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# Isolation of antagonistic fungi from local rice and rhizosphere as potential biocontrol agents against *Pyricularia oryzae*

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[3]Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, South Sulawesi, Indonesia. Tel./fax.: +62-411-586014, ♥email: sylviasjam@yahoo.com

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**Abstract.** *Sari DE, Sjam S, Rosmana A, Melina, Sulfiani. 2026. Isolation of antagonistic fungi from local rice and rhizosphere as potential biocontrol agents against* Pyricularia oryzae*. Biodiversitas 27 (4): d270419. https://doi.org/10.13057/biodiv/d270419*. Antagonistic fungi act as biocontrol agents for plant diseases. *Pyricularia oryzae*, the causative agent of blast disease, is one of the main pathogens affecting rice crops. In Indonesia, this pathogen is a major constraint in rice cultivation. The use of biocontrol agents can be an alternative to control *P. oryzae*. The purpose of this study was to test fungi isolated from local rice plants and their rhizosphere as control agents for *P. oryzae*. Samples were randomly collected from two local rice plants and their rhizospheres at different locations. Fungi from the rhizosphere were isolated using serial dilution techniques. At the same time, plant parts, namely roots, stems, and leaves, were sterilized using surface sterilization methods and grown on potato dextrose agar media. Pathogenicity tests used rice seeds as indicator plants. Antagonism tests used the dual-culture method with *P. oryzae* as the pathogen. The fungal isolates were identified to the genus level. The results revealed that 31 fungal isolates were obtained from local rice plants and their rhizosphere. Pathogenicity tests showed that 29 isolates were non-pathogenic, with seed germination rates on PDA medium averaging 73%-100% and on sterile soil medium averaging 60%-100%. Dual culture tests showed that 11 isolates inhibited the growth of *P. oryzae* by more than 50%. The most effective isolate was RH4C3, which exhibited up to 82.52% inhibition and showed a significant difference compared to the other fungi, except for isolates RH4C22 and HB3C1. Microscopic identification revealed several genera with the greatest potential as antagonists, namely *Trichoderma* sp., *Paecilomyces* sp., *Penicillium* sp., *Nigrospora* sp., and *Pythium* sp. These findings provide insights into the diversity and potential of local biological control agents that utilize antagonistic fungi, their further identification and field application should be undertaken.

**Keywords**: Antagonist, endophytic fungi*, Pyricularia oryzae*, rhizosphere microbiome, rice blast

## INTRODUCTION

Plant diseases are a major threat to global agriculture because they reduce crop productivity and cause substantial economic losses, estimated at US$220 billion annually (Hossain et al. 2024). Rice is one of the world's most important staple crops (Mohidem et al. 2022). However, its production is seriously constrained by blast disease caused by *Pyricularia oryzae*, one of the most destructive rice pathogens (Song et al. 2025).

In Indonesia, blast disease ranks as the fourth leading cause of crop failure, with an average affected area of 9,788 ha per year over the last decade and disease intensity reaching 90% on the panicle neck of susceptible varieties (Kuswinanti et al. 2023). The disease can attack rice plants at all growth stages and infect leaves, nodes, panicle necks, and grains (Sanuriza et al. 2024). Its effect on yield is severe. In Ciherang rice, blast intensity reached 55.60% on leaves and 37.75% on panicle necks, resulting in estimated yield losses of 3.65 tonnes/ha, or about 61% (Suganda et al. 2016). Blast pathogens have been reported to cause yield losses of up to 50% (Kongcharoen et al. 2020) and 90% (Neuphane and Bushal 2021), with losses of up to 80% under favorable conditions (Kongcharoen et al. 2020).

Blast control in the field still relies heavily on chemical fungicides. Farmers commonly use pesticides to reduce qualitative and quantitative losses (Kaiser and Burger 2022), and rice cultivation may involve 16-27 applications in a single growing season (Yulia et al. 2020). This practice raises production costs (Agustina and Rachman 2009) and causes environmental problems (Kaur 2024). In addition, synthetic fungicides have not provided durable control because they can select for resistant pathogen populations (Nguyen et al. 2022). The blast pathogen has been reported to be resistant to various groups of fungicides (Kongcharoen et al. 2020), including isoprothiolane (Hu et al. 2014) and phosphorothiolate (Kim and Kim 2009). Blast disease is difficult to control because *P. oryzae* has high genetic diversity and highly adaptive cell development and morphology, causing *P. oryzae* strains to change their virulence in a short time, depending on the host and changes in climatic conditions (Khemmuk 2017). Therefore, there is a need for sustainable alternatives, such as antagonistic fungi as biocontrol agents against rice blast.

Antagonistic fungi are increasingly recognized as an alternative for plant disease control (Thambugala et al. 2020; Kuswinanti et al. 2023). Beneficial fungi can colonize pathogen-exposed habitats such as the rhizosphere,

phyllosphere, and plant organs (Ghorbanpour et al. 2017). They naturally occur in the phyllosphere (Wilson and Widow 1994; Ginting et al. 2005), rhizosphere (Noviyanti et al. 2024), and plant tissues without causing symptoms (Raunsai et al. 2025). Some form mutualistic associations with plants (Li et al. 2025) and produce bioactive compounds that help defend their hosts (Anam et al. 2024). Several genera have shown potential for blast suppression, including *Penicillium* sp. (Liang et al. 2021), *Aspergillus* sp., and *Trichoderma* sp. (Chou et al. 2020; Prismantoro et al. 2024; Choudhir et al. 2025).

Rice plants harbor diverse microorganisms, some of which possess antagonistic properties (Naik et al. 2009). The rhizosphere in particular supports a richer and more diverse microbial community than non-rhizosphere soil (Liza et al. 2015). Local fungal isolates are of special interest because they are generally better adapted to their native environment than introduced fungi (Asniwita et al. 2025). This is relevant to Sinjai local rice, which has been cultivated for generations in the West Sinjai District and is now maintained in only one hamlet. Its cultivation does not use inorganic fertilizers or chemical pesticides, so it is considered organic rice (Yustisia 2021). Such conditions may support high fungal diversity through greater soil organic matter. Fungi are important in maintaining ecosystem balance (Hyde et al. 2019) and provide valuable ecological functions in agricultural systems (Heimpel and Mills 2017). However, fungi associated with local rice varieties remain underexplored, even though they may offer effective and adaptable biocontrol potential.

This research is important as an alternative technology for environmentally friendly plant disease control, supporting sustainable agricultural development. This study aimed to (i) isolate and characterize fungi associated with local rice and its rhizosphere, (ii) evaluate their pathogenicity on rice seeds, (iii) assess in vitro antagonistic activity and interaction mechanisms with *P. oryzae*.

## MATERIALS AND METHODS

### Fungal pathogen

The pathogenic fungal species used in this study was *P. oryzae*. The fungus was isolated from symptomatic rice plants collected from Bantaeng District, South Sulawesi, Indonesia, in June 2024. Leaves showing symptoms of blast disease were grown in potato dextrose agar medium and incubated. The identified *P. oryzae* (isolate code PoB1) fungal species was then re-cultured on potato dextrose agar medium (Figure 1).

### Sampling and isolation of fungi from the rhizosphere and local rice plants

Sampling was conducted in rice fields in Puncak Hamlet, Gunung Perak Village, West Sinjai Sub-district, Sinjai District, South Sulawesi, Indonesia, at altitudes of 800-1000 meters above sea level. Sampling was carried out in June 2024 in accordance with the rice planting schedule of the Puncak hamlet residents. The samples were local rice plants of the red rice and black sticky rice varieties. The plants used as samples were 35 days old after planting. Samples were randomly collected by selecting 5 points across the land. Each point was sampled by taking 1 rice plant and ±250 g of rhizosphere soil at a depth of 5-10 cm. Plant and soil samples were placed in sterile plastic bags and transported to the laboratory for isolation. The rice plant isolation process involved taking 5 stems per clump at one point, yielding a total of 25 isolated rice plants. We realize that one factor contributing to the lack of fungi isolated from plant tissue is that samples of each local rice variety were collected from only one field per variety. Therefore, for further research, samples will be taken from several farmers' fields with a larger number of plants.

Fungal isolation was performed on PDA medium. PDA (Potato Dextrose Agar) medium was prepared using 200 g of potatoes, 20 g of sugar, 17 g of agar-agar, and 1000 mL of distilled water. For sterilization, PDA medium was placed in an autoclave for 40-60 minutes at 121°C. To avoid the contamination of bacteria, 50 mg/L of antibiotics (Chloramphenicol) was added to the PDA medium.

In the isolation process, the plant parts were first disinfected with distilled water for 60 seconds with distilled water for 60 seconds, then a 70% ethanol solution for 60 seconds, 2.5% sodium hypochlorite for 60 seconds, 70% ethanol for 30 seconds, and finally rinsed with sterile distilled water, and then dried. The sterilized plant parts (roots, stems, and leaves) were cut into 1-2 cm pieces, placed on PDA media (4 pieces per plate), and incubated at 28°C for 7 days. To ensure that the sterile distilled water used in the final rinse did not contain microbes, the final rinse water was poured onto PDA medium and spread evenly. Observations were made for 7 days, and no fungal growth was observed, concluding that the surface sterilization performed was effective.

Isolation of fungi from the rhizosphere using the dilution method. For isolation, 1 g of soil from a rice field was placed in a test tube, then 9 mL of sterile distilled water was added and shaken until homogeneous. From this stock solution, 1 mL was poured into a second test tube and shaken until dissolved. This continued a dilution of 104. After that, 0.1 mL of suspension was taken from the fourth test tube, poured into a Petri dish containing PDA medium, spread using a triangular spreader, and incubated for 48 hours (2 days). PDA medium without inoculum was used as a control in plant tissue and rhizosphere isolation, and no contamination was detected. Mycelium grown from plant parts and rhizosphere samples was transferred to culture media to obtain pure cultures. Each isolate was labeled by variety and part from which it was isolated.

### Pathogenicity test

Pathogenicity test was carried out using two methods. This test aimed to determine whether the fungi obtained were pathogenic or non-pathogenic. The seeds used were Ciherang rice seeds (varieties susceptible to *P. oryzae*) as indicator plants.

The first method of pathogenicity test used the PDA medium. Fungal isolates were grown on PDA medium and incubated for 7 days. Rice seeds were sterilized with sterile distilled water for 60 seconds, followed by 70% ethanol for

60 seconds, 2.5% sodium hypochlorite for 60 seconds, and finally washed with sterile distilled water for 60 seconds, then dried on sterile filter paper. Ten rice seeds per dish were placed in fungal culture and incubated for 7 days at 28°C. Each isolate was repeated three times.

The second pathogenicity test method used a sterile soil medium. This test was conducted by soaking rice seeds in a fungal suspension for 30 minutes and planting them in a sterilized soil medium. The planted containers were placed in a room with adequate lighting at 28°C. Each container was planted with 10 seeds, and the experiment was repeated 3 times. Observations were made for 7 days. Observations in both pathogenicity tests were recorded according to Mirsam et al. (2021a). Fungal isolates showed germination and seed growth rates below 50%; leaves with necrosis were not selected for further testing and were classified as pathogenic fungi.

**In vitro evaluation of antifungal activity of rhizosphere and local rice plants against *P. oryzae***

The test was conducted using the dual-culture method, with fungal isolate colonies grown in pairs—the control treatment used *P. oryzae* grown singly on PDA medium. Purified fungal isolates aged 7 days were collected using a 4 mm-diameter cork borer. In the test, both fungal isolates (Pathogen and antagonist) were grown side by side on PDA medium in Petri dishes, 3 cm from the edge. The cultures were incubated at room temperature, and the colony radius was measured for 7 days. Each fungal treatment was repeated 3 times. The observation parameters in this test were as follows:

The percentage of fungal growth inhibition was measured and calculated using the formula of Lu et al. (2022).

$$\% \text{ Inhibition} = \frac{(R1 - R2)}{R1} \text{x } 100$$

Where: R1: Radius growth of *P. oryzae* towards the opposite in the control plates, R2: Radius growth of *P. oryzae* towards the antagonist fungi in test plates.

Percentage inhibition of pathogenic fungal growth is categorized into several classes: (Živković et al. 2010; Nuraini et al. 2017). Class 0: 0% (No antagonistic activity), Class 1: ≤30% (Low antagonistic activity), Class 2: 30 - ≤50% (Moderate antagonistic activity), Class 3 : 50 - ≤70% (High antagonistic activity), Class 4: ≥70% (Very high antagonistic activity).

Observation of antagonistic mechanisms based on Porter (1924) and Skidmore and Dickson (1976) was conducted macroscopically through direct observation of Dual Culture as follows: (i) Interaction type A shows a mutually intermingling type in isolates: No interaction. (ii) Interaction type B shows an overgrowing type: competition. (iii) Interaction type C shows that the growth of each fungus was inhibited but almost close to each other: antibiosis (slight inhibition). (iv) Interaction type D shows growth around the organism: the growth of the pathogen was faster than the antagonist. (v) Interaction type E shows inhibition at a distance: antibiosis (strong inhibition). (vi) Mycelium weight was determined after 7 days of observation by scraping it from the dual culture medium. The *P. oryzae* mycelium and the antagonistic fungus mycelium were scraped separately, and then *P. oryzae* mycelium was weighed.

**Morphological identification of isolated fungal isolates**

Fungal identification was performed macroscopically and microscopically. The identification process was carried out with the aid of Barnett and Hunter (1998) and Watanabe's Identification Book (2010). Macroscopic observations included colony color, texture, shape, and elevation. Microscopic observations were performed using a light microscope at 40× and 100× magnification. The characteristics observed included hyphae shape, conidiophores, and conidia. These characteristics were then compared with Watanabe's (2010) Identification Key. Fungal isolates could only be identified to the genus level. The collection was stored with a code for each isolate. The isolate from local black sticky rice was coded H (RH2C1-HD2C2), while the isolate from local red rice was coded M (RM0C1-MD2C2).

**Data analysis**

This study used a completely randomized design with fungal isolates as factors, with each treatment repeated three times in each test. Data transformation was not performed because the percentage data had a clear biological meaning with direct interpretation that 0% (seeds did not grow/no inhibition) and 100% (all seeds grow/inhibition). Statistical analysis was performed using IBM SPSS Statistics. The observation data on germination and growth rates, inhibition percentage, and mycelium weight were analyzed using Analysis of Variance (ANOVA) to test for significant differences between treatments. If there were significant differences among treatments, a post hoc test was performed using Duncan's test at the 5% significance level.

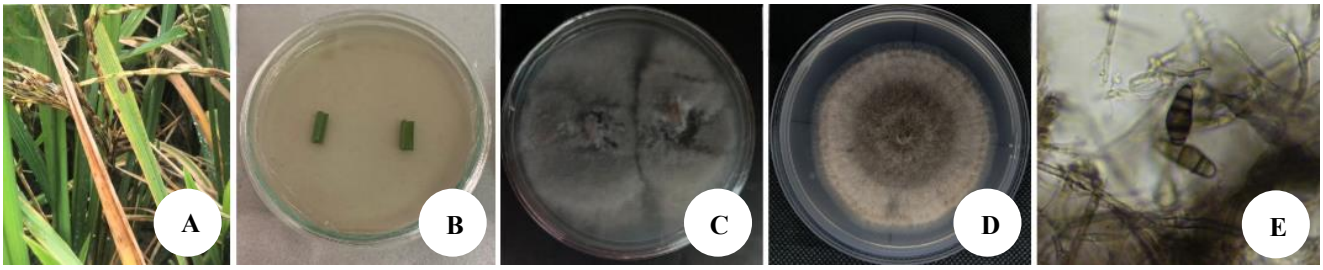


**Figure 1.** Isolation stages of *Pyricularia oryzae*: A. Symptoms of *Pyricularia oryzae*, B, C. Isolation *Pyricularia oryzae*, D, E. Characteristics of macroscopic and microscopic of the *Pyricularia oryzae*

## RESULT AND DISCUSSION

### Isolation of antagonistic fungi

Antagonistic fungi were found in the rhizosphere and on the surface of plant tissues. Isolation revealed that 31 fungal isolates were obtained from two local Sinjai rice varieties (local black glutinous rice and local red rice). Fungi were isolated from the rhizosphere, roots, stems, and leaves of two local Sinjai rice varieties. In black glutinous rice, 18 fungal isolates were obtained, of which 12 isolates were from the rhizosphere, 1 isolate from the roots, 4 isolates from the stems, and 1 isolate from the leaves. 13 fungal isolates were obtained from local red rice, including 6 from the rhizosphere, 3 from the roots, 3 from the stems and 1 from the leaves (Table 1). The isolates from black sticky rice were coded H (RH2C1-HD2C2), while the isolates from red rice were coded M (RM0C1-MD2C2).

The results of fungal isolation showed differences between different parts. Based on the table above, candidate antagonistic fungi were mostly found in the rhizosphere, including both local black rice and red rice varieties. Only two isolates of fungi were recovered from the leaves.

### Pathogenicity test

A total of 31 fungal isolates were successfully isolated and subjected to pathogenicity testing to determine the nature of the fungi. Pathogenicity testing was performed by growing rice seeds treated with the fungi. Fungal isolates that caused plant disease symptoms in both pathogenicity test methods, including seed germination and growth rates below 50% and leaf necrosis, were not advanced to the next testing phase. The observation parameters in the pathogenicity test were based on seed germination rates and necrosis symptoms. These serve as the basic morphological criteria for detecting plant pathogens. Those isolates exhibiting low germination rates and causing necrosis symptoms were classified as pathogens and excluded from further tests.

TThe pathogenicity test results showed that two isolates, namely RH3C1 and RH3C3, from two types of local rice were suspected to be pathogenic (Table 2). In the PDA media method, no seeds (0%) germinated with fungal isolates RH3C1 and RH3C3, indicating that these isolates were significantly different from the control and other isolates. On soil medium, the germination rates of rice seeds in RH3C1 and RH3C3 isolates were 6.67% and 73.33%, respectively. Fungal isolates capable of germinating rice seeds above 50% were mostly from the rhizosphere and plant tissues (roots, stems and leaves).

The difference between the pathogenicity tests using PDA and soil media was that a closed container was used in PDA medium, whereas an open container was used in soil medium. As can be seen in the table above, isolates that caused necrosis on PDA medium did not cause necrosis on soil medium (Table 2).

### Antagonistic activity

In this study, 29 fungal isolates were tested individually against *P. oryzae* using PDA medium. Significant differences in the percentage of growth inhibition were observed among the 29 isolated samples during the observation period. The inhibition percentage ranged from 24.49% to 82.52% (Table 3). On the 1st day, the highest percentage of inhibition occurred in isolate RH4C21 and was significantly different from other isolates. From day 2 to day 7, isolate showed higher inhibition than all other isolates, namely RH4C3, and was significantly different from all treatments. The inhibition percentage can be seen from the growth rate of the diameter of *P. oryzae* in the control, which was faster than that of *P. oryzae* in the dual culture (Figure 2). On day 7, the growth rate of *P. oryzae* mycelium treated with fungal isolates RH4C3, RH4C22, HB3C1, RH4C23, and RH4C21 showed a slower growth rate compared to other fungal isolates.

A dual culture test evaluating the antagonistic potential of 29 isolated fungi showed a continuously increasing inhibition rate up to day 7 (Table 3). Of these, 11 isolates had growth inhibition above 50% (Table 4). The inhibition levels of these isolates on day 7 were RH4C21 (61.43%), RH4C22 (79.14%), RH4C23 (72.05%), RH4C3 (82.52%), HB3C1 (73.97%), HB2C2 (50.44%), HD2C2 (50.65%), RM0C3 (54.83%), RM1C2 (50.89%), MA1C1 (52.32%), and MB2C1 (52.08%).

**Table 1.** Fungi isolated from local rice plants and rhizosphere

| Isolates code | Local plant | Parts |
|---|---|---|
| RH2C1 | Local black glutinous rice | Rhizosphere |
| RH2C2 | Local black glutinous rice | Rhizosphere |
| RH3C1 | Local black glutinous rice | Rhizosphere |
| RH3C2 | Local black glutinous rice | Rhizosphere |
| RH3C3 | Local black glutinous rice | Rhizosphere |
| RH4C21 | Local black glutinous rice | Rhizosphere |
| RH4C22 | Local black glutinous rice | Rhizosphere |
| RH4C23 | Local black glutinous rice | Rhizosphere |
| RH4C3 | Local black glutinous rice | Rhizosphere |
| RH5C1 | Local black glutinous rice | Rhizosphere |
| RH5C2 | Local black glutinous rice | Rhizosphere |
| RH6C2 | Local black glutinous rice | Rhizosphere |
| HA1C1 | Local black glutinous rice | Roots |
| HB3C1 | Local black glutinous rice | Stem |
| HB3C2 | Local black glutinous rice | Stem |
| HB2C1 | Local black glutinous rice | Stem |
| HB2C2 | Local black glutinous rice | Stem |
| HD2C2 | Local black glutinous rice | Leaves |
| RM0C1 | Local red rice | Rhizosphere |
| RM0C3 | Local red rice | Rhizosphere |
| RM1C1 | Local red rice | Rhizosphere |
| RM1C2 | Local red rice | Rhizosphere |
| RM3C1 | Local red rice | Rhizosphere |
| RM3C2 | Local red rice | Rhizosphere |
| MA1C1 | Local red rice | Roots |
| MA1C2 | Local red rice | Roots |
| MA2C1 | Local red rice | Roots |
| MB2C1 | Local red rice | Stem |
| MB2C2 | Local red rice | Stem |
| MB3C1 | Local red rice | Stem |
| MD2C1 | Local red rice | Leaves |

**Table 2.** Average germination and necrotic symptoms in rice seedling

| Isolates | Pathogenicity test method used PDA media | | Pathogenicity test method used sterile soil | |
|---|---|---|---|---|
| | Average seed growth (%) | Necrotic* | Average seed growth (%) | Necrotic* |
| Control | 100.00±0.00 $^{b}$ | - | 100.00±0.00 $^{e}$ | - |
| RH2C1 | 86.67±23.09 $^{b}$ | - | 93.33±11.54 $^{de}$ | - |
| RH2C2 | 73.33±30.55 $^{b}$ | - | 66.67±11.54 $^{bc}$ | - |
| RH3C1 | 0.00±0.00 $^{a}$ | - | 6.67±11.54 $^{a}$ | + |
| RH3C2 | 100.00±0.00 $^{b}$ | - | 100.00±0.00 $^{e}$ | - |
| RH3C3 | 0.00±0.00 $^{a}$ | - | 73.33±11.54 $^{bcd}$ | - |
| RH4C21 | 86.67 ±11.54 $^{b}$ | - | 80.00±20.00 $^{bcde}$ | - |
| RH4C22 | 86.67 ±11.54 $^{b}$ | - | 86.67±11.54 $^{cde}$ | - |
| RH4C23 | 100.00 ±0.00 $^{b}$ | - | 80.00±0.00 $^{bcde}$ | - |
| RH4C3 | 80.00±20.00 $^{b}$ | - | 100.00±0.00 $^{e}$ | - |
| RH5C1 | 93.33 ±11.54 $^{b}$ | - | 73.33±23.09 $^{bcd}$ | - |
| RH5C2 | 100.00 ±0.00 $^{b}$ | - | 93.33±11.54 $^{de}$ | - |
| RH6C2 | 93.33±11.54 $^{b}$ | + | 73.33±11.54 $^{bcd}$ | - |
| HA1C1 | 73.33±11.54 $^{b}$ | - | 60.00±20.00 $^{a}$ | - |
| HB3C1 | 93.33±11.54 $^{b}$ | - | 60.00±20.00 $^{a}$ | - |
| HB3C2 | 93.33±11.54 $^{b}$ | - | 100.00±0.00 $^{e}$ | - |
| HB2C1 | 86.67±23.09 $^{b}$ | - | 80.00±0.00 $^{bcde}$ | - |
| HB2C2 | 80.00±20.00 $^{b}$ | - | 80.00±20.00 $^{bcde}$ | - |
| HD2C2 | 93.33±11.54 $^{b}$ | - | 86.67±11.54 $^{cde}$ | - |
| RM0C1 | 80.00±0.00 $^{b}$ | - | 100.00±0.00 $^{e}$ | - |
| RM0C3 | 93.33±11.54 $^{b}$ | - | 100.00±0.00 $^{e}$ | - |
| RM1C1 | 93.33±11.54 $^{b}$ | - | 93.33±11.54 $^{de}$ | - |
| RM1C2 | 93.33±11.54 $^{b}$ | + | 80.00±0.00 $^{bcde}$ | - |
| RM3C1 | 80.00±20.00 $^{b}$ | - | 80.00±20.00 $^{bcde}$ | - |
| RM3C2 | 93.33±11.54 $^{b}$ | - | 93.33±11.54 $^{de}$ | - |
| MA1C1 | 86.67±11.54 $^{b}$ | - | 100.00±0.00 $^{e}$ | - |
| MA1C2 | 93.33±11.54 $^{b}$ | - | 100.00±0.00 $^{e}$ | - |
| MA2C1 | 86.67±11.54 $^{b}$ | - | 73.33±11.54 $^{bcd}$ | - |
| MB2C1 | 93.33±11.54 $^{b}$ | - | 100.00±0.00 $^{e}$ | - |
| MB2C2 | 86.67±23.09 $^{b}$ | + | 86.67±23.09 $^{cde}$ | - |
| MB3C1 | 100.00±0.00 $^{b}$ | - | 93.33±11.54 $^{de}$ | - |
| MD2C1 | 93.33±11.54 $^{b}$ | + | 80.00±0.00 $^{bcde}$ | - |

Note: Means in the same column followed by the same letter did not significantly different according to Duncan's test at 5% (α 0.05). *(+ Necrosis): Pathogenic, *(- No necrosis): Non pathogenic

The weight of antagonistic fungal mycelium was an indicator of the effectiveness of the agent in inhibiting pathogens. The weight of mycelium, when combined with the percentage of inhibition, determines the mechanism of interaction in antagonistic fungi. Table 5 shows that the lowest mycelium weight was observed for isolate RH4C3 (0.09 g), with an inhibition percentage of 82.52%, indicating a competitive interaction. Meanwhile, the highest mycelium weight was observed in isolate RH5C1 at 1.90 g, and its inhibition percentage was the lowest at 24.49%, indicating that the fungus lacked an interaction mechanism.

**Antagonistic mechanism**

Antagonistic fungal isolates exhibit various mechanisms of interaction. Macroscopic observations of the interaction types and antagonistic interaction mechanisms of 29 fungal isolates and *P. oryzae* are presented in Table 4. There are four types of interactions observed in this study based on Porter (1924), namely interaction types A, B, C, D and E. Interaction type A shows a mutually intermingling type in 11 isolates (Figures 3.C, 3.I, 3.J, 3.K, 3.N, 3.R, 3.S, 3.U, 3.V, 3.X, 3.Y, 3.Z). Interaction type B shows the overgrowing type in 8 isolates (Figures 3.D, 3.E, 3.F, 3.G, 3.L, 3.P, C.1). Type C shows that the growth of each fungus is inhibited but almost close to each other in 3 isolates (Figures 3.M, 3.O, 3.Q, A.1). Type D shows growth around the organism in 4 isolates (Figures 3.A, 3.B, 3.H, B.1). Type E shows inhibition at a distance in 2 isolates (Figures 3.T, 3.W).

In this study, two mechanisms can be observed macroscopically: antibiosis and competition. Antibiosis occurs when a clear zone forms between the pathogenic fungus and the antagonistic fungus, as in the case of the fungal isolates HB3C2, HB2C2, RM0C1, RM1C2, MA1C1, and MB2C2 (Figure 3, Table 6). The competition mechanism occurs when the antagonistic fungal colony covers the pathogen colony, and the antagonistic fungus grows faster, as seen in the dual culture of *P. oryzae* with fungal isolates RH4C21, RH4C22, RH4C23, RH4C3, HB3C1, HD2C2, RM0C3, MB2C1, and MD2C1 (Figure 4, Table 6).

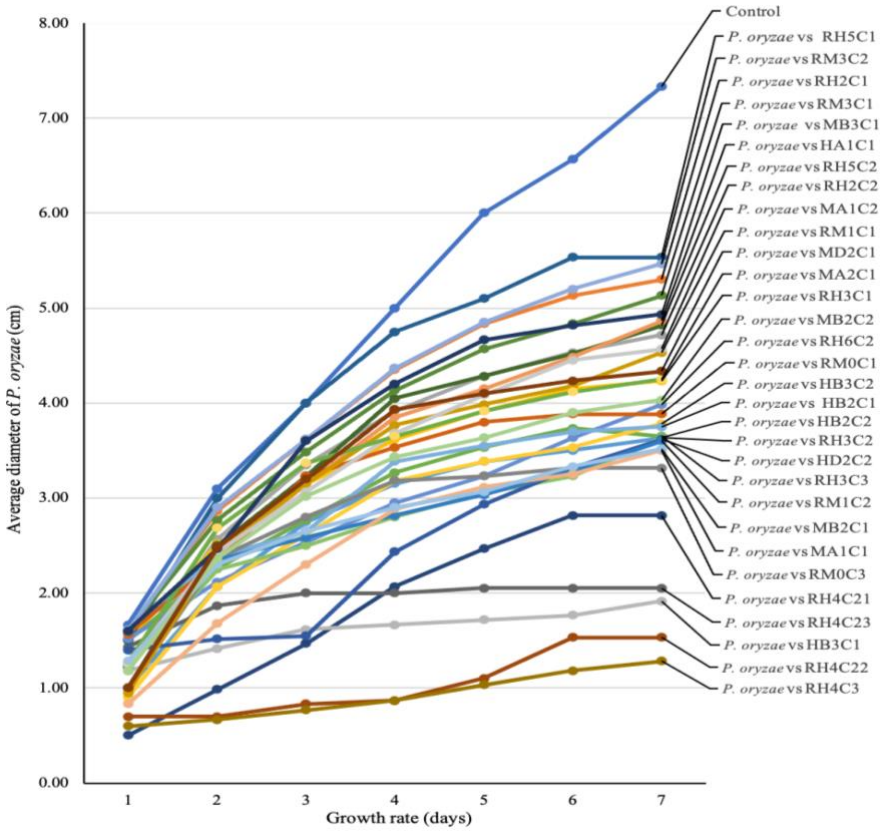


**Figure 2.** Daily cumulative growth rate of *Pyricularia oryzae*

**Table 3.** Percentage of growth inhibition of *Pyricularia oryzae* in dual culture test

| Isolates | Inhibition (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
| RH2C1 | 3.92$^{a}$ | 7.53$^{ab}$ | 9.58$^{ab}$ | 13.00$^{ab}$ | 19.44$^{ab}$ | 21.83$^{ab}$ | 27.75$^{abc}$ |
| RH2C2 | 25.92$^{e}$ | 17.20$^{cde}$ | 16.25$^{bcd}$ | 21.67$^{bcdefg}$ | 28.61$^{bcde}$ | 30.92$^{bcd}$ | 35.33$^{abcdef}$ |
| RH3C2 | 39.95$^{f}$ | 33.33$^{ij}$ | 31.25$^{defgh}$ | 37.00$^{hij}$ | 43.61$^{fghi}$ | 46.75$^{fghij}$ | 48.54$^{ghij}$ |
| RH4C21 | 69.98$^{j}$ | 68.28$^{m}$ | 63.33$^{k}$ | 58.67$^{lm}$ | 58.89$^{jk}$ | 57.10$^{j}$ | 61.43jk |
| RH4C22 | 57.97$^{hi}$ | 77.42$^{n}$ | 79.17$^{l}$ | 82.67$^{n}$ | 81.67$^{mn}$ | 76.61$^{kl}$ | 79.14$^{l}$ |
| RH4C23 | 13.91$^{bc}$ | 39.78$^{ij}$ | 50.00$^{ij}$ | 60.00$^{lm}$ | 65.83$^{kl}$ | 68.78$^{k}$ | 72.05$^{kl}$ |
| RH4C3 | 63.97$^{ij}$ | 78.49$^{n}$ | 80.83$^{l}$ | 82.67$^{n}$ | 82.78$^{n}$ | 81.93$^{l}$ | 82.52$^{l}$ |
| RH5C1 | 9.93$^{abc}$ | 3.23$^{a}$ | 0.00$^{a}$ | 5.00$^{a}$ | 15.00$^{a}$ | 15.71$^{a}$ | 24.49$^{a}$ |
| RH5C2 | 39.95$^{e}$ | 19.35$^{cdef}$ | 19.17$^{bcd}$ | 19.00$^{bcde}$ | 28.61$^{bcde}$ | 31.21$^{bcde}$ | 34.05$^{abcdef}$ |
| RH6C2 | 8.88$^{abc}$ | 31.72$^{hij}$ | 36.67$^{fgh}$ | 41.00$^{ijk}$ | 46.11$^{ghi}$ | 44.63$^{efghij}$ | 45.54$^{efghi}$ |
| HA1C1 | 39.95$^{f}$ | 22.58$^{efg}$ | 21.25$^{bcd}$ | 23.00$^{bcdefg}$ | 30.83$^{bcde}$ | 31.70$^{bcde}$ | 33.67$^{abcdef}$ |
| HB3C1 | 29.04$^{e}$ | 54.30$^{l}$ | 59.58$^{jk}$ | 66.67$^{hm}$ | 71.39$^{lm}$ | 73.13$^{kl}$ | 73.97$^{l}$ |
| HB3C2 | 45.96$^{fg}$ | 33.33$^{ij}$ | 34.58$^{efgh}$ | 36.33$^{hij}$ | 43.61$^{fghi}$ | 48.68$^{fghij}$ | 48.68$^{ghij}$ |
| HB2C1 | 2.88$^{a}$ | 23.12$^{efg}$ | 33.75$^{efgh}$ | 32.33$^{fghij}$ | 40.83$^{efghi}$ | 43.61$^{defghi}$ | 48.89$^{ghij}$ |
| HB2C2 | 26.96$^{e}$ | 27.42$^{fgh}$ | 37.50$^{gh}$ | 44.00$^{jk}$ | 49.17$^{hij}$ | 50.73$^{ij}$ | 50.44$^{ghij}$ |
| HD2C2 | 15.81$^{cd}$ | 51.08$^{kl}$ | 61.25$^{k}$ | 51.33$^{kl}$ | 51.11$^{ij}$ | 49.49$^{ghij}$ | 50.65$^{ghij}$ |
| RM0C1 | 5.82$^{ab}$ | 24.73$^{efgh}$ | 19.17$^{cde}$ | 29.33$^{defghi}$ | 36.67$^{efgh}$ | 40.93$^{defghi}$ | 46.98$^{fghi}$ |
| RM0C3 | 40.93$^{f}$ | 23.66$^{efg}$ | 30.00 $^{efgh}$ | 36.33$^{hij}$ | 46.11$^{ghi}$ | 49.56$^{ghij}$ | 54.83$^{ij}$ |
| RM1C1 | 43.01$^{fg}$ | 21.51$^{def}$ | 21.25$^{bcd}$ | 24.67$^{bcdefgh}$ | 33.61$^{cdefg}$ | 36.27$^{cdefg}$ | 38.21$^{bcdefg}$ |
| RM1C2 | 26.96$^{e}$ | 24.19$^{efg}$ | 35.42$^{efgh}$ | 43.67$^{jk}$ | 49.44$^{ij}$ | 50.04$^{hij}$ | 50.89$^{bcdefg}$ |
| RM3C1 | 3.92$^{a}$ | 10.75$^{bcd}$ | 12.92$^{bcd}$ | 17.33$^{abcd}$ | 23.89$^{abcd}$ | 26.29$^{abc}$ | 29.81$^{abcd}$ |
| RM3C2 | 3.92$^{a}$ | 6.45$^{ab}$ | 9.58$^{ab}$ | 12.67$^{ab}$ | 19.17$^{ab}$ | 20.82$^{ab}$ | 25.37$^{ab}$ |
| MA1C1 | 50.12$^{gh}$ | 45.70$^{jk}$ | 42.50$^{hi}$ | 42.33$^{ijk}$ | 48.06$^{hij}$ | 50.54$^{ij}$ | 52.32$^{hij}$ |
| MA1C2 | 25.06$^{e}$ | 21.51$^{def}$ | 23.33$^{cde}$ | 26.33$^{cdefgh}$ | 31.94$^{cdef}$ | 32.30$^{bcde}$ | 37.76$^{bcdefg}$ |
| MA2C1 | 23.84$^{de}$ | 13.44$^{bcd}$ | 15.83$^{bcd}$ | 27.00 $^{cdefgh}$ | 34.72$^{defg}$ | 37.34$^{cdefgh}$ | 42.27$^{defghi}$ |
| MB2C1 | 22.98$^{de}$ | 25.81$^{efgh}$ | 33.75$^{efgh}$ | 42.00$^{ijk}$ | 48.89$^{hij}$ | 49.06$^{ghij}$ | 52.08$^{efghi}$ |
| MB2C2 | 28.80$^{e}$ | 23.66$^{efg}$ | 24.58$^{cdef}$ | 31.33$^{efghij}$ | 39.44$^{efghi}$ | 40.57$^{defghi}$ | 45.03$^{efghi}$ |
| MB3C1 | 3.98$^{a}$ | 20.43$^{def}$ | 10.00$^{ab}$ | 16.00$^{abc}$ | 22.22$^{abc}$ | 26.65$^{abc}$ | 32.73$^{abcde}$ |
| MD2C1 | 39.95$^{f}$ | 19.89$^{def}$ | 20.00$^{bcd}$ | 21.33$^{bcdef}$ | 31.67$^{cdef}$ | 35.47$^{cdef}$ | 40.75$^{cdefgh}$ |

Note: Means in the same column followed by same letter did not significantly different according to Duncan's test at 5% (α 0.05)

**Table 4.** Antagonistic activity of fungal isolates on the 7th day

| Isolates | Inhibition (%) | Class | Antagonistic activity |
|---|---|---|---|
| RH2C1 | 27.75 | 2 | Moderate |
| RH2C2 | 35.33 | 2 | Moderate |
| RH3C2 | 48.54 | 2 | Moderate |
| RH4C21 | 61.43 | 3 | High |
| RH4C22 | 79.14 | 4 | Very high |
| RH4C23 | 72.05 | 4 | Very high |
| RH4C3 | 82.52 | 4 | Very high |
| RH5C1 | 24.49 | 1 | Low |
| RH5C2 | 34.05 | 2 | Moderate |
| RH6C2 | 45.54 | 2 | Moderate |
| HA1C1 | 33.67 | 2 | Moderate |
| HB3C1 | 73.97 | 4 | Very high |
| HB3C2 | 48.68 | 2 | Moderate |
| HB2C1 | 48.89 | 2 | Moderate |
| HB2C2 | 50.44 | 3 | High |
| HD2C2 | 50.65 | 3 | High |
| RM0C1 | 46.98 | 2 | Moderate |
| RM0C3 | 54.83 | 3 | High |
| RM1C1 | 38.21 | 2 | Moderate |
| RM1C2 | 50.89 | 3 | High |
| RM3C1 | 29.81 | 1 | Low |
| RM3C2 | 25.37 | 1 | Low |
| MA1C1 | 52.32 | 3 | High |
| MA1C2 | 37.76 | 2 | Moderate |
| MA2C1 | 42.27 | 2 | Moderate |
| MB2C1 | 52.08 | 3 | High |
| MB2C2 | 45.03 | 2 | Moderate |
| MB3C1 | 32.73 | 2 | Moderate |
| MD2C1 | 40.75 | 2 | Moderate |

**Table 5.** Average weight of *P. oryzae* mycelium in antagonistic test

| Isolates | Mycelium weight (g) | Inhibition (%) | Interaction type |
|---|---|---|---|
| Control | 2.70 | 0 | - |
| RH2C1 | 1.26 | 27.75 | - |
| RH2C2 | 0.57 | 35.33 | - |
| RH3C2 | 0.44 | 48.54 | - |
| RH4C21 | 0.12 | 61.43 | Competition |
| RH4C22 | 0.14 | 79.14 | Competition |
| RH4C23 | 0.36 | 72.05 | Competition |
| RH4C3 | 0.09 | 82.52 | Competition |
| RH5C1 | 1.90 | 24.49 | - |
| RH5C2 | 0.90 | 34.05 | - |
| RH6C2 | 0.34 | 45.54 | - |
| HA1C1 | 0.71 | 33.67 | - |
| HB3C1 | 0.37 | 73.97 | Competition |
| HB3C2 | 0.37 | 48.68 | Antibiosis |
| HB2C1 | 0.23 | 48.89 | - |
| HB2C2 | 0.47 | 50.44 | Antibiosis |
| HD2C2 | 0.96 | 50.65 | Competition |
| RM0C1 | 1.01 | 46.98 | Antibiosis |
| RM0C3 | 0.43 | 54.83 | Competition |
| RM1C1 | 0.91 | 38.21 | - |
| RM1C2 | 0.93 | 50.89 | Antibiosis |
| RM3C1 | 1.14 | 29.81 | - |
| RM3C2 | 1.02 | 25.37 | - |
| MA1C1 | 0.60 | 52.32 | Antibiosis |
| MA1C2 | 0.27 | 37.76 | - |
| MA2C1 | 0.60 | 42.27 | - |
| MB2C1 | 0.59 | 52.08 | Competition |
| MB2C2 | 0.38 | 45.03 | Antibiosis |
| MB3C1 | 0.84 | 32.73 | - |
| MD2C1 | 0.55 | 40.75 | Competition |

**Identification isolate fungi**

31 fungal isolates were successfully isolated from 2 types of local rice plants and their rhizosphere. Based on observations of colony color and morphology, most fungal colonies were white or green, with some orange, purple, and grey fungi. Microscopic identification of the fungi revealed the genera like *Gliocladium* (1 isolate), *Fusarium* (2 isolates), *Penicillium* (6 isolates), *Paecilomyces* (8 isolates), *Nigrospora* (1 isolate), *Aspergillus* (1 isolate), *Pythium* (1 isolate), *Rhizoctonia* (1 isolate), *Trichoderma* (4 isolates), and unidentified fungi (6 isolates) (Table 7; Figure 5). A total of 6 isolates could not be identified macroscopically because they exhibited only hyphae, with no conidia observed.

**Discussion**

This study isolated 31 fungi from two varieties of local Sinjai rice, collected from leaves, stems, roots, and the rhizosphere. The highest number of fungi was found in the rhizosphere, whereas their numbers were very low in plant tissues. The plant rhizosphere is an ideal habitat for fungal growth and development because it is supported by environmental factors and nutrients, including amino acids and sugars (Xiong et al. 2021; Liu et al. 2022). Fungi primarily inhabit the plant rhizosphere and are influenced by the roots, which serve as a gathering place for various microorganisms (da Silva et al. 2021). Some fungi originating from the rhizosphere are non-pathogenic and can support plant growth while also acting as biological control agents (Zhang et al. 2019).

Rhizosphere fungi form mutualistic symbioses with plants and do not cause plant diseases (Asniwita et al. 2025). Several types of antagonistic fungi can also live in plant tissues. Beneficial microbes can associate with plants on the surface of plants and within plant tissues (Thambugala et al. 2020). In this study, more fungi were isolated from the soil than from plant tissues. Different habitats provide different fungal population diversity (White et al. 2019; Putrie et al. 2020; Ratnawati et al. 2022). Antagonistic microorganisms can be found and isolated from plant tissues and are abundant in the plant root zone (rhizosphere) (Amaria et al. 2013). One factor contributing to the lack of fungi isolated from plant tissue is that samples of each local rice variety were collected from only one field per variety.

Pathogenicity tests on 31 fungal isolates showed significant differences in their ability to affect rice seed growth on PDA and soil media. Pathogenicity tests using the immersion method with fungal suspension identified pathogenic isolates that caused disease symptoms from the germination stage onward. Based on observations from pathogenicity tests using seeds, the isolated fungi can be classified as pathogenic or non-pathogenic. There were two fungal isolates tested that caused the seeds not to grow, so they were classified as pathogenic fungi. Pathogenic fungi can prevent seeds from germinating or cause abnormal growth (Irawati et al. 2017; Sari and Bella 2025).

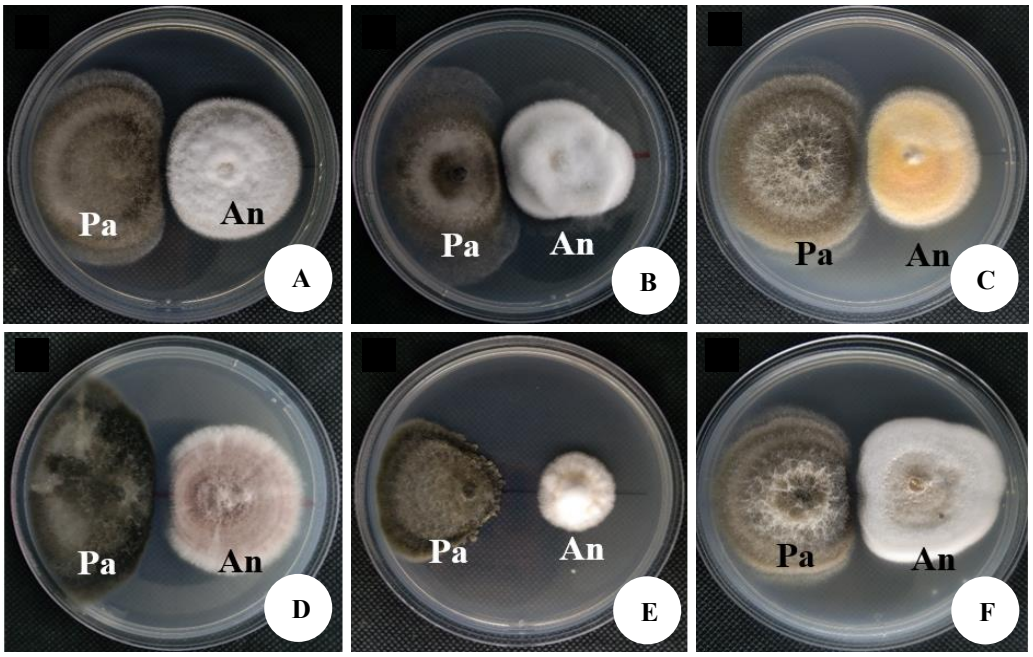


**Figure 3.** Antagonistic mechanism with antibiosis in dual culture tests. A. HB3C2, B. HB2C2, C. RM0C1, D. RM1C2, E. MA1C1, F. MB2C2. Pa: Pathogen, An: Antagonist

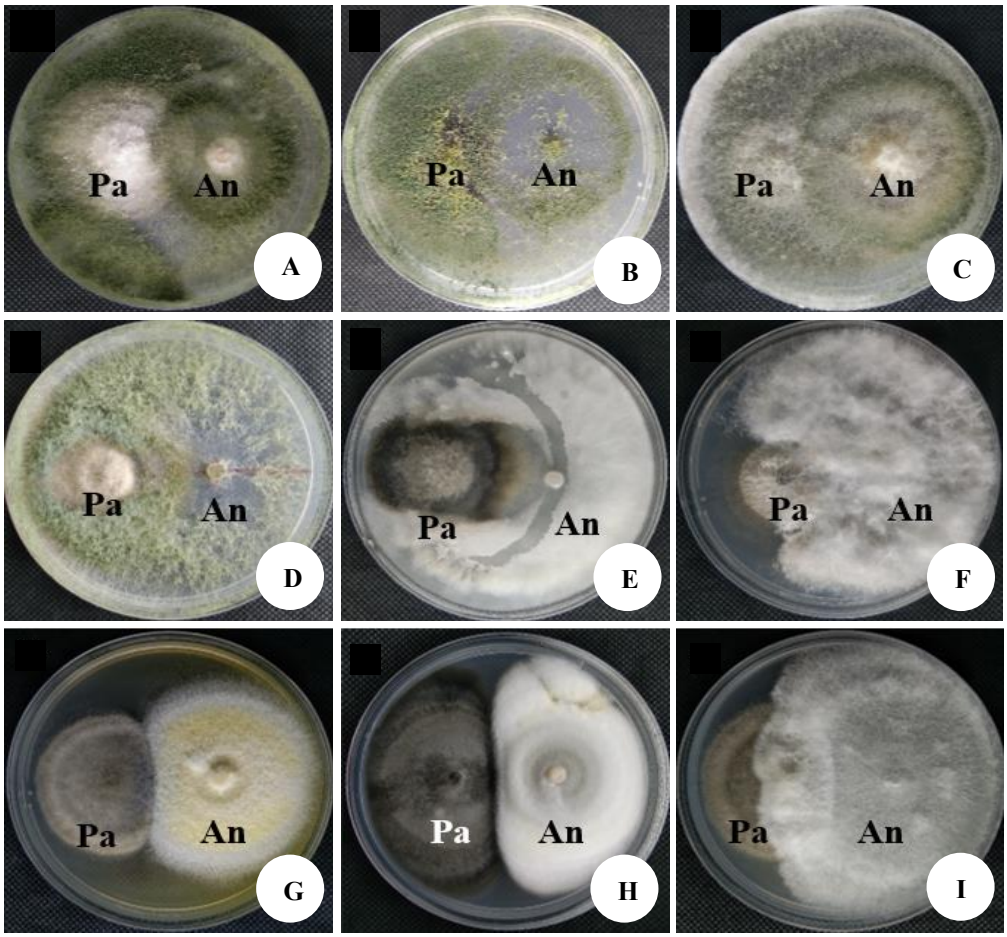


**Figure 4.** Antagonistic mechanism with competition in dual culture tests. A. RH4C21, B. RH4C22, C. RH4C23, D. RH4C3, E. HB3C1, F. HD2C2, G. RM0C3, H. MB2C1, I. MD2C1. Pa: Pathogen, An: Antagonist

**Table 6.** Interaction type and antagonistic mechanism

| Isolates | Interaction types | | | | | Antagonistic mechanism | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | No mechanism | Antibiosis | Competition |
| RH2C1 | | | | ✓ | | ✓ | | |
| RH2C2 | | | | ✓ | | ✓ | | |
| RH3C2 | ✓ | | | | | ✓ | | |
| RH4C21 | | ✓ | | | | | ✓ | |
| RH4C22 | | ✓ | | | | | ✓ | |
| RH4C23 | | ✓ | | | | | ✓ | |
| RH4C3 | | ✓ | | | | | ✓ | |
| RH5C1 | | | | ✓ | | ✓ | | |
| RH5C2 | ✓ | | | | | ✓ | | |
| RH6C2 | ✓ | | | | | ✓ | | |
| HA1C1 | ✓ | | | | | ✓ | | |
| HB3C1 | | ✓ | | | | | | ✓ |
| HB3C2 | | | ✓ | | | | ✓ | |
| HB2C1 | ✓ | | | | | ✓ | | |
| HB2C2 | | | ✓ | | | | ✓ | |
| HD2C2 | | ✓ | | | | | | ✓ |
| RM0C1 | | | ✓ | | | | ✓ | |
| RM0C3 | ✓ | | | | | | | ✓ |
| RM1C1 | ✓ | | | | | ✓ | | |
| RM1C2 | | | | | ✓ | | ✓ | |
| RM3C1 | ✓ | | | | | ✓ | | |
| RM3C2 | ✓ | | | | | ✓ | | |
| MA1C1 | | | | | ✓ | | ✓ | |
| MA1C2 | ✓ | | | | | ✓ | | |
| MA2C1 | ✓ | | | | | ✓ | | |
| MB2C1 | ✓ | | | | | | | ✓ |
| MB2C2 | | | ✓ | | | | ✓ | |
| MB3C1 | | | | | | ✓ | | |
| MD2C1 | | ✓ | | | | | | ✓ |

The results show that several isolates categorized as candidate antagonistic fungi exhibited necrotic symptoms in pathogenicity tests on PDA medium; however, only one isolate showed necrotic symptoms in pathogenicity tests on sterile soil medium. The preliminary hypothesis regarding the presence of these necrotic symptoms may be due to the following factors: (i) genotype/seed-borne factors. The necrosis is caused by a specific response to the genotype (Kumar et al. 2016; Ahmed and Palta 2017) or by other compounds carried by the seed (Kumar et al. 2016; da Silva et al. 2020), (ii) unbalanced environmental conditions within the container, particularly excessive moisture and poor air circulation. One of the factors causing necrosis in culture bottles is an imbalance of nutrients and high relative humidity inside the culture container (da Silva et al. 2020), (iii) the duration of the observation and the growing medium, so that fast-growing seeds require a lot of nutrients, and the growing medium is insufficient. Kishore et al. (2015) also reported that, in their studies, the percentage of necrosis in the shoot tips of Trichosanthes dioica propagated in culture bottles depended on the observation date; it was higher on day 42 (83%) than on day 14 (16%). The difference between pathogenicity tests conducted using PDA and soil media was that PDA was conducted in closed containers, whereas soil media was conducted in open containers. The results showed that the isolates, including those that induced necrosis on PDA media, did not produce necrosis on soil media.

The daily growth rate shows that *P. oryzae* has the highest growth rate because there are no obstacles to the growth of pathogens, whereas *P. oryzae* growth in dual culture testing has a low growth diameter. This is due to the presence of antagonistic fungi. Studies show that *P. oryzae* growth can be inhibited by fungi isolated from the rhizosphere or from rice plant tissue. Fungi associated with plants, whether living in plant tissue or in the rhizosphere, can control plant pathogens and enhance plant growth (Mirsam et al. 2021b). Fungi in the rhizosphere are among the microorganisms that can induce plant resistance to various diseases (Hyakumachi and Kubota 2004). Rhizosphere fungi such as *Trichoderma* sp. and *Gliocladium* sp. can suppress disease intensity (Istikorini and Budiman 2023). Fungi originating from plant tissue are endophytic fungi that do not cause disease symptoms, do not harm their host plants, and can produce secondary metabolites. Endophytic fungi can induce plant resistance to plant pathogens and abiotic stress (Fontana et al. 2021) and reduce the intensity of plant diseases (Sirikamonsathien et al. 2023).

The results show that isolates from the rhizosphere exhibited varying levels of antagonism against *P. oryzae* growth. The highest level of antagonism was observed in isolate RH4C3, identified as belonging to the genus

*Trichoderma*. This suggests that antagonistic properties may result from competition. Competition between microorganisms occurs when space and nutrients are limited, with antagonistic growth faster and pathogen growth slower due to nutrient depletion (El-Debaiky et al. 2017; Coulibaly et al. 2022). In addition, inhibition occurs due to the secondary metabolites produced by antagonistic fungi. Several *Trichoderma* species can inhibit the growth of plant pathogenic fungal mycelium by producing secondary metabolites in the form of fungal volatile organic compounds (Ruangwong et al. 2021). Tshilenge-Lukanda et al. (2024) state that *Trichoderma* sp. inhibits growth by 69%, concluding that *Trichoderma* sp. can be used to control *P. oryzae*. Antagonistic fungi can reduce the weight of pathogenic fungal mycelium by competing for nutrients and producing enzymes that directly inhibit pathogen growth. Isolate RH4C3 can suppress the growth of *P. oryzae* fungal mycelium.

**Table 7.** Identification of antagonist fungi from local rice and its rhizosphere

| Isolates | Genus | Colony color | Structure | Shape colony | Hyphae | Conidia |
|---|---|---|---|---|---|---|
| RH2C1 | *Gliocladium* sp. | Sage green | Granular | Irregular | Hyaline, erect, branched | Globose, often a few in a chain |
| RH2C2 | Unidentified | Grey | Absent | Circular | Hyaline | Not visible |
| RH3C1 | *Fusarium* sp. | Purplish white | Cottony | Irregular | Hyaline | Hyaline, globose |
| RH3C2 | *Fusarium* sp. | White | Cotton-like | Irregular | Hyaline, short | Hyaline, boat shaped, globose |
| RH3C3 | Unidentified | Dark brown | Cottony | Irregular | Hyaline | Not visible |
| RH4C21 | *Trichoderma* sp. | Green | Granular | Irregular | Hyaline, septate | Branched conidiophore, chained conidia |
| RH4C22 | *Trichoderma* sp. | Green | Granular | Irregular | Hyaline, septate | Branched conidiophore, chained conidia |
| RH4C23 | *Trichoderma* sp. | Green | Granular | Irregular | Hyaline, septate | Branched conidiophore, chained conidia |
| RH4C3 | *Trichoderma* sp. | Green | Granular | Irregular | Hyaline, septate | Branched conidiophore, chained conidia |
| RH5C1 | *Penicillium* sp. | White | Velvety | Irregular | Cylinder, septate | Elongated-cylindrical, chained |
| RH5C2 | *Paecilomyces* sp. | Orange | Woolly | Irregular | Hyaline, septate | Sickle-shaped, conidial cluster |
| RH6C2 | Unidentified | Grey | Absent | Irregular | Hyaline | Not visible |
| HAICI | *Paecilomyces* sp. | Orange whitish | Cotton-like | Irregular | Hyaline, branched | Sickle-shaped, conidial cluster |
| HB3C1 | *Nigrospora* sp. | Grey | Cotton-like | Irregular | Simple, hyaline, bearing single conidia | Black, subglobose |
| HB3C2 | *Penicillium* sp. | Yellowish white | Absent | Irregular | Hyaline, erect, branched | Hyaline, oval |
| HB2C1 | *Paecilomyces* sp. | White | Cotton-like | Irregular | Hyaline, branched | Hyaline, oval |
| HB2C2 | *Paecilomyces* sp. | White | Cotton-like | Irregular | Hyaline, branched | Hyaline, oval |
| HD2C2 | *Pythium* sp. | Translucent white | Downy | Irregular | Sporangia hypha like | Globose |
| RM0C1 | *Paecilomyces* sp. | Orange | Absent | Circular | Hyaline, branched | Hyaline, oval |
| RM0C3 | *Paecilomyces* sp. | Yellow | Cotton-like | Irregular | Hyaline, branched | Hyaline, oval |
| RM1C1 | *Rhizoctonia* sp. | White | Woolly | Rhizoid | Hyaline, branched, septate | Not formed |
| RM1C2 | *Penicillium* sp. | Purple | Granular | Irregular | Hyaline, erect, branched | Hyaline, oval |
| RM3C1 | Unidentified | Brown | Granular | Circular | Hyaline, septate | Not visible |
| RM3C2 | *Paecilomyces* sp. | Orange | Woolly | Circular | Hyaline, septate | Sickle-shaped, conidial cluster |
| MAIC1 | Unidentified | White | Cottony | Circular | Hyaline, septate | Not visible |
| MA1C2 | Unidentified | Yellowish white | Absent | Circular | Hyaline, septate | Not visible |
| MA2C1 | *Aspergillus* sp. | Black | Granular | Irregular | Hyaline, septate | Globose |
| MB2C1 | *Penicillium* sp. | White | Absent | Circular | Hyaline, erect, branched | Hyaline, oval |
| MB2C2 | *Penicillium* sp. | White | Woolly | Circular | Hyaline, erect, branched | Hyaline, oval |
| MB3C1 | *Paecilomyces* sp. | Orange | Woolly | Circular | Hyaline, branched | Hyaline, oval |
| MD2C1 | *Penicillium* sp. | Greyish white | Cottony | Circular | Hyaline, erect, branched | Hyaline, oval |

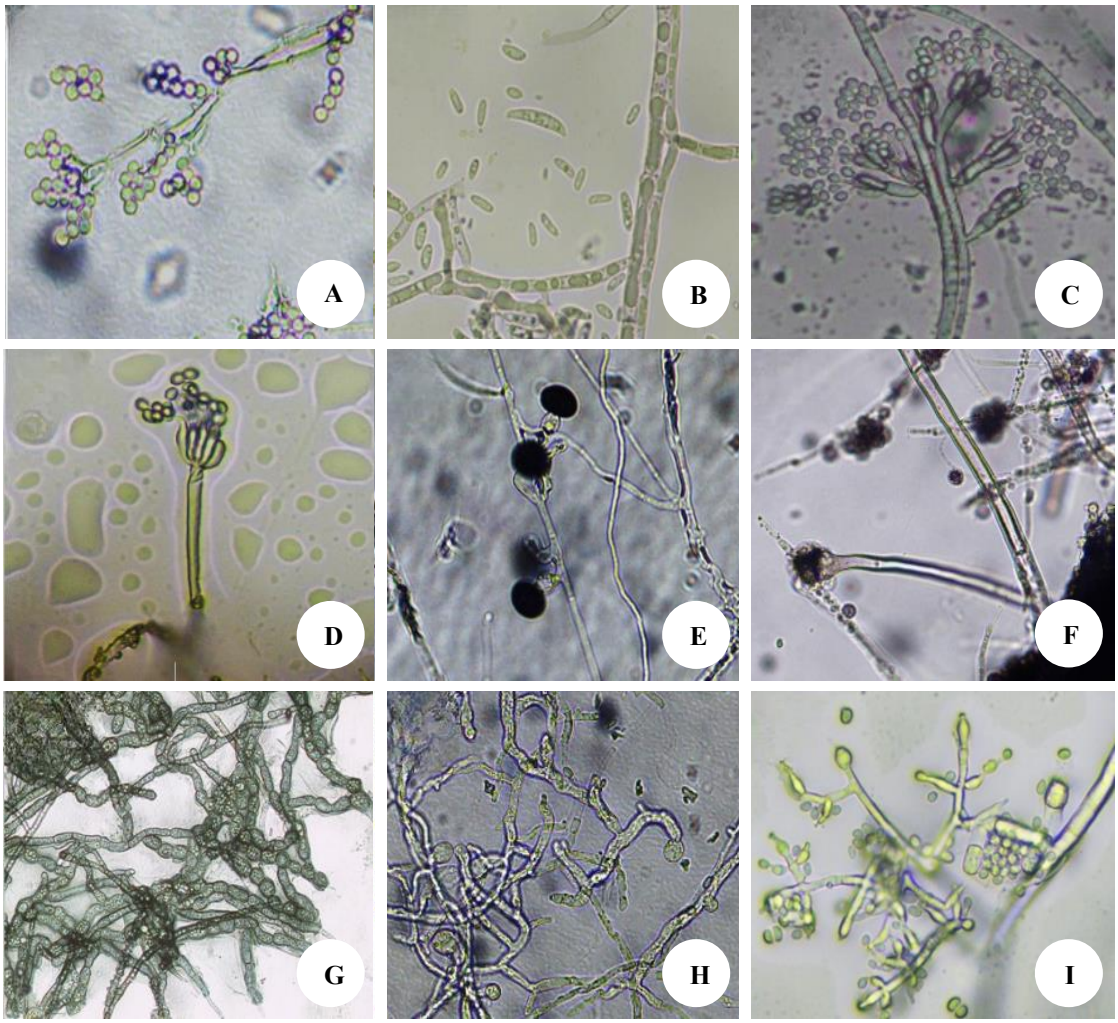


**Figure 5.** Microscopic characteristic of fungi isolated from local rice plants and rhizosphere: A. Genus *Gliocladium*, B. Genus *Fusarium*, C. Genus *Penicillium*, D. Genus *Paecilomyces*, E. Genus *Nigrospora*, F. Genus *Aspergillus*, G. Genus *Rhizoctonia*, H. Genus *Pythium*, I. Genus *Trichoderma*

Several *Trichoderma* species can inhibit the growth of plant pathogenic fungal mycelium by producing secondary metabolites in the form of fungal volatile organic compounds (Ruangwong et al. 2021). Fungal interactions can be observed based on inhibitory power and mycelium weight. The more effective the biological agent is at suppressing pathogen growth, the lower the pathogen mycelium dry weight is compared to the control. Antagonistic fungi can suppress pathogen growth (Trizelia et al. 2023). *Trichoderma* spp. effectively reduces the weight of *Phytophthora* mycelium, as indicated by the control having a heavier mycelium weight than the pathogen mycelium (Soesanto et al. 2013). Fungi in the rhizosphere directly and indirectly influence plant composition and productivity/biomass (Mohamed et al. 2022).

Based on the results of dual-culture tests, antagonistic mechanisms were classified into antibiosis, competition, and no mechanism. An interesting finding in this study was that several fungi successfully isolated exhibited antibiosis mechanisms, a characteristic of antagonistic fungi. Based on the observations, a competitive mechanism was observed when the antagonistic fungal colony overtook the pathogenic colony, and the antagonistic fungus grew faster until it filled the Petri dish. Antibiosis occurs when a clear zone forms between the pathogen fungus and the antagonist fungus, the pathogen fungus changes shape, and pigments are produced on the underside of the antagonist fungus colony. Antagonistic mechanisms are an important criterion for selecting fungal isolates with potential as biocontrol agents (Nassary 2025). The mechanism of antagonistic fungi can occur directly, namely, antagonism outside the host (Antagonism exterior to host). This mechanism occurs when fungi parasitize, suppress, and directly interfere with pathogens (Muslim and Suwandi 2023). This mechanism is known for its antagonistic properties, including antibiosis, mycoparasitism/hyperparasitism, and competition (Amaria et al. 2015; Kuswinanti et al. 2023; Muslim and Suwandi 2023; Mulyani et al. 2024). Fungal biocontrol agents can exhibit parasitism (Amaria et al. 2015; Ul Haq et al. 2024), antibiosis (Ul Haq et al. 2024; Zhang et al. 2015), competition for nutrients and space, and induce plant resistance to disease (Ul Haq et al. 2024).

In the observation of fungi with an antibiosis mechanism, a clear zone (inhibition zone) formed on the growth medium between the growth of the pathogenic fungus and the antagonistic fungus. Antibiosis occurs when metabolic compounds produced by antagonistic fungi inhibit the growth of pathogenic fungi, thereby forming an inhibition zone (Zhao et al. 2022). Isolate MB2C2 exhibits antibiosis mechanisms, but in dual culture, its growth was slow, resulting in low inhibition percentages. Inhibition activity is indicated by the appearance of a clear zone between the two fungi, causing *P. oryzae* to stop growing. In the inhibition observations in this study, a competition mechanism also occurred due to space and nutrient competition, inhibiting the growth of one of the fungi. The competition mechanism was characterized by the faster growth of the antagonistic fungus mycelium compared to that of the pathogenic fungus, leading to competition for space and nutrients. Slow growth in pathogenic fungi inhibits the pathogen, and the colony cannot develop properly because the space is filled with antagonistic fungal mycelium (Mulyani et al. 2024). In this interaction, the slow growth of antagonistic fungi can be compensated for by the biosynthesis and diffusion of antifungal compounds, thereby inhibiting pathogen growth (Coulibaly et al. 2022).

Microscopic identification revealed 3 predominant genera: *Penicillium*, *Paecilomyces*, and *Trichoderma*. *Penicillium* colonies are generally white and found in the rhizosphere and stems. Some *Penicillium* genera are white, yellow, or grey, with rapid growth (Sciortino 2017). *Penicillium* is commonly found across habitats, interacts well in soil by suppressing the growth of soil pathogens, exhibits antagonistic activity against plant pathogens, and is endophytic in plant tissues (Srinivasan et al. 2020). The genus *Paecilomyces* was characterized by white, yellow, and orange colonies found in the rhizosphere, roots, and stems. Colonies of the genus *Paecilomyces* can be yellow, light brown or white on the upper side, while the underside is cream, yellow or light brown (Sciortino 2017). *Paecilomyces* is also an endophytic fungus that can be isolated from soil (Constantin et al. 2022). What is interesting about the genus fungi in this study is that these fungi, which have been known as *Paecilomyces*, can act as biocontrol agents for nematodes and pests and can suppress the growth of *P. oryzae*. *Paecilomyces* sp., as an endophytic fungus, can suppress the growth of *Rhizoctonia solani* by 76.25% with an antagonistic level of 4 (Hawar et al. 2023). In this study, several unidentified fungi were isolated. These fungi possessed only sterile mycelium and did not produce conidia. The unidentified fungi were isolated from the rhizosphere and plant roots. Fungi from the rhizosphere are highly diverse and may require specific culture conditions to grow and sporulate; if these conditions are not met, the fungi remain unidentified.

To our knowledge, this is the first report showing the antagonistic activity of *Nigrospora* sp. and *Pythium* sp. isolates from local Sinjai rice and its rhizosphere against *P. oryzae* in vitro. This is based on the actual inhibition data and mechanisms. *Nigrospora* sp. is a grey-colored fungus isolated from rice stems. *Nigrospora* is a fungus that can colonize plants with different properties. It can be endophytic, pathogenic, or saprophytic depending on the habitat or host (Dutta et al. 2023). In this case, *Nigrospora* is endophytic because it was isolated from rice stem tissue, and dual culture showed that the isolate exhibits a competitive antagonism, with its growth faster than that of *P. oryzae*. *Nigrospora* sp. can inhibit the growth of *P. oryzae* with an inhibition percentage of 51.1% (Suada et al. 2012). It is capable of suppressing the severity of leaf blight by 3.0%-25.3% and neck blight by 49.5%-61.6% (Istikorini and Budiman 2023). It can also suppress the severity of leaf blight by 3.0%-25.3% and neck blight by 49.5%-61.6% (Purnomo et al. 2022).

The *Pythium* fungus isolate exhibited interaction mechanisms, including competition and antibiosis. Interestingly, there was antibiosis interaction with the formation of an inhibition zone and faster growth filling the space and covering the *P. oryzae* colony. Several *Pythium* species act as mycoparasites of pathogenic fungi, bacteria, and oomycetes in soil, thereby serving as beneficial biocontrol agents (Belonozníkova et al. 2022). *Pythium oligandrum* exhibits antagonistic and parasitic activity against pathogenic fungi (Majtan et al. 2024). *P. oligandrum* can attack plant pathogens by utilizing root exudates and the mycelium of pathogenic fungi to meet its nutritional needs (Brozova 2022).

In conclusion, 31 fungal isolates were obtained from local rice and its rhizosphere. 11 non-pathogenic isolates showed >50% inhibition of *P. oryzae* growth in vitro via antibiosis and competition, and were therefore selected as antagonist candidates. These 11 fungi belonged to the genus *Trichoderma* (RH4C21, RH4C22, RH4C23, RH4C3), *Nigrospora* (HB3C1), *Pythium* (HD2C2), *Paecilomyces* (HB2C2, RM0C3), *Penicillium* (RM1C2, MB2C1), and unidentified (MA1C1). The isolate that showed the highest inhibition was RH4C3 (*Trichoderma* sp.). This research is a preliminary, small-scale laboratory study as a basis for the development of biological control agents and local biocontrol agents. Therefore, further research is needed to identify fungi at the species level, characterize bioactive antifungal compounds, conduct in vivo testing, and evaluate field applications.

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