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## Formulation of the bark extract of *Michelia champaca* to control *Curvularia* leaf spot in rice (*Oryza sativa* L.)

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Bawa, I G. A. G., Bawa-Putra, A. A., Santi, S. R., Sukadana, I. M., Suryanadi, O. and Indyan, G. (2024). Formulation of the bark extract of *Michelia champaca* to control *Curvularia* leaf spot in rice (*Oryza sativa* L.). International Journal of Agricultural Technology 20(5):1787-1796.

**Abstract** The formulation of a botanical fungicide with the active ingredient of *Michelia champaca* bark extract to control the growth of *Curvularia verruculosa*, which causes leaf spot disease in rice plants in the greenhouse was investigated. The results showed that the formula for *M. champaca* bark extract at a concentration of 1.5% (w/v) was reduced the disease intensity of *Curvularia* leaf spot by up to 66.01% at 24 days after application. This formula was also able to increase the number of tillers and leaf chlorophyll levels by 40.05% and 41.18%, respectively at 63 days after planting. The formula of *M. champaca* bark extract at a concentration of 1.5% (w/v) increased the yield of harvested dry grain by 89.26%.

**Keywords:** Botanical fungicide, *Curvularia verruculosa*, Disease intensity, Rice production

### Introduction

The *Curvularia verruculosa* fungus has caused leaf spot disease in Ciharang rice plants (Bawa, 2019). This disease has caused losses, so it is very disturbing to the farmers. The control of this disease was carried out using the synthetic fungicide. However, the uncontrolled use of synthetic fungicides harms farmers and the environment (West *et al.*, 2003; Yoon *et al.*, 2013). Therefore, it is necessary to find alternative solutions to control this disease without causing negative effects on farmers and the environment. Today many botanical fungicides have been developed to control various plant diseases because they contain a wide variety of bioactive compounds with residues that are easily degraded in nature and are not phytotoxic (Kagale *et al.*, 2005; Omezzine *et al.*, 2011).

The utilization of plant extracts that have the potential as a botanical fungicide in controlling various diseases caused by *Curvularia* fungus in rice plants is still very little. The extract of *Polygonum hydropiper* L., *Allium sativum*,

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*Zingiber officinale*, and *Azadirachta indica* were effective in controlling the seed-borne rice *Curvularia lunata* fungi (Rahman, 1992). The plant extract of *Juniperus procera* was able to suppress the growth of *C. lunata* isolated from stored grain at a concentration of 3 mg/ml with an inhibition percentage of 88.42% (Abdel-Ghany *et al.*, 2015). The bark extract of *Michelia alba* was able to control the growth of *Curvularia verruculosa*, which causes the leaf spot disease in rice plant (Bawa, 2019). Bawa, (2021) found that the bark extract of *Michelia champaca* also showed a very strong inhibitory effect on the growth of *C. verruculosa* fungi with an inhibition zone of 30.01 mm. This extract was known to contain tributyl acetylcitrate and terephthalic acid, dodecyl-2-ethylhexyl ester, which synergistically inhibit the growth of the fungus *C. verruculosa* very strongly (inhibition zone 26.73 mm) (Bawa *et al.*, 2024).

To develop of *M. champaca* bark extract as a botanical fungicide, the formulation of *M. champaca* bark extract in controlling *Curvularia* leaf spot in rice plants was conducted. The aim of this research was to determine the ability of this formula to reduce the intensity of *Curvularia* leaf spot and to increase rice production yields.

## **Materials and methods**

The bark of *Michelia champaca* was collected in Bongkasa Village, Badung, Bali. The strain of *C. verruculosa* CI-MAP Og 22018 was deposited in Biopesticide Laboratory Udayana University.

### ***Extraction***

The extraction was carried out in the Natural Chemistry Laboratorium of *Chemistry Department, Udayana University* of Bali Province in April, 2023. The bark of *Michelia champaca* was cleaned in running tap water and air-dried for a day. The dried samples were cut into small pieces and dried again. Samples were blended to dry to a powder. As much as 1000 g of powder macerated with 2000 ml of methanol for 24 hours 3 times. The filtrates obtained were combined, then the solvent evaporated using a rotary vacuum evaporator. The obtained residues are called crude extracts.

### ***Formulation of Michelia champaca bark extract***

The formulation was conducted in the Natural Chemistry Laboratorium of *Chemistry Department, Udayana University* of Bali Province in May, 2023. The formulation of *M. champaca* bark extract was made in Emulsifiable Concentrate (EC). The composition of the formula is presented in Table 1. The formula was

made in 1000 ml capacity, with 20% active extract, 10% amylum (density 1.5 g/ml), 1% emulsifier (Tween-80), 1% teefol as aligners, and water as solvent.

**Table 1.** The formulation of *Michelia champaca* bark extract

Treatment (%)	Compositon of Formula				
	Active Extract* (ml)	Emulsifiers (ml)	Adhesives** (ml)	Aligners (ml)	Solvent *** (ml)
T <sub>0</sub> (0,0)	0	10	10	10	970
T <sub>1</sub> (0,5)	25	10	10	10	945
T <sub>2</sub> (1,0)	50	10	10	10	920
T <sub>3</sub> (1,5)	75	10	10	10	895
T <sub>4</sub> (2,0)	100	10	10	10	870

\* The bark extract of *Michelia champaca* 20%, \*\* Amylum 10% and \*\*\* Water as solvent

***The capability of M. champaca bark extract to inhibit the C. verruculosa fungal caused the leaf spot disease on the rice plant in the greenhouse***

The experiment was conducted in the greenhouse of *Agricultural Faculty, Udayana University* of Bali Province in June to October, 2023. The experiment using a Group Random Design consisting of five treatments; they were T<sub>0</sub> = control (without extract); T<sub>1</sub> = the extract formula with a 0.5 % concentration; T<sub>2</sub> = the extract formula with a 1.0% concentration; T<sub>3</sub> = the extract formula with a 1.5% concentration; T<sub>4</sub> = the extract formula with a 2.0 % concentration, and each treatment was repeated five times, meaning that there were 25 units of experiment. Each unit consisted of 10 pots and in each pot two rice seeds were planted. The experimental activities included the process of raising seedlings, preparing the planting medium, the seedlings planting, the fertilizing process, the plant raising, the inoculation of the pathogen fungus (*C. verruculosa*), the application of the botanical fungicide and harvest.

The parameters that were observed included the intensity of the leaf spot disease, the growth (the height of the plant, the number of tillers, the content of leaf chlorophyll), and the crop components (the number of productive tillers, the number of rice grain per panicle, the weight of the rice grain per clump, the percentage of empty grain, the weight of 1000 grains and the estimated yield per hectare by weighing the rice yielded). Then all the production was totaled and converted into tons/hectare. The intensity of the leaf spot disease can be measured using the formula developed by Sinaga (2006). All data obtained were analysed quantitatively using analysis of variance (ANOVA). If there was a significant effect between treatments on the observed variables, the Duncans Multiple Range Test (DMRT) was used at the 5% level.

## Results

### *Effect of various formulations on intensity of leaf spot disease*

The use of the formula of *M. champaca* bark extract at various concentrations distinctively affects the intensity of *Curvularia* leaf spot on rice when it is 24 days after application (Table 2).

**Table 2.** Effect of treatment on disease intensity in rice for 24 days after application

Treatment (%)	Disease Intensity (%)	Percent Inhibition (%)
T <sub>0</sub> (0,0)	26,62a*	-
T <sub>1</sub> (0,5)	25,77a	3,19
T <sub>2</sub> (1,0)	12,43b	53,31
T <sub>3</sub> (1,5)	9,05c	66,01
T <sub>4</sub> (2,0)	10,00c	62,44

\*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%.

### *Effect of various formulations on the growth components*

The impact of the formula of *M. champaca* bark extract on the growth components such as the number of tillers, the height of the plant, and the chlorophyll content at 63 days after planting is presented in Table 3.

**Table 3.** The effect of the bark extract of *M. champaca* on the growth components in rice at 63 days after planting

Treatment (%)	Number of Tillers (stalks)	Height of Plant (cm)	Chlorophyll Content (SPAD unit)
T <sub>0</sub> (0,0)	20,80a*	108,97a <sup>ns</sup>	30,67a*
T <sub>1</sub> (0,5)	21,73a	108,43a	31,78a
T <sub>2</sub> (1,0)	26,47b	117,67a	36,83b
T <sub>3</sub> (1,5)	29,13c	121,61a	43,30c
T <sub>4</sub> (2,0)	28,20c	120,67a	43,00c

\*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%.

### *Effect of various formulations on the yield components*

The effect of the *M. champaca* bark extract formula on yield components which include the weight of grainy grain per hill (g), the weight percentage of empty grain (%), and the yield of harvested dry grain (tons/hectare) is presented in Table 4.

**Table 4.** The effect of the formula of *M. champaca* bark extract on yield components of rice plants

Treatment (%)	Weight of Rice Grain per Hill (g)	Weight Percentage of Empty Grain (%)	Harvest Yields (tons/hectare)
T <sub>0</sub> (0,0)	41,52a*	14,98a*	4,19a*
T <sub>1</sub> (0,5)	44,09b	12,99b	4,73a
T <sub>2</sub> (1,0)	60,98c	10,05c	6,52b
T <sub>3</sub> (1,5)	69,92d	7,94d	7,93c
T <sub>4</sub> (2,0)	68,17d	8,23d	7,78c

\*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%.

### **Discussion**

The use of a botanical fungicide with the active ingredient derived from the bark extract of *M. champaca* at a concentration of 1.5% significantly influences ( $p<0.05$ ) the intensity of *Curvularia* leaf spot in 24 days after application in rice plants. The botanical fungicide with the active ingredient extracted from *M. champaca* bark at a concentration of 1.5% was significantly affected ( $p<0.05$ ) in reducing the intensity of *Curvularia* leaf spot compared to the control (T<sub>0</sub>) without extract, (T<sub>1</sub>) with extract concentration of 0.5%, and (T<sub>2</sub>) with extract concentration of 1.0%, but not significantly different from (T<sub>4</sub>) with extract concentration of 2.0%. The highest intensity of *Curvularia* leaf spot occurred in the control (T<sub>0</sub>) at 26.62%, while the lowest intensity of the disease occurred in (T<sub>3</sub>) with an intensity of 9.05%. This data indicated that the botanical fungicide with the active ingredient extracted from *M. champaca* bark at a concentration of 1.5% is most effective in reducing the intensity of *Curvularia* leaf spot in rice plants at 24 days after application, with a percentage reduction

of 66.01% compared to the control. These results were better than the findings of several researcher, such as Nguefack *et al.* (2013), who discovered that the use of *Callistemon citrinus* L. extracts at a concentration of 2% (w/v) could reduce brown spot disease attacks on rice plants by 36-42%. Manjappa (2015) found that methanol, acetone, and water extracts of *Chromolaena odorata* L. at a concentration of 15% could decrease the intensity of blast disease on rice plants by 54.18%, 58.54%, and 56.79%, respectively, at 50 days after spraying. Thi *et al.* (2016) found that *Houttuynia cordata* and *Artemisia indica* extracts at a concentration of 5% could reduce the intensity of blast disease caused by *Magnaporthe oryzae* by 74.11% and 87.67%, respectively, at 5 days after application. Choudhury *et al.* (2017) found that chloroform extract of *Clerodendrum infortunatum* L. could reduce sheath blight attacks on rice plants caused by *Rhizoctonia solani* by 55.10%, while methanol extract of *Polyalthia longifolia* Sonn. could only reduce it by 44.23%. Kumar *et al.* (2017) found that the use of *Azadirachta indica* leaf extract at a concentration of 2% could reduce the intensity of blast disease caused by *Pyricularia oryzae* by 41.41%. Bawa (2019) reported that the use of a botanical fungicide with the active ingredient from *Michelia alba* bark at a concentration of 1.5% could reduce the intensity of *Curvularia* leaf spot by 62.87%. However, these result still far from being compare the findings of Pandey (2018) reported that the synthetic fungicides Bavistin (Carbendazim 50% WP) and Hinosan (Ediphenphos 50% EC) at a concentration of 0.1% (w/v) could reduce the intensity of the leaf blast on rice plants by 44.79% and 40.51%, respectively, compared to the control. The use of commercial botanical fungicides with azadirachtin as the active ingredient, such as Achook, Neem Gold, Tricure, and Wanis, at a concentration of 0.5% (v/v) could reduce the intensity of the leaf blast on rice plants by 36.63%, 33.58%, 33.27%, and 26.42%, respectively.

The use of the formula of the bark extract of *M. champaca* distinctively affects the number of tillers at the age of 63 days after planting. It showed that the formula of the bark extract of *M. champaca* at a 1.5% concentration was the most effective formula for increasing the number of tillers by 22.45% compared to the control when the rice plant is 63 days after planting. The capability of the bark extract of *M. champaca* at a 1.5% concentration is comparable to the use of commercial botanical fungicides made from active azadirachtin such as Tricure and Wanis at a 0.5% concentration (v/v) can increase the number of productive tillers by 12 and 4% each compared to the control (Pandey, 2018); but much better than extract of the *Azadirachta indica* at a 2% concentration (b/v) used as

the botanical fungicide for controlling the disease of the leaf blast can increase the number of productive tillers by 9.88% (Kumar *et al.*, 2017).

The use of the formula of bark extract of *M. champaca* at various concentrations was not distinctively contributed to the height of the rice plant when it is 63 days after planting. However, the use of the formula of bark extract of *M. champaca* at different concentrations distinctively contributes to the leaf's chlorophyll content. This data showed that the chlorophyll content of the formula of bark extract of *M. champaca* at a 1.5% concentration (T<sub>3</sub>) was 37.04% higher than that of the control, resulting from the low intensity of the *Curvularia* leaf spot. The reason is that the existence of a pathogen affects the plant's physiological function as it decreases the efficiency of photosynthesis (Perez *et al.*, 2014; Rios *et al.*, 2014).

The use of the *M. champaca* bark extract formula at various concentrations was significantly affected ( $p < 0.05$ ) on the weight of rice grain per hill. The active extract formula with a concentration of 1.5% (T<sub>3</sub>) had significantly affected ( $p < 0.05$ ) in producing the weight of rice grain per hill compared to control (T<sub>0</sub>), T<sub>1</sub> treatment (0.5%), and T<sub>2</sub> (1.0 %), but not significantly different from the T<sub>4</sub> treatment (2.0%). The heaviest weight of rice grain per hill was obtained in the T<sub>3</sub> treatment (1.5%), namely 69.92 grams, while the smallest weight of grain per hill was produced by the control (T<sub>0</sub>), namely 41.52 grams. It showed that the *M. champaca* bark extract formula at a concentration of 1.5% was the most effective in increasing the weight of rice grain per hill with an increasing percentage reaching 68.40% as compared to the control.

The use of the *M. champaca* bark extract formula at various concentrations was significantly affected ( $p < 0.05$ ) on the weight percentage of empty grain produced. The active extract formula with a concentration of 1.5% (T<sub>3</sub>) was significantly affected ( $p < 0.05$ ) in producing the weight percentage of empty grain compared to control (T<sub>0</sub>), treatment T<sub>1</sub> (0.5%), and T<sub>2</sub> (1.0%) but not significantly different from the T<sub>4</sub> treatment (2.0%). The highest weight percentage of empty grain, namely 14.98%, was produced by the T<sub>0</sub> (control) treatment, while the lowest weight percentage of empty grain was produced by the T<sub>3</sub> treatment (1.5%), namely 7.94%.

The use of the active extract formula for *M. champaca* bark at various concentrations was significantly affected ( $p < 0.05$ ) on harvest yields. The active extract formula with a concentration of 1.5% (T<sub>3</sub>) was significantly affected ( $p < 0.05$ ) in producing harvest yields compared to control (T<sub>0</sub>), T<sub>1</sub> treatment (0.5%), and T<sub>2</sub> treatment (1.0%), but not significantly different from the T<sub>4</sub>

treatment (2.0%). The highest harvest yield 7.93 tons/hectare was shown in the T<sub>3</sub> treatment (1.5%), while the smallest harvest yield was shown in control (T<sub>0</sub>) 4.19 tons/hectare. It showed that the *M. champaca* bark extract formula at a concentration of 1.5% was the most effective in increasing harvest yields with an increasing percentage of 89.26%. The results of this study were better than the results of research by Nguefack *et al.* (2013) who reported that *Callistemon citrinus* L extract at a concentration of 2% increased harvest yields by around 25-55%. Kumar *et al.* (2017) reported that *Azadiractha indica* leaf extract at a concentration of 2% was increased rice yields by 2.03%. However, these result are still far from being compared the findings of Pandey (2018) reported that the use of synthetic fungicides Bavistin (Carbendazim 50% WP) and Hinosan (Ediphenphos 50% EC) at a concentration of 0.1% (w/v) increased rice yields by 39.99% and 34%, respectively, and the use of commercial vegetable fungicides containing the active ingredient azadirachtin such as Achook, Neem Gold, Tricure, and Wanis at a concentration of 0.5% (v/v) were able to increase rice yields by 29.97; 20.30; 18.82; and 17.00%.

The formula of *M. champaca* bark extract at a concentration of 1.5% (w/v) can reduce the intensity of *Curvularia* leaf spot by up to 66.01% at 24 days after application, increasing the number of tillers and leaf chlorophyll levels by 40.05% and 41.18% respectively at 63 days after planting, and also increasing the yield of harvested dry grain by 89.26%.

## Acknowledgements

The authors wish to acknowledge the Dean of Mathematics and Natural Sciences Faculty, Udayana University, Bali for funding this research.

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(Received: 27 April 2024, Revised: 7 September 2024, Accepted: 10 September 2024)