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APPLICATION OF *Rhizoctonia* Mycorrhiza ON *Dendrobium nindii* SEEDLINGS: THE RESISTANCE STUDY AGAINST *Rhizopus* sp.

APLIKASI *Rhizoctonia* MIKORIZA PADA BIBIT *Dendrobium nindii* : SEBUAH STUDI KETAHANAN TERHADAP *Rhizopus* sp.

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ABSTRAK

Dendrobium merupakan salah satu genus anggrek yang memiliki daya tarik paling banyak di masyarakat diantara jenis anggrek lainnya, salah satunya jenis *Dendrobium nindii*. Kendala terbaru dalam budidaya anggrek adalah serangan yang disebabkan patogen baru *Rhizopus* sp. Penelitian ini bertujuan untuk mengetahui ketahanan *seedling D. nindii* terhadap serangan *Rhizopus* sp. dengan teknik induksi ketahanan menggunakan *Rhizoctonia* sp. Penelitian dilakukan di Greenhouse Fakultas Pertanian Unversitas Tunas Pembangunan dari Januari sampai Juli 2023. Metode penelitian menggunakan Rancangan Acak Kelompok Lengkap (RAKL) terdiri dari 2 faktor perlakuan dan 5 ulangan. Faktor pertama yaitu aplikasi *Rhizoctonia* mikoriza(M1) dan tanpa aplikasi *Rhizoctonia* mikoriza(M0), Faktor kedua yaitu Inokulum *Rizhopus* sp dari roti tawar(R1), Inokulum *Rhizopus* sp. dari papaya busuk(R2), Inokulum *Rhizopus* sp. dari tempe busuk(R3). Hasil penelitian menunjukkan (1) Ciri morfologi *Rhizopus* sp. berupa koloni berwarna putih, stolon halus berwarna putih, sporangiospora bewarna hitam tumbuh dari stolon dan mengarah ke udara. (2) Aplikasi *Rhizoctonia* mikoriza berpengaruh sangat nyata pada ketahanan *D. nindii* yang ditunjukan pada parameter tinggi tanaman, panjang daun, jumlah daun, panjang akar, jumlah akar dan berat segar *seedling*. (3) Sedangkan pplikasi *Rhizopus* sp. berpengaruh nyata terhadap tinggi tanaman, panjang daun, jumlah akar. Sedangkan (4) interaksi perlakuan tidak berpengaruh nyata terhadap semua parameter.

Kata kunci : *Dendrobium nindii*, induksi ketahanan, *Rhizoctonia* Mikoriza, *Rhizopus sp*.

ABSTRACT

Dendrobium is a genus of orchid that most attractive to public among other orchids types, one is the *Dendrobium nindii*. The new problems orchid cultivation is attacks caused by the new pathogen *Rhizopus* sp. This research aims to determine resistance of *D. nindii* seedlings to attack by *Rhizopus* sp. with resistance induction techniques using *Rhizoctonia* mycorrhizae. The research was conducted at Tunas Pembangunan University Greenhouse from Januari to June 2023. The research method used a Complete Randomized Block Design (CRBD) consisting of 2treatment factors and 5replications. The first factor is *Rhizoctonia* mycorrhizae application(M1) and without *Rhizoctonia* mycorrhizae application(M0), the second factor is *Rizhopus* sp. inoculum from white bread(R1), rotten papaya(R2), rotten tempeh(R3). The results showed (1)Morphological characteristics of *Rhizopus* sp. Is white colonies, smooth white stolons, black sporangiospores grow stolons and point into the air. (2)The application of *Rhizoctonia* mycorrhizae has very significant effect on the resistance of *D. nindii* as shown in plant height, leaf length, number of leaves, root length, number of roots and fresh weight of seedlings parameters. (3)Meanwhile, the application of *Rhizopus* sp. has a significant effect on plant height, leaf length, number of roots. Meanwhile (4)treatment interactions have no significant effect on all parameters.

Key words: *Dendrobium nindii*, induction resistance, *Rhizoctonia* Mycorrhizae, *Rhizopus* sp.

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 this genus of orchids has high adaptation to its environment. Apart from adaptability, other advantages of this genus are that it has various types and colors, is long-lasting and does not fall off easily, and is easy to use in packaging cut flowers. Therefore, this genus is very interesting and is in great demand by many consumers, making this type of orchid one of the most popular orchid-loving consumers (Tuhuteru *et al*., 2012). One of the favorite Dendrobium orchids is *Dendrobium nindii*.

The *Dendrobium nindii* orchid, known as the blue antler orchid, is an epiphytic orchid species originating from northern to eastern Papua New Guinea. It has purplish white flowers with a purple labellum with strong false stems, usually dark in color, can reach a length of 2 m and a diameter of 4 cm and leaves that are oval in shape with a length of 5 to 15 cm (Puccio, 2018). The growth of nindii orchid plants is very slow, so special care is needed to increase the growth of nindii orchids. Using appropriate planting media and utilizing soil microbes such as *Rhizoctonia* mycorrhizae are efforts that can be made to increase the growth and flowering of orchids (Herliana *et a*l., 2018).

Rhizopus is a warehouse fungus or fungus in storage places so its distribution is very wide in nature. The spores are black, stalked sporagiophores grow upwards and contain hundreds of spores and are usually separated from the hyphae by a wall and partition. The shape of the spores is globose to oval, the conidia are black and measure 5-15µm. *Rhizopus* sp. can cause soft rot disease in plants. Plants attacked by *Rhizopus* sp. are characterized by the presence of white spots that gradually turn gray on the thallus, plant growth becomes slow, and the thallus on some branches rots (Agrios, 2023). In this research *Rhizopus* sp. those tested as pathogenic fungi were isolated from various sources as initial inoculum which infected *D. nindii* seedlings.

One way to deal with fungal diseases in orchids is to use the resistance induction method (Hatni 2017). Induction of resistance is one method used by providing induction in the form of chemicals to plants, so that later if a pathogen infects them by releasing chemical compounds, the plant will provide an immune response to these chemical compounds (Putri *et al*., 2022). However, the method is not environmentally friendly, so other methods are used that utilize non-pathogenic microorganisms to induce resistance. Previous research by Soelistijono, (2015) showed that the use of *Rhizoctonia* mycorrhizae was effective in curbing the rate of infection by *Fusarium* sp. However, this has never been done on *Rhizopus* sp. which is a new pathogen in orchids (Harris *et al*., 2023).

In nature, association of *Rhizoctonia* mycorrhizae with orchid roots that occurs when seeds begin to germinate to form roots and shoots (protocorm) (Balesrini et al. 2014). *Rhizoctonia* mycorrhizae found in orchid roots is a fungus that lives naturally and is in symbiosis with plant roots, which helps meet the needs of orchids for plant nutrients. Mycorrhizal fungi are beneficial for plants because they can increase the ability of plants to absorb water (Sari D.N. 2018), improve chemical, physical, and biological properties of soil, because external hyphae of mycorrhizal fungi are able to penetrate soil pore spaces, both micro and macros. The presence of external hyphae and roots is very important because they are able to absorb and store soil moisture. The aim of this research was to assess the resistance of *D. nindii* seedlings after being given *Rhizoctonia* mycorrhiza to *Rhizopus* sp. fungus which is a new pathogen of orchids (Harris et al., 2023).

MATERIALS AND METHODS

The research was carried out from January to July 2023. *Rhizoctonia* mychorrizae taken from *Dendrobium lasianthera* collection with Hossain (2022) methods in the tissue culture laboratory of Agriculture Faculty Universitas Tunas Pembangunan (UTP) Surakarta. The seedling *D. nindii* to be inoculated was obtained from tissue culture laboratory with George *et al*. (2008) methods and inoculation was carried out in the experimental garden of UTP.

Rhizoctonia mycorrhizae isolates were grown on Potato Dextrose Agar (PDA) media and incubated for 9 days and identification of the colony form and hyphal structure was carried out. After 9 days, five grams of *Rhizoctonia* mycorrhizae culture were mixed with 100ml of sterile water. Seedling of *D. Nindii* aged 8 months was placed in the pot containing moss. Each *D. nindii* seedling was sprayed with 1ml of *Rhizoctonia* mycorrhizae inoculum and was acclimatized in green house for 2 months. After 8 months, the seedling roots of *D. nindii* were cut and examined under a microscope to see the *Rhizoctonia* mycorrhizae associations in the form of *peloton* structure. Isolation of Rhizopus sp. carried out in the Microbiology laboratory of the Agriculture Faculty, UTP. The parts that were isolated were moldy white bread, rotten papaya and tempeh. Next, it is cultured in Potato Dextrose Agar (PDA) media which gives different colony shapes and colors. *D. nindii* seedlings were 8 months old, inoculated with *Rhizopus* sp. from various ingredients such as white bread, papaya, and rotten tempeh. The growth of *D. nindii* seedling was observed every week from 8 to 10 months of age.

The study used Complete Randomized Block Design (CRBD) research method with two factorial treatments, namely: M_0: without *Rhizoctonia* mycorrhizae application, M_1: with *Rhizoctonia* mycorrhizae application, and second factor was inoculated *Rhizopus* sp. from white bread (R1), rotten papaya (R2), rotten tempeh (R3). Each treatment was repeated five times. Observational data from each parameter in each observation were analyzed with 5% and 1% Analysis of Variance (ANOVA) tests. If there was significantly different or very significantly different calculation, then proceed with Duncan Multiple Range Test (DMRT) with a level of 5% to find out any differences between treatments. The parameter observed were plants 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 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). After subculture to new PDA media, in the first week the results showed that *Rhizoctonia* mycorrhizae which developed from the *D. lasiantera* orchid had a white color (Figure 1). However, in general, mycorrhizal *Rhizoctonia* isolates have different colors (Sneh *et al*., 2004). This is in accordance with Carling in Soelistijono *et al.* (2011), that of the 26 isolates of *Rhizoctonia solani* (part of *Rhizoctonia* spesies) collected from *Pterostylis acuminata* orchid plants, 20 isolates were dark brown while the other 6 isolates were light brown.

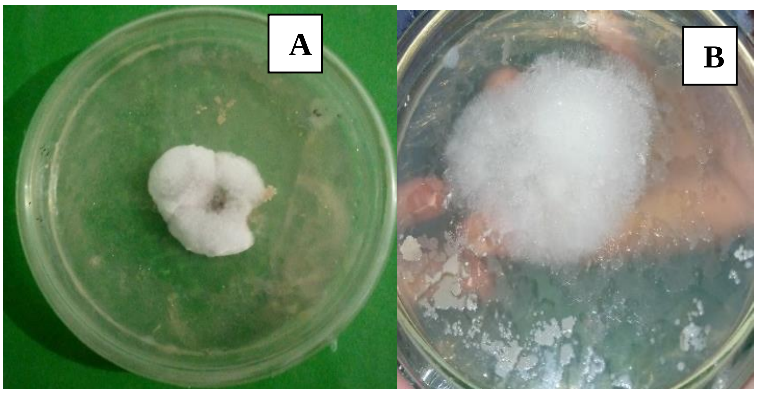

Figure 1. Growth and development of *Rhizoctonia* mycorrhizae colonies isolates from *Dendrobium lasianthera* on Potato Dextrose Agar media
Description : Development observation of *Rhizoctonia* mycorrhizae colonies on the 3rd day (A), and 9th day (B)

According to Agustini *et al*. (2009) stated that in the Cycloops Jayapura botanical garden, 10 orchid mycorrhizal isolated from orchids were obtained whose colony colors varied from white to black. This is also in accordance with the opinion of Dwiyanto *et al.* (2017) who stated that the colonies of *Rhizoctonia* mycorrhizae different depending on each group. The rapid growth rate of *Rhizoctonia* mycorrhizae is expected to accelerate formation of mycorrhizal associations with orchid seedlings and formation of peloton structures in the root cortex. To determine that the *Rhizoctonia* 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 (Sneh *et al*., 2004). Microscopic identification of *Rhizoctonia* mycorrhizae to identify branching shape and number of cell nuclei can be seen in Figure 2.

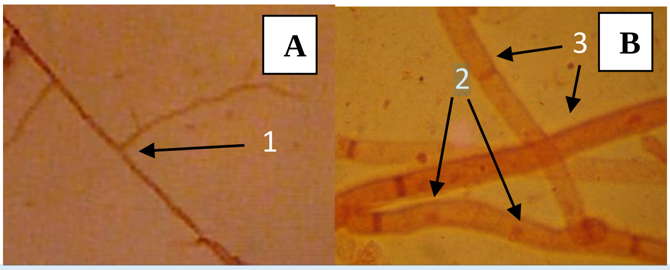

Figure 2. (A). *Rhizoctonia* mycorrhizae hyphae have angular branches at a magnification of 90 times (1), (B). Cell nucleus in *Rhizoctonia* mycorrhizae hyphae (2) and septa hyphae (3).

In Figure 2, the hyphae of *Rhizoctonia* mycorrhizae appear reddish brown in color and have branches that form right angles when observed at 40 times magnification. Based on the results of observations carried out microscopically, the results showed that the developing *Rhizoctonia* mycorrhizal colonies were clumped in the middle and spread to the edges over time. In the isolate there are hyphae that are angular in shape at the branches and are brownish in color. This is in accordance with what has been stated by Soelistijono *et al.* (2020) that the branching hyphae of *Rhizoctonia* mycorrhizae have a brownish color and are angular in shape. The characteristics of *Rhizoctonia* mycorrhizae are that it has two cell nuclei, brownish hyphae and the presence of right-angled branches in the fungal hyphae. Apart from that, hyphae with 2 cell nuclei are also another characteristic of *Rhizoctonia* sp.

(Kasiamdari. 2000). This can be seen in Figure 3 below.

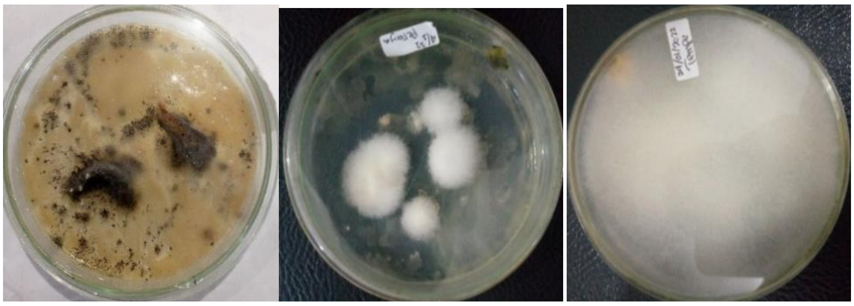

Figure 3: *Rhizopus* sp. colonies on PDA (Potato Dextrose Agar) media
Description: (A) Isolate from white bread, (B) Isolate from rotten papaya, (C) Isolate from rotten tempeh.

The results of observations in Figure 3 show that colony development is different. The fastest colony development was seen in isolates from white bread which showed zygospores, while the slowest was seen in rotten papaya isolates. The color of the colonies was also different, isolates from white bread colonies were brownish while isolates from temped and rotten papaya were white. This is in accordance with the opinion of Agrios (2023) that the characteristics of *Rhizopus* sp. are that the white colonies gradually become gray. To confirm the morphology of the hypha cells, microscopic observations were carried out which can be seen in Figure 4.

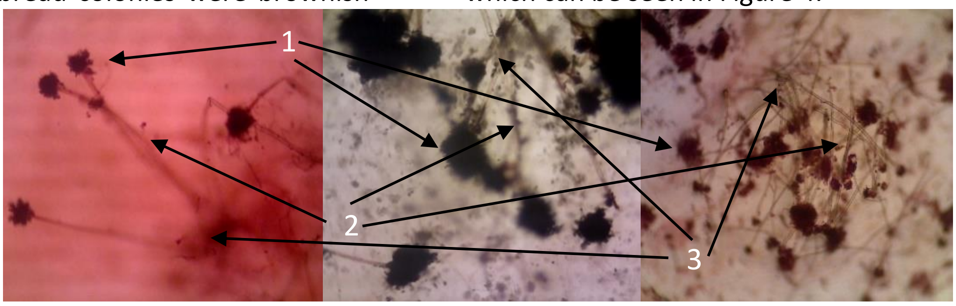

Figure 4. (A). Rhizopus sp. ari of Sari Roti white bread, (B) Rhizopus sp. from papaya, (C) Rhizopus sp. from tempeh
Description: (1). Sporangium, (2) Stolon Hyphae, (3) Rhizoid Hyphae

Microscopically, Figure 4 shows the rhizoids are brown, the stolons are smooth and yellowish brown. Certain parts of the sporangium appear in the form of black dots like pins. The spores are round or semi-spherical with dark brown walls. This sporangium grows upwards and contains hundreds of spores. This is in accordance with the characteristics stated by Agrios (2023), who stated that *Rhizopus* sp. has hyphae that are not insulated and the sporangium is round, has smooth walls and is in the opposite direction to the rhizoid and the shape of the spores is round. To determine the resistance of *D. nindii*, whether pre-inoculated with mycorrhizal rhizoctonia or not, it is necessary to carry out morphological observations. Morphologically, *D. nindii* seedlings pre-inoculated with *Rhizoctonia* mycorrhizae and what doesn't seem real, this can be seen in Figure 4.

Figure 4. Comparison of the morphological appearance of plants without the application of *Rhizoctonia* mycorrhizae and with the application of *Rhizoctonia* mycorrhizae.

From Figure 4, it can be seen that *D. nindii* seedlings was pre-inoculated with *Rhizoctonia* mycorrhizae (M1) have a greener leaf color compared to the M0 treatment. This happens because M1 plants obtain nutrients assisted by mycorrhizae and these can be utilized by the plants so that the plants can grow well. Sufficient nutrients will influence photosynthesis in plants so that the photosynthesis obtained will increase and be used by plants in leaf formation. According to Abdelghany (2016), the number of leaves will have a positive correlation with plant growth and development. Based on observations of root morphology, M1 plants have longer roots and have a large number of them compared to M0 plants. According to Bierman and Linderman (1983), roots that were given mycorrhiza had a higher auxin content than those that were not given mycorrhiza. *Rhizoctonia* mycorrhizae can produce hormones such as auxin which play a role in plant root growth. According to Bierman and Linderman (1983), mycorrhizae is able to play a role in plant rooting and can help the formation of the hormone auxin which plays a role in the elongation of root cells. Observation of the roots shows that all plant roots are white for mature roots, and for old roots they are light brown and young roots are greenish.

The results of the application of *Rhizoctonia* mycorrhizae and inoculation of *Rhizopus* sp. from various inoculum sources gave different responses, this can be seen in the plant morphology observation parameters carried out in the final week of the study by looking at plant height, leaf length, number of leaves, root length, number of roots, and fresh weight. plants that can be seen in Table 1.

Table 1. Results of the DMRT test (Duncan Multiple Range Test) with a level of 5% Effect of Rhizoctonia mycorrhiza application and the effect of *Rhizopus* sp application on the growth of *Dendrobium nindii* orchid seedlings

Treatments	Parameters					
	Plant heigh (cm)	Length leaf (cm)	Number of Leaf (sheet)	Number of root (sheet)	Length root (cm)	Weight of plants (g)
Aplication of *Rhizoctonia* michorrhizae (M)						
M0	3,23 a	4,03 a	2,53 a	7,27 a	9,11 a	2,71 a
M1	3,84 b	4,23 a	4,53 b	10,47 b	9,18 a	4,33 b
Aplication of *Rhizopus* sp. (R)						
R1	3,75	4,21	3,80	9,50	10,38	4,14
R2	3,33	3,97	3,30	9,70	10,63	3,28
R3	3,52	4,21	3,50	9,20	9,87	3,74
Combine application *Rhizoctonia* michorrhizae and *Rhizopus* sp. (M x R)						
M0R1	3,40	4,06	2,60	8,80	9,34	3,22
M0R2	3,00	3,80	2,40	8,20	9,52	2,76
M0R3	3,28	4,24	2,60	7,80	9,10	3,36
M1R1	4,10	4,36	5,00	10,20	11,42	5,06
M1R2	3,66	4,14	4,20	11,20	11,74	3,80
M1R3	3,76	4,18	4,40	10,60	10,64	4,12

Note: Treatments followed by the same letter in the same column are not significantly different in the DMRT test at the 5% level.

From Table 1, it can be seen that the growth of *D. nindii* that is applied with *Rhizoctonia* mycorrhizae will be better compared to plants that are not given *Rhizoctonia* mycorrhizae. This happens because *Rhizoctonia* mycorrhizae is able to help plants obtain the nutrients that orchids really need. According to Senthilkumar *et al.* (2001) Mycorrhizal Rhizoctonia is able to associate with orchids and form peloton structures. Peloton plays a role in providing the nutrients that orchids really need during the seedling period, especially during attacks by pathogens. Nitrogen nutrients in the planting medium are absorbed easily by plants with the help of *Rhizoctonia* mycorrhizae (Zimmer *et al.*, 2007) which suggests that nitrogen nutrients can improve plant growth, plants whose nitrogen needs are met will be greener. In treatments M0 and M1, the differences in number of leaves, leaf length, number of roots and root length were very clear.

CONCLUSION

The results showed that pre-inoculation of *Rhizoctonia* mycorrhizae increased the resistance of *D. nindii* to *Rhizopus* sp. which appeared in plant height, number and length of leaves, number and length, and fresh weight of plants compared to those without *Rhizoctonia* mycorrhizae pre-inoculation

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