E-ISSN-2580-7129
Print- ISSN-1978-6107

TECHNO: JURNAL PENELITIAN
Journal homepage: http://ejournal.unkhair.ac.id/index.php/Techno
Volume 11 Number 02 October 2022 DOI: http://dx.doi.org/10.33387/tjp.v11i2.3044

Study of Resistance on Orchids Induced *Rhizoctonia* to ORSV Based on Secondary Metabolite Analysis

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Received : 21-04-2021
Accepted : 27-08-2022
Available online : 30-10-2022

ABSTRACT

Dendrobium discolor and *Phalaenopsis amabilis* are in high demand because of their beautiful shape, color, texture, and flower arrangement. The current obstacle to orchid cultivation is infection with *Odontoglossum ringspot virus* (ORSV). One of the efficient ways to control the infection of this pathogen and does not cause an impact on the environment is to use *Rhizoctonia* mycorrhizae. Mycorrhizal relationship with plants will form induced resistance. Plants resulting from induced resistance will induce secondary metabolite, namely peroxidase enzymes. This type of secondary metabolites will prevent the growth and development of pathogens and plants become resistant to pathogenic infections. The purpose of this study was to determine differences in peroxidase enzyme activity and differences in resistance to *P. amabilis* and *D. discolor* after *Rhizoctonia* induction against ORSV infection. This research was carried out in several stages, namely plantlet preparation, mycorrhizal inoculation, mycorrhizal inoculation, and peroxidase enzyme activity analysis using a UV Vis spectrometer at a wavelength of 420 nm. The results showed that *D. discolor* had a higher peroxidase enzyme activity than *P. amabilis*. In the treatment application factor, the peroxidase enzyme activity was the highest in the Mycorrhizal Virus treatment compared to the Mycorrhizal and Virus treatments. This study showed that *D. discolor* was more resistant to ORSV infection than *P. amabilis*. and the application of Mycorrhizae proved to be successful in increasing the activity of the peroxidase enzyme as a response to plant protection against viral infections.

Keywords: *Dendrobium discolor*, ORSV, Peroxidase Enzyme, *Phalaenopsis amabilis*, *Rhizoctonia*

ABSTRAK

Dendrobium discolor dan *Phalaenopsis amabilis* banyak diminati karena keindahan bentuk, warna, tekstur, dan susunan bunganya. Kendala budidaya anggrek saat ini adalah infeksi *Odontoglossum ringspot virus* (ORSV). Salah satu cara yang efisien untuk pengendalian infeksi pathogen ini dan tidak menimbulkan dampak terhadap lingkungan adalah menggunakan mikoriza *Rhizoctonia.* Hubungan mikoriza dengan tanaman akan membentuk *induced resistance.* Tanaman hasil *induced resistance* akan menginduksi metabolit sekunder yaitu enzim peroksidase. Jenis metabolit sekunder ini akan mencegah pertumbuhan dan perkembangan patogen serta tanaman menjadi resisten terhadap infeksi pathogen. Tujuan penelitian ini untuk mengetahui perbedaan aktivitas enzim peroksidase dan perbedaan ketahanan pada *P. amabilis* dan *D. discolor* setelah di induksi *Rhizoctonia* terhadap infeksi ORSV. Penelitian ini dilakukan dalam beberapa tahapan yaitu persiapan planlet, inokulasi mikoriza, inokulasi mikoriza, dan analisis aktivitas enzim peroksidase menggunakan spektrotometer UV Vis pada panjang gelombang 420 nm. Hasil penelitian menunjukkan *D. discolor* mengalami peningkatan aktivitas enzim peroksidase yang lebih tinggi dibandingkan *P. amabilis.* Pada faktor aplikasi perlakuan, aktivitas enzim peroksidase terjadi peningkatan tertinggi pada perlakuan Mikoriza Virus dibandingkan dengan perlakuan Mikoriza

dan Virus. Penelitian ini menunjukkan bahwa *D. discolor* lebih tahan terinfeksi ORSV dibandingkan *P. amabilis*. serta aplikasi Mikoriza terbukti berhasil meningkatkan aktivitas enzim peroksidase sebagai respon perlindungan tanaman terhadap infeksi virus.

Kata kunci: *Dendrobium discolor,* Enzim Peroksidase, ORSV**,** *Phalaenopsis amabilis, Rhizoctonia*

INTRODUCTION

Orchids are included in the family Orchidaceae which has 800 genera and 25,000 species in the world and 5000 species live in the territory of Indonesia (Yusnita, 2012). The species richness is Indonesia's potential to cultivate orchids. Orchids have high economic value and are widely cultivated by the community because they have various patterns, shapes, textures, and flower colors such as *Dendrobium discolor* and *Phalaenopsis amabilis* (Putra, 2021).

One of the problems of orchid cultivation in Indonesia is infection with viral pathogens. The type of virus that infects this plant is *Odontoglossum ringspot virus* or abbreviated ORSV (Mahfut *et al*., 2017). ORSV is reported to have infected many orchid genera and has the widest distribution in the world, including in Indonesia. Symptoms of this viral infection are chlorotic, mosaic, and necrotic ringspot-shaped (Sari *et al*., 2021).

Biological control is an effort to protect plants against disease that is effective, efficient, and does not have an impact on the environment (Kaur, 2022). One form of biological control is using endophytic mycorrhizae such as *Rhizoctonia* (Nnamchi *et al*., 2021; Tsulsiyah *et al*., 2021). The results study of Soelistijono (2015) has reported that *Rhizoctonia* inoculated on *Phalaenopsis amabilis* in Surakarta was proven to be able to inhibit *Fusarium oxysporum* infection by 100%. This is due to the inoculation of *Rhizoctonia* producing secondary metabolites in the form of peroxidase enzymes. This compound is known to be a specific protein in plants that can show the mechanism of plant resistance to pathogen infection (Delyani *et al*., 2019). Furthermore, Anggreiny *et al* (2021) also reported that *Ceratorhiza* mycorrhizal induction was able to increase peroxidase activity in orchids infected with ORSV. The peroxidase activity of *D. discolor* was higher than that of *P. amabilis*. This indicates that *D. discolor* has a higher level of resistance than *P. amabilis*.

Research on the application of *Rhizoctonia* in inhibiting disease has only been carried out against fungal infections of Fusarium oxysporum. Until now there has been no research on the application of Rhizoctonia in inhibiting the severity of plant viral infections, including ORSV in orchids. This study aimed to reduce the severity of infection in leaves due to Rhizoctonia-induced ORSV infection based on peroxidase enzyme analysis. This research needs to be done as an effort to protect orchids against infectious diseases and to determine the effectiveness of endophytic mycorrhizal applications in their control.

METHODOLOGY

This research was conducted from January 2021 – March 2021 at the Botanical Laboratory 2, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung. The study was structured using a factorial Completely Randomized Design (CRD) with 2 treatment factors, the 1st factor being the type of orchid, consisting of *P. amabilis* or *D. discolor* and the 2nd factor being the treatment of Mycorrhizae (M), Virus (V), and Mycorrhizal Virus (MV) to obtain 2x3 = 6 treatment combinations. Each treatment was repeated 4 times to obtain 24 experimental units. Each experimental unit consisted of 1 plant *P. amabilis* or 1 plant *D. discolor* in each small plastic pot, as a comparison factor outside the design used control of

orchids without treatment. Parameters tested included peroxidase enzyme activity in plant samples.

The tools used in this study were mortar, test tube, dropper, petri dish, tweezers, filter paper, measuring flask, Erlenmeyer, spectrophotometer, and centrifuge. The materials used were orchid seeds, sterile moss media, Potato Dextrose Agar (PDA) medium, *Rhizoctonia* mycorrhiza, carborundum, ORSV inoculum, 2.5 ml Potassium Phosphate 0.5 M pH 7, 0.1 grams of Polyvinylpropyrolidone (PVP), 5 ml of Pyrogallol, and 0.5 ml of 1% H_2O_2.

This research consists of 5 stages, namely:

1. *Preparation of plantlets*
   Plantlets aged 8 months were planted in pots containing sterile moss media. After acclimatization, treatment is carried out regularly until 3-4 leaves appear on the orchid.
2. *Mycorrhizal inoculation*

   Rhizoctonia inoculation method was carried out using the method of Nuangmek *et al*., (2008). *Rhizoctonia* was grown on PDA medium in 9 cm diameter petridish. *Rhizoctonia* isolates were inoculated on the medium and incubated for 7 days. Next, the plantlets were placed in a petridish containing *Rhizoctonia* for 24 hours and then re-grown in sterile moss plant media.
3. *Virus inoculation*
   ORSV inoculation on plantlets was carried out using inoculum samples from virus propagation in tobacco plants. Tobacco leaves were weighed as much as 1 gram and then crushed using a mortar and pestle that had been sterilized. Furthermore, the crushed leaves are added with phosphate buffer in a ratio of 1:10 (m/v), modified (Irni, 2020). The ORSV inculation method was carried out using the method of Calvo *et al*. (2008). Before being inoculated, the surface of the plantlet leaves was sprinkled with carborundum in the direction of the leaf bone using fingers or a cotton bud until evenly distributed. Inoculation is done slowly. Furthermore, plantlets were reared on sterile moss growing media and observed for infection symptoms including necrosis, chlorosis, streak yellowing, mosaic, leaf malformations, and curling leaves during the incubation period until these symptoms appeared (15-30 days).
4. *Analysis of Peroxidase Enzyme Activity*
   Peroxidase activity was analyzed using the method of Saunders and McClure (Suawati *et al*., 2004). Plantlets were taken as much as 1 gram of leaves then cut into pieces and crushed using a mortar. The results of the scour were added with 2.5 ml of 0.5 M potassium phosphate pH 7 and 0.1 grams of PVP. Next, extraction was carried out by filtering the sample using 2 layers of gauze, then centrifuged at 6000 rpm at 40C for 15 minutes. To measure the activity of the peroxidase enzyme, a 0.2 ml supernatant solution was put into a test tube, then added 5 ml of pyrogallol (containing 0.631 grams of pyrogalol and 0.005 M phosphate buffer pH 6) then the absorbance value was measured using a Spectrophotometer UV Vis with a maximum wavelength of 420 nm. The buffer solution with enzyme extract was added with 0.5 ml of 1% H_2O_2 and incubated for 30 minutes, then the absorbance value was measured and the changes were observed.
5. *Data Analysis*
   Data analysis in this study used a factorial Completely Randomized Design (CRD). Quantitative data from each parameter was tested for homogeneity first using Levene's test at a significance level of 5%, after homogeneous data then analyzed by analysis of variance or ANOVA. The analysis was carried out at a 5% significance level and further test using the Tukey test at a 5% significance level.

RESULTS AND DISCUSSION

Based on the results of the analysis of Levene test data at a 5% significance level, the sample data obtained from the combination of Mycorrhizal (M), Virus (V), and Mycorrhizal Virus (MV) treatments with orchids were homogeneous, then ANOVA test was carried out with P-value = 0.002 < 0.05. These results indicate that the interaction between orchids with the combination treatment of Mycorrhizal (M), Virus (V), and Mycorrhizal Virus (MV) is significant. Thus, further tests can be carried out using the Tukey test. The results of the Tukey peroxidase enzyme activity are presented in Table 3.

Table 3. Tukey test results of peroxidase enzyme activity in combination treatment of *P. amabilis* and *D. discolor* with the application of mycorrhizae, viruses, mycorrhizae viruses

Factor B = Species	**Factor A = Treatment**			
	M	**V**	**MV**	**Marginal mean**
P. amabilis	0.781 a ± 0.01	0.958 b ± 0.02	1.144 c ± 0.01	0.961 a
D. discolor	0.793 a ± 0.01	1.047 d ± 0.02	1.224 e ± 0	1.021 b
Marginal mean	0.787 a	1.003 b	1.184 c	

HSD Cell[0.05] = 0.05 ; Columns[0.05] = 0.03 ; Rows[0.05] = 0.02. M= Mycorrhizae, V= Viruses, MV=Mycorrhizae Virus

Based on the results of the Tukey test at a 5% significance level, it showed that the combination of Mycorrhizal (M), Virus (V), and Mycorrhizal Virus (MV) treatments had a significant effect on the activity of the peroxidase enzyme. The combination of Mycorrhizal Virus (MV) treatment resulted in the highest average peroxidase enzyme activity compared to the Control (without treatment), M (Mycorrhizal), and V (Virus) treatment. The treatment of different types of orchids also had a significant effect on the activity of the peroxidase enzyme. It is known that *D. discolour* produces a very high average peroxidase enzyme activity compared to *P. amabilis*. The results of the analysis of the peroxidase enzyme activity obtained from the combination of Mycorrhizal (M), Virus (V), and Mycorrhizal Virus (MV) treatments with *P. amabilis* and *D. discolor* types can be seen in Figure 1.

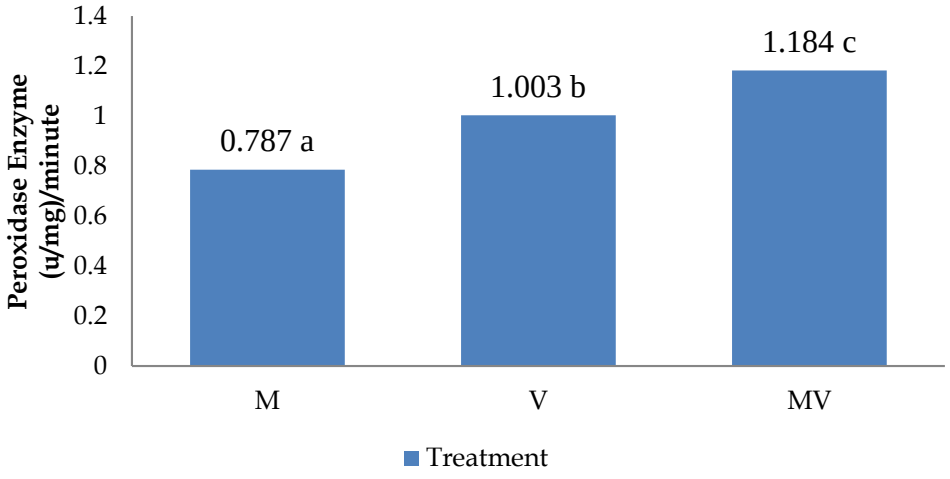

Figure 1. Peroxidase enzyme activity curve on treatment of mycorrhizae, viruses, and mycorrhizae viruses

Based on Figure 1, it can be seen that the treatment of Mycorrhizae (M) resulted in an average peroxidase enzyme activity of 0.787 (u/mg)/minute. In the treatment with Virus (V) the average peroxidase enzyme activity increased to 1,003 (u/mg)/minute, while the combination treatment with Mycorrhizal Virus (MV) resulted in the highest average peroxidase enzyme activity, which

was 1,184 (u/mg)/ minutes after being compared with Control, Mycorrhizal (M), and Virus (V) treatments.

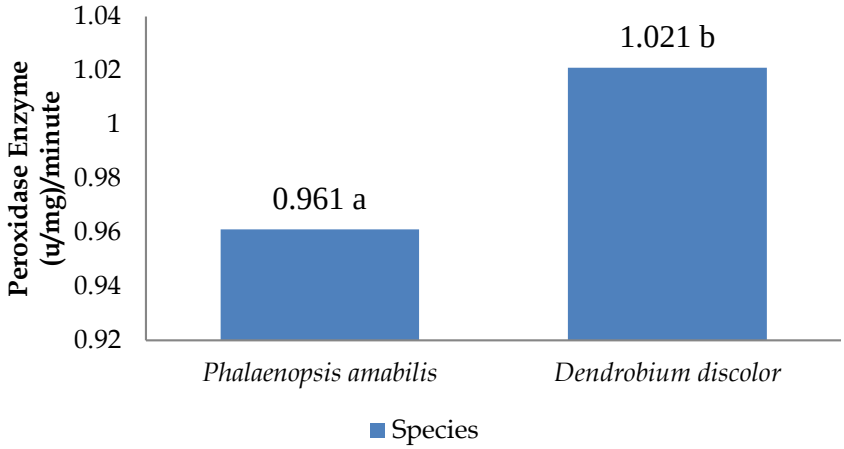

Figure 2. Peroxidase enzyme activity curve on type *dendrobium discolor* and *phalaenopsis amabilis*

Based on Figure 2, it can be seen that *D. discolor* produces the highest average peroxidase enzyme activity of 1.021 (u/mg)/minute while *P. amabilis* only produces an average peroxidase enzyme activity of 0.961 (u/mg)/minute.

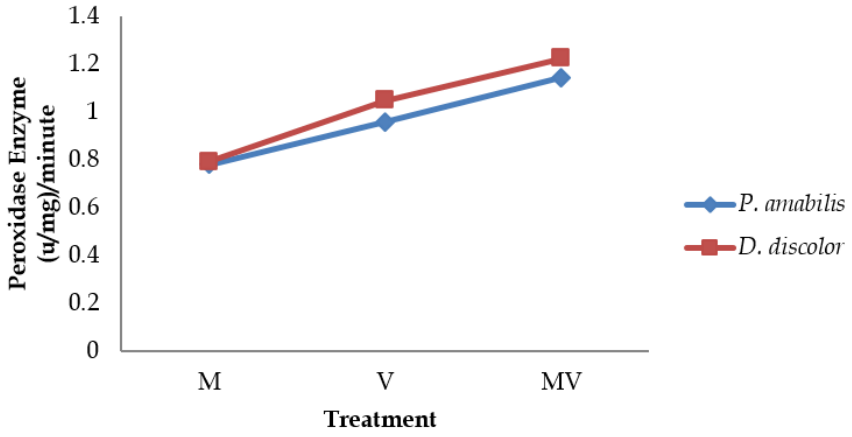

Figure 3. Interaction curve between *dendrobium discolor* and *phalaenopsis amabilis* with Mycorrhizal, Virus, and Mycorrhizal Virus treatment

Based on the results of the analysis curve of the peroxidase enzyme activity showed significantly different interactions . It can be seen that the combination of Mycorrhizal (M), Virus (V), and Mycorrhizal Virus (MV with P. amabilis and D. discolor) treatments had a positive effect on the peroxidase enzyme activity. higher than the Control, Mycorrhizal (M) and Virus (V) treatments alone, while the *D. discolor* species also produced a very high average peroxidase enzyme activity compared to the *P. amabilis* species.

Based on the results of the Tukey test at a 5% significance level, the treatment of Mycorrhizal (M), Virus (V), and Mycorrhizal Virus (MV) with the types of *D. discolor* and *P. amabilis* can affect the activity of the peroxidase enzyme in plants. The relationship between peroxidase enzyme

activity in plants treated with Mycorrhizal (M), Virus (V), and Mycorrhizal Virus (MV) is shown by the regression line equation in Figure 4-5.

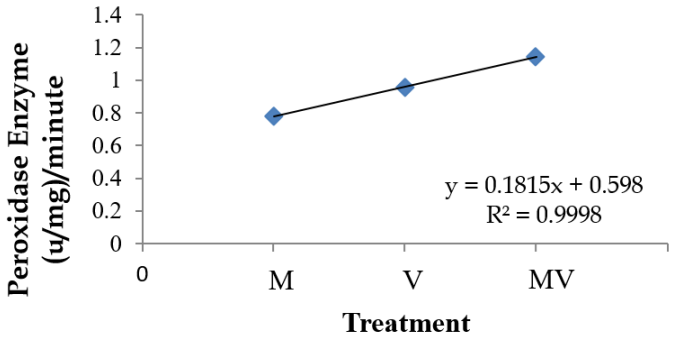

Figure 4. Peroxidase activity regression curve between combinations M, V, and MV treatment with *P. amabilis*

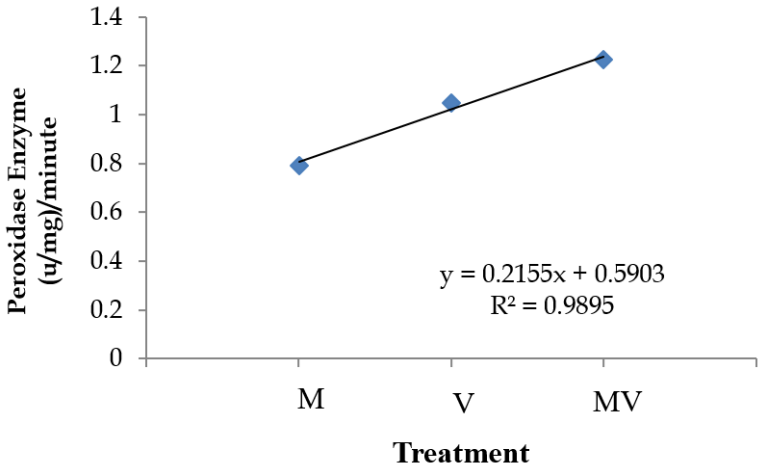

Figure 5. Regression curve of peroxidase enzyme activity between combination of treatments M, V, and MV with *D. discolor*

Based on Figure 4-5, it can be seen that the relationship between peroxidase enzyme activity between the treatment of Mycorrhizal (M), Virus (V), and Mycorrhizal Virus (MV) and *P. amabilis* is shown by a positive linear equation y = 0.1815x + 0.598 with a coefficient of determination (R2 = 0.9998) while in *D. discolor* obtained a linear equation y = 0.2155x + 0.5903 with a coefficient of determination (R2 = 0.9895). This indicates that there is a strong relationship between the treatment of Mycorrhizal (M), Virus (V), and Mycorrhizal Virus (MV) with different types of orchids on the activity of the peroxidase enzyme produced.

Based on the results of the study, the Mycorrhizal (M) treatment produced the least peroxidase enzyme activity compared to the Virus (V) and Mycorrhizal Virus (MV) treatments. This is because jasmonic acid and methyl jasmonic produced by mycorrhizae play a role in activating plant resistance signals so that mycorrhizae only reduce the severity of pathogenic infections in plants (Vieheilig et al., 2008). In this study, the mycorrhizal agent *Rhizoctonia* was used which can produce secondary metabolites in the form of peroxidase enzymes. This enzyme will catalyze the oxidation reaction of phenol compounds into quinone compounds by producing H2O2 which is toxic to pathogens (Do *et al*., 2003). This indicates that in the Mycorrhizal (M) treatment, the peroxidase enzyme activity produced is not too high because the peroxidase enzyme activity will increase if the plant is attacked by pathogens. Peroxidase enzyme is an

indicator of local and systemic resistance induction and will increase if the plant is infected with pathogens (EL-Mougy et al., 2013). In the Virus (V) treatment, the average peroxidase enzyme activity was higher than the Mycorrhizal (M) treatment. Increased activity of the peroxidase enzyme indicates the level of plant resistance to viruses. The higher the viral infection in plants, the higher the peroxidase enzyme activity in plants (Ferdhiani *et al*., 2015).

Mycorrhizal Virus (MV) treatment had a positive effect on the activity of the peroxidase enzyme. This treatment resulted in the highest average peroxidase enzyme activity compared to the Control, Mycorrhizal (M), and Virus (V) treatments. This was caused by the combination of Mycorrhizal Virus (MV) treatments which were inoculated on plants simultaneously producing peroxidase enzymes which were used for defense systems against ORSV attacks. Peroxidase enzymes will increase when plants are infected with pathogens. In addition, the activity of the peroxidase enzyme will increase when triggered by scavenging agents such as mycorrhizae.

The increase in peroxidase activity on *D. discolor* was higher than *P. amabilis*. This indicates that *D. discolor* is more resistant to ORSV attack because it has a thick cell wall structure. Peroxidase enzymes play a role in producing plant defense compounds such as lignin, chitin, and compounds that make up cell walls (Diez *et al*., 2016). The formation of lignin in plants causes the cell wall to become thicker so that it is difficult for pathogens to penetrate (Vicuna *et al*., 2011). Lignin is a plant defense system that functions as an inhibitor of pathogens and is formed due to the penetration of pathogens. The formation of lignin is driven by the activity of the peroxidase enzyme by strengthening the cell wall. Lignin is difficult to be degraded by pathogens, causing pathogens not to thrive in plant tissues (Andari *et al*., 2016). The higher the peroxidase enzyme activity in plants, the thicker the plant cell walls and the higher resistance of plants to ORSV attack.

P. amabilis is a plant that is susceptible to ORSV infection. The results of the study (Mahfut *et al*., 2016) detected ORSV with a DAS-ELISA serological test showing that from a total of 11 samples infected with ORSV, 9 of them were Phalaenopsis sp. Orchid Phalaenopsis sp. has a leaf texture that is thick, soft, and has a thin cell wall structure (Wang & Chang, 2017). Thin cell walls facilitate the spread and penetration of pathogens into plants (Firgiyanto *et al*., 2016). The cell wall serves as a structural defense of plants to prevent pathogens and block the spread of toxins (Nort & Dueck, 2015). In addition, *P. amabilis* contains more water than *D. discolor*. Plants that have high water content will cause them to be susceptible to pathogen attack (Firgiyanto et al., 2016). According to Cho *et al*. (2020) bacteria or pathogens that attack plants can grow rapidly following the flow of water.

Based on the results of the study, it was known that the *P. amabilis* orchid was more susceptible to viruses, so the resistance of these plants to ORSV was very low, so the activity of the peroxidase enzyme was lower than *D. discolor*. According to Shen *et al*. (2018), plants that are resistant to pathogenic infections will increase the activity of the peroxidase enzyme, while in plants that are sensitive or susceptible to pathogenic infections, there is no change in the activity of the peroxidase enzyme and can even decrease compared to healthy conditions.

CONCLUSION

The results of the highest peroxidase enzyme activity were obtained in the combination treatment of Mycorrhizal Virus (MV) with *D. discolor* of 1.144 (u/mg)/minute and *P. amabilis* of 1.224 (u/mg)/minute. *D. discolor* has better resistance than *P. amabilis* against ORSV infection.

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