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Rhizoctonia MYCORRHIZAE APPLICATION AND WATERING INTERVALS ON *Dendrobium violaceoflavens* SEEDLING : A STUDY OF ITS EFFECT ON DROUGHT STRESS

APLIKASI *Rhizoctonia* MIKORIZA DAN INTERVAL PENYIRAMAN BIBIT *Dendrobium violaceoflavens* : STUDI PENGARUHNYA TERHADAP CEKAMAN KEKERINGAN

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ABSTRAK

Dendrobium violaceoflavens merupakan spesies asli Papua, habitat alaminya adalah daerah dengan curah hujan tinggi sehingga akan menjadi masalah bila dibudidayakan dan terlambat dalam penyiraman. Penelitian bertujuan mengetahui pengaruh aplikasi *Rhizoctonia* mikoriza dan interval penyiraman pada pertumbuhan vegetatif bibit *D. violaceoflavens* terhadap cekaman kekeringan (faktor abiotik). Penelitian menggunakan Rancangan Acak Lengkap (RAL) dengan 2 faktor dan 5 ulangan. Faktor pertama: dengan dan tanpa aplikasi *Rhizoctonia* mikoriza, faktor kedua adalah interval penyiraman 2 hari, 4 hari dan 6 hari. Hasil penelitian menunjukkan [1] aplikasi *Rhizoctonia* mikoriza berpengaruh nyata terhadap tinggi tanaman dengan nilai tertinggi 2,43 cm, jumlah daun 3,17 helai, dan jumlah akar 4,6 helai; [2] Interval penyiraman berpengaruh nyata terhadap tinggi tanaman dengan nilai tertinggi 2, 85 cm, jumlah daun 3,35 helai, jumlah akar 5,27 helai, dan bobot segar 1,23 g; [3] Interaksi pemberian *Rhizoctonia* mikoriza dan interval penyiraman 4 hari berpengaruh nyata pada panjang daun 1,90 cm dan jumlah daun 3,70 helai, dan pada akar terbentuk struktur peloton. Hasil terbaik diperoleh pada aplikasi *Rhizoctonia* mikoriza dan interval penyiraman 4 hari dalam penanggulangan cekaman kekeringan.

Kata kunci : Cekaman kekeringan, *D. violaceoflavens*, Interval penyiraman, *Rhizoctonia* mikoriza.

ABSTRACT

Dendrobium violaceoflavens is a native species of Papua, its natural habitat is an area with high rainfall that will be a problem if it is cultivated and watering is delayed. The aim of this study was to determine the effect of *Rhizoctonia* mycorrhizae application and watering interval on vegetative growth of *D. violaceoflavens* seedlings on drought stress (abiotic factors).

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Research used a completely Randomized Design (CRD) with 2 factors and 5 replications. First factor was with and without application of *Rhizoctonia* mycorrhizae, second factor was watering interval of 2 days, 4 days and 6 days. The result showed that [1] the application of *Rhizoctonia* mycorrhizae had a significant effect on plant height with the highest value of 2.43 cm, number of leaves 3.17 leaves, number of roots 4.6 leaves; [2] watering interval significantly affected on plant height with the highest value of 2.85 cm, number of leaves 3.35 sheets , number of roots 5.27, fresh weight of 1.23 g; [3] Interaction between *Rhizoctonia* mycorrhizae and watering interval of 4 days significantly affected on leaf length 1.90 cm, leaf number 3.70 sheets and a peloton structure was formed on the root. Best results were obtained on the application of *Rhizoctonia* mycorrhizae and watering interval of 4 days in overcoming the drought stress.

Key words *: D. violaceoflavens, Rhizoctonia* mycorrhizae*, Watering Intervals, Water stress.*

INTRODUCTION

Dendrobium orchids are a great demand orchids by the public because they meet the consumer demands. The number of florets, the length of the panicles and the durability of the flowers determine consumer's appetite for interest in *Dendrobium* orchids, because *Dendrobium* has different levels of color. *Dendrobium violaceoflavens* is an orchid species native to Papua, which grows as an epiphyte in moist forests on high branches or as a lithophyte on rocks making it difficult to collect. Some orchids such as *Phalaenopsis javanica* a rare species that is highly sought after by collectors because it has beautiful flowers at last about 2 months. *D. violaceflavens* is often used as parental in crosses, because it has advantages of tall stems, deep roots, and are a rare species, so this species needs to be preserved in its natural life, this species is also reported in appendix II of CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) which is one of the species whose trade has been regulated internationally (Pietro, 2002).

Water is one of the most important physical components and is needed in large quantities for growth and development of orchids, like in Papua with where its often rains. About 85%-90% of the fresh weight of plant cells and tissues is water and has a function as nutrient dissolution, preparation of protoplasm, raw material in the process of photosynthesis. Plants have a mechanism to adjust it, in plants with moderate drought levels, plants can have mechanisms to avoid it, while at high drought levels, plants will have mechanisms for tolerance (Sukma, 2015). In general, orchids grow at an altitude 1000 meters above sea, so they are very suitable in areas with moderate to high humidity. However it will be problems if we move orchids from the highlands to the lowlands due to the humidity. Optimum growth of orchids also requires relative humidity ranging from 60% -90% (Ginting *et al.*, 2001). High humidity can reduce evaporation in orchids so they don't experience a shortage of water needed to translocate nutrients to all parts of the tissue (SARI, 2018).

Previous research was carried out in the laboratory to overcome the drought stress factor in the orchids so they have the same humidity conditions as ini nature by using the chemical Poly Ethylen Glycol (PEG). However, this method is rather difficult because it must be carried out under *in vitro* conditions using tissue culture techniques and this method had a negative effect on the percentage of plantlets that survived. In addition PEG stress also induced a visual change in plantlet color from green to brown so that it would be difficult for the photosynthesis process as evidenced by a decrease in chlorophyll levels in plantlets. Where total plantlet chlorophyll from medium with 15% and 20% PEG decreased significantly compared to plants under control conditions. Chlorophyll a, b, and stomatal index also showed a decrease in response to PEG 6000 administration, although the changes were not statistically significant (Nurcahyani & Sabatini, 2022). Therefore, another method was tried to overcome drought stress, namely by applying *Rhizoctonia* mycorrhizae to orchid seedling. So if orchids is removed from the highlands to the lowlands it can still adapted to environment with minimal water.

In plant productivity, mycorrhizae play an important role in the beneficial association of formation of a symbiotic structure between fungi and orchid roots (Siddiqui & Pichtel, 2008). In a lack of water environment, mycorrhizal fungi will be useful for plants because they can increase the ability of plant roots to absorb water (Cui et al., 2004). In nature, association of *Rhizoctonia* mycorrhizae with orchid roots that occurs when seeds begin to germinate to form roots and shoots (*protocorm*). Spread to other cell tissues after hyphae and *Rhizoctonia* mycorrhizae penetrate seed walls of orchids. The peloton structure is formed after protocorm has developed into a perfect plant (*plantlet*) then hyphae network of *Rhizoctonia* mycorrhizae will penetrate into the cortex of orchid root (Senthilkumar *et al.*, 2001). *Rhizoctonia* mycorrhizae found in orchid roots is a fungus that lives naturally and is in symbiosis with plant roots, which helps meet needs of orchids for plant nutrients. Mycorrhizal fungi are beneficial for plants in dry areas because they can increase ability of plants to absorb water (Cui *et al.*, 2004), improve chemical, physical, and biological properties of soil, because external hyphae of mycorrhizal fungi are able to penetrate soil pore spaces, both micro and macro. The presence of external hyphae and roots is very important because they are able to absorb and store soil moisture. The aim of the study was to determine the effect of *Rhizoctonia* mycorrhizae application and watering interval on the growth of *D. violaceoflavens* orchid seedling.

MATERIALS AND METHODS

The research was carried out from July 2021 to May 2022. *Rhizoctonia* mychorrizae taken from the Larat orchid (*Dendrobium phaleonopsis*) collection of Soelistijono et al., (2020) in the tissue culture laboratory of Agriculture Faculty Universitas Tunas Pembangunan (UTP) Surakarta. The seedling *D. violaceoflavens* to be inoculated was obtained from tissue culture laboratory and inoculation was carried out in the experimental garden of UTP. The cultivation of plantlets was carried out in the Borobudur Orchid Garden, Mungkid, Magelang Regency.

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. violaceflavens* aged 8 months was placed in the pot containing moss. Each *D. violaceoflavens* 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. violaceoflavens* were cut and examined under a microscope to see the *Rhizoctonia* mycorrhizae associations in the form of *peloton* structure. Seedlings were 8 months old, watered 2, 4, and 6 days for 2 months. The growth of seedling *D. violaceoflavens* was observed every week from 8 to 10 months of age.

The study used Completelt Randmized Design (CRD) research method with two factorial treatments, namely : M_0: without *Rhizoctonia* mycorrhizae application, M_1: with *Rhizoctonia* mycorrhizae application, and second factor was watering interval of 2 days (P1), 4 days (P2) and 6 days (P3). 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

After subculture to new PDA media, in first week white hyphae colonies were seen forming a circle in the middle of Petridish. After 3 days, hyphal colony was seen rapidly with increase in extent of hyphae growth on PDA media, there was no contamination in the Petrdish. On day 9^{th} *Rhizoctonia* mycorrhizae hyphae colonies began to slow down. Colonies showed white *Rhizoctonia* mycorrhizae hyphae, there were circles up to the edges (Figure 1).

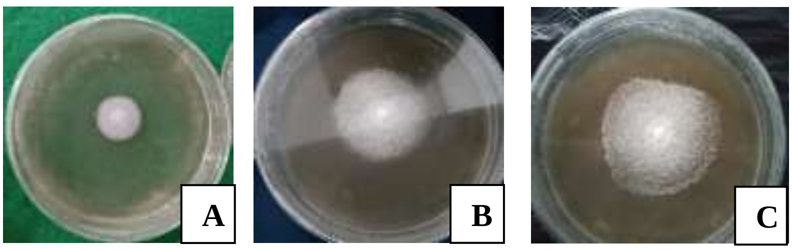

Figure 1. Growth and development of *Rhizoctonia* mycorrhizae colonies isolates from *Dendrobium phalaenopsis* on Potato Dextrose Agar media

Description : Development observation of *Rhizoctonia* mycorrhizae colonies on the 3^{rd} day (A), 6^{th} day (B), and 9^{th} day (C)

This is in accordance with results of Ningsih & Febrianti researchs, (2014) that *Rhizoctonia* mycorrhizae has morphological characteristics of colonies that have a white color above or below surface. On the surface, in middle of the colony, white clumps were found with wet conditions, a circle was formed at the edges, with very slow growth. Based on Sneh *et al*., (2004), growth rate of *Rhizoctonia* mycorrhizae hyphae in forming colonies will vary depending on each species. The rapid growth rate of *Rhizoctonia* mycorrhizae is expected to accelerate formation of mycorrhizal associations with orchid seedling and formation of peloton structures in the root cortex. Microscopic identification of *Rhizoctonia* mycorrhizae to identify branching shape and number of cell nuclei can be seen in Figure 2.

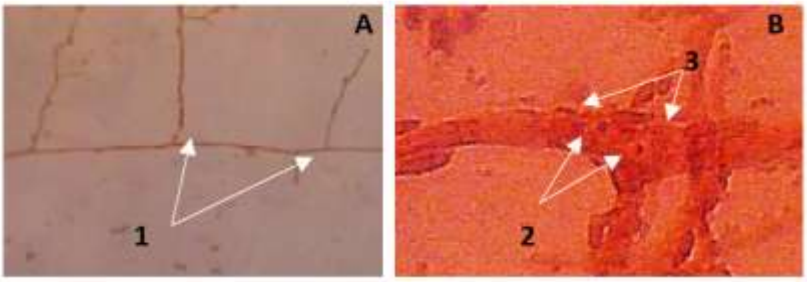

Figure 2. (A). *Rhizoctonia* mycorrhizae hyphae have angular branches at a magnification of 90 times (1), (B). Cell nucleus in *Rhizoctonia* mycorrhizae hyphae (2) and septa hyphae (3).

In Figure 2, hyphae have branches that form right angles on observation with a magnification of 90 times, branching of hyphae that form right angles is one of the characteristics of *Rhizoctonia* mycorrhizae, this is in accordance with Agrios, (2004) who stated that branching in hyphae of *Rhizoctonia* mycorrhizae form a right angle. Figure 2b shows *Rhizoctonia* mycorrhizae hyphae which have 2 cell nuclei. This indicates that hyphae belong to the type of *Rhizoctonia* binucleate (BNR) and is in accordance with the opinion of (Kasiamdari, 2000). Observations of plant morphology are used to provide a visual description of the research results, so that it is easier to understand and describe results which can be seen in Figure 3 and Figure 4.

M_0P_1 M_0P_2 M_0P_3

Figure 3. Morphological appearance of plants without *Rhizoctonia* mycorrhizae

Description : (M_0P_1): Watering every 2 days, (M_0P_2): Watering every 4 days, (M0P3): Watering every 6 days

M_1P_1 M_1P_2 M_1P_3

Figure 4. Morphological appearance of plants with *Rhizoctonia* mycorrhizae

Description : (M_1P_1): Watering every 2 days, (M_1P_2): Watering every 4 days, (M_1P_3): Watering every 6 days

From Figure 3 and Figure 4 it can be seen that mycorrhizal application treatment and watering intervals affected plant height, number of leaves, leaf length, tillers, number of roots and root length. Watering treatment every 2 days with the application of plant mycorrhizae could grow well (M_1P_1) compared to the treatment without application of plant mycorrhizae (M_0P_1). For watered treatment every 4 days, plants with mycorrhizal treatment (M_1P_2) had better growth than without mycorrhizae (M_0P_2). While watered treatment every 6 days either with application of mycorrhizae (M_1P_3) or without mycorrhizae (M_0P_3) had poor growth, this was because the role of water is very important in plant metabolism.

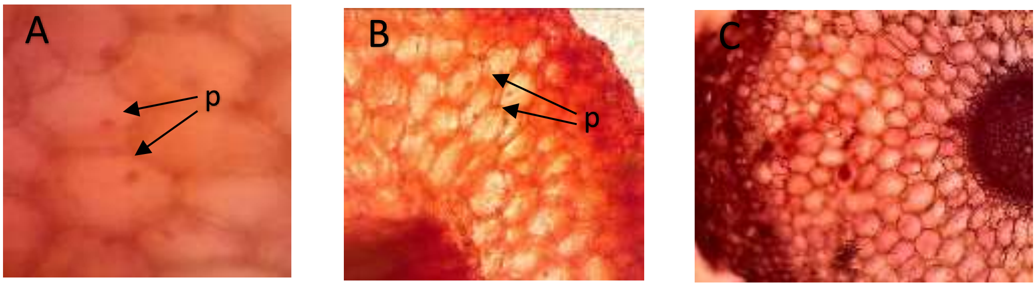

Figure 5. Peloton observation

Description : (A): Cross-sectional section of roots with a peloton 10X40 (B): Cross-section of roots with a peloton 10X10, (C): Cross-sectional area of roots without a peloton 10× 10. P = peloton

In the cross section of the roots of *D. violaceoflavens* with application of *Rhizoctonia* mycorrhizae, red clumps were seen in the middle cells or at the edges of the cells in the form of peloton. This is in accordance with what was stated by (George et al., 2008), namely that peloton are intracellular hyphae in form of clumps root cortex tissue and are usually found only for a certain period before being lysed and digested by plants. According to Nusantara, (2007), intracellular hyphae of *Rhizoctonia* on orchid roots have ability to penetrate cortical tissue at root and form dense, clumping coils known as peloton. Peloton are usually only found in a limited period before experiencing lysis. Infection and lysis occur repeatedly in interior of same cells and tissues. The formation of this structure is a characteristic of mycorrhizal fungi of orchid plants (Smith & Read *in* Dwiyanto et al., 2017).

To increase the capacity of orchids in absorbing nutrients in plants, *Rhizoctonia* mycorrhizae first infects roots and forms a network of hyphae in root cortex tissue (Hatni, 2017). Mycorrhizae in orchids have a nutrient flow where the fungus gets a direct supply of carbon from plants instead of phosphorus or as a substitute nitrogen for plants (Balestrini et al., 2014). However, according to (Zimmer et al., 2007) 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. There are approximately 400 species of orchids, no flow of carbon nutrients from plants, but fungi can supply nutrients to orchids so this proves that *Rhizoctonia* mychorrizae can reduce stress due to water stress.

Table 1. *Rhizoctonia* mycorrhizae application and watering interval on the growth of *Dendrobium violaceoflavens* orchid seedling

Treatment	Plants height (cm)	Leaf lenght (cm)	Number of leaves (sheet)	Root lenght (cm)	Number of root (pieces)	Fresh weight (g)
Rhizoctonia mycorrhizae application (M)						
M_0	2.43a	1.76	2.40a	4.88	3.90a	0.91a
M_1	2.76b	1.64	3.17b	5.07	4.86b	1.12a
Watering interval (P)						
P_1	2.57ab	1.63	2.61a	5.01	4.11a	0.97ab
P_2	2.85b	1.85	3.35b	5.54	5.27b	1.23b
P_3	2.38a	1.61	2.40a	4.39	3.75a	0.84a

Description : Numbers followed by same letter in the same columns are not significantly different based on Duncans Multiple Range Test (DMRT) 5% level. Description: M_0: Without *Rhizoctonia* mycorrhizae, M_1: Application with *Rhizoctonia* mycorrhizae, F_1: Watering every 2 days, F_2: Watering every 4 days, F3: Watering every 6 days

The parameters of plant height, number of leaves, and number of roots in application treatment of *Rhizoctonia* mycorrhizae gave very significant results on plants height, number of leaves, number of roots, and plant fresh weight. While watering interval treatment gave significant results on parameters of plant height, number of leaves, number of roots, and fresh weight (Table 1). This indicated that factor of giving *Rhizoctonia* mycorrhizae gave significant results on vegetative growth. By giving *Rhizoctonia* mycorrhizae in M_1 treatment, it was possible for plants to get nitrogen from mycorrhizal which is good for leaf growth. Mycorrhizal interactions with orchid has a unique nutrient flow, usually in mycorrhizal interactions, plant unidirectionally supplies fungus with carbon instead of phosphorus or nitrogen or both depending on environment (Balestrini *et al*., 2014).

From Table 1 it can be seen that P_2 factor had more influence on plant growth than P_3 factor even P_1 factor. This is show that watering every 2 days was more intensive (P_1), but did not give better results than watering every 4 days. According to Sessler in Ginting et al., (2001) orchids can grow well if their water needs are met. In addition, all physiological activities ranging from biochemical processes to the growth and development of plant tissues are determined by percentage of water they contain.

Table 2. Effect of *Rhizoctonia* mycorrhizae application and watering interval on the growth of *Dendrobium violaceoflavens* orchid seedling

Treatment	Plants height (cm)	Leaf lenght (cm)	Number of leaves (sheet)	Root lenght (cm)	Number of root (pieces)	Fresh weight (g)
M_0P_1	2.40	1.7ab	2.00a	5.02	3.40	0.83
M_0P_2	2.68	1.90c	3.00bc	5.30	4.80	1.10
M_0P_3	2.21	1.81bc	2.20a	4.32	3.50	0.79
M_1P_1	2.73	1.69bc	3.23c	4.99	4.83	1.11
M_1P_2	3.01	1.81bc	3.70c	5.77	5.75	1.35
M_1P_3	2.54	1.41a	2.60ab	4.46	4.00	0.89

Description : Numbers followed by same letter in the same columns are not significantly different based on Duncans Multiple Range Test (DMRT) 5% level. Description: M_0: Without *Rhizoctonia* mycorrhizae, M_1: Application with *Rhizoctonia* mycorrhizae, F_1: Watering every 2 days, F_2: Watering every 4 days, F3: Watering every 6 days

Based on Table 2 shows that factor of giving *Rhizoctonia* mycorrhizae and watering intervals gives significant results. Interaction between two factors gave significantly different results on parameters of leaf length and number of leaves. In number of leaves parameter, prove that there was an interaction between the application of *Rhizoctonia* mycorrhizae and the watering interval treatment every 4 days that gave good results on number of leaves. This means that application of *Rhizoctonia* mycorrhizae had an effect on drought stress factor as stated by Cui *et al.*, (2004). Herliana *et al.*, (2018) stated that the application of mycorrhizal biological fertilizers had no significant effect on root length but had a significant effect on the number of roots on the growth of *Dendrobium* sp. orchids, in plant growth if the environment did not affect its growth, the genetic factor was the genetic factor of the plant itself. The moss planting media factor also has a function as a place to grow and store nutrients and water for plant growth (Munir & Zulman, 2011). So it is recomended to uses moss on *D. violaceflavens* seedlings cultivated in areas with minimal water (drought stress).

CONCLUSION

Provision of *Rhizoctonia* mycorrhizae application and watering every 4 days gave the best results effect for growth of seedling *D. violaceflavens*, so it can still adapted on drought stress (abiotic factor).

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