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EFFECT OF LIQUID SMOKE APPLICATION INTERVAL ON PLANT BINTARO (*Carbera manghas*) AGAINST MUSHROOM GROWTH CAUSES OF DISEASE IN ANNUAL PLANT SEEDS

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Abstract

The objective of this study is to identify the most dominant pathogen fungi found in the seed, then to be able to analyze the level of effectiveness of bintaro plant liquid smoke, against plants affected by the pathogen fungus, and to analyze the time intervals of application of the liquid fume of binto plant that is most effective in controlling pathogen mushrooms in the sowing. The research was conducted at the Faculty of Agriculture, Mulawarman University, Samarinda, and in the planting of PT. Adindo Jaya Borneo for 3 months starting in October - December 2023. The research was carried out using a completely randomized design consisting of 4 treatments which were repeated 6 times, namely: p0 = without liquid smoke treatment as control; p1 = sprayed with 50 ml/l of liquid smoke once every 1 week; p2 = sprayed with 50 ml/l of liquid smoke once every 2 weeks; and p3 = sprayed with 50 ml/l of liquid smoke once every 3 weeks. The research results show thatthe dominant disease-causing pathogenic fungus found in annual plant nurseries is the fungus Rhizoctonia sp. which causes leaf spot disease;Application of liquid smoke from Bintaro plants has a very significant effect on the intensity of attacks by Rhizoctonia sp leaf spot disease. Application of liquid smoke from bintaro plants is quite effective in suppressing the growth of leaf spot disease by the herbal medicine Rhizoctonia sp. in the nursery and sprayed with 50 ml/l of liquid smoke once every week (p1) resulted in the lowest intensity of leaf spot disease attacks by the fungus Rhizoctonia sp with an average of 31.21%, and with an efficacy level of 27.56%.

Keywords

Liquid smoke, Bintaro plants, Pathogens in the seed.

1. INTRODUCTION

Liquid smoke is liquid smoke vapor condensate resulting from the pyrolysis of materials containing the main constituent compounds acid, phenol, and carbonyl resulting from the thermal degradation of cellulose, hemicellulose, and lignin components. Acid, phenol, and carbonyl compounds in liquid smoke contribute to aroma, color, and flavor characteristics. This phenolic compound has strong anti-microbial properties and one of its earliest uses was as an antiseptic.[1] Bintaro is a tropical mangrove plant that is often used as a shade tree found in Asia, Australia, Madagascar, and the islands of the Western Pacific Ocean. Bintaro plants have beneficial anticancer properties through apoptotic activity and plant leaf extracts are filtered to determine their antioxidant activity. Apart from that, it is also used as an insecticide, pesticide, or antifungal agent [2].

The use of quality plant seeds is one supporting factor. By using high-quality plant seeds, you will get plants with a very high level of adaptation, fairly fast initial growth, and good growth form [3]. This decrease in the quality of the seeds produced can be influenced by aspects of maintaining and protecting these plants in the nursery. The main factor that can limit the success of these plant seeds in being able to grow optimally is the attack of disease-causing pathogens [4]. Seedling damage resulting from disease attacks is relatively difficult to overcome when compared to attacks from pests.

In their application, inorganic pesticides have been proven to be able to reduce losses due to damage to agricultural products due to pest organisms. However, on the other hand, inorganic pesticides have a negative impact. Considering the side effects caused by the use of the above pesticides, it is necessary to develop pesticides that are easily degraded naturally, and are toxic to target plant pest organisms but are not toxic to humans and livestock in the surrounding area. does not pollute the environment and is not harmful to human health [5].

Research regarding the identification of diseases in nurseries has been carried out quite a lot in other locations but in nurseries owned by PT. Adindo Jaya Borneo has still not identified any cases of plant disease caused by pathogen attacks, according to the results of interviews and surveys at the PT nursery. Adindo Jaya Borneo, most of the seedlings in the nursery show characteristics and symptoms of pathogenic fungal attacks and there is still no control effort by the company.

Therefore, research was carried out to identify the pathogenic fungi that were found to be the most dominant in the nursery, then to be able to analyze the level of efficacy of Bintaro plant liquid smoke on plants attacked by pathogenic fungi, and to analyze the time interval for application of Bintaro plant liquid smoke that was most effective in controlling Pathogenic fungi in the nursery.

2. MATERIALS AND METHODS

2.1. Time and Location

This research was carried out from October to December 2023 at the Faculty of Agriculture, Mulawarman University, Samarinda, and the PT plant nursery. Adindo Jaya Borneo.

2.2. Materials and Tools

The materials used in this research were leaves from 4 types of plants, 2 types of fruit plants (Rambutan, and Durian) and 2 types of forest plants (Belangeran, and Gaharu) that had symptoms of being attacked by pathogenic fungi, plant samples taken that were 4 months old, liquid plant smoke. Bintaro grade 3, potato agar media (PDA), 70% alcohol, sterile distilled water, spirits, filter paper, tissue paper, label paper, cotton, plastic bags, aluminum foil, and newsprint. The tools used in this research are a camera, cutter, microscope, cover, deck glass, laminar air flow, cup, oven, analytical balance, tissue, tube needle, tweezers, Bunsen burner, calculator, hand sprayer, meter, tally set, rope, measuring cup, water bottle, bamboo, and writing utensils.

2.3. Experimental Design

This study used a Completely Randomized Design (CRD) with 4 treatment plots, 6 replications, and 3 application time intervals with the same dose given throughout the interval, namely 50 ml/l, namely:

p0 = without liquid smoke treatment
p1 = sprayed with 50 ml/l of liquid smoke once every 1 week
p2 = sprayed with 50 ml/l of liquid smoke once every 2 weeks
p3 = sprayed with 50 ml/l of liquid smoke once every 3 weeks

Each sample consists of 12 polybags in each plot, and each plot consists of 4 different types of plants, in each plot there are 3 polybags with the same type of plant, the polybags are closely spaced, the length of the plot is 65 cm, the width of the plot 50 cm, the distance between rows of plots is 1 meter and the distance between plots is 1.5 meters.

2.4. Research Procedure

2.4.1. Sterilization of tools and materials

Before sterilization is carried out, tools such as Erlenmeyer flasks and Petri dishes are washed and dried, then wrapped in wrapping paper. Then sterilized using an oven at a temperature of 121 °C for 1 hour. Tools such as needles and tweezers are sterilized by burning or heating with a Bunsen lamp before use. Meanwhile, the fruit is sterilized by spraying it with 90% alcohol.

2.4.2. Making PDA Media (Potato Dextrose Agar)

Prepare potatoes weighing 200-250 grams, then peel and wash them clean, then cut the potatoes into 1 cm pieces like dice. Boil the potatoes in 1 liter of distilled water until the potatoes are cooked, if they are soft, immediately filter the boiled potato water and then boil the filtered potato water again. Add 20 grams of powdered sugar and 20 grams of agar sticks into the boiled potato water while stirring until the stems dissolve evenly. After that, add 1 capsule of Chloramphenicol as an antibacterial. After everything is mixed evenly, turn off the stove then immediately put the solution into an Erlenmeyer tube, then cover it with cotton wool and then cover it with aluminum foil. Put the Erlenmeyer into the autoclave at 121 °C for 15 minutes. Storing the PDA in the refrigerator, if you want to use it, heat the PDA media first, then the PDA is ready for use.

2.4.3. Taking samples of plants attacked by fungi

Macroscopic research activity procedures were carried out in the PT nursery. Adindo Jaya Borneo where the beds containing seedlings are divided into 4 beds based on different types of plants with plant ages of 4 months. The plot size is the same, each bed consists of 1000 seeds. Then observe and identify symptoms of disease attacks on each plant seed. Next, take the part of the plant that has the clearest symptoms of the disease as a sample and put it in a plastic clip to take it to the laboratory and carry out microscopic observations.

2.4.4. Isolate plant parts attacked by pathogenic fungi

Isolation of plant parts attacked by pathogenic fungi begins with washing all parts of the plant with clean running water. After sterilizing the plant parts, the samples were cut into ± 1 cm lengths. The pieces were then washed with 5% NaOCl solution for 1 minute and then soaked in 90% alcohol for 1 minute and repeated 2 times. Next, rinse using distilled water for 1 minute and repeat 2 times, so that it is sterile from other pathogens so that it is hoped that the fungus that grows comes from parts of the plant that are attacked by pathogenic fungi. Next, the sample pieces are dried on sterile tissue. After drying, the sample pieces were placed on PDA media in a petri dish. Petri dishes containing sample pieces of plants attacked by pathogenic fungi were then wrapped in plastic cling wrap.

2.4.5. Purification of pathogenic fungi

Purification of pathogenic fungi is carried out from the results of fungal isolation that has been carried out previously. Fungal colonies that grow around the sample are purified based on macroscopic morphology. After isolation, on the PDA media the fungal hyphae will grow, then take 1 spore each from all the growing pathogenic fungi using a loop needle and place them into a petri dish containing PDA media using the scratch cup technique. The petri dish where the mushrooms have been placed is then wrapped in plastic cling wrap. Observations were carried out for 7-10 days and if macroscopically different colony growth was still found, then re-purification was carried out until a pure isolate was obtained. [6]

2.4.6. Characteristics of pathogenic fungal isolates

Characteristics of pathogenic fungal isolates were carried out by observing macroscopic and microscopic characteristics. Macroscopic characteristics were carried out by observing pure pathogenic fungal isolates including colony color, the reverse color of the colony, elevation, top shape, and edges. Meanwhile, direct microscopic characteristics were carried out by dripping methylene blue which had been inoculated with pathogenic fungal isolates and observed using a microscope. The microscopic characteristics observed include hyphal structures and reproductive structures.

2.4.7. Production of liquid smoke

Making liquid smoke is done using a tool in the form of a tightly closed chimney, at the top of which there is a smoke hole through a pipe and then it flows through an iron pipe soaked in water which functions as a coolant. The capacity of this tool is 200kg of organic material. After the bintaro shell and leaves are weighed, they are then put into the chimney which has previously been made into a burning fire, and then closed tightly with a cover made of iron plate. Burning lasts for 7 hours with temperatures reaching ± 400°C. The liquid smoke that is formed comes out of the cooling column through a smoke distribution pipe connected to the liquid smoke combustion chimney. Liquid smoke comes out through a distribution pipe and is then collected into a bucket. And then let it sit at room temperature. At this stage, the liquid smoke is called crude smoke liquid[7].

2.5. Data Collection

The data collected were observations of the intensity and level of efficacy of Bintaro fruit liquid smoke carried out at 1, 2, 3, 4, 5, 6, 7, 8, and 9 weeks.

2.6. Data Analysis

Data analysis was carried out descriptively and analysis of variance as well as further tests with the Least Significant Difference (LSD) test at 5% level.

3. RESULTS AND DISCUSSION

3.1. Result of Research

3.1.1 Making Liquid Smoke

Liquid smoke can be obtained by condensing or condensing biomass from the pyrolysis process. Liquid smoke is a mixture of solutions of wood smoke suspension in water which is made by condensing or condensing the results of wood pyrolysis. This pyrolysis process produces gas, solids, and liquid smoke. Smoke is produced by incomplete combustion which involves the decomposition reaction of polymer constituents into low molecular weight organic compounds due to the influence of heat which includes oxidation, polymerization, and condensation reactions.

During combustion, wood components, including cellulose, hemicellulose, and lignin, will undergo a pyrolysis process to produce various compounds, including phenols and their derivatives, carbonyls (ketones and aldehydes), acids, furans, alcohols, lactones, polycyclic aromatic hydrocarbons and so on. The most important components contributing to the smoking reaction are three compounds, namely phenol, acid, and carbonyl.

The pyrolysis process involves various reaction processes, namely decomposition, oxidation, polymerization, and condensation. The processes that occur during pyrolysis according to increasing temperature are removal of water from wood at a temperature of 120 - 150°C, pyrolysis of hemicellulose at a temperature of 200 - 250°C, pyrolysis of cellulose at a temperature of 280 - 320°C and pyrolysis of lignin at a temperature of 400°C. Pyrolysis at a temperature of 400°C produces compounds that have high organoleptic qualities and at even higher temperatures there will be a condensation reaction to form new compounds and oxidation of the condensation products followed by a linear increase in tar compounds and polycyclic aromatic hydrocarbons. [8]

The pyrolysis temperature greatly influences the yield of liquid smoke produced, from the trials that have been carried out show that the higher the temperature in the pyrolyzer will decompose the organic components in the material into liquid smoke to the maximum, so that the liquid smoke produced will also have a higher yield. The increase in liquid smoke yield for each increase in temperature is because each additional heat in the pyrolysis process will decompose many substances contained in the material so that the condensed smoke increases. [9]

3.1.2. Identification of Pathogenic Fungi

The results of the isolation and identification of the fungal pathogen that causes leaf spot disease in gaharu, belangeran, rambutan, and durian plants show that the leaves of all plants are infected by the fungus *Rhizoctonia* sp. Symptoms at the start of the attack are yellow-brown spots extending to the tips of the leaves, and gray-brown on the underside of the leaves, but some types of plant leaves show reddish and reddish-purple colors. The results of observations in the field characterized the symptoms of *Rhizoctonia* sp leaf spot disease. in the nursery can be seen in Figure 1.

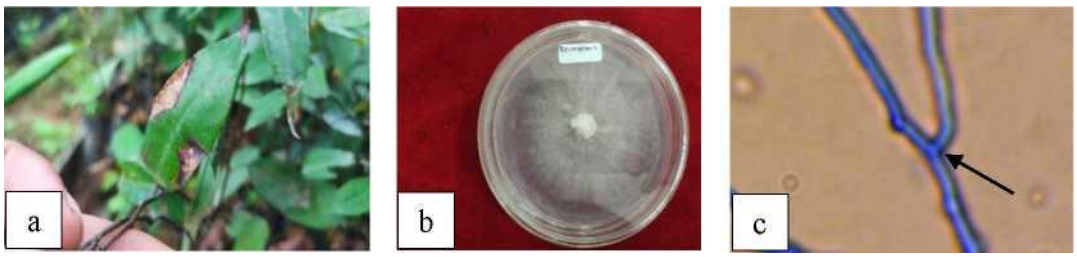

Figure 1. Leaf Spot Disease (Rhizoctonia sp.) on Belangeran Plants (a) Symptoms of attack in the field, (b) Mycelium *Rhizoctonia* sp. on PDA media, (c) *Rhizoctonia* sp. Microscopically, the hyphae branching shows a 90^{o} angle

Based on the characteristics of the fungus isolate *Rhizoctonia* sp. on PDA media, at the beginning of growth it will be pure white and spread thinly on the surface of the cup, then it will turn brown to black when it is old. Results of macroscopic observations of fungal isolates *Rhizoctonia* sp. on PDA media can be seen in Figure 2.

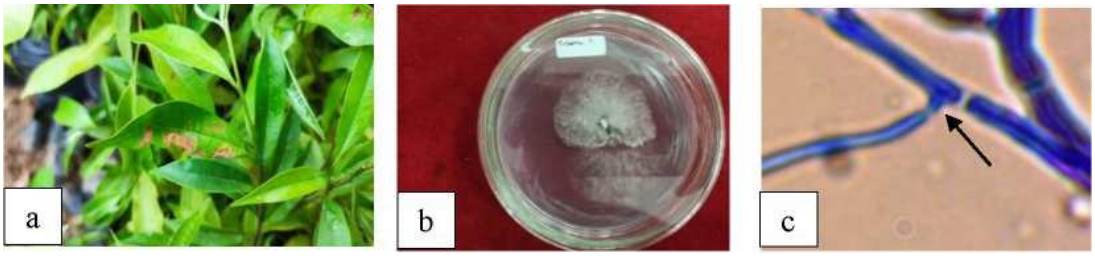

Figure 2. Leaf Spot Disease (*Rhizoctonia* sp.) on Agarwood Plants (a) Symptoms of attacks in the field, (b) Mycelium *Rhizoctonia* sp. on PDA media, (c) *Rhizoctonia* sp. Microscopically, the hyphae branching shows a 90º angle

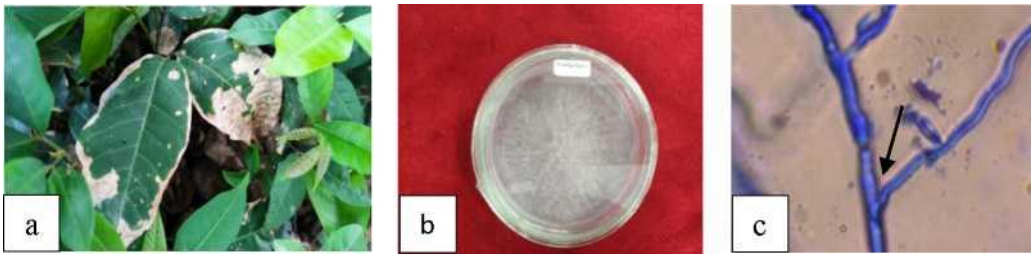

Figure 3. Leaf Spot Disease (*Rhizoctonia* sp.) on Rambutan Plants (a) Symptoms of attack in the field, (b) Mycelium of the fungus *Rhizoctonia* sp. on PDA media, (c) *Rhizoctonia* sp. Microscopically, the hyphae branching shows a 90º angle

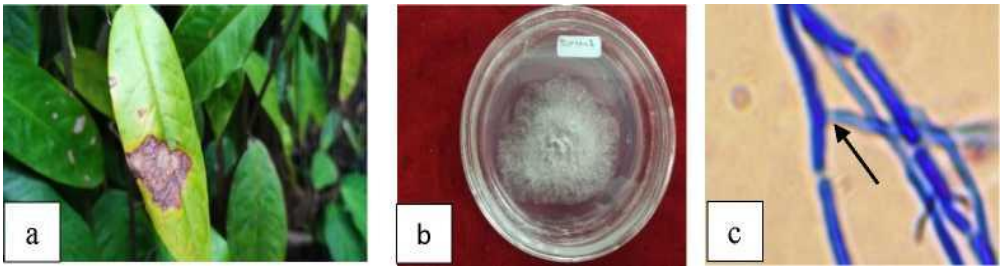

Figure 4. Leaf Spot Disease (*Rhizoctonia* sp.) on Durian Plants (a) Symptoms of attack in the field, (b) Mycelium of the fungus *Rhizoctonia* sp. on PDA media, (c) *Rhizoctonia* sp. Microscopically, the hyphae branching shows a 90ºangle

3.1.3. Intensity of Rhizoctonia Leaf Spot Disease Attacks. sp Before Application of Liquid Smoke to Bintaro Plants

Based on the analysis results of calculating the intensity of leaf spot disease attacks by the fungus *Rhizoctonia* sp. On plants in the nursery, before liquid smoke was applied to Bintaro plants, all plant samples were identified as being attacked by *Rhizoctonia* sp leaf spot disease. Data on calculating the intensity of disease attacks before applying liquid smoke to the Bintaro plant is presented in Table 1.

Table 1. Disease Intensity Analysis Before Liquid Smoke Application

Treatments	Average Attack Intensity (%)
p0 (Without liquid smoke treatment)	38,51
p1 (sprayed with 50 ml/l of liquid smoke once every 1 week)	39,81
p2 (sprayed with 50 ml/l of liquid smoke once every 2 weeks)	40,28
p3 (sprayed with 50 ml/l of liquid smoke once every 3 weeks)	40,63

Source: Primary data processed

Based on the data above, shows the intensity of attacks by *Rhizoctonia* sp leaf spot disease. before the application of liquid smoke, the Bintaro plant ranged between 38.51 – 40.63% (all included in the medium attack intensity category)

3.1.4. Intensity of Rhizoctonia Leaf Spot Disease Attacks. sp After Application of Liquid Smoke to Bintaro Plants

Observation of the intensity of this disease attack was carried out by observing the rate of spread of spots on plant leaves in the nursery after the application of liquid smoke to Bintaro plants during 9 weeks of observation. The results of the variance analysis showed that the application of liquid smoke to the Bintaro plant had a very significant effect on the intensity of the Rhizoctonia sp leaf spot disease attack. The results of the research on the effect of the application of liquid smoke on Bintaro plants had a very significant effect on the intensity of *Rhizoctonia* sp leaf spot disease attacks at 1 to 9 weeks of observation are presented in Table 2.

Table 2. Percentage of Disease Intensity after Application of Liquid Smoke to Bintaro Plants at 1 to 9 Weeks of Observation

Treatments	Average Disease Intensity in the Nursery (%)										
	Before Application	After Application (Weeks of Observation)									Average (%)
		1	2	3	4	5	6	7	8	9	
P0	38,51	38,87ab	42,58a	41,13a	41,94a	42,43a	41,61a	42,10a	39,47a	43,04a	41,46
P1	39,81	36,36b	38,36b	33,71c	31,73c	30,86c	28,02d	27,31d	26,46c	28,09c	31,21
P2	40,28	40,68a	39,07b	36,48bc	35,96b	34,55b	32,44c	32,43c	30,64b	30,48bc	34,75
P3	40,63	40,86a	40,96ab	39,61ab	40,94a	36,60b	38,30b	35,90b	34,34b	34,53b	38,00

Source: Primary data processed
Note: Numbers followed by different letters are significantly different based on the LSD test at the 5% level

Based on the data above, it shows that in various treatments the application of liquid smoke to Bintaro plants (p1, p2, and p3) resulted in a reduction in the intensity of leaf spot disease attacks by the fungus *Rhizoctonia* sp at all ages of observation (1 to 9 weeks). Treatment p1 (sprayed with 50 ml/l of liquid smoke once every week) produced the lowest intensity of leaf spot disease attacks by the fungus *Rhizoctonia* sp with an average of 31.21%, followed by treatment p2 (sprayed with 50 ml/l of liquid smoke every once every 2 weeks) namely 34.75% and the p3 treatment (sprayed with 50 ml/l liquid smoke once every 3 weeks) namely 38.00%, while the intensity of leaf spot disease attacks by the fungus *Rhizoctonia* sp was greatest in the p0 treatment (control) namely 41.46%.In the p0 treatment, it showed that the attack of leaf spot disease by the fungus *Rhizoctonia* sp increased in intensity as the observation time progressed from week 1 to week 9.

3.1.5. Analysis of the Efficacy Level of Liquid Smoke

Analysis of the level of efficacy of Bintaro plant liquid smoke was carried out to see the level of effectiveness of using Bintaro plant liquid smoke at several different application time intervals against leaf spot disease caused by the fungus *Rhizoctonia* sp. in the nursery. Data from the calculation of efficacy levels can be seen in Table 3 below:

Table 3. Analysis of the level of efficacy of liquid smoke against attacks by the fungus *Rhizoctonia* sp. in the Nursery

Treatments	Level Efficacy (%)
p0 (Without liquid smoke treatment)	-7,12
p1 (sprayed with 50 ml/l of liquid smoke once every 1 week)	27,56
p2 (sprayed with 50 ml/l of liquid smoke once every 2 weeks)	15,95
p3 (sprayed with 50 ml/l of liquid smoke once every 3 weeks)	6,92

Source: Primary data processed

Based on the data above, it shows that the efficacy level value at p0 (control) is -7.12%, at p1 it is 27.56%, at p2 treatment it is 15.95% and at p3 treatment it is 6.92%. The highest level of efficacy value indicates that the application of liquid smoke from the Bintaro plant in this treatment has a high level of effectiveness.

3.2. Discussion

3.2.1. Pathogenic fungus *Rhizoctonia* sp

Rhizoctonia sp. is a fungus that can be transmitted through soil and seeds. This pathogenic fungus usually infects plant roots causing wilting and damping off of seedlings in young plants, but it is not uncommon to find cases of leaf spot disease caused by the fungus *Rhizoctonia* sp. The most common symptom seen in leaf spot disease is*Rhizoctonia* sp. This is according to[10] is that the lower leaves change color to brown and brown net-like mycelium is visible attached to the leaves and stems. In more severe conditions, a cream-colored membrane can be seen forming on the infected leaves or plant tissue.

Symptoms of attack on the leaves develop about 48 hours after infection and will begin with symptoms of small, round, water-soaked spots with a diameter of about 2 to 3 millimeters. Lesions will expand more quickly during periods of warm temperatures ranging from 24-30 °C and at the highest humidity levels. The primary lesion will remain visible even though the lesion continues to expand. Widespread lesions will first become light green, almost transparent, with irregular edges, and then form large circular spots in the form of concentric rings, giving them a distinctive 'bullseye' appearance. The tissue in the center of the lesion is thin and may fall off, giving the leaf the appearance of a bullet hole. After germination, penetration by appressoria occurs and the invading hyphae also grow on the leaf surface and enter the stomata to create secondary lesion sites. Once lesions form, secondary inoculum is produced by the hymenia on the lower and sometimes upper leaf surfaces and is spread by wind and rain around the plant [10].

Color and isolate of the pathogenic fungus *Rhizoctonia* sp. At the beginning of the growth period, it will be white to brown, in fuller isolates the color will be darker in the middle. The mycelium will spread thinly on the surface of the cup [11].

The species Rhizoctonia sp. It also produces special hyphae consisting of compact cells called monilioid cells. Monilioid cells fuse to produce a hard structure called the sclerotium, which is resistant to environmental extremes, thereby allowing the fungus to survive in adverse conditions [12]. Sclerotium in *Rhizoctonia* sp. It has right angles in its branches is partitioned without forming conidia, and has a hyphae length of 8-12 pm [13].

3.2.2. Intensity of Plant Disease Attacks Before Liquid Smoke Application

Observing the intensity of disease attacks before applying this fungicide is one of the initial stages carried out in controlling disease in plants. By observing the intensity of the disease before applying this, it will be possible to add information about what type of pathogen is infecting, how

wide the spread is, and what the level is. damage caused by pathogens and look at the supporting factors that cause an increase in disease intensity in these plants. Based on

From these aspects, we will find out whether the intensity of the disease has crossed the economic threshold. If it has not exceeded the economic threshold, it will be suggested what prevention methods can be used, and if it exceeds the economic threshold, appropriate control methods will be carried out according to the type of pathogen that infects. and environmental conditions for growing plants attacked by pathogens.

3.2.3. Intensity of Plant Disease Attacks After Liquid Smoke Application

Observing the intensity of the disease after fungicide application is a very important activity to carry out as an effort to control and evaluate whether the application system has been implemented by procedures or not and whether it is on target or not. Apart from that, observations of the intensity of disease attacks after the application of this fungicide were also carried out to see the percentage reduction in the intensity of disease attacks between before application and after application, whether there was a significant percentage reduction or not and whether certain doses and treatments needed to be added to have a more significant effect. in controlling disease attacks on plants. Thus, the lower intensity value after application compared to before application, shows that the application is quite effective in controlling plant disease attacks.

3.2.4. Analysis of the Efficacy Level of Bintaro Plant Liquid Smoke

The activity of analyzing the level of efficacy of Bintaro plant liquid smoke is an activity carried out to see the level of effectiveness of Bintaro plant liquid smoke in controlling pathogenic fungi in nurseries. Analysis of the efficacy level is one of the important activities carried out to see the appropriate and effective use of doses and application time intervals in controlling pathogen attacks. The highest efficacy value is the best recommendation value to be applied in subsequent applications.

4. CONCLUSION

1. The dominant disease-causing pathogenic fungus found in annual plant nurseries is the fungus *Rhizoctonia* sp. which causes leaf spot disease.
2. The application of liquid smoke from Bintaro plants has a very significant effect on the intensity of attacks by Rhizoctonia sp leaf spot disease. The application of liquid smoke from Bintaro plants is quite effective in suppressing the growth of leaf spot disease by the herbal medicine *Rhizoctonia* sp. in the nursery.
3. Treatment p1 (sprayed with 50 ml/l of liquid smoke once every week) resulted in the lowest intensity of leaf spot disease attacks by the fungus *Rhizoctonia* sp with an average of 31.21%, and with an efficacy level of 27.56%.

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