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Characteristics of the Antagonistic Fungus Nigrospora Oryzae Against the Fungus Fusarium as a Source of Biological Learning in Chili Plants

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Abstract: One of the primary diseases that attack chilli plants *(Capsicum annum)* is *Fusarium wilt*, which is caused by the pathogenic fungi *F. oxysporum* and *F. solani*. This study aims to analyze the parasitism mechanism of the antagonistic fungus *N. oryzae* against the pathogen that causes *Fusarium wilt* in chilli. The method used was a dual culture technique antagonism power test with observations carried out every day from day 0 to day 5. The results of the study showed that the inhibition rate achieved was 33.3% against F. oxysporum and 18.18% against *F. solani* with the antagonism mechanism of *N.oryzae*, including 1) Mycoparasitism; 2) Degradation of pathogen cell wall; 3) competition for nutrients and space. The results showed that *N. oryzae* could inhibit the growth of *F. solani* and *F. oxysporum*. This proves that *N. oryzae* has the potential to be an effective antagonistic fungus in controlling both pathogens. The contribution of the results of the introduction of fungal antagonism is used as a learning resource to understand the concept of learning fungi through direct practical activities in the Laboratory.

Keywords*:* *F. oxysporum, F. solani, Fusarium Wilt*, Learning resources *N. oryzae.*

Introduction

Chilli plants *(Capsicum annuum L.)* are a horticultural commodity with high economic value in Indonesia (Agustina et al., 2022). In addition, chilli plants also have relatively good market opportunities with very high selling values, so the cultivation of chilli plants needs attention (Ulya et al., 2020). The implementation of cultivation and efforts to increase the production of chilli plants cannot be separated from the problem of pests and diseases (Wati et al., 2020). This disease can cause losses of 10-80%, and even severe attacks can cause crop failure (Gabrekiristos & Demiyo, 2020). According to the Central Bureau of Statistics of East Java Province (2022), data on the production of major horticultural commodities in East Java province in 2021-2022 showed that chilli production in 2021 was 976,848 tons, decreasing to 851,445 tons in 2022.

The development of chilli plants still faces various obstacles, especially low yield and disease attacks caused by fungi, viruses, and bacteria (Reddy et al., 2024). One of the leading causes of low chilli productivity is Fusarium fungal infection (Jamil et al., 2021). This fungus is very harmful because it can attack chilli plants from the germination phase to maturity (Muhammad et al., 2022). Although a soil-borne pathogen, Fusarium infects roots and can spread through wounds to other organs, such as stems, leaves, flowers, and fruits (Halimah et al., 2024). Some Fusarium species that often attack chilli plants include *F. oxysporum, F. solani, F. moniliforme, and F. chlamydosporum* (Ihkwanisa et al., 2023). The symptoms are that the surface of the bark on the stem becomes wrinkled or sunken inward, and the internal tissue is brown and decayed (Hong et al., 2020). The pathogenic fungus *Fusarium solani* can be transmitted through soil and planting material from diseased plants and infect host plants through root wounds (Girma, 2022). This pathogen can survive in the soil as long as chlamydospores, even though there are no host plants (Beka & Pichiah, 2021).

Control of Fusarium wilt disease has relied heavily on chemical fungicides. However, excessive use of chemicals can have negative impacts, such as pathogen resistance, environmental pollution, and chemical residues in agricultural products (Susanna et al., 2023). Therefore, an alternative, more environmentally friendly and sustainable control is needed, one of which is by utilizing biological agents. One potential biological agent is the antagonistic fungus *Nigrospora oryzae* (Sha et al., 2023). This fungus is known to have the ability to inhibit pathogen growth through antagonism mechanisms, such as competition for space and nutrients, production of antibiotic compounds, and induction of plant resistance. Several previous studies have reported the effectiveness of *Nigrospora oryzae* in controlling various plant diseases. However, studies on its antagonism characteristics against *Fusarium oxysporum* and *Fusarium solani* in chilli plants are still limited (Tao et al., 2024).

Antagonism between antagonistic and pathogenic fungi is a phenomenon in which certain fungi compete in the same environment, with the effect of inhibiting each other's growth or activity (Mustofa & Hastuti, 2024). This fungal antagonism research can also be used as a source for learning biology. One of the uses of learning resources originating from the environment is by using research results (Anggoroputro & Salamah, 2021). Learning resources also include communication, individuals, materials, equipment, techniques, and the surrounding environment that can be used to help students learn (Aliah et al., 2024) .Based on these problems, it is necessary to conduct research on *Nigrospora oryzae* in inhibiting *Fusarium wilt* in chili plants. The antagonism between antagonistic fungi and pathogenic fungi needs to be studied properly so that its properties can be known.

The purpose of this study was to analyze the mechanism of parasitism carried out by the antagonistic fungus *N. oryzae* against the pathogen that causes *Fusarium wilt* in chilies. This study also provides benefits regarding the effectiveness of the antagonistic fungus *Nigrospora oryzae* against *Fusarium wilt* of chili plants *(Capsicum annum)*, which can be used as a learning resource in the form of references or instructions for fungal antagonism practicums.

Research Methods

Time and place of implementation

This research was conducted in March 2025 at the Microbiology Laboratory of the Faculty of Teacher Training and Education, Universitas PGRI Ronggolawe Tuban, the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, State University of Malang, and the Central Laboratory of State University of Malang.

Tools and materials

The equipment used during the research included an autoclave, laminar air flow, oven, cork borer, analytical balance, culture rack, Petri dishes, cover glass, glass slides, scalpel, ose needle, beaker, clear plastic, cotton, aluminum foil, spirit lamp, tweezers, cutter, tape, scanning electron microscope (SEM), knife, pot, stove, sieve, stirrer, ruler, camera, and writing instruments. The materials used include local isolates of *Nigrospora oryzae* from the collection of the Microbiology Laboratory, FMIPA, University of Malang, Indonesia, isolates of *Fusarium oxysporum*, and isolates of *Fusarium solani* from chili stems, distilled water, Potato Dextrose Agar (PDA), and 70% alcohol.

Research procedure

Antagonism testing was carried out using a dual culture method, in which pure cultures of antagonistic fungi and pathogenic fungi were cut using a sterile cork drill and then placed in pairs on a PDA medium. After that, the culture was incubated at 25^o-27^o for 5x24 hours. The growth and interaction of the two moulds were observed every day from day 0 to day 5. Furthermore, the Mechanism of antagonistic interaction was analyzed using scanning electron microscopy (SEM) to observe changes in the structure of hyphae or cells in both fungi.

Data analysis

The data in this study were analyzed using quantitative description. Quantitative data analysis is used to The level of antagonism between *Nigrospora oryzae* and both pathogenic moulds (*Fusarium solani and Fusarium oxysporum*) was measured and calculated based on the formula developed by (Ihkwanisa et al., 2023).

$$I = \frac{r1 - r2}{r1} \times 100\% \qquad \textbf{(1)}$$

Notes:
I = antagonism
r1 = radius of pathogenic mould colonies growing away from *Nigrospora oryzae,*
r2 = radius of pathogenic mould colonies growing towards *Nigrospora oryzae*.

This data analysis was used to measure the level of antagonism and processed descriptively to explain, describe, and illustrate the data from the research results on the level of fungal antagonism that had been carried out.

Results and Discussion

Research on the antagonistic activity of *N. oryzae* was conducted using a dual culture approach. The data collected included morphological characteristics of the interaction between the antagonistic fungus *N. oryzae* and the pathogenic fungi *Fusarium oxysporum* and *Fusarium solani*. In addition, the level of antagonism between fungi was quantitatively calculated and evaluated. Histological observations of the structures of *N. oryzae* interacting and invading hyphae of pathogenic fungi were also included to analyze the Mechanism of mycoparasitism.

The results of morphological observations

Figure 1 shows the results of antagonism testing on the interaction of antagonistic fungi *N. oryzae* with pathogenic moulds *F. oxysporum* and *F. solani*.

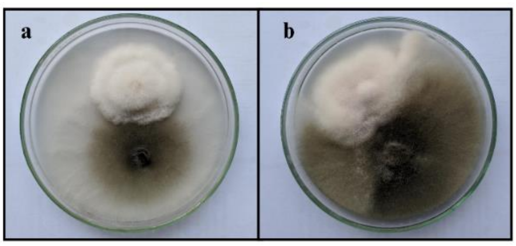

Figure 1 Colony surface between antagonistic fungus *Nigrosporae orizae* and *F. solani* (a). Colony surface between antagonistic fungus *Nigrosporae orizae* and *F. oxysporum* (b).

The results of the antagonism test indicated that *N. oryzae* could inhibit the development of *F. solani* and *F. oxysporum*. This can be seen from the growth of *N. oryzae*, which suppresses the growth of pathogenic fungi. These findings suggest that *N. oryzae* has the potential to be an effective antagonistic mould against *F. solani* and *F. oxysporum*. *N. oryzae* has been shown to have antifungal properties against various plant pathogens. For example, *N. oryzae* extracts showed a marked inhibitory effect against Cladosporium cladosporioides, a plant pathogen, which corroborates its potential as a biological control agent (Thanabalasingam et al., 2015). Furthermore, N. oryzae was also shown to inhibit the growth of Bipolaris maydis and Parastagonospora nodorum, which cause *Southern Leaf Blight* and *Septoria Nodorum Blotch* (Dutta et al., 2023).

N. oryzae also can inhibit the growth of various pathogenic moulds by producing bioactive compounds. This fungus has shown strong antimicrobial effects against several types of pathogens. Studies have shown that *N. oryzae* extract effectively inhibits Staphylococcus aureus and Candida albicans, indicating its potential as a biocontrol agent (Ghanem et al., 2025). In addition, the ethanol extract of *N. oryzae* also showed inhibitory activity against *Streptococcus pneumoniae* and *Salmonella typhi* with a clear zone of inhibition. One inhibitory Mechanism is the synthesis of bioactive compounds such as Aurofusarin, which can bind to proteins essential for pathogen survival, showing promising pharmacological potential. Compounds produced by *N. oryzae* can damage cell wall structure and disrupt fungal growth, key factors that support its effectiveness as a biological control agent (Ashwini & Rajagopal, 2016).

Antagonism power measurement results

The results of the antagonism test (Table 1) revealed that *N. oryzae* showed different levels of antagonism against the two pathogens. Against *F. oxysporum*, this fungus showed high inhibition (33.3%), while against *F. solani*, it only showed a low inhibitory effect (18.18%). In the dual culture test, the inhibitory ability *of N. oryzae* against both pathogens also varied. The highest inhibition value was achieved against *F. oxysporum* (77.2%), while the lowest was recorded at 46.5% (Mustofa et al., 2025). This difference indicates that the level of susceptibility of the two pathogenic fungi to antifungal compounds is different. *F. oxysporum* is known to be more susceptible to various antagonistic mechanisms, including production of antifungal compounds and induction of oxidative stress oksidatif (Al-Mutar, Alzawar, et al., 2023; Al-Mutar, Noman, et al., 2023). In contrast, *F. solani* exhibits different resistance mechanisms, including higher resistance to certain antifungal compounds and oxidative stress (Winn et al., 2003). This may explain why the antagonistic activity of *N. oryzae* against *F. solani* is lower than that of *F. oxysporum*.

Table 1. Antagonism of *Nigrospora oryzae* against pathogenic fungi *F. solani* and *F. Oxysporum*

No	Antagonistic fungi	Pathogenic fungi	R_1	R_2	Antagonism Power
1	*Nigrospora prize*	*F.solani*	22 mm	18 mm	18,18%
2	*Nigrospora orizae*	*F. oxysporum*	24 mm	16 mm	33,3%

Nigrospora oryzae has been shown to have broad antimicrobial effects, including against the pathogens *F. solani* and *F. oxysporum*. These findings indicate the ability of the fungus to produce bioactive compounds with potential antifungal characteristics (Ghanem et al., 2025). Furthermore, *N. oryzae* was reported to synthesize secondary metabolites with antifungal activity (S. Yang et al., 2022). The metabolites can markedly inhibit the mycelial development of *F. oxysporum*, showing a fairly strong antagonistic effect. It is suspected that the bioactive compounds produced by *N. oryzae* work by interfering with the growth and development process of the Fusarium pathogen. In a double culture study, *N. oryzae* showed effectiveness in suppressing Fusarium growth through a direct antagonism mechanism (Mustofa et al., 2025). This is evident from forming a clear zone of inhibition around the growth of Fusarium. These results support the hypothesis that *N. oryzae* has a competitive ability with Fusarium in terms of the struggle for growth space and nutrients, so it can significantly limit the proliferation of the pathogen.

Mechanism of mycoparasitism between *Nigrospora oryzae* moulds against *F. solani* and *F. oxysporum*

SEM testing results revealed the existence of a mycoparasitism mechanism (Figure 2). The fungus *N. oryzae* was able to attack *F. solani* and *F. oxysporum* through the Mechanism of 1) Mycoparasitism, *N. oryzae* acts as a parasite by penetrating, twining, and adhering to the hyphae of pathogenic moulds to compete for nutrients and space. This ability includes penetration, entanglement, and destruction of reproductive structures such as hyphae and pathogen spores (Sempere & Santamarina, 2008). 2) Pathogen cell wall degradation: *N. oryzae* secretes enzymes such as chitinase, β-1,3 glucanase, and β-1,6 glucanase that break down the cell wall of pathogenic moulds. The presence of the pathogen triggers the production of these enzymes during mycoparasitism (P. Yang, 2014). 3) competition for nutrients and space, *N. oryzae* competes with *F. solani* and *F. oxysporum* to obtain resources such as nutrients (Mustofa & Hastuti, 2024).

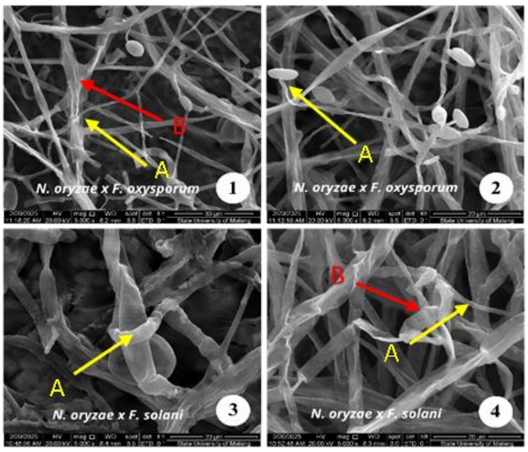

Figure 2 Micoparasistism mechanism of *N. oryzae* against *F. solani* and *F. oxysporum*

Image Caption: Figure 1. and Figure 4: Hyphae of antagonistic fungus *N. oryzae* attached to hyphae of pathogen (yellow arrow Hyphae of antagonistic fungus and red arrow Hyphae of pathogenic fungus. Figure 2. A, Conidia shape of *N. oryzae*. Figure 3. A, Mechanism of mycoparasitism, hyphae of *N. oryzae* wrapped around hyphae of pathogenic mould. This finding is to the results of a study (Mustofa et al., 2025), which reported that antagonistic fungi such as *Trichoderma* *harzianum* and *Nigrospora oryzae* can inhibit the growth of plant pathogens, including *Fusarium oxysporum* and *Fusarium solani,* through several mechanisms. These include competition for space and nutrients and synthesizing antifungal compounds from secondary metabolites.

Furthermore, *N. oryzae* also produces *volatile organic compounds* (VOCs) that have been shown to effectively block Fusarium development (Ting et al., 2010). These VOC compounds can diffuse through the air and act without requiring direct physical contact, thus expanding the scope of their effectiveness. However, further characterization of the specific type of VOCs and their mode of action still requires further exploration. Another advantage of *N. oryzae* is its tolerance to variations in environmental conditions, which strengthens its role as a biocontrol agent. It exhibits optimal growth over various temperatures and water availability, allowing it to stably suppress Fusarium in diverse cultivation systems (Sempere & Santamarina, 2008).

Research on the characteristics of antagonism of antagonistic fungi *Nigrospora oryzae* against *Fusarium oxysporum* and *Fusarium solani* that cause *Fusarium wilt* in chilli plants needs to be done to determine the effectiveness of antagonistic fungi *Nigrospora oryzae* against *Fusarium wilt* of chilli plants *(Capsicum annum).* Research can be used as a source of learning biology because research on the characteristics of fungal antagonism is still small. Therefore, this research can be used as a learning resource for students as one of the reference sources in learning materials and instructions in practicum activities of fungal antagonism. Learning resources for practicum instructions can be used as a guide and information for practicum activities (Pramana, 2024). The practicum guide itself is a guide that guides the steps in carrying out a practicum activity, which can utilize various learning resources available to students (Pramana, 2024).

Learning resources are all types of media, objects, data, facts, ideas, people, and others that can facilitate the learning process for students (Samsinar, 2019). In the context of this study, diverse learning resources refer to a variety of learning resources of various types, not only textbooks but also those sourced from the internet, not only in printed form but also in visual, audio, or audio-visual form (Salahuddin, 2022).

Conclusion

The results of the study concluded that *Nigrospora oryzae* has antimicrobial ability against several pathogens, including *Fusarium solani* and *Fusarium oxysporum*. The study showed that *N. oryzae* has high antagonistic power against *F. oxysporum* with a percentage of 33.3%, while against *F. solani*, its antagonistic power is lower, which is 18.18%. Biology learning resources can utilize the results of observations made in the laboratory, one of which is the observation of fungal antagonism. The contribution of the results of the identification of fungal antagonism is used as a learning resource to understand the concept in learning fungi through direct practical activities in the Laboratory.

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