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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 concentratiotn 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 Ciherang 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 futher 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).

$$\text{inhibition activity (\%)} = \frac{\text{diameter of control colony} - \text{diameter of treatment colony}}{\text{diameter of control colony}} \times 100\%$$

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 biomass formation. The determination was done by the growth of fungal in PDB media. The biomass was harvested through centrifugation at 5,000 rpm for 5 minutes. The pellet (biomass) was taken and placed on glass filter paper and dried in an oven at 60°C until constant weight. The inhibitory activity against the fungal biomass was calculated according to the formula :

$$\text{inhibition activity (\%)} = \frac{\text{weight of control biomass} - \text{weight of treatment biomass}}{\text{weight of control biomass}} \times 100\%$$

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 P_5 (0.5%) is the treatment which shows the smallest inhibiting capabality, 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 capabality 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 P_8 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 P_8, 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

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 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

*solvent :ethanol 5% in water + Tween-80 (0,5%) as control.
**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 (0.50)	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 showed 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 *Syzygiumaromaticum* at a 0.1% concentration can so strongly inhibit the growth of *Rhizoctoniasolani* 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 is 100%.This is supported by the result of the study conducted by Nguefack, *et al.* (2013) found out that the essential oils of *Callistemon citrinus* L. and *Cymbopogoncitratus* can completely inhibit the growth of fungal colonies of *Alternariapadwickii* and *Bipolarisoryzae* the cause of brown spot disease on the leaf of rice plant at a 0.50 and 0.05% (b/v), respectively. Srinivas, *et al*. (2014) reported that the extracts of *Allium sativum* and C*alotropisprocera* at 10% concentration can effectively inhibit the growth of fungal colony of *Rhizoctoniasolani* the cause of sheath blight disease on the rice plant. Their inhibiting percentages are 91.82 dan 84.75% respectively. Panley (2015) reported that the extract of the *Azadirachta indica* leaves with a 0.5% concentration can most effectively inhibit the growth of the mycelia of the fungus *Magnaporthe oryzae* as the fungus responsible for the leaf blast and the mycelia of the fungus *Bipolaris oryzae* as the fungus responsible for the brown spot on the rice plant. Their inhibiting percentages are 40.09% and 43.13% respectively.

The result of this current study shows that the bark extract of *M. alba* so effectively inhibits the biomass formation of the fungus *C. verruculosa* as the fungus responsible for the leaf spot disease on the rice plant. The bark extract of *M. Alba* with a2.0% concentration has been able to inhibit the biomass production of fungal by 100%.This result of the current study is supported by the result of the study conducted by Zargar (2014) who found out that the extract of *Rhazya stricta* Decne at a 1.0% concentration can inhibit the biomass formation of the fungi *Penicillium notatum* and *Aspergillus niger* by 91.8 % and 93.2% respectively for a 15-day period of incubation.

CONCLUSIONS

The results of this study can be concluded that the bark extract of *Micheliaalba* 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 capabality 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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