JURNAL
BIOS LOGOS
Accredited by Ministry of Research, Technology and Higher Education No. 105/E/KPT/2022

Vol. 13 (No.1), Februari 2023, 16 - 26
DOI: https://doi.org/10.35799/jbl.v13i1.46484
E-ISSN: 2656-3282
P-ISSN: 2088-9569

In Vitro Evaluation of the Antagonism of Saprophyte and Endophytic Fungi Isolated from Groundnut (*Arachis hypogaea*) Against Soil-Transmitted Diseases *Sclerotium rolfsii*

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(Article History: Received Dec 01, 2022; Revised 10 Jan, 2023; Accepted 31 Jan, 2023)

ABSTRACT

Certain soil-borne diseases can have a detrimental effect on the quality and quantity of peanut production. One of the soil-borne diseases is Sclerotium rolfsii, a fungus that is the primary limiting factor in peanut plants. An alternative to controlling this fungus is to use saprophytic and endophytic fungi. The purpose of this study was to evaluate several saprophytic and endophytic fungi isolated from peanut plants in vitro as antagonistic agents against S. rolfsii. Isolation of saprophytic and endophytic fungi was carried out using the stratified dilution method. The fungal antagonism test was carried out using a non-factorial completely randomized design with 3 replications. The parameters observed in this study included the inhibiting zone, the diameter of the isolate colonies, the growth area, and the interaction of saprophytic and endophytic fungi with S. rolfsii. Five families and eight species of soil fungi were identified using macroscopic and microscopic identification techniques. The type of soil fungus has a large impact on the growth rate and inhibition area. Mucor hiemalis grew at the fastest rate, while Rhizopus oryzae had the largest inhibition zone.

***Keywords**: antagonism; saprophytic fungi; endophytic fungi; soil-borne fungi; population level; ground peanut*

INTRODUCTION

Ground peanut (*Arachis hypogaea* L.) is a high-value food plant that contributes significantly to the Indonesian economy. Ground peanuts are economically significant because they are a source of vegetable fat and protein that are consumed on a daily basis by the Indonesian people. This plant is a source of vegetable protein, which is a necessary component of the average population diet. Ground peanut is a food crop that is prioritized for development and expansion in several areas of Indonesia, after rice and soybeans. This trend is being driven by rising demand for ground peanuts as food and industrial raw materials. The demand for ground peanuts continues to grow year after year, owing to the growing population, the community's nutritional requirements, and the capacity of Indonesia's feed and food industries (Fachruddin 2000).

Numerous obstacles arise during the cultivation of ground peanuts, including disturbances caused by pests and plant diseases. Important diseases that attack ground peanut plants are leaf spot caused by pathogens (*Cercospora arachidicola* and *Cercosporidium personatum*), leaf rust (*Puccinia arachidis*), bacterial wilt (*Ralstonia solanacearum*), striped virus (Peanut Mottle Virus), welt virus (Peanut Stripe Virus), root rot (*Meloidogyne* Spp.) and stem rot (*Sclerotium rolfsii*) (Soesanto 2013).

Attacks by *S. rolfsii* on elephant variety ground peanuts in the field can result in yield losses of up to 74.22% (Rahayu 2003). Stem rot disease caused by *S. rolfsii* is a serious problem in several ground peanut-producing countries,

including the United States, Latin America, China, India, Thailand, Egypt, Mali, Nigeria, Senegal, Bangladesh, Australia, and Indonesia (Mehan *et al*. 1995). Peanut yield losses due to *S. rolfsii* are quite high, ranging between 13 and 59% (Nautiyal 2002). *S. rolfsii* typically initiates infection on the surface of the planting hole or at the base of the host plant's stem. Stem rot-infected plants gradually wilt and turn yellow. The initial symptom of this stem rot disease is yellowing at the base of the stem, followed by the appearance of fine white threads called mycelium on the stem of the ground peanut plant, which causes the stem to rot. Chlorosis occurs when leaves are too close to the soil surface and they turn brown; sclerotia develop on the underside of the leaves and throughout the peanut plantation (Porter *et al*. 1984).

Numerous efforts have been made to control stem rot disease in ground peanuts, including soil sterilization, the use of resistant varieties, and the application of synthetic fungicides. The use of synthetic fungicides for disease control has proven to be very effective and practical but can have negative impacts on humans and the environment. This is consistent with the findings of Wasilah *et al*. (2005), who concluded that when synthetic pesticides are used improperly, they can cause harm to humans and the environment. Farmers continue to use synthetic pesticides to control plant pest organisms because they consider this method to be the simplest and most effective.

In light of the foregoing, it is critical to develop alternative methods of disease control that are both safe and environmentally friendly. One of them is through the use of endophytic fungi (Sinaga 2009) and saprophytic fungi capable of controlling soil-borne pathogens (Soesanto 2008). Alternative biological control methods, such as the use of antagonistic microbes, have been reported to be quite effective, and no report on the emergence of pathogenic fungi resistance to biological control agents (Freeman *et al*. 2002). Various biological control agents have been found and have shown the ability to inhibit the growth and development of plant diseases. The development of antagonists needs to be continued in order to create a balance of ecosystems, the realization of human health, and the preservation of the environment for the sustainability of future generations (Soesanto 2013).

Numerous fungal antagonist microbes such as *Trichoderma hamatum*, *T. viride*, *T. koningi*, *Gliocladium virens*, *G. roseum*, *Penicillium janthinellum*, *Epicocum purpureum*, and *Pythium nunn* are commonly used in biological control of plant diseases (Aryantha 2001). Agrios (2005) reported that, of the 25,000 species of soil fungi, approximately 10,000 are plant pathogens and 15,000 are saprophytic fungal species that act as antagonist microbes, suppressing disease development. These fungi may contribute to the conversion of organic matter into useful compounds (Sumarsih 2003).

Baker and Cook (1983) in Yulianto (2014) stated that biological control can be carried out without having a negative influence on the environment and its surroundings, one of which is the use of biological agents such as viruses, fungi, bacteria or actinomycetes. Biological control, such as the use of antagonistic fungi that live in root zones, has the potential to suppress disease and promote plant growth.

Given the importance of biological agents as antagonistic agents, it is necessary to test saprophytic and endophytic fungi as biological agents with the

potential to control plant pathogens, more specifically *S. rolfsii*, which causes ground peanut stem rot disease. The purpose of this study was to determine whether several saprophytic and endophytic fungi isolated from ground peanut plants act as antagonistic agents against the fungus *S. rolfsii*, which causes stem rot disease in the plants.

METHODS

Isolation, Purification, and Identification of Saprophytic Fungi

Following the dilution procedure, the isolation procedure using Umboh's method was performed (2016), by pouring 10 ml of PDA media which has been liquefied at 50^{o}C from each petri dish. To avoid bacterial contamination, cotrimoxazole was added. Then, using a syringe, 1 ml of fungus suspension was taken from each dilution and placed in a petri dish containing solidified PDA media. The cultures were then incubated for 72–96 hours at room temperature. Additionally, purification was accomplished by cultivating pure cultures of various fungi in each petri dish.

Following the establishment of a pure culture of each fungus for one week, identification was accomplished through microscopic examination (Sudarma and Suprapta 2011). The method of Indrawati and Fakhrudin (2016) is used for identification: first, first macroscopically by looking at the shape and color of the fungal colonies. Second, by examining the structure or arrangement of hyphae, conidia, and fungal spores microscopically. The microscopic identification step entails aseptically extracting a small portion of the fungus with an aseptic needle. The specimen was then mounted on a slide and dripped with methylene blue solution. After covering them with a cover slip, they were examined under a microscope for the presence of hyphae, spores, and conidia.

Identification of saprophytic fungi was carried out using the Pictorial Atlas of Soil and Seed Fungi identification book (Watanabe 2002), Compendium of Soil Fungi (Domsch *et al*. 1980 in Ilyas 2006), and Introduction to General Tropical Molds by Gandjar *et al.* (1999), and Introduction to Food–Borne Mushrooms (Samson *et al*. 1981 in Subowo 2012).

Isolation, Purification, and Identification of Endophytic Fungi

The isolation method was similar to that used by Surhatina *et al*. (2018), where fresh and unblemished leaves from the ground peanut plants were used (no stains/spots). A sterile knife was used to cut the leaves to a size of 1cm x 1cm. Additionally, surface sterilization was performed, in which the leaf pieces were washed for 5 minutes under running water. Leaf samples were first soaked in 70% alcohol for 1 minute, then in sodium hypochlorite solution for 5 minutes, then resoaked in 70% alcohol for 30 seconds before being rinsed with sterile distilled water for 3-5 seconds.

After sterilizing the leaf fragments, they were placed on filter paper and then into a petri dish containing PDA media. Each petri dish contained five pieces of leaves, and each sample was planted in duplicate. The inoculated media were kept at room temperature for 7-10 days after being inoculated with leaf pieces. Another PDA was used to isolate the last rinsed distilled water. This treatment was used to ensure that the leaf surface is sterilized.

Isolation, Purification, and Identification of Pathogenic Fungi

Isolation of the pathogen was accomplished by cutting the infected part (leaf) to a size of approximately 1x1cm, dipping it for 2 minutes in a glass beaker containing 70% alcohol to remove contamination on the outside, and then rinsing three times in sterile distilled water. It was then incubated for 5 days at 27-28^{o}C on the surface of Potato Dextrose Agar (PDA) media supplemented with the antibiotic chloramphenicol (100 mg/L) (Samson *et al*. 1995). The growing fungal mycelium was then isolated on new PDA media until an isolate of the fungus suspected to be responsible for stem rot disease in peanut plants was obtained..

In vitro Antagonism Test

The in vitro testing was performed using the dual culture method (Dharmaputra *et al*. 1999), in which each pure culture obtained from the characterization results of the test antagonist and pathogenic fungi was inoculated into a petri dish containing PDA media face to face at a distance of 3 cm (**Figure 1**).

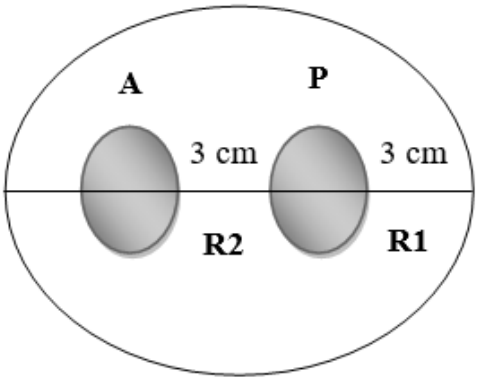

Figure 1. Multiple culture test. P= Pieces of pathogenic fungal colonies, A= Slice of tested antagonist fungus colonies, R1= Radius of the pathogenic colony away from the tested antagonist fungal colony, R2= Radius of pathogenic colonies that are close to the tested antagonist fungal colonies.

Following that, each petri dish was incubated at room temperature. The variables that were observed are as follows:

a. Fungal growth rate (cm)
   The rate of fungal growth was determined by measuring the colony diameter of each fungus daily until the fifth day after inoculation. A ruler was used to take measurements.
b. Inhibition percentage (%)
   Inhibition percentage (%) was calculated on the 7th day after inoculation with the formula:

$$\text{Inhibition (\%)} = \frac{R1 - R2}{R1} \text{x} 100\%$$

Where R1= Radius of the pathogenic colony away from the antagonist fungal colony being tested and R2= Radius of the pathogenic colony that is close to the antagonist fungal colony being tested

c. Antagonism mechanism

Identification of the mechanism of antagonism was carried out based on Farida (1992) which includes:

1. Competition for resources such as space, nutrients, and oxygen. The competition for space, nutrients, and oxygen between the tested fungi and pathogenic fungi was observed by observing which type of fungus filled the petri dish faster.
2. Antibiosis. Antibiotic observations were made by determining the width of the empty zone (inhibition) and determining whether or not the medium changed color as a result of the antibiotic compounds produced by the test fungus.
3. Lysis dan parasitism. Observation of the mechanism of lysis and parasitism was carried out by observing the hyphae of the test antagonist fungus growing on the pathogenic fungus by taking and growing the hyphae of the antagonist and pathogenic fungi using a loop needle, then placing it on an object glass for microscopic observation.

RESULTS AND DISCUSSION

Isolation, purification, and identification of saprophytic, endophytic and pathogenic fungi

Based on the results of isolation and macroscopic and microscopic observations, 5 families and 8 species were obtained (*Paecilomyces lilacinus*, *Penicillium citrinum*, *Geotricum* sp., *Aspergillus flavus*, *Rhizopus oryzae*, *Trichoderma harzianum*, *Rhizoctonia* sp., and *Mucor hiemalis*) with different characteristics.

Endophytic fungi were isolated and purified from healthy leaves. As was the case with saprophytic fungi, the purification results were identified. *Fusarium oxyporum* was identified as the endophytic fungus (**Figure 2**). Similar to endophytic fungi, pathogenic fungi were isolated from leaves infected with pathogenic fungi on peanut plants and then purified. The purification results were identified as performed on saprophytic and endophytic fungi. The identification results showed that the pathogenic fungus was *Sclerotium rolfsii* (**Figure 3**).

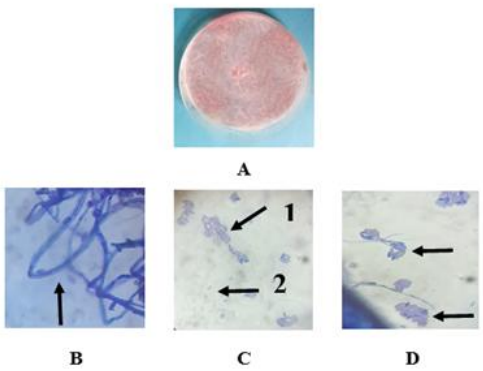

Figure 2. Morphological form of *Fusarium oxysporum* (A: Macroscopic form; B-D: Microscopic form; B: Septate hyphae (arrows); C: Macroconidia (arrow 1), Microconidia (arrow 2); D: Macroconidia clustered like pads (arrows).

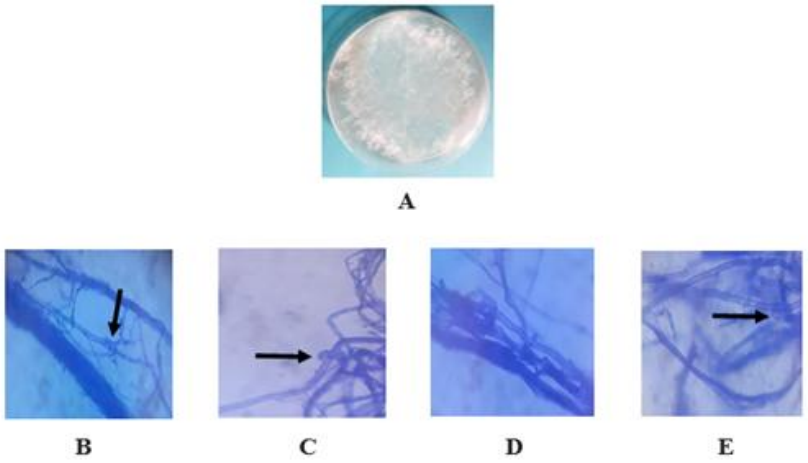

Figure 3. Morphological form of the fungus *Sclerotium rolfsii* (A: Macroscopic form; B-E: Microscopic form; B: Branching septate hyphae (arrows); C: Clamp connection (arrow); D: Hyphae without clamp connection (arrow); E: Sclerotia (arrow).

Based on macroscopic and microscopic observations, the fungus *S. rolfsii* showed the following morphological characteristics: the shape of the colony on the 3rd day was white, the 5th day white, and the 7th day white like cotton, with colony reverse: white, the surface of the colony like cotton. The sclerotia are round, the hyphae are branched, and there is a clamp connection.

These characteristics are consistent with the findings of Magenda *et al.* (2011), who discovered that *S. rolfsii* isolated from ground peanut plants with stem rot symptoms formed colonies with white mycelium similar to cotton or shaped like feathers after being grown on PDA media. Meanwhile, sclerotia germination on PDA media was dispersive (hyphae emanating from all corners of the sclerotia) and characterized by fine, branched threads resembling cotton and being white in color.

Saprophytic and endophytic fungi antagonism test

Percentage of inhibition zone

The results of the analysis of variance revealed that both saprophytic and endophytic fungi had a highly significant effect on the inhibition area for pathogenic fungi (**Table 1**). As can be seen in **Figure 4**, *Rhizopus oryzae* had the highest percentage of inhibition (55.36 %) and *Paecilomyces lilacinus* had the lowest (18.70 %) (**Figure 5**). The fungus *Rhizoctonia* sp. grew faster, inhibiting the growth of *S. rolfsii*. According to Soesanto (2008), each biological agent has its own ability and mechanism of inhibition. The mechanism of inhibition that occurs is antibiosis, as evidenced by the formation of clear zones, and hyperparasites, as evidenced by the growth of saprophytic fungi mycelium that covers the entire surface of the colony of pathogenic fungi. According to Soesanto (2008), antagonist agents can live as hyperparasites, produce antibiotics, and grow faster, allowing for competition for space and nutrients.

Table 1. Inhibition of Antagonist Fungi against Pathogenic Fungi

No	Jamur	Daya Hambat (%)
1.	*Paecilomyces lilacinus*	18,70
2.	*Penicillium citrinum*	31,37
3.	*Geotricum sp.*	42,86
4.	*Aspergillus flavus*	51
5.	*Rhizopus oryzae*	55,36
6.	*Trichoderma harzianum*	51,95
7.	*Rhizoctonia sp.*	45,46
8.	*Mucor hiemalis*	37,06

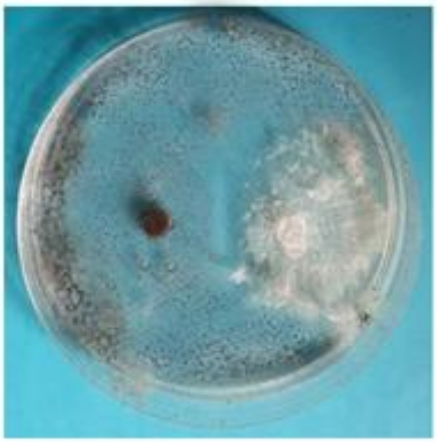

Figure 4. Inhibition of saprophytic fungus *R. oryzae* against pathogenic fungus *S. rolfsii* (Day 7, 2nd replication)

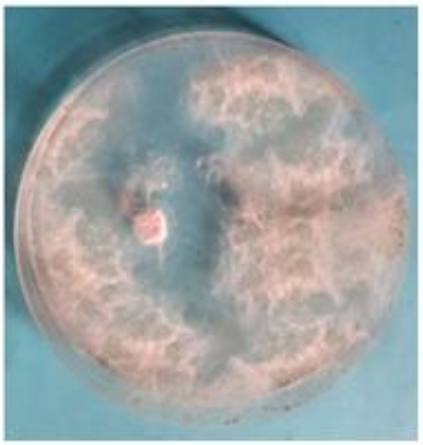

Figure 5. Inhibition of saprophytic fungus *P. lilacinus* against pathogenic fungus *S. rolfsii* (Day 8, 3rd replication)

Saprophytic and Endophytic Fungal Colony Diameter

The saprophytic fungi in this study were able to compete with the pathogenic fungus *S. rolfsii* for control of space, oxygen, and nutrients (**Figure 6**). Saprophytic fungi grow at a faster rate than pathogenic fungi, allowing them to outperform and suppress pathogenic fungi, in contrast to endophytic fungi, which outperform pathogenic fungi only slightly. The results of the analysis showed that of all the saprophytic fungi that had faster and higher growth rates were *M. hiemalis* (IJTK8) and *P. lilacinus* (IJTK1) after inoculation on day 5.

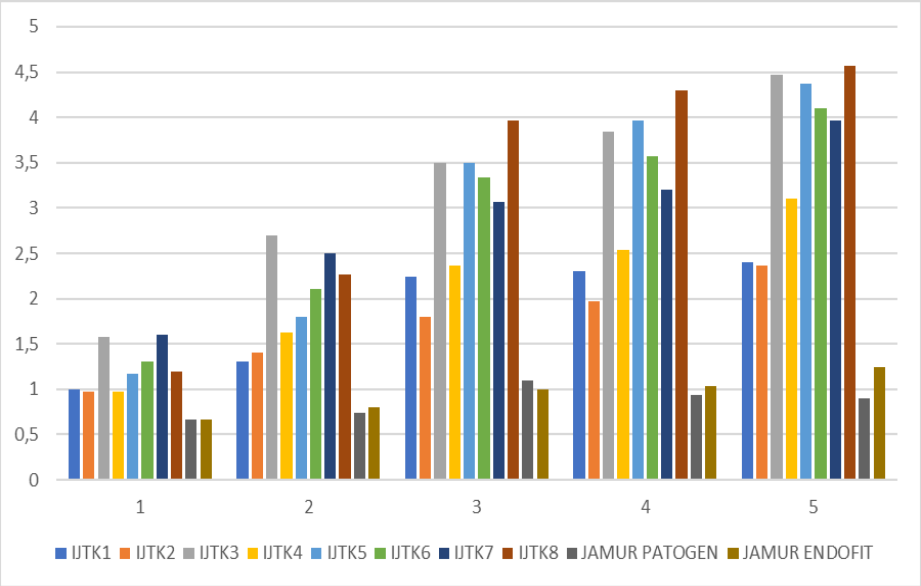

Figure 6. Comparison of growth between antagonistic fungi (saprophytic fungi), endophytic fungi, and pathogenic fungi *F. oxysporum.*

Pathogenic Hyphae Changes

Saprophytic fungi are able to compete with pathogenic fungi, as can be seen from their fast growth, so that the hyphae or mycelium of the pathogenic fungi are squeezed and do not get space to grow. Microscopic observations showed that the hyphae of the pathogen were damaged or lysed, dismembered, and destroyed (**Figure 7**). This is consistent with Sunarwati and Yoza (2010), who stated that another mechanism by which biological agents inhibit pathogens is through lysis, specifically the mycelium of biological agents capable of destroying or dismembering pathogens' mycelium and causing death.

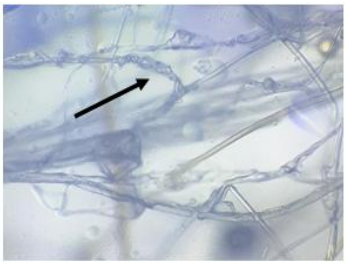

Figure 7. The lised hyphae of pathogenic fungal *S. rolfsii*).

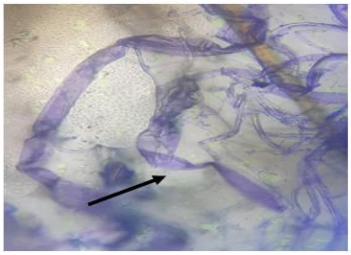

Figure 8. Deforming/malformation (crunching) of hyphae of pathogenic fungus *F. oxysporum*.

Based on observations, the hyphae of pathogenic fungi also changed shape/malformation (curling) (**Figure 8**). This is in accordance with Triharso (1996), which states that symptoms caused by infection with a microbe can be in the form of changes in color, as well as changes in shape.

CONCLUSION

Five families and eight species of soil fungi were identified based on the results of macroscopic and microscopic identification (*Paecilomyces lilacinus, Penicillium citrinum, Geotricum sp., Aspergillus flavus, Rhizopus oryzae, Trichoderma harzianum, Rhizoctonia* sp., and *Mucor hiemalis*). The type of soil fungus had a significant effect on the growth rate and area of inhibition; *M. hiemalis* grew the fastest and *R. oryzae* had the largest inhibition diameter.

REFERENCES

Carr, S., Francis, M., Rivlin, L. G., & Stone, A. M. (1992). *Public space*. Cambridge University Press.

Agrios, G.N. (2005). *Plant Pathology*. Edisi 5. Elsevier Academic Press, USA. 922p.

Aryantha, I.N.P. (2001). *Membangun Sistem Pertanian Berkelanjutan.* KPP Ilmu Hayati LPPM-ITB, Dept. Biologi - FMIPA-ITB. Ganesha, Bandung.

Dharmaputra, O.S., Gunawan, A.W., Wulandari, R., dan Basuki, T. (1999). Cendawan kontaminan dominan pada bedengan jamur merang dan interaksinya dengan jamur merang secara in vitro. *J. Mikro. Indonesia*, 4(1):14-18.

Fachruddin, L. (2000). *Budidaya Kacang-Kacangan.* Kanisius. Cetakan Ke-6. Yogyakarta.

Farida, S. (1992). Penggunaan Jamur Saprob Tanah Untuk Mengendalikan *Fusarium oxysporum* Pada Tanaman Tomat (*Lycopersicum esculenta*). *J. IPM*. 2(1):24-29.

Freeman, S., Zveibel, A., Vintal, H., and Maymon, M. (2002). Isolation of Nonpatogenic Mutants of Fusarium oxysporum f.sp. Melonis For Biological Control of Fusarium Wilts In Cucurbits. *Phytopathology*, 92:164-168.

Gandjar, I., R.A. Samson, K. van den Tweel-Vermeulen, A. Oetari and I. Santoso. (1999). *Pengenalan Kapang Tropik Umum.* Jakarta: Yayasan Obor Indonesia.

Ilyas, M. (2006). Isolation and Identification of Molds on Plant Rizosung in Gunung Mutis Nature Reserve, East Nusa Tenggara. *Biodiversity*, 7(3): 216-220.

Indrawati, I., dan Sarah, D. F. (2016). Isolasi Dan Identifikasi Jamur Patogen Pada Air Sumur Dan Air Sungai Di Pemukiman Warga Desa Karangwangi, Cianjur, Jawa Barat. *Jurnal Biodjati*, 1(1): 27-38.

Magenda, S., Febby, E.F.K., dan Stella, D.U. (2011). Karakteristik Isolat Jamur Sclerotium rolfsii dari Tanaman Kacang Tanah (Arachis hypogaea Linn.). Jurnal Bios Logos, 1(1):1-7.

Mehan, V.K., Y. J. Tan, and B.S. Liao. (1995). *Inoculation Techniques to Evaluate Resistance of Groundnut to Bacterial Wilt.* pp. 59–62 In V.K. Mehan and D. McDonald (eds.). Techniques For Diagnosis of P. solanacearum and for Resistance Screening Against Groundnut Bacterial Wilt. ICRISAT Technical Manual no.1.

Nautiyal, P.C. (2002). *Groundnuts: Post-harvest Operations. Research Centre for Groundnuts (ICAR)*. [www.icar.org.in] diakses 23 Mei 2013.

Porter, M.D., Smith, H.D. and Kabana, R.R. (1984). *Compendium of Peanut Diseases*. The American Phytipathological Society. United States of America. 73 hal.

Rahayu, B. (2003). *Uji Ketahanan Varietas Kacang Tanah Terhadap Penyakit Sclerotium rolfsii Sacc. di Lahan Petani (On Farm Research).* [Skripsi]. Fakultas Pertanian. Institut Pertanian Bogor. Bogor. 59 hal.

Samson, R.A., E.S. Hoekstra and C.A.N. Van Oorschot. (1995). *Introduction To Food-Borne Fungi*. Institute of The Royal Netherlands Academic of Arts and Sciences.

Sinaga, M.H. (2009). Pengaruh Bio Va-Mikorizadan Pemberian Arang terhadap Jamur *Fusarium oxysporum* f.sp. capsici pada Tanaman Cabai (*Capsicum annuum*) di Lapangan. Skripsi.Universitas Sumatera Utara, Medan.

Soesanto, L. (2008). *Pengantar Pengendalian Hayati Penyakit Tanaman*. Rajawali press. Jakarta.

Soesanto, L. (2013). *Pengantar Pengendalian Hayati Penyakit Tanaman*. Edisi kedua. Rajawali Pers. Jakarta.

Subowo. (2012). Pemberdayaan sumber daya hayati tanah untuk rehabilitasi tanah Ultisol terdegradasi. *Jurnal Sumberdaya Lahan*, 6(2): 79-88.

Sudarma, I.M., dan Suprapta, D.N. (2011). *Potensi Jamur Antagonis yang Berasal dariHabitat Tanaman Pisang dengan dantanpa Gejala Layu Fusarium untuk Mengendalikan Fusarium oxysporum f.sp. cubense Secara In Vitro*. Skripsi. Fakultas Pertanian Universitas Udayana. Bali.

Suhartina, Febby E.F. Kandou, Marina F.O. Singkoh. (2018). Isolasi dan Identifikasi Jamur Endofit Pada Tumbuhan Paku *Asplenium nidus*. Jurnal MIPA. https://ejournal.unsrat.ac.id/index.php/jmuo/article/view/20640 (Diakses pada tanggal 25 Oktober 2021).

Sunarwati, D., dan Yoza, R. (2010). Kemampuan *Trichoderma* sp. dan *Penicilium* sp. dalam Menghambat Pertumbuhan Cendawan Penyebab Penyakit Busuk Akar Durian (*Phytophtora palmivora*) Secara in-Vitro. Seminar Nasional Program & Strategi Pengembangan Buah Nusantara. Solok 10 Desember 2010.

Sumarsih, S. (2003). *Mikrobiologi Dasar*. Fakultas Pertanian UNP Veteran. Yogyakarta.

Triharso. (1996). *Dasar-dasar Perlindungan Tanaman*. UGM Press, Yogyakarta.

Umboh, S.D. (2016). *Analisis Kadar Residu Pestisida dan Bioakumulasinya Terhadap Laba-Laba Predator, Fisikokimia, dan Biologi Tanah Di Pertanaman Sayuran Kubis.* Disertasi. Universitas Sam Ratulangi. Pascasarjana. Manado.

Yulianto, E. (2014). *Evaluasi Potensi Beberapa Jamur Agen Antagonis Dalam Menghambat Patogen Fusarium sp. Pada Tanaman Jagung (Zea mays l.)* Skripsi. Program Studi Agroekoteknologi, Jurusan Budidaya Pertanian, Fakultas Pertanian, Universitas Bengkulu.