Utilization of *Rhizoctonia* Mycorrhizae for Orchid Late Blight Control in Sustainable Agricultural

R. Soelistijono[1*], Daryanti[1], Dian Rakhmawati[2], Prisma Aditya Rianto[1], Herry Utomo[3]

[1]Agricultural Faculty Tunas Pembangunan University Jl. Balekambang Lor No 1, Surakarta (57139) Indonesia
[2]Magelang District's Agriculture and Food Service, Magelang (56511) Indonesia
[3]LSU AgCenter H.Rouse Caffey Rice Research Station Crowley LA (70578) USA

[*]Corresponding Author : soelistijono@lecture.utp.ac.id

Abstract. *Vanda tricolor* is currently a protected species because is the occurrence of orchid late blight caused by the *Fusarium* sp. pathogenic fungus. The objective of this study was to investigate the effectiveness of *Rhizoctonia* mycorrhizae in inducing resistance against *Fusarium* sp., the causative agent of late blight, in *V. tricolor*. The research employed CRBD with three treatments and six replications. *Rhizoctonia* mycorrhizae were isolated and identified following the procedure outlined by Bayman. Pre-inoculation of *Rhizoctonia* mycorrhizae was conducted on PDA media, and mycorrhizae were subsequently inoculated onto the roots of *V. tricolor* seedlings derived from tissue culture. The presence of peloton structures on the roots was determined using the Nakano methods. The Saravanan method was employed to measure peroxidase activity. The results demonstrated that *V. tricolor* induced by *Rhizoctonia* mycorrhizae exhibited resistance against *Fusarium* sp. and displayed the formation of peroxidase enzymes, peloton structures, and lignification. In contrast, the non-induced orchids suffered significant damage to the epidermal tissue of the leaves, did not exhibit an increase in peroxidase enzymes, and failed to form peloton or lignified structures. The novelty of this research is the use of *Rhizoctonia* mycorrhizae to control orchid late blight diseases at Mount Merapi.

Keywords: *Fusarium* sp.; induced resistance; orchid late blight; *Rhizoctonia* mycorrhizae.

INTRODUCTION

Orchids are highly sought-after flowers worldwide due to their attractive shapes, vibrant colors, long-lasting nature, and high market value. They have become immensely popular among the general population. Efforts to tap into the potential of orchids as a non-oil and gas export commodity have been made by both the government and the private sector. However, these endeavors have faced challenges, particularly in the cultivation of terrestrial orchids. One of the main obstacles encountered is diseases caused by pathogens like viruses (Mursyidin & Saputra, 2023), bacteria, and fungi that can attack various parts of the plant, such as the stems, leaves, and roots. Among the fungal pathogens affecting orchids, *Fusarium* sp. deserves attention, particularly *Fusarium solani* (Tamrin et al., 2020; Fayaz et al., 2014). This fungus targets numerous plant species and also inflicts damage on orchids (Sweet CS. and Uchida,

2015; Srvastaka et al., 2018). Initially, it attacks *Phalaenopsis amabilis*, causing yellowing and rotting of the leaves. Subsequently, the pathogenic fungus spreads throughout the entire plant (Laurence et al., 2016). Apart from *P. amabilis*, *F. solani* also poses a threat to other orchid varieties (Soelistijono et al., 2017).

Vanda tricolor, a unique orchid species found exclusively in Kaliurang on the slopes of Mount Merapi in Java, faces the challenge of being rare due to its difficult cultivation and vulnerability to pathogenic fungi. *V. tricolor* is currently on the protected species list due to its declining presence in the wild. One of the contributing factors to this decline is the occurrence of orchid late blight caused by the *Fusarium* sp. pathogenic fungus. Traditional control methods for orchid late blight involve the use of chemical fungicides, which pose risks to both farmers and the environment. The main hindrance to successfully cultivating *V. tricolor* is the occurrence of late blight caused by

the pathogenic fungus *Fusarium* sp. While *Fusarium* attacks *Vanda* species, there have been no specific reports of its impact on *V. tricolor*. Traditionally, the approach to tackling such diseases has involved using fungicides, which are sprayed onto the affected parts of the plant. However, this method poses risks to the environment, as the fungicide persists in the soil for an extended period. Implementing chemical fungicides as a countermeasure can have adverse effects on farmers and the environment. Therefore, alternative methods need to be explored.

Sustainable agriculture is widely embraced due to its safer approach compared to fungicide usage, making it more environmentally conscious. This approach falls under the umbrella of organic farming, which involves the utilization of diverse organic fertilizers, resistant varieties, and biological agents to manage fungal, bacterial, and viral pathogens (Shreya, 2020). One of the strategies employed in sustainable agriculture to control pathogenic fungi is the induction of resistance. Resistance induction is a mechanism that enhances plant resistance to major pathogens or insects through pre-inoculation with non-pathogenic biological agents (Borges & Sandalio, 2015; Al-Saidi and Al-Obaidy, 2022). This mechanism finds widespread application in the realm of sustainable agriculture. One potential biological agent that can be explored for controlling late blight caused by *Fusarium* sp. in *V. tricolor* is the utilization of *Rhizoctonia* mycorrhizae as a resistance induction agent. *Rhizoctonia* mycorrhizae belongs to the *Rhizoctonia* group and was identified in orchids and their groups (Haryuni et al., 2022). Unlike *Rhizoctonia solani*, which is a plant pathogen (F Ahmad et al. 2014), *Rhizoctonia* mycorrhizae are mycorrhizal fungi that can establish a symbiotic relationship with the root tissue of orchids. They form hyphae that aggregate in the cortex tissue of the roots (Hossain , 2022). The association between *Rhizoctonia* mycorrhizae and orchids occurs during the early stages when the embryo develops roots and shoots, known as protocorms. As the protocorm matures into a fully formed plantlet, the hyphae network of *Rhizoctonia* mycorrhizae becomes established in the orchid's root cortex, forming a structure called a peloton (Calevo J. & Duffy, 2023). The mechanism through which *Rhizoctonia* mycorrhizae controls late blight caused by *Fusarium* sp. in orchids is known as induced resistance and is an integral part of sustainable agriculture.

The reason this research needs to be done is because this control method offers the advantages of being environmentally friendly and providing systemic resistance. The underlying mechanisms of induced resistance, encompassing both structural and chemical resistance, remain largely unknown in orchids, particularly those derived from tissue culture. The novelty of this research is based on a previous study (Soelistijono et al., 2018) that highlighted the capacity of *Rhizoctonia* mycorrhizae to mitigate drought stress in *Dendrobium macrophyllum*. However, this study merely identified *Rhizoctonia* mycorrhizae isolates capable of forming peloton structures, without elucidating the formation of resistance mechanisms (structural and chemical) against orchid late blight by *Fusarium* sp. Furthermore, the effectiveness of these mechanisms has not been evaluated in epiphytic orchids. Therefore, the objective of this study is to explore the application of *Rhizoctonia* mycorrhizae in controlling the latest orchid pathogen, *Fusarium* sp., in epiphytic orchids such as *V. tricolor*. Additionally, the resistance mechanisms observed in *V. tricolor* leaves affected by *Fusarium* sp. will be tested in other epiphytic orchids, including *Phalaenopsis* sp., *Cattleya* sp., and particularly *Vanda* sp. The purpose of this research is to overcome the problem of *V. tricolor* infected with *Fusarium* sp. with environmentally friendly *Rhizoctonia* mycorrhizae as part of sustainable agriculture.

METHODS

Propagation of *V. tricolor* seedlings.

As a model for epiphytic orchids, *V. tricolor* propagation is carried out in the tissue culture laboratory of the Faculty of Agriculture, Tunas Pembangunan University in vitro. The 6-month-old in vitro *V. tricolor* seedlings were then transferred to the greenhouse for the acclimatization process. The percentage of growth ratio plants can be calculated by the following formula:

$$GR = \frac{(\sum G - \sum NG) \times 100\%}{N}$$

GR   = Growth ratio of orchid seedlings
$\sum$ G   = seedling inoculated by *Rhizoctonia* mycorrhizae
$\sum$NG = nongrowing of orchid seedlings
N   = number of orchid seedlings

V. tricolor seedlings aged 7 months were then pre-inoculated with *Rhizoctonia* mycorrhizae

Identification of *Rhizoctonia* mycorrhizae

Rhizoctonia mycorrhizae were isolated and identified from healthy roots of *V. tricolor* collected from different locations on the slopes of Mount Merapi (i.e. Kaliurang and Kopeng). The isolation method followed the modified procedure of Xu-Hui Chen, et al. (2014), and the mycorrhizae were cultured on Potato Dextrose Agar (PDA) medium. Before inoculation, the *Rhizoctonia* mycorrhizae were cultured on PDA media and then introduced to the roots of *V. tricolor* seedlings obtained from tissue culture, which were 6 months old at the time. Successful pre-inoculation was confirmed by the presence of growth ratio and pelotons on the roots of *V. tricolor* seedlings after 1 month. The pelotons were observed through cross-sections of orchid roots stained with safranin (Xi et al., 2020). The observations of pelotons were conducted at 24, 48, 72, and 96 hours post-induction by *Rhizoctonia* mycorrhizae to verify their presence.

Fusarium sp. isolation and inoculation.

Fusarium sp. was isolated from *V. tricolor* leaves exhibiting symptoms of root rot. The isolation process involved placing the affected leaves on a PDA medium, and the identification was carried out using a microscope (Laurence et al., 2016). 8 months *V. tricolor* seedlings, which had been inoculated with *Rhizoctonia* mycorrhizae for 2 months, were subsequently inoculated with *Fusarium* sp., and the lignification of their leaves was observed.

Observation of lignin and analysis of peroxidase

The observation of lignification was performed on cross-sections of *V. tricolor* orchid leaves stained with phloroglucinol (Sharma et al., 2019). To measure peroxidase activity, which is a mechanism of orchid resistance to pathogenic fungi, the Saravanan method (Laurence et al., 2016) was employed. The method used in this research is factorial experimental design was a one-way ANOVA that assessed statistical differences between treatments while the mean differences were compared using the Duncan Multiple Range test (DMRT) with three treatments, one control, and six replications. Each replication consisted of 5 samples so a total of 120 plants were obtained.

RESULTS AND DISCUSSION

Rhizoctonia mycorrhizae were isolated from healthy *V. tricolor* roots collected from various locations, revealing the presence of irregularly shaped hyphae connected by brown mycelial threads. Subsequently, upon identification, a distinct forked angle was observed at the end of the hyphae (Figure 2a). These findings align with the research conducted by Gondal (2019), which indicates that *Rhizoctonia* mycorrhizae exhibit brown, irregularly shaped hyphae interconnected by brown mycelial threads, forming right angles. Notably, the *Rhizoctonia* mycorrhizal hyphae obtained from the Kaliurang area displayed slightly larger dimensions compared to those from the Kopeng area, despite the 100 km geographical separation (Figure 1).

Figure 1. (a) *Rhizoctonia* mycorrhizae Kaliurang hyphae
(b) *Rhizoctonia* mycorrhizae Kopeng hyphae

From Figure 1 above, it can be seen that the *Rhizoctonia* mycorrhizae isolated from the Kaliurang location has a thin hyphae diameter of around 2 µm and is thinner compared to the *Rhizoctonia* mycorrhizae isolated from the Kopeng location which is thicker at around 5 µm.

This indicates that *Rhizoctonia* mycorrhizae have different specific areas. It is possible that this is partly caused by different environmental humidity.

This observation suggests that *Rhizoctonia* mycorrhizae exhibit a plant-specific nature

(Soelistijono, et al., 2023). The results of seedlings growth of *V. tricolor* orchids plants were 4 plants out of 120 plants that nongrowing plants. So it can be concluded that inoculated *Rhizoctonia* mycorrhizae were not pathogenic to *V. tricolor* seedling.
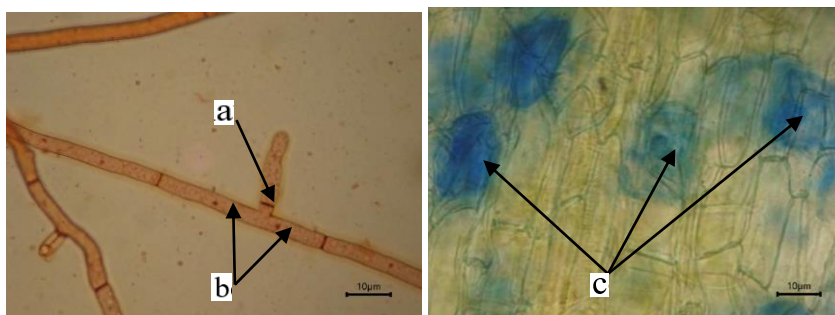

Figure 2. *Rhizoctonia* mycorrhizae form on the roots of *V. tricolor* orchids with brown mycelium.
(a): Branched elbow hyphae, (b): septae , and (c): peloton structure in root tissue

The pre-inoculation of *V. tricolor* seedlings with *Rhizoctonia* mycorrhizae leads to the penetration of *Rhizoctonia* mycorrhizal mycelium into the epidermal tissue, ultimately reaching the root cortex of *V. tricolor*. This interaction between the root cortex of *V. tricolor* and *Rhizoctonia* mycorrhizae facilitates the formation of a peloton structure. Within the root cortex, the mycelium of *Rhizoctonia* mycorrhizae enters the intracellular space, forming a peloton structure in the middle of the cell (Figure 2c). It is worth nothing that not all pelotons are positioned at the center. For instance, in *Vanda limbata* orchid roots, the pelotons are located between the cell spaces (Dwiyanto et al., 2017). However, regardless of their specific location, all peloton structures share the common characteristic of being present within the root cortex. These peloton structures serve as sites for the accumulation of organic matter, including proteins, glycogen, and fats, obtained from nutrient absorption in the soil. Eventually, the orchid embryo absorbs this organic matter for its growth, resulting in the lysis of the peloton structure. When the supply of nutrients is needed, the peloton structures reform within the root cortex. Therefore, the presence of pelotons indicates an association between *Rhizoctonia* mycorrhizae and orchids. To identify the pathogen responsible for late blight infection in *V. tricolor* leaves, it is necessary to conduct isolation procedures specifically targeting *Fusarium*.

Upon isolating the pathogen from the decayed leaves of *V. tricolor*, it was determined that *Fusarium* sp. is responsible for causing late blight. This particular pathogenic fungus leads to rapid deterioration of plant leaf tissue, typically occurring within a week after infecting the leaf epidermis tissue. This fungal pathogen, *Fusarium* sp., initially attacked *Phalaenopsis amabilis* orchids (Laurence et al., 2016). It poses a significant threat as it can infect various plant species, not limited to orchids, thereby having numerous hosts and making eradication efforts challenging. There are two groups of *Fusarium* sp. conidia: macroconidia and microconidia. The macroconidia of *Fusarium* sp. were found to be pathogenic (Ali et al., 2021), while the microconidia were non-pathogenic.

Figure 3. Macroconidias *Fusarium* sp. which was isolated from the sick *V. tricolor* orchid leaves tissue.M: 40 x 10. (a) Macroconidians of *Fusariums* sp.

Based on the findings presented in Figure 3, it is evident that the isolated conidia belong to the group of macroconidia, indicating their pathogenic nature. However, the extent of their virulence remains unknown due to the potential for mutation. Consequently, further in-depth studies are required to investigate the pathogenicity of *Fusarium* sp. on orchids. Recent research has identified a novel species within the *Fusarium* genus, specifically *Fusarium solani*, which has been found to infect *Phalaenopsis* sp. orchids (Laurence et al., 2016). This discovery raises concerns among orchid farmers as *F. solani* demonstrates the ability to not only attack *Phalaenopsis* sp. orchids but also pose a threat to *Vanda* orchids, including the endemic *V. tricolor* species found in the Kaliurang region on the slopes of Mount Merapi.

Table 1. Plant growth parameters of *V. tricolor*

Treatment	Parameter's				
	Plants height	Leafs length	Number of leaves	Number of roots	Plants fresh weight
Rhizoctonia mycorrhizae	6.72 a	4.69 a	5.0 a	11.7 a	4.31 a
Rhizoctonia mycorrhizae + *Fusarium* sp.	6,57 a	4,68 a	5,0 a	11,8 a	4,30 a
Fusarium sp.	5,42 b	3,56 b	4,3 b	8,2 b	3,05 b
Control	5,59 b	3,56 b	4,8 a	8,5 b	3,22 b

Description: Treatments followed by the same letter in the same column were not significantly different in Duncan's 5% follow-up test.

The application of *Rhizoctonia* mycorrhizae (Table 1) showed that had a very significant effect on parameters of plant height, leaves length, number of leaves, number of roots, and plant fresh weight. This shows that the application of *Rhizoctonia* mycorrhizae actively plays a role in stimulating the height growth of orchid plants. The results of this study are consistent with Huseini et al. (2013), who stated that the application of *Rhizoctonia* mycorrhizae on *Cymbidium georingii* orchids showed a significant difference in plant height. *Rhizoctonia* mycorrhizae which are applied to plants and associated with orchids play a role in providing nutrients for plant growth, especially in terms of leaf length which is closely related to more effective capture of light and CO_2 so that the rate of photosynthesis increases and is also related to the growth of orchid bulbs, the formation of new shoots on the bulb. The greater the number of roots in an orchid, the more nutrients will be absorbed. This is because *Rhizoctonia* mycorrhizal hyphae that have infected plant roots can help the roots absorb nutrients and water in areas that are not reached by plant roots. The growth parameters of plant height, leaf length, and number of roots will cause an increase in the weight of fresh orchid stover so that it is expected to survive the infection by *Fusarium* sp. Therefore it is necessary to look at the level of resistance by observing the formation of peroxidase enzymes (chemical resistance) and the formation of lignification (structural resistance).

Peroxidase enzymes, categorized as pathogen-associated proteins (PR proteins) (Appu et al., 2021; Sharma et al., 2019), possess the ability to facilitate the conversion of phenolic compounds into quinone compounds by generating peroxidase (H_2O_2). This peroxidase production exhibits toxicity towards *Fusarium* sp. (Yoonji et al., 2018). In the case of *V. tricolor*, peroxidase activity serves as a resistance mechanism against *Fusarium* sp. This activity was observed across various treatments: pre-inoculation treatment with *Rhizoctonia* mycorrhizae and inoculation with *Fusarium* sp. (A), inoculation with *Fusarium* sp. (B), and the untreated/control group (C). The measurement of peroxidase activity was conducted at different time intervals, including 0 days, as well as 1, 2, 3, 4, and 5 days after the inoculation of *Fusarium* sp., as depicted in Table 2.

Table 2. Peroxidase activity of *V. tricolor*.

Treatment		Peroxide enzyme unit (mnt/mg)				
		1st day	2nd day	3rd day	4th day	5th day
Rhizoctonia mycorrhizae	(A)	0.2 a	0.2 a	0.4 a	0.3 a	0.2 a
Rhizoctonia mycorrhizae + *Fusarium* sp.	(B)	0.7 c	1.2 b	0.9 b	1.1 b	0.7 b
Fusarium sp.	(C)	0.5 b	0.9 b	0.5 a	0.6 a	0.4 a
Control	(D)	0.0 a	0.2 a	0.4 a	0.4 a	0.3 a

Description: Treatments followed by the same letter in the same column were not significantly different in Duncan's 5% follow-up test.

In the A treatment, *V. tricolor* plants induced by *Rhizoctonia* mycorrhizae exhibited an initial increase in peroxidase content on the first and second day following inoculation with *Fusarium* sp. Subsequently, the peroxidase content decreased on the third day and then increased again on the fourth day (refer to Table 2). The peak peroxidase content was observed on the second day, measuring 1.2 min/mg protein, indicating the highest peroxidase production by *V. tricolor* on that day. This suggests that the leaves of *V. tricolor*, induced by *Rhizoctonia* mycorrhizae, displayed the highest peroxidase activity on the second day after inoculation with *Fusarium* sp., with subsequent decreases on the third day and subsequent increases on the fourth day. The notable increase in peroxidase activity on the second and fourth days corresponds to the incubation period of *V. tricolor*, which spans from the second to the fourth day. This indicates that during the incubation period of *Fusarium* sp. on *V. tricolor*, the plant mounts a response characterized by increased peroxidase activity, highlighting lignification as a mechanism of resistance against not only fungi but also bacteria. This finding aligns with Saravanan's research on banana plants, where the introduction of *Pseudomonas fluorescens* induced structural changes in banana roots and inhibited the development of diseases caused by *Fusarium* sp. (Nakkeeran S., et al., 2021). The treatment involving peroxidase activity solely comprised the inoculation of *Fusarium* sp. (Treatment B). Peroxidase levels increased on day 2 but subsequently declined on days 3 and 4. This decline in peroxidase activity indicates that the plant's ability to resist pathogens has diminished. In the control treatment (Treatment C), which involved *V. tricolor* unaffected by *Rhizoctonia* mycorrhizae and not inoculated with *Fusarium* sp., the leaves of *V. tricolor* did not exhibit resistance because no pathogens infect the plants. In addition to its toxic properties against pathogens, peroxidase enzymes can also act as catalysts for lignin formation. The presence of peroxidase enzymes stimulates lignification through the phenylpropanoid pathway, serving as a mechanism for plant resistance against other pathogenic infections. This is explained by Yadaw et al. (2019), that the intermediate metabolites and phenylpropanoid compounds produced during the lignin biosynthesis pathway display antimicrobial activities and are involved in plant defense. The genes involved in shikimate pathways are known and facts about their response regarding initial access to cell walls are demonstrated in some studies.

When *V. tricolor* orchids are pre-inoculated with *Rhizoctonia* mycorrhizae, their leaves exhibit resistance or immunity when confronted with *Fusarium* sp. The resistance observed in *V. tricolor* leaves is attributed to the development of lignification in the epidermis (Figure 4). Lignification occurs as a result of the accumulation of lignin compounds and serves as a form of structural resistance in plants (Saravanan et al., 2004).
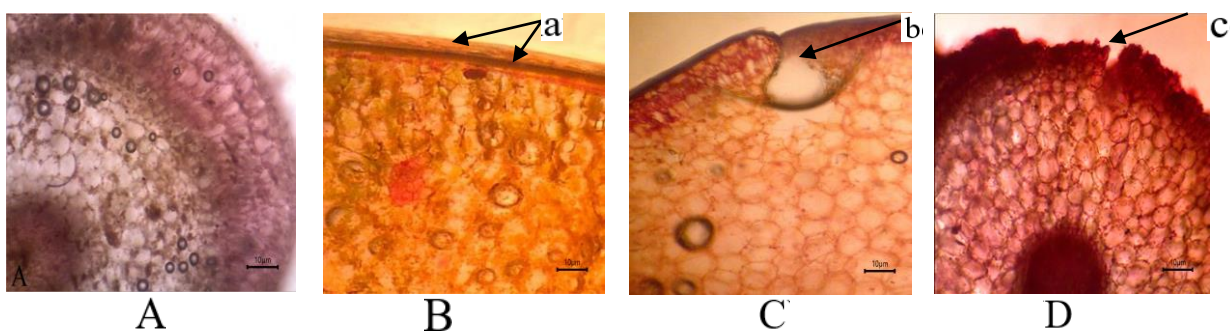

Figure 4. (A) Healthy leaf *V. tricolor*, (B) lignification in leaf *V. tricolor*, (C) mechanism of induced resistance in leaf *V. tricolor*, (D) Damage of leaves leaf *V. tricolor* . a. lignification cell on epidermic leaves *V. tricolor*, b. mechanism-induced resistance on epidermic leaves *V. tricolor*, and c. Damage to leaf cell tissue *V. tricolor* by *Fusarium* sp.

The leaves of *V. tricolor* that were inoculated with *Rhizoctonia* mycorrhizae displayed an accumulation of lignin in the epidermic (Figure 4B), resulting in their protection against damage when infected by *Fusarium* sp. the leaf cortex remained intact because it is induced by *Rhizoctonia* mycorrhizae through induced resistance systems (Figure 4C). In contrast, *V. tricolor* which was not inoculated with *Rhizoctonia* mycorrhizae showed damaged leaf

epidermis (Figure 4D) when exposed to *Fusarium* sp., making the plants vulnerable and prone to illness. The process of lignification occurs as a response to enhance the plant's structural resistance against *Fusarium* sp. infection. Lignin (4a), a polymer compound, poses difficulty for pathogenic fungal hyphae to penetrate due to its composition of various phenol compound monomers, such as coniferyl alcohol, coumaril, and sinapyl. These monomers are synthesized via the phenylpropanoid pathway starting from coumaric acid. The resistance induction mechanism can protect *V. tricolor* leaves even though the pathogen may have already entered and will be surrounded by resistant cells (5b).

During the early stage of pathogen infection in resistant plants, there is an accumulation of lignin or other phenolic substances in the plant cell wall region that comes into contact with the pathogen. Lignin production is increased significantly, which hampers the growth of pathogenic hyphae (Liu, et al., 2009). The ability of *V. tricolor* to undergo lignification is attributed to its association with *Rhizoctonia* mycorrhizae, as evidenced by the presence of peloton structures (Figure 3c). However, the presence of lignification alone does not guarantee structural resistance against *Fusarium* sp. The structure is not foolproof because the hyphae of *Fusarium* sp. can penetrate deeper into the root cortical tissue before lignification takes place in the epidermal tissue. The occurrence of lignification in the plant's epidermal tissue varies depending on its formation pathway, as explained by Liu et al. The expression of monolignol genes in plants is influenced by pathogenic fungal infections in both susceptible and resistant plants. In resistant plants, the expression of monolignol genes typically occurs 24 hours after pathogen inoculation. In the case of *V. tricolor* roots that are unaffected by *Rhizoctonia* mycorrhizae, inoculation with *Fusarium* sp. can cause severe damage to the leaf epidermis tissue (Figure 4c). The penetration of *Fusarium* sp. into the leaf cortex of *V. tricolor* takes around 3 days. After an incubation period of 3 days, hyphae can develop in the cortex tissue, leading to the degradation of leaf cell tissue and subsequent rotting of the leaf tissue on the 6th day.

The novelty of this research is the use of *Rhizoctonia* mycorrhizae in dealing with leaf blight on *V. tricolor* orchids which is caused by one of the fungal pathogens on orchids, namely *Fusarium* sp. In previous studies, control of leaf blight on *V. tricolor* orchids caused by *Fusarium* sp. was managed by spraying fungicides. This has a very detrimental effect on both farmer and the environment because high toxicity and heavy metals take a long time to decompose.

Control of orchid late blight disease traditionally involves the use of chemical fungicides, which pose risks to farmers and the environment and therefore need to be replaced with more environmentally friendly methods, namely by using *Rhizoctonia* mycorrhizae as a biological resistance inducer.

CONCLUSION

Rhizoctonia mycorrhizae were able to inhibit *Fusarium* against *V. tricolor* by an increase in peroxidase compounds in plants, as evident by a recorded degradation rate of 1.2 min/mg protein on the second day. The lignification process in the leaves of *V. tricolor* shows structural resistance facilitated by *Rhizoctonia* mycorrhizae which effectively inhibited the development of *Fusarium* sp. hyphae into the epidermal tissue leaves, ensuring their protection against late blight. This stage is characterized by the formation of peloton structures in the root cortex. Recommendations for further research: 1). The next research will test the application of *Rhizoctonia* mycorrhizae to control leaf blight by *Fusarium* sp. in other epiphytic orchids in Indonesia including *Phalaenopsis* sp., *Cattleya* sp., and *Dendrobium* sp. 2). Possibility of conducting a consortium of 2 / 3 *Rhizoctonia* mycorrhizae isolated from various types of orchids and various locations in Indonesia. The goal is that *Rhizoctonia* mycorrhizae is not specific to just one orchid but can also be used on various types of epiphytic orchids in Indonesia so that the production/manufacturing costs are cheaper/more efficient.

REFERENCES

Ahmad, F., Khan, H., Ali, R., & Ahmad, I. (2014). Growth Rate Of Different Isolates Of *Fusarium solani*, The Cause Of Root Rot Of Okra (Abelmoschus Esculentus L). *Asian J Agri Biol*,

2:114-118. https://www.asianjab.com/wp-content/uploads/2015/05/6-2-2-MS-No.-67.pdf

Ali, R., Khan, H., Ahmad, F., & Ahmad, N. (2013).Colony Colour and Texture of Different Isolates of *Fusarium solani*, the Cause of Root Rot Disease of Okra (Abelmoschus Esculentus l) in Peshawar. Asian J Agri Biol, 1:190-193. https://www.asianjab.com/wp-content/uploads/2015/05/6-1-4-MS-No.-34.pdf`

Al-Saidi, S. S., & Al-Obaidy, S. H. (2022). Induced Resistance of cucumber *Cucumis Sativus* L. To Whitefly Bemisia Tabaci By Silicon. *Int. J. Agric. Stat. Sci.*, 18: 2147-2152. https://www.researchgate.net/publication/366977632_Induced_Resistance_of_Cucumber_*Cucumis sativus* L to Whitefly_*Bemisia tabaci* by Silicon

Appu., Parthasarathy Ramalingam, Anusuya Sathiy anarayanan, Jianying Huang. (2021). An Overview of Plant Defense-related Enzymes Responses to Biotic Stresses. *Plant Gene* Vol.27,100302. https://www.sciencedirect.com/science/article/abs/pii/S2352407321000329

Mursyidin, D. H., Saputra, A.W. (2023). Phylogenetic Relationship of Cymbidium Mosaic Virus from the Native Orchids of South Kalimantan, Indonesia. *Biosaintifika* 15 (2) p: 185-193. https://journal.unnes.ac.id/nju/index.php/biosaintifika/article/view/41842/14693

Dwiyanto, W., Soelistijono, R., & Utami, D. S. (2017). Morphology and anatomical character of *Rhizoctonia* mycorrhiza in *Vanda limbata* orchid. *Agrineca,* 17: 30-39. http://ejournal.utp.ac.id/index.php/AFP/article/view/563

Gondal, A. S., Abdul, R., Farah, N. (2019). Anastomosis Groups of *Rhizoctonia Solani* associated with tomato foot rot in Pothohar Region of Pakistan. PubMed. 9 (1). https://www.nature.com/articles/s41598-019-40043-5

Haryuni H., Amin M., Suprapti E., Dewi T.S.K., Hartoyo E. (2022). Proline Reduction and Increasing Growth of Vanilla Plants Induced by BNR Fungus with Dose of Goat Manure and Husk Charcoal. *Biosaintifika* 14 (1) p:82-89. https://journal.unnes.ac.id/nju/biosaintifika/article/view/35618/12843

Hossain, M. M., Rahi, P., Gulati, A., & Sharma, M. (2013). Improved ex vitro survival of asymbiotically raised seedlings of cymbidium using mycorrhizal fungi isolated from distant orchid taxa. Scientia Horticulturae, 159, 109–

116. https://www.sciencedirect.com/science/article/abs/pii/S0304423813002203

Hossain M. M. (2022). Orchid mycorrhiza: Isolation, culture, characterization and application. South African Journal of Botany Volume 151, Part A, Pages 365-384. https://www.sciencedirect.com/science/article/abs/pii/S0254629922005300

Laurence, M. H., C. Howard, B.A. Summerell & E.C.Y. Liew. (2016). Identification of *Fusarium solani* f.Sp. *Phalaenopsis* in Australia. *Australasian Plant Disease Notes,* 11. https://link.springer.com/article/10.1007/s13314-016-0200-y

Liu Q.,Le Luo, and Luqing Zheng. (2018). Lignins: Biosynthesis and Biological Functions in Plants. *International Journal of Molecular Sciences*. Volume 19 (2) p: 335 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5855557/

Nakkeeran S. S. Rajamanickam, R. Saravanan, M. Vanthana, and K. Soorianathasundaram. (2021). Bacterial endophytome-mediated resistance in banana for the management of *Fusarium* wilt. 3 Biotech. Volume 11(6) p: 267. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8124033/

Jacopo Calevo & Karl J. Duffy.(2023). Interactions among mycorrhizal fungi enhance the early development of a Mediterranean orchid. *Mycorrhiza* 33:229–240. https://doi.org/10.1007/s00572-023-01118-4

Borges, A. A. and Luisa M. Sandalio. (2015). Induced Resistance for Plant Defense. Front Plant Sci. Vol. 6: 109. https://www.frontiersin.org/articles/10.3389/fpls.2015.00109/full

Rui Ding, Xu-Hui Chen,Li-Jun Zhang, Xiao-Dan Yu, Bo Qu, Ru Duan. (2014). Identity and Specificity of *Rhizoctonia*-Like Fungi from Different Populations of *Liparis japonica* (Orchidaceae) in Northeast China. PLoS One. 9 (8). https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0105573

Yu-Feng XuShreya, D., Mohanty, S., Sahu, G., & Sarkar, S. (2020). Sustainable agriculture: a path towards better future. *Food and Scientific Reports*, 1: 22-25. https://foodandscientificreports.com/details/sustainable-agriculture-a-path-towards-better-future

Sharma A.,Babar Shahzad, Abdul Rehman, Renu Bhardwaj, Marco Landi, and Bingsong Zheng.(2019). Response of Phenylpropanoid Pathway and the Role of Polyphenols in Plants

under Abiotic Stress. *Molecules*. Vol. 24(13) p: 2452. https://www.mdpi.com/1420-3049/24/13/2452

Soelistijono, Utami, D. S., & Priyatmojo, A. (2017). Identification of *Rhizoctonia* Mychorrizal and *Fusarium* on *Ascocentrum Miniatum* Orchid. *BIOTA*, 2: 7-13. https://ojs.uajy.ac.id/index.php/biota/article/view/1504

Soelistijono, Daryanti, & Handayani, M. T. (2018). Isolation of Mycorrhizal *Rhizoctonia* as Resistance Inducer of *Dendrobium Macrophyllum* to Drought. *IOP Cof. Series Earth and Enviromental Science,* 142: 12-13. https://iopscience.iop.org/article/10.1088/1755-1315/142/1/012013/pdf

Soelistijono, R., Jakti A. W., Budiyono A., Suprapti E., Daryanti. (2023). The Uses of *Rhizoctonia* Mycorrhizae As a Dry Resistance Induction of Dendrobium aggregatum Seedlings. *Malaysian Applied Biology*. 5 (2) 147-151. https://jms.mabjournal.com/index.php/mab/article/view/1994/794

Srivastava S, C Kadooka, JY Uchida (2018). *Fusarium* species as pathogen on orchids. *Microbiological Research*, 207: 188-195. https://www.sciencedirect.com/science/article/pii/S0944501317307218?via%3Dihub

Swett, C. S., & Uchida, J. Y. (2015). Characterization of *Fusarium* Diseases on Commercially Grown Orchids in Hawaii. *Plant Pathology,* 64: 648–654. https://bsppjournals.onlinelibrary.wiley.com/doi/full/10.1111/ppa.12290

Tamrin, A., Irwan , Tutik, K., Itji, D. D., Asman, Andi, N., & Nurariaty, A. (2020). Entomopathogenic fungi *isolated from* agro-ecosystem soil *in South Sulawesi, Indonesia. Asian J Agric & Biol,* 8: 17-23. https://www.asianjab.com/wp-content/uploads/2020/02/3.-AJAB-2019-05-197_17-23.pdf

Xia G., Jun Shia, Jingbao Lib, and Zhengmin Han. (2020). Isolation and identification of beneficial orchid mycorrhizal fungi in Bletilla striata (Thunb.) Rchb.f.(Orchidaceae). Plant Signaling & Behavior Vol. 15, No. 12. https://doi.org/10.1080/15592324.2020.1816644

Yadav V., Zhongyuan Wang, Chunhua Wei, Aduragbemi Amo, Bilal Ahmed, Xiaozhen Yang and Xian Zhang. (2020). Phenylpropanoid Pathway Engineering: An Emerging Approach towards Plant Defense. Pathogens*. Vol.9 (4) p: 312.* https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7238016/

Yoonji, L., Hokyoung, S., Ji-Young, S., Gyung, J. C., & Yin-Won, L. (2018). Genome-wide functional characterization of putative peroxidases *in the head blight fungus* Fusarium graminearum*. Molecular Plant Pathology, 19: 715-730.* https://bsppjournals.onlinelibrary.wiley.com/doi/10.1111/mpp.12557