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Biological Control of Wood Destroying Organism Using Plant Extracts Collected from Mt. Merapi National Park, Indonesia

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Abstract

Due to the climate change and global warming, the biodiversity database has gained the attention of the government. In line with the Indonesian Biodiversity Strategy and Action Plan (IBSAP), we have collected plants with insecticidal activity based on the local wisdom. This program aimed to protect Indonesian biodiversity from deforestation along with the loss of the number of species. This research's goal was to evaluate termiticidal and antifungal properties from some plant extracts collected from the Turgo forest area, Mt. Merapi National park, Java. Three potential plants were evaluated. Based on specimen identification, the three plant samples were Kina (*Cinchona* sp.), Kamadoh (*Dendrocnide stimulans* (L.f.) Chew), and Keremi (*Homalanthus populneus* (Geiseler) Pax). The phytochemical test showed that Kina contained alkoloid, falvonoid, saponin, and tannin, whereas Kamadoh and Keremi contained saponin and tanin, respectively. Overall, all plant extracts have the termiticidal activities and able to inhibit wood-decay fungi with the inhibition percentage around 60% - 100%. Leaf extracts of Kina (*Cinchona* sp) and Keremi (*Homalanthus populneus* (Geiseler) showed the highest activity as wood-decay fungi inhibitor. The disclosure of the potential of bioinsecticides from some plants originating from Mount Merapi is very important before being lost due to deforestation and the Mount Merapi disaster. Thus, the potential bioinsecticide in these plants can increase its economic value as a substitute for synthetic insecticides that are friendly to the environment.

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INTRODUCTION

Termites are well known as notorious pest of wooden structures, it is estimated that the economic loss by termites are more than $400 million per year in Southeast Asia only (Yeap et al, 2011). Besides termites, wood-decay fungi also cause serious damage to buildings and other wood structures. In fact, chemical treatment using insecticide is an effective method to be used extensively in daily life for wood preservatives. Several insecticides that are effective including organochlorine, organophosphorus, carbamates, pyrethroids and others. Nevertheless, the constant use of chemical insecticides has often led to the disruption of natural biological control systems as well as undesirable effects on humans, mammals, and other non-target organism. Researchers are developing new products for the management of mosquitoes that are safer for the environment, biodegradable, low in cost, reliable, and functional for preventive and remedial management. Researchers are seeking for safer treatment that is reliable and have functional application on preventive and remedial control (Tarmadi et al, 2014).

Plants can be used as an alternative source to control termite and wood-decay fungi because they are a rich in bioactive chemicals. The specific chemical content and the dose is related to the anti-termite and antifungal properties. A study reported that the dosage of 4-methyl-anthraquinone in the crude extract of *T. gandis* has toxicity and feeding deterrent effect independently towards subterranean termite, *Coptotermes formosanus* and *Reticulitermes speratus* (Ismayati et al., 2016). Meanwhile, the antifungal properties is exhibited by Mimosa (*Acacia mollissima*), quebracho (*Schinopsis lorentzii*) and pine (*Pinus brutia*) that can inhibit the growth of white rot fungi (*Trametes versicolor* and *Pleurotus ostreatus*) and two two types of brown rot fungi (*Fomitopsis* palustris and *Gloeophyllum trabeum*), i.e: (Tascioglu et al, 2013). All of these marker active compounds can be developed on an industrial scale through a chemical synthetic process. For example, some insecticide agents have been developed from plant extracts such as rotenone, nicotine, pyrethrin, sabadilla, and ryanodine, those chemicals were isolated from *Derris elliptica, Nicotiana tabacum, Chrysanthemum cinerariifolium, Schoenocaulon officinale,* and *Ryania speciosa,* respectively (Ahmad et al., 2018).

In this present study, we conducted the ethnobotany survey and collected the potential plants from Mt. Merapi National park, Java as a preliminary screening to discover the prospective plant extracts that can be used as insecticides against termites and wood decay fungi. We collected the potential plants based on the information from local people. The plants collected had not yet been reported regarding their ability to control termite and wood-decay fungi based on a literature study. This study was a part from flagship program of the Indonesian Biodiversity Strategy and Action Plan, IBSAP. One of the goals of IBSAP was to record the biodiversity and its benefit in Indonesia. The plant extract collected from this program is expected to become a database of extractive that have bioinsecticide properties. Furthermore, those plant can be development as a commercial bioinsecticide that effective and friendly environment.

METHODS

Collection and Identification of Sample Plants

Collection of plant samples was carried out in the Turgo forest area, Mt. Merapi National Park, Java (Figure 1). The sample was collected based on the interview with the local community. Approximately 5 kg (dry weight) of potential plants that have insecticide activities were collected based on their local wisdom information. Identification of the plant materials was performed by the Laboratory of Botany, Research center for Biology, Indonesian Institute of Sciences (LIPI), where voucher specimens are deposited.

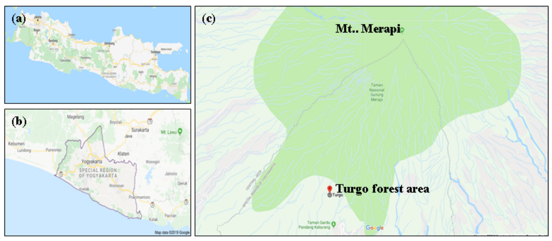

Figure 1. Sampling location in the Mt. Merapi national park, Java, Indonesia (Source: www.maps.google.com)

Preparation of Plant Extracts

Approximately 300 g of dried leaves and barks were powdered mechanically using a commercial blender and extracted with methanol (Merck, Darmstadt, Germany). Dried crude extracts were obtained using a rotary evaporator (RV 10 Digital, IKA Works GmbH & Co., Germany) at 40^0C. The phytochemical test was conducted for secondary metabolites, alkaloids, flavonoids, steroid, triterpenoid, saponin, and tannin.

Termiticidal efficacy of plant extracts

No-choice feeding test was conducted according to (Ohmura et al., 2000). A test container was made from a plastic petri dish (60 mm dia.) with a plaster bottom (3 mm high). Paper discs (13 mm dia., Whatmann International) were treated with 10 % (w/v) of each plant extracts, and the untreated paper discs were used for control. Those discs were dried for 12 hours in a vacuum desiccator. A treated paper disc was put into a test container, and then fifty workers and five soldiers of *C. curvignathus* obtained from a laboratory colony at the Research Center for Biomaterials, Indonesian Institute of sciences (LIPI) were fed on a treated paper disc. The number of live workers was recorded every two days for 12 days. The mass loss of paper discs was balanced in the end of the test period. The tests were performed in three times of repetition.

Preparation of wood-decay fungi isolates

This study used 9 types of wood-decay fungi (brown and white fungi). All the isolates were collections of Microbioloby laboratory, Research center for Biomaterial, LIPI and the result of isolation of the Batam Regional Botanical Garden (Lestari et al., 2018) (See Table 1). The isolates were inoculated and grown on PDA (Potato Dextrose Agar) media in a petri dish for 7 days.

Determination of antifungal activities

Determination of antifungal properties was carried out by growing wood-decay fungi on PDA media which had been added with plant extract. Decayed-wood fungi were grown on PDA, wherein about 2 ml extract with a concentration of 10,000 ppm dissolved in methanol was poured into 20 ml PDA media after being sterilized by autoclaving at 121 °C for 15 minutes. The media-extract mixture was poured into a Petri dish after solidifying the wood weathering fungus into it. Mushroom inoculum measuring 0.6 cm in diameter was placed right in the middle of the Petri dish (Mohareb et al., 2013). Then, it was incubated for 10 days and the diameter of mushroom growth was measured every 2 days. The test was replicated in three times. Diameter of colony growth and antifungal activity were determined using the following formula:

$$\text{Diameter of colony} = \frac{R1 + R2}{2}$$ (Elfirta et al., 2018)

Figure 2. Determination of diameter of rot fungi colony

$$\%\,(G) = \frac{A - B}{A} \times 100\%$$

A = diameter of growing isolates for control

B = diameter of growing isolates for treatment

G = inhibition percentage of isolates (%)

RESULTS AND DISCUSSION

The potential plants that have insecticidal activities from the Turgo forest area, Mt. Merapi national park have been collected based on information from the local community. Based on identification in LIPI, those plants are Kina (*Cinchona* sp.), Kamadoh (*Dendrocnide stimulans* (L.f.) Chew), and Keremi (*Homalanthus populneus* (Geiseler) Pax) (Table 2). In order to understand the insecticide activities from these plants, the chemical compounds such as: alkoloid, flavonoid,

Table 1. List of wood-decay fungi isolates

Species	Isolate code	Origin of
White rot		
Trametes versicolor	COR	Collection of RC of Biomaterial, LIPI
Schizophyllum commune	SC	Collection of RC of Biomaterial, LIPI
*Trametes*sp.	Gano	Collection of RC of Biomaterial, LIPI
Pycnoporus sanguineus	M3	Botanical Garden of Batam
Trametes ijubarskii	M4	Botanical Garden of Batam
Flavodon flavus	H2A	Botanical Garden of Batam
Phanerochaete chrysosporium	PC	Collection of RC of Biomaterial, LIPI
Brown rot		
Fomitopsis palustris	TP	Collection of RC of Biomaterial, LIPI
Antrodia wangii	M7	Botanical Garden of Batam

steroid, triterpenoid, saponin, and tannin were extracted and analyzed using phytochemical test, in which those compounds thought to have insecticidal effect (Ge et al., 2015; Ismayati et al., 2017; Meshram et al., 2019; Verma et al., 2009). As shown in Table 3, Kina contains alkoloid, falvonoid, saponin, and tannin, whereas Kamadoh and Keremi contain saponin and tanin, respectively. Saponin is commonly found in many parts of plants from roots to leaves (Dias et al., 2012).

Figure 3. Collecting and drying process of samples collected from Turgo forest area, Mt. Merapi national park, Jogyakarta.

Table 2. The identification result of some plants collected from Turgo forest area

Sample code	Local name	Part of sample	Species
B1	Kina	Leaf	*Cinchona* sp.
B2	Kina	Bark	*Cinchona* sp.
B3	Kamadoh	Leaf	*Dendrocnide stimulans* -
B4	Keremi	Leaf	*Homalanthus populneus* -

Tannins are defined as "water-soluble phenolic compounds having molecular weights between 500-3000 that has phenolic reactions and having special properties such as the ability to precipitate alkaloids, gelatin and other proteins" (Hagermann, 2002). Tannin is normally found in certain plant tissues like bark and wood. Tannin has been documented to be a natural wood preservative because of its ability to affect the insect growth and development by binding to the proteins, reduce nutrient absorption efficiency, and cause midgut lesions (Barbehenn & Constabel, 2011; Sharma et al., 2009). Tannins are also astringent (mouth puckering) bitter polyphenols and can act as feeding deterrents to many insect pests (War et al., 2012).

Termiticidal activity

The termiticidal effect of saponin has been documented by earlier studies in preliminary evaluation of anti-termite activity of *Prosopis juliflora* extract against *Macrotermes* sp. (Bezuneh et al., 2019), they reported that the presence of steroids, saponins, terpenoids, alkaloids and flavonoids in leaf extract of *P. juliflora* might be responsible for its insecticidal activity. In regards to alkaloid presence, earlier study showed that alkaloid such as Matrine, Oxymatrine, Antofine N-oxide, Nicotine, 5-Oxoproline, Methyl 5-oxo-2-pyrrolidinecarboxylate, 4-Morpholineethanol, 3-Amino-4-Hydroxybutanoic acid and 1H-1,2,4-Triazol-3-amine gave significant activities for repellence and mortality of termites (Ge et al., 2015; Meshram et al., 2019). On the other hand, the presence of flavonoid can also induce termiticidal effect. The common link among flavonoid compounds are the presence of two hydroxyl groups at C-5 and C-7 in A-rings that incite high antifeedant activity. Furthermore, the presence of a carbonyl group at C-4 in the pyran rings of the compounds is needed for the occurrence of high activity (Ismayati et al., 2018).

The phytochemical test (Table 3) shows the presence of alkaloid, flavonoid, saponin and tannin. Comparing the phytochemical test and termite mortality (Figure 4), it seems that tannin presence in plant may contributes to high mortality on termite (B4). Although the data showed such clear-cut indication, tannins as classes of polyphenol is too large of a group which made the identification of the chemical compounds responsible for termiticidal effect was difficult to pinpoint.

On the other hand, the combination of alkaloid and saponin (B1) can induce similar termiticidal effect just like tannin (B4). Saponin has been known to be termiticidal and alkaloid as termiticide agent is a newly discussed topic, with the latest research showed great termiticidal ef-

Table 3. The yield of extract and phytochemical test results of samples.

Sample code	Moisture content (%)	Yield (%)	Phytochemical test result					
			Alkaloid	Flavonoid	Steroid	Triterpenoid	Saponin	Tannin
B1	25.39	24.66	+	-	-	-	+	-
B2	12.59	14.30	+	+	-	-	+	+
B3	12.76	5.73	-	-	-	-	+	-
B4	13.33	6.92	-	-	-	-	-	+

Note: + = Available; - = Not available

fect (Antwi-Boasiako & Eshun, 2013; Ge et al., 2015; Meshram et al., 2019). Even then, since the ratio of alkaloid and saponin presence in B1 is unknown, the exact nature of chemical interaction still remains unknown.

In regard of this study, the finding that saponin-only (B3) can exert termiticidal effect with almost similar effect to B4 and B1 extract is very important. Through this finding, the chemical interaction on termiticidal effect in B1 can be deduced. Although the data suggest there is an increase of mortality rate when two class (alkaloid and saponin) are present (Figure 4, B1), the increase is negligible when t-test was conducted on point by point basis (not shown). In fact, past study showed that the presence of alkaloid and saponin in the same place is antagonistic in terms of antioxidants (DPPH method = alkaloid 63%; alkaloid and saponin 15%) (Milugo et al., 2013).

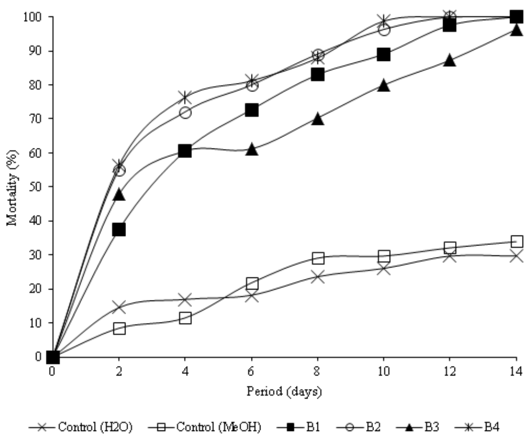

Figure 4. Percentage of the termite mortality observed for *C. curvignathus* in no-choice feeding test using sample extracts with controls (H_2O for water and MeOH for methanol) in 14 days

Another finding is how similar B2 and B4 actually progressed during the mortality test (Figure 4) while the chemical compounds among them are different. It is important to understand that tannin can be broken down into flavonoid. Since phytochemical test is only qualitative in nature, the further break down component have not been yet analyzed. This raises an interesting problem since the chemical compound in B4 was only tannin while B2 contained additional alkaloid, flavonoid, and saponin with each of them can be projected to yield almost similar mortality rate. The data suggested whether the tannin in B2 actually dominate the rest of compounds or there is a certain ration that yielded such result. This inference was made because of the mortality rate was almost parallel which means its general lethality for duration of the test was similar.

Comparing the phytochemical test against the weight loss, it was interesting that wood bait that was applied with extract containing alkaloid had lower weight loss in average although based on t-test is not statistically different compare to wood bait containing saponin and tannin-only. Although by comparing the weight loss with control resulted in a very significant result which showed the extract ability to be antifeedant for termite.

In the end, regarding the efficacy of plant extract B1-4, the presences of tannin, saponin, alkaloid, and flavonoid show significant termiticidal effect with 50% mortality achieved after 4 days of treatment. It is also important to note that, the extract of B4 (keremi) with tannin-only managed to achieve 100% mortality after 10 days.

Tabel 4. Percentage of termite mortality and weight loss in no-choice feeding test using filter paper discs treated by plant extracts.

Sample	Termite mortality (%)	Weight loss (%)
Control (H2O)	29.70 ± 2.08	21.78 ± 3.12
Control (MeOH)	33.94 ± 1.53	31.69 ± 3.95
B1	100.00 ± 0.00	1.51 ± 1.33
B2	100.00 ± 0.00	1.33 ± 1.42
B3	96.36 ± 3.46	2.96 ± 1.04
B4	100.00 ± 0.00	2.38 ± 1.71

Antifungal activity

In this study, the treatment of plant extracts from Mt. Merapi (Table 2) have a potential to inhibit the growth of white and brown rot fungi. Effect of four types of plant extracts on 7 types of white rot fungi (*T. versicolor* (COR), *S. commune* (SC), *Trametes* sp. (Gano), *P. sanguineus* (M3), *T. ijubarskii* (M4), *F. flavus* (H2A), *P. chrysosporium* (PC)) and 2 types of brown rot fungi (*F. palustris* (TP), *A. wangii* (M7)) was observed from the diameter of mushroom growth for 10 days. Antifungal activity was determined by comparing the growth of wood-decay fungi treated with the extracts with control.

As shown in Figure 5, the diameter of the growth of all fungal colonies in the control (without extract concentration) reached 9 cm on the 10^{th} day, except the diameter of *F. palustris* colony growth that reached 10 cm. Meanwhile, Kina bark and leaf, Keremi leaf and Kamadoh leaf extracts can cause the growth inhibition of all fungal colonies with colony diameters between 0 - 3.93 cm for 10 days of experiment.

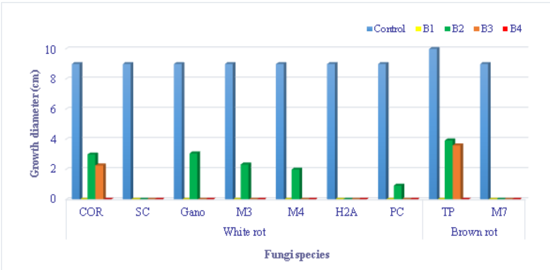

Figure 5. Growth diameter of wood rot fungi treated by plant extracts for 10 days

The comparison of all the plants extracts shows that Keremi and Kina leaf appear to be more toxic to the nine wood-decay fungi, where the fungus cannot grow until the 10th day of testing with a diameter of 0 cm colony growth. It is followed by Kamadoh leaf extract which can inhibit the growth of *S. commune, Trametes* sp., *P. sanguineus, T. ijubarskii, A. wangii, F. flavus*, and *P. chrysosporium* where all fungi did not grow (diameter 0 cm), but less effective against *T. versicolor* and *F. palustris* where fungi can still grow with diameters of 2.27 and 3.6 cm respectively. Meanwhile, the smallest inhibition of fungal growth is shown by Kina bark extract which could only inhibit the growth of *S. commune*, *A. wangii,* and *F. flavus* with a diameter of 0 cm colony growth, while six other fungi could still grow with a colony diameter of 0, 93 - 3.39 cm.

Based on the percentage of inhibitory activity against the growth of wood rot fungus, it was seen that the Kina leaf extract had the highest inhibitory activity against the growth of 9 types of fungus with an inhibition percentage of 100%, followed by Keremi leaf extract with inhibition percentage of 84.68% - 100%. Meanwhile, Kamadoh leaf extract can inhibit 100% growth of 7 types of fungi, but can only inhibit 72.80% growth of *T. versicolor* and 64% growth of *F. palustris*. The smallest inhibitory ability was shown by Kina bark extract which can only inhibit 100% growth of three types of wood-decay fungi, namely *S. commune, A. wangii, F. flavus*, while the growth of six other types of weathering fungi can only be inhibited by 60.67% - 89 , 63% (Table 5).

The growth inhibition of white and brown rot fungi in treatment groups was caused by plant extracts that were added to the growth media contain active compounds that are toxic to the growth of wood rot fungi. Kina (*Cinchona* sp.) Contains alkaloid compounds, one of which is quinine, quinidine, cinchonidine, and cinchonine which is anti-malaria, anti-parasitic (Ezekwesili et al., 2012; Kacprzak, 2013) and also has antibacterial activity especially against Gram-positive bacteria *Staphyllococcus aureus* (Kushwah et al., 2016). Meanwhile, Karei leaf extract (*H. populneus*) is commonly used as a traditional medicine especially as antipirurytic, fever and as worm medicine in livestock (Arbiastutie et al., 2017). In accordance with the previous literature, Mariani et al. (2014) have been confirmed the plants with antimicrobial activities from Indonesia urticacea (*Cypholophus lutescens, Dendrocnide stimulans, Dendrocnide microstigma, Debregeasia longifolia, Elatostema repens, Elatostema sinuatum, Elatostema parasiticum*)

Kina and Keremi leaf extracts have better antifungal activity against *T. versicolor* which is 92.78% and can inhibit growth of *F. palustris*

Table 5. Antifungal activity of plant extracts against wood rot fungi

Fungi	Antifungal activity (%)			
	B1	B2	B3	B4
White rot fungi				
Trametes versicolor (COR)	92.78 ± 0.15	61.58 ± 1.36	72.80 ± 0.52	92.78 ± 0.15
Schizophyllum commune (SC)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Trametes sp. (Gano)	100.00 ± 0.00	65.93 ± 0.74	100.00 ± 0.00	100.00 ± 0.00
Pycnoporus sanguineus (M3)	100.00 ± 0.00	74.07 ± 3.70	100.00 ± 0.00	100.00 ± 0.00
Trametes ijubarskii (M4)	100.00 ± 0.00	77.78 ± 0.00	100.00 ± 0.00	100 .00 ± 0.00
Flavodon flavus (H2A)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Phanerochaete chrysosporium (PC)	100.00 ± 0.00	89.63 ± 0.74	100.00 ± 0.00	100.00 ± 0.00
Brown rot fungi				
Fomitopsis palustris (TP)	100.00 ± 0.00	60.67 ± 1.76	64.00 ± 2.00	84.68 ± 0.71
Antrodia wangii (M7)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

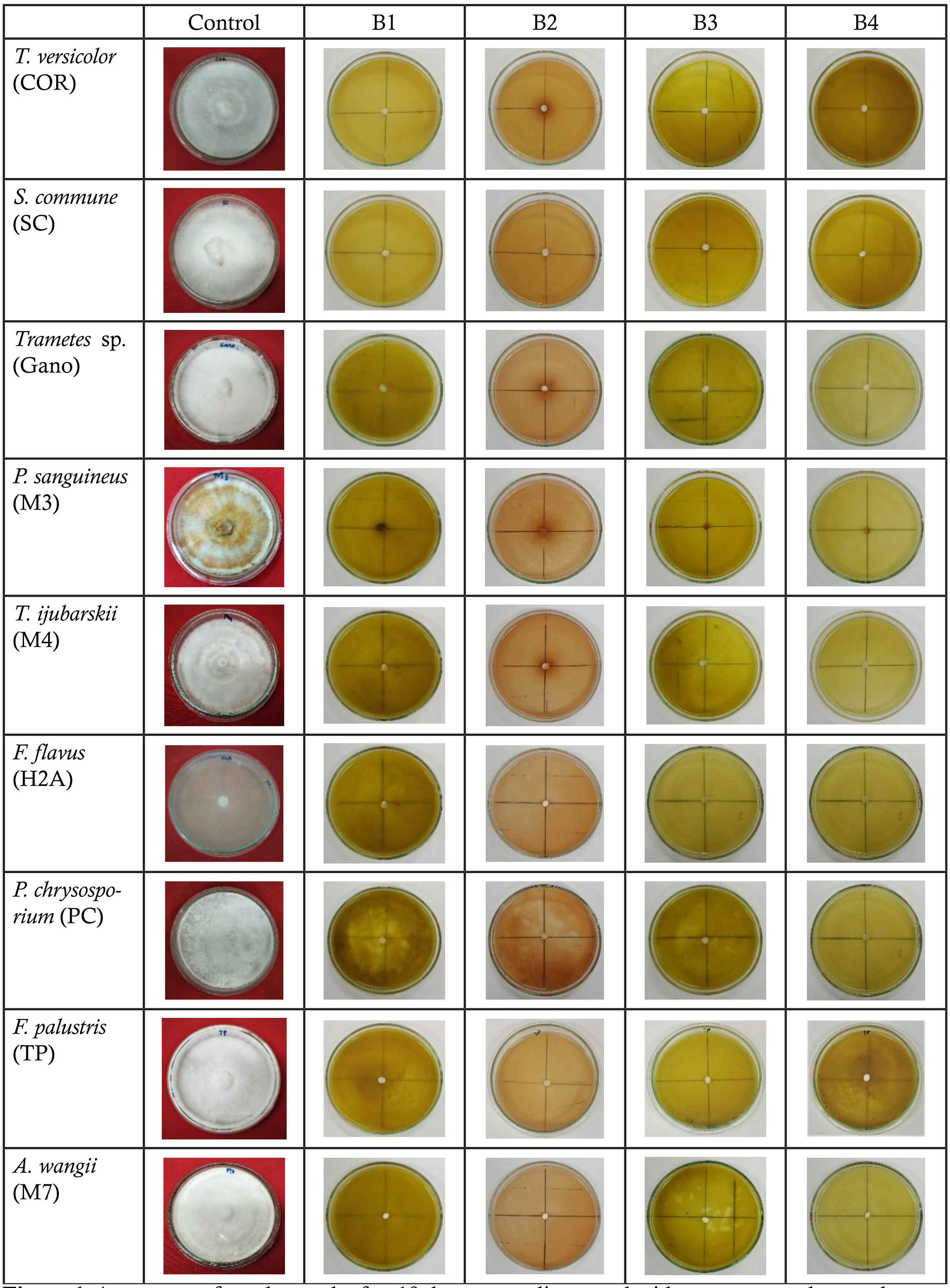

Figure 6. Appearance fungal growth after 10 days on media treated with treatment and control

by 100% and 84.68% respectively. This result is better when compared with the research of Celimene et al., (1999) which examined the ability of Pynosilvin compounds extracted from pine cones to white and brown rot fungi which were only able to inhibit *T. versicolor* growth by 11.1% and *Placental postia* by 11.5%. This result is also better than the ability of the Deoxylapachol compound which is one of the active compounds contained in Teak wood extract which can inhibit the growth of *T. versicolor* and *F. palustris* by 64-75% (Lukmandaru, 2013).

Based on these result, all plant extracts show the activity as biological control of wood-decay fungi. Thus, the knowledge of bioinsecticide activity from plant extract collected from Mt. Merapi can increase the utilization and economical values of those plants before the loss of species due to disaster or deforestation.

CONCLUSION

Three plant samples collected from the Turgo forest area, Mt. Merapi National Park, Java were Kina (*Cinchona* sp.), Kamadoh (*Dendrocnide stimulans* - and Keremi (*Homalanthus populneus* (Geiseler) Pax). All of the collected specimens showed a potential to be used as termiticide with 50% mortality achieved after 4 days and almost total mortality after 2 weeks. Meanwhile, as an inhibitor of wood-decay fungi, all the specimens showed a potency with percentage of inhibition about 60% - 100%. Leaves extracts of Kina and Keremi have the highest percentage of inhibitory power for all types of wood-decay fungi, while the lowest percentage of inhibitory power is showed by Kina bark extract.

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