Antagonism of rice phylloplane fungi against *Cercospora oryzae*

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**Abstract.** Narrow brown leaf spot (NBLS) caused by *Cercospora oryzae* Miyake is one of the important obstacle in rice cultivation that can decrease the productivity up to 40%. It has been known well that some phylloplane fungi are antagonistic to some leaf diseases. Phylloplane fungi of rice however haven’t been studied much and poorly understood as biological control agent of rice pathogen such *C. oryzae*. The research aimed to study the antagonism of some phylloplane fungi of rice against *C. oryzae*. At least 14 isolates of phylloplane fungi were collected which consisted of six pathogenic and eight nonpathogenic variants. All of nonpathogenic isolates were antagonistic against *C. oryzae* both in vitro and only one isolate could not inhibit the infection of the pathogen in vivo. Some isolates were identified as *Aspergillus*, *Mucor*, *Penicillium*, *Fusarium*, and *Trichoderma*. The isolate of *Mucor* and *Fusarium* could inhibit the highest growth of pathogen on potato dextrose medium that were at 36.0% and 35.5% respectively. Whereas on artificial inoculation on rice, some isolates such *Penicillium* and *Fusarium* could inhibit most effectively and were significantly different to Mencozeb application with dosage 5g L⁻¹.

1. Introduction
Rice is one of important cereal plants. National rice consumption of Indonesia in 2011 was 79.1 Kg capita. It was higher than consumptions of cassavas and sweet potatoes, that were 3.0 Kg capita and 0.1 Kg capita respectively [1]. According to BPS [2], rice consumption for a week in 2013 was about 1.626 Kg, and then it increased in the next year.

NBLS caused by *C. oryzae* is one of rice cultivation obstacle. The symptoms start with lesion 2-10 x 1 mm. On the resistant varieties, spot will be narrower, shorter, and dark brown. If the disease occurs in the susceptible varieties, the symptoms will be larger and has grey necrosis in the center [3]. The disease could decrease yield until 40% [4].

Planting resistant varieties is alternative way to control NBLS. However, resistant varieties are just able to stand for some years until the new virulent fungi will be developing [5]. Fungicides spraying are also able to control NBLS, but it has a lot of negative impact to human health [6]. Based on the reasons above, it needs other alternative to control the disease.

Phylloplane fungi is a part of microorganism living in the leaf surface [7, 8]. The fungi inhibited the growth of *Alternaria brassicaceae*. Evueh and Ogbebor [9] reported that phylloplane *Trichocladium* sp. and *Trichophyton* sp. had capability to inhibit *Colletotrichum*. Although it is effective as an antagonist, using phylloplane fungi to control *C. oryzae* is not discover yet. This research aimed to find antagonistic phylloplane fungi and their potency as antagonist against *C. oryzae*. 
2. Materials and methods
The researches were conducted at laboratory and greenhouse of Pests and Diseases Observation, Palur Surakarta from August 2016 until May 2017. Tool needed for laboratory research was inchase, while the materials were isolate of *C. oryzae*, isolates of phylloplane fungi, PDA medium (*Potato Dextrose Agar*), and PDB medium (*Potato Dextrose Broth*). In the greenhouse, materials needed were soil from rice field, polibag, and IR64 seed.

The researchers used Completely Randomized Design (CRD), three replications with one factor that was phylloplane fungi application. Antagonism in vitro test had 2 treatments that were control and application of phylloplane fungi + *C. oryzae*. In the antagonism in vivo test there were 4 treatments that were fungicide, without pathogen or phylloplane fungi application/no inoculation, application of *C. oryzae*, and application of phylloplane fungi+pathogen.

Observation variables were percentage of growth inhibition, spot area, inhibition percentage in antagonism in vitro test, incubation period, and disease inhibition effectiveness. Percentage of growth inhibition was measured with formula:

\[ H (\%) = \frac{R_2 - R_1}{R_2} \times 100\% \]

- \( H \) = inhibition percentage
- \( R_1 \) = the distance between pathogen colony center to antagonist colony
- \( R_2 \) = the distance of pathogen colony away from antagonist colony

Incubation period was measured from day of symptoms appearance. it was observed from day 1\(^{st}\) after *C. oryzae* inoculation. spot area were measured on day-7 after *C. boryzae* inoculation. Disease growth effectiveness was measured with formula:

\[ \frac{A - B}{A} \times 100\% \]

- \( A \) = spot area of *C. oryzae* inoculation
- \( B \) = spot area of no inoculation treatments

3. Results and discussion

3.1. Phylloplane fungi identification
Phylloplane fungi isolated from 3 parts, that were leaf (D), sheath (P), and stem (B). Different fungus were found in each parts of rice. Five fungus were found in the leaf, 7 fungus in sheath, and 2 fungus in the stem. Identification using morphological is the common way to identify. However, morphological identification is not accurate, microscopic observation is needed [10].

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Table 1. Identification and Pathogenicity of Rice Phylloplane Fungi Isolates

| Isolates | Species        | pathogenicity |
|----------|----------------|---------------|
| D 1      | *Aspergillus* sp. | -             |
| D 2      | -              | +             |
| D 3      | *Mucor* sp.    |               |
| D 4      | *Botrytis* sp. | +             |
| D 5      | *Penicillium* sp. | -           |
| P 1      | *Fusarium* sp. | -             |
| P 2      | -              | -             |
| P 3      | -              | -             |
| P 4      | *Curvularia* sp. | +           |
| P 5      | *Curvularia* sp. | +           |
| P 6      | *Botryosphaeria* sp. | +         |
| P 7      | -              | +             |
| B 1      | *Trichoderma* sp. | -           |
| B 2      | -              | -             |

3.2. Antagonism in vitro test of phylloplane fungi against *C. oryzae*
Phylloplane fungi impacted significantly to *C. oryzae* growth on PDA. Picture 1 about growth inhibition of *C. oryzae* by rice phylloplane fungi show the highest inhibition is *Mucor* sp. (36%). *Fusarium* sp. has 35.5% inhabitation that not significant different with *Mucor* sp. Phylloplane fungi with the lowest inhabitation is B3.2 (4.4%).

![Figure 1. Growth Inhibition of *C. oryzae* by Rice Phylloplane Fungi on PDA Medium](image)

Mechanisms of phylloplane fungi to inhibit pathogen are parasitism [9] antibiotics production, and volatile metabolites [11]. *Mucor* sp. has capability to grow rapidly [12], it is possible that the fungi inhibit *C. oryzae* through nutrition competition.

*Fusarium* sp. has been reported, can inhibit pathogen by nutrition competition, antibiotic production, and induced resistant [13]. Figure 2A indicate that *Fusarium* sp. grows rapidly and surrounds *C. oryzae*, so that, the growth of *C. oryzae* was limited.

Antagonistic *Trichoderma* sp. grew faster than pathogen [14]. However, *Trichodema* sp. colony has zone that not overgrown by both colonies (Figure 2B). It indicates that the zone is caused by certain compounds produced by *Trichoderma* sp. [15], revealed that *Trichoderma* sp. was able to inhibit pathogen growth by producing antibiotics and secondory metabolites.
3.3. Spot area appear on antagonism in vivo test of phylloplane fungi against C. oryzae

Application phylloplane fungi affected significantly to C. oryzae symptom. Control negative (C-), Penicillium sp., dan Fusarium sp. formed no spot (spot large=0.00 mm$^2$). C. oryzae inoculation has 2.34 mm$^2$, Mancozeb 2.11 mm$^2$. The largest spot was in P3, 7.00 mm$^2$.

The highest disease inhibition effectiveness were Penicillium sp. and Fusarium sp. The results showed that both fungi inhibited C. oryzae and were significantly more effective than Mancozeb 5 g L$^{-1}$. P3 had the lowest disease inhibition effectiveness that was 8.82%.

Table 2. Spot area, incubation Period, and Disease Inhibition Effectiveness of C. oryzae by Rice Phylloplane Fungi

| Isolates of Phylloplane Fungi | Spot Area (mm$^2$)* | Incubation Period (DAI) | Disease Inhibition Effectiveness (%) |
|-------------------------------|---------------------|-------------------------|-------------------------------------|
| Aspergillus sp.               | 2.89±2.46 bc        | 3                       | 48.28±42.81 abc                     |
| Mucor sp.                    | 2.56±1.90 bc        | 3                       | 53.02±34.84 abcd                    |
| Penicillium sp.              | 0.00±0.00 a         | -                       | 100.00±0.00 d                       |
| Fusarium sp.                 | 0.00±0.00 a         | -                       | 100.00±0.00 d                       |
| P2                           | 3.78±2.91 bcd       | 3                       | 40.11±37.96 abc                     |
| P3                           | 7.00±2.60 d         | 3                       | 8.82±15.28 a                        |
| Trichoderma sp.              | 5.33±1.73 cd        | 3                       | 13.56±11.74 ab                      |
| B2                           | 1.89±2.14 ab        | 3                       | 65.27±39.39 cd                      |
| Mancozeb**                   | 2.11±0.38 bc        | 3                       | 61.19±7.07 bcd                      |
| No inoculation               | 0.00±0.00 a         | -                       | 100.00±0.00 d                       |
| C. oryzae                    | 5.44±2.34cd         | 3                       | -                                   |

The values followed by different letter are non-different based on Duncan’s test at level of 5%.

*Data transformed into $\sqrt{(x + 0.5)}$. **Application with dosage 5 g L$^{-1}$

Penicillium sp. had capability to inhibit growth of Phytophthora capsici [16]. The fungi have mechanism of induced resistant [17]. It is also parasitism to the pathogen [18] and produce antibiotics [19, 20].

Treatment B2 had the lowest inhibition in antagonism in vivo test. However, B2 formed the lowest spot area in antagonism in vivo test (1.89 mm$^2$). This result was not significant compared with Penicillium sp., Fusarium sp., and no inoculation. It was assumed that the fungi induced resistant to C. oryzae.

Spot area of Trichoderma sp. was at about 5.33 mm$^2$. This is not significant compare with treatment of C. oryzae. P3 had 7.00 mm$^2$ in spot area. The opposite result is possibly caused by
different condition during antagonism in vitro and in vivo test. Uncontrolled environmental during antagonism in vivo made *Trichoderma* sp. and P3 could not grow optimally on the rice plant.

Spot appear in day-3 after inoculation, 3 treatments not form symptom, that were no inoculation, *Penicillium* sp., and *Fusarium* sp. The fungus effective for controlling *C. oryzae*. However, it can assumed caused by the loss of pathogen inoculum after inoculation.

### 4. Conclusions

There are 8 fungus isolated from rice, that are *Aspergillus*, *Mucor*, *Penicillium*, *Fusarium*, *Trichoderma*. P2, P3, and B2. Phylloplane fungus effective against *C. oryzae*. *Mucor* and *Fusarium* have the highest inhibition, that were 36% and 35.5%. in the artificial inoculation, *Penicillium* sp. And *Fusarium* sp. could inhibit most effectively.

After conducting the research, the recommendations to give are the need for research about phylloplane fungi application in the field.

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