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Utilization of *Rhizoctonia* Mycorrhiza in the Management of *Fusarium* Sp. Seedling Orchid *Dendrobium nindii*

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Abstrac *endrobium* is one of the largest orchid genera in the family *Orchidaceae*, and ~~includes~~ consists of more than 2,000 species. In nurseries, this orchid is easily attacked by the fungus *Fusarium* sp. ~~Countermeasures using fungicides will damage the environment and will also inhibit the growth of mycorrhizal fungi that are useful for plants.~~ *Rhizoctonia* mycorrhiza is a mycorrhizal fungus that is ~~able to~~prevalently ~~-~~associated with orchids. At In vitro this fungus ~~is able to~~ provides induced resistance ~~to~~ of orchids against pathogenic fungal attacks. The research was conducted in a laboratory and a greenhouse from August to June 202[illegible]. The research method used a Completely Randomized Block Design (CRBD) consisting of 2 treatment factors and 5 replications. The first factor is the application of *Rhizoctonia* mycorrhizae (M1), and the second factor is the origin of the *Fusarium* inoculum from garlic (F1), ~~from~~ potatoes (F2), and ~~from~~ chili (F3). ~~T~~the highest M1F1 6.62 cm, the number of leaves with the highest value M1F1 6.00 cm, the number of roots with the highest value M1F1 20.20 cm, root length with the highest value of 11.12 cm in M1F1, plant fresh weight with the highest value in M1F3 namely 9.85 cm. ~~And t~~The application of *Rhizoctonia* mycorrhiza had a significant effect on l[illegible]length with the highest value of 4.40 cm in the M1F1 treatment. The application of *Fusarium* sp also ~~had~~ significant effect on the number of leaves with the highest value of 6.00 cm on the M1F1 requirement, and the fresh weight of the plant with the highest value on the M1F3 [illegible]tment of 9.85 cm. The treatment interaction between *Rhyzoctonia* mycorrhizae very real different ~~had~~ significant effect on the number of roots with the highest value of 20.20 cm. This study aimed to use *Rhizoctonia* mycorrhizae in controlling *Fusarium* sp. attack on sedling *Dendrobium nindii* in experimental garden. ~~in the treatment~~

Key words : *Rhizoctonia* mycorrhiza, induced resistance, *Fusarium* sp, *Dendrobium nindii*

1. Introduction

endrobium is one of the largest orchid genera in the family Orchidaceae, and includes more than 2,000 species [1]. *Dendrobium* is one of Indonesia's natural resources, and the number is estimated at 275 species [2]. The best *Dendrobium* orchid species are mostly found in eastern Indonesia, such as Papua and Maluku. *Fusarium* sp. is a fungus that is capable of infecting various plants (polyphagous fungus) and commonly attacks orchids [3]. In orchids, *Fusarium* sp. will cause leaf blight symptoms. ~~Symptoms include y~~Yellowing, wrinkled, thin, and bent leaves and stems, rotting leaf necks reaching the base of the stem are other typical symptoms of infected orchids by *Fusarium*. In general, *Fusarium* sp. causes plants to rot and eventually die. Biological

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control by inoculation of various biological agents in plants will lead to increased resistance to subsequent inoculation by the main pathogen known as the induced resistance mechanism –[4]. This control measure can be tested on orchid seedlings that is attacked by *Fusarium* sp.

Orchids are known to have associations with fungi that act as mycorrhizae and are known as mycorrhizal orchids [5]. Research on the genus of *Pterostylis* orchids from Australia found 72 types of mycorrhizae associated with roots and 20 of them were *Rhizoctonia* sp. with a different Anostomosis Group (AG). Some *Rhizoctonia* sp. which functions as mycorrhizae in orchids is *Tulasnella* sp. [6]. Mycorrhizal associations in *Dendrobium* orchids ~~are interesting to study because they can~~ have a positive effect on nutrient absorption and host growth [7]. Mycorrhizal isolates taken from the roots of *Phalaenopsis lowii* Rchb.F. which grows in its natural habitat is thought to have the advantage of better compatibility than using mycorrhizal species from other plant species [8].

2. Methods

This research was carried out at the Green House, Faculty of Agriculture, Tunas Pembangunan University, in Surakarta, Central Java Province, ~~with~~ at an altitude of 105 meters above sea level. The implementation of this research began in November 2021 until July 2022. The materials used were: seedling *Dendrobium nindii*, *Rhizoctonia* mycorrhiza, and *Fusarium* sp. *Rhizoctonia* mycorrhiza was isolated from the roots of the orchid *Dendrobium Lasianthera* in the tissue culture laboratory of the Faculty of Agriculture, UTP Surakarta. *Rhizoctonia* mycorrhiza was ~~then~~ cultured in Potato Dextrose Agar (PDA) ~~media~~medium. After 5-6 days of incubation, the growth of white hyphae that formed a circle around the piece of root segments~~s~~ was seen [9].

Isolation of *Fusarium* sp. carried out from wilted garlic, potato, and chili leaves that were infected with *Fusarium* sp. The isolates obtained were ~~then~~ cultured in PDA ~~media~~ medium until the fungal mycelium filled Petri dish. Hyphae change color in the center of the colony from white to purple or pink. Furthermore, the isolates were propagated on PDA and incubated for 1 week. *Fusarium* isolates aged 1 week were taken from PDA, cut into 1 cm^2 and immersed in 100 ml of Potato Dextrose (PD/only contains potato extract and aquades) ~~media~~medium. *D. nindii* 7 months old was an application with 1 week old *Rhizoctonia* mycorrhiza on the roots [7], then at 12 ~~months~~ weeks *Fusarium* sp. was inoculated with PD media which was sprayed on the leaves. *D. nindii* as a control ~~is enough to be~~was sprayed with distilled water. The volume of liquid of PD ~~media~~ medium that was sprayed was 1 ~~cc~~ ml per plant.

The study used the CRBD research method (Completely Randomized Block Design) with two treatment factors, namely: M0: Without *Rhizoctonia* mycorrhizal application, M1: With *Rhizoctonia* mycorrhizal application, and the origin of the inoculum (F) consisted of three levels, namely: F1: Inoculum *Fusarium* sp. from onions white; F2 : Inoculum *Fusarium* sp. from potato; F3 : Inoculum *Fusarium* sp. from chili. ~~s~~So there were ~~that~~ 6 treatment combinations ~~were~~ obtained. The parameters observed were the percentage of plant growth, plant height, leaf length, number of leaves, number of roots, root length, plant fresh weight, and root peloton observations. Observational data from each parameter in each observation were analyzed with 5% and 1% ANOVA tests. If there are calculations that are significantly different or very significantly different, then proceed with DMRT (Duncan Multiple Range Test) with a level of 5% to find out any differences between treatments.

3. Results and Discusion

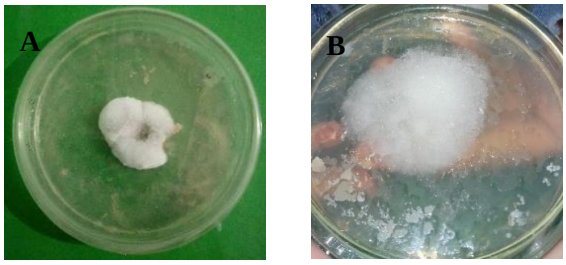

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FIGURE 1. Growth and development of *Rhizoctonia* mycorrhiza colonies on PDA ~~media~~ medium
Description: A. *Rhizoctonia* mycorrhiza 1 week old, B. *Rhizoctonia* mycorrhiza 2 weeks old.

The results of the isolation of *Rhizoctonia* mycorrhiza from the roots of *D. lineale* showed that there was a small white clump structure in the middle of petri plate, which meant that *Rhizoctonia* mycorrhiza was growing and forming colonies (Fig.1). This is ~~in accordance with~~following the results of previous research, ~~by Sari [9]~~ that *Rhizoctonia* mycorrhiza associated with orchids has a morphological characteristic of a white colony, in the middle of the colony a clump is formed with very slow growth [10~~9~~]. Microscopic observations of *Rhizoctonia* mycorrhizae showed that hyphae had branches that formed right angles which is one of the characteristics of *Rhizoctonia* mycorrhiza (Figure 2A).~~,~~ T~~t~~his ~~is~~finding is ~~in accordance with~~following ~~[4]~~another study ~~that who stated~~ that the branching of *Rhizoctonia* mycorrhizae formed a right angle [4]. ~~(Fig. 2A).~~ *Rhizoctonia* mycorrhiza has a common characteristic that is binucleate (Fig. 2B). This is what distinguishes the group of *Rhizoctonia* which are pathogenic such as *Rhizoctonia* solani which has ~~more than 2 nuclei (~~multinucleate (more than 2 nucleic acids) [11~~0~~]. *Rhizoctonia* sp. binucleic acid was also previously found in vanilla roots [12~~1~~].

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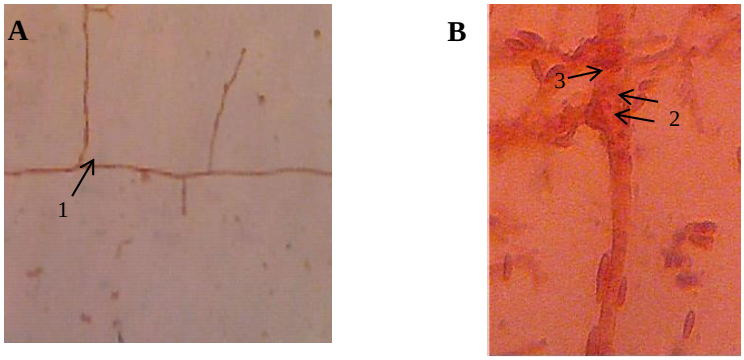

FIGURE 2. (A). *Rhizoctonia* mychorrhizal hyphae with right-angled branched (B) *Rhizoctonia* mycorrhizal hyphae 2
Description: (1) A typical ~~A~~angle ~~angle~~ on hyphae branching (→), (2) Cell nucleus (→), (3) Insulation between cells in hyphae (→)

Isolation of Fusarium sp. of various ingredients (garlic, potato, and chili) showed almost the same results (Fig. 3). Based on the above observations, it was shown that various *Fusarium* sp. colonies were white like cotton and purple or pink in the center of the colony.

FIGURE 3. Growth and development of *Fusarium* sp colonies on PDA ~~.~~ medium~~a~~
Description: a. Isolate of *Fusarium* sp. from Garlic, b. Isolate of *Fusarium* sp. from Potato, c. Isolate of *Fusarium* sp. from Chili

This is in accordance with the opinion ~~of Booth, C.~~ [13~~2~~] that *Fusarium* sp. have colonies that are white or accompanied by purple to pink in each colony. ~~In addition, t~~These fungal colonies

will produce different colors on isolates with the same growing media . ~~This is~~ because the fungus *Fusarium* sp. easily change the color of the colony so it cannot be used as an identification parameter [1~~3~~]. ~~For example, *Fusarium solani* is dominated by white colonies, *Fusarium verticillioides* is pink, and according to Booth, C. [12] *Fusarium oxysporum* is reddish reddish white in the middle and color variations in the medium can be caused by conditions and the culture medium used.~~ The results of observations of *Fusarium* isolates isolated from garlic, potatoes, and chilies were classified as microconidium (Figure 4).

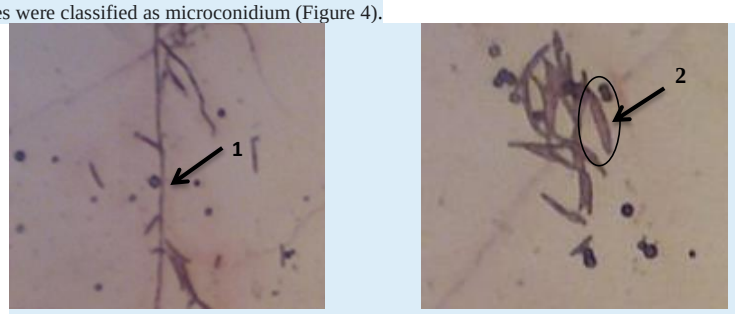

FIGURE 4. The morphology of the fungus *Fusarium* sp. which is identified by Microscopic
Description : 1. Hiphae, 2. Microconidia

The results showed that the microconidia were long, crescent-shaped with long and blunt conidiophores, 1-5 septa, single monophyalid and abundant in number. Microconidia are formed in clusters at the tips of conidiophores and are pathogenic to plants. This is in accordance with ~~Agrios~~ [4] that the genus *Fusarium* is a fungus that has ~~insulated hyphae, and produces~~ asexual spores in the form of microconidium and macroconidium. Observations of plant morphology were carried out at the end of the study, namely observing plants from roots to leaves in each treatment (Figure 8).

FIGURE 5A. Sighting of *D. nindii* without *Rhizoctonia* mycorrhiza
Information ; (M0F1): *Fusarium* sp. from garlic, (M)F2): *Fusarium* sp. from Potato, (M0F3): *Fusarium* sp. from chili

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FIGURE 5B. Plant Morphological Appearance With *Rhizoctonia* mycorrhiza Application Information ; (F1): *Rhizoctonia* mycorrhiza + *Fusarium* sp. from garlic, (F2): *Rhizoctonia* mycorrhiza + *Fusarium* sp from potato, (F3): *Rhizoctonia* mycorrhiza + *Fusarium* sp. from chili

From the picture, it can be seen that the application treatment of *Rhizoctonia* mycorrhiza first and subsequent inoculation with *Fusarium* sp. ~~effect~~ affect ~~on~~ the number of roots and root length. Whereas in the treatment without the application of *Rhizoctonia* mycorrhizae, the plant had poor root growth, ~~this was because~~ *Rhizoctonia* mycorrhiza was very important in the nutrient absorption process, ~~Zimmer et al.,~~ [154]. The association of Rhizoctonia mycorrhiza with the roots of *D. nindii* was indicated by the presence of a peloton structure (Fig. 6)

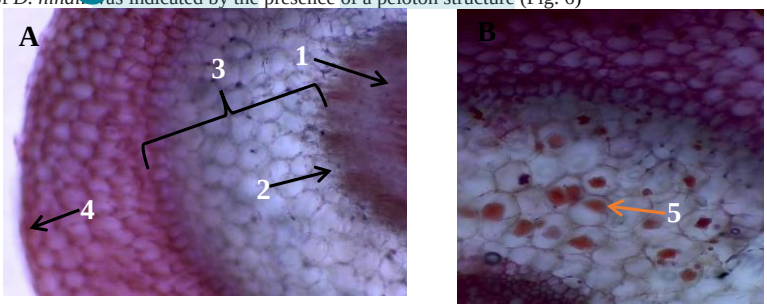

FIGURE 6. (A): Cross section of roots without application of *Rhizoctonia* mycorrhizae (B): Cross section of roots with application of *Rhizoctonia* mycorrhiza.
Description: (1) Endodermal tissue (→), (2) Central cylinder (→) (3) Cortical tissue, (4) Epidermal tissue (→), (5) Peloton (→).

In the cross section of the roots of *D. nindii* orchids that were inoculated with *Rhizoctonia* mycorrhiza first, they showed a red peloton structure in the center or the edge of the cortical cells (Figure 9A). This is ~~in accordance with~~following what was stated ~~by Kasiamdari~~ [165] and ~~Brundrett~~ [5] that the intracellular hyphae of *Rhizoctonia* mycorrhiza that infect orchid roots ~~have the ability to~~can penetrate into the cortical tissue at the root and form dense coagulation coils (peloton). There are several ways to see the association of *Rhizoctonia* mycorrhizae with orchids, namely: (1) looking at the mycorrhizal structure (peloton), (2) symbiotic germination test using established culture protocol (oat agar medium); and (3) molecular confirmation that *Rhizoctonia* mycorrhiza was detected in orchid root tissue [176]. Peloton appears ~~a~~ day after infection, which is about 30-36 hours after initial contact. When the fungus begins to enter the parenchyma cells of the orchid, the plasma membrane inside the cell is formed by a broad surface indentation to facilitate infection and fungal growth. The newly formed plasma membrane immediately surrounds the

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growing peloton and creates a large surface area through which nutrients are exchanged. The surrounding plant membrane becomes rough endoplasmic reticulum and there is evidence from electron microscopy showing exocytosis of this plant membrane [187]. Peloton is usually only found in a limited period when the orchid needs nutrients before undergoing lysis. Infection and lysis occur repeatedly in the interior of the same cells and tissues. The formation of this structure is characteristic of the association of *Rhizoctonia* mycorrhizae and orchids [198]. Mycorrhiza in orchids has a nutrient flow where the fungus gets a direct supply of carbon from plants instead of phosphorus or as a substitute for nitrogen for plants [6]. However, ~~according to Zimmer et al., [14] it is stated that~~ there is a frequent flow of carbon from fungi to plants or from plants to fungi alternately, where this flow involves nitrogen and phosphorus nutrients from fungi moving to plants [154]. There are approximately 200 species of orchids, there is no flow of carbon nutrients from plants to mycorrhizae, but mycorrhizae can supply nutrients to orchid plants. Based on the recapitulation data of variance, mycorrhizal application treatment had a significant effect on leaf length, and had a very significant effect on plant height, number of leaves, number of roots, root length, and plant fresh weight (Table1).

TABLE 1. Summary results of the effect of the application of *Rhizoctonia* mycorrhiza and *Fusarium* sp. inoculation on the growth of *Dendrobium nindii* orchid seedling

Number	Parameter	Rhizoctonia mycorrhizal application (M)	Fusarium Inoculation (F)	Interaction (M x F)	Score Highest	Score Lowest
1	Plant height (cm)	**	Ns	Ns	6,62 (M1F1)	5,68 (M0F3)
2	Leaf lenght (cm)	*	Ns	Ns	4,40 (M1F1)	3,86 (M0F2)
3	Number of leaves (cm)	**	*	Ns	6,00 (M1F1)	4,40(M0F2)
4	Number of roots (cm)	**	Ns	*	20,20 (M1F1)	15,00 (M0F2)
5	Root length (cm)	**	Ns	Ns	11,12 (M0F1)	9,24 (M0F3)
6	Plant fresh weight	**	*	Ns	9,85 (M1F3)	6,18 (M0F2)

Description: ns): No Significant
*) : Significant
**) : Very Significant

The application of *Rhizoctonia* mycorrhiza had a very significant effect on all parameters, and *Fusarium* inoculation had a significant effect on the number of leaves and plant fresh weight. While the interaction between the two ~~affects~~ affected the number of roots. ~~This shows that t~~The application of *Rhizoctonia* mycorrhiza on seedling *D. nindii* ~~which was~~ then followed by ~~infected with~~ *Fusarium* sp. infection affected the number of plant roots. This will have a positive effect on nutrient absorption and host growth. ~~The results of the DMRT (Duncan Multiple Range Test) test with a level of 5% Effect of application of *Rhizoctonia* mycorrhiza and Inoculum *Fusarium* sp. on the growth of *D. nindii* orchid seedling(~~ Table 21).

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TABLE 2 ~~Results of DMRT (Duncan Multiple Range Test) with a level of 5%~~ Effect of application of *Rhizoctonia* mycorrhiza and *Fusarium* sp. inoculation on the growth of *Dendrobium nindii* orchid seedling.

Treatment	Parameter					
	~~Applications~~ Inoculation	Plant height (cm)	Leaf ~~lenght~~ length (cm)	Number of leaves (sheet)	Number of root (sheet)	Leaf lenght (cm)	Plant fresh weight (g)
Rhizoctonia mycorrhizal application (M)							
M0		5,70 a	3,92	4,87 a	15,80 a	8,77	6,78 a
M1		6,23 b	4,21	5,80 b	18,07 b	10,80	8,80 b
	Fusarium sp. (F)						
F1		6,17	4,17	5,80	17,10	10,75	7,85
F2		5,85	3,93	4,9	16,30	8,74	7,08
F3		5,87	4,1	5,3	17,40	9,90	8,44
Combination application of *Rhizoctonia* mycorrhiza and *Fusarium* sp. inoculation (M x F)							
M0F1		5,68	3,94	5,40	15,00 a	10,4	7,13
M0F2		5,70	3,86	4,60	15,20 ab	9,62	6,18
M0F3		5,68	3,ad 96	4,60	17,20 bc	9,24	7,04
M1F1		6,62	4,40	6,20	19,20 c	11,12	8,57
M1F2		6,00	4,00	5,20	17,40 c	10,02	7,98
M1F3		6,06	4,24	6,00	17,60 c	10,22	9,85

Description:
(M0): Without *Rhizoctonia* mycorrhizal, (M1) : Application with *Rhizoctonia* mycorrhizal,
(F1): Inoculation *Fusarium* sp. from garlic, (F2): Inoculation *Fusarium* sp. from potato, (F3): Inoculation *Fusarium* sp. from chili.
(M0F1): Without *Rhizoctonia* mycorrhizal + Inoculation *Fusarium* sp. from garlic
(M0F2): Without *Rhizoctonia* mycorrhizal + Inoculation *Fusarium* sp. from potato
(M0F3): Without *Rhizoctonia* mycorrhizal + Inoculation *Fusarium* sp. from chili
(M1F1): Application with *Rhizoctonia* mycorrhizal + Inoculation *Fusarium* sp. from garlic
(M1F2): Application with *Rhizoctonia* mycorrhizal + Inoculation *Fusarium* sp. from potato
(M1F3): Application with *Rhizoctonia* mycorrhizal + Inoculation *Fusarium* sp. from chili
The treatment followed by the same letter in the same column, the description shows that there is no significant difference in DMRT with a level of 5%.

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~~Based on the results of the 5% DMRT test in the table above, it shows that t~~The parameter of the number of recapitulation results of variance shows that the application treatment of *Rhizoctonia* mycorrhizae and *Fusarium* sp. inoculation gave results that significantly affected the number of roots with the highest value of 19.20 pieces and the lowest value of 15.00 (Table 2). Overall, the application of *Rhizoctonia* mycorrhiza helped in the absorption of nutrients from the soil with a

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significantly increased number of roots when compared to that without the application of *Rhizoctonia* mycorrhiza. According to ~~Zimmer et al. (2007)~~[15] nitrogen supply will make plant pa green because it contains chlorophyll which plays a role in photosynthesis. These elements al so useful for accelerating plant growth in height, increasing the number of tillers, influencing leaf width and length and making them large, and i~~ncreasing~~ncreasing protein and fat levels for plants.

CONCLUSION

Application of *Rhizoctonia* mycorrhiza on seedling of *D. nindii* has an effect on increasing the number of roots, ~~so that they can survive when infected~~and increases its resistance against with *Fusarium* sp. ~~which comes from~~of garlic leaves, potatoes, and chilies ~~in seedling conditions~~.

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16. R. S. Kasiamdari, "Binucleate Rhizoctonia Isolate From Mycorrhizal Pot Cultures: Its Morphological Characteristics And Pathogenicity," *Berk. Ilm. Biol.*, vol. 2, no. 2~~2000~~, ~~2000.~~ ~~2000. [16]~~

~~[15]~~17. K. A. Kristiansen, D. L. Taylor, R. Kjøller, H. N. Rasmussen, and S. Rosendahl, ~~"Identification of mycorrhizal fungi from single pelotons of *Dactylorhiza majalis* (Orchidaceae) using single strand conformation polymorphism and mitochondrial ribosomal large subunit DNA sequences,"~~ *Mol. Ecol.*, vol. 10, no. 8, pp. 2089–2093, 2001.

~~[17]~~18. J. D. W. Dearnaley, ~~"Further advances in orchid mycorrhizal research,"~~ *Mycorrhiza*, vol. 17, no. 6, pp. 475–486, 2007.

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