cit. Hatni 2017). Ogura et al. (2021) suggests that *Rhizoctonia* mycorrhiza can increase water and nutrient uptake expressed by that endophytic fungi found in the Orchidaceae group function as saprophytic fungi (SAP) which provide the nutrient requirements for orchid seeds resulting from tissue culture (in vitro) which are still in heterotrophic conditions during the acclimatization process in the field.

Peloton plays a role in providing the nutrients that orchids really need during the seedling period, especially when they experience drought stress due to lack of water sources. The peloton structure also replaces the lack of nitrogen nutrients in the planting medium which are easily absorbed by plants with the help of *Rhizoctonia* mycorrhiza. Zimmer et al., (2007) stated that nitrogen nutrients can increase plant growth, plants whose nitrogen needs are met will be greener. Therefore, the presence of a peloton structure is important in increasing the vegetative growth of *D. nindii.*

CONCLUSION

The results showed that inoculation with *Rhizoctonia* mycorrhiza increased vegetative growth of *D. nindii* which was seen in plant height, number and length of leaves, number and length, and fresh weight of plants compared to plants without inoculation with *Rhizoctonia* mycorrhiza.

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