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Original Article

Activity of *Mikania micrantha* leaf extract against subterranian termite and wood decay

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Abstract

Mikania micrantha is a wild weed that thrives in tropical areas, grows rapidly in areas with high humidity and light intensity as well as in fertile soil. Meanwhile, Indonesia's tropical climate is also a suitable habitat for the breeding of termites and wood decay fungi. This study aims to determine the bioactivity of *M. micrantha* leaf extract against subterranean termite, *Coptotermes curvignathus* (Holmgren) and wood decaying fungus, *Schizopyllum commune* and its optimal extract concentration to prevent termite attack and inhibit fungal growth. The extracts were obtained by drying and mashing of *M. micrantha* leaves. The leaf powder was sieved with a 40-60 mesh filter and macerated with methanol for 3x24 h. The maceration results were filtered and the filtrate was concentrated using a rotary evaporator. Furthermore, identification of the secondary metabolites of the extract was carried out by phytochemistry. The bioactivity test included anti-subterranean termite and wood decay fungus using Potatos Dextrose Agar (PDA) medium. The results showed that *M. micrantha* leaf extract affected sample weight loss, termite mortality rates and the growth of wood decay fungi. Furthermore, the concentration of *M. micrantha* extract was inversely proportional to the sample weight loss and directly proportional to the termite mortality rate. In addition, the concentration of *M. micrantha* leaf extract had a negative correlation with the growth of the decaying fungus of *S. commune* therefore, the concentration of the extract was directly proportional to the growth inhibition of fungus. Meanwhile, the 4% extract concentration led to the increase in termite mortality rate above 80% and inhibited fungal growth by 100%. The results suggest that the bio-efficacy data from this study may be used to develop wood protecting systems based on wild weeds which are found in tropical areas.

Keywords:Termiticide, fungicide, *M. micrantha*, *Schizopyllum commune*, *Coptotermes curvignathus*

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Introduction

Tropical countries have high temperatures and humidity throughout the year and are very favorable for the growth of various kinds of wild weeds such as *Mikania micrantha,* which grows rapidly, produces vines and disturbs other plants. It has been reported to reduce the production of several plantation crops such as oil palm, rubber, coconut, tea orange, cassava, pineapple, banana, teak, acacia, eucalyptus and albasia (Sankaran, 2008). Due to its wide spread in Indonesia, it has the potential of been exploited.

Apart from *M. micrantha*, Indonesia's tropical climate is also a suitable habitat for termites and wood decay fungi to breed. *Coptotermes curvignathus* is a subterranean termite that causes economic losses, because it attacks buildings even multi-storey buildings and have also been found to attack oil palm trees in some cases. Controlling this termite is very tasking due to the difficulty in locating its nest. Meanwhile, the wood decay fungi widely found in Indonesia are the *Schizopyllum commune* Fries species, which are quite malignant and able attack more than 25 species of wood (Maryam, 2011). Therefore the attack caused by these wood-destroying pests needs serious attention. The use of synthetic pesticides to control termites and wood decay fungi have been in existence. However, it has a negative impact, such as polluting the environment which is dangerous to humans and non-target animals.

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Therefore, natural pesticide development has been carried out to tackle this situation. Furthermore, several studies have explored various natural ingredients to be used as natural pesticides. They include the extract of divine crown stem used against dry wood termites (Lukmandaru and Gazidy, 2016), *Euphorbia tirucalli* leaf extract is used for larvae-killer for cabbage leaf caterpillars (Toana and Nasir, 2010), Papaya leaf extract and kumis kucing were reported to be used against dry wood termites (Aziz et al., 2018) and Leaf extract of *Avicennia marina* VIERH to inhibit wood decaying fungi.

The widespread of weeds in Indonesia makes these plants potentially used as vegetable pesticides with the advantage of being organic, non-toxic, easy to obtain and cheap. Furthermore, the secondary metabolite mechanism of weeds is different from the synthetic pesticide. Several studies have reported the utilization of weed as a vegetable pesticide, which includes *Ageratum conyzoides* leaf extract (Isda et al, 2013, Kartika et al., 2016, Wardhiany et al., 2014), *Calopogonium mucunoides* (Sihombing et al., 2012), *Chromolaena odorata* (Thamrin et al., 2007, Fitriana et al., 2012, Huzni et al., 2015), *Cyperus rotundus* and *Imperata cylindrical* (Arie et al., 2015).

The utilization of *M. micrantha* weed has been reported to be used in the health sector for wound medicine (Fernandes et al., 2018), antibacterial and phytochemical (Matawali et al., 2016, Nasution et al., 2019), anti-cancer (Rahman et al., 2020) and antioxidants (Dev et al., 2015). Furthermore, its utilization as a vegetable herbicide has been reported by Pebriani et al. (2013). Although, there are many studies on its bioactivity, its role as an inhibitor against subterranean termites and wood decaying fungi has not

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been studied much. In addition, it contains secondary metabolites such as alkaloid compounds, triterpenoids and steroids (Fernandes et al, 2018 Polakit et al., 2017), terpenes and sesquiterpenes (Castro et al., 1986). These compounds are known to be toxic to termites and wood decay fungi therefore, its leaves have the potential to be developed as an anti-termite and anti-wood decay fungus.

This study was initiated to evaluate the bio-activity of *M. micrantha* leaf extract against termites and fungi and to determine the minimum effective concentration against termite mortality and growth of wood decay fungus. In addition, the effectiveness of its weed extracts against subterranean termites and wood decay fungi will be an important part of basic information for integrated pest management.

Methods

Producing *M. micrantha* leaf extracts

The *M. micrantha* leaves were first dried with indirect exposure to sunlight, crushed into powder and filtered with a mesh size of 40 and 60. Afterwards, the extraction process was carried out using either the maceration or soaking method. 200 g of *M. micrantha* leaf powder was soaked in 1000 mL of 96% methanol for 2x24 h and re-maceration on a shaker at a speed of 150 rpm for 1x24 h. Furthermore, the extract solution obtained was filtered using filter paper to obtain filtrate I and filtrate II from the first maceration and re-maceration results, respectively. These filtrates were evaporated using a rotary evaporator at a speed and temperature of 60 rpm and 45ºC, respectively until all solvent evaporated and a thick extract was obtained (Hajra et al., 2010). The thick extract was diluted to a concentration of 1%, 2%, 3%, 4%, 5% and 6%, respectively using methanol solvent.

Yield analysis

Yield analysis was determined according to Rahmah et al. (2018). Leaf and extract of *M. micrantha* were weighed then calculated by dividing the weight of extract with leaf and multiplied by 100%. Yield is calculated based on equations as follow:

$$\text{Yield (\%)} = \frac{\text{Extract weight}}{\text{Leaf weight}} \text{ X } 100\%$$

Moisture content analysis

Moisture content was calculated according to Rahmah et al. (2018). Empty vessel is oven-dried at 105 °C and cooled in a desiccators for 15 min. The empty vessel was weighed, two g of extract was added into the empty vessel. The vessel containing extract was oven-dried at 105 °C for three h then cooled in desiccators for 15 min. Moisture content is calculated based on equations as follow:

$$\text{Moisture content (\%)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Extract weight}} \text{ X } 100\%$$

Phytochemical Screening

Phytochemical screening of *M. micrantha* leaf extracts is carried out to determine the secondary metabolites compounds present in the extract qualitatively. Phytochemical identification such as alkaloids compound using Dragendroff's test, Mayer's test, and Wagner's test, steroids/triterpenoids compound using Liebermann Bouchard's test, flavonoids compound using NaOH 10%, H_2SO_4, and Mg+Cl, and tannins, saponins, phenolic, and terpenoids compounds as well.

Termite bioassay

Termite testing was carried out with the No Choice Test method, developed by Ohmura et al., (2000) with modifications. WhatmanTM No. filter paper. 1 Paint No. 1001-055 55 mm diameter was used as test sample. The filter paper was cut into a diameter of 30 mm, immersed in the extract solution according to the respective concentration for 1 h and air-dried for 24 h before testing. The test was carried out using a plastic cup measuring 50 mm in diameter and 60 mm in height. Each cup was filled with sand, size of 30 mesh and held by 10 g of 50 mesh which has previously been sterilized using an autoclave for 30 min at a temperature of 120 ºC and a pressure of 1 atm. Furthermore, the sand was moistened with 2 mL of water to maintain its moisture and a plastic gauze measuring 40 mm in diameter was placed on the sand to prevent the test sample from coming in direct contact with it (Fig. 1). One filter paper was put in a plastic cup for each concentration and 55 subterranean termites with a ratio of 50 workers to and 5 soldiers' caste termites were added. The study units were stored in a dark room at ± 26.9 °C - 28.3 °C and ± 70% - 82% humidity for 21 days. The study was conducted in 5 repetitions. The parameters observed include weight loss of filter paper and the mortality of termites. The weight loss of filter paper was obtained by calculating the percentage of weight loss before and after testing with the following formula:

$$\text{Weight Loss} = \frac{(W_1 - W_2)}{W_1} \text{ X } 100\%$$

Where;
W_1 = Filter paper weight before testing (g)
W_2 = Filter paper weight after testing (g)

Termite mortality is the ratio between the number of termites before and after testing expressed in percent. The formula for calculating termite mortality is as follows:

$$\text{Termite mortality} = \frac{N}{N_1} \text{ X } 100\%$$

Where;
N_1 = The number of termites that died in the i^{th} test sample (head)
N_2 = Number of early termites feed (head)

Figure 1. No-choice test method for termite testing

Fungal bioassay

The testing of fungal activity of the extracts produced was carried out by measuring mycelium growth in petri dishes (Khan and Zhihui, 2010), we used the wood-decay *S. commune*. *M. micrantha* leaf extract with each concentration was added to a PDA (Potato Dextrose Agar) solution until it reached 10 mL and poured into a Petri dish (Fig. 2). The seven-day-old *S. commune* isolate with a diameter of 5 mm was planted in the middle of a Petri dish and incubated for seven days at room temperature. The fungal mycelia growth was evaluated at the end of the incubation period and the diameter of the growing fungal colonies were measured using digital callipers. Meanwhile, the calculation of the inhibition percentage was carried out by measuring the colony on the 7th day after inoculation with the following formula (Khan and Zhihui, 2010):

$$AFA = \frac{GC - GT}{GC - A} X\ 100\%$$

Where;
AFA = Percentage of inhibition / anti-fungal activity (%)
GC = Diameter of control fungal colony (mm)
GT = Diameter of fungal colonies in media plus extract (mm)
A = Size of the initial incubation mycelium (mm)

Furthermore, based on the AFA value, the activity of each concentration was classified into activity category levels (Tab. 1). The AFA classification refers to the Mori et al., (1997) which is commonly used in testing wood decay.

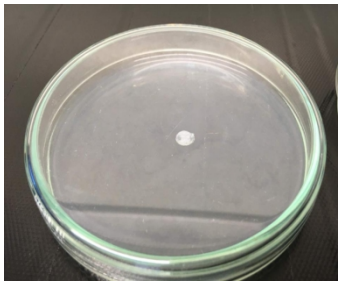

Figure 2. PDA media for wood-decay testing

Table 1. Classification of antifungal activity (AFA)

Anti-fungal activity (AFA)	Activity level
AFA ≥ 75%	Very strong (++++)
75% ≤ AFA < 50%	Strong (+++)
50% ≤ AFA < 25%	Medium (++)
25% ≤ AFA< 0	Weak (+)
0	Not active (-)

Statistical analyses

The analysis of variance (ANOVA) test was used as a statistical tool. Further analysis to compare between concentration levels uses Tukey's test according to Gaspersz (1991).

Results

Yield

The extraction process of *M. micrantha* leaves produced an extract yield of 24.35% with an average moisture content of 9.4%.

Phytochemical screening

Phytochemical screening is carried out to ensure the presence of secondary metabolites such as alkaloids, flavonoids, phenolics and other compounds potentially inhibit the growth of the wood decay *S. commune* as an anti-termite. This screening was carried out qualitatively to analyze the presence of compounds such as alkaloids, flavonoids, tannins, terpenoids, phenolics, anthraquinones and saponins. Based on the results obtained, it was known that *M. micrantha* leaf extracts were positive for alkaloids, flavonoids, steroids, tannins and phenolic compounds. However, saponins and terpenoids were not found in the extract. The results of the phytochemical screening are presented in Table 2.

Table 2. Phytochemical screening Results of *M. micrantha* leaf extract

Phytochemical Test	Reactor	Conclusion
Alkaloids	Mayer	(-)
	Wagner	(-)
	Dragendroff	(+++)
Flavonoids	NaOH 10%	(-)
	H2SO4	(+)
	Mg + HCl	(-)
Saponins	Air + HCl	(-)
Terpenoids	Liebermann-burchard	(-)
Steroids	Liebermann-burchard	(+)
Tannins	FeCl3 1%	(+)
Phenolic	FeCl3 1%	(+)

Description:
(-) does not contain
(+) low levels
(++) sufficient levels
(+++) high levels

Termite bioassay

Weight loss

Termite testing showed that the concentration of *M. micrantha* leaf extract influenced the weight loss of the filter paper after three weeks of feeding on the subterranean termite *C. curvignathus* (Fig. 3). The test results showed that the concentration of *M. micrantha* leaf extract was inversely proportional to the filter paper weight loss and vice versa. The highest weight loss of filter paper by 80.85% was discovered in the control, which was the filter paper without the *M. micrantha* leaf extract. Meanwhile, the lowest weight loss of filter paper was discovered in 6% *M. micrantha* leaf extract, which was 11.68%.

Although there was a decrease in filter paper weight loss with the addition of the *M. micrantha* leaf extract, Figure 3 also shows that the concentration of 2% *M. micrantha* leaf extract was not significantly different from 3% and 4%, but was different 5% and 6%. (Tukey`s test:P <0.01). Meanwhile, the 3% concentration

was not significantly different from the 4% and 5%, but was different from the 6% concentration (Tukey's test: P <0.01). Furthermore, the concentration of 4% *M. micrantha* leaf extract was not significantly different from the 5% and 6% and the 5% concentration was not different from 6% (Tukey's test: P <0.01).

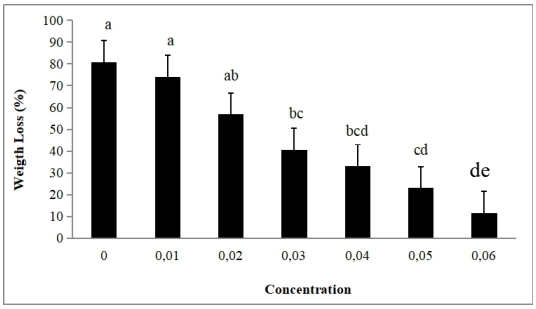

Figure 3. Weight loss of filter paper at various concentrations of *M. micrantha* leaf extract

Termite mortality

Increasing the concentration of the extract provided better properties to the parameters of weight loss and termites mortality. The effect of *M. Micrantha* leaf extract on termite mortality is presented in Figure 4. *M. micrantha* leaf extract at all concentrations were significantly different from the control (without extract). The concentration of 1% was not significantly different from 2% but was different from other concentrations. Meanwhile, the concentration of 4% was not different from 5%, which was not different from 6% (Tukey's test: P <0.01).

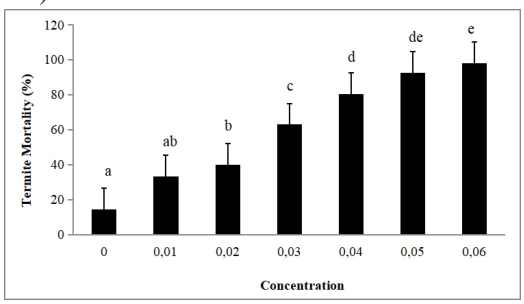

Figure 4. Termite mortality at various concentrations of *M. micrantha* leaf extract

Fungal bioassay

Anti-fungal activity (AFA)

M. micrantha leaf extract has inhibitory power against the growth of *S.commune* wood decay fungus. The anti-fungal activity value of *M. micrantha* leaf extract ranged from 78.36% at a concentration of 1% to 100% to a concentration of 4%, 5% and 6% (Tab. 3). At concentrations of 4%, 5% and 6% there was no growth in the fungus diameter (Tab. 3). Figure 5 shows that there is no significant difference between the concentrations of 4%, 5% and 6% (Tukey's test: P <0.01), indicating that at a concentration of 4%, it inhibits the growth of wood decay fungus by 100%.

Inhibition of fungal growth

Measurement of the fungus diameter was carried out on the seventh day because the fungi on the control media were full (Fig. 6). There was no visible growth of fungi at all concentrations on the first and second days of the incubation period. On the third day of incubation, fungal growth was seen on the control media. Meanwhile, on the fourth day of incubation period, fungal growth began to appear on media with 1%, 2% and 3% concentrations of *M. micrantha* leaf extract (Fig. 7). Fungal growth did not occur on media with the addition of *M. micrantha* leaf extract at a concentration of 4%, 5% and 6% during the incubation period (Fig. 7).

Table 3. Average anti-decay fungal activity of *S.commune* (AFA) on *M. micrantha* leaf extract at various concentration levels

Concentration (%)	AFA mean (%)	Activity level
0	0	Not active (-)
1	78.36	Very strong (++++)
2	91.70	Very strong (++++)
3	94.37	Very strong (++++)
4	100	Very strong (++++)
5	100	Very strong (++++)
6	100	Very strong (++++)

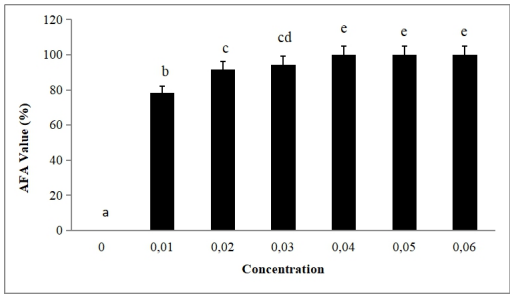

Figure 5. AFA value of *M. micrantha* leaf extract at various concentrations

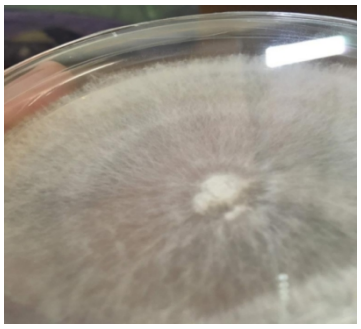

Figure 6. Fungal growth on the controlled media

Figure 6 shows that at the end of the incubation period, the fungi growth on the controlled media was 90 mm, while on the media with a concentration of 1%, 2% and 3%, were 19.99 mm, 12.05 mm and 9.79 mm, respectively. Conversely, no fungal growth was seen at concentrations of 4%, 5% and 6% (Fig. 7).

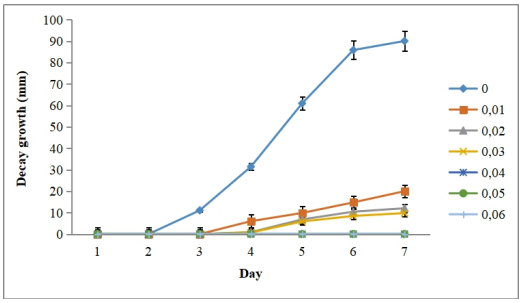

Figure 7. Growth of *S.commune* wood decay fungus after 7 days of incubation at various concentrations

Discussion

Extract content/yield

The methanol extract content of *M. micrantha* leaves in this study (24.35%) was higher than the ethanol extract content of *M. micrantha* by 9.37% (Perawati et al., 2018), and 3.8% (Tari et al., 2016). The high level of *M. micrantha* leaf extract in this study was thought to be due to the difference in the solvent used, which the greatly affected the yield of the extract produced. This is understandable since methanol solvents have a Polarity Index (PI) of 6.6, while ethanol solvents have PI of 5.2 (Abarca-Vargas et al., 2016). Fengel and Wegener (1995) clearly stated that the extraction method was a factor that caused variations in the content and composition of extractive substances. The methanol extract content of *M. micrantha* leaves was higher than the extract of other raw materials using the same solvent as lotus weevil with an extract content of 6.62% (Puspitasari et al., 2013) and *Citrus nobilis* lime leaves of 19.143%. (Nabu et al., 2015). This is assumed that the bioactive compounds contained in *M. micrantha* leaf are more than lotus weevil and *citrus nobilis* lime leaves. This results in line with Nurhayati et al. (2009) who states that a high extract content indicating higher in bioactive compounds.

Termite bioassay

Weight loss

Present study revealed that the concentration of *M. micrantha* leaf extract was inversely proportional to the filter paper weight loss and vice versa. Phytochemical test results of *M. micrantha* leaf extract showed that the extract contained alkaloids, flavonoids, steroids, tannins and phenolic chemical elements, which were responsible for the reluctance of termites to eat filter paper because of their anti-crawly properties. Vikery and Vikery (1981) supported the opinion that triterpenoids and their derivatives, including saponins and steroids in plants functioned as insect poisons. Meanwhile, Supriana and Jasni (2004) argued that tannins functions as an inhibitor of damage due to insect attack because they contain phenols which are toxic to wood-destroying organisms. Harborne (1987) added that the main function of tannins in plants is as a repellent for plant-eating insect.

The results obtained from this study were in line with Aziz et al., (2018) which showed that the lowest weight loss was on filter paper with the highest concentration of cat whiskers extract. The effect of concentration on weight loss has also been reported by Wahyudi et al. (2012), Kartal et al. (2012) and Tascioglu et al. (2012; 2013). The comparison of filter paper at each concentration of *M. micrantha* leaf extract after 21 days testing period can be seen in Figure 8.

Termite mortality

M. *micrantha* leaf extract had a positive correlation with termite mortality values, which was directly proportional to the extract concentration (Figure 4). These results were supported by Chieng et al. (2008) using *Piper sarmentosum* extract. The high mortality of termites at a concentration of 6% (98.18%) was inseparable from the role of the alkaloid, flavonoid, steroid, tannin and phenolic bioactive substances found in *M. micrantha* leaf extracts. Toxic materials were in direct proportion to the concentration.

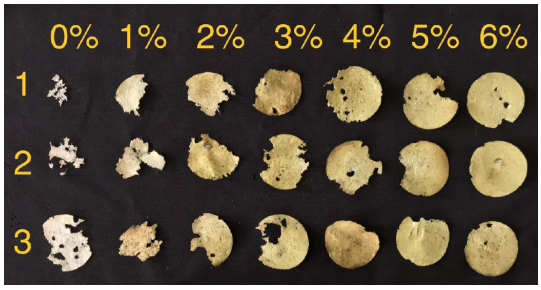

Figure 8. Filter paper condition at various concentrations after 21 days testing period

Furthermore, the high mortality of termites in the extract treatment indicated that it had an anti-termite effect. This was because the chemical compound, especially alkaloids, contents (Tab. 2) affected the eating activity of termites in the form of resistance in eating. Eventually, the termites run out of energy and die.

The weight loss value of filter paper due to *C. curvignathus* termite attack was inversely proportional to the termite mortality value. The low weight loss value of filter paper was followed by the high value of termite mortality. This was due to the reduced number of test termites, therefore, decreasing the physical damage condition or sample weight. *M. micrantha* leaf extract applied to the filter paper inhibited the termite's feeding rate.

Fungal bioassay

Anti-fungal activity (AFA)

Classification by Mori et al. (1997) showed that *M. micrantha* leaf extract has very strong anti-fungal activity (++++) with AFA values ranging from 78.36% to 100% (Table 3). Compared with the control, a concentration of 1% was able to inhibit the growth of *S. commune* wood decay fungus. It was suspected that *M. micrantha* leaves with high extractive content were more resistant to *S. commune* wood decay fungus. This opinion was supported by Lestari and Pari (1990) who stated that tree defence against wood decay fungi was largely dependent on the type and concentration of bioactive compounds in the extractive.

Furthermore, the test results showed that *M. micrantha* leaf extract affected the growth of *S. commune*. The extractive mechanism in inhibiting fungal growth was thought to be due to the presence of chemical compounds that interfered with / stopped the activity of fungal enzymes to break down carbohydrates into materials most easily absorbed for digestion and metabolism of fungi (Syafii et al., 1987; Jayasuriya et al., 2003; Mihara et al., 2005). The case of *M. micrantha* leaves in this study was that several bioactive compounds in the soluble methanol fraction affected the *S. commune* fungus by inhibiting the growth of their mycelium. Several studies suggest that phenolics and flavonoids inhibit fungal germination (Phongpaichit et al., 2004).

Inhibition of fungal growth

The results of this study indicated that an increase in leaf concentration was followed by an increase in inhibition of fungal growth. This proves that *M. micrantha* leaf extract has the potential to be used as a vegetable fungicide. Chemical compounds such as alkaloids, phenolics, flavonoids, steroids and tannins contained in *M. micrantha* leaves were thought to function as inhibitors of the *S. commune* wood decay fungus growth. This opinion was supported by Boulogne et al. (2012) which stated that phenolics, terpenoids and alkaloids are chemical substances with antifungal activity. Harborne (1987) added that tannins are phenolic compounds used as fungicides. The fungi growth on media with various concentrations after the incubation period is presented in Figure 9.

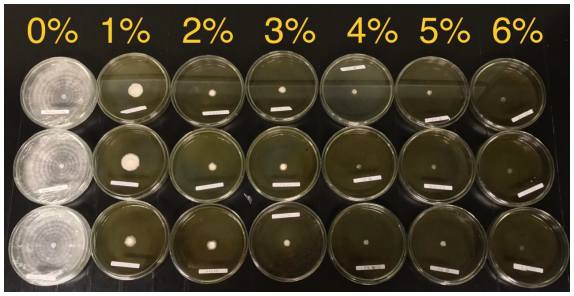

Figure 9. Growth of *S.commune* wood decay fungus after 7 days of incubation at various concentrations

This study concludes that *M. micrantha* leaf extract has a potential to be used as a natural anti-termite and anti-fungal biocide based on high termite mortalities and fungal inhibition rates. This result suggest that a higher concentration of *M. micrantha* leaf extract could give a better level of protection against termites and wood decay fungi that are less harmful to the environment and humans than recently available ones. Furthermore, the 4% extract was the optimum concentration for inhibiting the growth of wood decay fungi (very strong category AFA) and caused termite mortality by more than 80%. However, further study is needed to examine *M. micrantha* leaf extract using non-durable wood specimens to develop novel wood protecting biocides.

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