PAPER NAME

AIP Conf. Proc. 2957, 090027_Soelistijono et al., 2024 (1).pdf

AUTHOR

haryuni haryuni

WORD COUNT

3174 Words

CHARACTER COUNT

16865 Characters

PAGE COUNT

8 Pages

FILE SIZE

2.8MB

SUBMISSION DATE

Apr 24, 2024 12:48 PM GMT+7

REPORT DATE

Apr 24, 2024 12:48 PM GMT+7

12% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

- 7% Internet database
- 8% Publications database
- Crossref database
- Crossref Posted Content database
- 2% Submitted Works database

Excluded from Similarity Report

- Bibliographic material
- Quoted material
- Cited material
- Small Matches (Less then 10 words)
- Manually excluded sources

RESEARCH ARTICLE | FEBRUARY 06 2024

Utilization of *Rhizoctonia* mycorrhiza in the management of *Fusarium* sp. seedling orchid *Dendrobium nindii*

R. Soelistijono ✉; E. Puspitasari; H. Haryuni; S. Priyadi; A. F. Aziez; D. Rakhmawati

AIP Conf. Proc. 2957, 090027 (2024)
https://doi.org/10.1063/5.0184437

Utilization of *Rhizoctonia* Mycorrhiza in the Management of *Fusarium* sp. Seedling Orchid *Dendrobium nindii*

R. Soelistijono[1,a)], E. Puspitasari[2], H. Haryuni[1], S. Priyadi[1], A.F. Aziez[1], and D. Rakhmawati[3]

[1]*Faculty of Agriculture, Universitas Tunas Pembangunan. Jl. Balekambang No. 1, Manahan, Surakarta 57139, Central Java, Indonesia,*
[2]*Student of Faculty of Agriculture, Universitas Tunas Pembangunan. Jl. Balekambang No. 1, Manahan, Surakarta 57139, Central Java, Indonesia*
[3]*Magelang District's Agriculture and Food Service. Jl. Letnan Tukiyat, Mungkid, Magelang 56511, Central Java, Indonesia*

[a)] Corresponding author: sulistyo.utp@gmail.com

Abstract. *Dendrobium* is one of largest orchid genera in family *Orchidaceae*, and consists of more than 2,000 species. In nurseries, this orchid is easily attacked by the fungus *Fusarium* sp. *Rhizoctonia* mycorrhiza is mycorrhizal fungus that is prevantly associated with orchids. At in vitro this fungus provides induced resistance of orchids against pathogenic fungal attacks. The research was conducted in laboratory and green house from August to June 2022. The research method used a Completely Randomized Block Design consisting of two treatment factors and five replications. The first factor is application of *Rhizoctonia* mycorrhizae (M1), and second factor is origin of *Fusarium* inoculum from garlic (F1), potatoes (F2), and chili (F3). The application of *Rhizoctonia* mycorrhiza had a significant effect plant high with highest value 6.23 cm (M1F0), number of leaves with highest value 5.58 cm (M1F0), number of roots with highest value 18.07 cm (M1F0), and fresh weight of plant with highest value 8.80 g (M1F0). The application of *Fusarium* sp does not a give a significant different for all parameters. The treatment interaction between *Rhizoctonia* mycorrhizae and *Fusarium* sp is very real different on number of roots with highest value of 19.20 sheet (M1F1). This study aimed to use *Rhizoctonia* mycorrhiza in controlling *Fusarium* sp. attack on seedling *Dendrobium nindii* in experimental garden.

INTRODUCTION

Dendrobium is one of the largest orchid genera in the family Orchidaceae, and includes more than 2,000 species [1]. *Dendrobium* is one of Indonesia's natural resources, and the number is estimated at 275 species [2]. The best *Dendrobium* orchid species are mostly found in eastern Indonesia, such as Papua and Maluku. *Fusarium* sp. is a fungus that is capable of infecting various plants (polyphagous fungus) and commonly attacks orchids [3]. In orchids, *Fusarium* sp. will cause leaf blight symptoms. Yellowing, wrinkled, thin, and bent leaves and stems, rotting leaf necks reaching the base of the stem are other typical symptoms of infected orchids by *Fusarium*. In general, *Fusarium* sp. causes plants to rot and eventually die. Biological control by inoculation of various biological agents in plants will lead to increased resistance to subsequent inoculation by the main pathogen known as the induced resistance mechanism [4]. This control measure can be tested on orchid seedlings that is attacked by *Fusarium* sp.

Orchids are known to have associations with fungi that act as mycorrhizae and are known as mycorrhizal orchids [5]. Research on the genus of *Pterostylis* orchids from Australia found 72 types of mycorrhizae associated with roots and 20 of them were *Rhizoctonia* sp. with a different Anostomosis Group (AG). Some *Rhizoctonia* sp. which functions as mycorrhizae in orchids is *Tulasnella* sp. [6]. Mycorrhizal associations in *Dendrobium* orchids have a positive effect on nutrient absorption and host growth [7]. Mycorrhizal isolates taken from the roots of *Phalaenopsis lowii* Rchb.F. which grows in its natural habitat is thought to have the advantage of better compatibility than using mycorrhizal species from other plant species [8].

Proceedings of the 1st International Conference on Food and Agricultural Sciences (ICFAS) 2022
AIP Conf. Proc. 2957, 090027-1–090027-7; https://doi.org/10.1063/5.0184437
Published by AIP Publishing. 978-0-7354-4817-9/$30.00

METHODS

This research was carried out at the Green House, Faculty of Agriculture, Tunas Pembangunan University, in Surakarata, Central Java Province, at an altitude of 105 meters above sea level. The implementation of this research began in November 2021 until July 2022. The materials used were: seedling *Dendrobium nindii*, *Rhizoctonia* mycorrhiza, and *Fusarium* sp. *Rhizoctonia* mycorrhiza was isolated from the roots of the orchid *Dendrobium Lasianthera* in the tissue culture laboratory of the Faculty of Agriculture, UTP Surakarta. *Rhizoctonia* mycorrhiza was cultured in Potato Dextrose Agar (PDA) medium. After five to six days of incubation, the growth of white hyphae that formed a circle around the piece of root segments was seen [9].

Isolation of *Fusarium* sp. carried out from wilted garlic, potato, and chili leaves that were infected with *Fusarium* sp. The isolates obtained were cultured in PDA medium until the fungal mycelium filled Petridis. Hyphae change color in the center of the colony from white to purple or pink. Furthermore, the isolates were propagated on PDA and incubated for one week. *Fusarium* isolates aged one week were taken from PDA, cut into 1 cm^2, and immersed in 100 mL of Potato Dextrose (PD/only contains potato extract and aquades) medium. *D. nindii* 7 months old was an application with 1 week old *Rhizoctonia* mycorrhiza on the roots [7], then at 12 weeks *Fusarium* sp. was inoculated with PD media which was sprayed on the leaves. *D. nindii* as control was sprayed with distilled water. The volume of liquid of PD medium that was sprayed was 1 ml per plant.

The study used the CRBD research method (Completely Randomized Block Design) with two treatment factors, namely: M0: Without *Rhizoctonia* mycorrhizal application, M1: With *Rhizoctonia* mycorrhizal application, and the origin of the inoculum (F) consisted of three levels, namely: F1: Inoculum *Fusarium* sp. from onions white; F2: Inoculum *Fusarium* sp. from potato; F3: Inoculum *Fusarium* sp. from chili. So there were six treatment combinations obtained. The parameters observed were the percentage of plant growth, plant height, leaf length, number of leaves, number of roots, root length, plant fresh weight, and root peloton observations. Observational data from each parameter in each observation were analyzed with 5% and 1% ANOVA tests. If there are calculations that are significantly different or very significantly different, then proceed with DMRT (Duncan Multiple Range Test) with a level of 5% to find out any differences between treatments.

RESULTS AND DISCUSSION

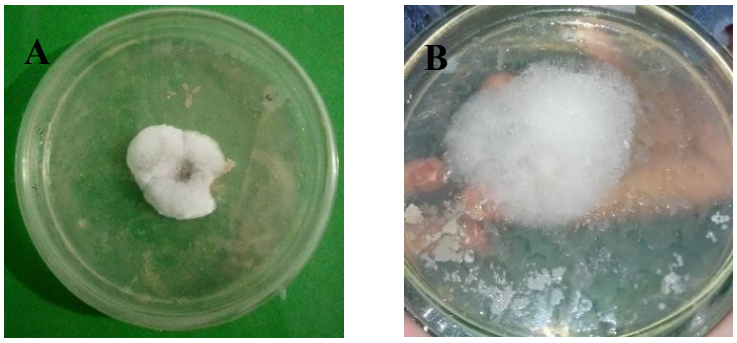

FIGURE 1. Growth and development of *Rhizoctonia* mycorrhiza colonies on PDA medium (A) *Rhizoctonia* mycorrhiza one week old, (B) *Rhizoctonia* mycorrhiza two weeks old

The results of the isolation of *Rhizoctonia* mycorrhiza from the roots of *D. lineale* showed that there was a small white clump structure in the middle of petri plate, which meant that *Rhizoctonia* mycorrhiza was growing and forming colonies (Fig. 1). This is following the results of previous research, that *Rhizoctonia* mycorrhiza associated with orchids has a morphological characteristic of a white colony, in the middle of the colony a clump is formed with very slow growth [10]. Microscopic observations of *Rhizoctonia* mycorrhizae showed that hyphae had branches that formed right angles which is one of the characteristics of *Rhizoctonia* mycorrhiza (Fig. 2A). This finding is following another study that the branching of *Rhizoctonia* mycorrhizae formed a right angle [4]. *Rhizoctonia* mycorrhiza has a common characteristic that is binucleate (Fig. 2B). This is what distinguishes the group of *Rhizoctonia* which are pathogenic such as *Rhizoctonia* solani which has multinucleate (more than two nucleic acids) [11]. *Rhizoctonia* sp. binucleic acid was also previously found in vanilla roots [12].

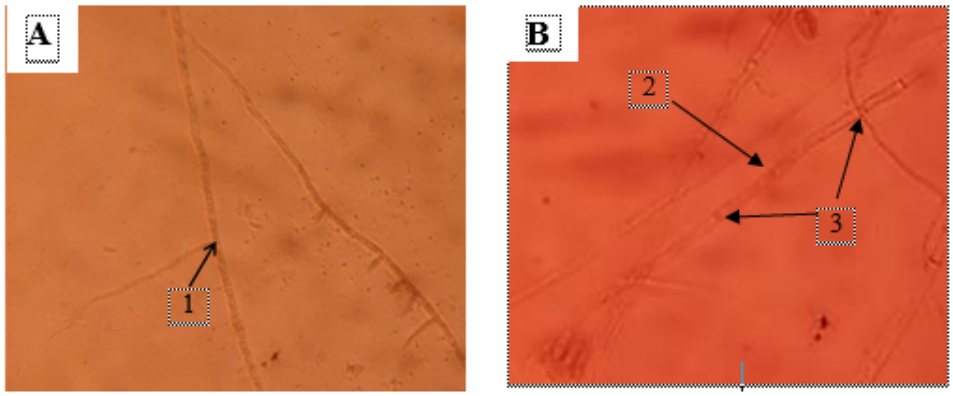

FIGURE 2. (A) *Rhizoctonia* mychorrhizal hyphae with right-angled branched, (B) *Rhizoctonia* mycorrhizal hyphae, (1) a typical angle on hyphae branching, (2) cell nucleus, (3) insulation between cells in hyphae

Isolation of Fusarium sp. of various ingredients (garlic, potato, and chili) showed almost the same results (Fig. 3). Based on the above observations, it was shown that various *Fusarium* sp. colonies were white like cotton and purple or pink in the center of the colony.

FIGURE 3. Growth and development of *Fusarium* sp colonies on PDA medium (a) isolate of *Fusarium* sp. from garlic, (b) isolate of *Fusarium* sp. from potato, (c) isolate of *Fusarium* sp. from chili

This is in accordance with the opinion [13] that *Fusarium* sp. have colonies that are white or accompanied by purple to pink in each colony. These fungal colonies will produce different colors on isolates with the same growing media because the fungus *Fusarium* sp. easily change the color of the colony so it cannot be used as an identification parameter [14]. The results of observations of *Fusarium* isolates isolated from garlic, potatoes, and chilies were classified as microconidium (Fig. 4).

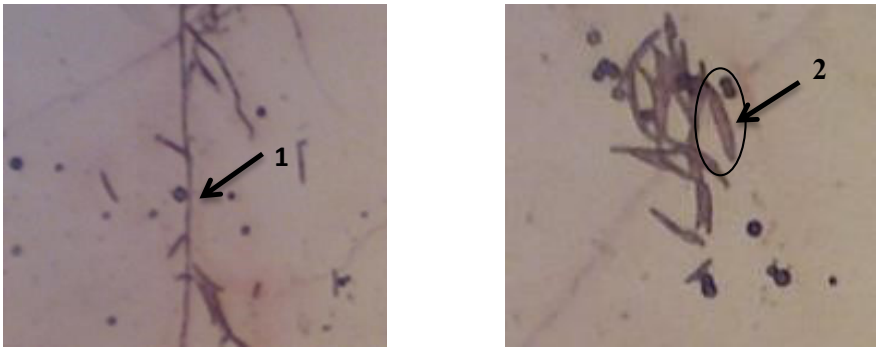

FIGURE 4. The morphology of the fungus *Fusarium* sp. which is identified by microscopic (1) hiphae, (2) microconidia

The results showed that the microconidia were long, crescent-shaped with long and blunt conidiophores, 1–5 septa, single monophyalid and abundant in number. Microconidia are formed in clusters at the tips of conidiophores and are pathogenic to plants. This is in accordance with Agrios [4] that the genus *Fusarium* is a fungus that has asexual spores in the form of microconidium and macroconidium. Observations of plant morphology were carried out at the end of the study, namely observing plants from roots to leaves in each treatment (Fig. 5A, 5B).

FIGURE 5A. Sighting of *D. nindii* without *Rhizoctonia* mycorrhiza (M0F1) *Fusarium* sp. from garlic, (M0F2) *Fusarium* sp. from Potato, (M0F3) *Fusarium* sp. from chili

FIGURE 5B. Plant Morphological Appearance With *Rhizoctonia* mycorrhiza application (F1) *Rhizoctonia* mycorrhiza + *Fusarium* sp. from garlic, (F2) *Rhizoctonia* mycorrhiza + *Fusarium* sp from potato, (F3) *Rhizoctonia* mycorrhiza + *Fusarium* sp. from chili

From the picture, it can be seen that the application treatment of *Rhizoctonia* mycorrhiza first and subsequent inoculation with *Fusarium* sp. affect the number of roots and root length. Whereas in the treatment without the application of *Rhizoctonia* mycorrhizae, the plant had poor root growth, *Rhizoctonia* mycorrhiza was very important in the nutrient absorption process [15]. The association of Rhizoctonia mycorrhiza with the roots of *D. nindii* was indicated by the presence of a peloton structure (Fig. 6).

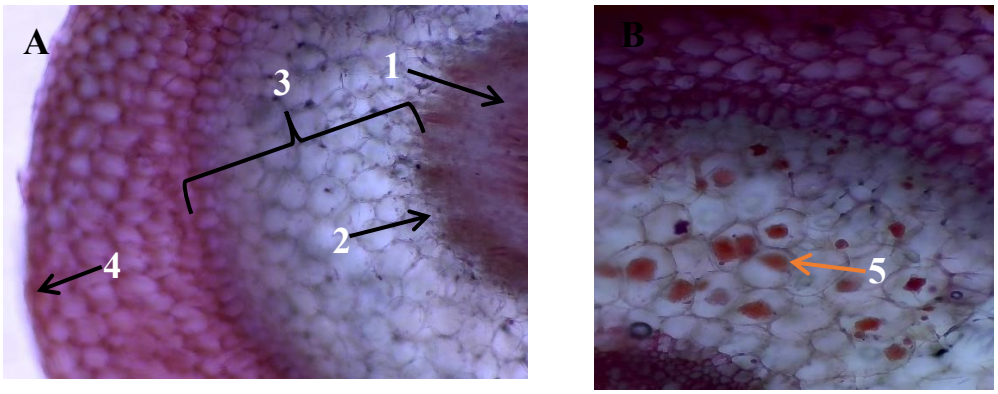

FIGURE 6. (A) cross section of roots without application of *Rhizoctonia* mycorrhizae, (B) cross section of roots with application of *Rhizoctonia* mycorrhiza, (1) endodermal tissue, (2) central cylinder, (3) cortical tissue, (4) epidermal tissue (5) peloton

In the cross section of the roots of *D. nindii* orchids that were inoculated with *Rhizoctonia* mycorrhiza first, they showed a red peloton structure in the center or the edge of the cortical cells (Fig. 6). This is following what was stated by Kasiamdari [16] and Brundrett [5] that the intracellular hyphae of *Rhizoctonia* mycorrhiza that infect orchid roots can penetrate into the cortical tissue at the root and form dense coagulation coils (peloton). There are several ways to see the association of *Rhizoctonia* mycorrhizae with orchids, namely: (1) looking at the mycorrhizal structure (peloton); (2) symbiotic germination test using established culture protocol (oat agar medium); and (3) molecular confirmation that *Rhizoctonia* mycorrhiza was detected in orchid root tissue [17]. Peloton appears only after infection, which is about 20–36 hours after initial contact. When the fungus begins to enter the parenchyma cells of the orchid, the plasma membrane inside the cell is formed by a broad surface indentation to facilitate infection and fungal growth. The newly formed plasma membrane immediately surrounds the growing peloton and creates a large surface area through which nutrients are exchanged. The surrounding plant membrane becomes rough endoplasmic reticulum and there is evidence from electron microscopy showing exocytosis of this plant membrane [18]. Peloton is usually only found in a limited period when the orchid needs nutrients before undergoing lysis. Infection and lysis occur repeatedly in the interior of the same cells and tissues. The formation of this structure is characteristic of the association of *Rhizoctonia* mycorrhizae and orchids [19]. Mycorrhiza in orchids has a nutrient flow where the fungus gets a direct supply of carbon from plants instead of phosphorus or as a substitute for nitrogen for plants [6]. However, there is a frequent flow of carbon from fungi to plants or from plants to fungi alternately, while this flow involves nitrogen and phosphorus nutrients from fungi moving to plants [15]. There are approximately 400 species of orchids, there is no flow of carbon nutrients from plants to mycorrhizae, but mycorrhizae can supply nutrients to orchid plants. Based on the recapitulation data of variance, mycorrhizal application treatment had a significant effect on leaf length and had a very significant effect on plant height, number of leaves, number of roots, root length, and plant fresh weight (Table 1).

TABLE 1. Summary results of the effect of the application of *Rhizoctonia* mycorrhiza and *Fusarium* sp. inoculation on the growth of *Dendrobium nindii* orchid seedling

No.	Parameter	Rhizoctonia mycorrhizal application (M)	Fusarium Inoculation (F)	Interaction (MxF)	Score	
					Highest	Lowest
1.	Plant height (cm)	**	ns	ns	6.62 (M1F1)	5.68 (M0F3)
2.	Leaf length (cm)	*	ns	ns	4.40 (M1F1)	3.86 (M0F2)
3.	Number of leaves (cm)	**	*	ns	6.00 (M1F1)	4.40(M0F2)
4.	Number of roots (cm)	**	ns	*	20.20 (M1F1)	15.00 (M0F2)
5.	Root length (cm)	**	ns	ns	11.12 (M0F1)	9.24 (M0F3)
6.	Plant fresh weight (g)	**	*	ns	9.85 (M1F3)	6.18 (M0F2)

Data followed by ns) no significant; *) significant; **) very significant

The application of *Rhizoctonia* mycorrhiza had a very significant effect on all parameters, and *Fusarium* inoculation had a significant effect on the number of leaves and plant fresh weight. While the interaction between the two affected the number of roots. The application of *Rhizoctonia* mycorrhiza on seedling *D. nindii* then followed by *Fusarium* sp. infection affected the number of plant roots. This will have a positive effect on nutrient absorption and host growth (Table 1).

The parameter of the number of recapitulation results of variance shows that the application treatment of *Rhizoctonia* mycorrhizae and *Fusarium* sp. inoculation gave results that significantly affected the number of roots with the highest value of 19.20 pieces and the lowest value of 15.00 (Table 2). Overall, the application of *Rhizoctonia* mycorrhiza helped in the absorption of nutrients from the soil with a significantly increased number of roots when compared to that without the application of *Rhizoctonia* mycorrhiza. According to [15] nitrogen supply will make plant parts green because it contains chlorophyll which plays a role in photosynthesis. These elements are also useful for accelerating plant growth in height, increasing the number of tillers, influencing leaf width and length and making them large, and increasing protein and fat levels for plants.

TABLE 2. Effect of application of *Rhizoctonia* mycorrhiza and *Fusarium* sp. inoculation on the growth of *Dendrobium nindii* orchid seedling

Treatment	Plants height (cm)	Leaf lenght (cm)	Number of leaves (sheet)	Root lenght (cm)	Number of root (pieces)	Fresh weight (g)
***Rhizoctonia* mycorrhizae application (M)**						
M0	2.43a	1.76	2.40a	4.88	3.90a	0.91a
M1	2.76b	1.64	3.17b	5.07	4.86b	1.12a
Watering interval (P)						
P1	2.57ab	1.63a	2.61a	5.01	4.11a	0.97ab
P2	2.85b	1.85a	3.35b	5.54	5.27b	1.23b
P3	2.38a	1.61a	2.40a	4.39	3.75a	0.84a
Interaction betwen *Rhizoctonia* mycorrhizae application and watering interval (M×P)						
M0P1	2.40	1.7ab	2.00a	5.02	3.40	0.83
M0P2	2.68	1.90c	3.00bc	5.30	4.80	1.10
M0P3	2.21	1.81bc	2.20a	4.32	3.50	0.79
M1P1	2.73	1.69bc	3.23c	4.99	4.83	1.11
M1P2	3.01	1.81bc	3.70c	5.77	5.75	1.35
M1P3	2.54	1.41a	2.60ab	4.46	4.00	0.89

The treatment followed by same letter in same column, shows that there is no significant difference in DMRT with a level of 5%, (M0) without *Rhizoctonia* mycorrhizae; (M1) application with *Rhizoctonia* mycorrhizae; (F1) watering every two days; (F2) watering every four days; (F3) watering every six days; (M0F1) without *Rhizoctonia* mycorrhizae + watering every two days; (M0F2) Without *Rhizoctonia* mycorrhizae + watering every four days; (M0F3) without *Rhizoctonia* mycorrhizae + watering every six days; (M1F1) application with *Rhizoctonia* mycorrhizae + watering every two days; (M1F2) application with *Rhizoctonia* mycorrhizae + watering every four days; (M1F3) application with *Rhizoctonia* mycorrhizae + watering every six days

CONCLUSION

Application of *Rhizoctonia* mycorrhiza on seedling of *D. nindii* has an effect on increasing the number of roots, and increases its resistance against with *Fusarium* sp. of garlic leaves, potatoes, and chilies.

ACKNOWLEDGMENTS

On this occasion, the author would like to thank the Institute for Research and Community Service, Tunas Pembangunan University which has funded the research according to the contract number 0031/PK-P/LPPM-UTP/XII/2021.

REFERENCES

1. R.L. Dressler, *The Orchids, Natural History and Classification* (Harvard University Press, Cambridge, 1981), pp. 352.
2. D. Gandawidjaya and S. Sastrapradja, Bull. Kebun Raya **4(4),** 113–125 (1980).
3. S. Srivastava, C. Kadooka, and J.Y. Uchida, Microbiol. Res. **207**, 188–195 (2018).
4. G.N. Agrios, *Plant Pathology*, (Elsevier Academic Press, Burlington, USA, 2005), pp. 922.
5. M. Brundrett, Biol. Rev. **79(3)**, 473–495 (2004).
6. R. Balestrini, L. Nerva, F. Sillo, M. Girlanda, and S. Perotto, Plant Signal. Behav. **9(11)**, e977707 (2014).
7. R. Soelistijono, D.S. Utami, M. Faizin, and R. Dian, Biodiversitas J. Biol. Divers. **21(3)**, 1007–1011 (2020).
8. Y. Bonnardeaux, M. Brundrett, A. Batty, K. Dixon, J. Koch, and K. Sivasithamparam, Mycol. Res. **111(1)**, 51–61 (2007).
9. P. Bayman, L. Lebron, R.L. Tremblay, and J. Lodge, New Phytologist **135**, 143–149 (1997).
10. D.N. Sari, "Identifikasi orchid mycorrhiza pada akar anggrek *Cattleya* sp. dan *Dendrobium* sp.,"Undergraduate thesis, UIN Raden Intan Lampung, 2018.
11. B. Sneh, L. Burpee, and A. Ogoshi, *Identification of Rhizoctonia Species,* (American Phytopathological Society (APS) Press, St. Paul, 1991), pp. 133.
12. Haryuni, Biosaintifica **5(1)**, 43–49 (2013).
13. C. Booth, *The Genus Fusarium*, (Commonwealth Mycological Institute, Kew, Surrey, 1971), pp. 237.
14. J.F. Leslie and B.A. Summerell, Mycotoxin Res. **22(2)**, 73–74 (2006).
15. K. Zimmer, N.A. Hynson, G. Gebauer, E.B. Allen, M.F. Allen, and D.J. Read, New Phytol. **175(1)**, 166–175 (2007).
16. R.S. Kasiamdari, Berkala Ilmiah Biologi, **2(2)**, 615–628 (2000).
17. K.A. Kristiansen, D.L. Taylor, R. Kjøller, H.N. Rasmussen, and S. Rosendahl, Mol. Ecol. **10(8)**, 2089–2093 (2001).
18. J.D.W. Dearnaley, Mycorrhiza **17(6)**, 475–486 (2007).
19. S.E. Smith and D.J. Read, *Mycorrhizal Symbiosis*, (Academic Press, London, 2008), pp. 787.

12% Overall Similarity

Top sources found in the following databases:

- 7% Internet database
- Crossref database
- 2% Submitted Works database
- 8% Publications database
- Crossref Posted Content database

TOP SOURCES

The sources with the highest number of matches within the submission. Overlapping sources will not be displayed.

1	**Zulwanis, Nintya Setiari, Jose Gutierrez-Marcos, Endang Semiarti. "The...** Crossref	2%
2	**Raden Soelistijono, Angga Wulindra Jakti, Agus Budiyono, Endang Sup...** Crossref	2%
3	**Roger S. Hegarty, Sinead C. Leahy. "Technologies for reducing enteric ...** Crossref	2%
4	**smujo.id** Internet	2%
5	**e3s-conferences.org** Internet	1%
6	**mafiadoc.com** Internet	1%
7	**en.wikipedia.org** Internet	1%
8	**Universitas Bangka Belitung on 2021-05-20** Submitted works	<1%

9	sersc.org Internet	<1%
10	B Pujiasmanto, A A Wicaksono, Supriyono, Nandariyah, Sutarno, Riya... Crossref	<1%
11	garuda.kemdikbud.go.id Internet	<1%

Excluded from Similarity Report

- Bibliographic material
- Cited material
- Manually excluded sources
- Quoted material
- Small Matches (Less then 10 words)

EXCLUDED SOURCES

repository.utp.ac.id Internet	84%
journal.uinsgd.ac.id Internet	16%
pubs.aip.org Internet	13%
Mitha Valentina Treesya Panjaitan, Mahfut Mahfut, Tundjung Tripeni Handaya... Crossref	1%