



## Anti-termite and anti-fungal activity test of ethanolic extract of *Melastoma malabathricum* leaves

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### ABSTRACT

Wood damage caused by the ground termite *Coptotermes curvignathus* and the rotting fungus *Schizophyllum commune* is a major problem in the tropics. This study aims to evaluate the anti-termite and anti-fungal activity of the ethanolic extract of *Melastoma malabathricum* leaves as a natural wood preservative. Extraction was carried out by the maceration method, and the extracts were tested at 2–10% concentration using a Complete Random Design with three replicates. Termite mortality and weight loss of the test paper were observed for 21 days, while inhibition of fungal growth was observed for 7 days. Data were statistically analyzed using the nonparametric Kruskal-Wallis test and the Pairwise Comparisons follow-up test with Bonferroni correction at 95% confidence level ( $p < 0.05$ ). The results showed that extract concentration significantly increased termite mortality ( $p = 0.005$ ) and anti-fungal activity ( $p = 0.006$ ). However, the treatment had no significant effect on test paper weight loss ( $p = 0.072$ ). The 10% concentration resulted in the highest termite mortality (36.36%) and the highest anti-fungal activity (88.23%), and was the only treatment to show a statistically significant difference compared with the control. Based on follow-up test results, a 10% concentration is recommended as optimal due to its clear effectiveness in providing biological protection. These results show that the ethanol extract of *Melastoma malabathricum* leaves has the potential to serve as an environmentally friendly natural wood preservative.

**Keyword:** Activity, Anti-fungal, Anti-termite, Ethanol Extract, *Melastoma malabathricum*

### 1. Introduction

Wood is widely utilized in construction, furniture, and various building applications. However, the availability of high-durability wood resources continues to decline, leading to increased use of low-durability wood species that are more susceptible to biological deterioration. Wood with low natural durability is particularly vulnerable to attack by wood-destroying organisms such as termites and decay fungi, which significantly reduce service life and structural integrity. Among subterranean termites, *C. curvignathus* is considered one of the most destructive species due to its ability to degrade cellulose and hemicellulose components of wood, resulting in severe structural damage [1]. In addition, *S. commune* is an aggressive white-rot fungus capable of rapidly decomposing lignocellulosic components through enzymatic activity, thereby accelerating wood decay processes [2].

Damage caused by termites and fungi not only compromises the strength and durability of wood but also leads to substantial economic losses in the construction and furniture industries. Conventional wood preservation strategies largely rely on synthetic chemical preservatives; however, their prolonged use raises concerns regarding environmental pollution and potential health risks [3]. Consequently, the development of environmentally friendly wood preservatives derived from natural resources has gained increasing attention. Numerous studies have demonstrated that plant-derived extracts containing bioactive secondary metabolites can inhibit wood-destroying organisms and may serve as potential alternatives to synthetic preservatives [4,5].

One plant species with promising potential is *M. malabathricum*, a fast-growing, widely distributed weed abundant in tropical regions [6]. Compared with other botanical sources that have been extensively investigated, *M. malabathricum* remains underutilized, particularly for wood protection. Previous studies on this plant have primarily focused on its pharmacological and antimicrobial properties [7,8]. At the same time, investigations into its efficacy against major wood-destroying organisms such as *C. curvignathus* and *S. commune* remain limited. This indicates a clear research gap regarding its application as a natural wood preservative.

Phytochemical analyses have revealed that *M. malabathricum* leaves contain various bioactive compounds, including flavonoids, tannins, saponins, steroids, and other phenolic compounds [7,9]. These secondary metabolites are known to exhibit biological activities relevant to wood protection mechanisms. Saponins are reported to disrupt cell membrane permeability in fungi, thereby inhibiting mycelial growth. At the same time, tannins act as antifeedants by binding proteins and digestive enzymes in termites, thereby reducing feeding activity [9,10]. Flavonoids have been shown to interfere with enzymatic systems and inhibit fungal sporulation, whereas steroids may disrupt cellular metabolism in insects and fungi [11]–[13]. Despite this phytochemical potential, the specific relationship between these compounds and their antitermite and anti-fungal activities against *C. curvignathus* and *S. commune* has not been comprehensively evaluated.

Therefore, this study aims to analyze the effects of the ethanolic leaf extract of *M. malabathricum* on the mortality and feeding activity of the subterranean termite *C. curvignathus*, as well as its inhibitory effect on the growth of *S. commune*. The findings of this research are expected to provide scientific evidence for the use of *M. malabathricum* as an environmentally friendly natural wood preservative and to contribute to the sustainable management of wood resources.

## 2. Method

### 2.1. Time and Place

This research was conducted at the Forest Products Chemistry Laboratory, Faculty of Forestry, Tanjungpura University. The study was conducted from April to September 2025, covering preparation, implementation, testing, and data processing.

### 2.2. Research Materials and Tools

The materials used are *M. malabathricum* leaves, spirits, amoxicillin, PDA (Potatoes Dextrose Agar), and 70% alcohol for hand and tool sterilization; *C. curvignathus* termites as research subjects; *Schizophyllum commune* fungi; and Whatman No. 1 paper with a diameter of 3.5 cm as termite food. In addition, sand serves as a medium for conditioning termites and conducting filter paper tests; cotton to maintain humidity during testing; aluminum foil as a medium for oven testing samples; distilled water to maintain sand humidity; and label paper.

The tools used in this study were aluminum foil, 1000 ml Erlenmeyer flask, 100 ml beaker glass, hotplate, magnetic stirrer, sprayer, oven, incubation cabinet, petri dish, loop needle, measuring pipette, bunsen, prastik wrap, digital caliper, measuring cup, analytical balance, mask, laminar air flow, matches, gloves, and camera for documentation of research activities, scissors for cutting Whatman No. 1 paper with a diameter of 3.5 cm which functions as termite food, desiccator for conditioning test samples, electric oven for drying samples, plastic cups as test containers, tweezers for separating live termites from dead ones, plastic gauze which functions as a barrier between test samples and sand, chicken feathers for moving termites from colonies to test containers, plastic containers for storing the test containers, black cloth for covering termites, mesh filters for filtering sand, autoclaves for sterilizing sand, analytical scales for measuring the weight of test samples, and stationery for recording the results obtained.

### 2.3. Research procedures

#### 2.3.1. Preparation of leaf extract *M. malabathricum*

Extraction was carried out using the maceration method with 96% ethanol as the solvent at a 1:7 (w/v) ratio. Maceration is carried out at room temperature for 48 hours by continuous shaking using a shaker. The solvent is removed using a rotary evaporator at 50°C, and the concentrated extract is stored in a dark glass bottle at 4°C before use. This extraction procedure was adapted from [14,15].

### 2.3.2. Testing for termites

The concentrated extract was accurately weighed according to the required amount for each concentration, dissolved in ethanol in a volumetric flask, and adjusted to a final volume of 10 ml. Extract concentrations of 2%, 4%, 6%, 8%, and 10% (w/v) were prepared using this procedure. Filter paper samples were immersed in the extract solutions for 1 hour, drained, air-dried for 24 hours, then oven-dried at 60 °C for 24 hours, and weighed prior to testing.

Termite testing involves two observation parameters: termite mortality and paper weight loss. Mortality testing uses the cellulose pad method [4]. The experiment was conducted with three replications. The test containers were specifically designed to ensure suitable environmental conditions for termites, thereby providing accurate and reliable results (Fig. 1).

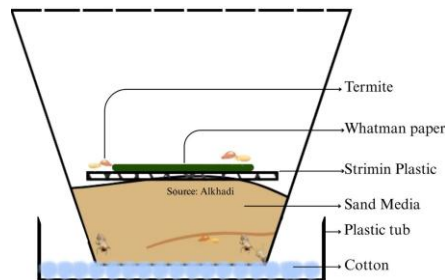


Figure 1. Termite Test Container

Termite testing is carried out under controlled laboratory conditions. The test was conducted at a temperature of 26–28°C with relative humidity maintained at 80–90% RH. Humidity is controlled by placing wet cotton in a sealed plastic container and is monitored daily during a 21-day trial period to ensure stable conditions.

The test sample container for termite testing used was 7cm high, and the diameter of the glass base was 5cm. The test container was filled with  $\pm 15$ g of sand that had been sterilized by autoclaving for 1 hour at 121 °C, then the sand was wetted with  $\pm 4$ ml of water. Stages of termite testing (1) test paper that had been treated with liquid smoke concentration according to the concentration was placed in the test glass, (2) subterranean termites were put into the test glass with a total of 33 termites (30 workers and 3 soldiers), (3) the test glass was placed in a humid container, where the container was filled with cotton and given water to maintain humidity, the test was carried out with 4 repetitions and the termite test was carried out for 21 days, the test involved checking every 3-day interval to remove dead termites, to prevent the growth of fungi that could potentially harm other living termites, and controlling the humidity conditions of the sand media. The parameters used in this study included termite mortality and weight loss of the test paper. Termite mortality and weight loss of the test paper were calculated after undergoing testing for a period of 21 days. Mortality values were calculated using the following formula [16].

$$\text{Mortality (\%)} = \frac{\text{Number of dead termites}}{\text{Initial number of termites}} \times 100 \quad (1)$$

The next parameter is the weight loss of the test paper to determine the level of consumption by *C. curvignathus* termites. Before weighing, the test paper samples were cleaned of any dirt attached due to termite activity. The samples were then dried in an oven at 60°C for 1 hour, placed in a desiccator for about 15 minutes, and weighed to obtain the oven-dry weight of the sample after testing. The percentage of weight loss was calculated using the formula referring to Andrie et al. [16] as follows.

$$\text{Weight loss (\%)} = \frac{\text{Initial weight of sample} - \text{Final weight of the sample}}{\text{Initial weight of sampel}} \times 100 \quad (2)$$

### 2.3.3. Testing Against *S. commune* Fungus

#### 2.3.3.1. Preparation of Potato Dextrose Agar (PDA) Media

A total of 39g of PDA was dissolved in 1000ml of distilled water, then homogenized using a magnetic stirrer and heated on a hot plate until the PDA solution was homogeneous. Afterward, 0.04g of amoxicillin antibiotic was added and homogenized. Then, it was sterilized using an autoclave at 121°C and 1 atm pressure for 15 minutes [17].

### 2.3.3.2. Propagation of *S. commune* Fungus

This fungus propagation was carried out using the method of Suresh et al. [18]. *S. commune* fungal isolates were obtained from the Forest Products Chemistry Laboratory, Faculty of Forestry, UNTAN, and then propagated using fresh PDA media. Propagation of *S. commune* fungi was carried out using an eyepiece needle to pick and plant *S. commune* into fresh PDA media in sterile Petri dishes. After inoculation, incubation was carried out at 22–32°C for 2–7 days.

### 2.3.3.3. *S. Commune* Fungal Testing Process

Fungal activity testing was carried out using a modified method of Suresh et al. [18] and Oramahi et al. [19]. The fungal growth medium used was PDA in Petri dishes, supplemented with extract concentrations of 2%, 4%, 6%, 8%, and 10%, with 0% as a control. Each treatment was conducted in three replications. Fungal growth assays were conducted under controlled laboratory conditions. All cultures were incubated at a temperature of 27±2°C for 7 days. The incubation temperature was monitored daily and maintained consistently across all replications to minimize environmental variation during fungal growth. The composition of the extract and the PDA medium for all treatments is shown in Table 1. Material requirements for each concentration treatment were calculated using the formula (%v/v) as described by Indrawati et al. [20].

Table 1. Extract composition with PDA media

Concentration (%)	Concentration treatment requirements	
	Extract (ml)	PDA (ml)
2	0.2	9.8
4	0.4	9.6
6	0.6	9.8
8	0.8	9.2
10	1	9.0

Next, a 7-day-old *S. commune* fungal isolate was taken with a diameter of 5mm using a needle and placed in the center of a petri dish containing PDA media treated with the concentration. The petri dish was covered with the lid, then the edge of the petri dish was covered using plastic wrapping, and stored in an incubation room. Observations were made every day on fungal growth by measuring the diameter of the fungal growth. Observations were stopped after 7 days or when fungal growth in the control had filled the petri dish. Incubation was carried out at 27±2°C for 2–7 days. All testing activities were carried out in a closed room using a laminar airflow device to maintain test sterility.

### 2.3.3.4. Anti-Fungal Activity (AFA)

Data collected to determine the AFA value were obtained by measuring fungal mycelial growth on the seventh day after inoculation or on the last day of testing. Measurements were made using digital calipers, drawing two perpendicular lines at the bottom of the petri dish (Fig. 2). This was done to obtain the average fungal growth rate and was calculated using the formula [21]. Average fungal growth was measured using the following formula.

$$P = \frac{d1+d2}{2} \quad (3)$$

Description: P = Average mushroom growth

d1 = Vertical growth

d2 = Horizontal growth

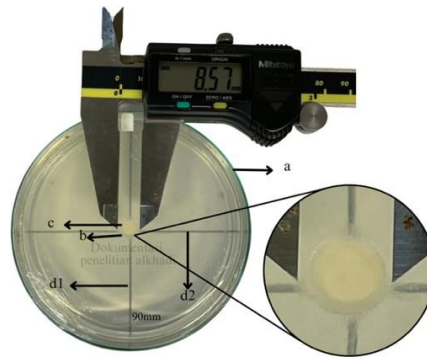


Figure 2. How to measure mushroom growth: Description: a: Petri dish; b: Mushroom growth; c: Early mycelium (5mm)

The AFA value calculation uses the measurement method of [22]. AFA data uses the average diameter on the last day of observation. The AFA formula is as follows.

$$\text{AFA (\%)} = \frac{(A-B)-(C-B)}{A-B} \times 100 \quad (4)$$

Description: AFA = Antifungal Activity (%)

A = Control mycelium growth (mm)

B = Initial mycelium size at incubation (mm)

C = Mycelium growth in the treatment medium (mm)

#### 2.4. Experimental Design

This study used a one-factor Complete Random Design (CRD) with six levels of concentration and three replicates. Data were analyzed using the nonparametric Kruskal-Wallis test (Shapiro-Wilk test,  $p < 0.05$ ). If there was a significant effect ( $p < 0.05$ ), a follow-up test of Pairwise Comparisons was performed to determine the difference between treatment groups at a 95% confidence level, using SPSS v.27 software.

### 3. Results and Discussion

#### 3.1. *M. malabathricum* leaf extract

The results of the study showed that the yield of *M. malabathricum* leaf extract obtained was 9.93% with a water content of 12.8%, due to the influence of water content in the simplicia of 12.8%. Compared with previous studies, the yield results in this study were within a relatively comparable range. A study by Diris et al. [7] reported that the yields of *M. malabathricum* and *Melastoma beccarianum* were 32% and 8%, respectively. Another study, Fajrina et al. [8], reported a yield of 15.05% for the ethanol extract of *M. malabathricum* L. leaves using a maceration method with 70% ethanol. This indicates a fairly wide variation in results between studies. This difference in yield can be caused by differences in solvents, extraction methods, plant parts used, water content of the simplicia, geographical conditions, and the plant's growing environment.

Regarding the water content of the herbal medicine, the value of 12.8% is slightly higher than the generally recommended quality standard of  $\leq 10\%$  to ensure metabolite stability and prevent microbial growth [23]. Higher water content can affect the quality of herbal medicine and potentially reduce the stability of bioactive compounds, although in this study the yield remained within an acceptable range. To improve quality, it is recommended that the herbal medicine be dried to a water content of  $\leq 10\%$  before extraction.

The extract yield obtained in this study, 9.93%, is quite good and consistent with previous studies. The difference in yield compared to several literature studies underscores the importance of standardizing extraction methods, selecting solvents, and controlling the water content of medicinal plants to achieve optimal yield.

### 3.2. Termite Mortality

The results showed that administration of *M. malabathricum* leaf extract affected the mortality rate of the subterranean termite *C. curvignathus*. The results showed an increase in mortality rate as the extract concentration increased. In the control treatment, mortality was only 8.08%, suggesting that natural factors or environmental conditions caused it. However, at a 2% extract concentration, mortality increased to 13.13%, then gradually increased to 36.36% at a 10% concentration. The overall treatment is presented in Fig. 3.

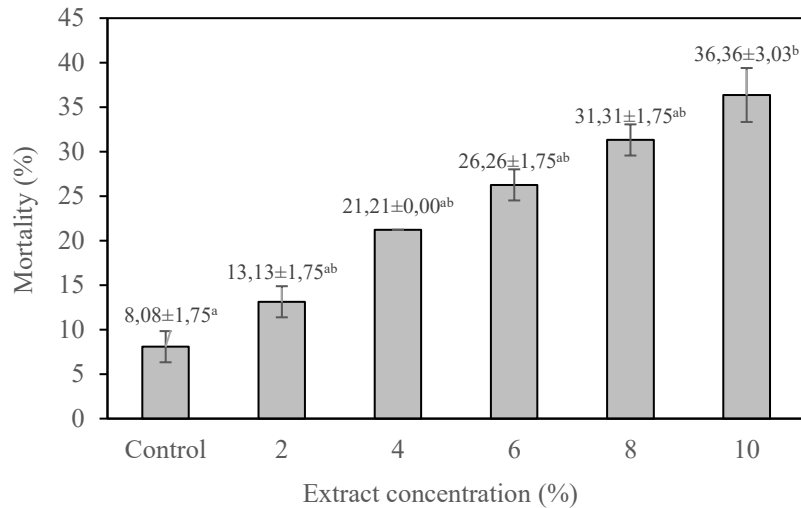


Figure 3. Termite mortality after 21 days of feeding

This pattern of increasing mortality indicates a relationship between extract concentration and termite mortality: the higher the extract concentration, the greater the termite mortality rate. This indicates that *M. malabathricum* leaf extract contains bioactive compounds that are toxic or disrupt termite physiology. Common phytochemical compounds found in *M. malabathricum* include flavonoids, tannins, saponins, and terpenoids. Flavonoids and tannins are thought to act as antifeedants and digestive toxins, inhibiting termite feeding activity, while saponins can damage insect cell membranes, disrupting metabolism and causing death.

Termite mortality in the treatment compared to the control increased mortality by more than 4 times at the highest concentration, proving the potential of the botanical insecticide from *M. malabathricum* leaf extract. However, the mortality rate at 10% (36.36%) was not very high (>50%), so to achieve a greater lethal effect, increasing the extract concentration, developing a more effective formulation, or combining with other plant extracts is required.

Overall, the results of this study support the potential use of *M. malabathricum* extract as an environmentally friendly biological control agent against the subterranean termite *C. curvignathus*. Its advantages include its natural origin, its environmental safety, and its potential as an alternative to synthetic insecticides.

Several previous studies have demonstrated the potential of plant extracts to suppress *C. curvignathus* populations. For example, research by Wattimena and Latumahina [5] reported that extracts of several local plants (papaya, soursop, and lemongrass leaves) at various concentrations significantly increased termite mortality compared with controls, warranting their consideration as biopesticides. Similarly, research by Adfa et al. [24] on *Azadirachta excelsa* leaf extract with acetone, hexane, and methanol solvents showed decreased wood consumption and increased mortality of *C. curvignathus*. These effects are thought to be due to the limonoid and terpenoid content, which act as antifeedants and digestive toxins.

Based on research by Arinana et al. [25], they found that the n-hexane extract from *Melaleuca cajuputi* has bioactivity against subterranean termites, both as an attractant and as a potentially toxic substance. This indicates that volatile and nonpolar compounds from plants, such as terpenoids, can influence termite behavior and survival. Another study by Diba et al. [26] using *Ocimum basilicum* extract in a paper waste-based bait formulation also showed reduced wood consumption and higher mortality compared to the control. Meanwhile, research by Hassan et al. [27] emphasized that biological control strategies, including the use of botanical biopesticides and entomopathogenic fungi, can be integrated into termite control for more sustainable results.

Although empirical evidence on the use of *M. malabathricum* against subterranean termites remains very limited, phytochemical studies indicate that this plant contains various secondary metabolites, including flavonoids, tannins, saponins, triterpenoids, and polyphenols [9]. These compounds are known to act as antifeedants, growth inhibitors, and digestive toxins in insects. Based on the results of this study, it can be concluded that research on plant extracts against subterranean termites has been widely conducted, with positive results, but the specific use of *M. malabathricum* remains rarely reported. Therefore, this study is important for strengthening the scientific basis for the potential of *M. malabathricum* leaf extract as a botanical insecticide for subterranean termite control.

The results of the homogeneity test showed that the data were not homogeneous due to constant values across certain treatments. Therefore, a comparative analysis between treatments was performed using the nonparametric Kruskal-Wallis test. The Shapiro-Wilk normality test showed that the mortality data in the majority of the treatment groups (controls, 2%, 6%, and 8%) had a p-value of  $<0.05$  (.000), indicating that the data were not normally distributed. In addition, there is constant data on the 4% and 10% treatments, which violate the assumption of homogeneity of variances in the parametric test. The comparative analysis was continued using the nonparametric Kruskal-Wallis test. The test results showed a statistically significant difference in mortality rates among the six treatment groups ( $\chi^2(5) = 16.634$ ;  $p = 0.005$ ). Significance values below 0.05 indicate that variations in treatment levels significantly influence termite mortality.

The results of the follow-up test, Pairwise Comparisons with Bonferroni correction, showed that a statistically significant difference was found only between the control group and the treatment with the highest dose (10%), with a p-value of 0.009. Meanwhile, comparisons among other treatment groups did not show statistically significant differences ( $p > 0.05$ ), although there was a trend toward increased mortality as the extract concentration increased.

### 3.3. Weight Loss

The weight loss of the test paper after feeding against the subterranean termite *C. curvignathus* at various extract concentrations showed that the weight loss of the test paper in the control was the highest. In contrast, in the treatment with increasing extract concentration, there was a decrease in weight loss, which indicated a protective effect of the extract against termite attacks. Fig.4 is the percentage of weight loss of the test paper after 21 days of feeding against termites.

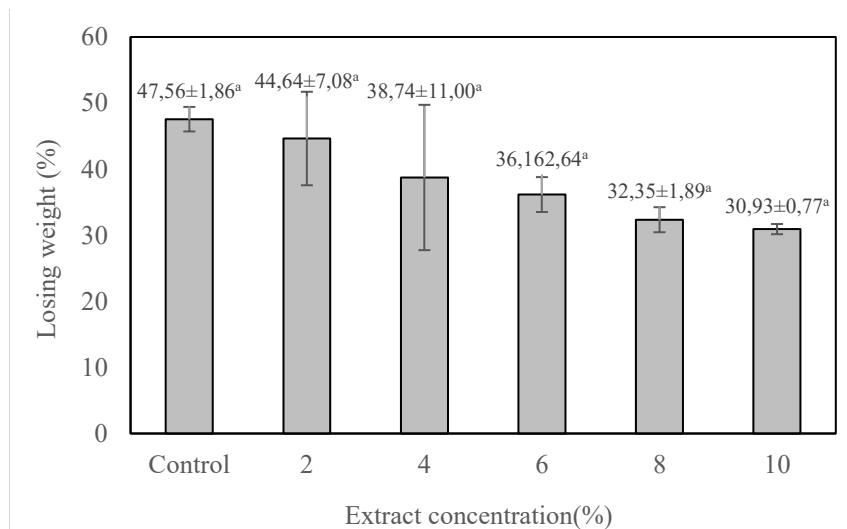


Figure 4. Percentage weight loss of the test paper after 21 days of feeding

Based on Figure 4, the percentage of weight loss of the test paper decreased with increasing concentration of *M. malabathricum* leaf extract. In the control (without extract), weight loss reached 47.56%, indicating a fairly high attack by the subterranean termite *C. curvignathus* due to the absence of inhibitory compounds. However, as the extract concentration increased, the weight loss of the paper gradually decreased, reaching a minimum of 30.93% at a concentration of 10%.

The decrease in the weight of the test paper indicates that the *M. malabathricum* extract has an anti-termite effect, inhibiting termite feeding activity. This mechanism is likely influenced by the content of secondary metabolites in the extract, such as flavonoids, tannins, saponins, and phenolics, which are known to be toxic,



anti-feedant, or disrupt the insect digestive system. These compounds can reduce the rate of termite consumption. These results are consistent with Luth's [10] research, which reported that plant extracts rich in phenolic compounds can suppress subterranean termite feeding activity and reduce damage to test samples. Furthermore, *M. malabathricum* is known to contain bioactive compounds with natural insecticidal properties, which can act as an environmentally friendly alternative in termite control. Fig.5 shows the final condition of the test paper after termite feeding.

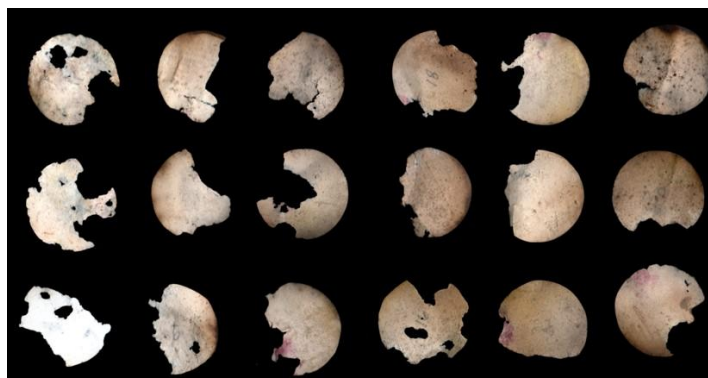


Figure 5. Condition of the test paper after 21 days of termite feeding

Based on Figure 5, the higher the concentration of the extract, the greater the protection against termite attack on the test paper. Although at a concentration of 10%, weight loss was still around 30%, it was significantly lower than in the control. This suggests the potential use of *M. malabathricum* leaf extract as a botanical bioinsecticide for subterranean termite control, though its effectiveness still needs improvement.

The test results showed a consistent inverse correlation between *C. curvignathus* mortality and the percentage of test paper weight loss in the *M. malabathricum* extract treatment. The higher the extract concentration, the higher the mortality rate, while the paper weight loss decreased. This phenomenon indicates a dual effect of the extract, namely a toxic effect that causes the death of individual termites and an antifeedant effect that reduces termite feeding activity [9].

A possible biochemical mechanism underlying these observations is the phytochemical profile of *M. malabathricum*, which is rich in flavonoids, tannins, saponins, triterpenoids, and other phenolic compounds. These compounds have been reported to act as antifeedants (reducing palatability), interfere with nutrient digestion and absorption, or cause systemic toxicity in insects by disrupting enzymes and damaging cell membranes. Therefore, the decrease in paper weight loss that coincided with the increase in mortality at higher concentrations can be explained by a combination of sublethal (reduced feeding) and lethal (death) effects. This statement aligns with modern phytochemical studies on the bioactive components of *M. malabathricum* [9].

The difference between the highly variable group (2%) and the nearly uniform group (10%) is what usually makes the significance value of the Levene's Test  $p < 0.05$  (Not Homogeneous). Based on the Shapiro-Wilk test, the variable weight loss in the control group, up to 8%, showed a normal distribution ( $p > 0.05$ ). However, in the 10% group, a significance value of 0.000 ( $p < 0.05$ ) was found, so the normality assumption for the parametric test was not fully fulfilled. Therefore, data analysis was performed using the nonparametric Kruskal-Wallis test. The test results showed a statistical value of 10.133 with an asymptomatic significance of 0.072. Because the p-value was 0.05, it can be concluded that there was no significant difference in weight loss across the treatment groups. This indicates that the variation in treatment given (P1-P5) did not have a significant impact on the subject's weight change when compared to controls. Pairwise comparisons were not performed because the main test showed non-significant results. This finding differs from the mortality variable, which previously showed a significant effect ( $p = 0.005$ ), suggesting that the treatment was more effective at influencing mortality rates than at influencing weight loss in the test paper.

### 3.4. Anti-fungal Activity

The results of the anti-fungal activity test showed that increasing the concentration of *M. malabathricum* extract was directly proportional to the ability to inhibit fungal growth in PDA media that had been added with the extract treatment. At the lowest concentration (2%), the anti-fungal activity was 30.83%; at 6% it increased to 72.84%; and at 10% it reached the highest activity of 88.23%. These findings indicate that the secondary metabolite content of the extract, including flavonoids, tannins, and phenolics, plays an active role in inhibiting



the growth of the tested fungal mycelium. Fig. 6 shows the value of the inhibitory activity of fungal growth in the test media with *M. malabathricum* extract treatment.

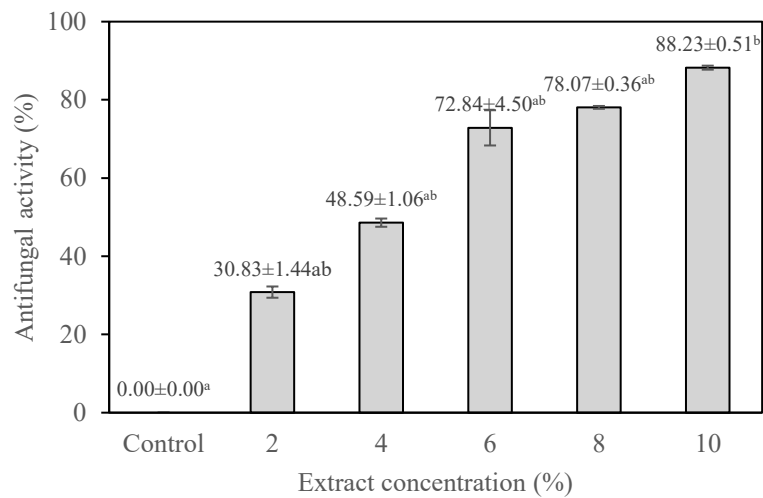


Figure 6. Percentage of anti-fungal activity of *M. malabathricum* extract

These results are in line with the research of Chattri et al. [11], who reported that *M. malabathricum* leaf extract has different anti-fungal activity against *Fusarium oxysporum* and *Sclerotium rolfsii*. Their results showed that increasing the extract concentration significantly affected the inhibition of fungal colony growth. Another study by Gholib et al. [12] also showed that the ethanol extract of *M. malabathricum* leaves inhibited the growth of *Trichophyton mentagrophytes* and *Candida albicans*, with the inhibition zone increasing with increasing extract concentration. Documentation of fungal growth after treatment with the extract concentration can be seen in Fig. 7.

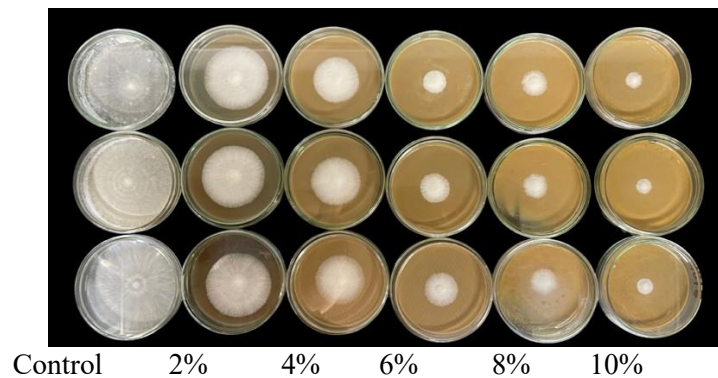


Figure 7. Documentation of the growth inhibition of the *S. commune* fungus

Anti-fungal effectiveness indicates that the higher the concentration of the applied extract, the greater the content of bioactive compounds that interact with fungal cells. Compounds such as flavonoids, tannins, and phenolics work by damaging cell walls, disrupting membrane permeability, and inhibiting intracellular enzyme activity. At low concentrations, the number of active compounds interacting is still limited, resulting in only partial inhibition of fungal growth. While at high concentrations, the accumulation of these compounds can significantly suppress fungal growth [13].

Based on the results of the homogeneity analysis, this anti-fungal data are inhomogeneous (due to zero variance in the control and differences in variation across the other groups), so ANOVA is invalid. Furthermore, the normality test on the AFA variable showed that the 2% group had abnormal data distribution ( $p = 0.036$ ). Therefore, data analysis was carried out using the nonparametric Kruskal-Wallis test. The analysis showed a significance value of 0.006 ( $X^2 = 16.460$ ), indicating a significant effect of treatment on anti-fungal activity. The results of the Pairwise Comparisons follow-up test confirmed that a statistically significant difference was found between the control group and the 10% group ( $p = 0.008$ ). Similar to the mortality variable, although the 8% treatment began to show an increase in inhibition activity, only treatment 10 was

statistically significantly different from the control ( $p < 0.05$ ). The daily growth data of mushrooms is presented in Fig. 8.

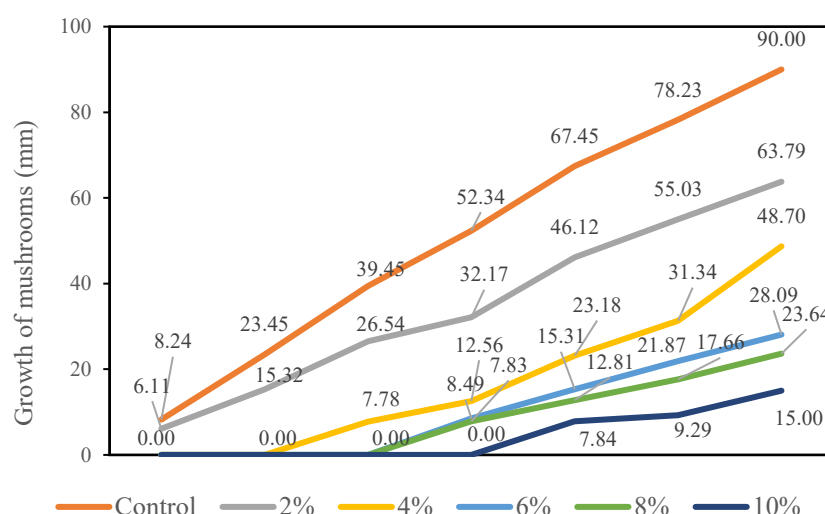


Figure 8. Daily growth data of mushrooms

Figure 8 shows that fungal growth increases over time across treatments, but with different growth rates at different extract concentrations. Control showed the highest and most consistent growth until day 7, indicating optimal conditions without the inhibition of active compounds. On the other hand, the addition of *Melastoma malabathricum* extract results in a gradual decrease in fungal growth as the concentration increases, indicating anti-fungal activity.

#### 4. Conclusion

Ethanol extract of *M. malabathricum* leaves significantly affected termite mortality ( $p = 0.005$ ) and anti-fungal activity ( $p = 0.006$ ), but had no significant effect on weight loss ( $p = 0.072$ ). The 10% concentration was established as the optimal concentration because it was the only treatment that had a statistically significant effect on the control variables of mortality ( $p = 0.009$ ) and anti-fungal activity ( $p = 0.008$ ). Thus, this extract has the potential to be a powerful, environmentally friendly natural wood preservative. Further studies should evaluate higher extract concentrations to determine the  $LC_{50}$  value and better characterize the concentration–response relationship. As the present findings were obtained under laboratory conditions, field trials are required to validate their effectiveness under natural environments. In addition, assessment of extract formulation and potential effects on non-target organisms is necessary to ensure environmental safety prior to large-scale application.

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#### Conflict of Interest

All authors declare that they have no conflicts of interest.

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