**Fungicidal effect of rolled tobacco and binahong extracts against rice sheath blight pathogen: in vitro studies**

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**Abstract**

The sheath blight disease of rice is becoming an increasingly important problem, especially in regions with intensive rice cultivation. Rice is a staple food and a major source of calories for many communities. There are various challenges to controlling this disease, including the lack of resistant varieties. The objective of this research was to examine rolled tobacco (*Nicotiana* sp) and “binahong” (*Andredera cordifolia*) leaf extracts' ability as a plant-based fungicide against *Rhizoctonia solani* sheath blight fungus, pathogenic to rice. An ethanol-based and water-based extraction was used in this work, and organized according to a Completely Randomized Design using 3 replications. The results showed that all extracts significantly affected the growth of sheath blight fungus *R. solani*. The best concentration in inhibiting thefungus was obtained in “binahong” extract at a concentration of 3% with ethanol solvent, while the best concentration of tobacco was 3% in ethanol solvent with an inhibition percentage of 48,52% and 24.82% respectively. Phytotoxicity occurred when using binahong extract 3 % in water and ethanol solvent, 14 days after application. On the contrary, rolled tobacco extract did not cause any side effects or toxicity to the plants. This will bring insight into further benefits of rolling tobacco in managing plant diseases

**Keywords**: ethanol, staple food, *Rhizoctonia solani*, water, widespread

**Introduction**

Sheath blight, caused by the fungus *Rhizoctonia solani* Kuhn, is one of the most common diseases, currently developing and widespread in rice-producing areas in Indonesia. The widespread planting of high-yielding short-type rice varieties with high doses of fertilizers, especially urea, can increase the severity of the disease. It is becoming increasingly important in the rice production systems, especially in areas with intensive rice cultivation as rice is a staple food and a major source of calories, and contains good magnesium, phosphorus, manganese, selenium, iron, folic acid, thiamin, and niacin (Fukagawa and Ziska, 2019). This disease is caused by a pathogen that has a wide host range so genetic resistance traits are challenging to find. Most rice varieties show a non-resistant reaction to this disease (Nuryanto, 2017). Pathogenic spread is more extensive than saprotrophic spread, as the fungus uses resources from the infected host to spread through translocation processes (Kleczkowski et al. 1997; Simon et al. 2014)

To control the fungus, synthetic pesticides are often used. While chemicals are intended to eliminate pests, they also pose a risk to non-target organisms. Pesticides can also cause human health problems in addition to environmental issues. Inappropriate and injudicious application techniques can harm the environment by killing beneficial microbes. Consequently, risk-free and environmentally friendly control strategies are needed. Research on the antibacterial and antifungal activities of plant extracts has attracted the attention of researchers.

Biopesticides, such as compounds found in plants, have promising prospects. This is because raw materials are readily available and easily decompose (Lengai et al. 2022; Kursheed et al., 2022). Mother nature has made available various phytochemicals within plants, which are shown to resist pests with great effectiveness. Utilizing plants and wastes from the agro-process industry is one of the natural pest control approaches. The active ingredients of plant-based pesticides are natural compounds derived from plants that contain a group of secondary metabolites containing bioactive compounds such as alkaloids, terpenoids, phenolics, and other secondary chemical substances. Bioactive compounds are secondary metabolites found in plants that have a protective role against biotic and abiotic stress. Recently, some primary metabolites have also been recognized as bioactive compounds and used to prevent and treat a wide range of diseases (Konstantinidou-Doltsinis 2006; Banožić et al. 2020). While plants have enormous benefits as pesticides, the potential is not widely exploited (Kala 2020). This study proposed to test the potential effectiveness of tobacco rolls (*Nicotiana* sp) and binahong (*Andredera cordivolia*) leaves (Figure 1) in inhibiting the growth of *R. solani*, which causes leaf blight disease in rice. There was not much information regarding these sources as a bio-fungicidal use against disease in rice.



Figure 1**.** Plant materials used in this study. On the left are rolled tobacco; On the right are Binahong leaves

**Materials and Methods**

**Isolation of *R. solani* from rice field**

Samples for fungal isolation were collected from rice local variety “Mawar”, grown in Senaning Village, Pemayung District, Batanghari Regency (-1.578582, 103.355562). Rice leaves and sheath were cut into 1 x 1 cm pieces and sterilized for 10 seconds in a 2% NaOCl solution. The leaves were then rinsed three times with sterile distilled water before being isolated on Potato Dextrose Agar (PDA) media. The fungal cultures were then subsequently transferred to a freshly developed PDA medium for pathogen determination and then incubated. The culture plates showing typical *R. solani* growth were selected after 3 days and the mycelium was observed under a microscope to confirm the pathogen (Figure 2). Mycelium derived from these plant parts was purified and identified using Barnett and Hunter's key (1998).

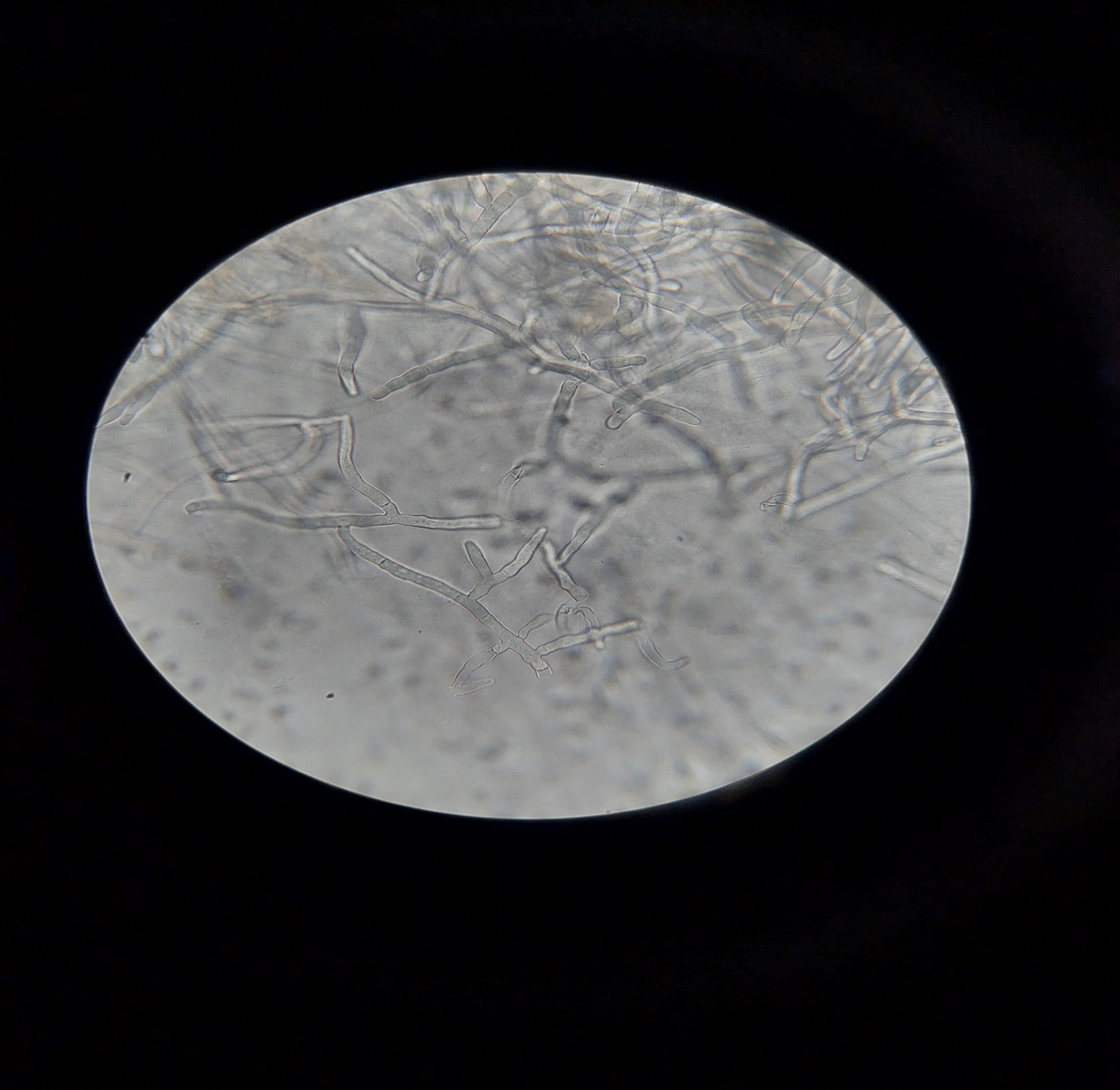
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Figure 2. *Rhizoctonia solani* growing in the PDA medium used in this study. Left: *R. solani* colony; Right: the appearance of septated hypha and right-angle branching

**Plant-based pesticide extract preparation**

In this experiment, we used 2 kinds of solvent for each plant; water solvent and ethanol solvent. Plant-based or biopesticides were derived from dried tobacco rolls and fresh binahong leaves. Clean rolled tobacco and “binahong” leaves were weighed and sterilized by soaking in a 2% NaClO solution for 2 minutes before being rinsed with running water. The rolled tobacco and binahong leaves were then cut into 2-3 mm pieces before being oven-dried for 24 hours at 45-50 C. Tobacco roll and binahong leaves Simplicia were then crushed until smooth with a mortar and pestle. Tobacco rolling and binahong Simplicia powder were weighed and placed in the first infundation pot, which was then filled with 100 ml of distilled water. The decocted method, which is an extraction method with hot water, was used to create extracts with water solvents (Health Department, 2000; Dono et al, 2008).

The decoction was started by placing the Erlenmeyer in the infusion pot, and then 10 g of each Simplicia was added to 100 ml of distilled water in the Erlenmeyer. Enough water was added to the poured pot to cover the Erlenmeyer. The next step was to heat the extraction pot to 90°C for 30 minutes. After standing the brewing results for 24 hours, they were filtered through Whatman #1 filter paper to separate the residue and extract Simplicia. The preparation of rolled tobacco and binahong leaf extracts with ethanol solvent was carried out by grinding the dried leaves using a mortar and pestle until a fine powder was obtained. The fine powder was then macerated in a 70% ethanol solution at a ratio of 1:10 (w/v). The macerated solution was left for 2 days, then filtered with Whatman filter paper and evaporated with a rotary evaporator (B-one series 1000 - RE 1000 HN**)** at 50°C and 100 rpm to separate and extract (Dono et al., 2008)

The concentrated extract was poured into pre-weighed glass bottles. The filtered solvent was allowed to dry at room temperature. The mass of the extract was determined and the extract was dissolved in ethanol (100%) to a final concentration of 10 mg/mL. The dissolved extract (10 mg/mL) was used as a stock solution to prepare when performing the food poison test (Utomo, 2016; Malada et al. 2022).

**Inhibition power of plant extracts**

The bioassay used the food poisoning technique to evaluate the biofungicidal effectiveness of the extracts. A pre-calculated amount of extract was poured into a petri dish, followed by Potato Dextrose Agar medium and incubation at room temperature. The tested *R. solani* fungus was picked up using a cork borer from the periphery of the developing stored colony. This 5 mm diameter disc was placed in the center of the premix extract PDA of each concentration and then incubated at room temperature (Minz et al., 2014). The medium with the tested extracts and fungal isolates was then allowed to settle at room temperature and the diameter of colonies was counted following incubation of the plates every day (Ramadas et al. 1998)

Observations were taken daily to measure the growth of the *R. solani* colony diameter. The observation ended when the colony in the control treatment covered the entire surface of the agar media on the Petri dish. The percentage of inhibition of fungal colonies was calculated using the formula by Lira-de Leon et al. (2014).

Note: The percentage of inhibition ability (I) is calculated using the mean diameter of the control (Dc) and the mean diameter of the treatment (Dt).

**Phytotoxicity test**

Phytotoxicity testing was performed on rice seeds by sterilizing them with 70% alcohol, immersing them in 2% NaClO solution, washing them with sterile distilled water, and then placing them in Petri dishes with assigned concentration treatments. Testing was carried out with 3 replications over 7 to 14 days, and any extract that prevents seed germination or shows symptoms is considered poisonous and cannot be further used as a biological agent (Tefa, 2017; Ferraz et al. 2022).

**Research Design and Data Analyses**

The treatment involved administering extracts of rolled tobacco and binahong leaves to inhibit the growth of *R. solani*. The extracts were obtained using water and ethanol solvents at concentrations of 1.5%, 2%, 2.5%, and 3%, and were arranged in a complete randomized design with 3 replications. There were 2 controls used; positive using synthetic pesticides, and negative using distilled water. ANOVA was used to analyze the data, and if significant, followed by the 5% DMRT test.

**Results**

**Inhibition power**

Figure 3. Effect of tobacco roll and “binahong” leaves extract on the colony growth of *R. solani* fungus at various concentrations. (NC), negative control=distilled water; (PC), positive control=difenoconazole; (AT1), tobacco water extract 1.5%; (AT2), tobacco water extract 2%; (AT3), tobacco water extract 2.5%; (AT4), tobacco water extract 3%; (AB1), binahong water extract 1.5%; (AB2), binahong water extract 2%; (AB3), binahong water extract 2.5%; (AB4), binahong water extract 3%; (ET1), tobacco ethanol extract 1.5%; (ET2), tobacco ethanol extract 2%; (ET3), tobacco ethanol extract 2.5%; (ET4), tobacco ethanol extract 3%; (EB1), binahong ethanol extract 1.5%; (EB2), binahong ethanol extract 2%; (EB3), binahong ethanol extract 2.5%; (EB4), binahong ethanol extract 3%.

The study found that a 3% concentration of binahong ethanol extract had the highest inhibitory ability against *R. solani* fungus colonies on a medium, with a 48.52% inhibition rate (Figure 3,4).



**AB4**

**EB4**

**ET4**

**AT4**

**PC**

**NC**

Figure 4. Representation of fungal colony growth of *R. solani* at 7 days after inoculation at various concentration levels of tobacco and “binahong” leaf extracts and negative and positive control treatments: (NC), negative control distilled water; (PC), positive control of difenoconazole fungicide; (AT4), 3% tobacco water extract; (AB4), 3% binahong water extract; (ET4), 3% tobacco ethanol extract; (EB4), 3% binahong ethanol extract. Sclerotia emerged from negative control.

**Plant extract phytotoxicity test**

The germination of rice seeds treated with water and ethanol extracts of rolled tobacco and “binahong” leaves at different concentrations was observed for 7 days. All seeds germinated without tobacco extract causing toxicity. However, the “binahong” extract showed phytotoxicity symptoms at 14 days at a concentration of 3% water and ethanol solvent, reaching 6.67% and 10.00% (Figure 5).

Figure 5. Phytotoxicity of rolled tobacco and binahong leaves extract on the rice seed at various concentrations. (NC), negative control=distilled water; (PC), positive control=difenoconazole; (AT1), tobacco water extract 1.5%; (AT2), tobacco water extract 2%; (AT3), tobacco water extract 2.5%; (AT4), tobacco water extract 3%; (AB1), binahong water extract 1.5%; (AB2), binahong water extract 2%; (AB3), binahong water extract 2.5%; (AB4), binahong water extract 3%; (ET1), tobacco ethanol extract 1.5%; (ET2), tobacco ethanol extract 2%; (ET3), tobacco ethanol extract 2.5%; (ET4), tobacco ethanol extract 3%; (EB1), binahong ethanol extract 1.5%; (EB2), binahong ethanol extract 2%; (EB3), binahong ethanol extract 2.5%; (EB4), binahong ethanol extract 3%. Data 14 DAI where Arcsin transformed

**Discussions**

The fungicidal effect of a compound against pathogenic fungi is usually tested in vitro through its ability to inhibit colony growth on culture medium, conidia or spore production, and conidia germination. This research’s observations were limited to the growth of visible fungal colonies on the culture medium, and it was noted that the colony morphology of the *R. solani* treated with the compounds was slightly different from the control treatment. It was worth mentioning that only the control treatment produced sclerotia. While many fungi are tested for plant-based fungicides for their ability to inhibit conidia production or germination, this is not possible with *R. solani* due to its unique life cycle (Datta et al. 2022).

This study employed rolled tobacco, the processed tobacco green leaf, through the formation of local tobacco traditional blends to tobacco smoke. We observed that the application of rolled tobacco leaf and “binahong” leaf extracts inhibited the growth of *R. solani* colonies in vitro. Similar findings by Sela-Buurlage (1993) of tobacco vacuolar chitinase isoforms and β-1,3-glucanase were effective against *Fusarium solani*. The 3% binahong ethanol extract showed the greatest inhibitory ability, which was influenced by the extract concentration and solvent used. Higher concentrations of extracts have a higher content of active ingredients that function as anti-fungi (Popova 2015).

Plant extracts are being used for fungicidal action owing to their content of secondary metabolites. Terpenoids are one of the most notable categories of secondary metabolites (Kennedy and Whitman 2011). The biogenic compound triterpenoid (limonoids) can control sheath blight disease in rice by inhibiting the pectate lyase enzyme. It is soluble in alcohols, ketones, and esters, and slightly hydrophilic (Mahanty et al. 2021). The plant bioactive compounds may prevent cell wall polymer synthesis by interfering with the (1,3)-ß glucan synthase enzyme (Hector 1993). Extracts of tobacco were also investigated for their antimicrobial properties against Gram-positive and Gram-negative bacteria and yeast. The results demonstrated variations in efficacy based on the product and type of tobacco used, indicating its potential applications (Popova et al., 2015).

The germination of rice seeds treated with aqueous and ethanol extracts of tobacco rolling and binahong leaves was observed for 7 days. All seeds germinated without any indication of toxicity. The lower part of the sprouts near the roots did not show any symptoms of shriveling or yellowing. However, after 14 days of tobacco extract application, the seeds turned yellow and rotted. This illustrated that the extract did not retain its longevity and the effect on disease control was less effective. Previous studies have shown that excessive use of vegetable pesticides can be toxic to plants, causing changes in shape, yellowing, necrosis, leaf dropping, and abnormal growth (Aktar et al. 2009). According to Eppo (2007), seed germination should be tested immediately after treatment with pesticides, although the period between seed treatment and phytotoxicity test can be after long-term storage. Tests are also carried out after a suitable storage interval depending on the possible storage period of the seeds ("12 months for cereals", i.e.). In the case of plant-based pesticides, it has been found that phytotoxicity occurs in the short term (14 days). For this reason, the seeds must be sown within the period before the onset of phytotoxicity.

We successfully extracted tobacco rolling and binahong leaves using two solvents, water and ethanol. Our primary focus was to determine the simplest extraction method, and we found that water was the most effective. This method can easily be executed by farmers independently if the results are satisfactory. We found exploring water extraction to be a valuable effort, as plant water extracts have been proven to be highly efficient in extracting active ingredients from plants. The exploration of extracting water is still worthwhile (Amadioha 2000; Onejeme 2021; Suganda and Wahda 2021). Hubert et al (2015) also reported that with only aqueous solvents some plant extracts, such as tobacco, aloe vera, and chrysanthemum, were effective in inhibiting more than 80% of *Pyricularia oryzae* colony growth and preventing powdery mildew (Konstantinidou-Doltsini et al 1998). Further research is required to ascertain the extent to which this affects its performance in practical applications.

**Conclusions**

The research discovered that a 3% concentration of “binahong” extract in ethanol effectively inhibited the growth of *R. solani* by almost 50 %. The most effective rolled tobacco extract was identified as 3% in ethanol with a 24.82% inhibition rate, and it was proven to be non-toxic to rice seeds. However, the use of “binahong” extract caused light phytotoxicity symptoms after 14 days of treatment at 3% water and ethanol extracts with a phytotoxicity percentage of 6.67% and 10.00%, respectively.

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**Author contributions**

IH designed and supervised the experiment. LR performed the experiment, analyzed the data, and wrote the manuscript with IH. IH and SM collaborated in the reviewing of the manuscript. N, and HM supervised data analysis. All authors read and approved the final manuscript.

**Conflict of Interest**

The authors declare that there is no conflict of interest regarding the publication of this article.

**Data Availability**

Data presented in this study will be available on fair request to the corresponding author.

**Ethics approval**

Not applicable.

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