

Antagonism and mycoparasitism mechanism of *T. harzianum* against pathogenic fungus species of *F. oxysporum* and *Capnodium sp.*

Ali Mustofa¹, Utami Sri Hastuti^{2,*}

¹Universitas PGRI Ronggolawe Tuban, Indonesia

²Universitas Negeri Malang, Indonesia

*Corresponding author, email: utami.sri.fmipa@um.ac.id

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Abstract: The purpose of this study was to test the ability of *T. harzianum* to inhibit the growth of pathogenic mold species and to determine the effectiveness of *T. harzianum* mold species in inhibiting the growth of pathogenic molds, as well as investigate how *T. harzianum* and pathogenic molds interact with each other. The Microbiology Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Malang, is where this research was conducted. The dual culture method was used for this test by using Czapek Agar (CA) medium. The isolated molds were then incubated for 4x24 hours at 25°–27°C. After that, the antagonism power was calculated. The results of macroscopic and microscopic observations were used to assess the mechanism of *T. harzianum* mold antagonism against pathogenic molds. The results showed that *Trichoderma* mold species were more resistant to the pathogenic mold *Fusarium oxysporum* than the pathogenic mold *Capnodium*. The antagonistic power of *T. harzianum* was 80%, with the antagonistic power of *Capnodium sp.* at 66.7%. The mechanism of mycoparasitism occurs when the hyphae of *T. harzianum* attach or entangle the hyphae of pathogenic molds, causing damage to the hyphal structure and inhibiting the growth of pathogenic molds.

Keywords: Antagonistic fungi, *Capnodium sp.*, *F. oxysporum*, pathogenic fungi, *T. harzianum*

Abstrak: Tujuan dari penelitian ini adalah untuk menguji kemampuan *T. harzianum* dalam menghambat pertumbuhan spesies kapang patogen dan untuk menentukan efektivitas spesies kapang *T. harzianum* dalam menghambat pertumbuhan kapang patogen serta menyelidiki bagaimana kapang *T. harzianum* dan kapang patogen berinteraksi satu sama lain. Laboratorium Mikrobiologi Jurusan Biologi FMIPA Universitas Negeri Malang adalah tempat penelitian ini dilakukan. Metode dual culture digunakan untuk pengujian ini dengan menggunakan medium Czapek Agar (CA). kapang yang telah diisolasi kemudian diinkubasi selama 4x24 jam pada suhu 25°–27° C. Setelah itu, dilakukan perhitungan daya antagonisme. Hasil pengamatan makroskopis dan mikroskopis digunakan untuk menilai mekanisme antagonisme kapang *T. harzianum* terhadap kapang patogen. Hasil penelitian ini menunjukkan bahwa spesies kapang *Trichoderma* lebih tahan terhadap kapang patogen *Fusarium oxysporum* daripada kapang patogen *Capnodium*. Daya antagonis kapang *T. harzianum* adalah 80%, dengan daya antagonis *capnodium sp.* 66,7%. Mekanisme mikoparasitisme terjadi ketika hifa *T. harzianum* menempel atau membelit hifa kapang patogen, menyebabkan kerusakan struktur hifa dan menghambat pertumbuhan kapang patogen.

Kata kunci: Antagonistic fungi, *Capnodium sp.*, *F. oxysporum*, pathogenic fungi, *T. harzianum*

INTRODUCTION

Synthetic pesticides are typically used to treat plant diseases, but their use contaminates the environment and poses health risks to humans and animals. Biological

control can be achieved without the use of synthetic pesticides through the utilization of antagonistic microbes (Lahlali et al., 2022). Fungal biological control agents have gained significant traction in their prospective application against plant infections due to their specialized targets, short generation durations, and relatively high rates of both sexual and asexual reproduction. Furthermore, they can persist in the environment without a host by switching from parasitism to saprotrophism, which preserves sustainability. According to Thambugala et al., (2020), a variety of fungal species possess defense mechanisms that allow them to effectively shield plants from diseases brought on by plant pathogenic fungi. It has been demonstrated that antagonistic microorganisms, which may be obtained from a variety of settings, are more effective than synthetic pesticides at managing plant diseases (Droby et al., 2022).

Antagonism between antagonistic and pathogenic molds is a phenomenon in which certain molds compete in the same environment, with the effect of inhibiting each other's growth or activity (Mazaro et al., 2022). Microorganisms with the capacity to prevent or regulate the growth of harmful molds are referred to in this context as antagonistic molds. By stimulating increased plant growth and systemic resistance, antagonistic fungi prevent the growth of pathogenic fungi. They also compete with other harmful microbes and produce a variety of secondary metabolites (lipopeptides, antibiotics, and enzymes) through colonization (Akram et al., 2023). Antagonism between antagonistic molds and pathogenic molds needs to be well studied, so that its properties can be known (Rahmawati et al., 2018).

In this study, the pathogenic fungi used were *F.oxysporum* and *Capnodium sp.* These two fungi are pathogenic fungi that attack plants. *F.oxysporum* causes wilt disease in plants characterized by decay of roots and tubers. *F.oxysporum* usually attacks tomato, chili and other agricultural production plants (Michielse & Rep, 2009). In addition, *Capnodium* is a mold that attacks leaves, characterized by sooty dew on the leaves. The symptom of this disease is that on the surface of the leaves and stems there is an evenly distributed black layer that is easily peeled off the leaves but the leaf tissue underneath remains green. Although *Capnodium sp.* is not a parasite because it only covers the surface of the leaves, it is still dangerous for plants because it can inhibit metabolism, especially the photosynthesis process (Nurfalinda, 2023).

A significant portion of their parts are assimilated by fungi in a systematic and biological manner to prevent the establishment of other pathogenic fungi that are detrimental to the growth and development of plants. The creation of fungal strains as biocontrol agents for plant diseases has received a lot of attention. The members of the genus *Trichoderma* have been the subject of the greatest investigation (Tariq et al., 2020). It has been documented that *Trichoderma* fungi have antagonistic action against a number of plant fungal diseases, such as *Botrytis*, *Alternaria*, *Pythium*, *Aspergillus*, *Fusarium*, *Rhizoctonia*, *Phytophthora*, and *Gaeumannomyces* (Pal and Gardener 2006). *Trichoderma* is a crucial biocontrol agent that helps manage another fungus that causes phytopathies. In field or

greenhouse tests, *Trichoderma*, a mycoparasite, can be employed as a biopesticide to combat a variety of soil- and air-borne plant infections (Tariq et al., 2020).

Antagonism of molds must be observed and measured because knowledge of the antagonism between antagonistic molds and pathogenic molds has significant benefits in mycology and agriculture (Ainy et al., 2015). Antagonistic molds can inhibit the growth of pathogenic molds that cause disease in plants. The use of antagonistic molds as biological agents helps reduce dependence on synthetic chemicals that have the potential to damage the environment, so antagonistic molds are a more environmentally friendly alternative to chemical fungicides. This can help maintain the balance of the soil ecosystem (J. Chen et al., 2021). In addition, the presence of antagonistic molds can inhibit the growth of pathogenic molds, thereby reducing human health risks associated with mycotoxin exposure. Based on this, knowledge of the antagonism of antagonistic molds and pathogenic molds needs to be studied and analyzed in order to be useful for agriculture and biological control (Martin-Sanchez et al., 2022). The purpose of the study was to determine the antagonistic power of *T.harzianum* mold against *F.oxysporum* and *Capnodium sp.*

METHOD

The research was conducted in April 2023 in the Microbiology Laboratory of the Biology Department, FMIPA UM. The tools used are: Petri dish, cork drill, incubator, spiritus lamp, scalpel, glass objects, cover glass, microscope, laminar air flow, dry oven, autoclave. The materials used were: pure culture of *T. harzianum*, *F. oxysporum*, and *Capnodium sp*, lactophenol cotton blue solution, Czapek Agar (CA) plate medium.

Antagonism testing of *T. harzianum* against several species of pathogenic molds

Pure cultures of the molds *T. harzianum*, *F. oxysporum*, and *Capnodium sp* were inoculated on Czapek Agar plate medium, then incubated at 25°-26°C for 5x24 hours. A sterile cork drill with a diameter of 5 mm was used to cut the mold culture aseptically. Then inoculated on the surface of CA plate medium in pairs between *T. harzianum* and 2 pathogenic mold species. The distance between the two pieces of mold culture was 3 cm. Then incubated at 250-27°C for 4x24 hours. Furthermore, the antagonism between *T. harzianum*. and the two pathogenic mold species was observed and calculated using the formula according to Sudantha (2009).

$$I = \frac{r_1 - r_2}{r_1} \times 100\% \quad (1)$$

Notes:

I = antagonism, r1 = radius of pathogenic mold colonies growing away from *T. harzianum*, and r2 = radius of pathogenic mold colonies growing toward *T. harzianum*.

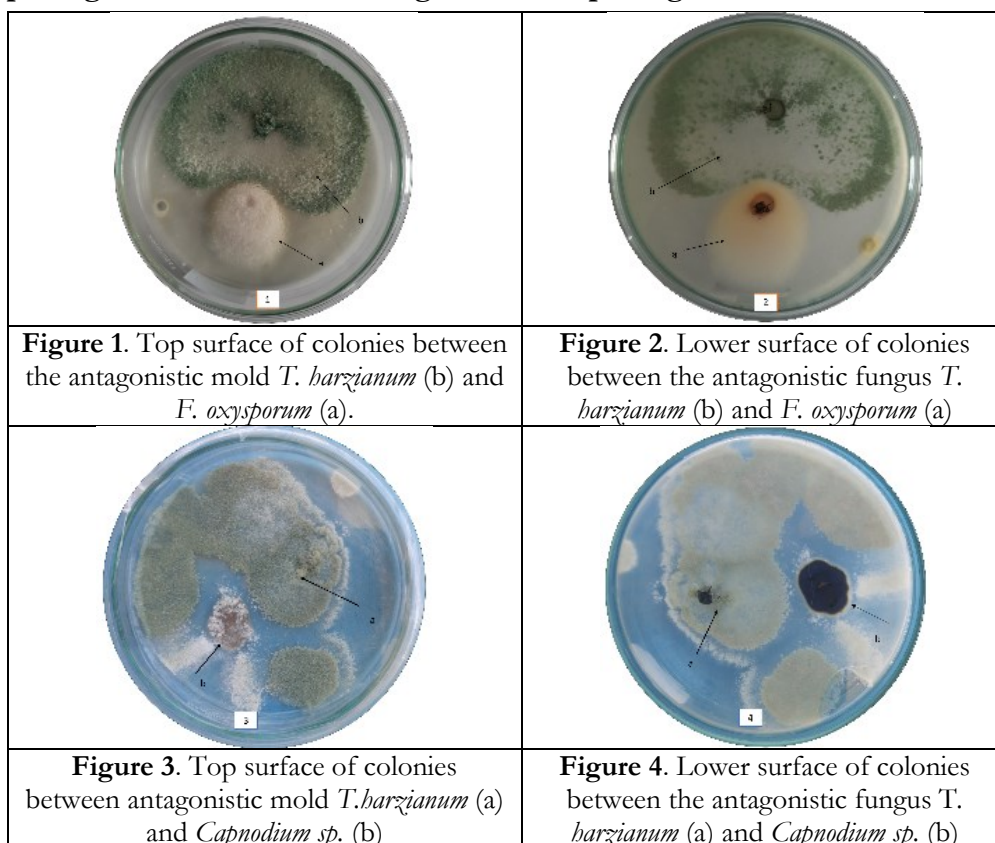
Observation of macroscopic and microscopic antagonism mechanisms between *T. harzianum* with pathogenic mold species

Observations were made on the growth of *T. harzianum* mold colonies with pathogenic mold colonies grown together. The mold culture was cut aseptically at the border area between the *T. harzianum* mold colony and the pathogenic mold colony with a sterile scalpel with a size of 1 x 1 cm². The pieces of mold culture were placed on a glass slide, dripped with lactophenol cotton blue solution and covered with a glass cover, then the antagonistic mechanism between *T. harzianum* and pathogenic molds was observed under a microscope.

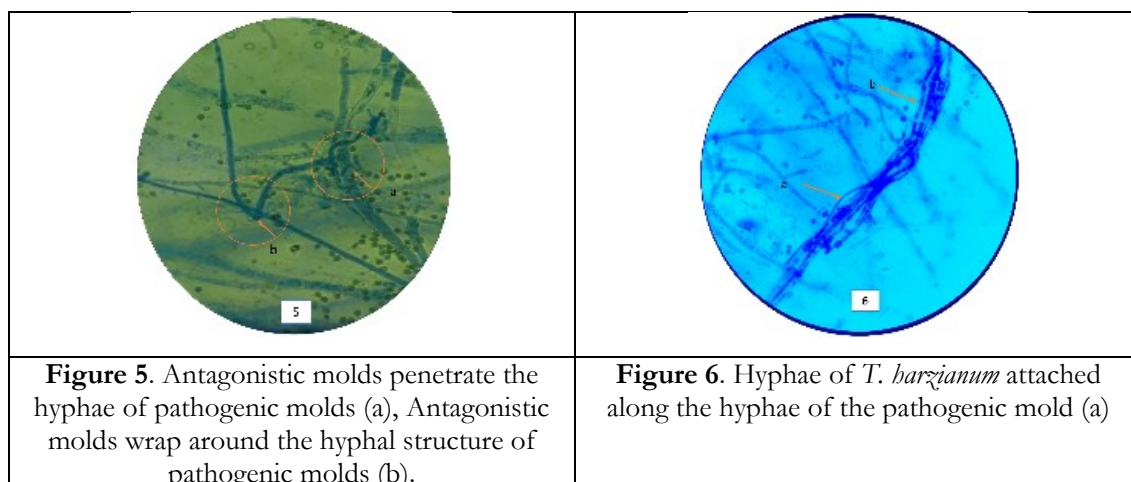
RESULTS AND DISCUSSION

Testing the antagonism power of *T. harzianum* was carried out using the dual culture method. The data in this practicum report consists of morphological data of the antagonism of the antagonistic mold *T.harzianum* with the pathogenic mold *F.oxysporum* and the mold *T.harzianum* with the pathogenic mold *Capnodium sp.* The measurement of antagonism power between these molds is also presented. In addition, histological observation data of the structure of the antagonistic molds attacking the pathogenic molds are presented.

Morphological observation of antagonistic and pathogenic molds



Antagonism activity of the mold *T. harzianum* against the pathogenic mold *F. oxysporum*



Antagonism power measurement results (day 6)

Table 1. Measurement of mold antagonism power

No.	Antagonistic Molds	Pathogenic Molds	R ₁	R ₂	Antagonism Power
1	<i>T.harzianum</i>	<i>F.oxysporum</i>	25 mm	5mm	80%
2	<i>T.harzianum</i>	<i>Capnodium sp.</i>	9 mm	3 mm	66.7%

The analyzed *T. harzianum* mold inhibits the formation of pathogenic mold colonies. Based on macroscopic and microscopic investigations, it is clear that the mechanism of antagonism for *T. harzianum* mold is mycoparasite and competition. The mechanism of competition antagonism can be seen from the macroscopic observations shown by the faster growth rate of *T. harzianum* mold in filling the petri dish compared to the growth of the test pathogenic mold (Figure 1 and Figure 3). In addition, macroscopic observations also showed that the colonies of *T. harzianum* molds were able to grow from sticking to covering some of the colonies of the test pathogenic molds, namely *F. oxysporum* and *Capnodium sp.*, so that this could inhibit the growth of the test pathogenic molds (Figure 2 and Figure 4). Microscopic observations showed that the antagonism of the antagonistic mold *T. harzianum* caused the hyphae of the pathogenic mold to coagulate protoplasm, as well as the size of hyphal cells that became shorter when compared to the size of normal hyphae. The mycoparasitic mechanism exhibited by *T. harzianum* was by penetrating the hyphae of pathogenic fungi (Figure 5a), coiling the hyphae of pathogenic fungi (Figure 5b) and growing along the hyphae of pathogenic fungi (Figure 6a).

Antagonism was calculated as the percentage of growth inhibition of pathogenic molds by antagonistic molds. The antagonism between *T. harzianum* and *F. oxysporum* was 80%. The antagonism between *T. harzianum* and *Capnodium sp.* was 66.7%. These results indicate that *T. harzianum* has significant antagonism against both pathogenic molds tested.

In the soil there are antagonistic soil molds and also pathogenic molds that live together, so naturally the interaction between these two molds will produce a balance in the soil ecosystem. The *T. harzianum* mold is one of the antagonistic molds, so it can be used to control pathogenic molds (Rahmawati et al., 2018). The results of testing the antagonism power of 2 isolates of *T. harzianum* mold. *T. harzianum* mold has significant antagonism against both pathogenic molds tested. *T. harzianum* antagonism against *F. oxysporum* was 80% while against *Capnodium sp.* was 67% (Table 1).

T. harzianum is a type of fungus known to be effective as a biological control agent against various plant pathogens, including *F. oxysporum*. The effectiveness of *Trichoderma harzianum* against *F. oxysporum* is attributed to several mechanisms, including: Mycoparasitism: *T. harzianum* can attack and destroy hyphae of *F. oxysporum*. Production of Antifungal Enzymes and Compounds: *T. harzianum* produces various lysing enzymes and volatile organic compounds that have antifungal activity. Enhancement of Plant Resistance: *T. harzianum* can increase the systemic resistance of plants to *F. oxysporum* (Chen et al., 2019; Lakhdari et al., 2018; Meddad-Hamza et al., 2023). However, the effectiveness of *T. harzianum* against *Capnodium* fungi may differ and depends on several factors, such as the strain of *T. harzianum* used, environmental conditions, and the specific interaction between *T. harzianum* and *Capnodium* fungi. Although *T. harzianum* is generally effective in controlling various fungal diseases, it may not be effective against all types of fungi or under all conditions. Therefore, it is possible that *T. harzianum* may not be effective in killing *Capnodium* fungi under certain conditions (Sharma & Sharma, 2020).

The difference in antagonism power can be caused by the characteristics of each mold, the type of antibiotic compounds produced and also the antagonistic mechanism of each species of *T. harzianum* mold. The mechanism of antagonism between *T. harzianum* and *Capnodium sp.* can involve various factors, such as competition for nutrients, production of antimicrobial compounds, and physical interactions between the hyphae of the molds (Rahmawati et al., 2018). The *T. harzianum* mold can inhibit the growth of pathogenic molds, this can be seen in the results of macroscopic observations showing the existence of a mycoparasitic mechanism, namely the hyphae of the *T. harzianum* mold entangle, penetrate the hyphae of pathogenic molds or grow attached along the hyphae of pathogenic molds (Figure 5 and Figure 6).

Trichoderma molds attack pathogenic molds through various complex mechanisms. Some of these are: 1) Mycoparasitism, *Trichoderma* directly parasitizes and attacks pathogenic molds, competing for nutrients and space. 2) pathogen cell wall degradation, *Trichoderma* produces enzymes that degrade the cell wall of pathogenic molds. 3) competition for nutrients and space, *Trichoderma* competes with pathogenic molds for resources. 4) induction of plant resistance, *Trichoderma* induces systemic resistance in plants to pathogenic molds. 5) production of antimicrobial compounds (Antibiosis), *Trichoderma* secretes antimicrobial compounds that inhibit pathogen growth. These techniques all contribute to *Trichoderma*'s

efficacy as a biological control agent for plant diseases (Saldaña-Mendoza et al., 2023; Tyśkiewicz et al., 2022; Yao et al., 2023).

The findings of this study suggest that *Trichoderma* molds have the capacity to inhibit the growth of harmful molds on plants. Antagonistic molds have the capacity to promote nutrient uptake and nitrogen usage in plants, as well as biocontrol capabilities (Pandit et al., 2022). Antagonistic molds can help resist pests like nematodes and microbial pathogens that infect many sections of the plant, including roots, leaves, and fruits. Antagonistic molds protect against diseases by processes such as mycoparasitism, resource rivalry with pathogens, antibiosis, and mycovirus-mediated cross-protection (Singh & Giri, 2017).

Some well-known mold biocontrol agents include *Trichoderma* species, *ectomycorrhiza*, *arbuscular mycorrhiza* (AMF), yeasts, and endophytic fungi. Thambugala et al. (2020) recognized *Trichoderma* as the genus with the greatest biocontrol potential, with 25 species utilized as biocontrol agents to combat a variety of mold-borne diseases. The majority of plant growth-promoting fungi (PGPR), namely *Trichoderma*, *Penicillium*, and *Aspergillus*, have been shown to stimulate plant immune responses against pathogenic mold attack and are considered one of the safest ways to induce systemic resistance (ISR) and enhance crop plant growth (Divya et al., 2021; Jogaiah et al., 2018). Furthermore, PGPR is known to help plants resist the effects of numerous fungus, bacteria, viruses, and nematodes. *Trichoderma* species are soil-borne filamentous fungus that are recognized to be effective in numerous plant health benefits applications (Patra & Baek, 2014; Rai, 2016). The pathogen control mechanisms involve various intricate processes such as colonizing the soil and roots of the host, occupying physical spaces, preventing the multiplication of phytopathogens, and producing enzymes that degrade cell walls. Additionally, they produce antimicrobial substances to eliminate pathogens, stimulate plant defense mechanisms, enhance plant growth, and improve plant resistance to both biotic and abiotic stresses (Zin & Badaluddin, 2020).

CONCLUSION

T. harzianum demonstrates a mycoparasitic mechanism by the penetration, coiling, and growth along the hyphae of pathogenic fungi. The hostility between *T. harzianum* and *F. oxysporum* was 80%. The level of antagonism between *T. harzianum* and *Capnodium* sp. was 66.7%. The data suggest that *T. harzianum* exhibits substantial antagonistic activity against both tested pathogenic molds.

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