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Antagonist Test of *Trichoderma harzianum* Against *Ganoderma boninense* Causes of Oil Palm Basal Stem Rot Disease

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Abstract
This study aims to determine the type of *Trichoderma* fungus that can inhibit the growth of *G. boninense* and determine the percentage of inhibition of *Trichoderma* on the growth and development of *G. boninense*. This research was conducted at the Central Laboratory of the STIPER Agricultural Institute Yogyakarta in February-March 2022. *G. boninense* samples were taken from three different plantation locations belonging to residents of Aek Paing Atas Village and Aek Paing Bawah Satu Village, Kec. Rantau Utara, Kab. Labuhan Batu and Aek Janji Village, Kec. Rantau Selatan, Kab. Labuhan Batu, North Sumatra. *Trichoderma* samples were taken from Naga Soppah Village, Naga Harjo Village and Bahung Kahean Village, Kab. Simalungun, North Sumatra. The research method used is descriptive by identifying antagonistic (*Trichoderma*) and pathogenic (*G. boninense*) fungi. Data on the percentage of inhibition of fungi were analyzed quantitatively. Fungi macroscopic and microscopic characterizations were analyzed qualitatively. The results showed that the *Trichoderma* fungus that could inhibit the growth of *G. boninense* was *T. harzianum*. The highest percentage of inhibition power of *T. harzianum* (Naga Soppah Village) against *G. boninense* (Aek Paing Bawah Satu village) was 30%.
Keywords— Oil palm, Basal stem rot, *Ganoderma boninense*, *Trichoderma harzianum*, antagonist test

Introduction

Oil palm, as a crop that produces crude palm oil (CPO) and palm kernel (PK), is one of the primadonnas of plantation crops which is a source of non-oil and gas foreign exchange for Indonesia. The bright prospects for the commodity of palm oil and its derivatives worldwide have encouraged the Indonesian government to increase its productivity. Productivity is the keyword in increasing national palm oil production. Moreover, productivity can increase the income of plantations primarily owned by farmers (Pardamean, 2017).

One of the problems faced in increasing the productivity of oil palm crops, both CPO and PKO, is stem rot disease caused by the fungus *Ganoderma boninense*. The fungus is very detrimental in oil palm plantations. Therefore, control measures are needed.

The most widely used control of stem rot disease today is chemical fungicides to treat cuttings and spray plants (Ruhnayat, 2004). One of the control alternatives is biologically using

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antagonistic endophytic fungi. Endophytic fungi live in healthy plant tissues without causing symptoms or damage to the host plant (Petrini, 1991). One of the endophytic fungi is *Trichoderma* sp. The advantages of having endophytic fungi in host plants are increased tolerance to heavy metals, increased resistance to drought, suppression of pest attacks, and systemic resistance to pathogens (Saikkonen et al., 1998 in Arnold et al., 2003).

Research on Trichoderma mushroom antagonist test against *G. boninense* from Labuan Batu District, North Sumatra, has never been reported. Therefore it is necessary to research this matter.

Research Method

This research was conducted at the Central Laboratory of the Yogyakarta Instiper campus, Maguwoharjo, Kab. Sleman, Province of the Special Region of Yogyakarta. The sampling location for *Ganoderma* sp. was conducted in three different garden locations. Samples were taken at the base of the oil palm stems. The first plantation in Aek Paing Atas Village, Kec. Rantau Utara, Kab. Labuhan Batu. The second plantation is in Aek Paing Bawah Satu Village, Kec. Rantau Utara, Kab. Labuhan Batu. The third plantation in Aek Janji Village, Kec. Rantau Selatan, Kab. Labuhan Batu, North Sumatra.

Fungal isolation was carried out by taking samples of the fruiting bodies of Ganoderma sp. The isolation method used is the same as the Ganoderma mushroom isolation method by Purnamasari et al. (2012). The fruit body is cut to about 1 cm x 1 cm and then washed with sterile water. Pieces of fruit bodies were transferred to 0.5% NaOCI solution for 1 minute and then immersed in 96% alcohol for 30 seconds. Pieces of fruit bodies were cut again into smaller sizes (0.5 cm x 0.5 cm). Soak in sterile distilled water 3 times for 30 seconds. After that, air dry with sterile filter paper. After that, the sample is placed on Potato Dextrose Agar (PDA) media. PDA media has been mixed with Chloramphenicol solution as an antibiotic, so bacteria do not contaminate it. After that, it was incubated at room temperature 25ºC. Observations were made by paying attention to the growth speed, the fungal colonies' colour and the spores produced. Furthermore, the hyphae and fungal spores were observed under a microscope.

In the purification process of Trichoderma sp., the first thing to do was to move the mushroom colonies in the form of the mushroom mycelium Trichoderma sp. obtained from the previous isolate media into a petri dish containing PDA media. Purification is carried out in the Laminar Air Flaw cabinet to prevent contamination of bacteria or other unwanted fungi.

Observation of Trichoderma fungus macroscopically was carried out by observing the colour of the colony and the diameter of the mushroom colony. Microscopic observation was carried out by observing the type of hyphae, the shape of the conidia, the presence or absence of phyllids, the presence or absence of conidiophores, and the presence or absence of sporangiophores.

Pure cultures of antagonistic fungi were grown using PDA media with pathogenic fungi in 9 cm diameter Petri dishes. Each culture piece was 4 mm in diameter. The spacing between the antagonistic and pathogenic fungi in the petri dish was 3 cm.

Observations of Ganoderma are carried out daily by measuring the colony's radius using a millimetre ruler. Calculations were carried out using the formula to determine the inhibition power of each Trichoderma sp. (Widyastuti et al., 1998).

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$$I = \frac{(R1 - R2)}{R1} \; x \; 100\%$$

Information:
I : Percentage of inhibition
R1 : The radius of the pathogen in the opposite direction with *Trichoderma*
R2 : Radius of the pathogen in the direction towards Trichoderma colony center

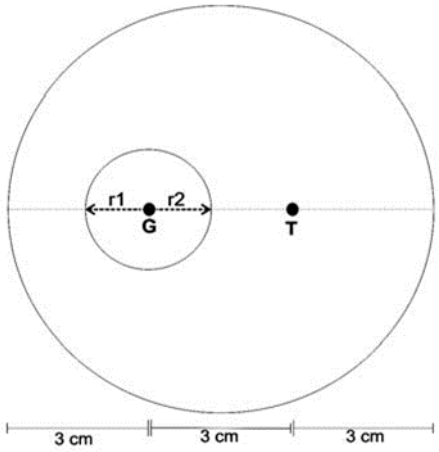


Figure 1. Position of inoculation of the antagonist fungus Trichoderma sp. with the pathogenic fungus Ganoderma sp. during the inhibition test in a petri dish using the direct opposition method

Results and Discussion

Symptoms of stem rot disease of oil palm plant stems are growth retardation, leaf colour turns pale green, all leaves and midrib dry up, spear leaves do not open, rot occurs at the base of plant stems, and basidiomata growth. The following shows the appearance of Ganoderma fruiting bodies in three different garden locations presented in Figure 2.

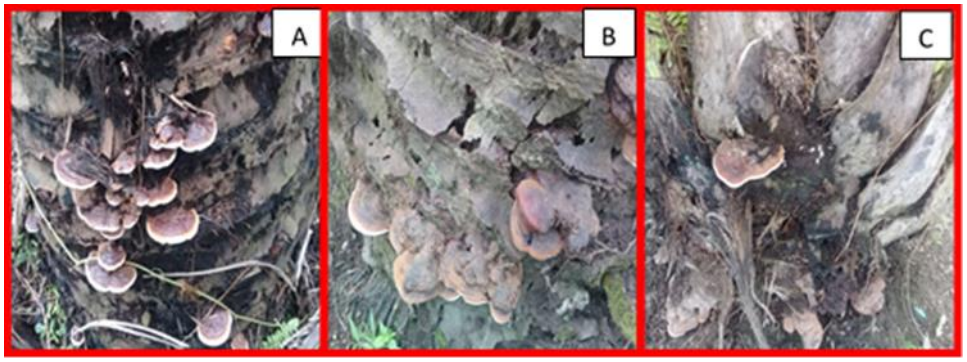


Figure 2. The fruiting bodies of *G. boninense* on oil palm stems. Notes: A. Aek Paing Atas Village Plantation, B. Aek Paing Bawah Satu Village Plantation, C. Aek Janji Village Plantation

To ensure that stem rot disease in oil palm plants from each of the three different plantation locations was caused by the pathogenic fungus G. *boninense*, macroscopic and microscopic observations of the fungus were carried out based on the writings of Dedek et al. (2019). It is proven that the fungus that causes stem rot in this study is caused by the fungus G.

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boninense. The following is the appearance of the fungus G. *boninense* at the three garden locations macroscopically and microscopically presented in Figures 3, 4 and 5.

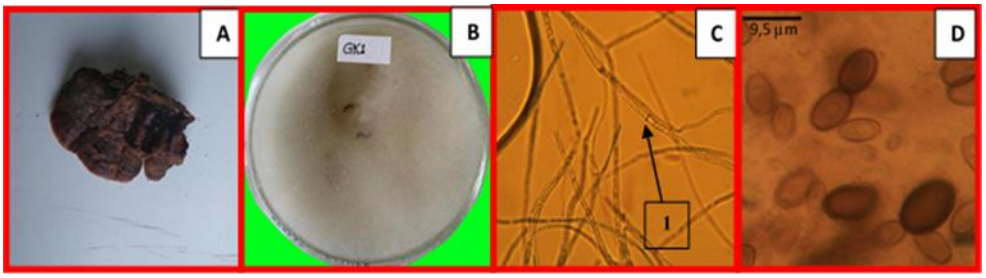


Figure 3. Macroscopic and microscopic appearance of the fungus G. *boninense* in Aek Paing Atas Village. Description: A. Basidiomata, B. Fungal colonies, C. Hyphae insulated (1), D. Basidiospores

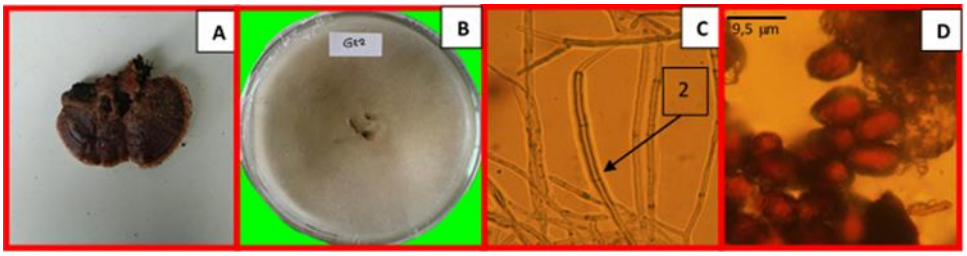


Figure 4. Macroscopic and microscopic appearance of the fungus G. *boninense* in Aek Paing Bawah Satu Village. Description: A. Basidiomata, B. Fungal colonies, C. Hyphae insulated (2), D. Basidiospores

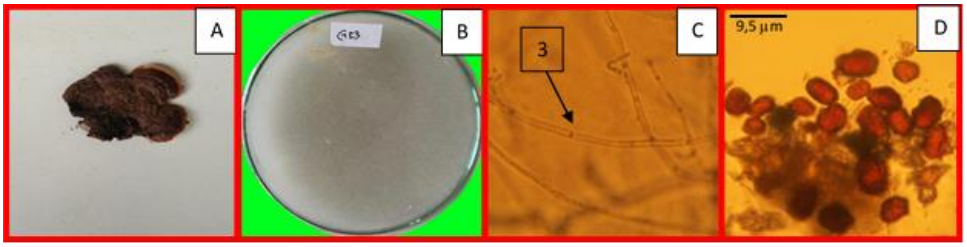


Figure 5. Macroscopic and microscopic appearance of the fungus G. *boninense* in Aek Janji Village. Description: A. Basidiomata, B. Fungal colonies, C. Hyphae insulated (3), D. Basidiospores

Figures 3, 4 and 5 show the macroscopic and microscopic appearance of the fungus G. *boninense* at the three different garden locations. Macroscopically, the colour of the isolate mycelium is white, like cotton. The mycelium grows for about 7-10 days in a petri dish with a diameter of about 8-9 cm. In this study, basidiospores were observed directly from the Ganoderma fruit bodies. Basidiospores *G. boninense* has a length and width of 9.5 µm and 4.5 µm at 1000 x magnification, respectively.

Fungal isolation was carried out according to the isolation method used by Purnamasari et al. (2012). In this study, the mycelium of the fungus G. *boninense* grew on the ninth day after planting. This is also following research conducted by Dendang (2015) that Ganoderma mycelium grows around 7-12 days. To ensure that the isolated Ganoderma mushroom is G. *boninense*, macroscopic and microscopic observations were made. This study proved that the pathogenic fungus that causes stem rot in oil palms is G. *boninense*. Macroscopically, the colony of G. *boninense* is creamy white, like cotton. Microscopically the hyphae of G. *boninense* have

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partitions and oval-shaped spores with an average spore length of 9.2 µm and a spore width of 4.5 µm with 1000x magnification (Dedek et al., 2019).

Rosa et al. (2009) said that Trichoderma is a fungus commonly used as a biocontrol agent for plant diseases. Trichoderma has several types, including T. *harzianum*, T. *koningi*, T. *viride* and T. *harzianum* (Gusnawati et al, 2014). Of the four types of Trichoderma above, T. *harzianum* is the fungus that has the greatest antagonistic effect on the growth of G. *boninense* (Benyamin, 2015). Therefore, to ensure that the type of Trichoderma fungus in this study was T. *harzianum*, macroscopic and microscopic observations of the fungus were carried out. The following shows the macroscopic and microscopic appearance of T. *harzianum* on the three different isolates shown in Figures 6, 7 and 8.

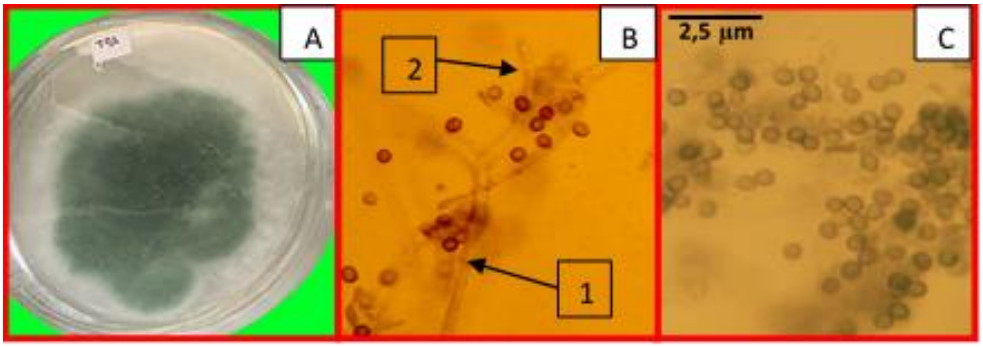


Figure 6. Macroscopic and microscopic appearance of the fungus Trichoderma *harzianum* Isolate 1: (A) Fungal colonies, (B) insulated hyphae (1) conidiophores (2), conidia (C)

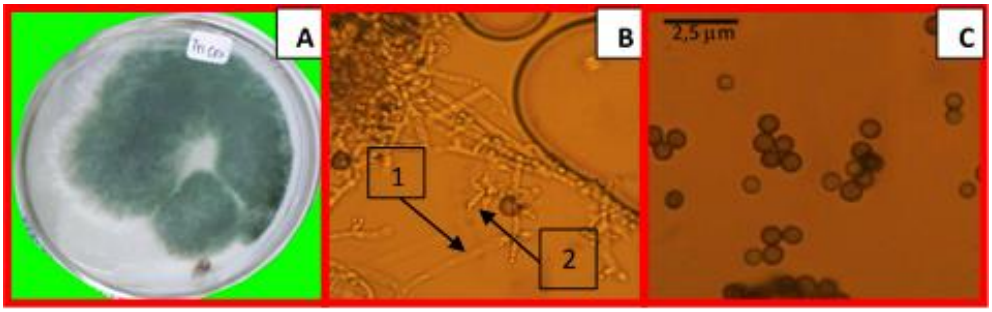


Figure 7. Macroscopic and microscopic appearance of the fungus Trichoderma *harzianum* Isolate 2: (A) Fungal colonies, (B) insulated hyphae (1) conidiophores (2), conidia (C)

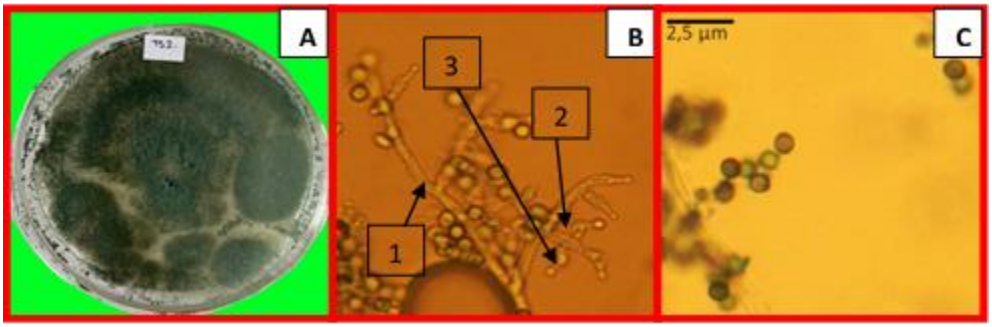


Figure 8. Macroscopic and microscopic appearance of the fungus Trichoderma *harzianum* Isolate 3: (A) Fungal colonies, (B) insulated hyphae (1) conidiophores (2), fialids (3), conidia (C)

In this study, the mycelium of T. *harzianum* grew on the third day in the petri dish. On the sixth day, the mycelium began to fill the petri dish with a diameter of 7-9 cm. This was also

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explained in a study by Gusnawaty et al. (2014), which stated that colonies on the fungus T. *harzianum* grew on the second day and reached more than 9 cm in 5 days. To determine the type of *Trichoderma* fungus, macroscopic and microscopic observations were carried out. Macroscopically, on the third day, T. *harzianum* colonies were translucent white with a small quantity of blue in the middle. Then on the sixth day, the colonies appeared bluish-green and grew to fill the petri dish. Microscopically T. *harzianum* has insulated hyphae, spore green and round shape with a diameter of 2-3 µm at 400x magnification and grows on Phyllida. This follows Gusnawaty et al. (2014) that T. *harzianum* grows to fill the petri dish on days 5-7 and has a bluish-green colony with a greenish spore colour.

The antagonist test of the two fungi was carried out after each fungus was isolated. The results of the antagonist test experiment showed that after the first day of planting, there was no inhibition. However, on the third day, the mycelium development of the two fungi began. On the third day, the growth of the G. *boninense* mushroom colonies looked bigger. This is because the antagonist process of the G. *boninense* culture was planted earlier in a petri dish. This is following research conducted by Widyastuti et al. (1998), namely the culture of the fungus G. *boninense* was grown 48 hours earlier than Trichoderma because the growth of the Ganoderma fungus was slower.

The following shows the appearance of the two fungal cultures during the antagonist test on the third, fifth, and ninth days after planting. Shown in figure 9.

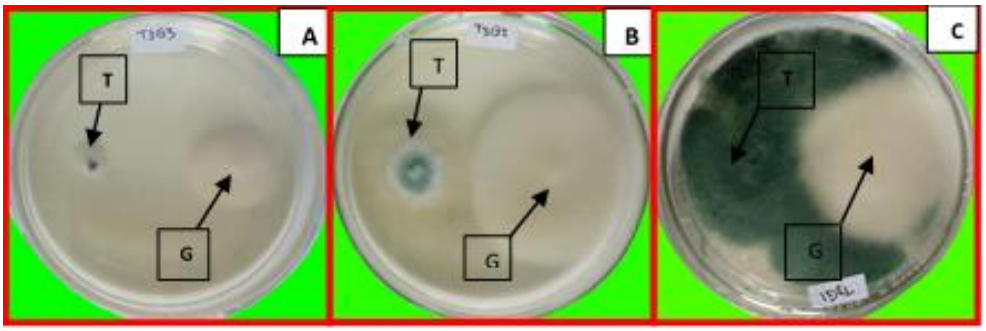


Figure 9. Antagonist test of T. *harzianum* (T) against G. *boninense* (G). Description: A. culture after the third day, B. culture after the fifth day, C. culture after the ninth day

Growth inhibition of G. *boninense* began to appear on the fifth day after planting. The distance between the mycelium of T. *harzianum* and G. *boninense* is close to each other. Indications of inhibition are visible macroscopically. If seen from the shape of the T. *harzianum* colony, it is wider towards the centre of the G. *boninense* colony. The percentage of growth inhibition of G. *boninense* can be seen in Table 2.

Table 2. Average inhibition percentage of T. *harzianum* on the growth of G. *boninense*

Antagonist test	Inhibitory percentage (%)
T1 vs. G1	13%
T2 vs. G1	14%
T3 vs. G1	18.7%
T1 vs. G2	30%
T2 vs. G2	5.9%
T3 vs. G2	11%
T1 vs. G3	17.7%
T2 vs. G3	15.8%

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T3 vs. G3	12.5%

Table 2 shows the highest average percentage of inhibition of T. *harzianum* on the growth of G. *boninense*, namely the combination of T1 vs G2 is 30%, followed by T3 vs G1, which is equal to 18.7%, the lowest inhibition percentage is at T2 vs G2 is 5.9%.

If seen from the percentage of inhibition above, Trichoderma from Naga Soppah Village (T1) is an antagonistic fungus with greater potential to control G. *boninense* in Petri dishes. This is because the growth response of T. *harzianum* in Naga Soppah Village was faster than that of T. *harzianum* from other villages. Environmental conditions also support the growth response. Domsch et al. (1993), in their writing, said that the optimum temperature for the growth of the fungus Trichoderma sp., i.e. 15-35ºC. This can also be proven by previous research conducted by Saragih et al. (2022), that environmental conditions in Naga Soppah Village support the growth of Trichoderma where the air temperature in Naga Soppah Village is 28.7 ºC, soil pH is 4.5-5. This means that these conditions support the growth of T. *harzianum*.

Trichoderma can produce the enzymes β-1,3 gluconase (linamarin) and chitinase, which can hydrolyze chitin from the hyphae walls of pathogenic fungi and penetrate the pathogenic hyphae resulting in the process of degradation of the pathogenic cell walls of G. *boninense* (Habazar and Yaherwandi, 2006). Trichoderma sp. can also parasitize hyphae by entwining pathogenic fungal hyphae to penetrate the cell wall to take nutrients from the cell that causes the pathogen to die.

Conclusion

Based on the results of the research conducted, it can be concluded that the antagonistic fungus used in this study was Trichoderma *harzianum*; the highest to lowest percentage of fungal inhibition, namely T1 vs G2 by 30%, T3 vs G1 at 18.7%, T1 vs G3 at 17.65%, T2 vs G3 at 15.8%, T1 vs G1 at 13%, T3 vs G3 at 12.5%, T2 vs G1 was 7.14%. The lowest percentage of inhibition was T2 vs G2 at 5.9%, and T. *harzianum* from Naga Soppah Village had the highest inhibition in suppressing the growth of the fungus G. *boninense*.

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