Antifungal Activity of the Bark Extract of *Michelia Alba* against *Curvularia Verruculosa* Fungal the Cause of Leaf Spot Disease on Rice

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The purpose of this study was observe the antifungal activity of the bark extract of *Michelia alba* against *Curvularia verruculosa* fungal of the cause of leaf spot disease in rice. The antifungal activities was carried out using the diffusion well, colony, biomass formation methods. The bark extract of *Michelia alba* has showed the antifungal activity against *Curvularia verruculosa* fungal with a minimum inhibition concentration value to be 0.5%. The bark extract of *Michelia alba* with 2.0% concentration can strongly inhibit the growth of *C. Verruculosa* with inhibiting capability is 33.17 mm. This extract at 0.6% concentration was able to completely inhibit the growth of fungal colony and at 2.0% concentration has been able to inhibit completely the biomass formation of *C. Verruculosa* fungal for a 14-day period of incubation.

**Keywords:** Bark Extract of *Michelia Alba*; Biomass formation; *C. Verruculosa*; Fungal colony; Minimum inhibition concentration.

*Curvularia verruculosa* fungal has caused leaf spot disease in Ciharang rice plants (Bawa, 2019). This disease has caused losses, so it is very disturbing for farmers. The leaf spot disease in rice is still controlled using the synthetic fungicide. It is true that the use of the synthetic fungicide to control the leaf spot disease on rice has been effective enough, but being chemical, its excessive and repeated use has polluted the environment and disturbed the nature’s biological system. In addition, its excessive use has also caused the fungus to be getting resistant to it and to increase the production cost (West *et al*., 2003; Yoon *et al*., 2013). Therefore, the alternative fungicides, which are cheap and environmentally friendly, are being developed. The botanical fungicide seems to fulfill such criteria as it does not pollute the environment, and preparing and using it are not dangerous (Rout and Tiwari, 2012).

The use of plant extract which has potential as botanical fungicide to control various diseases causing by *Curvularia* on rice is still limited. Rahman (1992) reported that the extract of bishkatali (*Polygonum hydropiper L*.), the extract of garlic (*Allium sativum*), the extract of ginger (*Zingiber officinale*) and the extract of neem (*Azadirachta indica*) can be effectively used to control the fungus *Curvularia lunata* carried by the rice seeds. Abdel-Ghany *et al*. (2015) reported that the plant extract of *Juniperus procera* could reduce
the growth of *Curvularia lunata* isolated from the unhulled rice stored at 3 mg/ml concentration by 88.42%.

The antifungal active compound for various plants is explored to obtain the botanical fungicide which can be used to control the leaf spot disease caused by *Curvularia* sp. From 51 types of plants extracted using different solvents, it was found that the methanol extract of the bark of *Michelia alba* could highly strongly inhibit the growth of the fungal colony in the amount of 36 mm.

**MATERIALS AND METHODS**

**Materials**

The bark of *Michelia alba* and *C. verruculossa* fungal.

**Methods**

**Sampling and Extraction Methods**

The bark of *Michelia alba* were collected at one of the wood cutting place in Jehem, Bangli, Bali, Indonesia. Samples are washed in tap water and air dried for a day, then cut into small pieces and re-dried. The samples were ground using a blender to powder form. Dry powder as much as 1000 gram was macerated with 2000 ml methanol (p.a grade) for 24 hours 3 times. The filtrates were combined and evaporated in a rotary vacuum evaporator, so that crude extract of methanol was obtained. The extract was used for further testing.

**Antifungal Test of Bark Extract of *Michelia alba* by Diffusion Well Method**

Fungus suspension about 1.0 ml added 10 ml melted PDA medium in a laminar flow. After the medium become solid, a diffusion well was made in the center of PDA using cork borer (5 mm diam.). Into the well, 20 µl bark extract of *Michelia alba* was applied using a micro pipette at concentration 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.5% and 2.0% (w/v). For control, 20 µl ethanol 5% in sterile distilled water containing 0.5% Tween-80 was used. Five Petri dishes were prepared for each concentration. The cultures were incubated for 48 h in the dark under room temperature. The formation of inhibition zone around the diffusion well was observed to determine the antifungal activity.

**Antifungal Test of Bark Extract of *Michelia alba* by Colony Method**

The bark extract of *Michelia alba* at various concentration (0.1%, 0.2%, 0.3%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.5%, 2.0% and 0% (w/v) as control) were tested for inhibition to growth of fungal colony in PDA media. The diameter of fungal colony was measured daily. The inhibitory activity to the radial growth was determined according to the following formula : (Rai, 2006).

\[
\text{inhibition activity(\%) = } \left( \frac{\text{diameter of control colony} - \text{diameter of treatment colony}}{\text{diameter of control colony}} \right) \times 100\%
\]

**Determination of the Effect of Extract on Fungal Biomass**

Five concentration of the bark extract of *Michelia alba*, that is 0% as control, 0.5%, 1.0%, 1.5%, and 2.0% (w/v) were tested to inhibition to growth of fungal colony in PDA media. The diameter of fungal colony was measured daily. The inhibitory activity to the radial growth was determined according to the following formula : (Rai, 2006).

\[
\text{inhibition activity(\%) = } \left( \frac{\text{weight of control biomass} - \text{weight of treatment biomass}}{\text{weight of control biomass}} \right) \times 100\%
\]

**Analysis of Statistic**

The result data analyzed by statistics. The difference between the average levels of the test groups was tested with One Way ANOVA followed by the Duncan’s Multiple Range Test at p<5%.

**RESULTS AND DISCUSSION**

The treatment of bark extract of *Michelia alba* significantly inhibited the growth of *Curvularia verruculosa* using PDA as the medium (Table 1). The treatment of P1 (0.5%) is the treatment which shows the smallest inhibiting capability, causing the minimum value of the inhibitory concentration (MIC) to be 0.5%. Apart from that, the bark extract of *M. alba* with a 2.0% concentration can so strongly inhibit the growth of *C. verruculosa* as the fungus responsible for the leaf spot disease on
the rice plant. Its inhibiting capability is 33.17 mm, showing that the bark extract of *M. alba* effectively inhibits the growth of *C. verruculosa* as the fungus responsible for the leaf spot disease on the rice plant.

In fact, the treatment of the bark extract of *Michelia alba* significantly inhibited the growth of the colony of the fungus *C. verruculosa* made to grow on the PDA used as the medium (Table 2). The higher the concentration of the bark extract of *M. alba* the smaller the growth of the fungal colony will be. However, in the treatment of P8 in which the concentration of the bark extract of *M. alba* is 0.6%, the fungal colony does not grow at all.

Apart from that, the data in Table 2 shows that, in fact, the bark extract of *M. alba* can increase the inhibiting capability of the growth of the fungal colony. The higher the concentration of the bark extract of *M. alba*, the greater capability it will have to inhibit the growth of the colony of the fungus *C. verruculosa*. In the treatment of P8 in which the concentration of the bark extract of *M. alba* is 0.6% its capability to inhibit the growth of fungal colony is 100%.

The treatment of the bark extract of *M. alba* can distinctively inhibit the formation of the biomass of the fungus *C. verruculosa* as the fungus responsible for the leaf spot disease on the rice plant (Table 3). The higher the concentration of the bark extract of *M. alba*, the higher its capability of inhibiting the formation of the biomass will be. The bark extract of *M. alba* at a 2.0% concentration has been able to inhibit completely the biomass formation of the fungus *C. verruculosa* as the fungus responsible for the leaf spot disease on the rice plant for a 14-day period of incubation.

The result of this current study shows that

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**Table 1. Inhibition of bark extract of *Michelia alba* to the growth of *C. verruculosa* the cause of leaf spot disease in rice**

| No | Extract Concentration (%) | Average Diameter of Inhibition Zone (mm) |
|----|---------------------------|-----------------------------------------|
| 1  | P_0 (0.0)                 | 0.00a**                                 |
| 2  | P_1 (0.1)                 | 0.00a                                   |
| 3  | P_2 (0.2)                 | 0.00a                                   |
| 4  | P_3 (0.3)                 | 0.00a                                   |
| 5  | P_4 (0.4)                 | 0.00a                                   |
| 6  | P_5 (0.5)                 | 5.11b                                   |
| 7  | P_6 (0.6)                 | 9.83c                                   |
| 8  | P_7 (0.7)                 | 11.17d                                  |
| 9  | P_8 (0.8)                 | 12.94e                                  |
| 10 | P_9 (0.9)                 | 22.94f                                  |
| 11 | P_{10} (1.0)              | 26.50g                                  |
| 12 | P_{11} (1.5)              | 31.67h                                  |
| 13 | P_{12} (2.0)              | 33.17i                                  |

*values followed by the same letter in the same column are not significantly different according to the Duncan’s Multiple Range Test at p<5%.

**Table 2. The growth of *C. verruculosa* colony and percent inhibition of bark extract of *M. alba* at various concentration in two weeks**

| No | Extract Concentration (%) | Average of the Growth of Fungal Colony (mm) | Average of Inhibition of the Growth of Fungal Colony (%) |
|----|---------------------------|---------------------------------------------|--------------------------------------------------------|
| 1  | P_0 (0.00)                | 59.05a*                                     | 0.00                                                   |
| 2  | P_1 (0.01)                | 49.50b                                      | 13.73a*                                                |
| 3  | P_2 (0.05)                | 50.50b                                      | 13.81a                                                 |
| 4  | P_3 (0.10)                | 49.21b                                      | 16.29a                                                 |
| 5  | P_4 (0.20)                | 46.07bc                                     | 20.55b                                                 |
| 6  | P_5 (0.30)                | 38.69c                                      | 31.84c                                                 |
| 7  | P_6 (0.40)                | 27.19d                                      | 52.18d                                                 |
| 8  | P_7 (0.50)                | 15.64e                                      | 70.79e                                                 |
| 9  | P_8 (0.60)                | 0.00f                                       | 100.00f                                                |

*values followed by the same letter in the same column are not significantly different according to the Duncan’s Multiple Range Test at p<5%.

**Table 3. Biomass formation and percent inhibition of the bark extract of *M. alba* in various concentration for a 14-day period of incubation**

| No | Extract Concentration (%) | Average of Biomass Formation (g) | Average of Inhibition of Biomass Formation (%) |
|----|---------------------------|---------------------------------|-----------------------------------------------|
| 1  | P_0 (0.00)                | 0.2040a*                        | 0.00a*                                        |
| 2  | P_1 (0.10)                | 0.1985a                         | 2.64a                                         |
| 3  | P_2 (0.20)                | 0.0661b                         | 67.80b                                        |
| 4  | P_3 (1.00)                | 0.0261c                         | 87.23c                                        |
| 5  | P_4 (1.50)                | 0.0138c                         | 93.24cd                                       |
| 6  | P_5 (2.00)                | 0.0000c                         | 100.00d                                       |

*values followed by the same letter in the same column are not significantly different according to the Duncan’s Multiple Range Test at p<5%.
the bark extract of *M. alba* so effectively inhibits the growth of *C. verruculosa* as the fungus responsible for the leaf spot disease on the rice plant. These are shown by the minimum inhibitory concentration to be 0.5% and at a 2.0% has inhibiting capability is 33.17 mm. This result of the current study is supported by the result of the study conducted by Sehajpal (2009) found out that the extracts of *Allium sativum* and *Syzygium aromaticum* at a 0.1% concentration can so strongly inhibit the growth of *Rhizoctonia solani* as the fungus responsible for the sheath blight disease on the rice plant. The diameters of the inhibited zone are 5.75 mm and 7.50 mm each. Pandey (2015) found out that the extract of the *Azadirachta indica* leaves at a 0.5% concentration can effectively inhibit the growth of the mycelia of the fungus *Magnaporthe oryzae* as the fungus responsible for the leaf blast and the growth of the mycelia of the fungus responsible for the brown leaf spot disease on the rice plant. The diameters of the inhibited zone are 28.35 mm and 27.12 mm each.

This result of the current study shows that the bark extract of *M. alba* can so effectively inhibit the colony of the fungus *C. verruculosa*. The bark extract of *M. alba* with a 0.6% concentration has been able to inhibit the growth of fungal colony. The bark extract of *M. alba* at a 2.0% concentration has been able to inhibit completely the biomass formation of the fungus *C. verruculosa* for a 14-day period of incubation.

### CONCLUSIONS

The results of this study can be concluded that the bark extract of *Michelia alba* has showed the antifungal activity against *Curvularia verruculosa* fungal with a minimum inhibition concentration value to be 0.5%. This extract at a 2.0% concentration can so strongly inhibit the growth of *C. verruculosa* with inhibiting capability is 33.17 mm. The bark extract of *M. alba* at a 0.6% concentration was able to completely inhibit the growth of fungal colony. The bark extract of *M. alba* at a 2.0% concentration has been able to inhibit completely the biomass formation of the fungus *C. verruculosa* for a 14-day period of incubation.

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### Conflict of Interest

Non-financial interest.

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