In Vitro Biological Control of Fungus *Botryodiplodia* sp. Using Plant-based Pesticide

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Abstract. *Botryodiplodia* sp. is a pathogenic fungus with a wide range of hosts, including agricultural and forestry crops. *Botryodiplodia* sp. attacks have been reported to cause symptoms of dry rot, branch rot, fruit rot, and shoot death resulting in decreased productivity. Efforts to control diseases caused by the fungus *Botryodiplodia* sp. are needed to avoid further infection in healthy plants. Control using chemical pesticides will cause several negative problems for the environment and human health. Alternative control of *Botryodiplodia* sp. is using biopesticide. This study aimed to analyze the ability of plant extract-based pesticides to inhibit the growth of *Botryodiplodia* sp. in vitro. The method used was the food poisoning method on PDA and PDB with concentrations of biopesticide 0%, 0.25%, 0.5%, 1%, 4%, 7%, and 10%. The result of this research is the concentration of 0.5% is sufficient to inhibit the growth of *Botryodiplodia* sp by 11.73% in PDA and 21.96% in PDB. Biopesticides are suspected to have fungistatic characteristics so that they only inhibit but do not stop fungal growth.

1. Introduction

Integrated pest and disease management is an important thing in the practice of forestry crop cultivation. Chemical compounds commonly used in integrated pest and disease management are pesticides. Pesticide is a translation of pesticide which comes from the Latin *pestis* and *caedo* which means poison to control intruders[1]. Based on experience in Latin America, using pesticides can increase production by up to 40% in cocoa plants. Sugarcane cropping increased by 33% in Pakistan with pesticides, and according to FAO records, pesticide use can save yields of 50% in cotton crops[2].

The use of synthetic pesticides can cause resistance to pests and diseases, the development of new pests or diseases (resurgence), and pollute the environment. The application of chemical pesticides also interferes with human health when applying and pesticide residues on crop yields interfere with consumer health[3]. Therefore, alternative pesticides that are environmentally friendly and do not harm human health are needed to overcome these problems. Biopesticides derived from the extraction of certain parts of plants, such as leaves, fruit, seeds, or roots that have secondary compounds or metabolites, which are toxic to certain pests and diseases are called biopesticides[4]. The effectiveness of biopesticides varies depending on the type and dose or concentration.

Jabon (*Neolamarckia cadamba* (Roxb.) Miq.) is a fast-growing species that is currently widely chosen in forestry planting practices. This species is widely used as shade trees, plywood, construction wood, pulp and paper, carving, traditional medicine, and for restoration[5]. The problem that occurs in Jabon cultivation in nurseries is the presence of pests and diseases. Diseases that often occur in
nurseries are shoot death, leaf blight, and leaf spot caused by *Botryodiplodia* sp. Research on the application of synthetic pesticides to *Botryodiplodia theobromae* in vitro has been reported to affect inhibiting the growth of mycelium colony diameter[6]. This study aimed to analyze the ability of plant extract-based pesticides to inhibit the growth of *Botryodiplodia* sp. in vitro. This research is expected to provide basic information regarding the effectiveness of biopesticide in inhibiting the growth of *Botryodiplodia* sp.

2. Methods

The research was carried out in the form of in vitro laboratory experiments using the food poisoning method which was tested by mixing biopesticide and growing media of fungi. The experiments were located at Forest Pathology Laboratory in the Department of Silviculture Faculty of Forestry and Environment IPB University from May to June 2021. The data used are the results of observations of *Botryodiplodia* sp. diameter growth on Potato Dextrose Agar (PDA) media and biomass growth on Potato Dextrose Broth (PDB) media. The biopesticide content information was collected from the product package.

2.1. Preparation and Rejuvenation of Pathogen Isolates

Isolates of *Botryodiplodia* sp. used in this study was a collection from the Laboratory of Forest Pathology, Faculty of Forestry and Environment, IPB University. The pure isolate was then rejuvenated on PDA media in a petri dish. Work was carried out in a sterile cabin in Laminar Air Flow.

2.2. Biopesticide Solution Dilution

The dilution of the biopesticide solution was carried out based on the concentration level in the test. The calculation of the concentration was carried out using the concentration formula in units of percent volume (v/v). The biopesticide solution was diluted in 7 concentration levels, namely 0%, 0.25%, 0.5%, 1%, 4%, 7%, and 10%. The concentration of 0% was used as a control.

2.3. Isolate Inoculation

The rejuvenated isolates that had filled the Petri dishes were used as a source of inoculum. In the first stage, the isolates were printed using a 0.8 cm cork borer. The isolate pieces were then transferred to the growing media using a spatula. Cork borers and spatulas have been sterilized in a bunsen flame before use. Petri dishes and jars filled with isolate were sealed with plastic wrap to prevent contamination.

2.4. Diameter and Biomass Observation

The growth of isolate diameter was measured using a ruler every 12 hours until the control filled the dishes. Observation of diameter growth was carried out by measuring the x-axis and y-axis on a petri dish. the lengths of the two axes are averaged. Isolates of *Botryodiplodia* sp. incubated for 7 days in PDB medium. During the incubation period, PDB was shaken in a stirrer at 100 rpm. After the incubation period, the mycelium was filtered using filter paper which had been previously oven-baked for 24 hours at 60°C. Dry weight was weighed using an analytical balance.

2.5. Calculation of Inhibition Percentage

The calculation of the percentage of inhibition was carried out by comparing the treatment and control. The percent inhibition value of mycelium colony diameter and mycelium biomass was calculated using the formula.

\[
\text{Inhibition Diameter} = \frac{Dc - Dt}{Dc} \times 100\% \\
\text{Inhibition Biomass} = \frac{Bc - Bt}{Bc} \times 100\% \\
Dc = \text{Mycelium’s colony diameter of control (cm)}
\]
3. Discussion

3.1. Morphology of Botryodiplodia sp.

Botryodiplodia sp. is a plant pathogenic fungus that is a facultative parasite. This fungus is classified as belonging to the Ascomycetes class, the order Dothideales, and the Botryosphaeraceae family[7]. Fungal morphology in general can be seen based on its macroscopic and microscopic morphology. The macroscopic characteristics that can be seen in this study are the color and shape of the colony (Figure 1).

**Figure 1.** The appearance of *Botryodiplodia* sp. in a petri dish on Potato Dextrose Agar (PDA). (A) Colony on day 7. (B) Colony on day 30.

Isolates of *Botryodiplodia* sp. which were rejuvenated on Potato Dextrose Agar (PDA) media and incubated for one month had a color change. Fungal isolates one week after inoculation showed grayish-white colonies, while fungal isolates one month after inoculation showed blackish colonies. Macroscopic appearance also has aerial colony character. Asnani[8] stated that hyphae are divided into two types, namely vegetative hyphae (substrate) and aerial hyphae (air). Colony growth initially developed as substrate hyphae which were then followed by the development of aerial hyphae.

**Figure 2.** Microscopic appearance of *Botryodiplodia* sp. (A) Hyphae. (B) Young conidia. Scale = 50 m.

Microscopic appearances that can be seen under a microscope are hyphae and conidia (Figure 2). The observed hyphae had brownish hyaline characteristics, were insulated, and branched, while the conidia were brown and not insulated. Faizah[9] stated that in his research the fungus *Botryodiplodia theobromae* had insulated and branched hyphae, and conidia in the form of ellipsoids or ovoids. Hyaline young hyphae that grow will become brown when old. Young conidia were 9.49 x 22.33 m and were hyaline in color, while older conidia were 10.05 x 22.36 m and dark brown[10]. Initially, conidia are not insulated (aseptate), then when old conidia have septa.
3.2. Biopesticide Content

The biopesticide which used in this research is included in the biopesticide in the form of insecticides, bactericides, and fungicides that play a role in disturbing pests and inhibiting the growth of fungi and bacteria that cause plant diseases.

The biopesticide is made from natural ingredients so they are safe for human health and the environment. The use of synthetic pesticides can cause resistance to pests and diseases, the development of new pests or diseases (resurgence), and pollute the environment. The composition of the plantes solution was synthesized from plant ingredients containing active ingredients, such as neem (Azadirachta indica), cloves (Syzygium aromaticum), citronella (Cymbopogon nardus L.), and liquid smoke from charcoal coconut shell.

3.3. Inhibition test on PDA

*Botryodiplodia* sp isolates without application of biopesticides (control) filled the dishes at 72 hours or 3 days after inoculation (Figure 3). Other fungi, such as *Pestalotia* sp., filled the dishes at 7 days after inoculation[11]. This indicates that the radial growth of the fungus is relatively fast.

![Figure 3](image-url)

**Figure 3.** The average diameter growth of mycelium colony

The average growth rate of the control treatment and the application of biopesticides has a relatively similar pattern. The treatment of giving botanical pesticides with a level of 0.5% gave different trends at 24, 36, and 48 hours after inoculation. Diameters at 24, 36, and 48 hours after inoculation were slightly lower than the other concentrations. Then the growth increased again at 60 hours after inoculation to approach 0% treatment or control. This was caused by a delay in growth or temporary growth inhibition, after which growth takes place again. This condition illustrates that Plantes biopesticides are more fungistatic than fungicidal. The hyphae of the fungus *Botryodiplodia theobromae* can continue their growth again after the inhibitory effect of liquid smoke disappears.

![Figure 4](image-url)

**Figure 4.** Inhibition percentage of biopesticide
The graph of the percentage of inhibition is inversely proportional to the growth rate of the pathogen (Figure 4). The highest percentage of inhibition was indicated by the 0.5% treatment level of 25.25% at 48 hours after inoculation. Meanwhile, the lowest percentage of inhibition was indicated by the 4% treatment level at 48 hours after inoculation. A positive value in the graph indicates an inhibition, while a negative value indicates an increase in growth. Based on the graph, all treatment levels gave diameter inhibition on PDA media. This indicates that the growth inhibition that occurs at this concentration is due to the active substance in Plantses which has antifungal activity. The suspected antifungal content comes from each plant extract in the pesticide.

Neem (A. indica) which has the main content of azadirachtin can inhibit the production of mycotoxins by pathogenic fungi and has Nimbin and nimbicin compounds that play an active role in inhibiting the growth of pathogenic mycelium[12]. Clove (S. aromaticum) and citronella (C. nardus) essential oils can dissolve fat in cell walls so that cell walls are damaged and as a result, fungal cells are not selective and can cause tissue damage and death[13]. Phenol compounds produced from liquid smoke can affect the mitochondrial function of fungi so that cellular respiration is disrupted, as well as damaging cell membranes in fungal body tissues and inactivating enzymes secreted by fungi[14].

Observations on PDA media were carried out until the control isolate filled the cup. Radial growth of isolates tended to fill the dish at 72 hours after inoculation. This pattern was in contrast to Akromah’s study[11], which reported that the diameter of the fungus on PDA media will get smaller as the concentration level increases, which means that antifungal compounds are inhibited.

The calculation of the percentage of inhibition was carried out to find out how much diameter inhibition was due to the treatment. The calculation of the inhibition value was carried out by comparing the treatment diameter with the control diameter at every 12 hours of measurement. Figure 5 shows the percentage value of the inhibition of the fungus Botryodiplodia sp. at each concentration level of the treatment against the control on PDA media. The highest diameter inhibition value was found at the 0.5% level of 11.73%, followed by concentrations of 0.25%, 7%, 4%, 10%, and 1% with inhibition values of 5.21%, 3.70%, 3.17%, 1.86%, and 1.76%, respectively. The graph does not show a correlation between the concentration level and the percentage of inhibition.

This is not by Putri[15] statement which states that the lower the concentration of the extract, the less the amount of active substance dissolved in the extract so that the ability to inhibit fungi is lower. On the other hand, the higher the concentration, the more active substances that function as antifungals, so that the ability to inhibit fungal growth is greater.

The magnitude of the different inhibitory power on PDA media is thought to be due to the lack of ability of the agar medium to diffuse at high concentrations of biopesticides. According to Dewi[16], the increase and decrease in the inhibition zone were due to the solubility of the active substance in the

![Figure 5. Biopesticide inhibition percentage average on PDA](image-url)
extract and the difference in the rate of diffusion in the agar medium. Extract concentrations that are too high can cause saturation to cause the active compounds contained cannot be dissolved completely[17].

3.4. Inhibition test on PDB
An inhibition test on the PDB medium was carried out at the end of the observation, which was the 7th day after inoculation. The calculation of the inhibition of biomass was carried out by comparing the treatment biomass and the control biomass. The percentage of inhibition for each treatment level can be seen in Figure 6.

Based on the calculation of the percentage of inhibition of the fungus Botryodiplodia sp. In PDB media, the application of Plantes biopesticides with a level of 0.5% resulted in the greatest inhibition of biomass, namely 21.96%. Giving treatment with a level of 7% and 1% also resulted in inhibition of 13.90% and 3.92%, respectively. Meanwhile, the levels of 0.25%, 4%, and 10% gave the opposite result.

![Figure 6. Biomass inhibition percentage average on PDB](image-url)

The application of Plantes botanical pesticides increased the growth rate by 0.87%, 4.14%, and 6.42%, respectively. The inhibition produced in PDB media is quite fluctuating. However, the concentration of 0.5% still produced the highest inhibition as was the case with PDA media.

Plantes biopesticides contain many natural chemical components derived from plant extracts. Pratiwi[18] stated that the main component can affect the antifungal properties of a solution, but chemical compounds in the solution also play an important role in synergistic or antagonistic against pathogen inhibition.

4. Conclusion
A plant-based pesticide with food poisoning method was able to give 11.73% inhibition of mycelium’s colony diameter at 0.5% concentration and 21.96% inhibition of mycelium’s colony biomass at the same concentration against the fungus Botryodiplodia sp. The increase in concentration did not indicate an increase in inhibition. This is presumably because the solution is too concentrated and there are antagonistic factors between the components of biopesticides.

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