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Antagonism of *Trichoderma spp*. Against *Colletotrichum gloeosporioides* and *Colletotrichum aotearoa* on Avocado (*Persea americana* Mill) and Mechanisms of Antagonism Based on Electron Microscopy Observations

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Abstract

The objective of this study is to ascertain the pathogenic and antagonistic fungi that induce disease in avocado plants (Persea americana Mill), identify the antagonistic fungi with the greatest antagonism potential among a variety of antagonistic fungi, and elucidate the mechanism by which antagonistic fungi inhibit the growth of pathogenic fungi. From January to May 2023, this study was conducted in vitro at the Microbiology Laboratory, State University of Malang, employing a completely randomized design (CRD) comprising six replications and four interventions. The identification of mold was accomplished through macroscopically and microscopically descriptive analysis, followed by a comparison with the mold identification key book. The antagonism process was executed utilizing the dual culture method on PDA medium, and the electron microscope (SEM) was employed to witness the antagonism mechanism. The research findings revealed that T. harzianum spp. and T. viride were identified as antagonist fungi, whereas C. aotearoa and C. gloeospoiroides were identified as pathogenic fungi. The antagonistic fungus T. viride exhibited the greatest degree of antagonism, impeding the growth of C. gloeospoiroides by 77.8%. Observed antagonism occurs when the hyphae of the antagonistic mold ensnare, entangle, and pierce those of the pathogenic mold, causing harm to the region surrounding the pathogenic mold's hyphae and subsequently impeding its growth

Keywords: *Antagonism, Mechanisms of antagonism, Trichoderma sp., C. gloeospoiroides, C. Aotearoa*

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INTRODUCTION

The avocado (*Persea americana* Mill) is a highly valuable agricultural commodity. In comparison to other fruits, avocados contain twice as much protein as other fruits. Additionally, avocados are abundant in minerals and vitamins (Dreher & Davenport, 2013). A rise in avocado market demand has resulted from the fruit's application in a variety of industries. The global avocado industry produces in excess of 3.5 metric tons (Valle-Aguirre *et sal.*, 2016). The Central Statistics Agency (2021) stated that avocado production in Indonesia was 669.3 thousand tons in 2020 and increased the following year by 31.9%.

In Indonesia, avocado is available year-round; however, its quality remains constant despite fluctuations in quantity. In contrast to their restricted distribution in large-scale plantation systems, avocado plants are typically observed in unmaintained household gardens (Lestari *et al.*, 2016). Anthracnose on avocado is a disease caused by pathogenic molds from the genus *Colletorichum* and *Pestalotiopsis*. Kimaru *et al.* (2018) stated that the high susceptibility of avocado fruit to anthracnose can result in a reduction in yield, expiration life, and overall quality. Kenya documented a loss of marketability of over 60% of avocado production as a result of anthracnose-induced injury and substandard fruit quality.

In the realm of avocado disease control, synthetic fungicides are frequently and heavily applied throughout the growth phase and after harvest (María *et al.*, 2014). As a consequence of fungicide application, hazardous compounds that are detrimental to both human health and the environment may accumulate (Purwantisari & Evendi, 2015; Muhibuddin *et al.*, 2022) and can increase pathogen resistance (Živković *et al.*, 2010). Numerous studies have demonstrated that pathogenic molds, which are responsible for inducing plant diseases, are amenable to biocontrol with the assistance of soil microorganisms, particularly mold species (Hastuti *et al.*, 2008; Purwantisari & Evendi, 2015; Swain *et al.*, 2018; Sood *et al.*, 2020; Es-Soufi *et al.*, 2020). The mechanism by which *Trichoderma sp.* inhibits the proliferation of pathogenic fungi as a biocontrol agent may be due to their antagonistic capability (Živković *et al.*, 2010).

Additional research is required to determine the extent to which various species of antagonistic fungi (*Trichoderma* sp.) can inhibit pathogenic fungi that cause avocado leaf diseases characterized by brown patches. On avocado plants, the differentiation between antagonistic and pathogenic microorganisms is essential. Different species of pathogenic molds can differ in their antagonistic potency. Hence, quantifying the antagonistic potency of individual species of antagonistic mold against pathogenic mold is imperative. In addition, it is necessary to investigate the mechanism by which antagonistic fungi inhibit pathogenic molds by analyzing the outcomes of light microscope and electron microscope (SEM) observations. If the efficacy of the antagonistic mold Trichoderma sp. in impeding the growth of pathogenic fungi on avocado plants is validated via this research, then the findings may have practical implications within the agricultural industry.

METHOD

This research consisted of 2 stages, namely: The first research stage was descriptive research which aims to identify species of pathogenic fungi that cause spots on avocado leaves and skin, as well as *Trichoderma sp.* The second research stage was experimental research which aimed to determine the highest antagonistic power of several *Trichoderma* species in inhibiting the growth of pathogenic fungi that cause spots on avocado leaves and fruit. The research design used was a completely randomized design where each treatment was carried out in 6 replications. Through this research, we also observed the mechanism of antagonism of several *Trichoderma* species against pathogenic fungi on spots on avocado leaves and fruit. This research was carried out at the Microbiology Laboratory, Department of Biology, FMIPA, Universitas Negeri Malang. The research was carried out in January-May 2023.

Identification of antagonistic molds and pathogenic molds was carried out by isolating the rhizosphere soil of avocado plants, parts of the leaves and skin of avocado fruit. Rhizosphere soil was taken at a depth of 20 cm from the soil surface then dissolved in 0.1% peptone water and diluted to a dilution level of 10-5 then inoculated on PDA media then incubated for 7x24 hours. Isolation of mold on leaves and fruit skin was carried out by cutting parts of the leaves and fruit skin that contained spots measuring 1x1 cm, then disinfecting using 1% NaOCl, rinsing with sterile distilled water, soaking in 70% alcohol and rinsing again using sterile distilled water. Pieces of leaves and fruit skin were placed in PDA media, then incubated. After that, the mold was purified and a slide culture was made to simplify the identification process. Mold identification was done by describing the morphology microscopically and macroscopically and then adjusting it to the fungus identification key book.

The antagonism between antagonistic molds and pathogenic molds was measured using the dual culture method. Cultures of antagonistic molds and aptogenic molds were taken using a sterile cork drill, then inoculated on PDA plate medium at a distance of 3 cm and incubated for 4 days and the antagonism power was calculated using the formula (Dharmaputra et al., 1999):

$$P = \frac{R1 - R2}{R1} x100\%$$

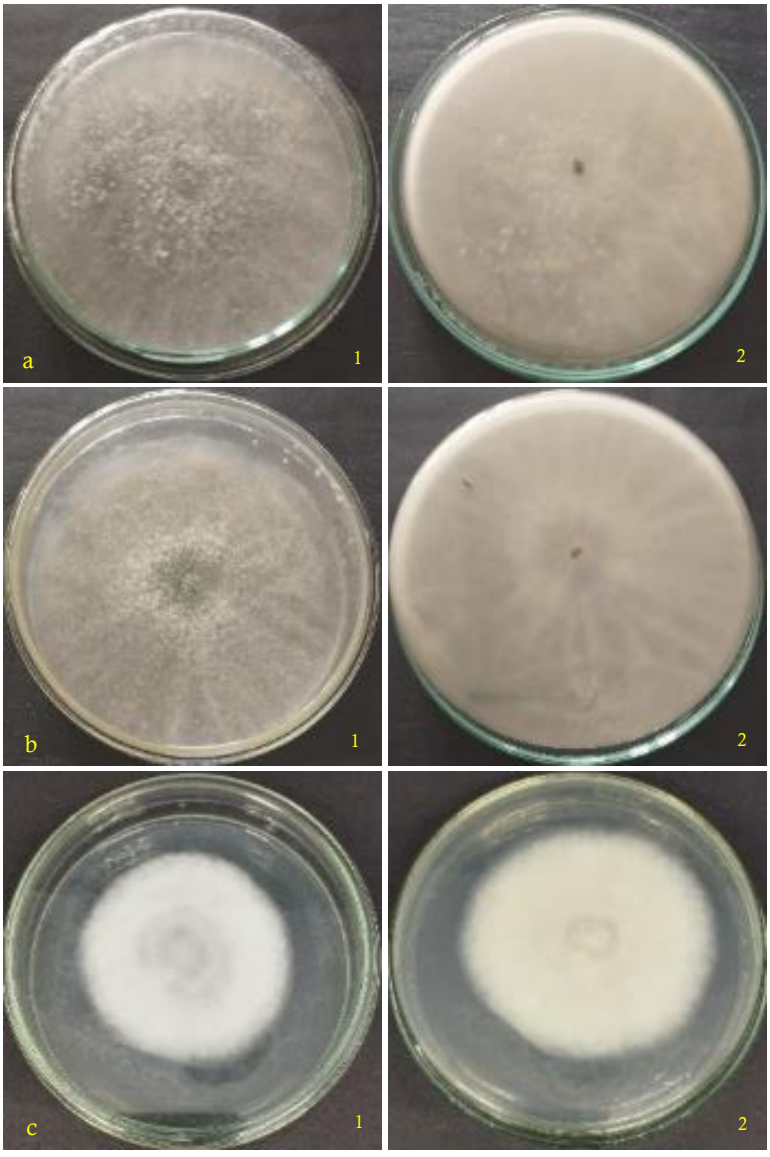
Information:
P : percentage of inhibition
R1 : the radius of the pathogenic mold colony that moves away from the antagonist mold
R2 : the radius of the pathogenic mold colony approaching the antagonist mold

The data obtained in this research were: 1) colony morphology and results of microscopic observations of identified antagonistic molds and pathogenic molds; 2) results of measurements of the radius of pathogenic mold colonies that move away from and approach the antagonistic molds, then calculate the percentage of antagonism power of the antagonistic molds towards pathogenic mold; 3) antagonism mechanism based on microscopic observation (SEM). The data obtained were subjected to parameter tests including normality tests and homogeneity tests, followed by using oneway Anova to determine differences in the antagonism power of each treatment, then a 5% BNT test was carried out.

RESULTS AND DISCUSSION

The Morphology of Tubuca forcipata

The identification results showed the presence of antagonistic molds, namely *Trichoderma harzianum*, and pathogenic molds *Colletotrichum gloeospoiroides*, *Colletotrichum aotearoa*. Only 1 species of *Trichoderma* was found, so another antagonistic mold was added, namely the *Trichoderma viride* isolate, which is a collection of molds in the Microbiology laboratory to test the antagonism of two different antagonistic mold species. The colony morphology of each mold can be seen in Figure 1, while microscopically it can be seen in Figure 2.



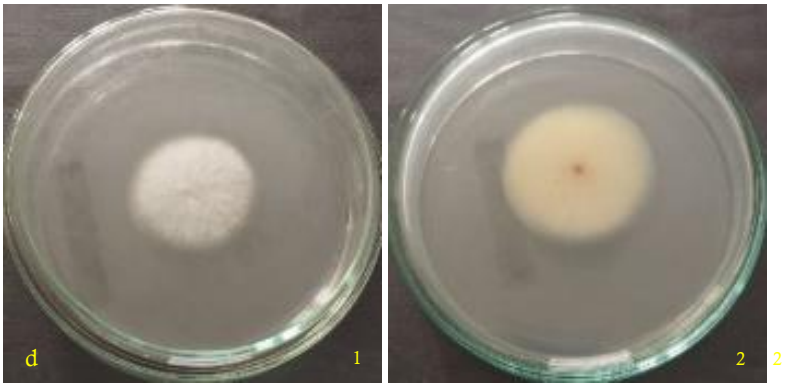


Figure 1. Mold Morphology
Image caption: (a) *Trichoderma harzianum*, (b) *Trichoderma viride*, pathogenic mold (c) *Colletotrichum gloeospoiroides* and (d) *Colletotrichum aotearoa*. Information number 1 = top view, number 2 = bottom view

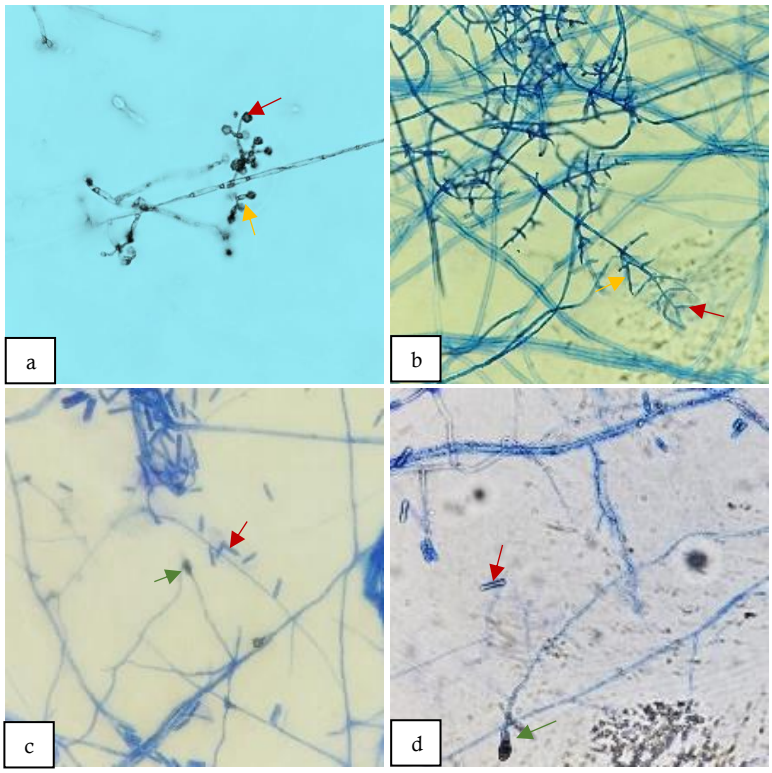


Figure 2. Microscopic View of Antagonistic Mold
(a) *Trichoderma harzianum*, (b) *Trichoderma viride*, pathogenic mold (c) *Colletotrichum gloeospoiroides* and (d) *Colletotrichum aotearoa*. Description: (red arrow) conidia, (yellow arrow) *phialida*, (green arrow) *appressorium*

Antagonism of *T. harzianum* and *T. viride* against *C. gloeospoiroides* and *C. aotearoa*

The results of these calculations were analyzed further using Oneway Anova in the SPSS version 25 application. The Anova result was p= 0.007, this value shows that there is a difference in the antagonism of *Trichoderma sp.* against *C. gloeospoiroides* and *C. aotearoa* significantly. If we compare the antagonistic power of the antagonistic fungi *T. harzianum* and *T. viride* against *C. gloeospoiroides*, the fungus *T. viride* has a higher antagonistic power.

When comparing *T. harzianum* and *T. viride* against *C. aotearoa*, there is no significant difference between the two antagonistic fungi. The results of measuring the power of antagonism can be seen in Table 1.

Table 1. Antagonism power measurement results (%)

No	Treatment	Average
1	TV x CA	62,5[a]
2	TH x CA	63,9[a]
3	TH x CG	71,42[a]
4	TV x CG	77,8[b]

Information: TH = *Trichoderma harzianum*; TV = *Trichoderma viride*;
CA = *Colletotrichum aotearoa*; CG = *Colletotrichum gloeosporioides*

The research results above are in accordance with research by Triasih, Abadi, et al. (2022) demonstrated that *T. harzianum* and *T. viride* fungi have an inhibitory power of 79.86% and 53.62% against the growth of the pathogenic fungus *C. gloeospoiroides* which causes *Manalagi* apple rot disease. This research also shows the high inhibitory power of the mold *Trichoderma sp.* against *C. gloeospoiroides* which causes anthracnose in oranges, namely 63.24%. Mold *Trichoderma sp.* Not only is it able to inhibit the growth of *C. gloeospoiroides*, but it can also inhibit several pathogenic fungi that cause disease in plants such as Fusarium oxysporum. (Ningsih et al., 2016), *Neoscytalidium dimidiatum* (Tanama et al., 2020), *Sclerotium rolsfii, Alternaria solani* and *Rhizoctonia solani* (Muhibbudin et al., 2021).

Antagonism Mechanism of Molds *T. harzianum* and *T. viride* against *C. gloeospoiroides* and *C. aotearoa*

The mechanism of antagonism of antagonistic fungi consists of: antibiosis mechanism, mycoparasitism mechanism and competition mechanism (Schubert et al., 2008, Hastuti et al., 2008). The antagonism mechanisms examined in this research are competition mechanisms and mycoparasitism mechanisms. The results of the research in the form of a parasitism mechanism can be seen macroscopically, namely the growth of *Trichoderma sp.* which grows rapidly so that it dominates the media and encourages the growth of *C. aotearoa* and *C. gloeospoiroides* mold colonies (Figure 3).

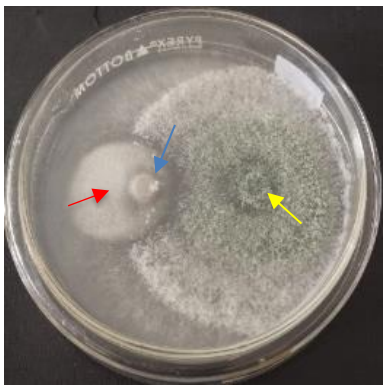


Figure 3. Competition mechanism between *T. viride* and *C. gloeospoiroides*
Description: (red arrow) *C. gloeospoiroides* mold colony, (yellow arrow) *T. viride* mold colony, (blue arrow) clear area indicating inhibited growth of *C. gloeospoiroides*.

This is in accordance with the research by Triasih (2022) that mold *Trichoderma sp.* has fast growth and fills the petri dish, causing the pathogen growth diameter to remain small. The competition that occurs between antagonistic molds and pathogenic molds is a struggle for space and nutrients. The mechanism of antagonism in the form of mycoparasitism shows the presence of antagonistic mold hyphae that entangle, stick to and pierce the hyphae of pathogenic molds. A research by Khare (2018) stated that hyphae of the mold Trichoderma sp. entangling or coiling around the hyphae of the Fusarium oxysporum mold which results in changes in the morphology of the Fusarium oxysporum hyphae. Changes in the morphology of pathogenic mold hyphae or damage to the hyphae due to Trichoderma sp. produces several hydrolytic enzymes, namely β-1,4-glucanase, chitinase, cellulase, and protease (Kumar et al., 2012., Hastuti, 2017). *T. harzianum* has a chitinase enzyme activity of 3.68% while *T. viride* has 1.96% (Triasih et al., 2022). Several enzymes produced by *Trichoderma sp.* degrades the compounds that make up the cell walls of pathogenic mold hyphae, causing inhibition of the growth of pathogenic mold colonies and damage to the cell walls of pathogenic mold hyphae (Hastuti, 2017).

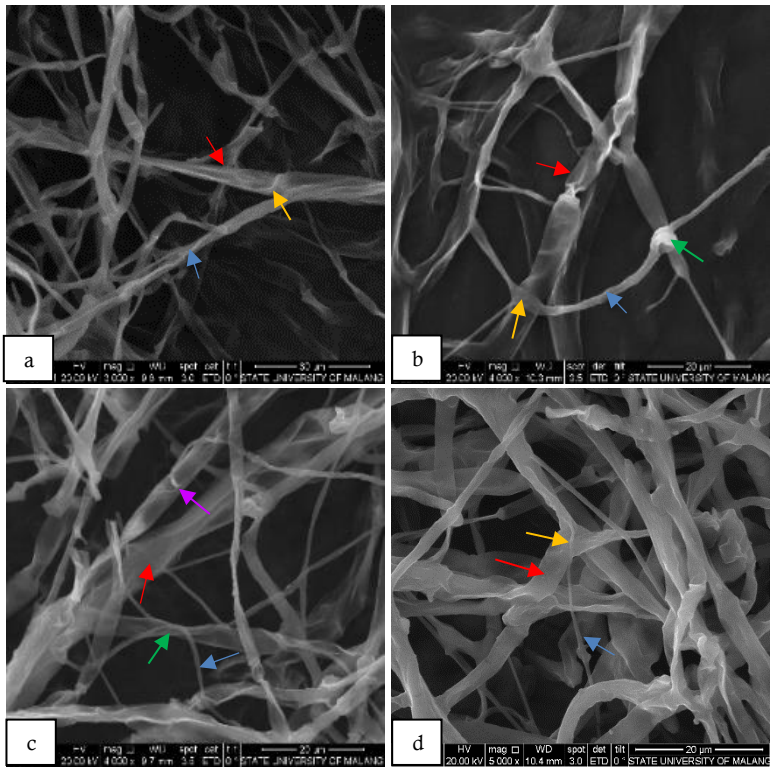


Figure 4. Antagonism Mechanism
a). *T. harzianum* against *C. aotearoa*, b). *T. harzianum* against *C. gloeospoiroides*, c). *T. viride* against *C. gloeospoiroides*, d). *T. viride* against *C. aotearoa*
Figure information: (red arrow) pathogenic mold hyphae, (blue arrow) antagonistic mold hyphae, (green arrow) antagonistic mold hyphae that entangle pathogenic mold hyphae, (purple arrow) antagonistic mold hyphae that pierce pathogenic mold hyphae, (yellow arrow) mold hyphae antagonists that attach to the hyphae of pathogenic molds.

The results of this research were the identification of antagonistic fungi *T. harzianum* and *T. viride* as well as pathogenic fungi on avocado plants, namely *C. gloeospoiroides* and *C. aotearoa*. The highest antagonism was produced by T. viride which inhibited the growth of *C. gloeospoiroides* (77.8%). The mechanism of antagonism that occurs is that the hyphae of the antagonistic mold entangle, stick to and pierce the hyphae of the pathogenic mold.

CONCLUSION

T. harzianum was identified as the antagonistic mold present in the rhizosphere soil of avocado plants. *C. Gloeospoiroides* and *C. aotearoa* were identified as the pathogenic molds. When examining the antagonistic potency of *T. viride* in comparison to *T. harzianum* against *C. gloeospoiroides*, it becomes evident that *T. viride* exhibits a more pronounced antagonistic capability. Regarding the antagonistic fungi *T. viride* and *T. harzianum* against *C. aotearoa*, no statistically significant distinction can be observed. Competition for nutrients and space is the mechanism of antagonism; antagonistic mold hyphae mycoparasitism entangles, adheres to, and pierces the pathogenic mold hyphae, thereby causing cell wall injury to the pathogenic mold hyphae.

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