Surface morphology of composite from mahogany leaves waste with citronella oil (*Cymbopogon winterianus* Jowitt) as a natural coating for antifungal of *Pleurotus ostreatus*

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**Abstract.** In this work, citronella oil's activity as a natural coating for antifungal on mahogany's surface morphology (*Swietenia mahagoni*) leaves waste as composite was investigated. The composite material from mahogany leaves requires a coating that can inhibit fungi growth. Citronella oil was a natural material that can be used as an antifungal for coating this composite. The concentration of citronella oil used for the MIC (Minimum Inhibitory Concentration) assay was 0.1-2.0%. Based on the MIC of citronella oil can inhibit the growth of the fungus *Pleurotus ostreatus* at the optimum concentration of 0.2% with inhibition of 33.33%. Coating of citronella oil on composites using several concentrations ranging from 0.0, 0.4, 0.8, 1.2, 1.6 and 2%, respectively. The surface morphology on composite with citronella oil as the natural coating was observed using a CCD camera microscope with a magnification of 50x showed that the growth of fungal spores on the composite surface began to be inhibited a concentration of 0.4%. This surface morphology of composite can prove that citronella oil can be used as an antifungal.

1. **Introduction**

One way to deal with waste problems is by processing leaves litter into a lightweight and strong material at low cost through composite engineering materials as a substitute for wood [1-6]. Mahogany leaves are natural materials that are organic waste that can be developed into the composite material. The mahogany leaves were chosen because of their abundant availability and suitable quality to be used as a wood substitute particleboard [4]. The abundant cellulose content in mahogany leaf waste material can continue to be developed into new, more efficient materials [2-6].

However, one of the shortcomings of composite materials is that they are not durable against fungi. The content of cellulose and lignin can trigger rot fungi' growth when water infiltrates the composite pores. Also, rotting fungal spores outside the composite will enter the composite material, eventually leading to fungi' growth. This weathering fungus can degrade composites, triggering weathering, which
causes the material to become brittle and lose its mass [2]. Therefore, it is necessary to have a follow-up to overcome the resistance to fungi in composite materials. In several studies that have been conducted, the essential oil has properties antifungal. The use of essential oils as a composite coating, primary cellulose, was chosen because it is a natural ingredient and abundant in Indonesia [7]. In Indonesia, essential oils such as clove oil and fragrant lemongrass oil are not only enhanced through refinings, such as research by Eden et al. (2018) and Alighiri et al. (2018), efforts were also made to diversify their products [8,9]. The application of essential oils as a natural coating on composites is something new and a breakthrough to increase the diversification of Indonesia's essential oil products.

Various studies on the benefits of essential oils have been carried out, especially those closely related to the broad biological activity such as antioxidants, anti-inflammatory, antimicrobial, antifungal, antiviral, mosquito repellent, and antineoplastic [10-14]. The antifungal activity of citronella oil, lemongrass oil, clove oil, betel oil, eucalyptus oil, and peppermint has been shown to inhibit the fungi *Penicillium sp.*, *Aspergillus niger*, and *A. versicolor* [15]. Essential oil from *Citrus spp.* It has also been shown to have antifungals found to inhibit *P. digitatum*, *P. italicum*, and *A. niger* [16].

Therefore, in this study, citronella oil, one of Indonesia's essential oils and has antifungal activity, used a natural coating to composite mahogany leaves waste. The coating aims to protect the material against water, weather, high humidity, and microorganisms such as bacteria and fungi [17]. In this research, we studied citronella oil as a natural coating on the surface morphology of mahogany leaves waste composites and expected to produce composite materials that are lightweight, strong, and resistant to fungi or bacteria.

2. Methods
2.1. Material
The mahogany (*Swietenia mahagoni*) leaves waste was collected from Universitas Negeri Semarang, Semarang, Indonesia. Commercial polyurethane polymers and glass fiber were purchased from Bratachem, Semarang, Indonesia. *Pleurotus ostreatus* was purchased from the Indonesian Institute of Sciences (LIPI). Citronella oil (*Cymbopogon winterianus* Jowitt) was purchased from PT. Eterische International. All the chemicals used in these studies were pure analytical reagents purchased from Merck (Germany).

2.2. Sample preparation
The mahogany (*Swietenia mahagoni*) leaves waste was cleaned with water, then dried at 50-60 °C temperature for 60 minutes. The dried mahogany leaves were pulverized using a grinder machine [2-6].

2.3. Fabrication of composite
15 g powder of Mahogany leaves waste was weighed and mixed polyurethane and glass fiber. Waste leaves are mixed with 1.2 gram of polyurethane and 0.4 gram of glass fiber until evenly distributed. Composites are pressed using a hydraulic press with a pressure of 4 tons for 15 minutes. The finished sample is dried naturally for 24 hours [4].

2.4. Determination of MIC (Minimum Inhibitory Concentration)
The assessment of the MIC of *Pleurotus ostreatus* was maintained on potato dextrose agar (PDA) media and stored at 4 °C. 10 mL PDA was poured into the petri dish. Then, citronella oil was added with concentrations from 0.2 to 2.0%, respectively. Then, the petri dish was shaken simultaneously for 15 seconds until evenly distributed and allowed to solidify. The *Pleurotus ostreatus* fungus that has been cultured for 5 days in a petri dish was taken a little using a sterile loop needle or tweezers and transferred to a petri dish containing PDA media and citronella oil. After the oil concentrations have been filled with fungus, then, the petri dishes were put in the incubator, and the observations were carried out until the seventh day.

The absence of fungus growth with the lowest concentration was interpreted as the MIC. The MIC was determined by sub-culturing, which showed no fungus growth on agar plates after incubated at 37°C for 24 h in an incubator. The lowest concentration that did not show fungus growth was defined as the MIC value. The experiment was conducted in triplicate, and the mean diameter in millimeters (mm) of
the zone of inhibition was recorded. 2% dimethyl sulfoxide (DMSO) solution was used as a negative control. All experiments were performed in triplicate [18].

2.5. Composite coating
The concentration of citronella oil used for composite coating was taken from the MIC assay. Several coating variations, namely concentrations of 0.2-1%, respectively. 2% DMSO solution was used as a negative control. The composites’ coating was carried out by immersing the composites in various citronella oil concentrations for ±30 seconds. The composites that have been coated are then placed in the spores of the cultured fungus on PDB media, then observed for 1 month using a CCD camera microscope.

2.6. Characterization of samples
Characterization of surface morphology and elements in the composite was carried out using SEM-EDX (Scanning Electron Microscopy-Energy Dispersive X-ray) Phenom Pro X, manufactured by the Netherlands. Fungal growth in the composite surface was observed with a CCD (Charge-Coupled Device) camera microscope with a magnification of 50x.

2.7. Statistical analysis
The experimental data was performed using a one-way analysis of variance (ANOVA). Data were expressed as the mean ± standard deviation (n = 3). Homogenous groups and the least significant difference (LSD) were determined at the significance level of p ≤ 0.05. All statistical analyses were performed using SPSS 24 software.

3. Results and Discussion
3.1. Surface morphology of mahogany leaves waste
Mahogany leaves waste composites were successfully fabricated using a simple mixing process, mixed with polyurethane polymer binder and fiberglass reinforcement. Leave mahogany composites to have denser pores and a smooth surface, with a diameter ±4.5 cm, as shown in Figure 1.

![Figure 1](image1.png)

**Figure 1.** Pictures of leave mahogany composite (a) without coating citronella oil and (b) with coating citronella oil

Surface scanning electron microscope (SEM) analysis was performed to characterize the composite’s surface structure (Figure 2). When compared to Figures 2a and 2b, it showed that the use of glass fiber in the composite of leave mahogany/polyurethane as reinforcement, it was clear that the composite’s pores were getting tighter. The cavities in the composite of leave mahogany/polyurethane were covered by glass fiber. The SEM micrograph reinforced this in the cross-section in Figure 2d. Figure 2c shown SEM of glass fiber as raw material. As evidenced in Figure 2d, the glass fiber has entered the cavity on the composite of leaves mahogany/polyurethane. The cavity in the composite will undoubtedly have implications for mechanical strength. This was evidenced by Masturi et al.’s (2020) research that the compressive strength on the composite of leaves mahogany/polyurethane with the addition of glass fiber increased from 41.59 to 57.68 MPa [4].


Figure 2. SEM Micrograph of (a) composite of mahogany leaves waste/polyurethane, (b) glass fiber, (c) composite of mahogany leaves waste/polyurethane/glass fiber in surface section, and (d) composite of mahogany leaves waste/polyurethane/glass fiber in cross-section

Also, this addition increased the density of composite of leaves mahogany/polyurethane from 1,060 to 1,140 g/cm³ [4]. The large cavity caused the possibility of water entering the pores of the composite. The addition of glass fiber has resolved the research of Masturi et al. (2020). Even though the addition still has open pores that can be entered by water. The presence of water will undoubtedly cause the composite to have the potential to be exposed to mold, which will cause the composite to rot. This research proves that by studying the surface area on the composite surface using SEM, it is proven that the composite surface is coated with citronella oil.

The SEM micrograph reinforced this in Figure 3. Figure 3 showed the raw material’s initial surface morphology, namely, mahogany leave waste (Figure 3a), then a composite was made with the addition of polyurethane as a binder (Figure 3b). Figure 3c showed the addition of glass fiber on the composite of mahogany leave/polyurethane. Each treatment showed a closed pore in each composite. The pore size becomes small because the cavity was closed by polyurethane and glass fiber, resulting in increased mechanical strength and density. Then, it was treated with citronella oil as a natural coating shown in Figure 3d. Citronella oil as natural coatings on the composite of mahogany leaves/polyurethane/glass fiber was seen in Figure 3d. Although, it looks like the pores and density do not differ much. However, there is a difference in opacity in Figures 3c and 3d. The opacity in Figure 3d was decreased, and the composite SEM micrograph becomes brighter. The success of citronella oil as a natural coating on the
composite of mahogany leave/polyurethane/glass fiber was also evidenced by the EDX results shown in Figure 4.

**Figure 3.** SEM Micrograph of (a) mahogany (Swietenia mahagoni) leaves waste, (b) composite of mahogany leaves waste/polyurethane, (c) composite of mahogany leaves waste/polyurethane/glass fiber, and (d) composite of mahogany leaves waste/polyurethane/glass fiber/citronella oil

It was seen that the content of mahogany leaves (Figure 4a) consists mainly of C 51.51 wt% and O 47.36 wt%, which can be derived from cellulose. The addition of polyurethane into the composite reduced the element C and O contents to 48.08 wt% and 40.21 wt%, respectively. This decrease occurred due to the addition of polyurethane polymer into the mahogany leaves waste composite, which caused 10.25 wt% of the element N in the composite (Figure 4b). Figure 4c showed the addition of glass fiber as evidenced by the element Si at 19.09%. The presence of citronella oil coating on the composites was proven by adding element C to 54.63% (Figure 4d) from 23.76% (Figure 4c). The addition of this element can be analyzed from the molecular formula of citronella oil is C_{10}H_{16}O.
Figure 4. EDX of (a) mahogany (Swietenia mahagoni) leaves waste, (b) composite of mahogany leaves waste/polyurethane, (c) composite of mahogany leaves waste/polyurethane/glass fiber, and (d) composite of mahogany leaves waste/polyurethane/glass fiber/citronella oil.

3.2. MIC (Minimum Inhibitory Concentration) Assay
The MIC determination of citronella oil against Pleurotus ostreatus fungus is shown in Table 1. Table 1 indicated the activity of different concentrations of citronella oil from 0.2 to 1.0% on Pleurotus ostreatus fungus's growth using 2% dimethyl sulfoxide (DMSO) solution as a negative control.

Table 1. Percentage of inhibition of citronella oil on Pleurotus ostreatus fungus on the 7th day using 2% dimethyl sulfoxide (DMSO) solution as negative control*

| Citronella oil concentration (%) | Fungi diameter colony (cm) | Inhibition (%) |
|---------------------------------|----------------------------|----------------|
| 0.0 ± 0.00                      | 1.5 ± 0.20                 | 0.0 ± 0.00     |
| 0.2 ± 0.03                      | 1.0 ± 0.30                 | 33.33 ± 0.50   |
| 0.4 ± 0.02                      | 0.8 ± 0.15                 | 46.67 ± 0.43   |
| 0.6 ± 0.01                      | 0.7 ± 0.08                 | 53.33 ± 0.05   |
| 0.8 ± 0.01                      | 0.5 ± 0.10                 | 66.67 ± 0.02   |
| 1.0 ± 0.01                      | 0.0 ± 0.00                 | 100 ± 0.00     |

*Treatment means of the ANOVA test
Values were expressed as the mean ± standard deviation of three replications.
The mean difference is significant at the $p \leq 0.050$.
Means are not significantly different in the different place in Indonesia at the $p=0.000$
Based on the percentage of inhibition, it is known that citronella oil can be inhibited fungus at a minimum concentration of 0.2%. In other words, the MIC value of *Pleurotus ostreatus* fungus was 0.2%. This type of white-rot fungus can be inhibited 100% of its growth at a citronella oil concentration of above 0.8% for the *Pleurotus ostreatus* fungus. This showed that the more citronella oil used, the greater its ability to inhibit fungal spores' growth, especially white-rot fungus of *Pleurotus ostreatus*. However, using too much citronella oil from an economic point of view is not profitable.

3.3. Antifungal assay on composite coating with citronella oil

The citronella oil coating process on the composite has been completed. The concentration of citronella oil used in the coating process is based on the MIC assay concentration. The growth of the spores of the *Pleurotus ostreatus* fungus that had been dripped on the composite surface was observed microscopically using a CCD camera microscope with a magnification of 50x. Figure 5 showed the activity of citronella oil, which was expected to function as an antifungal in the composite mahogany leaves/polyurethane/glass fiber.

![Figure 5](image)

**Figure 5.** The observation of citronella oil activity on the composite of mahogany leaves/polyurethane/glass fiber using a CCD (Charge-Coupled Device) camera microscope with a magnification of 50x at the concentration of citronella oil (a) 0.0% (2% dimethyl sulfoxide (DMSO) solution as negative control), (b) 0.4%, (c) 0.8%, (d) 1.2%, (e) 1.6%, and (f) 2.0%

Based on observations using a CCD camera microscope with a magnification of 50x, it can be seen that the spores of the *Pleurotus ostreatus* fungus were dripped on the composite of mahogany leaves/polyurethane/glass on their surface were being grown, and some were be stunted. In Figures 5a, 5b, and 5c, with a citronella oil concentration of 0.4%, it can be seen that the fungal spores can still grow and spread to the composite surface.

However, this did not occur in composites coated with citronella oil with concentrations of 1.2%, 1.6%, and 2%. Fungal growth was strictly inhibited so that the fungus did not spread over the composite surface. This proves that citronella oil can be inhibited the growth of wood rot fungus on the composite material.

4. Conclusion

Citronella oil has an activity that can effectively inhibit the growth of white-rot fungus of *Pleurotus ostreatus*. The MIC value of citronella oil, which can be inhibited the growth of white-rot fungus
Pleurotus ostreatus, was 0.2% with inhibition of 33.33%. This MIC value was used as a basis for coating citronella oil on the composite of mahogany leaves waste/polyurethane/glass fiber. Based on the observations, it turns out that citronella oil has the most optimal activity at a concentration of 0.8%. This slight difference may be due to the matrix factors in composites such as cellulose on mahogany leave, polyurethane binder, and glass fiber reinforcement compared to the MIC assay, which only uses PDA media. This research proves that through surface morphological studies on the composite of mahogany leaves waste/polyurethane/glass fiber, citronella oil has been successfully used as a natural coating with an effective antifungal function.

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