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Antagonism Capability *invitro* of *Trichoderma harziaum* and *Trichoderma viride* against Pathogenic Fungi *Colletotrichum gloeosporioides* in *Mangifera caesia* Jack

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A B S T R A C T

Mangifera caesia plant was a plant from Kalimantan which was now rarely found, due to the pathogenic fungi infection found on the leaves, thus it was able to obstruct photosynthesis process on the plant. One of attempts to hamper this pathogenic fungi growth was to present antagonistic fungi. The aims of this research: (1) to identify the highest antagonism effect of *Trichoderma harzianum* and *T. viride*, (2) to analyze the amount of enzyme produced from *Trichoderma harzianum* and *T. viride*, (3) to examine antagonism mechanism of *Trichoderma harzianum* and *T. viride* on *Collectotrichum gloeosporioides* fungi based on microscopic examination. The research method was multiple culture method inoculating antagonistic fungi: *Trichoderma harzianum* and *T. viride* also pathogenic fungi: *Colletotrichum gloeosporioides* on media of paired PDA plate, and then incubated at 25^0-27^0C for 4x24 hours. Next, the antagonism effect of *Trichoderma harzianum* and *T. viride* on *Colletotrichum gloeosporioides*, and the mechanism of antagonism would be examined by Scanning Electron Microscope (SEM), the results were analyzed descriptively and quantitatively. This research results referred that: (1) antagonistic fungi of *Trichoderma harzianum* has the highest antagonism power with the average percentage of antagonism 78,39% rather than *T. Viridae* fungi on *Colletotrichum gloeosporioides*, (2) cellulose enzyme produced by *Trichoderma harzianum* was greater, around 143,562 U/,L than *T. Viridae* fungi, (3) antagonism mechanism of *Trichoderma* spp. indicated that three mycoparasitism mechanisms in three methods: sticking, twisting, and piercing on hyphae fungi of *Colletotrichum gloeosporioides.*

Introduction

Mangifera caesia is a type of plant that is often used by people and has economic value. Moreover, *Mangifera caesia* is one of multipurpose commodities among researchers because of its content of secondary metabolite compounds within varied plant structures. Specifically, this kind of plant is now rarely found in East Kalimantan, whereas this plant is a seasonal plant that brings a lot of advantages. Many plant taxonomists have stated that this plant is initially an endemic plant in

Kalimantan. *M. caesia* is only able to grow perfectly in Kalimantan. Further, *M. caesia* is now often found in some regions like Bali, West Java, and Sumatra (Darsono *et al.,* 2022; Kartawinata *et al.,* 2014; Fitmawati and Hayati, 2018).

The problem of scarcity of *Mangifera caesia* in Kalimantan is affected by several factors like damage to plantation land that can cause this plant is damaged and died, this condition then causes a decrease of crop yields. However, the cultivation of *Mangifera caesia* plant has high risk that is caused by pest and disease attack of pathogenic fungi, one of *Colletotrichum* sp. fungi. The symptoms indicated on plants attacked by fungi are shown on the leaf top, twigs, and branches turn dried and blackish brown in color, and the plant died. The attack of fungi is aroused from plant root, and spread to the stems, branches, twigs, and leaves.

The farmer exerts a specific method to control pathogenic fungi by using compound of chemical fungicide (Agustyarini *et al.,* 2017), which sometimes it does not fulfill the dose and application time recommended, and lessen control efforts to be less effective with the dose and application time recommended, therefore it is less effective in the fungi control.

The negative impact of this chemical fungicide use can harm health and the residue will be spread to the surrounding area, so it needs a solution of pathogenic fungi control in the different method. The fungicide means a material containing compounds that are used to prevent the growth of pathogenic fungi, but those compounds might affect negatively especially to the environment (Bernardes *et al.,* 2015; Aktar *et al.,* 2008; Qin *et al.,* 2014; Miraglia *et al.,* 2009).

The residue resulted by synthetic fungicide is in the form of chemical compound that can spread over cultivated plants such as vegetables and fruits consumed by human. Moreover, the residue can also kill microbes that live in the soil which it plays a role in biological controlling of pathogenic fungi (Teli *et al.,* 2017). Based on that condition, it needs to consider about the use of eco-friendly biological fungicide. One of alternatives that can be used to substitute synthetic fungicide is to use antagonistic fungi as a biological control of pathogenic fungi (Adnan *et al.,* 2019; Yusnawan *et al.,* 2019; Poudel *et al.,* 2023). The use of antagonistic fungi is able to hamper the growth of pathogenic fungi, so the cultivated plants are avoided from the infection caused by those pathogenic fungi. The use of antagonistic fungi does not bring negative impact, since it cannot result harmful residue for the environment (Pellerin *et al.,* 2007; Karim *et al.,* 2022). Furthermore, the antagonistic fungi are naturally contained in soil ecosystem and take role as the agents of biological control (Thambugala *et al.,* 2020; Knudsen *et al.,* 2014). The interaction between antagonistic fungi and pathogenic fungi can prevent the growth of pathogenic fungal hyphae in *Mangifera caesia* naturally.

Based on the previous study, the species of *Colletotrichum gloeosporioides* fungi is a pathogenic fungi that is able to harm leaves structure in *M. caesia*, so it can hamper photosynthesis process. This kind of fungi usually attacks the roots, stems, and fruits. The indication that might be seen in the field on *Mangifera caesia* morphology is having white, brown and black spot marks on stems, leaves, and fruits. It requires a concern, so *Mangifera caesia* commodity can be preserved.

Trichoderma harzianum and *T. viride* fungi are soil saprophytic fungi that are naturally able to be used as the agents of biological controller of pathogenic fungi (Haouhach *et al.,* 2020; Zheng *et al.,* 2021; Wang *et al.,* 2022). The fungi are antagonistic fungi that have antagonistic features to the pathogenic fungi in the form of space and nutrition, mycoparasites and antibiosis competitions.

The species of *Trichoderma harzianum* and *T. viride* fungi can be the biological agents that are very appropriate to handle pest and disease in plants caused by pathogenic fungi (Vicente *et al.,* 2020; Abbas *et al.,* 2022). In line with this condition, it

needs to conduct a research on antagonism between antagonistic fungi and pathogenic fungi in the plantation land of *Mangifera caesia*.

Moreover, it can determine *Trichoderma harzianum* and *Trichoderma viride* species that might contain the highest antagonistic power to oppose *Colletotrichum gloeosporioides*, so it can be benefitted in the attempt of biological control of pathogenic fungi in *Mangifera caesia*. This attempt can be certainly used as an alternative to substitute synthetic fungicide in order to preserve *Mangifera caesia* commodities in the future.

Materials and Methods

Antagonism Testing on *Trichoderma harziaum* and *Trichoderma viride* Fungi for *Colletotrichum gloeosporioides*

In this research, antagonism test used dual culture method. The pure culture of *Trichoderma harzianum*, *T. viride*, and *Colletotrichum gloeosporioides* fungi rebred on medium of Potato Dextrose Agar plate, and it was incubated at 27^0C for 7 x 24 hours. Next, the culture of antagonistic fungi of *Trichoderma harzianum*, *T. viride*, and *Colletotrichum gloeosporioides* fungi were cut by a sterile cork drill with diameter of 5 mm aseptically, and then placed in pairs between *Trichoderma harzianum* and *Colletotrichum gloeosporioides* fungi also *T. viride* and *Colletotrichum gloeosporioides* fungi on the surface medium of PDA plate, and they were incubated for 4 x 24 hours. Further, measurement and calculation of antagonism percentage was conducted. The following formula was used to find out the percentage of antagonism power:

$$P = \frac{R1-R2}{R1} X\ 100\%$$

Description:

R1 = Fingers of pathogenic fungi colonies that looked away from antagonistic fungi.

R2 = Fingers of pathogenic fungi colonies that looked close to antagonistic fungi.

P = Antagonistic power was calculated on each antagonistic fungi.

Observation on Antagonism Mechanism of *Trichoderma harzianum* and *Trichoderma viride* Fungi for *Colletotrichum gloeosporioides* Fungi

Observation on the antagonism mechanism between *Trichoderma harzianum* and *Colletotrichum gloeosporioides* also *Trichoderma viride* and *Colletotrichum gloeosporioides* was conducted macroscopically and microscopically. The macroscopic observation was done by implementing a direct observation technique on the competition mechanism between *Trichoderma harzianum* and *Colletotrichum gloeosporioides* fungi also *T. viride* and *Colletotrichum gloeosporioides*, while microscopic observation was conducted by thinly slicing the surface of medium on the border zone between both fungi colonies by using a razor blade, and then made into preparation and observed by using electron microscope or Scanning Electron Microscope (SEM).

Results and Discussion

Antagonism Power of *Trichoderma harzianum* and *T. viride* for *Colletotrichum gloeosporioides* Fungi

Based on the figure 1, it showed that antagonistic fungi of *T. harzianum* has the higher antagonism level on *Colletotrichum gloeosporioides*, comparing to *T. viride* fungi which has the lower antagonism level on the same fungi of *Colletotrichum gloeosporioides* during the incubation time of 4 x 24 hours.

This result was affected by the growth speed of antagonistic fungi mycelium on *T. harzianum* was faster than on *T. viride* fungi, so it could build an interaction that resulted the highest antagonism level on the growth of *Colletotrichum gloeosporioides* fungi. The following table 2 would show the

measurement result in the form of percentage of the isolate obstruction between antagonistic fungi of *T. harzianum* and *T. viride* fungi for *Colletotrichum gloeosporioides* fungi.

Based on the table of antagonism level percentage, it showed average of the highest obstruction by antagonistic *T. harzianum* fungi on *C. gloeosporioides* (Penz.) Sacc. of 78,39%, while antagonism level percentage of antagonistic *T. viride* (Pers.) ex Fries on *C. gloeosporioides* (Penz.) Sacc of 62,17%. This condition was due to the faster growth speed of *T. harzianum* fungi than comparing to *T. viride.*

This result was supported by the research result stated by Matroudi *et al.*, (2009) that *T. harzianum* fungi has the faster mycelium growth, so it was really appropriate to be used for controlling the growth of pathogenic fungi. Moreover, the faster growth speed of *T. harzianum* because this kind of fungi could produce a lot of hydrolytic enzymes, thus it was able to push pathogenic fungi growth through mycoparasitism activity. Several factors that might explain the higher level of antagonism obstruction on *Trichoderma harzianum* comparing to *T. viride* : (1) *Trichoderma harzianum* has enzyme activities like chitinase, β-1,3-glucanase, protease, and other enzymes with the greater activity level than *T. viride.*

Those enzymes could be more effective in destroying pathogenic cell walls and result the stronger growth of obstruction, (2) *Trichoderma harzianum* could produce secondary metabolite with the stronger antimicrobial and antifungal characteristic than *T. viride.* This metabolite could contain the greater obstruction level for pathogenic growth and progress like *Colletotrichum gloeosporioides*, (3) *Trichoderma harzianum* could contain the higher level of competition capacity in order to fight over resources such as nutrition, space, or spot to attach to the plants. The better competitiveness might result a domination of *Trichoderma harzianum* in order to hamper pathogenic growth, (4) *Trichoderma harzianum* could be more effective to hamper or interfere penetration process and specific structure formation such as appressoria in pathogenic fungi, so it hampered pathogenic ability to attack plants, (5) the difference of genetic structure and gene expression between *Trichoderma harzianum* and *T. viride* fungi could cause difference in metabolite, enzyme, and antimicrobial compound production that might have potentials of pathogen inhibition (Lerran *et al.*, 2020; Saravanakumar *et al.,* 2017; Sanchez-espinoas *et al.,* 2020; Chaverri *et al.,* 2015).

Antagonism Mechanism of *Trichoderma harzianum* and *T. viride* for *Colletotrichum gloeosporioides*

Trichoderma harzianum and *T. viride* were two species of antagonistic fungi that could control pathogenic fungi. The mechanism of antagonistic *Trichoderma harzianum* and *T. viride* fungi for *Colletotrichum gloeosporioides* fungi was consisted of competition, mycoparasite, and antibiosis. In this research, the mechanism of competition was referred by colonies of *Trichoderma harzianum* and *T. viride* fungi that grew faster than pathogenic fungi of *Colletotrichum gloeosporioides* on PDA medium.

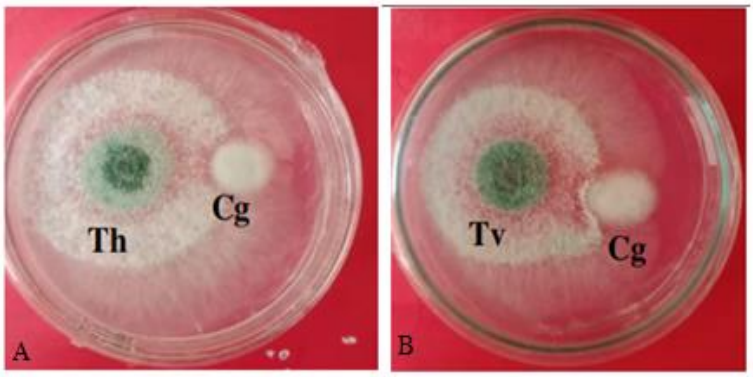
This result was in line with the result of secondary metabolite analysis that cellulose enzyme resulted from *Trichoderma harzianum* was about 143,562 U/mL, while *T. viride* 111,914 U/mL. This result referred that cellulose secondary metabolite contained in *Trichoderma harzianum* was greater than *T. viride.* The following figure 1 presented growth mechanism of antagonistic *Trichoderma harzianum* and *T. viride* fungi for pathogenic *Colletotrichum gloeosporioides* fungi.

The figure above was the antagonism process of antagonistic fungi on pathogenic fungi which have been observed macroscopically. Meanwhile, the mycoparasitism mechanism of *Trichoderma harzianum* and *T. viride* for pathogenic *Colletotrichum gloeosporioides* fungi observed by using SEM with intensification of 2500 X would be presented in the following figure 2.

Table.1 Percentage of Antagonism Power between Antagonistic *T. harzianum* and *T. viride* Fungi for *Colletotrichum gloeosporioides* Fungi

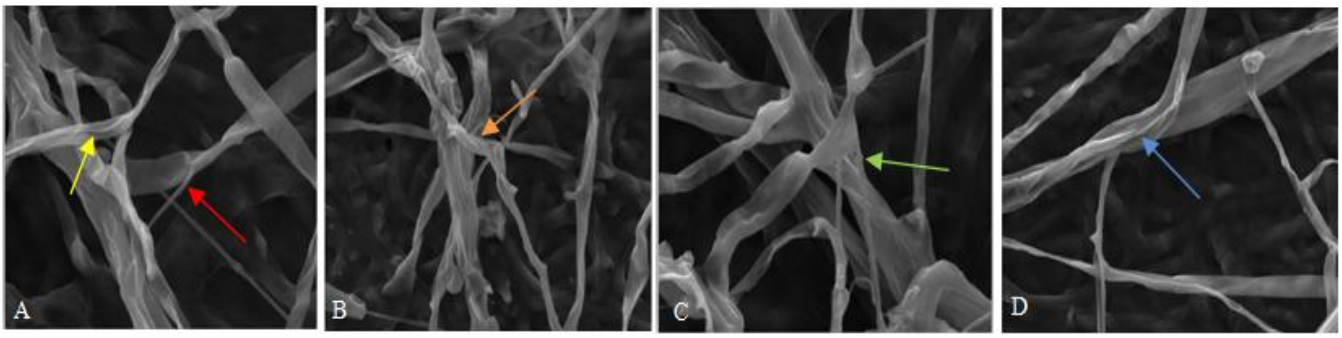
S.No	Treatment	Antagonism Power (%) on repetition to			Results (%)	Mean
		1	2	3		
1	*T. harzianum* Rifai., vs *C. gloeosporioides* (Penz.) Sacc.	78,26	80,00	76,92	235,18	78,39
2	*T. viride* (Pers.) ex Fries vs *C. gloeosporioides* (Penz.) Sacc.	66,66	61,53	58,33	186.52	62,17

Fig.1 Result of Antagonism Observation after Incubation for 4 x 24 hours



Description: A. Antagonism of *T. harzianum* (Th) on *Colletotrichum gloeosporioides* (Cg), B. Antagonism of *T. viride* (TV) on *Colletotrichum gloeosporioides* (Cg)

Fig.2 Mycoparasitism Mechanism of Antagonistic Fungi on Pathogenic Fungi



Description: (A) *T. harzianum* fungal hyphae that pierce (red arrow) and attach (yellow arrow) to *C. gloeosporioides* fungal hyphae. (B) *T. harzianum* fungal hyphae twist *C. gloeosporioides* fungal hyphae (orange arrow). (C) *T. viride* fungal hyphae pierce on *C. gloeosporioides* fungal hyphae (green arrow). (D) *T. viride* fungal hyphae attach to *C. gloeosporioides* fungal hyphae (blue arrow).

Based on the figure 2, it referred three mycoparasitism mechanisms of *Trichoderma harzianum* and *T. viride* on pathogenic *Colletotrichum gloeosporioides* fungi such as attaching, twisting, and piercing on the plant. Mycoparasitism was defined as an interaction

between two fungi, in which the first antagonistic fungi (mycoparasite) would attack and seize nutrition of pathogenic fungi (host). Mycoparasite has a lot of ways to attack pathogenic fungi, but three basic mechanisms of physical interaction between mycoparasite and its host: (1) attachment: the mechanism was started before antagonistic fungi could attack pathogenic fungi, it should be attach to the surface of pathogenic fungi. This process was generally mediated by specific structures and compounds on the mycoparasite cell surface. The components involved in this process were hydrophobin and other adhesion proteins that were frequently involved in this attachment process. In some cases, for instance in *Trichoderma* fungi, hydrophobin would play a significant role in facilitating the initial contact with pathogenic fungi (Benítez *et al.*, 2004; Contreras-Cornejo *et al.*, 2009), (2) Twisting, after being attached to the plant, mycoparasite often emitted hyphae grown around the plant and twisted hyphae or pathogenic fungi organ. This process was aimed to isolate parts of pathogenic fungi which would be attacked and facilitate the further penetration process. The component involved in this process was active mycoparasite hyphae that grew and differentiated into specific structures (like appressoria) that would facilitate penetration into pathogenic fungi (Druzhinina *et al.*, 2011; Hoyos-Carvajal *et al.*, 2009; Reino *et al.*, 2016) (3) piercing, after being attached and twisted, antagonistic fungi would then penetrate into pathogenic fungi cell wall. This piercing process was mechanical, in which the antagonistic fungal hyphae would grow with pressure and physically penetrate into pathogenic fungi, or through enzymatic activities (Gruber *et al.*, 2008; Gruber *et al.*, 2012; Zeilinger *et al.*, 2007). The component involved in this process was mycoparasite that resulted cell wall enzymes such as chitinase, glucanase, and protease for degrading the components of pathogenic fungi cell wall (Gruber *et al.*, 2011; Benítez *et al.*, 2004). The specific structure like appressoria was able to increase force and facilitate mechanical penetration. After antagonistic fungi has penetrated into pathogenic fungi, it would grow inside, degrade pathogenic fungi tissue, and seize the nutrition for its growth. This mycoparasitism process could cause pathogenic fungi death or growth inhibition, therefore this type of antagonistic fungi was often used in biological control of pathogenic fungi.

The researcher concluded: (1) antagonistic *Trichoderma harzianum* fungi has the highest antagonism level with average antagonism percentage of 78,39% from *T. Viridae* fungi on *Colletotrichum gloeosporioides,* (2) cellulose enzyme resulted by *Trichoderma harzianum* was higher around 143,562 U/mL comparing to *T. Viridae* fungi, (3) antagonism mechanism of *Trichoderma* spp. has referred the following three mycoparasitism mechanisms: attaching, twisting, and piercing *Colletotrichum gloeosporioides* fungal hyphae.

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