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Effect of water decoction of Eucalyptus Grandis clone CGP 066 leaf as a Biofungicide to Curvularia sp. on oil palm seeds in vitro

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Abstract: Oil palm growth during the nursery is always attacked by leaf spot disease caused by the fungus Curvularia sp..Leaf spot disease can be controlled by application of biofungicides. A plant that can be used as a biofungicide is E. grandis. This research aims to determine the effect of E. grandis leaf boiled water in inhibiting the growth of Curvularia sp. and get the best concentration of E. grandis leaf boiled water in inhibiting the growth of Curvularia sp. colonies by in vitro. This study used a Completely Randomized Design with 4 treatments and 5 replications, namely: B0 (control/without treatment), B1 (0.5 ml of E. grandis leaf boiled water), B2 (1 ml of E. grandis leaf boiled water), and B3 (1.5 ml of E. grandis leaf boiled water). The observed parameters are the growth of Curvularia sp. colonies, the growth area of Curvularia sp. colonies, the percentage of accelerated growth of Curvularia sp. colonies. and the percentage of inhibition of Curvularia sp. colonies. The results showed that the of E. grandis leaf boiled water had an effect on inhibiting the growth of Curvularia sp. colonies and the best concentration of E. grandis leaf boiled water was in treatment B3 (1.5 ml of E. grandis leaf boiled water) with the percentage of inhibition of Curvularia sp. 57% at 5 HSI and the inhibitory activity level is quite strong.
Keywords: Biofungicides; Boiled Water; Curvularia sp; Eucalyptus grandis; Oil Palm Seeds

INTRODUCTION

The growth and development of oil palm plants during the nursery period which experience disease problems is caused by pathogens, such as Natuna wilt disease (Lethal Yellowing), stem base rot, anthracnose leaf rot, leaf spot, and stripe disease (Budi & Hadie 2015). One of the diseases that often attacks oil palm seedlings is leaf spot disease (Curvularia sp.). This disease attacks dead leaves that have opened. The initial symptoms of leaf spot disease (Curvularia sp.) are small round yellow spots appearing on the edges of the leaves and translucent which can be seen on both leaf surfaces, the spots enlarge, the shape is round, the color gradually changes to light brown and the center of the spots settles (curves). After that, the color of the spots changes to dark brown. Leaf spot disease is caused by pathogenic fungi from the species Curvularia sp. can be better known as leaf spot. Spread can be through soil, carried by gusts of wind, rain splashes, and possible infection from insects (Lalang et al. 2016).

Control of leaf spot disease in oil palm nurseries caused by the fungus Curvularia sp. Using synthetic fungicides is often used, because it is considered more efficient and easier to use or apply. Sinaga et al. (2017) stated that the use of fungicides Synthetic with the active ingredient Tekunazole with a concentration of 2 ml/L can inhibit the growth of leaf spot disease on oil palm seedlings (Curvularia sp.). Continuous use of synthetic fungicides can cause pathogen resistance, poisoning in humans and pollute the environment (Hadizadeh et al. 2009). An environmentally friendly alternative for controlling pathogens is to use natural fungicides or biofungicides from plant extracts (Apriani et al. 2014). The use of biofungicides has promising

prospects because the raw materials for biofungicides are abundant in nature and the manufacturing process only requires simple technology. Biofungicide materials are relatively easier to obtain, safe for non-target organisms, environmentally friendly, safe for human health and easily decomposed so they do not cause environmental pollution (Trisawa & Iwa 2014).

Biofungicides can be obtained from various types of plants which contain compounds to inhibit the growth of diseases in plants (Tjahyani et al. 2015). Plants used as biofungicides have distinctive characteristics such as a pungent odor and a bitter taste (Elvana 2022). One plant that can be used as a biofungicide is the Eucalyptus sp. According to Koswandy and Ramadhania (2016), E. globulus leaves can be used as an antiseptic, disinfectant, antibacterial, antifeedant, anti-fungal, antioxidant, antimicrobial and insect repellent.

Eucalyptus sp. leaf extract. contains active compounds that can be used as raw materials for biofungicides to control plant diseases. The active compounds contained in the leaves of Eucalyptus sp. including flavonoids, terpenoids and tannins (Setianingsih et al. 2017). Kurniawan (2022) reported that administering methanol extract of acacia leaves at a concentration of 1 ml was able to inhibit the development of the fungus Curvularia sp. In vitro. The E. grandis plant is one of the essential oil producing plants which is widely processed and used to produce oil from its leaves (Salam 2018). The E. grandis species has been widely developed at PT. Riau Andalan Pulp & Paper (PT. RAPP) includes clone CGP 066, E. grandis clone CGP 066 is the result of a cross between E. grandis and E. pellyta. (KCN 2 PT. RAPP 2021). The leaves of E. grandis have narrower, more bell-shaped leaves with prominent valves (Hollyday and Ivan 1989). E. grandis leaves have a lower water content compared to Eucalyptus sp varieties. other. E. grandis leaves contain quite high levels of phenolic compounds, terpenoids, flavonoids and tannins, which are secondary compounds that are anti-fungal and antioxidant, which compounds can damage fungal cell membranes and can inhibit fungal cell growth (Setyowati et al. 2019), so that E. grandis leaves can be used as a biofungicide. The aim of this research was to determine the effect of boiled water from the leaves of E. grandis clone CGP 066 as an inhibitor of the growth of Curvularia sp. on oil palm seedlings in vitro and obtained the best concentration of boiled water from E. grandis clone CGP 066 leaves to inhibit the growth of Curvularia sp. in vitro.

METHODS

The research was carried out in May – June 2023, at the Integrated Laboratory, Pelalawan Indonesian Institute of Plantation Technology (ITP2I), Building I Pangkalan Kerinci, Pelalawan, Riau. The experimental design used was a non-factorial Completely Randomized Design (CRD), consisting of 4 treatments with 5 replications. The total number of experiments carried out was 20 units. The treatment tested in the Eucalyptus grandis leaf boiled water test research as a biofungicide against Curvularia sp. on Oil Palm Seedlings in Vitro based on research by Kurniawan (2022) which has been modified. The treatments in this experiment are as follows:

B0 : Control (No Treatment)
B1 : Give 0.5 ml of boiled water from E. grandis leaves
B2 : Give 1 ml of boiled water from E. grandis leaves
B3 : Give 1.5 ml of boiled water from E. grandis leaves

The research implementation included sterilizing material tools, taking leaf samples, making boiled water from E. grandis leaves, making PDA, taking samples of oil palm seedling leaves, isolating and purifying Curvularia sp, and testing boiled leaf water on the growth of Curvularia sp.

Observation Parameters

Colony Growth of Curvularia sp.

Observation of colony growth began on day 2 HSI until 7 days after inoculation (HSI). Observations were made macroscopically by looking at the color, growth direction and structure of the colony mycelium in each treatment and compared with the control (Ningsih et al. 2016).

Colony Growth Area of Curvularia sp.

Observation of the area of colony growth was carried out by measuring the diameter starting on days 2 to 7 DAP. Diameter growth observations were carried out vertically and horizontally. Diameter growth is measured using Formula 1 (Wahyuningtyas 2013):

$$\text{DAR} = \frac{Øx + Øy}{2} \quad \text{..... (1)}$$

Information :
DAR = Diameter in Radial Direction
Øx = x-axis diameter (cm)
Øy = Diameter of the y axis (cm)

Percentage of Colony Growth Acceleration of Curvularia sp.

Observation of the percentage of accelerated growth of the Curvularia sp fungus colony. carried out on days 2 to 7 of HSI (Silangit 2015). The comparison of growth acceleration was calculated from the last observation of the control colony which had filled the petri dish, then compared for each treatment using Formula 2.

$$PPT = \frac{\text{PDKP}}{\text{PDKK}} \text{ x } 100\% \text{.....(2)}$$

Information :
PPT = Growth Acceleration Growth
PDKP = Treatment Colony Diameter Growth
PDKK = Control Colony Diameter Growth

Percentage of Colony Inhibition of Curvularia sp.

Observation of the growth of the fungus colony Curvularia sp. on PDA media carried out on days 2 to 7 HSI. The Relative Resistance Level (HR) of each treatment on PDA media can be calculated using Formula 3 (Nurafidah 2014):

$$\text{HR} = \frac{\text{D1}-\text{D2}}{\text{D1}} \text{ x } 100\% \text{ .....(3)}$$

Information :
HR = Relative Barrier
D1 = Diameter of control colony (cm)
D2 = Diameter of treatment colony (cm)

The level of activity of the inhibitory power of vegetable biopesticides is grouped based on the inhibitory power which can be seen in Table 1 (Novriyanti et al. 2010).

The data analysis used in this research is quantitative and qualitative analysis techniques. Quantitative data obtained included the area of colony growth, the percentage of colony growth acceleration, and the percentage of colony inhibition of the growth of the fungus Curvularia sp. processed using Excel software. The qualitative data obtained was macroscopic observation of colony growth, and presented in the form of images (Yudistina et al. 2013).

Table 1. Active level of inhibitory power of vegetable biopesticides

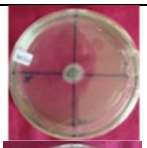
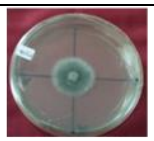
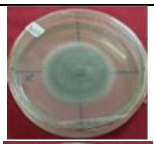
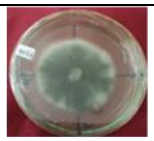
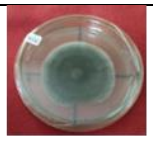
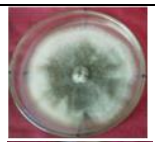
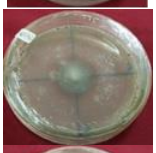
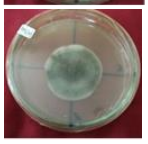
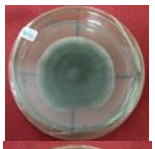
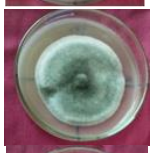
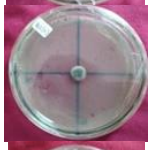
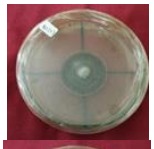
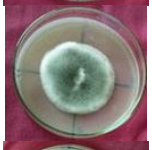
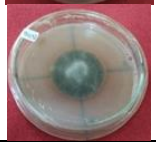
Inhibitory Power Activity (IPA)	Level of inhibitory activity
>75%	Very Strong
50% - 75%	Strong
25% - 50%	Currently
0% - 25%	Weak
0%	Not Active

RESULTS AND DISCUSSION

Colony Growth of Curvularia sp.

Growth of the fungus colony Curvularia sp. macroscopically, including colony color, colony surface and growth of the colony tip. Based on macroscopic observations, B0 experienced thickening of the colonies, had a blackish gray colony color, a smooth surface, the reverse side was black and the growth direction was uneven from 3 DAP to 7 DAT. Treatment B1 showed that the colonies were thickened, had a blackish gray color, a smooth surface, the reverse side was black and the direction of colony growth was uneven from 4 DAS to 7 DAS. Treatment B2 showed thickening of the colonies, the colony color was blackish gray, the surface was smooth, the reverse side was black and the growth direction was uneven from 4 DAS to 7 DAS. Treatment B3 showed thickening of the colonies, the colony color was blackish gray, the surface was smooth, the reverse side was black and the growth direction was uneven from 4 DAP to 7 DAT (Table 2).

Table 2. Colony growth of Curvularia sp. from 2 DAI to 7 DAI

Treatment	Days After Inoculation					
	2	3	4	5	6	7
B0						
B1						
B2						
B3						

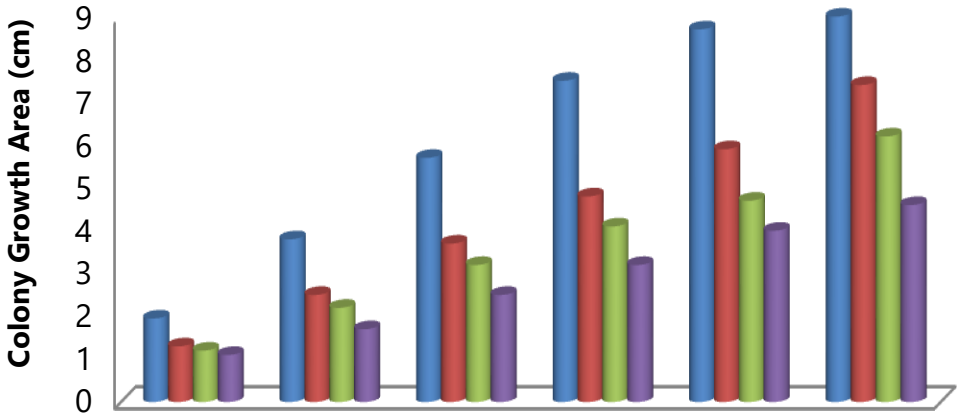
Information: B0 (control/no treatment), B1 (giving boiled water from E. grandis leaves 0.5 ml), B2 (giving boiled water from E. grandis leaves 1ml), and B3 (giving boiled water from E. grandis leaves 1.5 ml)

This shows that treatments B0, B1, B2 and B3 both have blackish gray colonies, a smooth surface, the reverse side is black and the growth direction is uneven. This is in accordance with research by Mujahid (2018) which stated the results of macroscopic observations of Curvularia sp. on PDA media, it shows that the Curvularia lunata fungus colony is blackish gray, the surface is smooth, the reverse side is black and grows concentrically. Butarbutar et al. (2019) stated that macroscopically, at first the colonies were white, at the age of 3 days they started to turn blackish brown and on the fifth day they started to turn black. Curvularia sp. has a brown colony

color and resembles velvet or cotton. The same thing was stated by Kalpajar et al. (2015) that the macroscopic characters of the fungus Curvularia sp. has round colonies, the top of the colony is blackish gray and the bottom of the colony is black with tapered colony edges, and the texture of the colony is like cotton.

Colony Growth Area of Curvularia sp.

Colony growth area of Curvularia sp. on PDA growth media which had been mixed with boiled water from E. grandis leaves, observed from 2 DAP to 7 DAP. Colony growth area of Curvularia sp. in each treatment can be seen in Figure 1. Based on Figure 1, the area of growth of the Curvularia sp colony. in treatments B1, B2 and B3 were lower than in treatment B0 on PDA growth media which was observed up to 7 DAT. This explains the effect of giving boiled water from E. grandis leaves on the growth of Curvularia sp colonies.



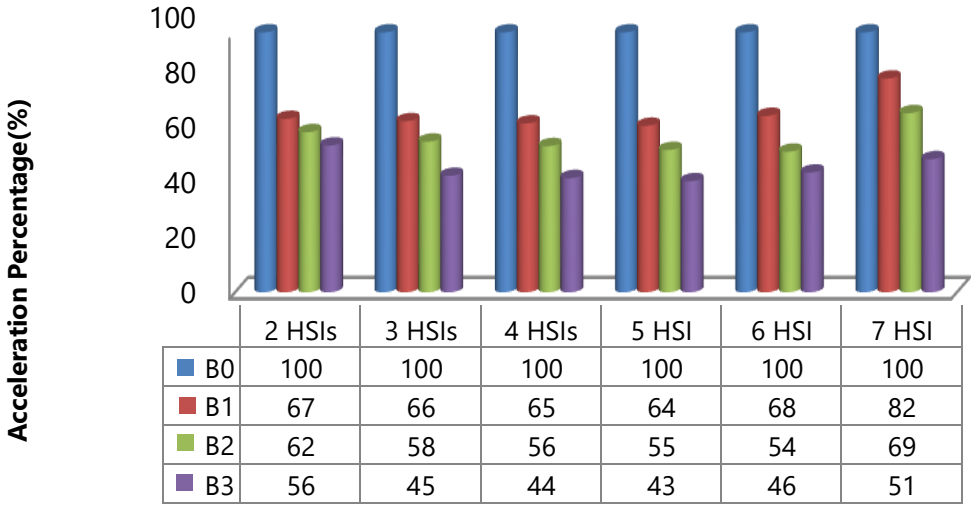
	2 HSIs	3 HSIs	4 HSIs	5 HSI	6 HSI	7 HSI
B0	1.95	3.8	5.7	7.5	8.7	9
B1	1.3	2.5	3.7	4.8	5.9	7.4
B2	1,2	2,2	3,2	4.1	4.7	6.2
B3	1.1	1.7	2.5	3,2	4	4.6

Figure 1 Colony growth area. B0 (control/no treatment),
B1(giving boiled water from E. grandis leaves 0.5 ml),
B2 (giving water decoction of E. grandis leaves 1 ml) and grandis1.5 ml)
B3 (giving boiled water from E leaves.

Based on Figure 1, observations from 2 DAS to 7 DAS, it is known that B0 treatment shows extensive growth of Curvularia sp colonies. ranges from 1.95 cm to 9 cm. Treatment B1 showed extensive growth of Curvularia sp colonies. ranges from 1.3 cm to 7.4 cm. Treatment B2 showed extensive growth of Curvularia sp colonies. ranges from 1.2 cm to 6.2 cm. Treatment B3 showed extensive growth of Curvularia sp colonies. ranges from 1.1 cm to 4.6 cm. These results show that the higher the treatment given with boiled water from E. grandis leaves, the wider the growth of the Curvularia sp colony. lower on PDA media. This occurs due to the suppression of colony diameter caused by secondary metabolite compounds contained in E leaves grandis which metabolite compounds can damage the functioning of fungal cell membranes. This refers to the statement by Mailoa et al. (2014) who stated that secondary metabolite compounds can cause fungi to experience damage to their cell membranes and inhibit fungal growth and even cause fungal cell death. The metabolite compounds contained in E. grandis leaves include phenolics and flavonoids (Soegianto 2019) and were added by Setianingsih et al. (2017) stated that the leaves of Eucalyptus sp. has flavonoid, terpenoid and tannin metabolite compounds.

Percentage of Colony Growth Acceleration of Curvularia sp.

Accelerated growth of the fungus Curvularia sp. on the PDA growth medium which had been mixed with E. grandis boiled water, it was observed from 2 DAP to 7 DAT. Figure 2 shows that treatment B1, B2 and B3 was able to influence the accelerated growth of the Curvularia sp fungus colony. Based on Figure 2, it is known that all treatments experienced an increase in the growth of Curvularia sp fungus colonies. which was observed until 7 HSI. Figure 2 can also be observed that treatment B1 shows the percentage value of accelerated growth of Curvularia sp colonies. by 64% to 82% compared to treatment B0. Treatment B2 shows the percentage value of accelerated growth of Curvularia sp colonies. by 54% to 69% compared to treatment B0.



	2 HSIs	3 HSIs	4 HSIs	5 HSI	6 HSI	7 HSI
B0	100	100	100	100	100	100
B1	67	66	65	64	68	82
B2	62	58	56	55	54	69
B3	56	45	44	43	46	51

Figure 2. Percentage of colony growth acceleration. B0 (control/no treatment), B1 (giving boiled water from E. grandis leaves 0.5 ml), B2 (giving waterdecoction of E. grandis leaves 1 ml) and grandis1.5 ml) B3 (giving boiled water from E leaves.

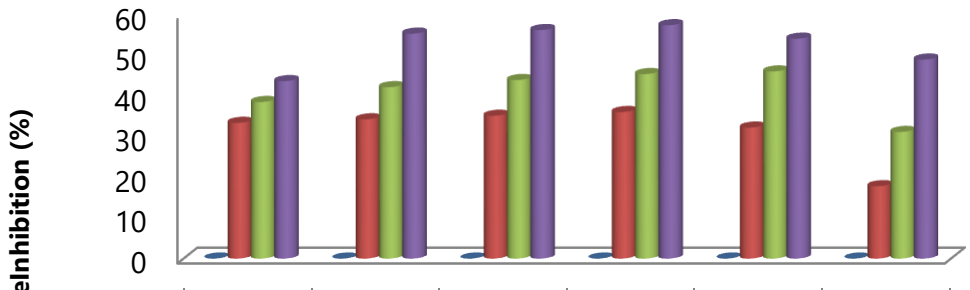
The best treatment was obtained by B3 which showed the percentage of inhibition of Curvularia sp colonies. by 43% to 56% compared to treatment B0. This states that the provision of boiled water from E. grandis leaves has an effect in slowing down the percentage of accelerated growth of the Curvularia sp fungus colony. Especially the B3 treatment showed a greater influence compared to the B2 and B1 treatments from 2 DAS to 5 DAS. Furthermore, at 6 and 7 DAT in treatments B1, B2, and B3 there was an increase in the percentage of accelerated fungal colony growth. It is suspected that secondary metabolite compounds in boiled water are only able to inhibit up to 5 HSI (Hidayat 2022). These results also explain that the more treatment with boiled water from E. grandis leaves, the wider the growth of the Curvularia sp colony. lower on PDA media. Kurniawan (2022) stated that the content of metabolite compounds increases as the concentration of boiled water increases so that its ability to inhibit microbial growth becomes higher.

The low percentage of growth acceleration for colonies treated with B3 is thought to be due to the secondary metabolite compounds contained in E. grandis leaves which are able to inhibit and damage the work of the Curvularia sp colony cells. so that the percentage of colony growth becomes low. Hidayat (2022) stated that secondary metabolite compounds are able to inhibit and damage the work of Curvularia sp colony cells. Ragasa et al. (2015) reported that the leaves of Eucalyptus sp. generally contain phenolic chemical compounds, flavonoids, terpenoids and tannins. The chemical compounds contained in the leaves of Eucalyptus sp. is a compound that

can damage fungal cells and fungal cell membranes. Phenolic compounds have a tendency to bind proteins, thereby disrupting fungal metabolic processes (Dalimunthe et al. 2016). Flavonoid compounds can cause damage to the permeability of fungal cell walls, microsomes and lysosomes. Flavonoid compounds can also cause changes in organic components and nutrient transport which will ultimately result in toxic effects on fungi. Disruption of cell membrane permeability can cause the cell to be unable to carry out living activities so that its growth is hampered (Malik et al. 2019).

Percentage of Colony Inhibition of Curvularia sp.

Observation of the percentage of colony inhibition of Curvularia sp. observed from 2 to 7 DAP. The results of the data on the percentage of colony inhibition of Curvularia sp. can be seen in Table 2. Based on Table 2, it shows that treatments B1, B2 and B3 experienced an increase in the percentage of colony inhibition up to 5 DAP and then decreased at 6 and 7 DAT. The B3 treatment percentage showed the highest percentage in inhibiting the growth of Curvularia sp colonies. compared to treatment B2, and B1 up to 7 DSI. Treatment B3 showed an inhibition percentage value of 57% at 5 DAP. Treatment B2 shows the percentage value of inhibition of growth of Curvularia sp colonies. amounted to 45% at 5 DAT and treatment B1 showed the percentage value of inhibition of growth of Curvularia sp colonies. by 36% at 5 HSI. Furthermore, at 6 and 7 DAT in treatments B1, B2, and B3 there was a decrease in the percentage of fungal colony growth inhibition. This is thought to be because the difference in growth between the treated colonies and the control colonies was not very significant. The percentage parameter of colony growth acceleration is in line with the percentage of colony growth inhibition, where the growth percentage increases so that the inhibition percentage decreases.



	2 HSIs	3 HSIs	4 HSIs	5 HSI	6 HSI	7 HSI
B0	0	0	0	0	0	0
B1	33	34	35	36	32	18
B2	38	42	44	45	46	31
B3	44	55	56	57	54	49

Figure 3 Percentage of colony growth inhibition. B0 (control/no treatment), B1 (giving boiled water from E. grandis leaves 0.5 ml), B2 (giving boiled water from E. grandis leaves 1 ml) and B3 (giving boiled water from E. grandis leaves 1.5 ml)

Curvulariasp. in vitro. The B0 treatment did not inhibit the growth of Curvularia sp. colonies, whereas the treatment with the addition of boiled water from E. grandis leaves had a growth inhibitory power value on Curvularia sp. colonies. A large percentage of colony growth inhibition power was obtained in treatment B3. Parameters of colony growth area of Curvularia sp. is very closely related to the percentage parameter of the inhibitory power of Curvularia sp colony growth. that the higher the treatment given, the higher the percentage of inhibition of growth of Curvularia sp colonies and the lower the area of growth of Curvularia sp colonies.

In Figure 3, B3 treatment shows a strong level of inhibitory activity, namely 57% at 5 DAP. According to Novriyanti et al. (2010) an inhibitory activity level of 50% - 75% is considered strong. This explains that B3 treatment is the best treatment capable of inhibiting the growth of Curvularia sp colonies. The secondary metabolite content in B3 treatment is thought to cause high inhibition of Curvularia sp. colonies. Setianingsih et al. (2017) reported that the compounds contained in the leaves of Eucalyptus sp. It is antifungal because it contains essential oils and contains chemical compounds flavonoids, tannins and terpenoids. Soegianto (2019) stated that phenolic secondary metabolite compounds are also contained in the leaves E. grandis, phenolic compounds can inhibit the growth of fungal cells.

Nuria (2009) stated that flavonoid compounds can be called antifungal, because these compounds denature proteins, disrupt the lipid layer, and cause cell wall damage. The substance dissolves easily so it can damage fungal cell membranes and is followed by the release of intracellular compounds. Meanwhile, tannin compounds are compounds that can damage the main components of cell walls consisting of chitin, glucan and lipids so that they can inhibit the growth of fungi. Ismaini (2011) also reported that terpenoid compounds are compounds that have an antifungal function by inhibiting fungal growth, either through the cytoplasmic membrane or disrupting the growth and development of fungal spores.

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that boiled water from the leaves of E. grandis clone CGP 066 has an effect in inhibiting the growth of the fungus colony Curvularia sp. from 2 HSI to 7 HSI. The best concentration of boiled water from E. grandis clone CGP 066 leaves to inhibit the growth of Culvularia sp colonies. is treatment B3 concentration 1.5 ml) with an average percentage value of inhibition of the fungal colony Curvularia sp. 57% at 5 HSI and the level of inhibitory activity is classified as strong.

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