The Potency of *Anredera cordifolia* as Botanical Pesticide for Sustainable Blast Disease (*Pyricularia oryzae*) Management on Paddy

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**Abstract.** Blast disease (*Pyricularia oryzae*) is one of the most important diseases of rice that can significantly reduce paddy production. This disease causes a potential loss of 3.65 tonnes/ha with loss percentages ranging from 60-100% if the disease is not handled properly. In spite of the issue of the negative effects of synthetic pesticide usage, the blast disease is commonly controlled using synthetic fungicides. Therefore, the exploration of potential botanical pesticides may provide safer and environmentally friendly blast disease management. This study aimed to test the potential of binahong plant (*Anredera cordifolia*) as botanical fungicide to inhibit the growth of *P. oryzae* and the development of blast disease in laboratory testing. The experiments were arranged in a Completely Randomized Design (CRD) consisting of 7 treatments of 5 extract concentrations and 2 controls. All treatments were repeated 5 times. The effectiveness of binahong leaf extract in controlling the pathogen and the disease was tested using poisoned food technique and detached leaf assay. The binahong leaf extract tested in the concentration range of 0.25-2% showed the ability to inhibit the growth of *P. oryzae* with the highest colony growth inhibition of 25.11%. Characteristics of *P. oryzae* mycelium and colony in the binahong leaf extract treatment were different from the control treatment. The treatment of paddy leaves with binahong leaf extract suppressed the blast disease development through the lessening of diseased paddy leaf area percentages. This study indicates the potency of binahong leaf extract for sustainable rice blast disease management.

1. **Introduction**

The decline in rice production is reported to be caused by the reduction of rice cultivation area in addition to the decrease in rice productivity. Rice production and rice productivity in Java are reduced by 0.83 million tons and 0.17 quintal/ha (0.33%) in 2014 [1]. Several factors contribute to the decline of rice productivity, one of which is the presence of plant pathogens. In some cases, plant pathogen has become a major obstacle in increasing rice production. Pathogenic fungus *Pyricularia oryzae* Cav. is the causal agent of rice blast disease that the infection of this pathogen can result in high rice yield loss or crop failure [2].

Blast disease is a worldwide major disease in rice plants [3,4]. Yield loss in several countries such as Kenya, China, the Philippines, India and Korea can be above 50% or even reach up to 100% [5,6]. In Indonesia, the disease has considerably reduced rice yields in several regions, especially in West Java, Central Java and East Java [7]. Previous research described that *P. oryzae* infection on Ciherang variety
in endemic areas (Sukabumi, West Java) can cause yield loss of 3.65 tons/ha, equivalent to 61% yield loss compared to the average production of the variety [8].

Due to its potential to cause high yield loss, it is necessary to control blast disease seriously. Control of blast disease can be done including by applying particular cultivation techniques, the use of resistant varieties and the application of fungicides [7]. However, blast disease control is predominantly carried out chemically using synthetic fungicides. Some active ingredients of synthetic fungicides commonly used in controlling blast disease are reported to be effective in reducing the disease development or inhibiting P. oryzae infection [9,10]. Nevertheless, continuous application of synthetic chemicals is believed to have negative effects on the environment and human health. Likewise, in recent time there has been considerable pressure from consumers to farmers to reduce or eliminate synthetic pesticides in agriculture [11], so that there is an urge to develop alternative control methods to synthetic pesticides such as the use of botanical pesticides.

Botanical pesticides are natural chemicals extracted from plants which are mainly secondary metabolites that are used to control pests through inhibition of pathogen growth or suppression of disease development [12,13]. Botanical pesticides can be recommended as an alternative because they can be degraded more quickly and have low toxicity to the environment and humans [11,14,15]. The control of rice plant diseases using plant extracts has been widely reported and provides good and effective results in suppressing targeted pathogens. Achalypha indica weed extract had a potential as an antifungal of several rice pathogens such as P. oryzae, Cercospora oryzae, and Sclerotium oryzae in vitro [16]. The use of other plant extracts such as garlic and ginger extracts was found to reduce the pathogen infection of Pyricularia grisea in a field trial (Shafuallah and Khan, 2016). In addition, Azadirachta indica seed oil extract reduced the growth of P. oryzae in vitro and reduced the development of blast disease in rice plants in greenhouse trial [17]. The ethanol extract of Citrus medica was also reported to inhibit S. oryzae radial growth in vitro with inhibition reached up to 90% [18].

Plants with the potency as botanical pesticides are considered to contain secondary metabolites or phytochemical compounds [18]. These secondary metabolites are the active ingredients of botanical pesticide which are generally obtained through the extraction of plant parts such as seeds and leave [20]. Binahong (Anredera cordifolia (Ten.) Steenis) has been widely used as traditional medicinal ingredients and informed to have antifungal and antibacterial properties. In human health, binahong has been reported to suppress the growth of fungal pathogens as well as accelerates wound healing by stimulating more granulation tissue formation [21,22]. Binahong plant extracts showed to have antibacterial substances that can inhibit the growth of Streptococcus mutans and Escherichia coli bacteria in vitro [23,24]. In some countries such as China, Korea, or Taiwan, binahong plant has been used in healing various diseases and has been consumed for more than hundreds of years [25]. This plant is commonly used to treat wounds, smooth the skin, eliminate body aches, increase body stamina, as an antioxidant, and as an antibacterial drug [23]. Binahong plants are reported to have properties for health and treatment of various diseases because of its antimicrobial compounds [26,27]. Some researchers reported that binahong plants contain alkaloids, polyphenols, flavonoids, saponins, tannins and anthraquinones [25,28,22]. Studies of binahong extracts as antimicrobials of plant pathogens confirmed that binahong leaf water extract inhibited the growth of Collectotrichum spp. and Fusarium oxysporum with the inhibition percentage of 66.95% and 38.67%, respectively [29,30]. This study aimed to examine the potential of binahong plants as botanical fungicide to inhibit the growth of P. oryzae colony and suppress the development of blast disease in vitro.

2. Materials and methods

2.1. Pyricularia oryzae isolation

Pyricularia oryzae was isolated from rice plant leaves that showing symptoms of blast disease. The plant tissue was cut between healthy and diseased appearance. These small pieces were dipped in 70% alcohol solution for 10 seconds then dipped in 1% sodium hypochlorite solution for 1 minute, rinsed with sterile distilled water and dried on sterile filter paper. The leaf tissue pieces are then placed in a Petri dish
containing Potato Dextrose Agar (PDA) medium and incubated at room temperature. The obtained isolates were purified and identified as *P. oryzae* using identification book of [31].

2.2. *Binahong* leaf extractions

*Binahong* leaves were washed and dried using oven for 12 hours at 40°C to reduce their water content. The oven dried leaves are then prepared in powder form by blending. *Binahong* leaf powder was then macerated with methanol with a ratio of leaves powder and methanol 1:10 (w/v). The maceration was allowed to stand for two days in a closed container, filtered using filter paper, and evaporated with a rotary evaporator at a temperature of 55-60°C and a pressure of 580-600 mmHg [32]. Obtained crude extract was then prepared in five tested extract concentrations of 0.25, 0.5, 1, 1.5 and 2% w/v using sterile Tween-80 0.01% solution. The extract solution was sterilized by filtering using a 0.2 µm microfilter.

2.3. *Inhibition test of binahong leaf extract on the growth of P. oryzae colony (Poisoned food technique)*

The inhibition test of *binahong* leaf extract on the growth of *P. oryzae* colony was performed on PDA medium. Each tested concentration of *binahong* leaf extract was mixed with sterilized molten PDA (1 ml: 9 ml) aseptically in sterile Petri dishes. The mixtures were then swirled gently to mix content evenly. Mycelial discs (Ø = 5 mm) were taken with a sterile cork-bore from the 7 days old cultures of the *P. oryzae* and were placed centrally on the cooled extract-medium plates and incubated at 28°C. Two control treatments were carried out as described above but with sterile aquadest and Tween-80 0.01% solvent were added to each of the plates. The radial mycelial growth of both test and control plates were then measured. Observations on the diameter of fungal colonies were carried out at 24-hour intervals until the control treatment reached maximum diameter. The percentage of inhibition of the growth of *P. oryzae* colonies was calculated by the following formula:

\[
\text{Inhibition} = \frac{(\text{ØC} - \text{ØT})}{\text{ØC}} \times 100\% = \frac{(\text{ØC} - \text{ØT})}{\text{ØC}} \times 100\% \quad (1)
\]

\(\text{ØC} = \) Colony diameter in control treatment (cm); \(\text{ØT} = \) Colony diameter in extract treatment (cm)

The study was carried out using Completely Randomized experimental design with 7 treatments that were repeated 5 times. All data were subjected to ANOVA, and significant means were separated by Duncan Multiple Range Test of SPSS Ver. 16.

2.4. *Inhibition test of binahong leaf extract on the blast disease suppression in vitro (Detached leaf method)*

Healthy rice plant leaves were cut and washed using sterile distilled water. In the meantime, plastic boxes were prepared with sterile tissue was placed on the bottom surface of the boxes and straws were then arranged vertically on the tissue layer. The tissue was moistened with sterile distilled water until saturated. To facilitate the infection, the leaf area was wounded using a small needle. Four pieces of leaves that have been cleaned and wounded were arranged in each plastic box horizontally with the both ends of the leaf pieces were inserted under the wet tissue [33] After that, 20 µl of *binahong* leaf extract at each tested concentration was dripped over the wounded leaf area and left around 60 seconds. The mycelium block (Ø = 5 mm) of *P. oryzae* was then inoculated above the surface of the wounded leaf area and incubated at room temperature. Observations are made up to 7 days after inoculation. Observations were made on the occurrence of symptoms of blast disease, the length and width of the formed lesion, and the characteristics of the lesion.

3. Results and discussion

The results showed that *binahong* leaf extract was able to inhibit the growth of *P. oryzae* colony at all tested concentrations except the lowest concentration of 0.25%. The diameter of the *P. oryzae* colonies on observation 14 days after incubation at 0.5-2% concentration treatments was significantly shorter.
than that in the control treatment (Table 1; Figure 1). The result indicates the potential of binahong leaf extract to suppress the growth of *P. oryzae*. The highest inhibition of *P. oryzae* colony growth was shown in the treatment of 2% extract concentration with the inhibition percentage of 25.11% that differed significantly with 0.5% and 1% concentration treatments. The results showed that the higher the extract concentration used, the higher the inhibition of *P. oryzae* growth.

**Table 1.** Inhibitions of the growth of *P. oryzae* colonies in binahong leaf extract treatments

| Treatments                  | Colony diameter (cm)* | Inhibition (%) |
|-----------------------------|-----------------------|----------------|
| 0.25% extract concentration | 9.00 a                | 0              |
| 0.5% extract concentration  | 8.60 a                | 4.44           |
| 1% extract concentration    | 7.90 b                | 12.22          |
| 1.5% extract concentration  | 7.40 b                | 17.78          |
| 2% extract concentration    | 6.74 c                | 25.11          |
| Tween-80 0.01% solution     | 9.00 a                | -              |
| Sterile aquadest            | 9.00 a                | -              |

* Means in the same column with the same letter are not significantly different (Duncan Multiple Range Test α = 0.05).

In addition to cause *P. oryzae* growth inhibition, the treatment of binahong leaf extract affected the characteristics of *P. oryzae* colony or mycelium. The condition of *P. oryzae* colony or mycelium in the extract treatment was different from the control treatment. The growth of mycelium in the control treatment appeared as normal conditions of lesser and thinner colony or mycelium with dark grey in color. Meanwhile, the colony or mycelium in the binahong leaf extract treatments was seen as thickening (dense) colony or mycelium with brighter colour and even dried (crusty) in some parts. The effect of plant antimicrobial compounds on the characteristics of fungal colonies or mycelium has also been reported. Essential oils of some plants that inhibited the colony growth of *Colletotrichum gloeosporioides* also caused mycelium agglomeration of the fungus and acervulus reduction [35]. Changes in fungal mycelium were also reported in the testing of several fungi such as *Alternaria humicola*, *Rhizoctonia solani* and *Fusarium solani* on the use of *Asarum heterotropoides* essential oil [34]).

The inhibition of fungal colony growth and abnormal growth of *P. oryzae* mycelium suggest that binahong leaf extract has the potential to suppress the growth of *P. oryzae*. The ability to inhibit growth and affect the characteristics of the mycelium of *P. oryzae* is considered to be caused by secondary metabolites contained in binahong leaf extract. It is reported that the ability of plant extracts in suppressing pathogens or disease development is caused by the metabolite compounds contained in the plants. Some bioactive compounds such as flavonoids, saponins, triterpenoids, coumarin and phenolic acids are reported to be present in binahong plants [25,28,36]. Secondary metabolite compounds such as alkaloids, flavonoids, tannins and saponins have properties as antimicrobials. Alkaloids produce allelopathy with characteristics as colourless, toxic, and bitter compounds [37]. According to [23], flavonoid compounds have the function as antibacterial and antivirus for plants. Saponin compounds function as antimicrobials by inhibiting fungal and bacterial growth and protecting from pests [38]. Tannin compounds are mentioned to have a bitter taste and function as an antifungal and antibacterial [39]. In plants, phenol compounds function as plant defence mechanisms against disturbances of plant pests and pathogens [19].
The inhibition test of colony growth of *P. oryzae* using binahong leaf extract at several tested concentrations.

In this test, crude extract of binahong leaves was dissolved using Tween-80 0.01% solution. Addition of Tween-80 0.01% as a carrier was intended to improve the solubility of the extract. However, the inhibition of *P. oryzae* growth with the highest inhibition percentage of 25.11% is still categorized to be low. Several factors might cause this low effectiveness, one of which was the possibility of less dissolved of binahong leaf extract in the PDA medium. In addition, it is reported that the quality of plant extracts was influenced by several factors, including plant materials used, types of solvents and extraction methods [13]. Therefore, the effectiveness of the binahong plant extract as a botanical fungicide can still be improved. In this study, crude binahong leaf extract was prepared through the extraction using methanol solvent but other solvents or emulsifiers were then not added in the tests. Thus, the addition of these substances is believed to be able to increase the ability of the extract of binahong plants in suppressing the growth of pathogens.

In detached leaf assay, binahong leaf extract treatments suppressed the development of blast disease symptoms. In the control treatment, typical blast disease lesions were clearly formed and developed. The lesions were longer and wider than in the extract treatments. Some lesions formed in binahong leaf extract treatments were considered not to be disease lesions but stains of leaf wounding effect or due to phytotoxic especially in the use of high extract concentrations (Figure 2). The result of this test indicated that the application of binahong leaf extract on rice plant leaves can prevent the development of blast disease symptoms. It is likely that the presence of extracts on the leaf surface can eliminate *P. oryzae* inoculum or prevent the infection. In general, the results of this study indicate the potential of binahong leaf extract to control blast disease which can be used as an alternative for disease control in environmentally friendly way.
Figure 2. (A) Symptoms of blast disease in control treatment. (B) Symptoms of blast disease in binahong leaf extract treatment.

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