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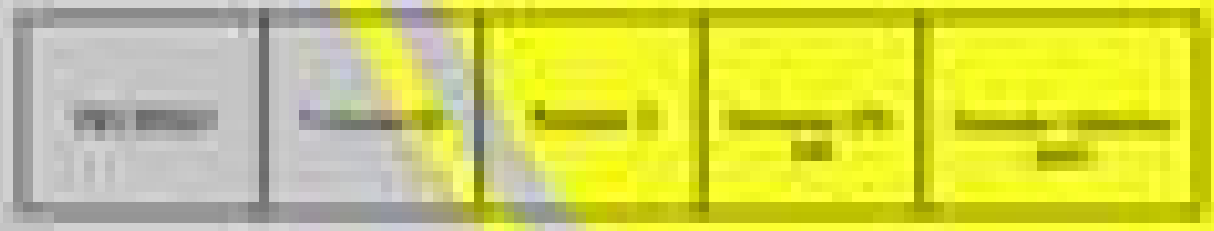

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Table of Contents

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Ceratorhiza Induction towards ORSV Infection on Viability of Phalaenopsis and Dendrobium

Valentina Dwi Anggita Sari, Mahfut Mahfut, Sri Wahyuningsih, Tundjung Tripeni Handayani

ABSTRACT

Orchids have a high level of biodiversity, such as *Dendrobium* and *Phalaenopsis*, which are epiphytes. *Dendrobium* can adapt to the condition of where it lives while *Phalaenopsis* can grow in highlands and depends on sunlight and humidity. Virus infection has become one of the obstacles in cultivating *Dendrobium* and *Phalaenopsis*. Efforts to increase fitness and control in *Dendrobium* and *Phalaenopsis* cultivation can be done by inducing the plant's fitness using a mycorrhiza, such as Ceratorhiza. A mycorrhiza is a form of mutualism between fungi and the plant's root. This research is aimed to give information related to the utilization of Cerathoriza for inducing orchids to suppress *Odontoglossum ringspot virus* (ORSV) infection, giving it better growth. The research was done in February-March 2021 at Botany Laboratory University of Lampung. A completely randomized factorial design was used on two factors, kind of orchid and mycorrhiza treatment (M), virus (V), and mycorrhiza virus (MV). Variables examined in this research are the amount of living and dead roots and leaves. Data obtained is homogenized using Levene's test and continued by ANOVA with the significance level of 5% and further testing using Tukey's test with the significance level of 5%. From this research, it is known that interaction between *Phalaenopsis* and *Dendrobium* exists during virus and mycorrhiza administration. It is concluded that *Phalaenopsis anabilis* is more vulnerable than *Dendrobium discolor*.

KEYWORDS

Ceratorhiza, Dendrobium, ORSV, Phalaenopsis

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Ceratorhiza Induction towards ORSV Infection on Viability of *Phalaenopsis* and *Dendrobium*

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ABSTRACT

Orchids have a high level of biodiversity, such as *Dendrobium* and *Phalaenopsis*, which are epiphytes. *Dendrobium* can adapt to the condition of where it lives while *Phalaenopsis* can grow in highlands and depends on sunlight and humidity. Virus infection has become one of the obstacles in cultivating *Dendrobium* and *Phalaenopsis*. Efforts to increase fitness and control in *Dendrobium* and *Phalaenopsis* cultivation can be done by inducing the plant's fitness using a mycorrhiza, such as Ceratorhiza. A mycorrhiza is a form of mutualism between fungi and the plant's root. This research is aimed to give information related to the utilization of Cerathoriza for inducing orchids to suppress *Odontoglossum ringspot virus* (ORSV) infection, giving it better growth. The research was done in February-March 2021 at Botany Laboratory University of Lampung. A completely randomized factorial design was used on two factors, kind of orchid and mycorrhiza treatment (M), virus (V), and mycorrhiza virus (MV). Variables examined in this research are the amount of living and dead roots and leaves. Data obtained is homogenized using Levene's test and continued by ANOVA with the significance level of 5% and further testing using Tukey's test with the significance level of 5%. From this research, it is known that interaction between *Phalaenopsis* and *Dendrobium* exists during virus and mycorrhiza administration. It is concluded that *Phalaenopsis anabilis* is more vulnerable than *Dendrobium discolor*.

Keywords: *ceratorhiza*, *dendrobium*, ORSV, *phalaenopsis*

ABSTRAK

Anggrek merupakan salah satu tumbuhan yang memiliki keanekargaman sangat tinggi diantaranya *Dendrobium* dan *Phaleonopsis* yang merupakan anggrek epifit. Anggrek *Dendrobium* adalah salah satu anggrek yang mampu beradaptasi dengan kondisi tempat tumbuh anggrek. *Phalenopsis* merupakan anggrek epifit yang dapat tumbuh di daerah ketinggian dan membutuhkan cahaya serta kelembaban. Kendala dalam budidaya anggrek *Dendrobium* dan *Phaleonopsis* salah satunya karena adanya infeksi virus. Upaya peningkatan ketahanan dan pengendalian budidaya anggrek *Dendrobium* dan *Phaleonopsis* dapat dilakakukan dengan menginduksi ketahanan tanaman menggunakan mikoriza *Ceratorhiza*. Mikoriza merupakan suatu bentuk simbiosis mutualistik antara jamur dan akar tanaman. Tujuan dari penelitian ini dapat memberikan informasi mengenai pemanfaatan mikoriza *Ceratorhiza* untuk menginduksi tanaman anggrek agar dapat menahan infeksi virus *Odontoglossum ringspot virus* (ORSV), sehingga pertumbuhannya lebih baik. Waktu dan tempat penelitian dilakukan pada bulan Februari-Maret 2021 di Laboratorium Botani Universitas Lampung. Pada penelitian ini menggunakan Metode rancangan acak lengkap faktorial (RALF) yang terdiri dari dua faktor, yaitu jenis anggrek dan perlakuan mikoriza (M), virus (V), dan mikoriza virus (MV). Variabel

yang diamati pada penelitian ini adalah jumlah akar yang hidup dan mati, jumlah daun yang hidup dan mati. Data yang diperoleh dihomogenkan dengan menggunakan uji Levene kemudian dianalisis ragam pada taraf nyata 5% menggunakan ANOVA dan uji lanjut dengan Tukey pada taraf nyata 5%. Hasil penelitian menunjukkan bahwa jenis anggrek *Phalaenopsis amabilis* lebih rentan dibandingkan *Dendrobium discolor* pada perlakuan mikoriza (M), virus (V), dan mikoriza virus (MV). Terdapat interaksi antara *Phalaenopsis* dan *Dendrobium* pada variabel jumlah daun pada perlakuan mikoriza (M) dan virus (V).

Kata Kunci: *ceratorhiza, dendrobium, ORSV, phalaenopsis*

INTRODUCTION

Orchids is one of the biggest family in Indonesia (Soetopo, 2009). It is widely-spread and can be found in tropical forests in West Sumatera, Java, Kalimantan, Sulawesi, Nusa Tenggara, Maluku, and Papua (Rukmana, 2000). It is categorized into two based on the way of living, namely terrestrial and epiphyte. Terrestrial orchids are orchids that grow and develop on land (Dina and Soetopo, 2019) while epiphyte orchids are orchids that live on host plants but are not parasites. They depend on their hosts. The loss of orchids' host plants will significantly ruin orchids' life cycle (Ambari, 2016). Population of orchids in their natural habitat has been decreasing due to deforestation and forest over-exploitation (Johanis et al., 2001). This condition has led to the efforts of cultivation.

Phalaenopsis and *Dendrobium* are two of the most cultivated orchids (Yudistira *et al*., 2011). Unfortunately, virus infection has become one of the biggest challenges in orchids' cultivation and potential development (Kumalawati et al., 2011). Odontoglossum ringspot virus (ORSV) infects most orchids. Based on previous research by Mahfut (2016), symtomps caused by ORSV include mosaic, streak, chlorotic, and necrosis. Orchids protection measures can be done by genetic recombination resulting ORSV resistant orchids. Therefore, a mycorrhiza, such as *Cerathoriza* sp., is used as it is eco-friendlier and more effective.

Mycorrhiza is a mutualistic symbiosis between fungi and root. Plants obtain nutrients absorbed by fungi while fungi obtain nutrients resulted from assimilations done by plants. Mycorrhiza infected the root system of the plant hosts, producing hyphae intensively. *Cerathoriza*'s role as a biofertilizer can help the plant's growth and development in increasing root length, root amount, leaf amount, and leaf width (Brundrett, 2008). *Cerathoriza* as biocontrol can decrease virus infection levels on plants. Orchids have different resistance levels towards virus infection. Orchids' resistance increases as root amount and root length have increased after *Cerathoriza* induction. This research is done to find out the effectiveness of *Cerathoriza* induction towards *Phalaenopsis* and *Dendrobium* and their root viability after infected by ORSV.

According to previous studies, it was reported that the results of Ceratorhiza induction on orchids were known to be effective in inhibiting pathogens (Mahfut, 2019). The results of the study also reported that the early stages of *Ceratorhiza* induction will cause rotting in roots. The best time for *Ceratorhiza* inoculation is on the third and fourth day. It can be concluded that the faster *Ceratorhiza* contact with the orchids, the higher its ability to absorb nutrients and enhance growth (Mahfut *et al.,* 2016).

MATERIALS AND METHOD

Completely randomized factorial design was used on two factors. The first factor was the orchid type used, *Phalaenopsis amabilis* and *Dendrobium discolor* while the second factor was mycorrhiza induction (M), virus inoculation (V), and mycorrhiza induction and virus inoculation (MV), resulting in 6 treatments. Each treatment combination was repeated four times, resulting in 24 experimental units. Each experimental unit was a cup consists of growing media, one orchid plant (*Phalaenopsis amabilis* or *Dendrobium discolor)* based on treatment combination given. Variables examined are mycorrhiza effectiveness, leaf length, leaf width, leaf amount, root length, and root amount. Positive controls for *Phalaenopsis amabilis* and *Dendrobium discolor*, which are not included in the research design, also exists.

Tools used in this research were petri dish, beaker measuring glass 100 ml and 500 ml, mortar and pestel, pen, label, gloves, tissue, and camera. Materials used in this research were bottled seedlings of *Phalaenopsis amabilis* and *Dendrobium discolor*, moss sterile media, Potato Dextrose Agar media, Ceratorhiza sp., inoculum of *Odontoglossum ringspot virus* (ORSV), karborondum, phosphate buffer, water, alcohol.

This research was done through five steps below.

1. Acclimatization
   Bottled orchids are grown in the greenhouse for 2 months. After acclimatization, orchid treatment was done until 3-4 leaves have grown.
2. *Cerathoriza* inoculation
   Cerathoriza inoculation was done by growing *Cerathoriza* isolates on PDA that had been added by chloramphenicol. Isolates on petri dish was taken ± 0.5 cm and was put on the media through three spotting. Incubation was done at room temperature for 5-7 days. Isolates rejuvenation was done on 8-10 petri dishes.
3. ORSV inoculation
   ORSV inoculation towards planlet was done through sample, an ORSV infected tobacco. Tobacco leaves was ground and added with phosphate buffer with the ratio of 1:10 (m/v). Phosphate buffer was used to break down the cells, releasing the virus inside. Inoculation towards the orchid was done by rubbing carborundrum evenly, slowly, and manually by finger or cotton bud based on the vein (Calvo et al., 2020). Planlets were replanted on the media and symtomps, including necrosis, chlorosis, mosaic, leaf malformation, streak yellowing, and curling leaf, were examined during incubation time until they appear (15-30 days).
4. Examination on mycorrhiza effectiveness
   Examination on mycorrhiza effectiveness was done on the acclimated *Phalaenopsis amabilis* and *Dendrobium discolour*. Rejuvenation on *Cerathoriza* was done in 3 days and inoculation was done on the first until the fourth day. The examination was done in 30 days.
5. Examination on root viability
   Root viability examination was done by examining root length growth and root amount.

RESULTS AND DISCUSSION

Result

Mycorrhiza Effectiveness

The results of mycorrhiza inoculation performed for up to 4 days showed that hyphae enveloped the plant roots. The inoculation period indicates the thickness of the hyphae covering the plant. Myculation inclusions of *Phalaenopsis amabilis* and *Dendrobium discolor*

orchids are shown in Figure 1. The efficacy of *Ceratorhiza* performed by observing orchid root growth for post-inoculation survival is shown in Table 1.

Figure 1. Inoculation of *Ceratorhiza* on orchids (A) *Phalaenopsis amabilis* and (B) *Dendrobium discolor*.

Table 1. The number of orchid roots *Phalaenopsis* and *Dendrobium*

Orchid kind	Incubation time (Days)	Sunday				
		0	**1**	**2**	**3**	**4**
Phalaenopsis	1	7	7	7	7	7
	2	6	5	5	5	1
	3	5	4	4	4	4
	4	5	5	5	2	2
Dendrobium	1	10	10	9	8	0
	2	12	12	12	9	0
	3	10	10	9	6	6
	4	9	8	8	9	9

Based on the observations, there was a significant difference between the effectiveness of mycorrhiza and the incubation time. More effective results were obtained on the third day. The results are shown in Table 1 and Figure 2.

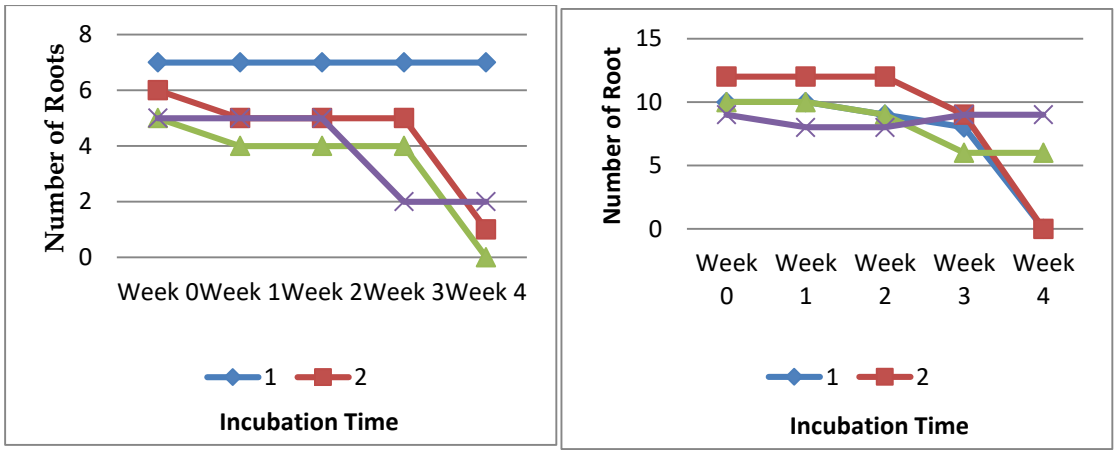

Figure 2. Mycorrhizal efficacy and its effect on the number roots

Based on the data above, there is no difference between *Phalaenopsis amabilis* and *Dendrobium discolor*. On the third day of incubation, both orchids showed their presence against

mycorrhiza. This may occur because mycorrhiza absorption has reached its maximum limit on the third day until it decreases by the 4th week.

After the mycorrhiza efficacy test was performed, observation was done from week 0 to week 5 with parameters namely, leaf length, leaf width, leaf number, root length, and root number shown in Figure 6-9. The results of the analysis using the ANOVA method are shown in Table 2-3.

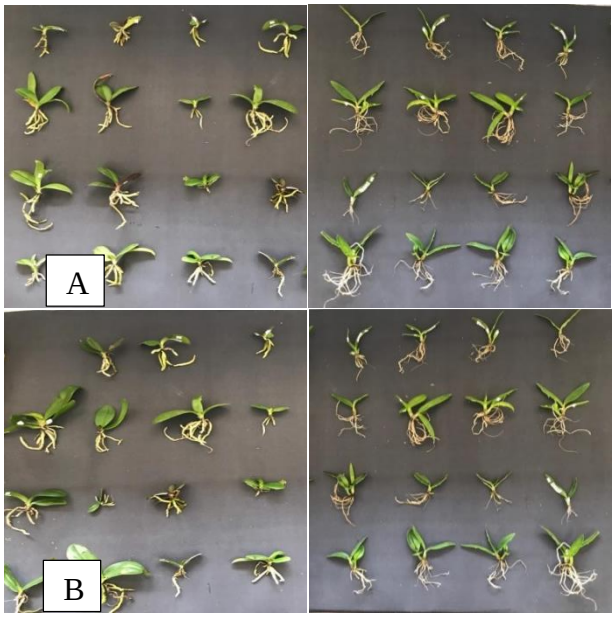

Figure 3. Development of small plants Phalaenopsis amabilis (left) and Dendrobium discolor (right) from mycorrhizal virus inoculation (MV) (A) week 0 (B) week 4.

Root Length

The initial stage of analyzing the root length observation data performed data homogeneity using the Levene test at 5%stage. The test results showed that the diversity of orchid samples were homogeneous. Further, the analysis was continued using ANOVA test, which showed that mycorrhiza (M), virus (V), and mycorrhizal virus (MV) inoculations on orchids gave significantly different results. The analysis was continued using the Tukey test at the 5% real level shown in Table 2. While the interaction between the two samples is seen on the interaction curve of Figure 6.

Based on the Tukey's test, there was a significant difference from the first factor that is shon in Figure 4. The second factors, mycorrhiza treatment, viruses, and mycorrhiza virus is not visible, this is because in the 0th week of mycorrhiza administration treatment is done after 0 week root length calculation and the result of mycorrhiza administration can be seen in week 1. While in the combination of first and second factor treatment (interaction) is also not visible.

At week 1, it can be seen that in the first factor, *Phalaenopsis amabilis* and *Dendrobium discolor* orchid types statistically did not show significant differences explained by Figure 4. In the second factor, treatment of mycorrhiza (M), virus (V), and MV mycorrhiza virus) have not been seen, this is because in the first week only mycorrhiza treatment is performed. Virus administration was performed after root length calculations for the first week and results were only seen in the 2nd week. Whereas in the combination of first and second factor treatment

(interaction) it was not visible, because at week 1 it was only visible for mycorrhiza treatment and could not be compared with other treatments.

Table 2. Tukey root length test in combination treatment of *Phalaenopsis amabilis* and *Dendrobium discolor* orchids by giving Mycorrhizae, Virus, and Mycorrhizae Virus at 0-5 weeks of age.

Sunday-	Factor B = Species	Factor A = Treatment			Marginal mean	K
		M	V	MV		
0	*Phalaenopsis*	3.45a ± 0.79	3.85a ± 0.58	2.76a ± 0.32	3.35a	3.66
	Dendrobium	3.61a ± 0.34	4.57a ± 0.38	4.80a ± 0.25	4.32b	4.64
	Marginal mean	3.53a	4.21a	3.78a		
	HSD Cell [0.05] = 2.17 ; Columns[0.05] = 1.23 ; Rows[0.05] = 0.83					
1	*Phalaenopsis*	3.37a ± 0.92	3.81a ± 0.28	2.42a ± 0.36	3.2a	3.65
	Dendrobium	3.51a ± 0.32	4.45a ± 0.15	4.59a ± 0.16	4.18a	4.66
	Marginal mean	3.44a	4.13a	3.50a		
	HSD Cell[0.05] = 2.02 ; Columns[0.05] = 1.14 ; Rows[0.05] = 0.77					
2	*Phalaenopsis*	2.88 ± 1.17	3.57 ± 0.47	2.43 ± 0.33	2.96a	3.45
	Dendrobium	3.41 ± 0.16	4.27 ± 0.25	4.63 ± 0.13	4.10a	4.01
	Marginal mean	3.14a	3.92a	3.53a		
	HSD Cell [0.05] = 2.47 ; Columns[0.05] = 1.4 ; Rows[0.05] = 0.94					
3	*Phalaenopsis*	2.74 ± 1.03	3.59 ± 0.53	2.19 ± 0.47	2.84a	3.16
	Dendrobium	2.87 ± 0.29	4.37 ± 0.27	4.63 ± 0.2	3.95a	3.63
	Marginal mean	2.80b	3.98a	3.41a		
	HSD Cell [0.05] = 2.44 ; Columns[0.05] = 1.39 ; Rows[0.05] = 0.93					
4	*Phalaenopsis*	2.74 ± 1.03	3.20 ± 0.54	2.18 ± 0.46	2.70a	3.20
	Dendrobium	2.39 ± 0.7	3.46 ± 0.1	4.27 ± 0.35	3.37a	2.82
	Marginal mean	2.56a	3.33a	3.22a		
	HSD Cell [0.05] = 2.72 ; Columns[0.05] = 1.54 ; Rows[0.05] = 1.04					
5	*Phalaenopsis*	2.70 ± 1.05	3.22 ± 0.53	2.21 ± 0.47	2.71a	3.21
	Dendrobium	2.03 ± 0.75	3.48 ± 0.1	4.28 ± 0.34	3.26a	2.83
	Marginal mean	2.36a	3.35a	3.24a		
	HSD Cell [0.05] = 2.78 ; Columns[0.05] = 1.58 ; Rows[0.05] = 1.06					

From week 2 to week 5, it can be seen that in the first factor, the orchid varieties *Phalaenopsis amabilis* and *Dendrobium discolor* statistically did not show significant differences explained by Figure 4.In the second factor, mycorrhiza (M) treatment, virus (V), and viral mycorrhiza (MV) did not show significant differences described in Figure 5. Whereas in the combination of the first and second factors the treatment (interaction) showed insignificant results, described in Figure 6.

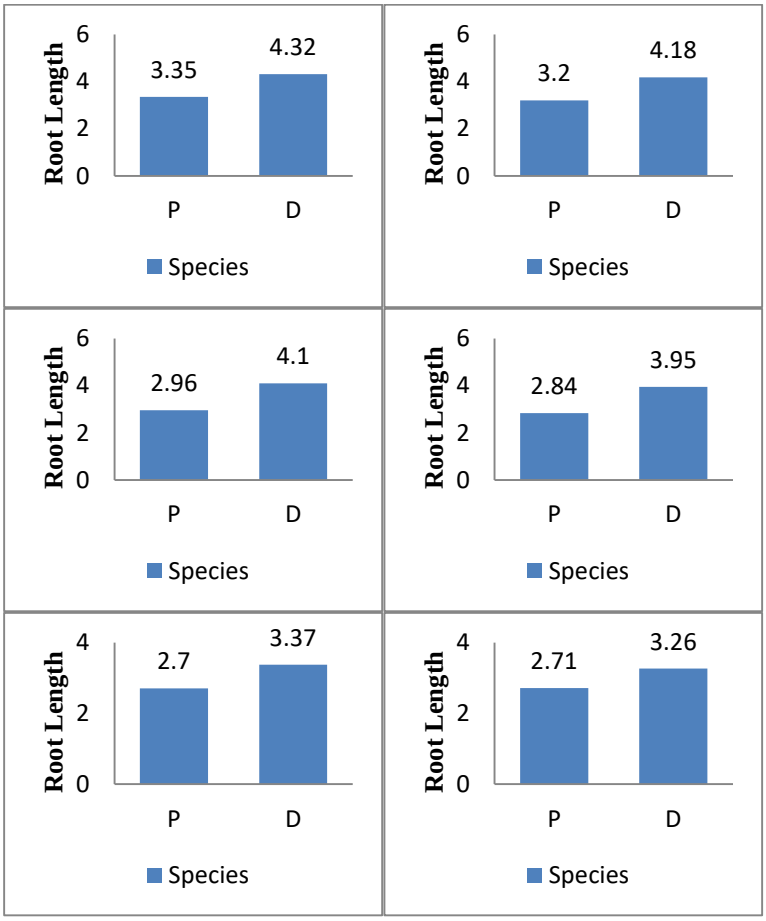

Figure 4. Comparative diagram of root length for Phalaenopsis amabilis and Dendrobium discolor

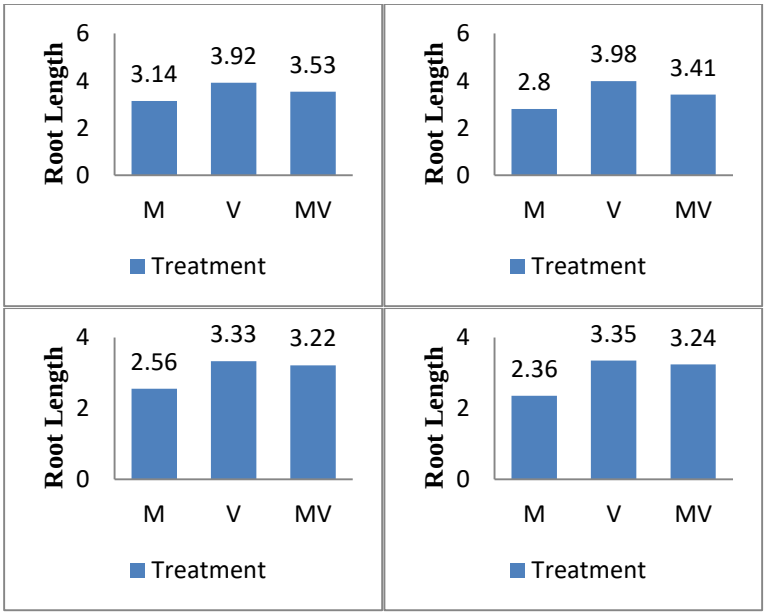

Figure 5. Comparative diagram of root length against the treatment of Phalaenopsis amabilis and Dendrobium discolor

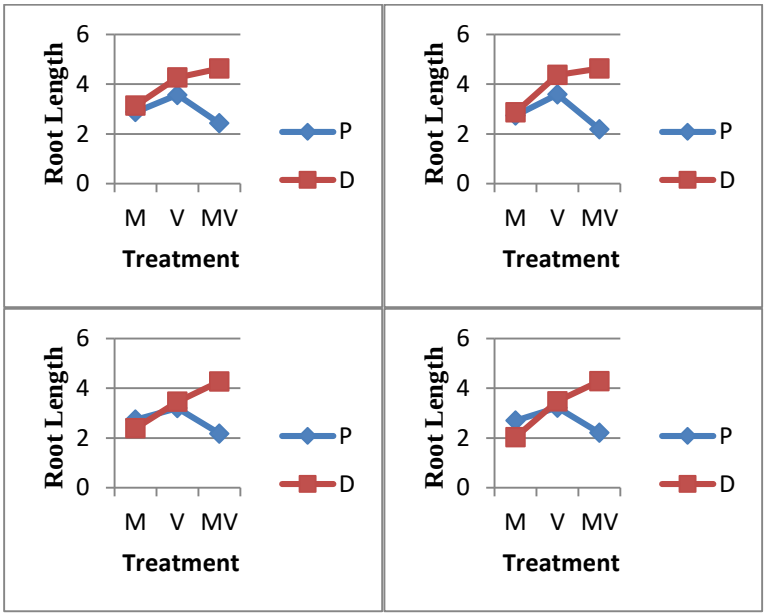

Figure 6. Interaction of Root Length with Mycorrhizae, Viruses, and Viral Mycorrhizae

Number of Roots

The initial stage of data analysis, observation of the number of roots, perform data homogeneity using Levene test at 5%stage. The test results showed that the diversity of samples on orchids was homogeneous. The analysis was continued using ANOVA test, which showed that mycorrhizal (M), virus (V), and mycorrizhal virus (MV) inoculations on orchids gave significantly different results. The analysis was continued using the Tukey test at the actual level of 5% as shown in Table 3. While the interaction between the two samples is seen on the interaction curve of Figure 9.

Based on the Tukey test at the actual level of 5% conducted (Table 3), it can be seen that on the first factor, *Phalaenopsis amabilis* and *Dendrobium discolor* orchids types statistically showed significant differences explained in Figure 7. factors, mycorrhiza treatment, virus, and mycorrhiza did not can be seen, this is because in week 0 mycorrhiza administration treatment is done after counting the number of roots in week 0 and the result of mycorrhiza administration can be seen in week 1. While in the combination of first and second factor treatment (interaction) is also not visible.

At week 1, it can be seen that in the first factor, Phalaenopsis amabilis and Dendrobium discolor orchid types statistically did not show significant differences explained by Figure 7. In the second factor, treatment of mycorrhizal (M), virus (V), and mycorrhizal virus (MV) has not been seen, this is because in the first week only mycorrhizal treatment is done. Virus administration was performed after the calculation of the number of roots in the 1st week and the results were only visible in the 2nd week. Whereas in the combination of first and second factor treatment (interaction) it was not visible, because at week 1 it was only visible for mycorrhizal treatment and could not be compared with other treatments.

From week 2 to week 5, it can be seen that on the first factor, the orchid types *Phalaenopsis amabilis* and *Dendrobium discolor* statistically did not show significant differences explained by Figure 7. In the second factor, mycorrhiza (M), virus (V) treatment, and viral mycorrhiza (MV) did not show the significant differences described in Figure 8. Although the treatment

combination of the first and second factors (interactions) showed insignificant results, it was explained in Figure 9.

Table 3. Total Root Tuky Test in combination treatment of Phalaenopsis amabilis and Dendrobium discolored orchids by administering Mycorrhiza Virus, Virus, Mycorrhizae at the age of 0-5 weeks

Sunday -	Factor B = Species	Factor A = Treatment			Marginal mean	K
		M	V	MV		
0	*Phalaenopsis*	5.25a ± 1.18	5.75a ± 0.75	5.75a ± 0.63	5.58a	5.25
	Dendrobium	6.5a ± 1.66	7.75a ± 0.48	8.75a ± 0.63	7.66a	8
	Marginal mean	5.87a	6.75a	7.25a		
	HSD Cell [0.05] = 4.4 ; Columns[0.05] = 2.5 ; Rows[0.05] = 1.68					
1	Anggrek *Phalaenopsis*	4.75a ± 1.31	6a ± 0.91	5.75a ± 0.48	5.5a	6.5
	Dendrobium	6.25a ± 1.55	7.75a ± 0.48	8a ± 0.41	7.33b	8.5
	Marginal mean	5.5a	6.87a	6.87a		
	HSD Cell[0.05] = 4.35 ; Columns[0.05] = 2.47 ; Rows[0.05] = 1.66					
2	*Phalaenopsis*	4.75a ± 1.31	6.5a ± 0.65	6a ± 0.71	5.75a	6.5
	Dendrobium	7a ± 1.87	8.25a ± 0.63	8.25a ± 0.75	7.83b	8
	Marginal mean	5.87a	7.37a	7.12a		
	HSD Cell [0.05] = 4.9 ; Columns[0.05] = 2.78 ; Rows[0.05] = 1.87					
3	*Phalaenopsis*	5a ± 1.47	6.5a ± 0.65	6a ± 0.71	5.83a	7.5
	Dendrobium	7.75a ± 1.65	8.25a ± 0.63	8.25a ± 0.85	8.08b	8.25
	Marginal mean	6.37a	7.37a	7.12a		
	HSD Cell [0.05] = 4.85 ; Columns[0.05] = 2.75 ; Rows[0.05] = 1.85					
4	*Phalaenopsis*	5a ± 1.47	6.5a ± 0.65	6a ± 0.71	5.83a	7
	Dendrobium	6.75a ± 2.02	7,25a ± 0.48	7.5a ± 0.65	7.16a	6.5
	Marginal mean	5.87a	6.87a	6.75a		
	HSD Cell [0.05] = 5.14 ; Columns[0.05] = 2.91 ; Rows[0.05] = 1.96					
5	*Phalaenopsis*	5.25a ± 1.38	6.5a ± 0.65	6a ± 0.71	5.91a	7
	Dendrobium	7a ± 1.78	7.25a ± 0.48	7.5a ± 0.65	7.25a	6
	Marginal mean	6.12a	6.87a	6.75a		
	HSD Cell [0.05] = 4.74 ; Columns[0.05] = 2.69 ; Rows[0.05] = 1.8					

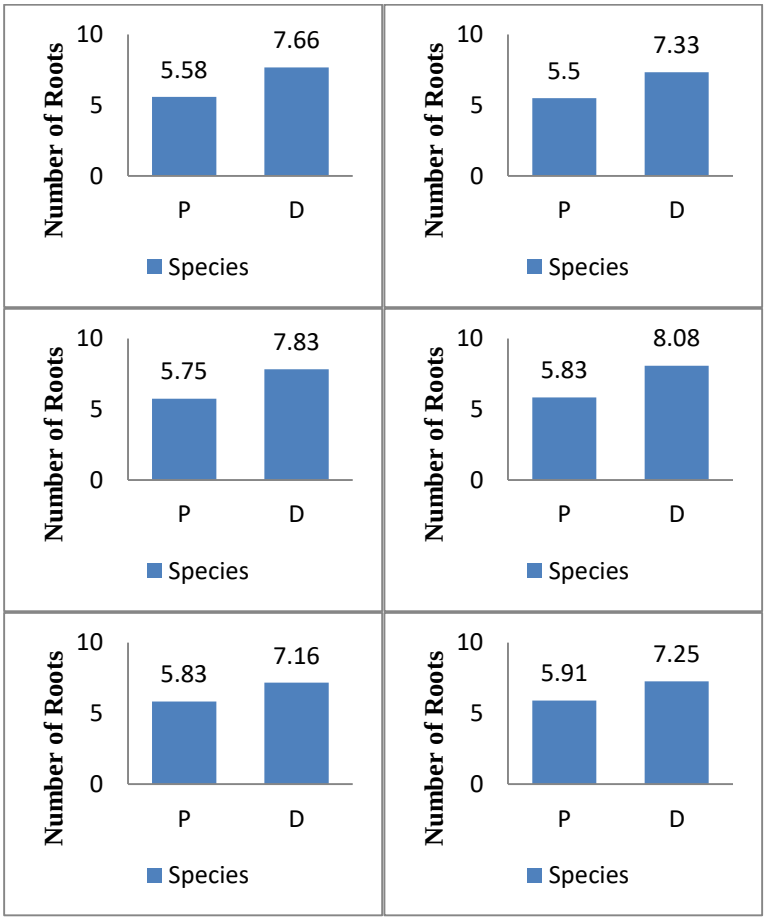

Figure 7. Comparative diagram of the number of roots of Phalaenopsis amabilis and Dendrobium discolor orchids.

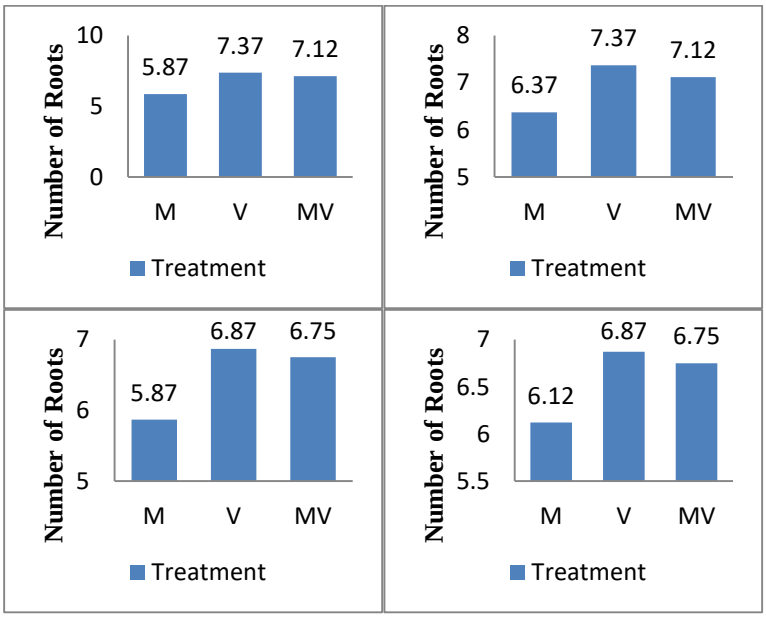

Figure 8. Comparative diagram of the number of treatment roots of Phalaenopsis amabilis and Dendrobium discolor orchids

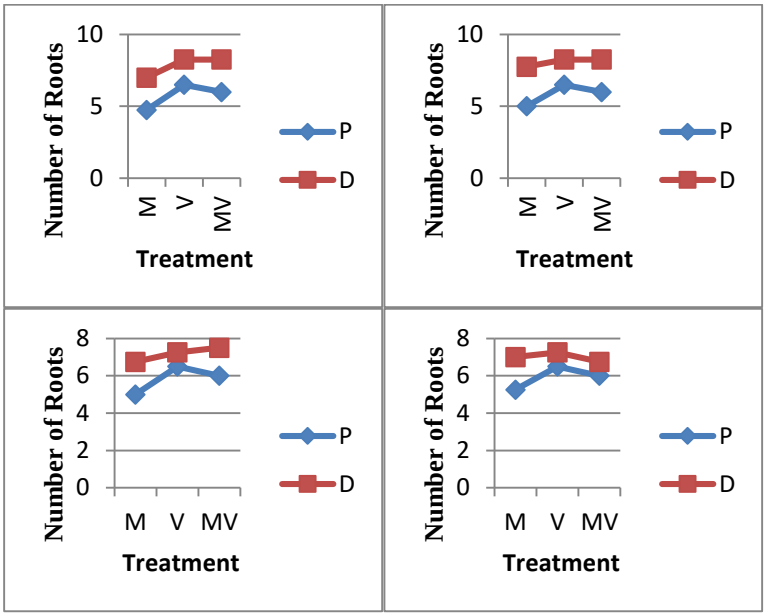

Figure 9. Graph of Number of Root Interactions Against Mycorrhizae Viruses, Viruses, and Mycorrhizae

Discussion

The research was conducted with observation for 4 weeks. The parameters observed were mycorrizhal efficacy test, leaf length, leaf width, number of leaves, root length, and number of roots on *Phalaenopsis amabilis* and *Dendrobium discolor* orchids. *Phalaenopsis* and *Dendrobium* orchids have been inoculated with *Ceratorhiza* and viruses. Khaterine (2016) explained that live orchids are associated with endophytic fungi. Mycorrhiza treatment can help improve the soil structure around the plant and can increase the length, width, and number of leaves as well as increase the length and number of roots. Meanwhile, viral treatment was used to see the resistance of mycorrhizas inoculated in orchids. In previous research by Lakani (2015), it is explained that the resistance of orchid plants can be seen on inoculated and uninoculated leaves.

Effectiveness of Mycorrhizae

Ceratorhiza efficacy test results on Phalaenopsis and Dendrobium were performed by observing growth and survival skills. Based on observations it is known that *Ceratorhiza* inoculation is most effective on the 3rd day. At the 3rd and 4th week observations the efficacy of *Ceratorhiza* showed mortality. Until the entire data was used only up to the second week of observation. This was done to avoid biased data if the entire observational data was applied up to the 4th week. Observations made from week 0 to week 2 could answer questions about the effectiveness of *Ceratorhiza* inoculation. This is supported by previous research by Mahfut (2020) who explained that mycorrhiza of *Trichoderma* and *Ceratorhiza* types showed that the best inoculation time was found on the 3rd and 4th day of inoculation.

The effectiveness of *Ceratoriza* can also be seen in the increase in the number of roots and leaves. *Ceratorhiza* treatment can increase the absorption of nutrients to enhance plant growth. Mahfut (2019) explained that induction of mycorrhiza types Ceratorhiza and *Trichoderma* can be used as *biofertilizers*. *Ceratorhiza* treatment on the 3rd and 4th day had more effect on the increased root volume. This is supported by a previous study by Mahfut (2019) who explained that *Ceratorhiza* has an influence on the number of dead roots compared to *Trichoderma* inoculation.

Ceratorhiza mycorrhizae can assist orchids in their growth and life cycle. Mahfut (2019) explains that *Ceratorhiza* mycorrhizae can infect the roots of orchid plants and produce intensive hyphae and can increase plant capacity. At week 3 and week 4 mycorrhiza inoculation the number of roots decreased, this may be due to environmental factors. According to a previous study by Kurnia (2019) regarding mycorrhiza characteristics, it was said that soil type is a factor that affects the types of mycorrhizae. Other factors that can affect plant growth are temperature, light, water, nutrients, and soil.

Root Length

Based on the data obtained from the analysis results of week 0 to week 5 on the root length variable, there was no significant difference. From week 1 to week 5, the number of leaves has decreased. This is due to treatment of mycorrhiza (M), virus (V), and mycorrhiza virus (MV), giving the virus causing damage to the leaves. If with *Phalaenopsis* control (3.38) (K); (2.96) (P) and *Dendrobium* (3.76) (K); (3.86) (P) on treatment showed higher outcomes than control. However, if viewed based on the type between the two orchids, Dendrobium better maintain the long growth of its roots compared to *Phalaenopsis*.

Mycorrhiza inoculated at the roots play a role in the growth and development of orchids for survival. Decreased root length growth can be caused by poor physiological processes. This is reinforced by the study of Lakit (2000) who explained that plant growth is a network of plant physiological processes in forming complex organ units with the addition of plant weight and size.

Mycorrhiza inoculated at the roots play a role in the growth and development of orchids for survival. Decreased root length growth can be caused by poor physiological processes. This is reinforced by the study of Lakitan (2000) who explained that plant growth is a network of plant physiological processes in forming complex organ units with the addition of plant weight and size.

Number of Roots

Based on the data obtained from the results of the analysis of week 0 to week 5 on the number of variable causes there was no significant difference. From week 1 to week 5, the number of leaf decreased. If with *Phalaenopsis* control 6.62 (K); 5.73 (P) and *Dendrobium* 7.54 (K); 7.55 (P) is similar to the root length variable indicating that *Phalaenopsis* treatment showed lower results compared to controls. Whereas in *Dendrobium*, when compared with control, the treatment showed higher results. This is due to treatment of mycorrhiza (M), virus (V), and mycorrhiza virus (MV), giving the virus causing leaf damages. However, if viewed based on the type between the two orchids, Dendrobium better maintains the long growth of its roots compared to Phalaenopsis.

Rianti (2017) explained that the number of plant roots indicates how wide the plant's reach is to absorb nutrients and nutrients in mycorrhiza (M) treatment, indicating that the ability of orchids to form roots is because they already have leaflets. This is supported by Bey et al., (2006), who explained that radicles will turn into roots with the help of auxin processed by leaves.

CONCLUSION

Cerathoriza induction can increase the effectiveness of *Phalaenopsis amabilis* and *Dendrobium discolor* with 3 days of incubation. Root viability drops in *Phalaenopsis amabilis* with the root

length of 2.96 cm and root amount of 5.73, while rises in *Dendrobium discolor* with the root length of 3.86 cm and root amount of 7.55.

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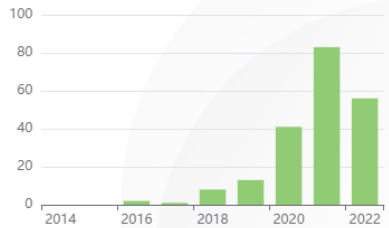

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TITLE AND ABSTRACT

Title: Ceratorhiza Induction towards ORSV Infection on Viability of Phalaenopsis and Dendrobium

Abstract

Orchids have a high level of biodiversity, such as *Dendrobium* and *Phalaenopsis*, which are epiphytes. *Dendrobium* can adapt to the condition of where it lives while *Phalaenopsis* can grow in highlands and depends on sunlight and humidity. Virus infection has become one of the obstacles in cultivating *Dendrobium* and *Phalaenopsis*. Efforts to increase fitness and control in *Dendrobium* and *Phalaenopsis* cultivation can be done by inducing the plant's fitness using a mycorrhiza, such as Ceratorhiza. A mycorrhiza is a form of mutualism between fungi and the plant's root. This research is aimed to give information related to the utilization of Cerathoriza for inducing orchids to suppress *Odontoglossum ringspot virus* (ORSV) infection, giving it better growth. The research was done in February-March 2021 at Botany Laboratory University of Lampung. A completely randomized factorial design was used on two factors, kind of orchid and mycorrhiza treatment (M), virus (V), and mycorrhiza virus (MV). Variables examined in this research are the amount of living and dead roots and leaves. Data obtained is homogenized using Levene's test and continued by ANOVA with the

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significance level of 5% and further testing using Tukey's test with the significance level of 5%. From this research, it is known that interaction between *Phalaenopsis* and *Dendrobium* exists during virus and mycorrhiza administration. It is concluded that *Phalaenopsis anabilis* is more vulnerable than *Dendrobium discolor*.

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Ceratorhiza Induction towards ORSV Infection on Viability of *Phalaenopsis* and *Dendrobium*

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ABSTRACT

Orchids are one of the plants with high level of biodiversity, such as *Dendrobium* and *Phalaenopsis*, which are epiphytes. *Dendrobium* is able to adapt to the condition of where it lives while *Phalaenopsis* can grow in highlands and depends on sunlight and humidity. Virus infection has become one of the obstacles in cultivating *Dendrobium* and *Phalaenopsis*. Efforts to increase fitness and control in *Dendrobium* and *Phalaenopsis* cultivation can be done by inducing the plant's fitness using a mycorrhiza, such as Ceratorhiza. Mycorrhiza is a form of mutualism between fungi and the plant's root. This research is aimed to give information related to the utilization of Cerathoriza for inducing orhcids to suppress *Odontoglossum ringspot virus* (ORSV) infection, giving it a better growth. The research was done in February-March 2021 in Botany Laboratory University of Lampung. Completely randomized factorial design was used on two factors, kind of orchid and mycorrhiza treatment (M), virus (V), and mycorrhiza virus (MV). Variables examined in this research are amount of living and dead roots, amount of living and dead leaves. Data obtained is homogenized using Levene's test and continued by ANOVA with the significance level of 5% and further testing using Tukey's test with the significance level of 5%. From this research it is known that an interaction between *Phalaenopsis* and *Dendrobium* exists during virus and mycorrhiza administration. It is concluded that *Phalaenopsis anabilis* is more vulnerable than *Dendrobium discolor*.

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Keywords: *Ceratorhiza*, *Dendrobium*, ORSV, *Phalaenopsis*

ABSTRAK

Anggrek merupakan salah satu tumbuhan yang memiliki keanekargaman sangat tinggi diantaranya *Dendrobium* dan *Phaleonopsis* yang merupakan anggrek epifit. Anggrek *Dendrobium* adalah salah satu anggrek yang mampu beradaptasi dengan kondisi tempat tumbuh anggrek. *Phalenopsis* merupakan anggrek epifit yang dapat tumbuh di daerah ketinggian dan membutuhkan cahaya serta kelembaban. Kendala dalam budidaya anggrek *Dendrobium* dan *Phaleonopsis* salah satunya karena adanya infeksi virus. Upaya peningkatan ketahanan dan pengendalian budidaya anggrek *Dendrobium* dan *Phaleonopsis* dapat dilakakukan dengan menginduksi ketahanan tanaman menggunakan mikoriza *Ceratorhiza*. Mikoriza merupakan suatu bentuk simbiosis mutualistik antara jamur dan akar tanaman. Tujuan dari penelitian ini dapat memberikan informasi mengenai pemanfaatan mikoriza *Ceratorhiza* untuk menginduksi

tanaman anggrek agar dapat menahan infeksi virus *Odontoglossum ringspot virus* (ORSV), sehingga pertumbuhannya lebih baik. Waktu dan tempat penelitian dilakukan pada bulan Februari-Maret 2021 di Laboratorium Botani Universitas Lampung. Pada penelitian ini menggunakan Metode rancangan acak lengkap faktorial (RALF) yang terdiri dari dua faktor, yaitu jenis anggrek dan perlakuan mikoriza (M), virus (V), dan mikoriza virus (MV). Variabel yang diamati pada penelitian ini adalah jumlah akar yang hidup dan mati, jumlah daun yang hidup dan mati. Data yang diperoleh dihomogenkan dengan menggunakan uji Levene kemudian dianalisis ragam pada taraf nyata 5% menggunakan ANOVA dan uji lanjut dengan Tukey pada taraf nyata 5%. Hasil penelitian menunjukkan bahwa jenis anggrek *Phalaenopsis amabilis* lebih rentan dibandingkan *Dendrobium discolor* pada perlakuan mikoriza (M), virus (V), dan mikoriza virus (MV). Terdapat interaksi antara *Phalaenopsis* dan *Dendrobium* pada variabel jumlah daun pada perlakuan mikoriza (M) dan virus (V).

Kata Kunci: *Ceratorhiza*, *Dendrobium*, ORSV, *Phalaenopsis*

INTRODUCTION

Orchids is one of the biggest family in Indonesia (Soetopo, 2009). Orchids are widely-spread and can be found in tropical forests in West Sumatera, Java, Kalimantan, Sulawesi, Nusa Tenggara, Maluku, and Papua (Rukmana, 2000). Population of orchids in their natural habitat have been decreasing due to deforestation and forest over-exploitation (Johanis et al., 2001). Orchids are categorized as epiphytes and depend on their hosts. The loss of orchids' host plants will significantly ruin orhcids' life cycle (Ambari, 2016).

Comment [S2]: add the results of t
analysis to previous research tha
is relevant to this study.

give an overview regarding the
originality of this research

Comment [S3]: Use Mendeley Application

There are two types of orchids, namely terrestrial and epiphyte. Terrestrial orchids are orchids that grow and develop on land (Dina and Soetopo, 2019) while epiphyte orchids are orchids that live on host plants but are not parasite. Most cultivated orchids are *Phalaenopsis* and *Dendrobium* (Yudistira *et al.*, 2011). Virus infection has become the biggest challenge in orchids' cultivation and potential development (Kumalawati et al., 2011). Odontoglossum ringspot virus (ORSV) infects most orchids. Based on previous research by Mahfut (2016), symptomps caused by ORSV include mosaic, streak, chlorotic, and necrosis. Orchids protection measures can be done by genetic recombination resulting ORSV-resistant orchids. Therefore, mycorrhiza, such as *Cerathoriza* sp., is used as it is more eco-friendly and effective.

Mycorrhiza is a mutualistic symbiosis between fungi and root. Plants obtain nutrient absorbed by fungi while fungi obtain nutrient resulted from assimilations done by plants. Mycorrhiza infected the root system of the plant hosts, producing hyphae intensively. *Cerathoriza*'s role as biofertilizer can help the plant's growth and development in increasing root length, root amount, leaf amount, and leaf width (Brundrett, 2008). *Cerathoriza* as biocontrol can decrease virus infection level on plants. Orchids have different resistance level towards virus infection. Orchids' resistance increases as root amount and root length have increased after *Cerathoriza* induction. This research is done to find out the effectivity of *Cerathoriza* induction towards *Phalaenopsis* and *Dendrobium* and their root viability after infected by ORSV.

MATERIALS AND METHOD (12, bold)

Completely randomized factorial design was used on two factors. The first factor was the orhcid type used, *Phalaenopsis amabilis* and *Dendrobium discolor* while the second factor was

mycorrhiza induction (M), virus inoculation (V), and mycorrhiza induction and virus inoculation (MV), resulting in 6 treatments. Each treatment combination was repeated four times, resulting in 24 experimental units. Each experimental unit was a cup consists of growing media, one orchid plant (*Phlaenopsis amabilis* or *Dendrobium discolor)* based on treatment combination given. Variables examined are mycorrhiza effectivity, leaf length, leaf width, leaf amount, root length, and root amount. Positive controls for *Phlaenopsis amabilis* and *Dendrobium discolor*, which are not included in the research design, also exists.

Tools used in this research were petri dish, beaker measuring glass 100 ml and 500 ml, mortar and pestel, pen, label, gloves, tissue, and camera. Materials used in this research were bottled seedlings of *Phalaenopsis amabilis* and *Dendrobium discolor*, moss sterile media, Potato Dextrose Agar media, Ceratorhiza sp., inoculum of *Odontoglossum ringspot virus* (ORSV), karborondum, phosphate buffer, water, alcohol.

This research was done through five steps below.

1. Acclimatization
   Bottled orhcids are grown in the greenhouse for 2 months. After acclimatization, orchid treatment was done until 3-4 leaves have grown.
2. *Cerathoriza* inoculation
   Cerathoirza inoculation was done by growing *Cerathoriza* isolates on PDA that had been added by chloramphenicol. Isolates on petri dish was taken ± 0.5 cm and was put on the media through three spotting. Incubation was done at room temperature for 5-7 days. Isolates rejuvenation was done on 8-10 petri dishes.
3. ORSV inoculation
   ORSV inoculation towards planlet was done through sample, an ORSV-infected tobacco. Tobacco leaves was grinded and added with phosphate buffer with the ratio of 1:10 (m/v). Phosphate buffer was used to break down the cells, releasing the virus inside. Inoculation towards the orchid was done by rubbing carborondrum evenly, slowly, and manually by finger or cutton bud based on the vein (Calvo et al., 2020). Planlet was replanted on the media and symptomps, including necrosis, chlorosis, mosaic, leaf malformation, streak yellowing, and curling leaf, were examined during incubation time until they appear (15-30 days).
4. Examination on mycorrhiza effectivity
   Examination on mycorrhiza effectivity was done on the aclimated *Phalaenopsis amabilis* and *Dendrobium discolour*. Rejuvenation on *Cerathoriza* was done 3 days and inoculation was done on the first until the fourth day. The examination was done in 30 days.
5. Examinaiton on root viability
   Root viability examination was done by examining root length growth and root amount.

RESULTS AND DISCUSSION (12, bold)

Result

1. Effectiveness of Mycorrhizae
   The results of mycorizal inoculation performed up to 4 days showed that hyphae enveloped the plant roots. The inoculation period indicates the thickness of the hyphae covering the plant. Myculation inclusions of Phalaenopsis amabilis and Dendrobium discolor orchids are shown in Figure 1.

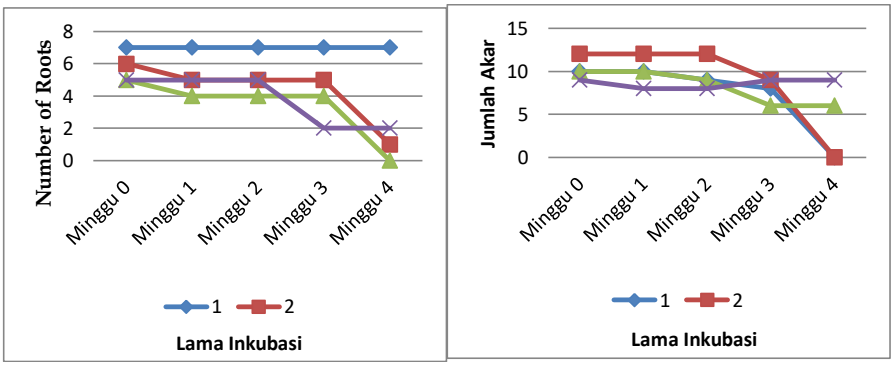
Figure 1. Inoculation of Ceratorhiza on orchids (A) *Phalaenopsis amabilis* and (B) *Dendrobium discolor*.

The efficacy of Ceratorhiza performed by observing orchid root growth for post-inoculation survival is shown in Table 1.

Tabel 1. The number of orchid roots *Phalaenopsis* and *Dendrobium*

Jenis Anggrek	Lama Inkubasi	Minggu				
		0	1	2	3	4
Phalaenopsis	1	7	7	7	7	7
	2	6	5	5	5	1
	3	5	4	4	4	4
	4	5	5	5	2	2
Dendrobium	1	10	10	9	8	0
	2	12	12	12	9	0
	3	10	10	9	6	6
	4	9	8	8	9	9

Based on the results of the observations that have been made, this indicates a significant difference in the effectiveness of mycorrhiza based on the incubation time. The data showed that on the third day mycorrhizae showed more effective results in mycorrhizal inoculation of orchid roots. The results are shown in Table 1 and Figure 2.



Figur 2. Graph of mycorrhizal efficacy on the number of orchid roots

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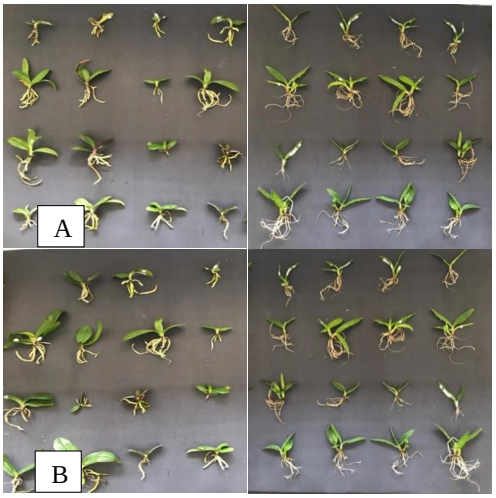
Based on the above data, there is no difference between the root chart of Phalaenopsis amabilis and Dendrobium discolor. On the 3rd day of incubation, both orchids showed their presence against mycorrhiza. This may occur because mycorrhizal absorption has reached its maximum limit on the third day until it decreases by the 4th week.

After mycorrhizal efficacy test was performed, observations were made from week 0 to week 5 with parameters namely, leaf length, leaf width, leaf number, root length, and root number shown in Figure 6-9. The results of the analysis using the ANOVA method are shown in Table 2-3.



Figur 3. Development of small plants Phalaenopsis amabilis (left) and Dendrobium discolor (right) from mycorrhizal virus inoculation (MV) (A) week 0 (B) week 4.

2. Root Length

   The initial stage of analyzing the root length observation data performed data homogeneity using Levene test at 5%stage. The test results showed that the diversity of samples on orchids was homogeneous. Further, the analysis was continued using ANOVA test, which showed that mycorizal (M), virus (V), and mycorizal virus (MV) inoculations on orchids gave significantly different results. The analysis was continued using the Tukey test at the 5% real level shown in Table 2. While the interaction between the two samples is seen on the interaction curve of Figure 6.

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Table 2. Tukey root length test in combination treatment of *Phalaenopsis amabilis* and *Dendrobium discolor* orchids by giving Mycorrhizae, Virus, and Mycorrhizae Virus at 0-5 weeks of age.

Minggu ke-	Faktor B = Spesies	Faktor A = Perlakuan			Marginal mean	K
		M	V	MV		
0	*Phalaenopsis*	3.45a ± 0.79	3.85a ± 0.58	2.76a ± 0.32	3.35a	3.66
	Dendrobium	3.61a ± 0.34	4.57a ± 0.38	4.80a ± 0.25	4.32b	4.64
	Marginal mean	3.53a	4.21a	3.78a		
	HSD Cell [0.05] = 2.17 ; Columns[0.05] = 1.23 ; Rows[0.05] = 0.83					
1	*Phalaenopsis*	3.37a ± 0.92	3.81a ± 0.28	2.42a ± 0.36	3.2a	3.65
	Dendrobium	3.51a ± 0.32	4.45a ± 0.15	4.59a ± 0.16	4.18a	4.66
	Marginal mean	3.44a	4.13a	3.50a		
	HSD Cell[0.05] = 2.02 ; Columns[0.05] = 1.14 ; Rows[0.05] = 0.77					
2	*Phalaenopsis*	2.88 ± 1.17	3.57 ± 0.47	2.43 ± 0.33	2.96a	3.45
	Dendrobium	3.41 ± 0.16	4.27 ± 0.25	4.63 ± 0.13	4.10a	4.01
	Marginal mean	3.14a	3.92a	3.53a		
	HSD Cell [0.05] = 2.47 ; Columns[0.05] = 1.4 ; Rows[0.05] = 0.94					
3	*Phalaenopsis*	2.74 ± 1.03	3.59 ± 0.53	2.19 ± 0.47	2.84a	3.16
	Dendrobium	2.87 ± 0.29	4.37 ± 0.27	4.63 ± 0.2	3.95a	3.63
	Marginal mean	2.80b	3.98a	3.41a		
	HSD Cell [0.05] = 2.44 ; Columns[0.05] = 1.39 ; Rows[0.05] = 0.93					
4	*Phalaenopsis*	2.74 ± 1.03	3.20 ± 0.54	2.18 ± 0.46	2.70a	3.20
	Dendrobium	2.39 ± 0.7	3.46 ± 0.1	4.27 ± 0.35	3.37a	2.82
	Marginal mean	2.56a	3.33a	3.22a		
	HSD Cell [0.05] = 2.72 ; Columns[0.05] = 1.54 ; Rows[0.05] = 1.04					
5	*Phalaenopsis*	2.70 ± 1.05	3.22 ± 0.53	2.21 ± 0.47	2.71a	3.21
	Dendrobium	2.03 ± 0.75	3.48 ± 0.1	4.28 ± 0.34	3.26a	2.83
	Marginal mean	2.36a	3.35a	3.24a		
	HSD Cell [0.05] = 2.78 ; Columns[0.05] = 1.58 ; Rows[0.05] = 1.06					

the first factor, the types of Phalaenopsis amabilis and Dendrobium discolor orchids statistically showed significant differences explained in Figure 4. factors, mycorrhizal treatment, viruses, and mycorrhizal virus is not visible, this is because in the 0th week of mycorrhizal administration treatment is done after 0 week root length calculation and the result of mycorrhizal administration can be seen in week 1. While in the combination of first and second factor treatment (interaction) is also not visible.

At week 1, it can be seen that in the first factor, Phalaenopsis amabilis and Dendrobium discolor orchid types statistically did not show significant differences explained by Figure 4. In the second factor, treatment of mycorrhiza (M), virus (V), and MV mycorrhiza virus) have not been seen, this is because in the first week only mycorrhizal treatment is performed. Virus administration was performed after root length calculations for the first week and results were only seen in the 2nd week. Whereas in the combination of first and second factor treatment (interaction) it was not visible, because at week 1 it was only visible for mycorrhizal treatment and could not be compared with other treatments.

From week 2 to week 5, it can be seen that in the first factor, the orchid varieties Phalaenopsis amabilis and Dendrobium discolor statistically did not show significant differences explained by Figure 4.In the second factor, mycorrhizal (M) treatment, virus (V), and viral mycorrhiza (MV) did not show significant differences described in Figure 5. Whereas in the combination of the first and second factors the treatment (interaction) showed insignificant results, described in Figure 6.

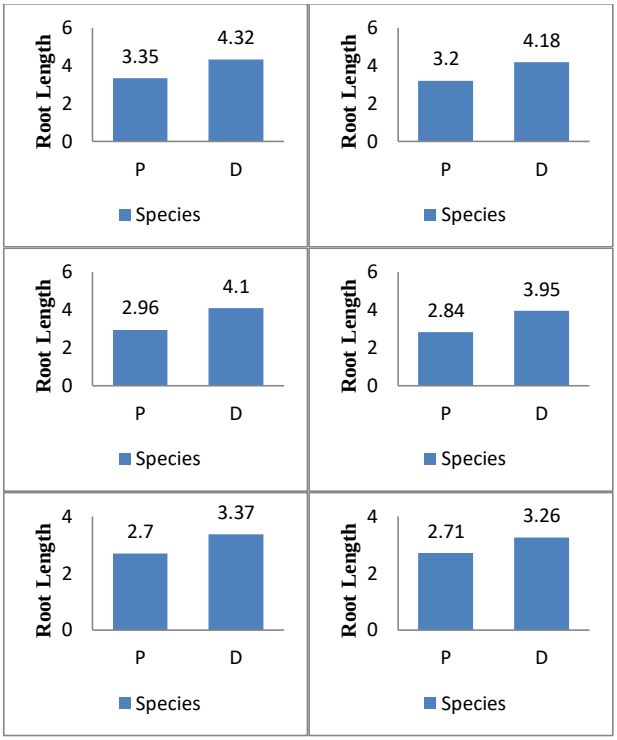

Figure 4. Comparative diagram of root length for Phalaenopsis amabilis and Dendrobium discolor orchids

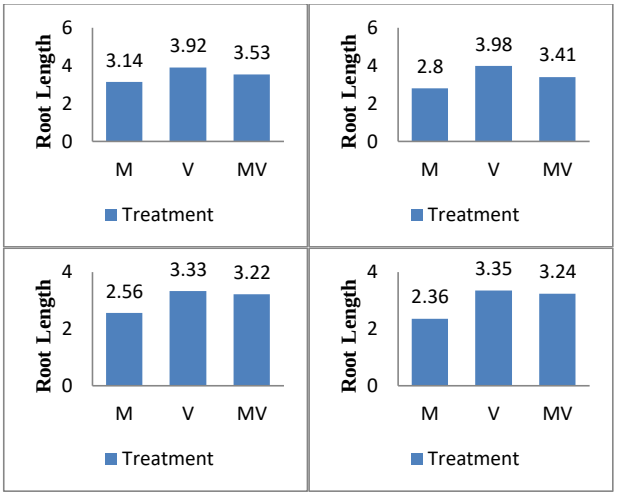

Figure 5. Comparative diagram of root length against treatment of Phalaenopsis amabilis and Dendrobium discolor orchids

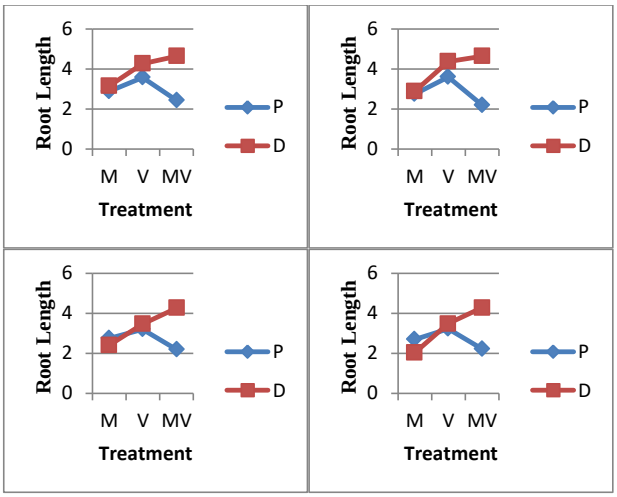

Figure 6. Graph of Root Length Interaction with Mycorrhizae, Viruses, and Viral Mycorrhizae

1. **Number of Roots**

The initial stage of data analysis, observation of the number of roots, perform data homogeneity using Levene test at 5%stage. The test results showed that the diversity of samples on orchids was homogeneous. Further, the analysis was continued using ANOVA test, which showed that mycorizal (M), virus (V), and mycorizal virus (MV) inoculations on orchids gave significantly different results. The analysis was continued using the Tukey test at the actual level of 5% as shown in Table 3. While the interaction between the two samples is seen on the interaction curve of Figure 9.

Table 3. Total Root Tuky Test in combination treatment of Phalaenopsis amabilis and Dendrobium discolored orchids by administering Mycorrhizae Virus, Virus, Mycorrhizae at the age of 0-5 weeks

Sunday-	Factor B = Spesies	Factor A = Treatment			Marginal mean	K
		M	V	MV		
0	*Phalaenopsis*	5.25a ± 1.18	5.75a ± 0.75	5.75a ± 0.63	5.58a	5.25
	Dendrobium	6.5a ± 1.66	7.75a ± 0.48	8.75a ± 0.63	7.66a	8
	Marginal mean	5.87a	6.75a	7.25a		
	HSD Cell [0.05] = 4.4 ; Columns[0.05] = 2.5 ; Rows[0.05] = 1.68					
1	Anggrek *Phalaenopsis*	4.75a ± 1.31	6a ± 0.91	5.75a ± 0.48	5.5a	6.5
	Dendrobium	6.25a ± 1.55	7.75a ± 0.48	8a ± 0.41	7.33b	8.5
	Marginal mean	5.5a	6.87a	6.87a		
	HSD Cell[0.05] = 4.35 ; Columns[0.05] = 2.47 ; Rows[0.05] = 1.66					
2	*Phalaenopsis*	4.75a ± 1.31	6.5a ± 0.65	6a ± 0.71	5.75a	6.5
	Dendrobium	7a ± 1.87	8.25a ± 0.63	8.25a ± 0.75	7.83b	8
	Marginal mean	5.87a	7.37a	7.12a		
	HSD Cell [0.05] = 4.9 ; Columns[0.05] = 2.78 ; Rows[0.05] = 1.87					
3	*Phalaenopsis*	5a ± 1.47	6.5a ± 0.65	6a ± 0.71	5.83a	7.5
	Dendrobium	7.75a ± 1.65	8.25a ± 0.63	8.25a ± 0.85	8.08b	8.25
	Marginal mean	6.37a	7.37a	7.12a		
	HSD Cell [0.05] = 4.85 ; Columns[0.05] = 2.75 ; Rows[0.05] = 1.85					
4	*Phalaenopsis*	5a ± 1.47	6.5a ± 0.65	6a ± 0.71	5.83a	7
	Dendrobium	6.75a ± 2.02	7,25a ± 0.48	7.5a ± 0.65	7.16a	6.5
	Marginal mean	5.87a	6.87a	6.75a		
	HSD Cell [0.05] = 5.14 ; Columns[0.05] = 2.91 ; Rows[0.05] = 1.96					
5	*Phalaenopsis*	5.25a ± 1.38	6.5a ± 0.65	6a ± 0.71	5.91a	7
	Dendrobium	7a ± 1.78	7.25a ± 0.48	7.5a ± 0.65	7.25a	6
	Marginal mean	6.12a	6.87a	6.75a		
	HSD Cell [0.05] = 4.74 ; Columns[0.05] = 2.69 ; Rows[0.05] = 1.8					

Based on the Tukey test at the actual level of 5% conducted (table 3) it can be seen that on the first factor, Phalaenopsis amabilis and Dendrobium discolor orchids types statistically showed significant differences explained in Figure 7. factors, mycorrhizal treatment, virus, and mycorrhizal did not can be seen, this is because in week 0 mycorrhizal administration treatment is done after counting the number of roots in week 0 and the result of mycorrhizal administration can be seen in week 1. While in the combination of first and second factor treatment (interaction) is also not visible.

At week 1, it can be seen that in the first factor, Phalaenopsis amabilis and Dendrobium discolor orchid types statistically did not show significant differences explained by Figure 7. In the second factor, treatment of mycorrhizal (M), virus (V), and mycorrhizal virus (MV) has not been seen, this is because in the first week only mycorrhizal treatment is done. Virus administration was performed after the calculation of the number of roots in the 1st week and the results were only visible in the 2nd week. Whereas in the combination of first and second factor treatment (interaction) it was not visible, because at week 1 it was only visible for mycorrhizal treatment and could not be compared with other treatments.

At week 2 to week 5, it can be seen that on the first factor, the orchid types Phalaenopsis amabilis and Dendrobium discolor statistically did not show significant differences explained by Figure 7. In the second factor, mycorizal (M), virus (V) treatment, and viral mycorrhizae (MV) did not show the significant differences described in Figure 8. Although the treatment combination of the first and second factors (interactions) showed insignificant results, it was explained in Figure 9.

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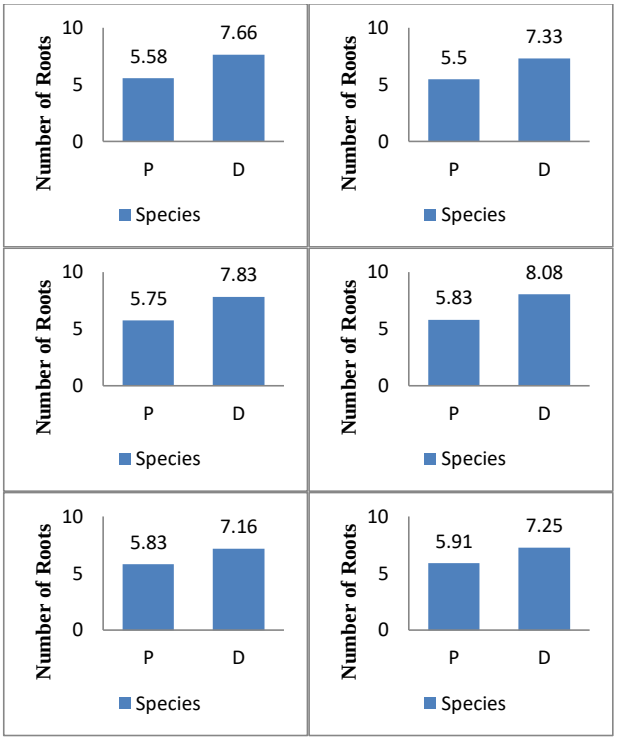

Figure 7. Comparative diagram of the number of roots of Phalaenopsis amabilis and Dendrobium discolor orchids.

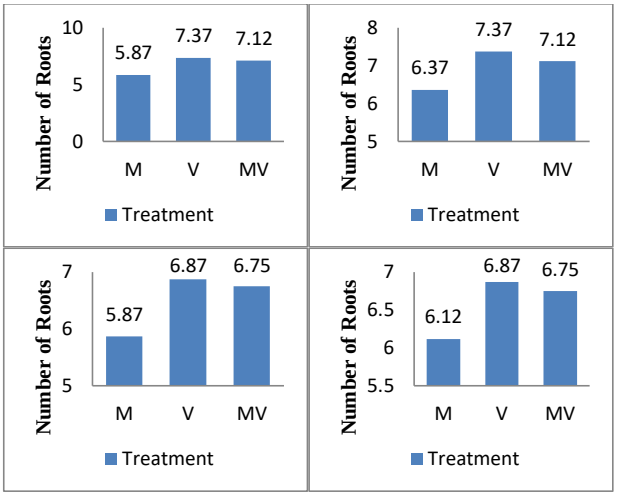

Figure 8. Comparative diagram of the number of treatment roots of Phalaenopsis amabilis and Dendrobium discolor orchids

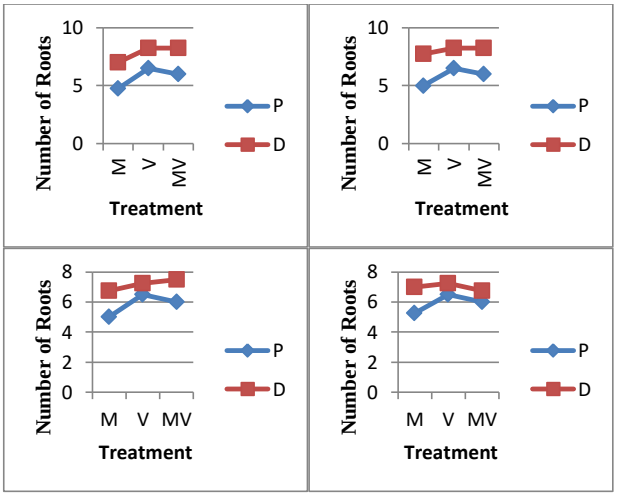

Figure 9. Graph of Number of Root Interactions Against Mycorrhizae Viruses, Viruses, and Mycorrhizae

Discussion

The research was conducted with observation for 4 weeks. The parameters observed were micorizal efficacy test, leaf length, leaf width, number of leaves, root length, and number of roots on Phalaenopsis amabilis and Dendrobium discolor orchids. Phalaenopsis and Dendrobium orchids have been inoculated with Ceratorhiza and viruses. Khaterine (2016) explained that live orchids are associated with endophytic fungi. Mycorizal treatment can help improve the soil structure around the plant and can increase the length, width and number of leaves as well as increase the length and number of roots. Meanwhile, viral treatment was used to see the resistance of mycorrhizes inoculated in orchids. In a previous research, Lakani (2015) explained that the resistance of orchid plants can be seen in inoculated leaves and in uninoculated leaves.

1. **Effectiveness of Mycorrhizae**

   Ceratorhiza efficacy test results on Phalaenopsis and Dendrobium were performed by observing growth and survival skills. Based on observations it is known that Ceratorhiza inoculation is most effective on the 3rd day. At the 3rd and 4th week observations the efficacy of Ceratorhiza showed mortality. Until the entire data was used only up to the second week of observation. This was done to avoid biased data if the entire observational data was applied up to the 4th week. Observations made at week 0 to week 2 could answer questions about the effectiveness of Ceratorhiza inoculation. This is supported by previous research by Mahfut (2020) who explained that mycorrhiza of Trichoderma and Ceratorhiza types showed that the best inoculation time was found on the 3rd and 4th day of inoculation.

   The effectiveness of Ceratoriza can also be seen in the increase in the number of roots and leaves. Ceratorhiza treatment can increase the absorption of nutrients to enhance plant growth. Mahfut (2019) explained that induction of mycorrhiza types Ceratorhiza and Trichoderma can be used as biofertilizers. Ceratorhiza treatment on the 3rd and 4th day had more effect on the increased root volume. This is supported by a previous study by Mahfut (2019) who explained that Ceratorhiza has an influence on the number of dead roots compared to Trichoderma inoculation.

   Ceratorhiza mycorrhizae can assist orchids in their growth and life cycle. Mahfut (2019) explains that Ceratorhiza mycorrhizae can infect the roots of orchid plants and produce intensive hyphae and can increase plant capacity. At week 3 and week 4 mycorrhizal inoculation the number of roots decreased, this may be due to environmental factors. According to a previous study by Kurnia (2019) regarding mycorrhizal characteristics, it was said that soil type is a factor that affects the types of mycorrhizae. Other factors that can affect plant growth are temperature, light, water, nutrients, and soil.

2. Root length

   Based on the data obtained from the analysis results of week 0 to week 5 on the root length variable, there was no significant difference. By week 1 to week 5 the number of leaves has decreased. This is due to treatment of mycorizal (M), virus (V), and mycorizal virus (MV), giving the virus causing damage to the leaves. If with Phalaenopsis control (3.38) (K); (2.96) (P) and Dendrobium (3.76) (K); (3.86) (P) on treatment showed higher outcomes than control. However, if viewed based on the type between the two orchids, Dendrobium better maintains the long growth of its roots compared to Phalaenopsis.

   Mycorrhizae inoculated at the roots play a role in the growth and development of orchids for survival. Decreased root length growth can be caused by poor physiological processes. This is reinforced by the study of Lakit (2000) who explained that plant growth is a network of plant physiological processes in forming complex organ units with the addition of plant weight and size.

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3. Number of Roots
Based on the data obtained from the results of the analysis of week 0 to week 5 on the number of variable causes there was no significant difference. At week 1 to week 5 the number of leaves had a pe If with Phalaenopsis control 6.62 (K); 5.73 (P) and Dendrobium 7.54 (K); 7.55 (P) is similar to the root length variable indicating that Phalaenopsis treatment showed lower results compared to controls. Whereas in Dendrobium, when compared with control, treatment showed higher results. descent. This is due to treatment of mycorizal (M), virus (V), and mycorizal virus (MV), giving the virus causing damage to the leaves. However, if viewed based on the type between the two orchids, Dendrobium better maintains the long growth of its roots compared to Phalaenopsis.

Rianti (2017) explained that the number of plant roots indicates how wide the plant's reach is to absorb nutrients and nutrients in mycorizal (M) treatment, indicating that the ability of orchids to form roots is because they already have leaflets. This is supported by Bey et al., (2006), who explained that radicles will turn into roots with the help of auxin processed by leaves.

CONCLUSION

Conclusions taken based on this research include:

1. *Cerathoriza* induction can increase the effectivity of *Phalaenopsis amabilis* and *Dendrobium discolor* with 3 days of incubation.
2. Root viability drops in *Phalaenopsis amabilis* with the root length of 2.96 cm and root amount of 5.73, while rises in *Dendrobium discolor* with the root length of 3.86 cm and root amount of 7.55.

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Ceratorhiza Induction towards ORSV Infection on Viability of *Phalaenopsis* and *Dendrobium*

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ABSTRACT

Orchids are one of the plants with high level of biodiversity, such as *Dendrobium* and *Phalaenopsis*, which are epiphytes. *Dendrobium* is able to adapt to the condition of where it lives while *Phalaenopsis* can grow in highlands and depends on sunlight and humidity. Virus infection has become one of the obstacles in cultivating *Dendrobium* and *Phalaenopsis*. Efforts to increase fitness and control in *Dendrobium* and *Phalaenopsis* cultivation can be done by inducing the plant's fitness using a mycorrhiza, such as Ceratorhiza. Mycorrhiza is a form of mutualism between fungi and the plant's root. This research is aimed to give information related to the utilization of Cerathoriza for inducing orhcids to suppress *Odontoglossum ringspot virus* (ORSV) infection, giving it a better growth. The research was done in February-March 2021 in Botany Laboratory University of Lampung. Completely randomized factorial design was used on two factors, kind of orchid and mycorrhiza treatment (M), virus (V), and mycorrhiza virus (MV). Variables examined in this research are amount of living and dead roots, amount of living and dead leaves. Data obtained is homogenized using Levene's test and continued by ANOVA with the significance level of 5% and further testing using Tukey's test with the significance level of 5%. From this research it is known that an interaction between *Phalaenopsis* and *Dendrobium* exists during virus and mycorrhiza administration. It is concluded that *Phalaenopsis anabilis* is more vulnerable than *Dendrobium discolor*.

Keywords: *Ceratorhiza*, *Dendrobium*, ORSV, *Phalaenopsis*

ABSTRAK

Anggrek merupakan salah satu tumbuhan yang memiliki keanekargaman sangat tinggi diantaranya *Dendrobium* dan *Phaleonopsis* yang merupakan anggrek epifit. Anggrek *Dendrobium* adalah salah satu anggrek yang mampu beradaptasi dengan kondisi tempat tumbuh anggrek. *Phalenopsis* merupakan anggrek epifit yang dapat tumbuh di daerah ketinggian dan membutuhkan cahaya serta kelembaban. Kendala dalam budidaya anggrek *Dendrobium* dan *Phaleonopsis* salah satunya karena adanya infeksi virus. Upaya peningkatan ketahanan dan pengendalian budidaya anggrek *Dendrobium* dan *Phaleonopsis* dapat dilakakukan dengan menginduksi ketahanan tanaman menggunakan mikoriza *Ceratorhiza*. Mikoriza merupakan suatu bentuk simbiosis mutualistik antara jamur dan akar tanaman. Tujuan dari penelitian ini dapat memberikan informasi mengenai pemanfaatan mikoriza *Ceratorhiza* untuk menginduksi

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tanaman anggrek agar dapat menahan infeksi virus *Odontoglossum ringspot virus* (ORSV), sehingga pertumbuhannya lebih baik. Waktu dan tempat penelitian dilakukan pada bulan Februari-Maret 2021 di Laboratorium Botani Universitas Lampung. Pada penelitian ini menggunakan Metode rancangan acak lengkap faktorial (RALF) yang terdiri dari dua faktor, yaitu jenis anggrek dan perlakuan mikoriza (M), virus (V), dan mikoriza virus (MV). Variabel yang diamati pada penelitian ini adalah jumlah akar yang hidup dan mati, jumlah daun yang hidup dan mati. Data yang diperoleh dihomogenkan dengan menggunakan uji Levene kemudian dianalisis ragam pada taraf nyata 5% menggunakan ANOVA dan uji lanjut dengan Tukey pada taraf nyata 5%. Hasil penelitian menunjukkan bahwa jenis anggrek *Phalaenopsis amabilis* lebih rentan dibandingkan *Dendrobium discolor* pada perlakuan mikoriza (M), virus (V), dan mikoriza virus (MV). Terdapat interaksi antara *Phalaenopsis* dan *Dendrobium* pada variabel jumlah daun pada perlakuan mikoriza (M) dan virus (V).

Kata Kunci: *Ceratorhiza*, *Dendrobium*, ORSV, *Phalaenopsis*

INTRODUCTION

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Orchids is one of the biggest family in Indonesia (Soetopo, 2009). Orchids are widely-spread and can be found in tropical forests in West Sumatera, Java, Kalimantan, Sulawesi, Nusa Tenggara, Maluku, and Papua (Rukmana, 2000). Population of orchids in their natural habitat have been decreasing due to deforestation and forest over-exploitation (Johanis et al., 2001). Orchids are categorized as epiphytes and depend on their hosts. The loss of orchids' host plants will significantly ruin orhcids' life cycle (Ambari, 2016).

There are two types of orchids, namely terrestrial and epiphyte. Terrestrial orchids are orchids that grow and develop on land (Dina and Soetopo, 2019) while epiphyte orchids are orchids that live on host plants but are not parasite. Most cultivated orchids are *Phalaenopsis* and *Dendrobium* (Yudistira *et al.*, 2011). Virus infection has become the biggest challenge in orchids' cultivation and potential development (Kumalawati et al., 2011). Odontoglossum ringspot virus (ORSV) infects most orchids. Based on previous research by Mahfut (2016), symptomps caused by ORSV include mosaic, streak, chlorotic, and necrosis. Orchids protection measures can be done by genetic recombination resulting ORSV-resistant orchids. Therefore, mycorrhiza, such as *Cerathoriza* sp., is used as it is more eco-friendly and effective.

Mycorrhiza is a mutualistic symbiosis between fungi and root. Plants obtain nutrient absorbed by fungi while fungi obtain nutrient resulted from assimilations done by plants. Mycorrhiza infected the root system of the plant hosts, producing hyphae intensively. *Cerathoriza*'s role as biofertilizer can help the plant's growth and development in increasing root length, root amount, leaf amount, and leaf width (Brundrett, 2008). *Cerathoriza* as biocontrol can decrease virus infection level on plants. Orchids have different resistance level towards virus infection. Orchids' resistance increases as root amount and root length have increased after *Cerathoriza* induction. This research is done to find out the effectivity of *Cerathoriza* induction towards *Phalaenopsis* and *Dendrobium* and their root viability after infected by ORSV.

MATERIALS AND METHOD (12, bold)

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Completely randomized factorial design was used on two factors. The first factor was the orhcid type used, *Phalaenopsis amabilis* and *Dendrobium discolor* while the second factor was

mycorrhiza induction (M), virus inoculation (V), and mycorrhiza induction and virus inoculation (MV), resulting in 6 treatments. Each treatment combination was repeated four times, resulting in 24 experimental units. Each experimental unit was a cup consists of growing media, one orchid plant (*Phlaenopsis amabilis* or *Dendrobium discolor)* based on treatment combination given. Variables examined are mycorrhiza effectivity, leaf length, leaf width, leaf amount, root length, and root amount. Positive controls for *Phlaenopsis amabilis* and *Dendrobium discolor*, which are not included in the research design, also exists.

Tools used in this research were petri dish, beaker measuring glass 100 ml and 500 ml, mortar and pestel, pen, label, gloves, tissue, and camera. Materials used in this research were bottled seedlings of *Phalaenopsis amabilis* and *Dendrobium discolor*, moss sterile media, Potato Dextrose Agar media, Ceratorhiza sp., inoculum of *Odontoglossum ringspot virus* (ORSV), karborondum, phosphate buffer, water, alcohol.

This research was done through five steps below.

1. Acclimatization
   Bottled orhcids are grown in the greenhouse for 2 months. After acclimatization, orchid treatment was done until 3-4 leaves have grown.
2. *Cerathoriza* inoculation
   Cerathoirza inoculation was done by growing *Cerathoriza* isolates on PDA that had been added by chloramphenicol. Isolates on petri dish was taken ± 0.5 cm and was put on the media through three spotting. Incubation was done at room temperature for 5-7 days. Isolates rejuvenation was done on 8-10 petri dishes.
3. ORSV inoculation
   ORSV inoculation towards planlet was done through sample, an ORSV-infected tobacco. Tobacco leaves was grinded and added with phosphate buffer with the ratio of 1:10 (m/v). Phosphate buffer was used to break down the cells, releasing the virus inside. Inoculation towards the orchid was done by rubbing carborondrum evenly, slowly, and manually by finger or cutton bud based on the vein (Calvo et al., 2020). Planlet was replanted on the media and symptomps, including necrosis, chlorosis, mosaic, leaf malformation, streak yellowing, and curling leaf, were examined during incubation time until they appear (15-30 days).
4. Examination on mycorrhiza effectivity
   Examination on mycorrhiza effectivity was done on the aclimated *Phalaenopsis amabilis* and *Dendrobium discolour*. Rejuvenation on *Cerathoriza* was done 3 days and inoculation was done on the first until the fourth day. The examination was done in 30 days.
5. Examinaiton on root viability
   Root viability examination was done by examining root length growth and root amount.

RESULTS AND DISCUSSION (12, bold)

Result

1. Effectiveness of Mycorrhizae
   The results of mycorizal inoculation performed up to 4 days showed that hyphae enveloped the plant roots. The inoculation period indicates the thickness of the hyphae covering the plant. Myculation inclusions of Phalaenopsis amabilis and Dendrobium discolor orchids are shown in Figure 1.

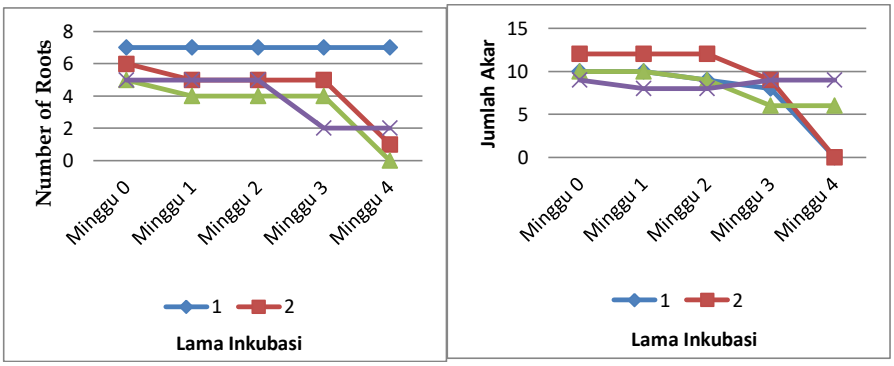
Figure 1. Inoculation of Ceratorhiza on orchids (A) *Phalaenopsis amabilis* and (B) *Dendrobium discolor*.

The efficacy of Ceratorhiza performed by observing orchid root growth for post-inoculation survival is shown in Table 1.

Tabel 1. The number of orchid roots *Phalaenopsis* and *Dendrobium*

Jenis Anggrek	Lama Inkubasi	Minggu				
		0	1	2	3	4
Phalaenopsis	1	7	7	7	7	7
	2	6	5	5	5	1
	3	5	4	4	4	4
	4	5	5	5	2	2
Dendrobium	1	10	10	9	8	0
	2	12	12	12	9	0
	3	10	10	9	6	6
	4	9	8	8	9	9

Based on the results of the observations that have been made, this indicates a significant difference in the effectiveness of mycorrhiza based on the incubation time. The data showed that on the third day mycorrhizae showed more effective results in mycorrhizal inoculation of orchid roots. The results are shown in Table 1 and Figure 2.



Figur 2. Graph of mycorrhizal efficacy on the number of orchid roots

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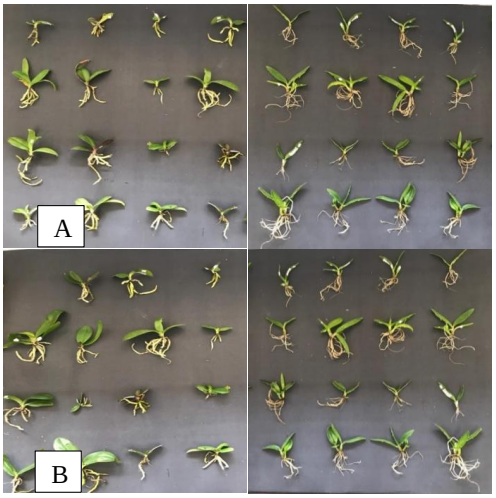
Based on the above data, there is no difference between the root chart of Phalaenopsis amabilis and Dendrobium discolor. On the 3rd day of incubation, both orchids showed their presence against mycorrhiza. This may occur because mycorrhizal absorption has reached its maximum limit on the third day until it decreases by the 4th week.

After mycorrhizal efficacy test was performed, observations were made from week 0 to week 5 with parameters namely, leaf length, leaf width, leaf number, root length, and root number shown in Figure 6-9. The results of the analysis using the ANOVA method are shown in Table 2-3.



Figur 3. Development of small plants Phalaenopsis amabilis (left) and Dendrobium discolor (right) from mycorrhizal virus inoculation (MV) (A) week 0 (B) week 4.

2. Root Length

   The initial stage of analyzing the root length observation data performed data homogeneity using Levene test at 5%stage. The test results showed that the diversity of samples on orchids was homogeneous. Further, the analysis was continued using ANOVA test, which showed that mycorizal (M), virus (V), and mycorizal virus (MV) inoculations on orchids gave significantly different results. The analysis was continued using the Tukey test at the 5% real level shown in Table 2. While the interaction between the two samples is seen on the interaction curve of Figure 6.

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Table 2. Tukey root length test in combination treatment of *Phalaenopsis amabilis* and *Dendrobium discolor* orchids by giving Mycorrhizae, Virus, and Mycorrhizae Virus at 0-5 weeks of age.

Minggu ke-	Faktor B = Spesies	Faktor A = Perlakuan			Marginal mean	K
		M	V	MV		
0	*Phalaenopsis*	3.45a ± 0.79	3.85a ± 0.58	2.76a ± 0.32	3.35a	3.66
	Dendrobium	3.61a ± 0.34	4.57a ± 0.38	4.80a ± 0.25	4.32b	4.64
	Marginal mean	3.53a	4.21a	3.78a		
	HSD Cell [0.05] = 2.17 ; Columns[0.05] = 1.23 ; Rows[0.05] = 0.83					
1	*Phalaenopsis*	3.37a ± 0.92	3.81a ± 0.28	2.42a ± 0.36	3.2a	3.65
	Dendrobium	3.51a ± 0.32	4.45a ± 0.15	4.59a ± 0.16	4.18a	4.66
	Marginal mean	3.44a	4.13a	3.50a		
	HSD Cell[0.05] = 2.02 ; Columns[0.05] = 1.14 ; Rows[0.05] = 0.77					
2	*Phalaenopsis*	2.88 ± 1.17	3.57 ± 0.47	2.43 ± 0.33	2.96a	3.45
	Dendrobium	3.41 ± 0.16	4.27 ± 0.25	4.63 ± 0.13	4.10a	4.01
	Marginal mean	3.14a	3.92a	3.53a		
	HSD Cell [0.05] = 2.47 ; Columns[0.05] = 1.4 ; Rows[0.05] = 0.94					
3	*Phalaenopsis*	2.74 ± 1.03	3.59 ± 0.53	2.19 ± 0.47	2.84a	3.16
	Dendrobium	2.87 ± 0.29	4.37 ± 0.27	4.63 ± 0.2	3.95a	3.63
	Marginal mean	2.80b	3.98a	3.41a		
	HSD Cell [0.05] = 2.44 ; Columns[0.05] = 1.39 ; Rows[0.05] = 0.93					
4	*Phalaenopsis*	2.74 ± 1.03	3.20 ± 0.54	2.18 ± 0.46	2.70a	3.20
	Dendrobium	2.39 ± 0.7	3.46 ± 0.1	4.27 ± 0.35	3.37a	2.82
	Marginal mean	2.56a	3.33a	3.22a		
	HSD Cell [0.05] = 2.72 ; Columns[0.05] = 1.54 ; Rows[0.05] = 1.04					
5	*Phalaenopsis*	2.70 ± 1.05	3.22 ± 0.53	2.21 ± 0.47	2.71a	3.21
	Dendrobium	2.03 ± 0.75	3.48 ± 0.1	4.28 ± 0.34	3.26a	2.83
	Marginal mean	2.36a	3.35a	3.24a		
	HSD Cell [0.05] = 2.78 ; Columns[0.05] = 1.58 ; Rows[0.05] = 1.06					

the first factor, the types of Phalaenopsis amabilis and Dendrobium discolor orchids statistically showed significant differences explained in Figure 4. factors, mycorrhizal treatment, viruses, and mycorrhizal virus is not visible, this is because in the 0th week of mycorrhizal administration treatment is done after 0 week root length calculation and the result of mycorrhizal administration can be seen in week 1. While in the combination of first and second factor treatment (interaction) is also not visible.

At week 1, it can be seen that in the first factor, Phalaenopsis amabilis and Dendrobium discolor orchid types statistically did not show significant differences explained by Figure 4. In the second factor, treatment of mycorrhiza (M), virus (V), and MV mycorrhiza virus) have not been seen, this is because in the first week only mycorrhizal treatment is performed. Virus administration was performed after root length calculations for the first week and results were only seen in the 2nd week. Whereas in the combination of first and second factor treatment (interaction) it was not visible, because at week 1 it was only visible for mycorrhizal treatment and could not be compared with other treatments.

From week 2 to week 5, it can be seen that in the first factor, the orchid varieties Phalaenopsis amabilis and Dendrobium discolor statistically did not show significant differences explained by Figure 4.In the second factor, mycorrhizal (M) treatment, virus (V), and viral mycorrhiza (MV) did not show significant differences described in Figure 5. Whereas in the combination of the first and second factors the treatment (interaction) showed insignificant results, described in Figure 6.

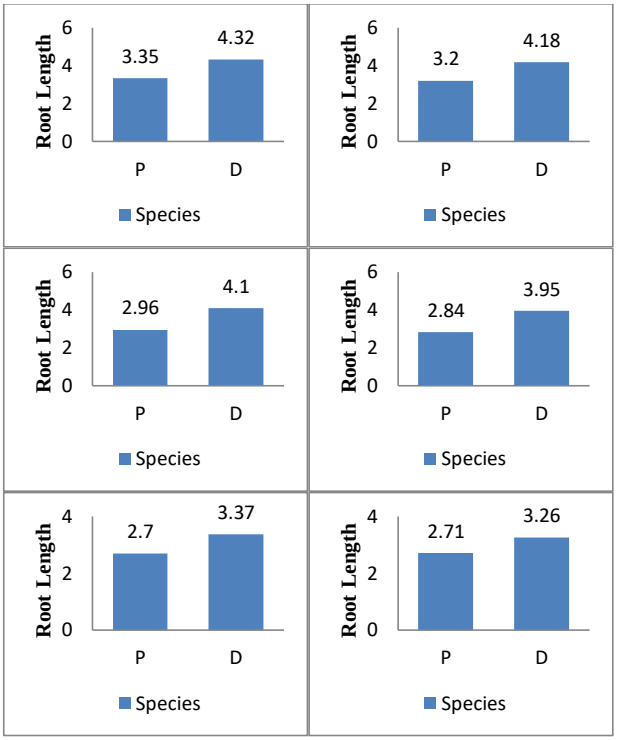

Figure 4. Comparative diagram of root length for Phalaenopsis amabilis and Dendrobium discolor orchids

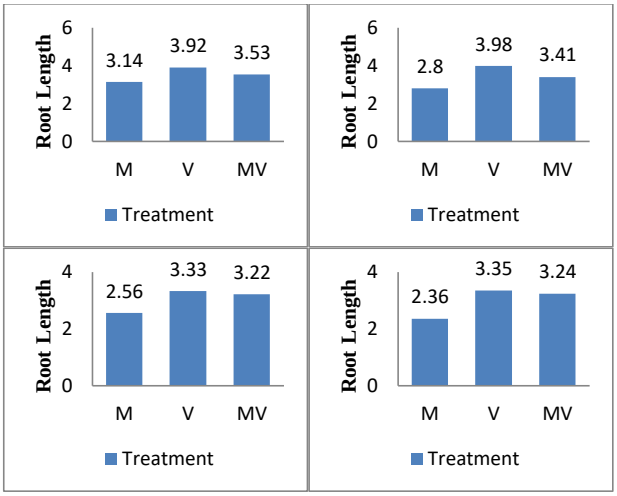

Figure 5. Comparative diagram of root length against treatment of Phalaenopsis amabilis and Dendrobium discolor orchids

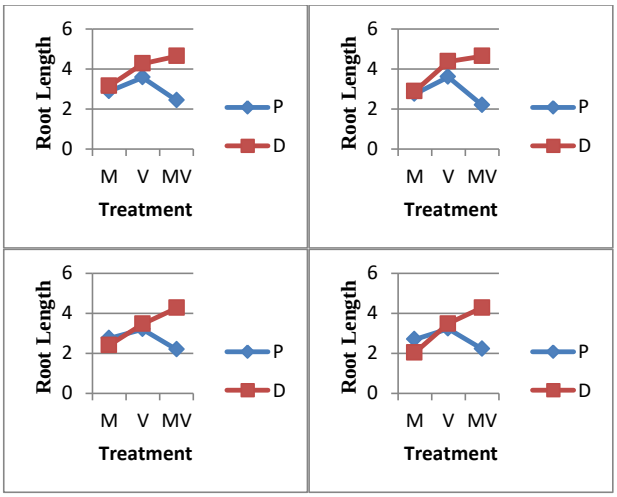

Figure 6. Graph of Root Length Interaction with Mycorrhizae, Viruses, and Viral Mycorrhizae

1. **Number of Roots**

The initial stage of data analysis, observation of the number of roots, perform data homogeneity using Levene test at 5%stage. The test results showed that the diversity of samples on orchids was homogeneous. Further, the analysis was continued using ANOVA test, which showed that mycorizal (M), virus (V), and mycorizal virus (MV) inoculations on orchids gave significantly different results. The analysis was continued using the Tukey test at the actual level of 5% as shown in Table 3. While the interaction between the two samples is seen on the interaction curve of Figure 9.

Table 3. Total Root Tuky Test in combination treatment of Phalaenopsis amabilis and Dendrobium discolored orchids by administering Mycorrhizae Virus, Virus, Mycorrhizae at the age of 0-5 weeks

Sunday-	Factor B = Spesies	Factor A = Treatment			Marginal mean	K
		M	V	MV		
0	*Phalaenopsis*	5.25a ± 1.18	5.75a ± 0.75	5.75a ± 0.63	5.58a	5.25
	Dendrobium	6.5a ± 1.66	7.75a ± 0.48	8.75a ± 0.63	7.66a	8
	Marginal mean	5.87a	6.75a	7.25a		
	HSD Cell [0.05] = 4.4 ; Columns[0.05] = 2.5 ; Rows[0.05] = 1.68					
1	Anggrek *Phalaenopsis*	4.75a ± 1.31	6a ± 0.91	5.75a ± 0.48	5.5a	6.5
	Dendrobium	6.25a ± 1.55	7.75a ± 0.48	8a ± 0.41	7.33b	8.5
	Marginal mean	5.5a	6.87a	6.87a		
	HSD Cell[0.05] = 4.35 ; Columns[0.05] = 2.47 ; Rows[0.05] = 1.66					
2	*Phalaenopsis*	4.75a ± 1.31	6.5a ± 0.65	6a ± 0.71	5.75a	6.5
	Dendrobium	7a ± 1.87	8.25a ± 0.63	8.25a ± 0.75	7.83b	8
	Marginal mean	5.87a	7.37a	7.12a		
	HSD Cell [0.05] = 4.9 ; Columns[0.05] = 2.78 ; Rows[0.05] = 1.87					
3	*Phalaenopsis*	5a ± 1.47	6.5a ± 0.65	6a ± 0.71	5.83a	7.5
	Dendrobium	7.75a ± 1.65	8.25a ± 0.63	8.25a ± 0.85	8.08b	8.25
	Marginal mean	6.37a	7.37a	7.12a		
	HSD Cell [0.05] = 4.85 ; Columns[0.05] = 2.75 ; Rows[0.05] = 1.85					
4	*Phalaenopsis*	5a ± 1.47	6.5a ± 0.65	6a ± 0.71	5.83a	7
	Dendrobium	6.75a ± 2.02	7,25a ± 0.48	7.5a ± 0.65	7.16a	6.5
	Marginal mean	5.87a	6.87a	6.75a		
	HSD Cell [0.05] = 5.14 ; Columns[0.05] = 2.91 ; Rows[0.05] = 1.96					
5	*Phalaenopsis*	5.25a ± 1.38	6.5a ± 0.65	6a ± 0.71	5.91a	7
	Dendrobium	7a ± 1.78	7.25a ± 0.48	7.5a ± 0.65	7.25a	6
	Marginal mean	6.12a	6.87a	6.75a		
	HSD Cell [0.05] = 4.74 ; Columns[0.05] = 2.69 ; Rows[0.05] = 1.8					

Based on the Tukey test at the actual level of 5% conducted (table 3) it can be seen that on the first factor, Phalaenopsis amabilis and Dendrobium discolor orchids types statistically showed significant differences explained in Figure 7. factors, mycorrhizal treatment, virus, and mycorrhizal did not can be seen, this is because in week 0 mycorrhizal administration treatment is done after counting the number of roots in week 0 and the result of mycorrhizal administration can be seen in week 1. While in the combination of first and second factor treatment (interaction) is also not visible.

At week 1, it can be seen that in the first factor, Phalaenopsis amabilis and Dendrobium discolor orchid types statistically did not show significant differences explained by Figure 7. In the second factor, treatment of mycorrhizal (M), virus (V), and mycorrhizal virus (MV) has not been seen, this is because in the first week only mycorrhizal treatment is done. Virus administration was performed after the calculation of the number of roots in the 1st week and the results were only visible in the 2nd week. Whereas in the combination of first and second factor treatment (interaction) it was not visible, because at week 1 it was only visible for mycorrhizal treatment and could not be compared with other treatments.

At week 2 to week 5, it can be seen that on the first factor, the orchid types Phalaenopsis amabilis and Dendrobium discolor statistically did not show significant differences explained by Figure 7. In the second factor, mycorizal (M), virus (V) treatment, and viral mycorrhizae (MV) did not show the significant differences described in Figure 8. Although the treatment combination of the first and second factors (interactions) showed insignificant results, it was explained in Figure 9.

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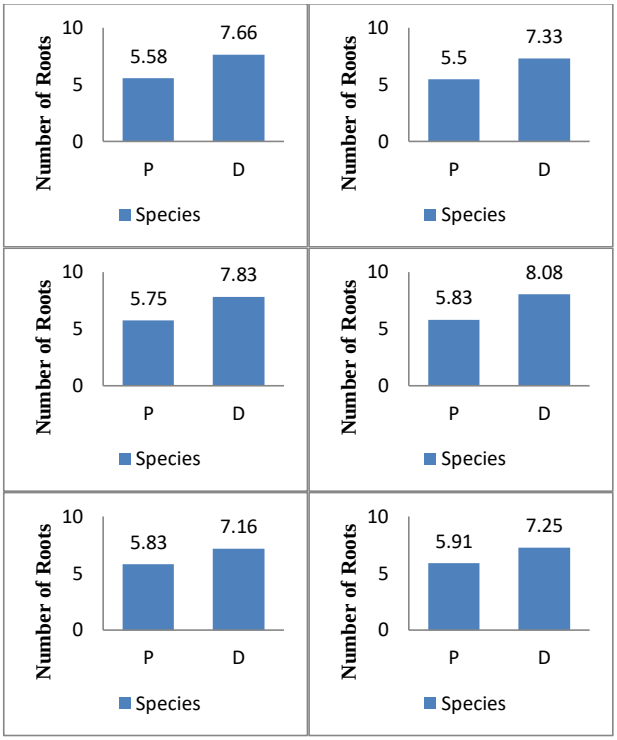

Figure 7. Comparative diagram of the number of roots of Phalaenopsis amabilis and Dendrobium discolor orchids.

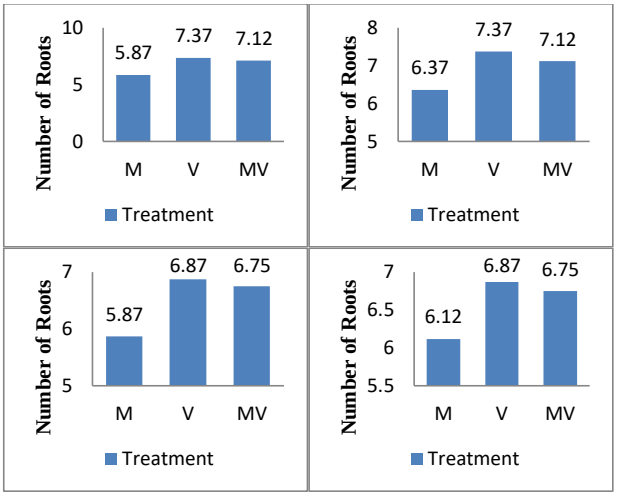

Figure 8. Comparative diagram of the number of treatment roots of Phalaenopsis amabilis and Dendrobium discolor orchids

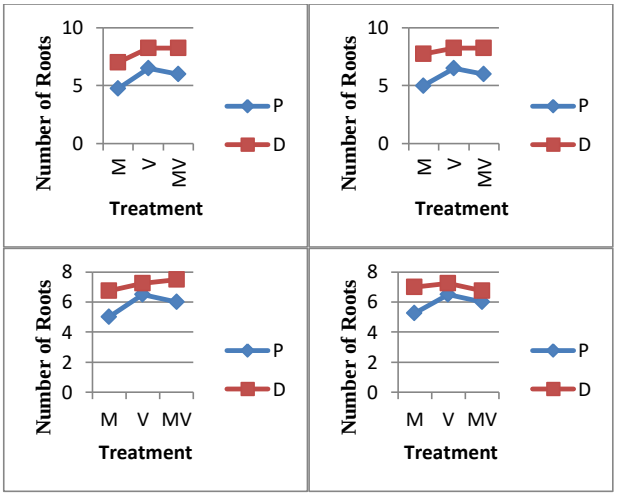

Figure 9. Graph of Number of Root Interactions Against Mycorrhizae Viruses, Viruses, and Mycorrhizae

Discussion

The research was conducted with observation for 4 weeks. The parameters observed were micorizal efficacy test, leaf length, leaf width, number of leaves, root length, and number of roots on Phalaenopsis amabilis and Dendrobium discolor orchids. Phalaenopsis and Dendrobium orchids have been inoculated with Ceratorhiza and viruses. Khaterine (2016) explained that live orchids are associated with endophytic fungi. Mycorizal treatment can help improve the soil structure around the plant and can increase the length, width and number of leaves as well as increase the length and number of roots. Meanwhile, viral treatment was used to see the resistance of mycorrhizes inoculated in orchids. In a previous research, Lakani (2015) explained that the resistance of orchid plants can be seen in inoculated leaves and in uninoculated leaves.

1. **Effectiveness of Mycorrhizae**

   Ceratorhiza efficacy test results on Phalaenopsis and Dendrobium were performed by observing growth and survival skills. Based on observations it is known that Ceratorhiza inoculation is most effective on the 3rd day. At the 3rd and 4th week observations the efficacy of Ceratorhiza showed mortality. Until the entire data was used only up to the second week of observation. This was done to avoid biased data if the entire observational data was applied up to the 4th week. Observations made at week 0 to week 2 could answer questions about the effectiveness of Ceratorhiza inoculation. This is supported by previous research by Mahfut (2020) who explained that mycorrhiza of Trichoderma and Ceratorhiza types showed that the best inoculation time was found on the 3rd and 4th day of inoculation.

   The effectiveness of Ceratoriza can also be seen in the increase in the number of roots and leaves. Ceratorhiza treatment can increase the absorption of nutrients to enhance plant growth. Mahfut (2019) explained that induction of mycorrhiza types Ceratorhiza and Trichoderma can be used as biofertilizers. Ceratorhiza treatment on the 3rd and 4th day had more effect on the increased root volume. This is supported by a previous study by Mahfut (2019) who explained that Ceratorhiza has an influence on the number of dead roots compared to Trichoderma inoculation.

   Ceratorhiza mycorrhizae can assist orchids in their growth and life cycle. Mahfut (2019) explains that Ceratorhiza mycorrhizae can infect the roots of orchid plants and produce intensive hyphae and can increase plant capacity. At week 3 and week 4 mycorrhizal inoculation the number of roots decreased, this may be due to environmental factors. According to a previous study by Kurnia (2019) regarding mycorrhizal characteristics, it was said that soil type is a factor that affects the types of mycorrhizae. Other factors that can affect plant growth are temperature, light, water, nutrients, and soil.

2. Root length

   Based on the data obtained from the analysis results of week 0 to week 5 on the root length variable, there was no significant difference. By week 1 to week 5 the number of leaves has decreased. This is due to treatment of mycorizal (M), virus (V), and mycorizal virus (MV), giving the virus causing damage to the leaves. If with Phalaenopsis control (3.38) (K); (2.96) (P) and Dendrobium (3.76) (K); (3.86) (P) on treatment showed higher outcomes than control. However, if viewed based on the type between the two orchids, Dendrobium better maintains the long growth of its roots compared to Phalaenopsis.

   Mycorrhizae inoculated at the roots play a role in the growth and development of orchids for survival. Decreased root length growth can be caused by poor physiological processes. This is reinforced by the study of Lakit (2000) who explained that plant growth is a network of plant physiological processes in forming complex organ units with the addition of plant weight and size.

Mycorrhizae inoculated at the roots play a role in the growth and development of orchids for survival. Decreased root length growth can be caused by poor physiological processes. This is reinforced by the study of Lakit (2000) who explained that plant growth is a network of plant physiological processes in forming complex organ units with the addition of plant weight and size.

3. Number of Roots
Based on the data obtained from the results of the analysis of week 0 to week 5 on the number of variable causes there was no significant difference. At week 1 to week 5 the number of leaves had a pe If with Phalaenopsis control 6.62 (K); 5.73 (P) and Dendrobium 7.54 (K); 7.55 (P) is similar to the root length variable indicating that Phalaenopsis treatment showed lower results compared to controls. Whereas in Dendrobium, when compared with control, treatment showed higher results. descent. This is due to treatment of mycorizal (M), virus (V), and mycorizal virus (MV), giving the virus causing damage to the leaves. However, if viewed based on the type between the two orchids, Dendrobium better maintains the long growth of its roots compared to Phalaenopsis.

Rianti (2017) explained that the number of plant roots indicates how wide the plant's reach is to absorb nutrients and nutrients in mycorizal (M) treatment, indicating that the ability of orchids to form roots is because they already have leaflets. This is supported by Bey et al., (2006), who explained that radicles will turn into roots with the help of auxin processed by leaves.

CONCLUSION

Conclusions taken based on this research include:

1. *Cerathoriza* induction can increase the effectivity of *Phalaenopsis amabilis* and *Dendrobium discolor* with 3 days of incubation.
2. Root viability drops in *Phalaenopsis amabilis* with the root length of 2.96 cm and root amount of 5.73, while rises in *Dendrobium discolor* with the root length of 3.86 cm and root amount of 7.55.

Comment [S8]: describe in paragrap form

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Induksi Ketahanan *Ceratorhiza* Terhadap Infeksi ORSV Pada Viabilitas Anggrek *Phalaenopsis* dan *Dendrobium*

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ABSTRACT

Orchids are one of the plants with high level of biodiversity, such as *Dendrobium* and *Phalaenopsis*, which are epiphytes. *Dendrobium* is able to adapt to the condition of where it lives while *Phalaenopsis* can grow in highlands and depends on sunlight and humidity. Virus infection has become one of the obstacles in cultivating *Dendrobium* and *Phalaenopsis*. Efforts to increase fitness and control in *Dendrobium* and *Phalaenopsis* cultivation can be done by inducing the plant's fitness using a mycorrhiza, such as Ceratorhiza. Mycorrhiza is a form of mutualism between fungi and the plant's root. This research is aimed to give information related to the utilization of Cerathoriza for inducing orhcids to suppress *Odontoglossum ringspot virus* (ORSV) infection, giving it a better growth. The research was done in February-March 2021 in Botany Laboratory University of Lampung. Completely randomized factorial design was used on two factors, kind of orchid and mycorrhiza treatment (M), virus (V), and mycorrhiza virus (MV). Variables examined in this research are amount of living and dead roots, amount of living and dead leaves. Data obtained is homogenized using Levene's test and continued by ANOVA with the significance level of 5% and further testing using Tukey's test with the significance level of 5%. From this research it is known that an interaction between *Phalaenopsis* and *Dendrobium* exists during virus and mycorrhiza administration. It is concluded that *Phalaenopsis anabilis* is more vulnerable than *Dendrobium discolor*.

Keywords: *Ceratorhiza*, *Dendrobium*, ORSV, *Phalaenopsis*

ABSTRAK

Anggrek merupakan salah satu tumbuhan yang memiliki keanekaragaman sangat tinggi diantaranya *Dendrobium* dan *Phaleonopsis* yang merupakan anggrek epifit. Anggrek *Dendrobium* adalah salah satu anggrek yang mampu beradaptasi dengan kondisi tempat tumbuh anggrek. *Phalenopsis* merupakan anggrek epifit yang dapat tumbuh di daerah ketinggian dan membutuhkan cahaya serta kelembaban. Kendala dalam budidaya anggrek *Dendrobium* dan *Phaleonopsis* salah satunya karena adanya infeksi virus. Upaya peningkatan ketahanan dan pengendalian budidaya anggrek *Dendrobium* dan *Phaleonopsis* dapat dilakakukan dengan menginduksi ketahanan tanaman menggunakan mikoriza *Ceratorhiza*. Mikoriza merupakan suatu bentuk simbiosis mutualistik antara jamur dan akar tanaman. Tujuan dari penelitian ini dapat memberikan informasi mengenai pemanfaatan mikoriza *Ceratorhiza* untuk menginduksi tanaman anggrek agar dapat menahan infeksi virus *Odontoglossum ringspot virus* (ORSV), sehingga pertumbuhannya lebih baik. Waktu dan tempat penelitian dilakukan pada bulan Februari-Maret 2021 di Laboratorium Botani Universitas Lampung. Pada penelitian ini menggunakan Metode

rancangan acak lengkap faktorial (RALF) yang terdiri dari dua faktor, yaitu jenis anggrek dan perlakuan mikoriza (M), virus (V), dan mikoriza virus (MV). Variabel yang diamati pada penelitian ini adalah jumlah akar yang hidup dan mati, jumlah daun yang hidup dan mati. Data yang diperoleh dihomogenkan dengan menggunakan uji Levene kemudian dianalisis ragam pada taraf nyata 5% menggunakan ANOVA dan uji lanjut dengan Tukey pada taraf nyata 5%. Hasil penelitian menunjukkan bahwa jenis anggrek *Phalaenopsis amabilis* lebih rentan dibandingkan *Dendrobium discolor* pada perlakuan mikoriza (M), virus (V), dan mikoriza virus (MV). Terdapat interaksi antara *Phalaenopsis* dan *Dendrobium* pada variabel jumlah daun pada perlakuan mikoriza (M) dan virus (V).

Kata Kunci: *Ceratorhiza*, *Dendrobium*, ORSV, *Phalaenopsis*

PENDAHULUAN

Anggrek merupakan salah satu famili terbesar di Indonesia (Soetopo, 2009). Tanaman anggrek tersebar luas dan dapat dijumpai di hutan-hutan tropis di Sumatera Barat, Pulau Jawa, Kalimantan, Sulawesi, Nusa Tenggara, Maluku, dan Papua (Rukmana, 2000). Populasi anggrek di habitat asli sudah berkurrang. Hal ini disebabkan adanya kerusakan hutan dan eksploitasi yang berlebihan (Johanis *et al.*, 2001). Tanaman anggrek merupakan tanaman epifit dan bergantung dengan tumbuhan inang. Hilangnya tanaman inang akan merusak daur hidup tanaman anggrek secara signifikan (Ambari, 2016).

Terdapat dua jenis anggrek yaitu terrestrial dan epifit. Anggrek terrestrial merupakan salah satu jenis anggrek yang tumbuh dan berkembang di tanah (Dina dan Soetopo, 2019). Anggrek epifit merupakan anggrek tumbuh menumpang pada tanaman lain tetapi tidak parasite. Anggrek yang sering dibudidaykan adalah anggrek *Phalaenopsis* dan *Dendrobium* (Yudistira *et al.*, 2011). Saat ini kendala utama dalam budidaya dan pengembangan potensi anggrek alam dengan adanya infeksi penyakit yang disebabkan oleh virus (Kumalawati *et al.*, 2011). *Odontoglossum ringspot virus* (ORSV) merupakan virus yang banyak menginfeksi tanaman anggrek. Berdasarkan penelitian sebelumnya (Mahfut, 2016) gejala infeksi ORSV, yaitu *mosaic*, *streak*, klorotik, dan nekrosis. Upaya perlindungan anggrek terhadap infeksi virus dapat dilakukan menggunkaan teknik rekayasa genetik dengan menghasilkan anggrek yang tahan terhadap ORSV. Oleh karena itu digunakan fungi mikoriza yang lebih ramah lingkungan dan efektif salah satunya *Ceratorhiza* sp.

Mikoriza merupakan simbiosis yang menguntungkan antara jamur dan akar tanaman. Tumbuhan mampu memperoleh sumber nutrisi dari jamur yang mampu menyerap unsur hara, sedangkan mikoriza memperoleh nutrient hasil asimilasi dari tumbuhan. Mikoriza menginfeksi sistem perakaran tanaman inang, memproduksi jalinan hifa secara intensif. Peran *Ceratorhiza* sebagai biofertilizer dapat membantu pertumbuhan dan perkembangan tanaman dalam menambah panjang akar, jumlah akar, jumlah daun, dan lebar daun (Brundrett, 2008). *Ceratorhiza* sebagai biokontrol dapat menurunkan infeksi virus pada tanaman. Anggrek memiliki tingkat ketahanan yang berbeda dalam infeksi virus. Ketahanan *Ceratorhiza* pada anggrek menunjukkan peningkatan jumlah akar akhir, akar hidup, dan akar mati (Mahfut, 2019). Penelitian ini dilakukan untuk mengetahui efektivitas anggrek *Phalaenopsis* dan *Dnedrobium* hasil induksi *Ceratorhiza* dan mengetahui viabilitas organ akar anggrek *Phalaenopsis* dan *Dendrobium* hasil induksi ketahanan *Ceratorhiza* terhadap infeksi ORSV.

METODE PENELITIAN

Penelitian ini dilaksanakan pada bulan Januari – Maret 2021 di Laboratorium Botani 2, Jurusan Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Lampung. Penelitian ini disusun dengan menggunakan Rancangan Acak Lengkap Faktorial (RALF) yang terdiri dari 6 perlakuan dan 2 kontrol. Faktor 1 berupa jenis anggrek, yang terdiri dari *Phalaenopsis* dan *Dendrobium* dan faktor 2 merupakan perlakuan mikoriza (M) virus (V), dan mikoriza virus (MV). Masing-masing perlakuan terdiri dari 4 kali ulangan sehingga diperoleh 24 satuan percobaan. Setiap satuan percobaan terdiri dari *Phlaenopsis amabilis* dan *Dendrobium discolor*. Parameter yang diuji yaitu efektivitas mikoriza, panjang akar, dan jumlah akar.

Alat-alat yang digunakan dalam penelitian ini adalah cawan petri, gelas ukur bervolume 100 ml dan 500 ml, mortar dan pestel, pena, label, sarung tangan, tisu, dan kamera. Bahan yang digunakan dalam penelitian ini adalah bibit anggrek *Phalaenopsis amabilis* botolan, bibit anggrek *Dendrobium discolor* botolan, media moss steril, medium *Potato Dextrose Agar* (PDA) bubuk, Ceratorhiza sp., inokulum *Odontoglossum ringspot virus* (ORSV), karborondum, bufferfosfat, air, alkohol.

Penelitian ini dilakukan dengan 5 tahapan: 1). Aklimatisasi, 2). Inokulasi *Ceratorhiza*, 3). Inokulasi Virus, 4). Pengamatan efektivitas mikoriza, panjang akar, dan jumlah akar.

Aklimatisasi: Anggrek dalam botol berumur 2 bulan dilakukan di *greenhouse*. Setelah aklimatisasi perawatan anggrek dilakukan secara rutin sampai ada pertambahan daun 3-4 daun pada anggrek.

Inokulasi *Ceratorhiza*: Inokulasi *Ceratorhiza* dilakukan dengan menumbuhkan isolat *Ceratorhiza* pada media PDA yang sudah ditambahkan antibakteri klramfenikol. Isolat pada cawan petri diambil kurang lebih 0,5 cm, selanjutnya diletakkan pada media PDA dengan 3 penitiikan, kemudian diinkubasi pada suhu ruang selama 5-7 hari. Isolat *Ceratorhiza* yang diremajakan sebanyak 8-10 cawan.

Inokulasi ORSV: Inokulasi ORSV pada planlet dilakukan dengan menggunakan sampel yaitu daun tembakau yang terinfeksi ORSV. Daun tembakau digerus dan ditambahkan bufferfosfat pada perbandingan 1:10 (m/v), bufferfosfat berperan untuk menghancurkan sel, sehingga virus terlepas dari sel. Tahap awal inokulasi, daun planlet ditaburi karborondum secara merata searah pertulangan daun dengan jari tangan atau cotton bud dan inokulasi dilakukan secara perlahan (Calvo *et al.*, 2020). Selanjutnya planlet ditanam kembali pada media moss steril dan dilakukan pengamatan gejala infeksi yang meliputi nekrosis, klorosis, mosaik, malformasi daun, *streak yellowing*, dan *curling leaf* selama masa inkubasi sampai gejala tersebut muncul (15-30 hari).

Pengamatan uji efektivitas

Pengamatan uji efektivitas *Ceratorhiza* dilakukan dengan aklimatisasi anggrek *Phalaenopsis amabilis* dan *Dendrobium discolour*. Selanjutnya dilakukan peremajaan *Ceratorhiza* selama 3 hari dan dilakukan inokulasi *Ceratorhiza* selama 1 hari, 2 hari, 3 hari, dan 4 hari. Pengematan uji efektivitas dilakukan selama sebulan.

Pengamatan viabilitas organ akar anggrek

Prosedur melanjutkan efektivitas *Ceratorhiza*. Inokulasi *Ceratorhiza* pada anggrek *Phalaenopsis amabilis* dan *Dendrobium discolour* selama 3 hari. Inokulasi ORSV pada anggrek dilakukan selama sebulan. Pengamatan viabilitas organ akar *Phalaenopsis amabilis* dan *Dendrobium discolor* dilakukan dengan mengamati pertumbuhan panjang akar dan jumlah akar.

HASIL DAN PEMBAHASAN

Hasil

1. Efektivitas Mikoriza

Hasil inokulasi mikoriza yang dilakukan sampai 4 hari menunjukkan bahwa hifa menyelubungi akar planlet. Lama inokulasi menunjukkan ketebalan hifa yang menyelubungi planlet. Inokulasi mikoriza pada anggrek *Phalaenopsis amabilis* dan *Dendrobium discolor* ditunjukkan pada Gambar 1.

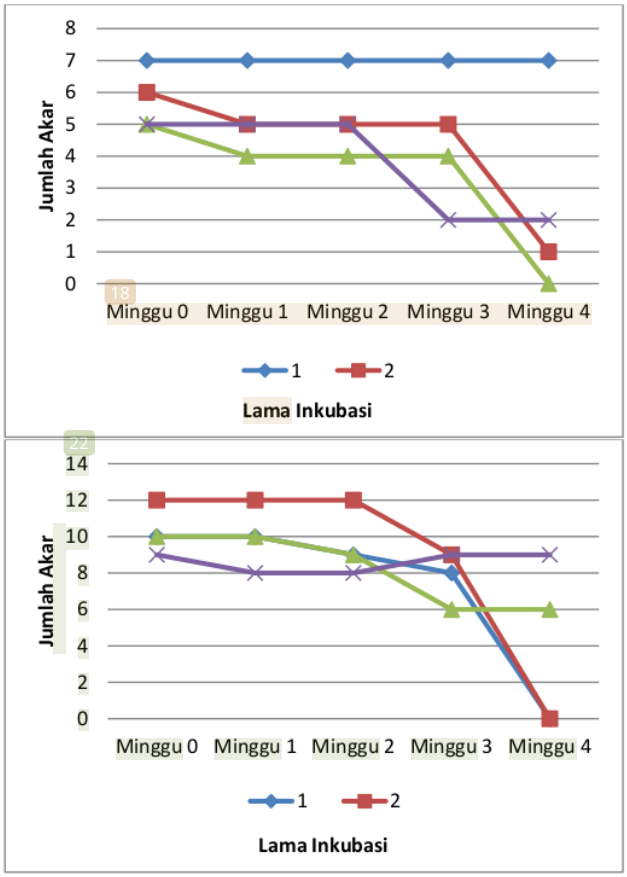
Gambar 1. Inokulasi mikoriza anggrek (A) *Phalaenopsis amabilis* dan (B) *Dendrobium discolor* dalam cawan petri pada perlakuan 4 hari

Efektivitas *Ceratorhiza* dilakukan dengan mengamati pertumbuhan akar anggrek untuk bertahan hidup pasca inokulasi ditunjukkan pada tabel 1.

Tabel 1. Pertambahan Jumlah Akar Anggrek *Phalaenopsis* dan *Dendrobium*

Jenis Anggrek	Lama Inkubasi	Minggu				
		0	1	2	3	4
Phalaenopsis	1	7	7	7	7	7
	2	6	5	5	5	1
	3	5	4	4	4	0
	4	5	5	5	2	2
Dendrobium	1	10	10	9	8	0
	2	12	12	12	9	0
	3	10	10	9	6	6
	4	9	8	8	9	9

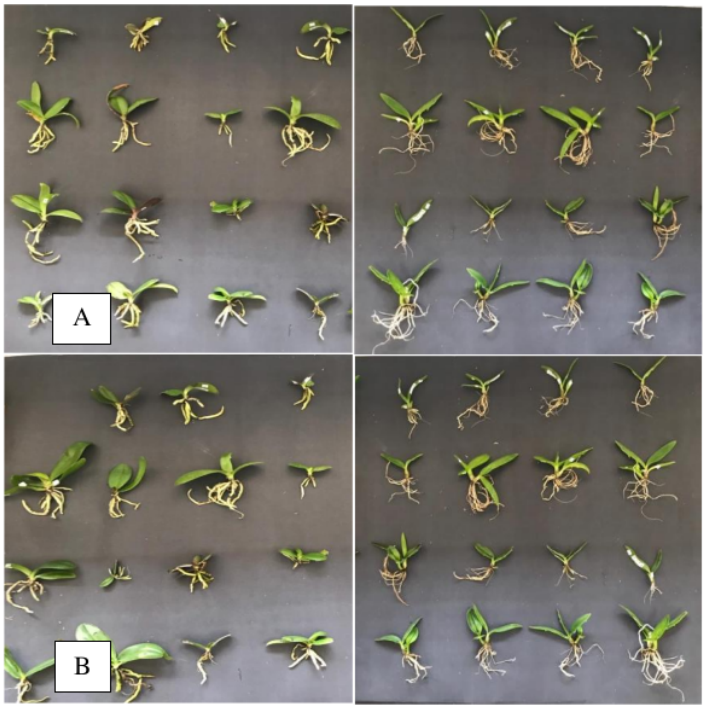
Berdasarkan hasil pengamatan yang telah dilakukan menunjukkan perbedaan yang nyata pada keefektivitasan mikoriza berdasarkan lama inkubasi. Data menunjukkan pada hari ketiga mikoriza memperlihatkan hasil yang lebih efektif dalam inokulasi mikoriza pada akar anggrek. Hasil ditampilkan pada Tabel 1 dan Gambar 2.



Gambar 2. Grafik efektivitas mikoriza terhadap jumlah akar Anggrek *Phalaenopsis amabilis* dan *Dendrobium discolor*

Berdasarkan data di atas tidak menunjukkan adanya perbedaan antara grafik akar *Phalaenopsis amabilis* dan *Dendrobium discolor*. Pada inkubasi hari ke-3 kedua anggrek memperlihatkan keberadaannya terhadap mikoriza. Hal tersebut dapat terjadi karena penyerapan mikoriza telah mencapai batas maksimum pada hari ketiga sehingga mengalami penurunan pada minggu ke-4.

Setelah dilakukan uji efektivitas mikoriza, dilakukan pengamatan dari minggu ke-0 sampai minggu ke-5 dengan parameter yaitu, panjang daun, lebar daun, jumlah daun, panjang akar, dan jumlah akar yang ditunjukkan pada Gambar 6-9. Hasil analisis dengan metode ANOVA disajikan pada Tabel 2-3.



Gambar 3. Perkembangan Planlet *Phalaenopsis amabilis* (kiri) dan *Dendrobium discolor* (kanan) hasil inokulasi mikoriza virus (MV) (A) Minggu ke-0 (B) Minggu ke-4

2. Panjang Akar

Tahap awal analisis data pengamatan panjang akar dilakukan homogenitas data menggunakan uji Levene pada taraf 5%. Hasil uji menunjukkan ragam sampel pada tanaman anggrek adalah homogen. Selanjutnya analisis dilanjutkan menggunakan uji ANOVA yang menujukkan bahwa perlakuan inokulasi mikoriza (M), virus (V), dan mikoriza virus (MV) pada tanaman anggrek memberikan hasil yang berbeda nyata. Analisis dilanjutkan menggunakan uji Tukey pada taraf nyata 5% yang ditampilkan pada Tabel 2. Sedangkan adanya interaksi pada kedua sampel dilihat pada kurva interaksi Gambar 6.

Tabel 2. Uji Tukey Panjang Akar pada kombinasi perlakuan Jenis Anggrek *Phalaenopsis amabilis* dan *Dendrobium discolor* dengan pemberian Mikoriza, Virus, Mikoriza Virus pada umur 0-5 minggu.

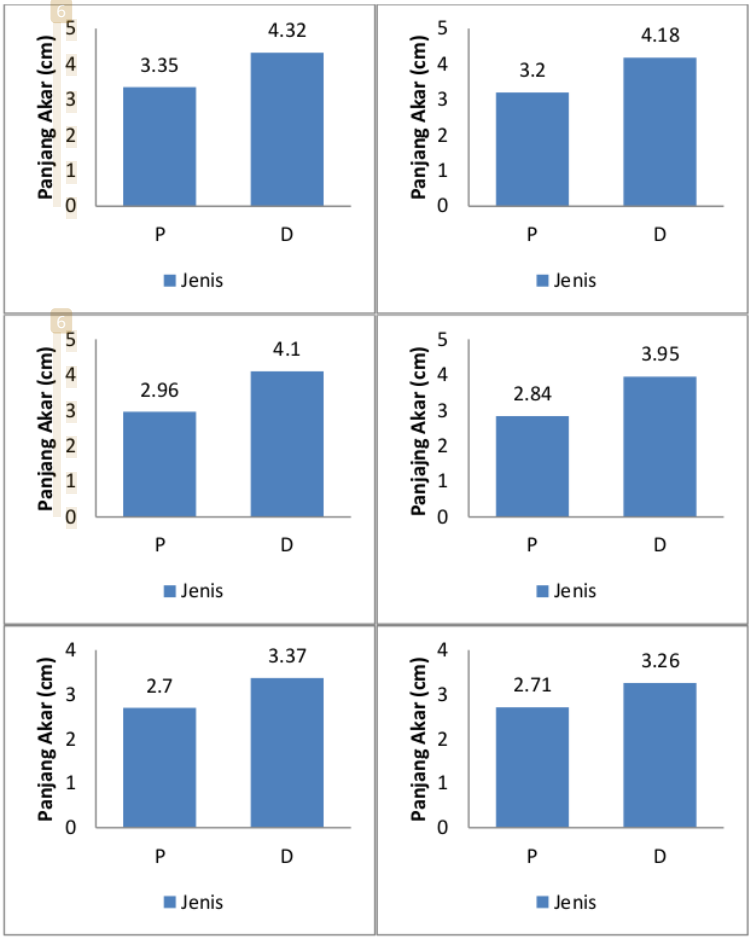
Minggu ke-	Faktor B = Spesies	Faktor A = Perlakuan			Marginal mean	K
		M	V	MV		
0	*Phalaenopsis*	3.45a ± 0.79	3.85a ± 0.58	2.76a ± 0.32	3.35a	3.66
	Dendrobium	3.61a ± 0.34	4.57a ± 0.38	4.80a ± 0.25	4.32b	4.64
	Marginal mean	3.53a	4.21a	3.78a		
	HSD Cell [0.05] = 2.17 ; Columns[0.05] = 1.23 ; Rows[0.05] = 0.83					
1	*Phalaenopsis*	3.37a ± 0.92	3.81a ± 0.28	2.42a ± 0.36	3.2a	3.65
	Dendrobium	3.51a ± 0.32	4.45a ± 0.15	4.59a ± 0.16	4.18a	4.66
	Marginal mean	3.44a	4.13a	3.50a		
	HSD Cell[0.05] = 2.02 ; Columns[0.05] = 1.14 ; Rows[0.05] = 0.77					
2	*Phalaenopsis*	2.88 ± 1.17	3.57 ± 0.47	2.43 ± 0.33	2.96a	3.45
	Dendrobium	3.41 ± 0.16	4.27 ± 0.25	4.63 ± 0.13	4.10a	4.01
	Marginal mean	3.14a	3.92a	3.53a		
	HSD Cell [0.05] = 2.47 ; Columns[0.05] = 1.4 ; Rows[0.05] = 0.94					
3	*Phalaenopsis*	2.74 ± 1.03	3.59 ± 0.53	2.19 ± 0.47	2.84a	3.16
	Dendrobium	2.87 ± 0.29	4.37 ± 0.27	4.63 ± 0.2	3.95a	3.63
	Marginal mean	2.80b	3.98a	3.41a		
	HSD Cell [0.05] = 2.44 ; Columns[0.05] = 1.39 ; Rows[0.05] = 0.93					
4	*Phalaenopsis*	2.74 ± 1.03	3.20 ± 0.54	2.18 ± 0.46	2.70a	3.20
	Dendrobium	2.39 ± 0.7	3.46 ± 0.1	4.27 ± 0.35	3.37a	2.82
	Marginal mean	2.56a	3.33a	3.22a		
	HSD Cell [0.05] = 2.72 ; Columns[0.05] = 1.54 ; Rows[0.05] = 1.04					
5	*Phalaenopsis*	2.70 ± 1.05	3.22 ± 0.53	2.21 ± 0.47	2.71a	3.21
	Dendrobium	2.03 ± 0.75	3.48 ± 0.1	4.28 ± 0.34	3.26a	2.83
	Marginal mean	2.36a	3.35a	3.24a		
	HSD Cell [0.05] = 2.78 ; Columns[0.05] = 1.58 ; Rows[0.05] = 1.06					

Berdasarkan uji Tukey pada taraf nyata 5% yang dilakukan (tabel 2) dapat diketahui bahwa pada faktor pertama jenis anggrek *Phalaenopsis amabilis* dan *Dendrobium discolor* secara statistik menunjukkan adanya perbedaan nyata yang diperjelas dengan Gambar 4. Pada faktor kedua pemberian perlakuan mikoriza, virus, mikoriza virus belum dapat dilihat, hal ini dikarenakan pada minggu ke-0 perlakuan pemberian mikoriza dilakukan setelah perhitungan panjang akar minggu ke-0 dan hasil pemberian mikoriza dapat dilihat pada minggu ke-1. Sedangkan pada kombinasi perlakuan faktor pertama dan kedua (interaksi) juga belum dapat dilihat.

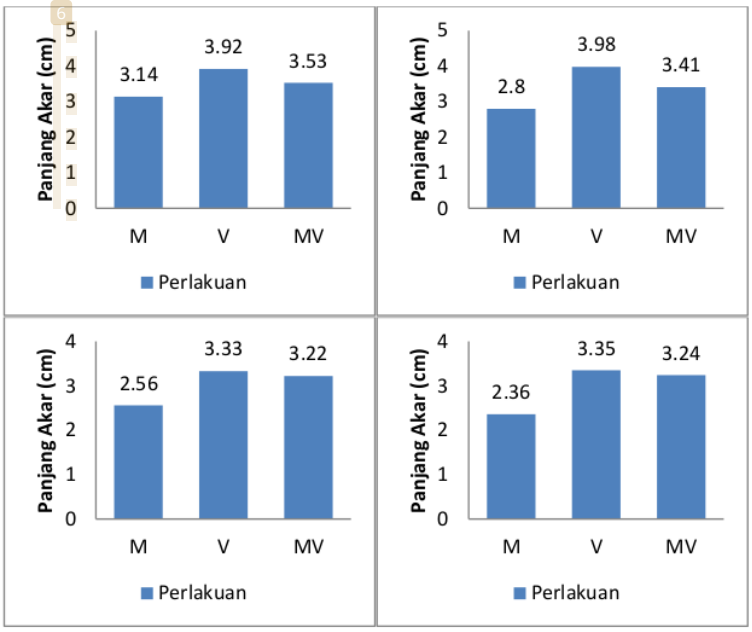
Pada minggu ke-1 dapat diketahui bahwa pada faktor pertama jenis anggrek *Phalaenopsis amabilis* dan *Dendrobium discolor* secara statistik menunjukkan tidak adanya perbedaan

nyata yang diperjelas dengan Gambar 4. Pada faktor kedua pemberian perlakuan mikoriza (M), virus (V), dan mikoriza virus (MV) belum dapat dilihat, hal ini dikarenakan pada minggu ke-1 baru dilakukan perlakuan pemberian mikoriza saja. Pemberian virus dilakukan setelah perhitungan panjang akar minggu ke-1 dan baru terlihat hasilnya pada minggu ke-2. Sedangkan pada kombinasi perlakuan faktor pertama dan kedua (interaksi) belum dapat dilihat, karena pada minggu ke-1 baru dapat dilihat untuk perlakuan mikoriza saja dan belum dapat dibandingkan dengan perlakuan yang lain.

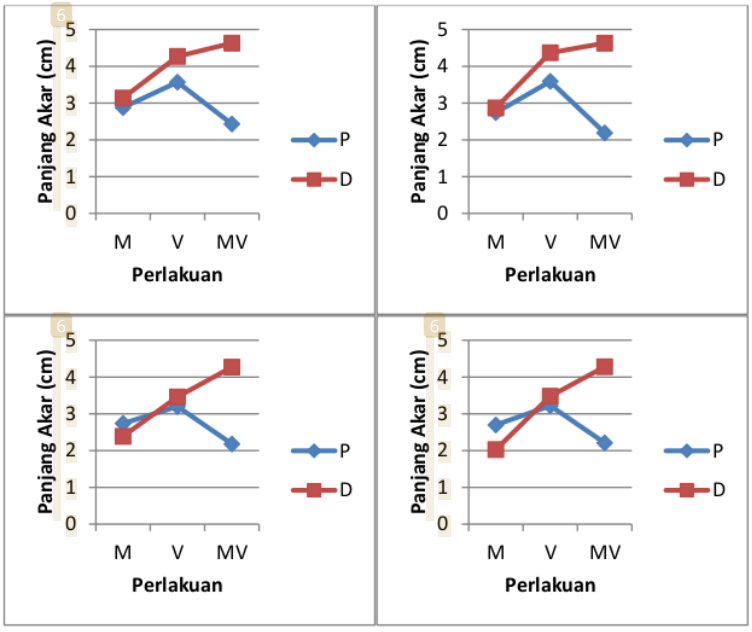
Pada minggu ke-2 sampai minggu ke-5 dapat diketahui bahwa pada faktor pertama jenis anggrek *Phalaenopsis amabilis* dan *Dendrobium discolor* secara statistik menunjukkan tidak adanya perbedaan nyata yang diperjelas dengan Gambar 4. Pada faktor kedua pemberian perlakuan mikoriza (M), virus (V), dan mikoriza virus (MV) tidak menunjukkan perbedaan nyata yang diperjelas dengan Gambar 5. Sedangkan pada kombinasi perlakuan faktor pertama dan kedua (interaksi) menunjukkan hasil yang non signifikan diperjelas pada Gambar 6.



Gambar 4. Diagram Pembanding Panjang Akar Terhadap Jenis Anggrek *Phalaenopsis amabilis* dan *Dendrobium discolor*



Gambar 5. Diagram Pembanding Panjang Akar Terhadap Perlakuan Anggrek *Phalaenopsis amabilis* dan *Dendrobium discolor*



Gambar 6. Grafik Interaksi Panjang Akar Terhadap Mikoriza, Virus, dan Mikoriza Virus

3. Jumlah Akar

Tahap awal analisis data pengamatan jumlah akar dilakukan homogenitas data menggunakan uji Levene pada taraf 5%. Hasil uji menunjukkan ragam sampel pada tanaman anggrek adalah homogen. Selanjutnya analisis dilanjutkan menggunakan uji ANOVA yang menujukkan bahwa perlakuan inokulasi mikoriza (M), virus (V), dan Mikoriza virus (MV) pada tanaman anggrek memberikan hasil yang berbeda nyata. Analisis dilanjutkan menggunkaan uji Tukey pada taraf nyata 5% yang ditampilkan pada Tabel 3. Sedangkan adanya interaksi pada kedua sampel dilihat pada kurva interaksi Gambar 9.

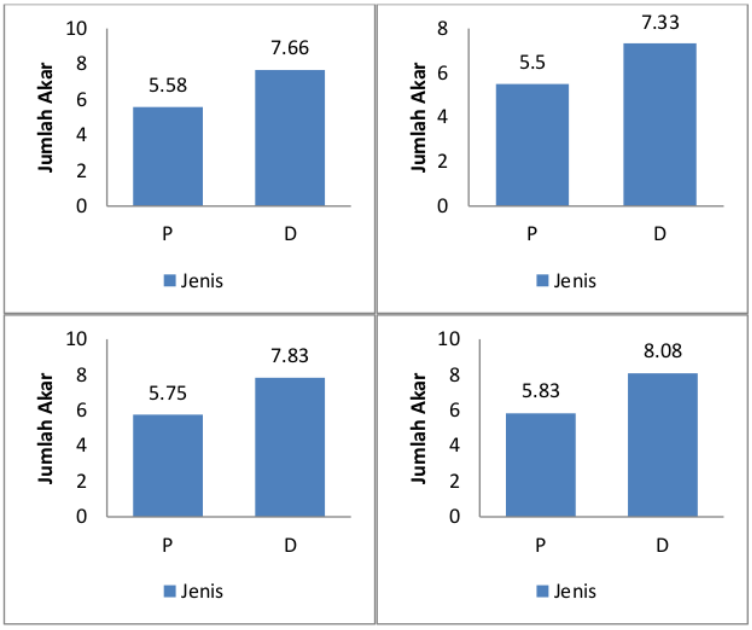
Tabel 3. Uji Tukey Jumlah Akar pada kombinasi perlakuan Jenis Anggrek *Phalaenopsis amabilis* dan *Dendrobium discolor* dengan pemberian Mikoriza, Virus, Mikoriza Virus pada umur 0-5 minggu

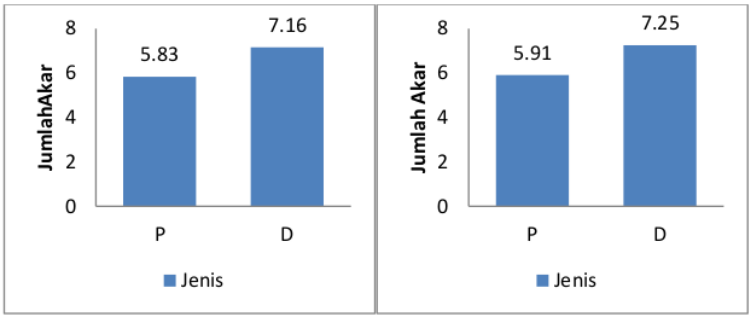
Minggu ke-	Faktor B = Spesies	Faktor A = Perlakuan			Marginal mean	K
		M	V	MV		
0	*Phalaenopsis*	5.25a ± 1.18	5.75a ± 0.75	5.75a ± 0.63	5.58a	5.25
	Dendrobium	6.5a ± 1.66	7.75a ± 0.48	8.75a ± 0.63	7.66a	8
	Marginal mean	5.87a	6.75a	7.25a		
	HSD Cell [0.05] = 4.4 ; Columns[0.05] = 2.5 ; Rows[0.05] = 1.68					
1	Anggrek *Phalaenopsis*	4.75a ± 1.31	6a ± 0.91	5.75a ± 0.48	5.5a	6.5
	Dendrobium	6.25a ± 1.55	7.75a ± 0.48	8a ± 0.41	7.33b	8.5
	Marginal mean	5.5a	6.87a	6.87a		
	HSD Cell[0.05] = 4.35 ; Columns[0.05] = 2.47 ; Rows[0.05] = 1.66					
2	*Phalaenopsis*	4.75a ± 1.31	6.5a ± 0.65	6a ± 0.71	5.75a	6.5
	Dendrobium	7a ± 1.87	8.25a ± 0.63	8.25a ± 0.75	7.83b	8
	Marginal mean	5.87a	7.37a	7.12a		
	HSD Cell [0.05] = 4.9 ; Columns[0.05] = 2.78 ; Rows[0.05] = 1.87					
3	*Phalaenopsis*	5a ± 1.47	6.5a ± 0.65	6a ± 0.71	5.83a	7.5
	Dendrobium	7.75a ± 1.65	8.25a ± 0.63	8.25a ± 0.85	8.08b	8.25
	Marginal mean	6.37a	7.37a	7.12a		
	HSD Cell [0.05] = 4.85 ; Columns[0.05] = 2.75 ; Rows[0.05] = 1.85					
4	*Phalaenopsis*	5a ± 1.47	6.5a ± 0.65	6a ± 0.71	5.83a	7
	Dendrobium	6.75a ± 2.02	7,25a ± 0.48	7.5a ± 0.65	7.16a	6.5
	Marginal mean	5.87a	6.87a	6.75a		
	HSD Cell [0.05] = 5.14 ; Columns[0.05] = 2.91 ; Rows[0.05] = 1.96					
5	*Phalaenopsis*	5.25a ± 1.38	6.5a ± 0.65	6a ± 0.71	5.91a	7
	Dendrobium	7a ± 1.78	7.25a ± 0.48	7.5a ± 0.65	7.25a	6
	Marginal mean	6.12a	6.87a	6.75a		
	HSD Cell [0.05] = 4.74 ; Columns[0.05] = 2.69 ; Rows[0.05] = 1.8					

Berdasarkan uji Tukey pada taraf nyata 5% yang dilakukan (tabel 3) dapat diketahui bahwa pada faktor pertama jenis anggrek *Phalaenopsis amabilis* dan *Dendrobium discolor* secara statistik menunjukkan adanya perbedaan nyata yang diperjelas dengan Gambar 7. Pada faktor kedua pemberian perlakuan mikoriza, virus, mikoriza virus belum dapat dilihat, hal ini dikarenakan pada minggu ke-0 perlakuan pemberian mikoriza dilakukan setelah perhitungan jumlah akar minggu ke-0 dan hasil pemberian mikoriza dapat dilihat pada minggu ke-1. Sedangkan pada kombinasi perlakuan faktor pertama dan kedua (interaksi) juga belum dapat dilihat.

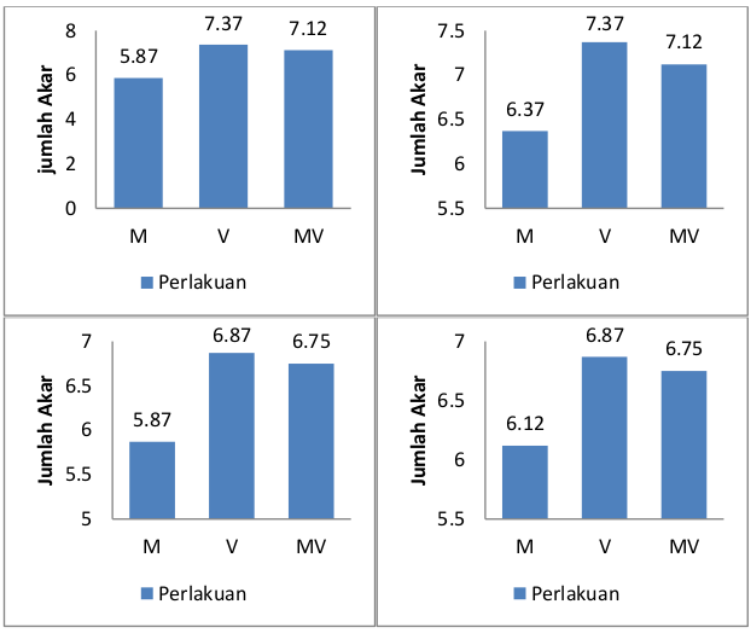
Pada minggu ke-1 dapat diketahui bahwa pada faktor pertama jenis anggrek *Phalaenopsis amabilis* dan *Dendrobium discolor* secara statistik menunjukkan tidak adanya perbedaan nyata yang diperjelas dengan Gambar 7. Pada faktor kedua pemberian perlakuan mikoriza (M), virus (V), dan mikoriza virus (MV) belum dapat dilihat, hal ini dikarenakan pada minggu ke-1 baru dilakukan perlakuan pemberian mikoriza saja. Pemberian virus dilakukan setelah perhitungan jumlah akar minggu ke-1 dan baru terlihat hasilnya pada minggu ke-2. Sedangkan pada kombinasi perlakuan faktor pertama dan kedua (interaksi) belum dapat dilihat, karena pada minggu ke-1 baru dapat dilihat untuk perlakuan mikoriza saja dan belum dapat dibandingkan dengan perlakuan yang lain.

Pada minggu ke-2 sampai minggu ke-5 dapat diketahui bahwa pada faktor pertama jenis anggrek *Phalaenopsis amabilis* dan *Dendrobium discolor* secara statistik menunjukkan tidak adanya perbedaan nyata yang diperjelas dengan Gambar 7. Pada faktor kedua pemberian perlakuan mikoriza (M), virus (V), dan mikoriza virus (MV) tidak menunjukkan perbedaan nyata yang diperjelas dengan Gambar 8. Sedangkan pada kombinasi perlakuan faktor pertama dan kedua (interaksi) menunjukkan hasil yang non signifikan diperjelas pada Gambar 9.

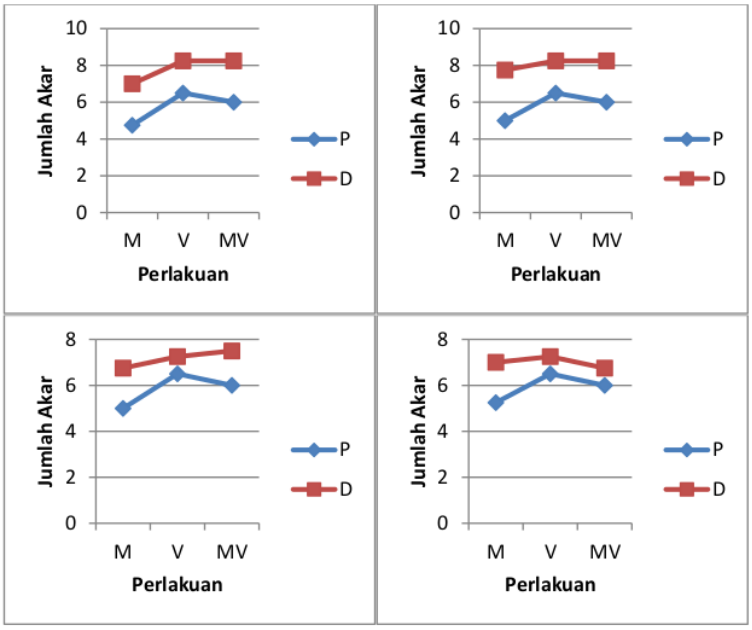




Gambar 7. Diagram Pembanding Jumlah Akar Terhadap Jenis Anggrek *Phalaenopsis amabilis* dan *Dendrobium discolor*



Gambar 8. Diagram Pembanding Jumlah Akar Terhadap Perlakuan Anggrek *Phalaenopsis amabilis* dan *Dendrobium discolor*



Gambar 9. Grafik Interaksi Jumlah Akar Terhadap Mikoriza, Virus, dan Mikoriza Virus

Pembahasan

Penelitian ini dilakukan dengan pengamatan selama 4 minggu. Parameter yang diamati yaitu, uji efektivitas mikoriza, panjang daun, lebar daun, jumlah daun, panjang akar, dan jumlah akar pada anggrek *Phalaenopsis amabilis* dan *Dendrobium discolor*. Anggrek *Phalaenopsis* dan *Dendrobium* telah diinokulasi *Ceratorhiza* dan virus. Khaterine (2016) menjelaskan bahwa anggrek hidup berasosiasi dengan fungi endofit. Perlakuan mikoriza dapat membantu memperbaiki struktur tanah di sekitar tanaman dan dapat meningkatkan panjang, lebar, dan jumlah daun serta meningkatkan panjang dan jumlah akar. Sedangkan pada perlakuan virus digunakan untuk melihat ketahanan mikoriza yang diinokulasi pada anggrek. Pada peneltian sebelumnya Lakani (2015) menjelaskan bahwa ketahanan tanaman anggrek dapat dilihat pada daun inokulasi dan pada daun yang tidak diinokulasi.

1. **Efektivitas Mikoriza**

   Hasil uji efektivitas *Ceratorhiza* pada *Phalaenopsis* dan *Dendrobium* dilakukan melalui pengamatan pertumbuhan dan kemampuan bertahan hidup. Berdasarkan hasil pengamatan diketahui bahwa inokulasi *Ceratorhiza* yang paling efektif pada hari ke-3. Pada pengamatan minggu ke-3 dan ke-4 efektivitas *Ceratorhiza* menunjukkan kematian. Sehingga data yang digunakan keseluruhan hanya sampai pengamatan minggu ke-2 saja. Hal ini dilakukan untuk menghindari data yang bias jika memaksakan keseluruhan data pengamatan sampai minggu ke-4. Pengamatan yang dilakukan pada minggu ke-0 sampai minggu ke-2 sudah dapat menjawab pertanyaan mengenai waktu efektivitas inokulasi *Ceratorhiza* tersebut. Hal tersebut didukung oleh penelitian sebelumnya Mahfut (2020) menjelaskan bahwa pada mikoriza jenis *Trichoderma* dan *Ceratorhiza* menunjukkan waktu inokulasi terbaik terdapat pada inokulasi hari ke-3 dan ke-4.

   Efektivitas *Ceratoriza* juga dapat dilihat pada pertambahan jumlah akar dan daun. Perlakuan *Ceratorhiza* mampu meningkatkan penyerapan unsur hara untuk meningkatkan

pertumbuhan tanaman. Mahfut (2019) menjelaskan bahwa induksi mikoriza jenis *Ceratorhiza* dan *Trichoderma* dapat digunakan sebagai *biofertilizer*. Perlakuan *Ceratorhiza* pada hari ke-3 dan ke-4 memberikan efek lebih pada jumlah akar yang bertambah. Hal tersebut didukung oleh penelitian sebelumnya Mahfut (2019) menjelaskan bahwa *Ceratorhiza* memberikan efek lebih jumlah akar yang mati dibandingkan dengan inokulasi *Trichoderma*.

Mikoriza jenis *Ceratorhiza* dapat membantu anggrek dalam pertumbuhan dan siklus hidupnya. Mahfut (2019) menjelaskan bahwa Mikoriza jenis *Ceratorhiza* dapat menginfeksi akar tanaman anggrek dan menghasilkan hifa intensif dan dapat meningkatkan kapasitas tanaman. Pada inokulasi mikoriza minggu ke-3 dan minggu ke-4 jumlah akar mengalami penurunan, hal ini dimungkinkan karena adanya faktor lingkungan. Menurut penelitian sebelumnya Kurnia (2019) tentang karakterisasi mikoriza mengatakan bahwa jenis tanah merupakan faktor yang berpengaruh terhadap jenis mikoriza. Faktor lain yang dapat mempengaruhi pertumbuhan tanaman yaitu, suhu, cahaya, air, nutrisi, dan tanah.

2. **Panjang Akar**

Berdasarkan data yang diperoleh dari hasil analisis minggu ke-0 sampai minggu ke-5 pada variabel panjang akar tidak ada perbedaan yang nyata. Pada minggu ke-1 sampai minggu ke-5 jumlah daun mengalami penurunan. Hal ini disebakan karena perlakuan mikoriza (M), virus (V), dan mikoriza virus (MV), pemberian virus mengakibatkan daun mengalami kerusakan. Jika dengan kontrol *Phalaenopsis* (3.38) (K); (2.96) (P) dan *Dendrobium* (3.76) (K); (3.86) (P) pada perlakuan menunjukkan hasil yang lebih tinggi dibandingkan dengan kontrol. Namun jika dilihat berdasarkan jenisnya diantara kedua anggrek tersebut, *Dendrobium* lebih baik dalam mempertahankan pertumbuhan panjang akar dibandingkan dengan *Phalaenopsis*.

Mikoriza yang diinokulasi pada akar berperan dalam pertumbuhan dan perkembangan anggrek untuk bertahan hidup. Pertumbuhan panjang akar yang mengalami penurunan dapat disebabkan karena proses fisiologis yang kurang baik. Hal ini diperkuat dengan penelitian Lakitan (2000) yang menjelaskan bahwa pertumbuhan tanaman merupakan serangkaian proses fisiologis tanaman dalam membentuk suatu kesatuan organ yang kompleks dengan adanya penambahan bobot dan ukuran tanaman.

3. **Jumlah Akar**

Berdasarkan data yang diperoleh dari hasil analisis minggu ke-0 sampai minggu ke-5 pada variabel jumlah akar tidak ada perbedaan yang nyata. Pada minggu ke-1 sampai minggu ke-5 jumlah daun mengalami pe Jika dengan kontrol *Phalaenopsis* 6.62 (K); 5.73 (P) dan *Dendrobium 7.54* (K); 7.55 (P) sama dengan variabel panjang akar yang menunjukkan pada perlakuan *Phalaenopsis* memperlihatkan hasil yang lebih rendah dibandingkan dengan kontrol. Sedangkan pada Dendrobium jika dibandingkan dengan kontrol pemberian perlakuan menmperlihhatkan hasil yang lebih tinggi. nurunan. Hal ini disebakan karena perlakuan mikoriza (M), virus (V), dan mikoriza virus (MV), pemberian virus mengakibatkan daun mengalami kerusakan. Namun jika dilihat berdasarkan jenisnya diantara kedua anggrek tersebut, *Dendrobium* lebih baik dalam mempertahankan pertumbuhan panjang akar dibandingkan dengan *Phalaenopsis*.

Rianti (2017) menjelaskan bahwa jumlah akar pada tanaman mengindikasikan seberapa luas jangkauan tanaman dalam menyerap nutrisi dan unsur hara pada perlakuan mikoriza (M) menunjukkan bahwa kemampuan anggrek untuk membentuk akar karena sudah

memiliki bakal daun. Hal ini didukung oleh Bey *et al.,* (2006), yang menjelaskan bahwa radikula akan berubah menjadi akar dengan bantuan auksin yang diproses oleh daun

KESIMPULAN

Kesimpulan yang diperoleh dari hasil penelitian ini meliputi:

1. Hasil induksi *Ceratorhiza* dapat meningkatkan efektivitas anggrek *Phalaenopsis amabilis* dan *Dendrobium discolor* dengan lama waktu inkubasi 3 hari
2. Hasil viabilitas organ akar anggrek *Phalaenopsis amabilis* mengalami penurunan pada panjang akar (2.96 cm) dan jumlah akar (5.73 buah), sedangkan pada *Dendrobium discolor* mengalami peningkatan panjang akar (3.86 cm) dan jumlah akar (7.55 buah).

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