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THE EFFECT OF USING *RHIZOCTONIA* MYCORRHIZAE AND WATERING INTERVALS ON VEGETATIVE GROWTH OF *Dendrobium violaceoflavens SEEDLING* AGAINST WATER STRESS

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Abstract. *Dendrobium violaceoflavens* is a rare species native of Papua, where natural habitat is an area that has a high rainfall so it will be a problem when cultivated in the lowlands. This study aims to determine the effect of *Rhizoctonia* mycorrhizae application and watering interval on the vegetative growth of iron orchid seedling against water stress (abiotic factors).

This research was carried out in the green house and laboratory of Agriculture Faculty, Tunas Pembangunan University from July 2020 to July 2021 used a Completely Randomized Block Design (CRBD). The first factor is application *Rhizoctonia* mycorrhizae with stage M0: no application *Rhizoctonia* mycorrhizae and M1: application *Rhizoctonia* mycorrhizae, the second factor is the interval watering with a level: watering 2 days (P1), watering 4 days (P2) and watering 6 days (P3).

The results showed (1) the application of *Rhizoctonia* mycorrhizae had a significant effect on plant height with the highest value 2.43 cm, the number of leaves (3.17 strands), the number of roots (4.6 pieces) and significant effect on fresh weight (1.12 g). (2) Interval watering 4 days significant effect on plant height with value leaf length (2.85) cm, the number of leaves (3.35 strands), the number of roots (5.27 pieces), fresh weight (1.23 pieces), and significantly affected leaf length (1.85 cm). (3) The treatment interaction of *Rhizoctonia* mychorrizae and watering 4 days had a significant effect on leaf length (1.90 cm) and the number of leaves (3.70 strands). In orchids induced by *Rhizoctonia* mycorrhizae a peloton structure is formed.

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

INTRODUCTION

From year to year *Dendrobium* orchids meet consumer demands. The number of florets, the length of the panicles and the durability of the flowers determine the 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 and is a rare species that is highly sought after by collectors because it has beautiful flowers that last about 2 months. This orchid is often used as a broodstock in crossbreeding, so because it has these advantages but is included in a rare species, this species needs to be preserved in its natural life, this species is also reported in appendix II of CITES 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 plant growth and development. About 85%-90% of the fresh weight of plant cells and tissues is water and has a function as nutrient dissolution, preparation of

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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). 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).

In plant productivity, mycorrhizae play an important role in the beneficial association of the formation of a symbiotic structure between fungi and orchid roots (Siddiqui & Pichtel, 2008). In an environment that lacks water, mycorrhizal fungi will benefit plants because they can increase the ability of plant roots to absorb water *(Cui et al*., 2004). Association of *Rhizoctonia* mycorrhizae with orchid roots that occurs when the seeds begin to germinate to form roots and shoots (*Protocorm*). Spread to other cell tissues after hyphae and *Rhizoctonia* mycorrhizae penetrate the seed walls of orchids. The peloton structure is formed after the protocorm has developed into a perfect plant (Plantlet) then the hyphae network of *Rhizoctonia* mycorrhizae will penetrate into the cortex of the 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 the needs of orchids for plant nutrients. Mycorrhizal fungi are beneficial for plants in dry areas because they can increase the ability of plants to absorb water (Cui *et al.*, 2004), improve the chemical, physical, and biological properties of the soil, because the 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 on the growth of *D. violaceoflavens* orchid *seedling* and watering intervals on its growth.

MATERIALS AND METHODS

The research was carried out from July 2020 to March 2021. *Rhizoctonia* michorrizae taken from the Larat orchid (*Dendrobium phaleonopsis*) by previous research conducted in the tissue culture lab of the Faculty of Agriculture UTP Surakarta (Soelistijono *et al*., 2020). The *seedling D. violaceoflavens* to be inoculated was obtained from the Tunas Pembangunan University tissue culture laboratory.

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, 5 grams of *Rhizoctonia* mycorrhizae culture were mixed with 100 ml of sterile water. *Seedling D. violaceoflavens* aged 8 months was placed in the pot

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containing the moss. Each *D. violaceoflavens seedling* was sprayed with 1 ml of *Rhizoctonia* mycorrhizae inoculum and was acclimatized in the green house for 2 months. After 8 months the *seedling* roots of *D. violaceoflavens* were cut and examined under a microscope to see *Rhizoctonia* mycorrhizae associations in the form of *peloton* structure. Seedlings are 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, both those inoculated with *Rhizoctonia mycorrhizae* or not.

RESULTS AND DISCUSSION

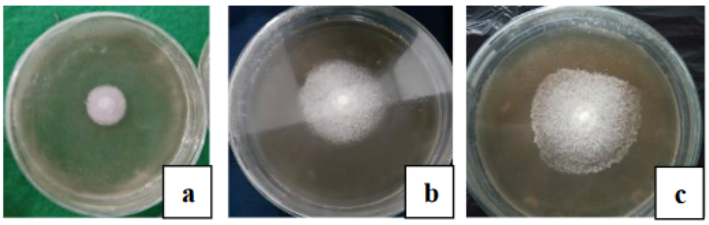

Figure 1. Growth and development of *Rhizoctonia* mycorrhizae colonies isolates from *Dendrobium phalaenopsis* on PDA media
Description : Observation of the development of *Rhizoctonia* mycorrhizae colonies on the third day (a), (b) sixth day, and ninth day (c)

After isolation from the old PDA media to the new PDA media, in the first week white hyphae colonies were seen forming a circle in the middle of the petridish. After 3 days the hyphal colony was seen rapidly with the increase in the extent of hyphae growth on the PDA media, there was no contamination in the petrdish. On day 9 *Rhizoctonia* mycorrhizae hyphae colonies began to slow down. Colonies showed white *Rhizoctonia* mycorrhizae hyphae, there were circles up to the edges. This is in accordance with the results of research (Ningsih & Febrianti, 2014) that *Rhizoctonia* mycorrhizae has morphological characteristics of colonies that have a white color above or below the surface. On the surface, in the 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), the 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 the formation of mycorrhizal associations with orchid seedling and the formation of peloton structures in the root cortex. Microscopic identification of *Rhizoctonia* mycorrhizae to identify the branching shape and number of cell nuclei can be seen in Figure 2.

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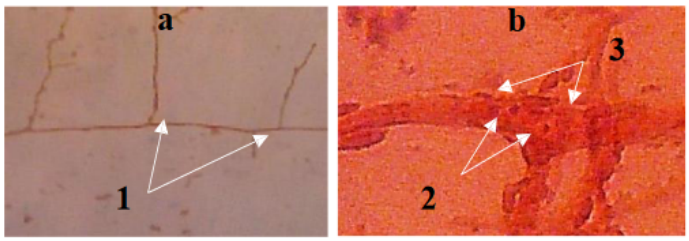

Figure 2. (a). *Rhizoctonia* mycorrhizae hyphae have angular branches at a magnification of 40 times. (b). cell nucleus in *Rhizoctonia* hyphae with a magnification of 40 times

In Figure 2, hyphae have branches that form right angles on observation with a magnification of 40 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 the hyphae belong to the type of *Rhizoctonia* Binucleate 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 the results which can be seen in Figure 3 and Figure 4.

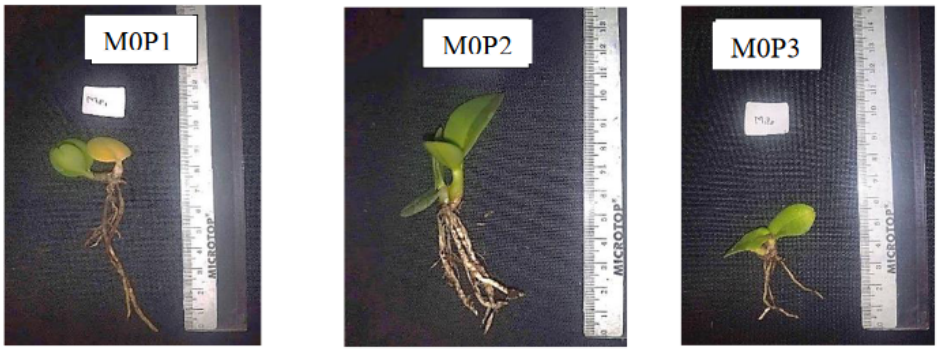

Figure 3. Morphological appearance of plants without *Rhizoctonia* mycorrhizae
Description : (P1): Watering every 2 days, (P2): Watering every 4 days, (P3): Watering every 6 days

Figure 4. Morphological appearance of plants with Rhizoctonia mycorrhiza
Description : (P1): Watering every 2 days, (P2): Watering every 4 days, (P3): Watering every 6 days

Figure 4. Morphological appearance of plants with Rhizoctonia mycorrhiza
Description : (P1): Watering every 2 days, (P2): Watering every 4 days, (P3): Watering every 6 days

From Figure 3 and Figure 4 it can be seen that the 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 can grow well (M1P1) compared to the treatment without the application of plant mycorrhizae (M0P1). For watering treatment every 4 days, plants with mycorrhizal treatment (M1P2) had better growth than without mycorrhizae (M0P2). While the watering treatment every 6 days either with the application of mycorrhizae (M1P3) or without mycorrhizae (M0P3) has poor growth, this is because the role of water is very important in plant metabolism.

Peloton Observation

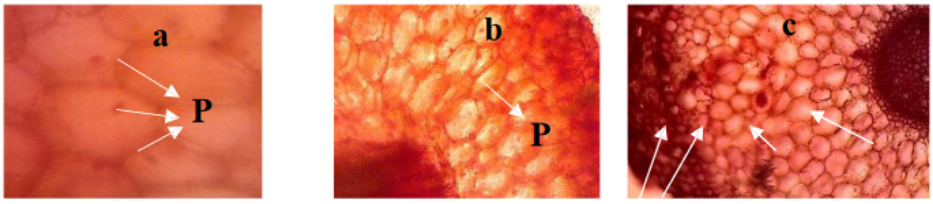

Figure 9. (a): Cross-sectional section of roots with a peloton of 40× magnification (b): Cross-section of roots with a peloton of 10× magnification (c): Cross-sectional area of roots without a peloton of 10× magnification.
Description : P = peloton

In the cross section of the roots of *D. violaceoflavens* with the 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 the form of clumps in the root cortex tissue and are usually found only for a certain period before being lysed and digested by plants. According to Nusantara, (2007), the intracellular hyphae of *Rhizoctonia* on orchid roots have the ability to penetrate the cortical tissue at the 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 the interior of the 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 to absorb nutrients in plants, *Rhizoctonia* mycorrhizae first infects the roots and forms a network of hyphae in the 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 for 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

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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.

Tabel 2. Duncans Multiple Range Test (DMRT) 5% level the effect of *Rhizoctonia* mycorrhizae application and watering interval on the growth of *Dendrobium violaceoflavens* orchid *seedling*

Treatment	Tall plants (cm)	Leaf lenght (cm)	Number of leaves (sheet)	Root lenght (cm)	Number of root (fruit)	Fresh weight (g)
Rhizoctonia mycorrhizae application (M)						
M0	2,43a	1,76	2,40a	4,88	3,90a	0,91a
M1	2,76b	1,64	3,17b	5,07	4,86b	1,12a
Watering interval (P)						
P1	2,57ab	1,63a	2,61a	5,01	4,11a	0,97ab
P2	2,85b	1,85a	3,35b	5,54	5,27b	1,23b
P3	2,38a	1,61a	2,40a	4,39	3,75a	0,84a
Interaction betwen Rhizoctonia mycorrhiza application and watering interval (M×P)						
M0P1	2,40	1,7ab	2,00a	5,02	3,40	0,83
M0P2	2,68	1,90c	3,00bc	5,30	4,80	1,10
M0P3	2,21	1,81bc	2,20a	4,32	3,50	0,79
M1P1	2,73	1,69bc	3,23c	4,99	4,83	1,11
M1P2	3,01	1,81bc	3,70c	5,77	5,75	1,35
M1P3	2,54	1,41a	2,60ab	4,46	4,00	0,89

The parameters of plant height, number of leaves, and number of roots in the application treatment of *Rhizoctonia* mycorrhizae gave very significant results, while the watering interval treatment gave significant results on the parameters of plant height, number of leaves, number of roots, and fresh weight. This shows that the factor of giving *Rhizoctonia* mycorrhizae and watering intervals gives significant results. The correlation of the two factors gave significantly different results on the parameters of leaf length and number of leaves. By giving *Rhizoctonia* mycorrhizae in M1 treatment, it is possible for plants to get nitrogen from mycorrhizal which is good for leaf growth. Mycorrhizal interactions Orchid has a unique nutrient flow, usually in mycorrhizal interactions, the plant unidirectionally supplies the fungus with carbon instead of phosphorus or nitrogen or both depending on the environment (Balestrini *et al*., 2014). 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 the percentage of water they contain.

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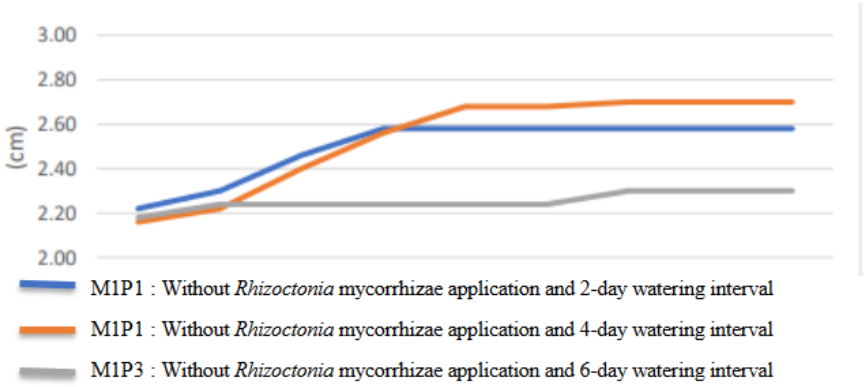
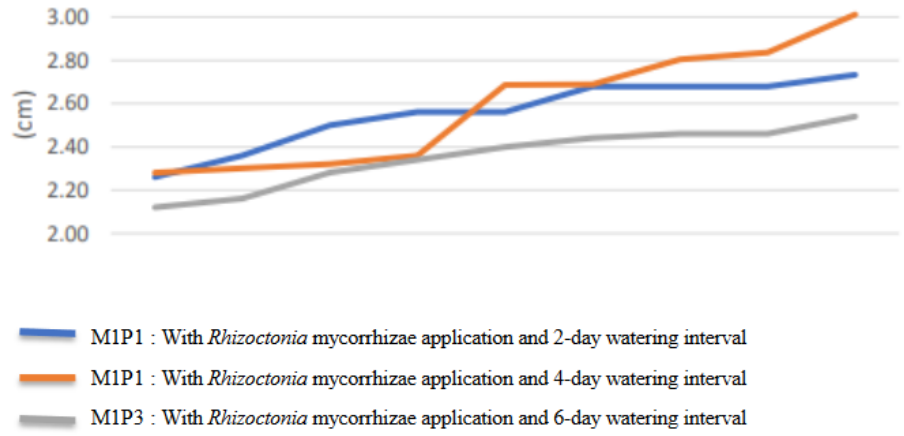
Without *Rhizoctonia* mycorrhizae



With *Rhizoctonia* mycorrhizae



The watering interval treatment showed that the P2 treatment with a value of 3.35 strands was very significantly different from the P1 treatment with a value of 2.61 strands and very significantly different from the P3 treatment with a value of 2.40 strands. These results prove that there is an interaction between the application of *Rhizoctonia* mycorrhizae and the watering interval that the watering treatment every 4 days with the application of *Rhizoctonia* mycorrhizae gives good results on the number of leaves. (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).

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CONCLUSION

Provision of *Rhizoctonia* mycorrhizae and watering every 2 days had an effect on the growth of *seedling D. violaceflavens* on abiotic factors in the form of drought stress / water stress.

ACKNOLEDGEMENTS

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