

Research Article

Isolation of Dark Septate Endophyte (DSE) from Ferns (*Pteris Vittata*) Roots

Dalia Sukmawati^{1,2*}, Alza Kirana Thaharah¹, Esti Komariah¹, Herawati Herawati¹, Vicky Theodora¹, Atin Supiyani¹, Shabrina Nida Al Husna³, Nuniek Ina Ratnaningtyas⁴, Hesham El Enshasy^{5,6} and Daniel Joe Dailin⁵

¹Biology Department, Laboratory of Microbiology, 9th Floor Hasyim Ashari Building, Faculty of Mathematics and Natural Sciences, Universitas Negeri Jakarta, Jakarta, Indonesia

²Universitas Negeri Jakarta Culture Collection, Laboratory of microbiology, 9th Floor Hasyim Ashari Building, Faculty of Mathematics and Natural Sciences, Universitas Negeri Jakarta, Jakarta, Indonesia

³Department of Microbiology, School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia

⁴Department of Biology, Faculty of Biology, Jenderal Soedirman University, Dr. Suparno Road, Number 63, Karangwangkal, North Purwokerto, Banyumas Regency, Central Java, 53122, Indonesia

⁵Institute of Bioproduct Development, Universiti Teknologi Malaysia (UTM), Skudai, Johor Bahru, Malaysia

⁶City of Scientific Research and Technology Applications, New Burg Al Arab, Alexandria, Egypt

Corresponding Author: Dalia

Sukmawati; email:

dalia-sukmawati@unj.ac.id

Published: 27 March 2024

Publishing services provided by
Knowledge E

© Dalia Sukmawati et al. This article is distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use and redistribution provided that the original author and source are credited.

Selection and Peer-review under the responsibility of the ICMSCE Conference Committee.

ORCID

Dalia Sukmawati: <https://orcid.org/0000-0001-9641-9321>

Atin Supiyani: <https://orcid.org/0000-0002-2279-568X>

Nuniek Ina Ratnaningtyas: <https://orcid.org/0000-0002-7841-1999>

Hesham El Enshasy: <https://orcid.org/0000-0002-9712-2033>

Daniel Joe Dailin: <https://orcid.org/0000-0001-9392-1041>

Abstract.

Dark Septate Endophytes (DSE) are a group of ascomycetes that live in intracellular and extracellular root tissue to facilitate plant growth and stress tolerance in extreme environments. However, little is known about the DSE fungi isolated from certain plant roots such as *Pteris vittata*, especially under drought condition. *Pteris vittata* is known for its ability to live in various types of substrates and ecosystems. In this study, we obtained DSE fungi from the fern roots *Pteris vittata* collected from the area of Universitas Negeri Jakarta. DSE isolation was carried out by inoculating the *Pteris vittata* fern roots with a size of 0.5 cm on the surface of sterilized PDA media for 5-7 days at 27°C. Observations were made every 24 hours using a stereo microscope to see the first hyphae appeared from the plant roots. The results exhibited 13 samples of roots with hyphae colonization and were suspected to be DSE fungi. Of the 13 root isolates, only 3 isolate (PP2, PP4A, and PPB) showed the DSE growth (23%) with melanin pigment. The morphological characteristics of endophytic DSE fungi collected from *Pteris vittata* roots represented septate hyphae, brownish to black colony color, a growing zone, and a velvety texture. For the isolate PP2, it showed sclerotia while for the isolate PP4, it exhibited light brown colonies.

Keywords: isolation, DSE, ferns, *pteris vittata*, roots

OPEN ACCESS

1. INTRODUCTION

The symbiotic relationship between Dark Septate Endophytes (DSEs) and host plants has been reported previously. DSEs live in the intracellular or extracellular part of the live plant roots to help them efficiently absorb soil nutrients [1, 2] without causing negative effects to plant tissue [3]. The colonization of land by plants may not have been possible without the ability of DSEs to make them available to the plants. The symbiosis between DSE and plants is often called symbiotic mutualism in which the fungus benefits by obtaining carbohydrates from the host and the host benefits from better growth and more efficient nutrient acquisition [4]. In addition, it was also reported that mycorrhizae can increase the investment in host reproduction as seen from the results of nutritional content of seeds. Other studies have revealed that DSE has a wide range of enzymatic abilities where it can utilize several major organic nutrient groups [3].

DSEs constitute a very heterogeneous group of Ascomycetes characterized by a septate, a darkly pigmented hyphae, and melanized mycelium [4]. DSEs have a wide distribution and high root colonization in various terrestrial ecosystems, especially in extreme environments, including arid and semiarid environments [5, 6], which are characterized by low water availability, high salinity, high irradiance, and nutrient deprivation [7]. DSEs can also survive in alpine environments [8] and are even found extensively distributed in polluted areas around the world [9]. These environmental conditions generate a series of adverse effects on the physical and chemical properties of the soil, as well as on microbial processes and plant growth [10, 11].

Various types of DSE have been collected from several types of hosts. *Picea glauca* and *Potentilla fruticosa* have been successfully isolated from the plant *Leptodontidium orchidicola*, and several others from the classes Rosaceae and Salicaceae [12]. Research also proved that there are types of DSE in ferns (Pteridophytes) [13, 14]. Ferns (Pteridophyta) plays an important role in the forest ecosystem and found diversely in Indonesia. Ferns can live in various places, ranging from soil, rocks, to sticking to other plant stems. Ferns have a variety of types and potential for extraordinary use for feed ingredients, medicine and ornamental plants. Besides being found as a wild plant, several types of ferns are used as ornamental plants and have been widely cultivated. Ferns also have many other potentials, including those that have been used by the community, namely as antibacterial drugs, malaria drugs, laxatives, bleeding stop drugs, postpartum drugs, skin diseases and anti-inflammatory drugs [15].

One type of fern that is easily found is *Pteris vittata*, which usually grows on almost all substrates such as calcareous substrates, in savanna ecosystems, mixed tropical

forest ecosystems and mountains [16]. The existence of ferns is very easy to find but research on DSE in ferns is still little done. Therefore, this study aims to isolate and identify the endophytic fungi collected from the ferns *Pteris vittata* roots in Universitas Negeri Jakarta area.

2. RESEARCH METHOD

2.1. Sampling

This research was conducted at the Microbiology Laboratory, Universitas Negeri Jakarta in May–June 2019. The sample was taken from the root of the fern *Pteris vittata* obtained from the surrounding area of Universitas Negeri Jakarta, Rawamangun, Indonesia. 16 pieces of root were chosen and the sampling was done using purposive sampling technique. The isolates were then named by the codes PP1, PP2, PP3 and PP4.

2.2. Root Surface Sterilization

Isolation of endophytic fungi was carried out based on the method of Irawati *et al.*, [17]. Sterilization of the root surface of the *Pteris vittata* plant sample was carried out by cleaning the root surface from the soil with running water, then sterilizing the root surface by soaking the roots in 70% alcohol for 5 minutes, followed by immersing 5% NaOCl for 5 minutes and then rinsing three times using sterile distilled water. Finally, the roots were dried with sterile tissue for 15 minutes.

2.3. DSE Isolation from *Pteris vittata* Root

Root samples were cut with a size of 1 x 1 cm which were randomly selected and then inoculated into PDA medium containing the antibiotic chloramphenicol to avoid bacterial growth or contaminants. It was then incubated at 27°C in the dark for 5-7 days [17]. Observations were made every 24h using a stereo microscope to see the growth of hyphae. The hyphae that first grew were then inoculated onto new PDA media.

2.4. Macroscopic and Microscopic Observations

During this period, the growth rate of the endophytic mold was observed. If the endophytic mold showed morphological characteristics, the mold could be transferred to a

new PDA medium to obtain pure isolates and observed microscopically and macroscopically. Macroscopic observations included color and surface of the colony (granular, powdery, mountainous, and slippery), texture, zoning, growing area, radial and concentric stripes, and colony turning color [18]. Microscopic observation of DSE was carried out by making preparations on glass objects using one drop of 70% alcohol and one mold culture tube inoculated on alcohol and then covered with a cover glass. Microscopic observations included microscopic observations of molds including the shape and type of spores or conidia, types of hyphae, size of hyphae, hyphae insulation, and the presence or absence of medulla, and vesicles [19][20].

3. RESULTS AND DISCUSSION

3.1. Isolation of Dark Septate Endophyte (DSE)

The DSE mold was isolated from the roots of the fern *Pteris vittata*. *Pteris vittata* are found to grow easily in pots with other plants. DSE can be found in various plant species and most DSE colonize various plants in the root system [21].

Isolation of DSE from the roots was carried out by separating the roots from the base of the stem, which was then cleaned of the remaining adhering soil. It was followed by a sterilization process and cutting the roots into small pieces. Root pieces were inserted into PDA medium with chloramphenicol antibiotic added in 4 petri dishes. Each petri dish contains 4 *Pteris vittata* roots. Petri dishes containing roots were incubated in a box in the dark at 27°C for 5-7 days of observation and their growth was observed everyday. Observation on the growth of DSE hyphae was carried out by looking at the characteristics of the hyphae microscopically using a stereo microscope. According to [22], DSE differs from other fungal groups in that it has septate hyphae and melanised hyphae. According to [23], it is also stated that the growth of DSE hyphae on plant roots had hyphal growth characteristics which were generally septate, hyaline or dark in color and melanized.

During the 7 days of observation, the highest hyphae growth on *Pteris vittata* roots was shown on the first day of incubation. The hyphae that grow are thin and slightly dark in color on the root surface. On the first day, there were 13 root samples from a total of 16 roots that were incubated with a percentage of 81.25%. On the second day to the seventh day of incubation, 12 root samples did not show any regrowth of hyphae, but only one root sample that exhibited hyphal growth on the second to the fourth day of incubation. The hyphae in these root samples colonized more than half of the roots

(Figure 1). The growing hyphae on the first day to the fourth day of incubation were inoculated into new PDA media and incubated at 27°C.

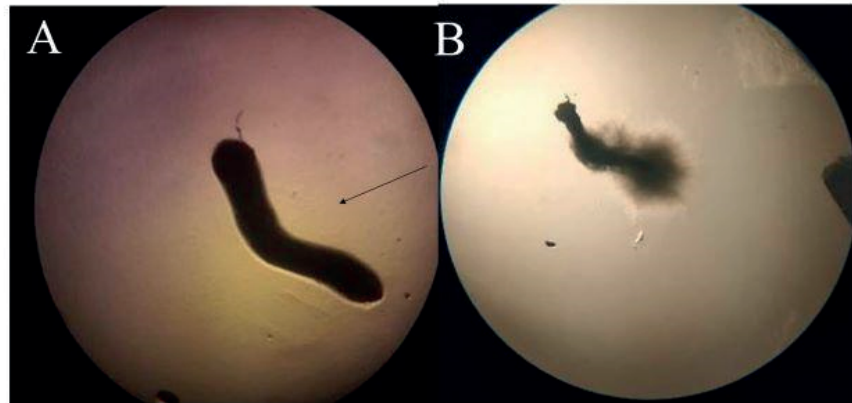


Figure 1: Root samples on the first day of incubation (A), root samples on days 2-4 of incubation (B). 100x magnification. Incubation at a temperature of 27°C.

From the 13 inoculated root samples, it resulted 3 isolates showed the growth in the new PDA medium, namely PP2, PP4-A and PP4-B isolates (Figure 2 and 3). The unsuccessful growth of inoculated DSE isolates could be due to the influence of environmental temperature at the time of incubation which was not suitable, resulting in the inability of DSE to grow. This requires further research on optimizing a good environment for DSE growth. Based on the isolation results, the percentage of roots that were isolated was 81.25% and the survival rate of roots suspected of DSE was only 23.07%.

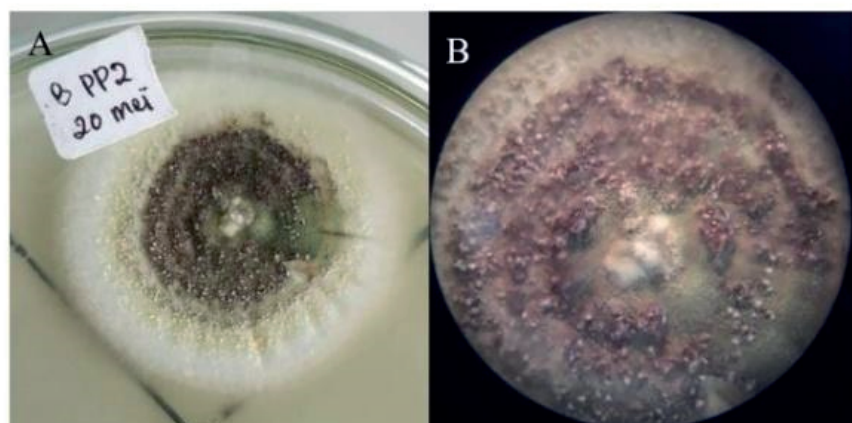


Figure 2: Isolate PP2 within 4 days of incubation at 27°C (A) DSE colony top view (B) Sclerotia at 100x magnification with a stereo microscope.

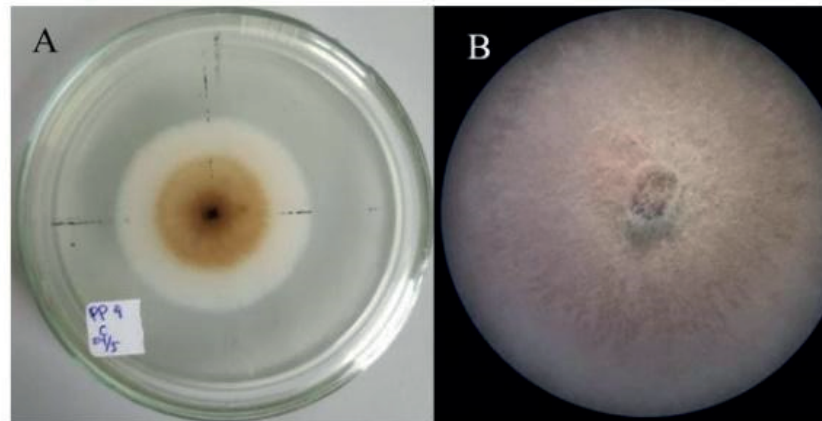


Figure 3: Isolate PP4 within 4 days of incubation at 27°C. (a) Reverse DSE colonies, (b) DSE colonies seen at 100x magnification with a stereo microscope.

3.2. Macroscopic and Microscopic Observation

The macroscopic observation shown in Figure 4, isolate PP2 has white-brown colonies, with light brown mycelium at the edges. The texture of the colonies is velvety, having a growing zone and a black exudate drop, while the color behind the colony is gold. From the microscopic observation, isolate PP2 has a septate hyphae, while the conidiophores could not be observed. The spores are in a sac containing 4-5 spores and are reddish in color.

The macroscopic observation shown in Figure 5, isolate PP4B has a brownish colony color, with light brown mycelium around the edges. The texture of the colony is velvety, has a growing zone, has exudate drop and the reverse color of the colony is black with white edges. From the microscopic observation, isolate PP4 has a septate hyphae, while the conidiophores and spores could not be observed.

From the macroscopic observation shown in Figure 6, isolate PP4A has a faded brown colonies from the center to the edges of the colonies. The texture of the colony is velvety, has a growing zone, has no exudate drop and the reverse color of the colony is black with brown radiance with white edges. From the microscopic observation, isolate PP4A has a septate hyphae, while the conidiophores and spores could not be observed.

Of the three DSE isolates, hyphae of isolates PP4A and PP4B showed a dark pigment. This result is in accordance with the statement of [24] which defines DSE colonizes plant roots with a characteristic internal hyphae structure in the form of dark-colored melanized hyphae and microsclerosia.

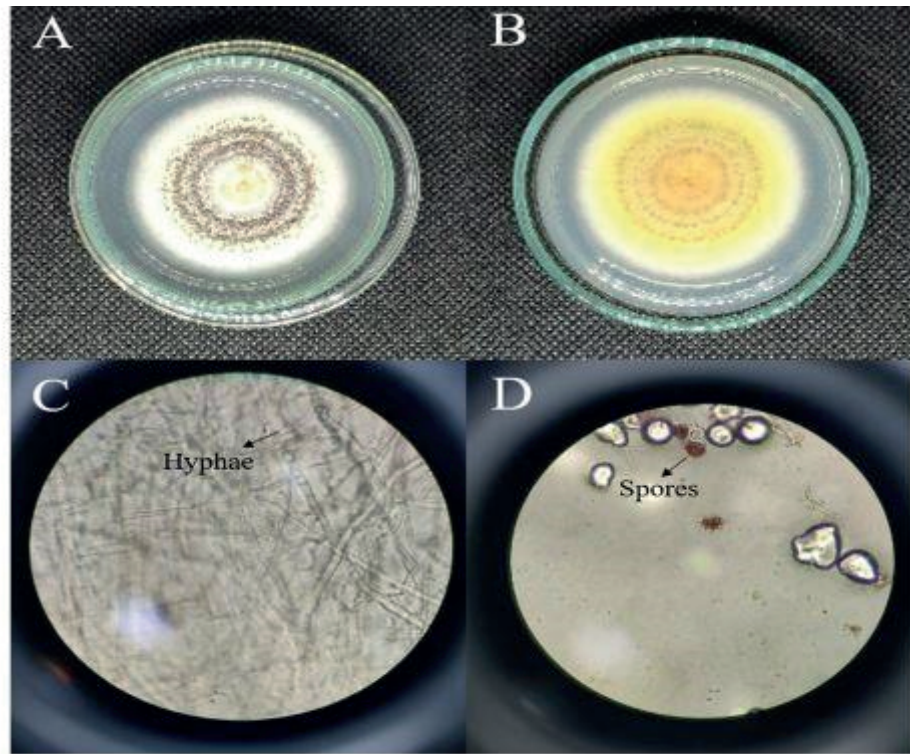


Figure 4: Isolate PP2 within 15 days of incubation at 24°C. (A) DSE colonies top view, (B) DSE colonies bottom view (C) mycelium 1000x magnification, (D) PP2 spores 1000x magnification.

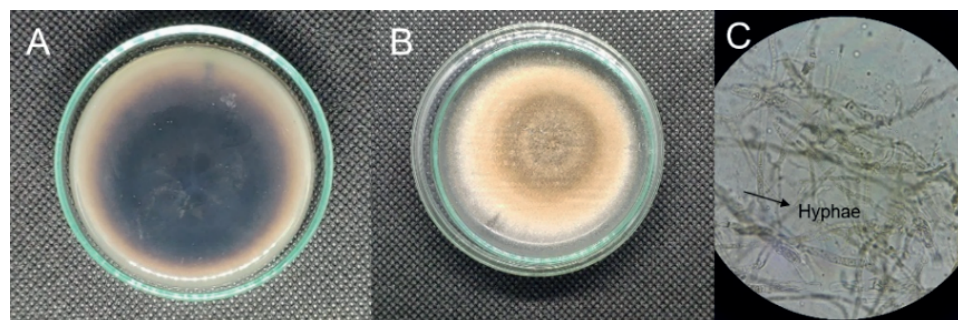


Figure 5: Isolate PP4B within 15 days of incubation at 24°C on PDA media. (a) DSE colony bottom view, (b) DSE colony top view, (c) DSE PP4B mycelium 1000x magnification.

4. CONCLUSION

Three isolates of DSE mold were obtained with the characteristics of having melanin pigment. The isolates were coded as PP4A and PP4B. DSE growth from *Pteris vittata* roots had a percentage of about 81.25%, but growth from the inoculation was only 23.07% (3 isolates from 13 isolates). Based on the morphological characteristics of endophytic fungi, it is suspected that the isolates were categorized as DSE. The characteristics include having septate hyphae, brownish to black colony color, and having a growing zone, and a velvety texture.

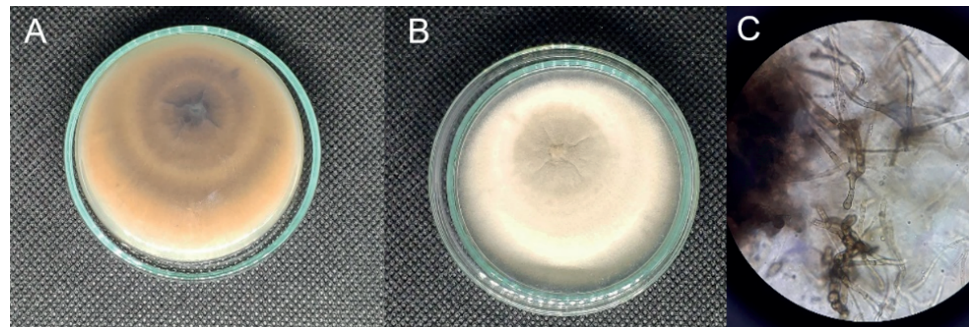


Figure 6: Isolate PP4A within 15 days of incubation at 24°C on PDA media. (a) DSE image bottom view, (b) DSE image top view, (c) DSE PP4A mycelium 1000x magnification.

Acknowledgments

The authors are very grateful to Hibah Penelitian Kolaboratif Internasional Universitas Negeri Jakarta Nomor:7/KI/IV/2021, Tanggal 14 April 2022 on behalf Dalia Sukmawati with title: “Dark Septate Endophyte: Its Potential As Immunity Agent For Health”. We thank the Lab. Microbiology and Universitas Negeri Jakarta Culture Collection (UNJCC) for the facilities provided to run this study.

References

- [1] Pirozynski KA, Malloch DW. The origin of land plants: a matter of mycotrophism. *Biosystems*. 1975 Mar;6(3):153–64.
- [2] Wang B, Qiu YL. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza*. 2006 Jul;16(5):299–363
- [3] Jumpponen A, Trappe JM. Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. *New Phytol*. 1998 Oct;140(2):295–310.
- [4] Barrow JR. Atypical morphology of dark septate fungal root endophytes of *Bouteloua* in arid southwestern USA rangelands. *Mycorrhiza*. 2003 Oct;13(5):239–47.
- [5] Postma JW, Olsson PA, Falkengren-Grerup U. Root colonisation by arbuscular mycorrhizal, fine endophytic and dark septate fungi across a pH gradient in acid beech forests. *Soil Biol Biochem*. 2007;39(2):400–8.
- [6] Zhi-wei Z. The arbuscular mycorrhizas of pteridophytes in Yunnan, southwest China: evolutionary interpretations. *Mycorrhiza*. 2000;10(3):145–9.
- [7] Read DJ, Haselwandter K; D.J. READ and K. HASELWANDTER. OBSERVATIONS on the mycorrhizal status of some alpine plant communities. *New Phytol*. 1981;88(2):341–52.

- [8] Agigal EF. Dark Septate Endophytes (DSE) in polluted areas. endophytic Fungi: Diversity, characterization and biocontrol. Polluted Areas. Endophytic Fungi: Diversity, Characterization and Biocontrol. Nova Science Publishers, Inc; 2017.
- [9] Chesson P, Gebauer RL, Schwinning S, Huntly N, Wiegand K, Ernest MS, et al. Resource pulses, species interactions, and diversity maintenance in arid and semi-arid environments. *Oecologia*. 2004 Oct;141(2):236–53.
- [10] Tejada M, Garcia C, Gonzalez JL, Hernandez MT. Use of organic amendment as a strategy for saline soil remediation: influence on the physical, chemical and biological properties of soil. *Soil Biol Biochem*. 2006;38(6):1413–21.
- [11] Fernando AA, Currah RS. A comparative study of the effects of the root endophytes *Leptodontidium orchidicola* and *Phialocephala fortinii* (Fungi Imperfecti) on the growth of some subalpine plants in culture. *Can J Bot*. 1996;74(7):1071–8.
- [12] Fernández NV, Messuti MI, Fontenla SB. Occurrence of arbuscular mycorrhizas and dark septate endophytes in pteridophytes from a Patagonian rainforest, Argentina. *J Basic Microbiol*. 2013 Jun;53(6):498–508.
- [13] Thangavelu M, Raji M. Arbuscular mycorrhizal and dark septate endophyte fungal associations in *Asparagus*. *Turk J Bot*. 2016;40:662–75.
- [14] Arini DI, Kinho J. Keragaman jenis tumbuhan paku (Pteridophyta) di Cagar Alam Gunung Ambang Sulawesi Utara. *Info BPK Manado*. 2012;2(1):17–40.
- [15] Mumpuni M. Variasi morfologi *Pteris vittata* L. (Pteridaceae; Pteridophyta) dan korelasinya dengan ketinggian lokasi tempat tumbuhnya di Jawa [Jurnal Biologi Lingkungan Industri Kesehatan]. *Biolink*. 2017;2(2):100–9.
- [16] Irawati AF, Mutaqin KH, Suhartono MT, Sastro Y, Sulastris N, Widodo N. Eksplorasi dan pengaruh cendawan endofit yang berasal dari akar tanaman cabai terhadap pertumbuhan benih cabai merah. *Jurnal Hortikultura*. 2017;27(1):105.
- [17] Sukmawati D, Setyaningsih A. T. Handayani K, et al., “Isolation and characterization of aflatoxigenic *Aspergillus spp.* from maize of livestock feed from Bogor.” *IOP Conference Series: Materials Science and Engineering*. vol. 434, no. 1, p. 012105, 2018.
- [18] Klich MA, Cary JW, Beltz SB, Bennett CA. Phylogenetic and morphological analysis of *Aspergillus ochraceoroseus*. *Mycologia*. 2003;95(6):1252–60.
- [19] Sukmawati D, Larasati RP, Kurniati TH, Arman Z, El Enshasy HA. Molds isolated from chicken feed as potential amylase resources. *International Journal of Scientific & Technology Research*. 2019;8(11):188–96.
- [20] Mandyam K, Jumpponen A. Seeking the elusive function of the root-colonising dark septate endophytic fungi. *Stud Mycol*. 2005;53(1):173–89.

- [21] Yu T, Nassuth A, Peterson RL. Characterization of the interaction between the dark septate fungus *Phialocephala fortinii* and *Asparagus officinalis* roots. *Can J Microbiol.* 2001 Aug;47(8):741–53.
- [22] Handayani D. Keberadaan cendawan Dark septate endophyte (Dse) pada sistem perakaran benih *Shorea selanica*. *EKSAKTA.* 2016;1:38–44.
- [23] Mandyam KG, Jumpponen A. Mutualism-parasitism paradigm synthesized from results of root-endophyte models. *Front Microbiol.* 2015 Jan;5:776.