

**SCREENING AND CHARACTERIZATION OF SOIL FUNGAL ISOLATES TO INHIBIT THE GROWTH OF *Ganoderma boninense*****Penapisan dan Karakterisasi Isolat Fungi Tanah untuk Menghambat Pertumbuhan *Ganoderma boninense*****Zhafira Amila Haqqa<sup>1\*</sup>, Bedah Rupaedah<sup>1</sup>, Indri Handayani<sup>1</sup>, Abdul Wahid<sup>1</sup>, Mahmud Sugianto<sup>2</sup>**<sup>1</sup> Research Center for Applied Microbiology, National Research and Innovation Agency, Cibinong Science Center, Soekarno Science and Technology Park, Bogor 16911, Indonesia<sup>2</sup> Deputy for Infrastructure Research and Innovation, National Research and Innovation Agency, Jakarta 10340, Indonesia

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**ABSTRACT**

*Ganoderma boninense*, a fungus recognized as a causative agent of basal stem rot and upper stem rot, is primarily found in oil palm plantations (*Elaeis guineensis* Jack.). This study aimed to identify soil fungal isolates with the greatest potential for inhibiting the pathogenic fungus *G. boninense*. The research employed curative antagonist testing using in vitro dual culture. Fungal isolates demonstrating the highest inhibition percentages were characterized through macroscopic and microscopic observation, and their hemolysis properties were assessed using blood agar media. Soil fungal isolates FA 3.8 and FA 2.8 exhibited the highest inhibition percentages, reaching 91% and 88%, respectively. Based on morphological characterization at both macroscopic and microscopic levels, FA 3.8 displayed similarities to *Trichoderma*, while FA 2.8 exhibited similarities to *Penicillium*. Hemolysis testing results on blood agar media indicated that both isolates exhibited gamma hemolysis or non-hemolysis, as they lacked red blood lysis properties.

**Keywords:** Antagonistic test, characterization, *Ganoderma boninense*, hemolysis test, soil fungi**ABSTRAK**

Fungi *Ganoderma boninense* merupakan parasit yang dikenal sebagai patogen penyebab penyakit busuk pangkal batang maupun busuk batang atas yang dominan ditemukan pada perkebunan tanaman kelapa sawit (*Elaeis guineensis* Jack.). Penelitian ini bertujuan untuk mengetahui isolat fungi tanah yang memiliki potensi penghambatan fungsi patogen *G. boninense* tertinggi. Penelitian menggunakan metode pengujian antagonis kuratif secara in vitro dengan dual kultur. Pada isolat fungi dengan persentase penghambatan tertinggi dilakukan karakteristik morfologi koloni baik secara makroskopis maupun mikroskopis, serta dilakukan pula pengujian hemolisis dengan media agar darah. Isolat fungi tanah FA 3.8 dan FA 2.8 memiliki persentase penghambatan tertinggi, masing-masing sebesar 91% dan 88%. Berdasarkan karakterisasi morfologi secara makroskopis dan mikroskopis fungsi FA 3.8 memiliki kemiripan dengan fungi *Trichoderma* dan FA 2.8 memiliki kemiripan dengan *Penicillium*. Hasil pengujian hemolisis pada media agar darah menunjukkan kedua isolat sebagai gamma hemolisis atau non hemolisis karena kurangnya sifat melisis darah merah.

**Kata Kunci:** Fungi tanah, *Ganoderma boninense*, karakterisasi, uji antagonis, uji hemolisis

## INTRODUCTION

Certain species of *Ganoderma* possess pathogenic, therapeutic, and aesthetic qualities (Kumar et al. 2022). *G. boninense* is a fungus that exhibits both saprophytic and parasitic properties (Edy and Lakani 2022). In its saprophytic form, this fungus obtains nutrients from decaying organisms in the soil by breaking down their lignin and cellulose (Surendran et al. 2021). However, it can also behave parasitically by extracting nutrients from living organisms (Ramzi et al. 2019). *G. boninense*, as a parasitic pathogen, is primarily associated with stem base rot and scion rot diseases, which greatly impact oil palm (*Elaeis guineensis* Jack.) plantations. Stem base rot caused by *G. boninense* is particularly prevalent in both mature and young plants, especially in sandy soil conditions (Susanto et al. 2013; Siddiqui et al. 2021). On the other hand, according to Flood et al. (2001), *G. boninense* spreads through airborne basidiospores, leading to the development of stem top rot disease.

*G. boninense*, as a parasite, has caused productivity losses in *Acacia mangium* monoculture on industrial plantations in Sumatra and Kalimantan (Nurrohmah et al. 2020), as well as in the sengon community forest in Yogyakarta (Maryono 2020). *Ganoderma* is known as a "silent killer" because the disease symptoms appear only when the plant is severely infected. The symptoms include wilting and drying leaves due to root rot (Suryantini and Wulandari 2018).

Plant pathogens, such as fungi like *G. boninense*, can reduce productivity, resulting in economic losses due to decreased yields (Deden and Heryanto 2021). Diseases caused by microorganisms, especially fungi, can spread rapidly. This is because fungi produce spores that can be easily dispersed by the wind, accelerating the spread of diseases in plants (Shafira and Suwandi 2020). Therefore, treatment is necessary to inhibit the spread and growth of *G. boninense* as a pathogen in plantation crops.

To ensure the preservation of plant health and the environment, it is crucial to adopt safe handling practices that promote sustainability. One effective approach is the utilization of biological agents and biological pesticides. These formulations consist of

specific microorganisms such as bacteria, fungi, or viruses, which act antagonistically against plant pathogens. Moreover, these microorganisms have the ability to produce compounds that can serve as toxins against insect pests and disease-causing nematodes (Fenibo et al. 2021).

The soil itself harbors a diverse array of microorganisms, many of which have untapped potential. Fungi, in particular, play a crucial role as primary decomposers, maintaining ecological equilibrium within the environment. However, only a fraction of the vast potential held by soil fungi has been explored by experts so far (Manoharachary 2005). Several studies have focused on the potential of soil microbes, mostly as antibacterials, such as swamp soil fungi as antibacterials against *Pseudomonas aeruginosa* (Pamungkas et al. 2021), and estuarine soil fungi as antibacterials against *Staphylococcus aureus* (Millah et al. 2022; Rosyadi et al. 2022). Therefore, further research is needed to explore other potentials, such as biopesticides. In this study, screening was conducted to identify plantation soil fungi isolates with the potential to inhibit the pathogenic fungus *G. boninense*, which causes stem base rot disease in oil palm.

## MATERIALS AND METHODS

### Location and time

Soil samples were obtained from oil palm plantations belonging to PTPN VIII, located in Cimulang, Bogor. The research was conducted at the Laboratory for Biotechnology, National Research and Innovation Agency (BRIN). The research was carried out from January to March 2023.

### Isolation of soil fungi from oil palm plantation

Isolation of fungi from soil derived from PTPN VIII oil palm plantations was conducted by collecting soil samples from five random locations: point 1 (6.51927° S, 106.73590° E), point 2 (6.51819° S, 106.73580° E), point 3 (6.51882° S, 106.73630° E), point 4 (6.51881° S, 106.73634° E), and point 5 (6.51876° S, 106.73617° E). Soil samples were obtained from a depth of 0 to 10 cm, with approximately 1 kg of soil collected from each point and placed in individual plastic containers. Subsequently, the soil samples from each

collection point were thoroughly mixed, and 10 g of the composite sample was added to 90 mL of sterile water in an Erlenmeyer flask. The soil suspension was then horizontally shaken for a duration of 1 hour. Following this, the sample was diluted to achieve a suspension concentration of  $10^3$  and subsequently inoculated with 1 mL of the suspension onto Potato Dextrose Agar (PDA) medium in Petri dishes. The Petri dishes were then incubated for a period of 2-3 days, during which all visible fungi were carefully observed and subjected to purification processes.

**Antagonistic testing**

Antagonistic testing was performed against isolates of the pathogenic fungus *G. boninense* obtained from the collection of the Indonesian Oil Palm Research Institute in Medan. The test utilized sterile Potato Dextrose Agar (PDA) media as the growth substrate. The antagonistic testing employed the dual culture method. In this method, the *G. boninense* isolate and chips were placed on opposite sides of the media in a Petri dish. The chips were obtained using a cork bore with a diameter of approximately 0.5 cm, positioned around 2 cm away from the edge of the Petri dish on each side. This procedure was conducted for all fungal isolates obtained from the soil samples.

To perform the curative antagonist test, the pathogenic fungus *G. boninense* was first cultured and allowed to incubate for 2 days. Subsequently, the antagonist isolates were introduced to the culture. After a period of 14 days, the growth of *G. boninense* mycelium was measured to determine the percentage of growth inhibition, using the following formula (Sharfuddin et al. 2012):

$$\text{Inhibition Percentage (IP)} = \frac{(R1 - R2)}{R1} \times 100 \%$$

Description:

R1 = Control radius of *G. boninense*

R2 = Radius of *G. boninense* in dual-culture

**Characterization of fungal isolates**

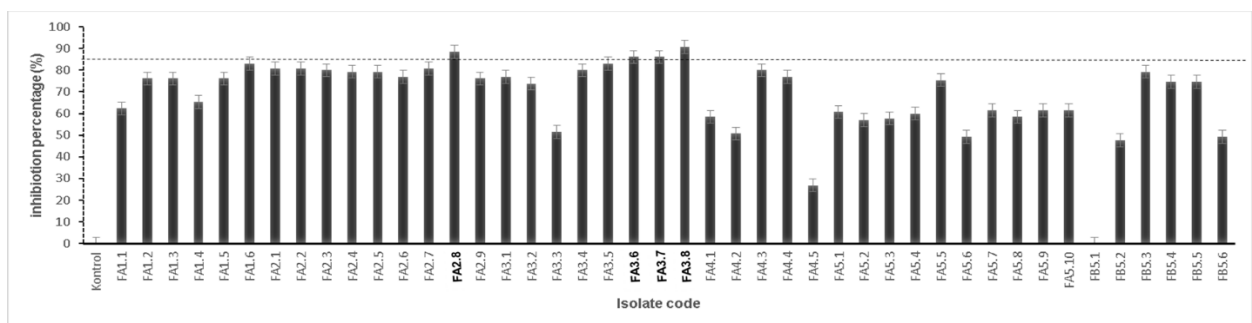
Characterization of fungi was conducted through the examination of the obtained isolates. The morphological characteristics were observed both macroscopically and microscopically. Macroscopic observations involved the assessment of colony color and appearance on the upper and lower surfaces of the media Petri dish. Microscopic observations were performed by preparing simple slides of the isolates and applying fungal staining using lactophenol cotton blue. The prepared slides were then examined using a light microscope (Olympus CX41) at the highest magnification of 1000x, and immersion oil was applied to enhance visualization. Microscopic observations focused on the examination of hyphae, conidia, and conidiophores present in the fungal isolates.

**Hemolysis testing of fungal isolates**

Hemolysis testing was conducted using blood agar media. The testing procedure involved taking a fungal isolate with a cork bore diameter of approximately 0.5 cm and placing it on the blood agar media Petri dish, about 2 cm away from the dish's edge. The isolate was then incubated in a 30 °C incubator. Daily observations were carried out for a period of 7 days.

**RESULTS AND DISCUSSION**

The assessment of the antagonistic potential of fungal isolates obtained from soil samples of oil palm plantations against *G. boninense* was performed under in vitro



**Figure 1.** The percentage of inhibition exhibited by 44 fungal isolates against *G. boninense* evaluated after 14 days of incubation (DAI)

conditions. The results of the antagonistic testing conducted on 44 fungal isolates against *G. boninense* are presented graphically in Figure 1. The observations were made 14 days after incubation (DAI). In addition, curative antagonist testing was conducted to simulate plant management when infected with *G. boninense*. In this test, the pathogenic fungus *G. boninense* was inoculated and incubated for two days on the growth medium.

Out of the 44 fungal isolates examined, four soil fungal isolates demonstrated a remarkable inhibitory capacity against the growth of the pathogenic fungus *G. boninense* in vitro, with a percentage of inhibition equal to or greater than 85%. Table 1 presents these four soil fungus isolates, showcasing their corresponding inhibition percentages. Among them, FA 3.8 exhibited

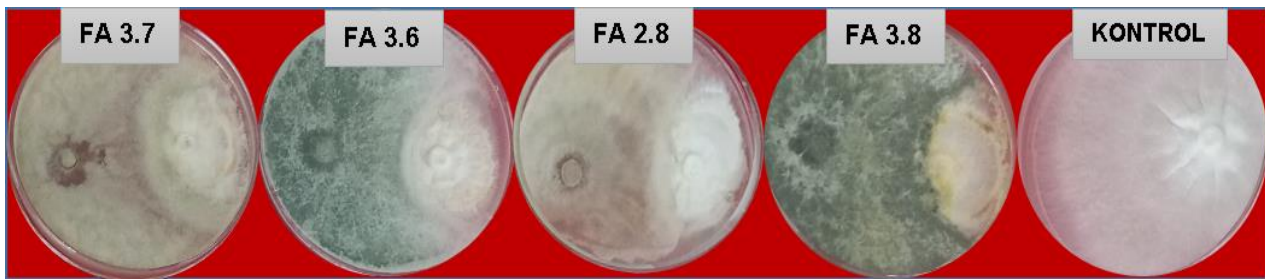
the highest inhibition percentage at 91%, followed by FA 2.8 at 88%, and FA 3.6 and FA 3.7 both at 86%.

Figure 2 illustrates that the inhibitory mechanism employed by four isolates is through growth dominance. The in vitro antibiosis mechanism is observed by inhibition of *G. boninense* growth. The predominant inhibitory mechanism is characterized by competition between *G. boninense* and the potential antagonistic microorganisms. This competition manifests in the form of dominant mycelium growth, effectively suppressing the growth of the pathogen (Hallman 2001, Putra et al. 2020).

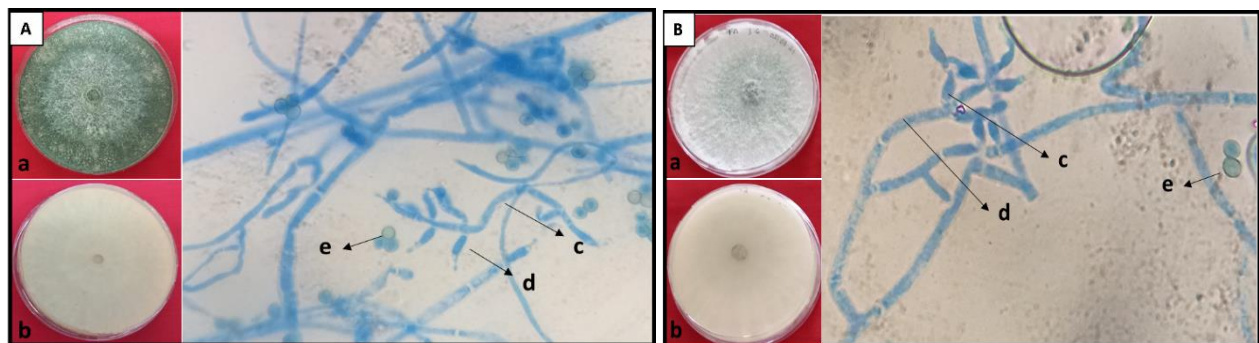
In the antagonist test, three inhibitory mechanisms were observed, namely competition, antibiotics, and hyperparasites (Nandung et al. 2018). The competition mechanism was identified by the thickening of

**Table 1.** Isolate of soil fungi with the highest percentage of inhibition against *G. boninense*

No	Code	Inhibition Percentage 7 DAI (%)	Inhibition Percentage 14 DAI (%)	Color of Colonies After Speciation	Inhibitory Mechanism
1	FA3.8	82	91	Green	Hyperparasitic
2	FA2.8	81	88	Light grayish green	Hyperparasitic
3	FA3.6	76	86	White green	Competition
4	FA3.7	83	86	Greenish gray	Competition



**Figure 2.** Dual-culture antagonistic testing of four highest percentage (IP>85%) fungal isolates with the highest inhibition percentage



**Figure 3.** Soil fungal isolate (A) FA 3.8 and (B) FA 3.6. Macroscopic: (a) upper view and (b) bottom view; microscopic: (c) conidiophores, (d) phialids, and (e) conidia.

mycelium at the interface between the colonies of the pathogenic fungus and the antagonistic fungus in the dual culture setup (Saputra et al. 2022). The antibiotic mechanism was evident through the formation of a clear zone surrounding the colony of the antagonist fungus, preventing the pathogenic fungus mycelium from approaching or restraining its growth. On the other hand, the hyperparasitic mechanism was characterized by the overlapping of antagonistic fungal colonies, effectively covering the colonies of the pathogenic fungus (Nehra et al. 2022).

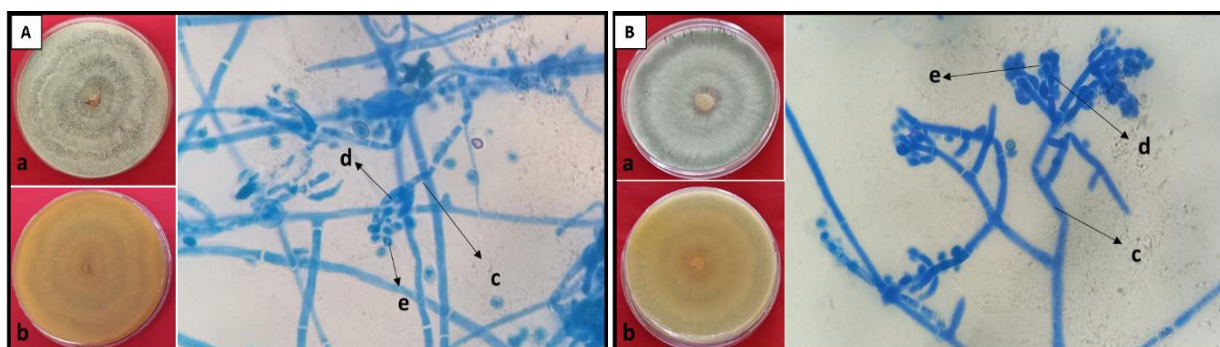
The macroscopic and microscopic characteristics of the four fungal isolates exhibiting the highest inhibitory potential against the pathogenic fungus *G. boninense* can be observed. Isolates FA 3.8 and FA 3.6 (Figure 3) share similarities with the characteristics of *Trichoderma* spp. Isolate FA 3.8 displays a white color on the upper part with a prominent dark green dominance, featuring long and thickened hyphae that resemble wool. The branched conidiophores bear phialides on each branch. Similarly, the colony morphology of Isolate FA 3.6 on the upper part exhibits a dominant greenish-white color with hyphae throughout. Microscopically, the hyphae show septation, and branched conidiophores with phialides on each branch producing green, globular/round conidia.

Macroscopically, *Trichoderma* spp. colonies exhibit a white color on the upper part, gradually transitioning to older green shades, while the reverse side remains white. By the fourth day, the colonies expand to fill the entire petri dish, displaying denser mycelium and forming distinctive concentric rings of green and white. Septa are present on the hyphae, and branching hyphae form

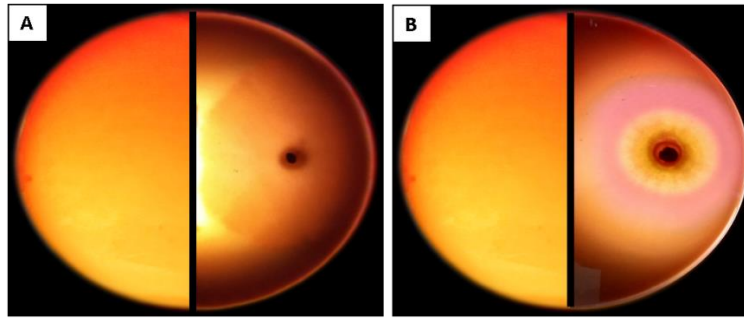
right angles from the main branches. Conidiophores branch out in a pyramid-like fashion, with longer branches positioned below. The phialids are arranged in various groups, with each group containing 2-3 phialids. The conidia appear green and take the form of globules formed at the tips of the conidiophores (Patty and Uruilal 2021).

*Trichoderma* spp. are renowned as dominant fungi that possess the ability to inhibit various pathogenic fungi. *Trichoderma* inhibited *G. boninense* by causing lysis of the pathogen hyphae (Ali and Samosir 2021). In terms of the competition mechanism, *Aspergillus* sp. and *Trichoderma* sp. have shown dominance against the pathogen *Sclerotium rolfsii* (Zuhria et al. 2016) as well as the pathogen *Fusarium* sp. (Lelana et al. 2015). *Trichoderma* spp. have also exhibited antagonistic potential against the pathogens *Phytophthora palmivora* and *Colletotricum gloeosporioides*. Another mechanism utilized by *Trichoderma* spp. involves antibiosis, wherein they produce antibiotics and secrete degrading enzymes that result in the formation of a zone surrounding the antagonistic microbes (Hallman 2001, Putra et al. 2020).

Isolate FA 2.8 (Figure 4) exhibits an upper morphology characterized by an olive-gray color with distinct concentric lines. The texture of the colony is cottony, with relatively long and spread-out hyphae. The reverse side of the colony appears cream in color, leaning towards orange. The isolated mycelium consists of septate hyphae and clustered conidia. Similarly, isolate FA 3.7 displays a colony morphology that is greenish-gray with a cottony texture of the mycelium. The reverse colonies of FA 3.7 are cream in color. Macroscopically, both FA 2.8 and FA 3.7 exhibit radial colonies with an



**Figure 4.** Soil fungal isolate (A) FA 2.8 and (B) FA 3.7. Macroscopic: (a) upper view and (b) bottom view; microscopic: (c) conidiophores, (d) phialids, and (e) conidia



**Figure 5.** Blood agar test of fungal isolate: (A) FA 3.8 and (B) FA 2.8 (left: reference, right: tested isolate)

upper part displaying a dartmouth green color and a cream-colored reverse. These isolates share similarities with the characteristics of *Penicillium* spp.

The *Penicillium* is characterized by its septate and hyaline hyphae; they also differ in color, internal structure, and colony characteristics (Saif et al. 2020). According to the identification by Anggraeni and Usman (2015), *Penicillium* sp. colonies start as white, then turn to blue-green, gray-green, olive-gray, sometimes yellow or reddish, and the reverse color is usually pale yellow. The microscopic morphology of *Penicillium* fungus consists of hyaline hyphae, spherical to ellipsoidal conidia, and a set of phialides (Ristiari et al. 2018).

*Penicillium* spp. has been reported to provide protection to plants against pathogenic attacks while promoting plant growth (Rozali 2015, Olowe et al. 2020). They can stimulate induced systemic resistance (ISR) against wilt disease by *Rhizoctonia solani* (El-Maraghy et al. 2020). Furthermore, *Penicillium* also functions as a decomposer, contributing to soil fertility enhancement (Altaf et al. 2018; Purwati and Hamidah 2018; Farooq et al. 2021). As one of the soil microorganisms, *Penicillium* also plays a crucial role in nutrient provision, particularly as a phosphate-solubilizing microbe (P), converting insoluble inorganic phosphate compounds into dissolved forms such as  $H_2PO_4^-$  and  $HPO_4^{2-}$ , facilitating their absorption by plants, stimulating the antioxidant system, and promoting the photosynthesis of quinoa (Kalayu 2019, Jin et al. 2022). Microbes with a high capacity for phosphate solubilization often exhibit a similar ability for potassium (K) solubilization (Yuleli 2009; Wulandari et al. 2013; Pantigoso et al. 2023).

Therefore, based on the inhibition test and the characterization of the four isolates, further hemolysis testing was conducted on the two isolates with the highest inhibition percentages. The purpose was to assess the ability of these fungi to degrade blood components in the media. Fungal isolates FA 3.8 and FA 2.8 (Figure 5) were tested on blood agar, and no clear zones were observed around the fungal mycelium, indicating the absence of blood lysis process. Consequently, it can be concluded that both isolates, FA 3.8 and FA 2.8, are non-hemolytic and do not possess hemolytic activity. These findings suggest that the two isolates can be considered safe for use in biotechnology product development, such as biofertilizers or biopesticides, as they do not pose a risk to users.

There are three types of hemolysis of red blood by microbes, namely beta hemolysis ( $\beta$ ), alpha hemolysis ( $\alpha$ ), and gamma hemolysis ( $\gamma$ ). Beta hemolysis ( $\beta$ ) is complete lysis of red blood cells so that the media forms a clear zone. Alpha hemolysis ( $\alpha$ ) is partial red blood cell lysis or reduced methemoglobin which causes a change in the color of the media around the microbes into gray until yellow. The last hemolysis is gamma hemolysis ( $\gamma$ ) or also called non-hemolysis due to the lack of red blood lysis properties so that the media does not change, as displayed by FA 3.8 and FA 2.8 (Hikmawati et al. 2019).

## CONCLUSION

Soil fungal isolates showing potential in combating the pathogenic fungus *G. boninense* are identified based on the highest inhibition percentages, with isolate FA 3.8 exhibiting 91% inhibition and isolate FA 2.8 with 88% inhibition. Macroscopic and

microscopic morphological characterization suggests that FA 3.8 shares similarities with fungi belonging to the *Trichoderma* genus, while FA 2.8 exhibits similarities to the *Penicillium* genus.

## ACKNOWLEDGMENT

We would like to express our gratitude to several entities that have provided invaluable support for this research. We extend our sincere appreciation to PTPN VIII, the Indonesian Oil Palm Research Institute in Medan, and the funders of this research, the Ministry of Finance of the Republic of Indonesia, under the RIIM - Technical Program No. PRN-018512262.

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