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Natural preservative from the liquid smoke of ebony wood as anti-subterranean termites (*Coptotermes curvignathus* Holmgren)

Ariyanti[*1,3], Edy Budiarso[2], Agus Sulistyo Budi[2], Irawan Wijaya Kusuma[2]

[1]*Department of Forestry, Tadulako University, Central Sulawesi, Indonesia*

[2]*Departement of Forestry, Mulawarman University, East Kalimantan, Indonesia*

[3]*Post Graduate Program of Forestry Science, Mulawarman University, East Kalimantan, Indonesia*

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Abstract

Chemicals have been used as wood preservatives because they prevent the attack of termites and fungi. However, since these chemicals can harm the environment and humans, their use is limited. This study introduces a natural preservative that is not harmful to humans and also environmentally friendly. This study aims to identify and produce lignocelluloses bio-oil biomass and also evaluate lignocellulosic bio-oil biomass from Ebony wood smoke as a wood preservative to prevent subterranean termite attack (*Coptotermes curvignathus* Holmgren) on Red Jabon wood with observed parameters is wood retention, wood weight loss, and termite mortality. This study uses a complete randomized design of 3 factors and 5 replications. There are 3 levels of treatment: different concentrations of preservatives (ebony lye + BAE), pyrolysis temperature and preservative duration. The treatment of the concentration of preservatives consists of 5 compositions, namely: control with solvent from liquid smoke of ebony wood = K, ebony wood smoke + BAE 2% = E1, ebony wood smoke + BAE 3% = E2, ebony wood smoke + BAE 4 % = E3, ebony liquid smoke + BAE 5% = E4. The temperature of pyrolysis is 400°C and the immersion time is 24 hours. The result shows that the compounds contained in ebony liquid smoke consist of Phenol, 2,6-dimethoxy-4- (2-propenyl) (CAS) 4-Allyl-2,6-dimethoxyphenol; Hexanoic acid, 1-methylethyl ester (CAS) isopropyl hexanoate; Pentanoic acid, 4-oxo, ethyl ester (CAS) Ethyl levulinate; Acetaldehyde (CAS) Ethanal; 4-Methoxy-3 - (methoxymethyl) phenol. Wood retention on wood samples of red Jabon from 55.77-126.604 g/cm3, decreased weight of wood is 7.40 - 8.92% and termite mortality from 95.5% - 100%.The best solution to prevent of wood from attact of termite and fungi without harmful to humans and also environment by using biochemical extract of lignocellulosic bio-oil biomass from Ebony Wood.

***Corresponding Author:** Ariyanti ✉ ariyanti.bohari@gmail.com

Introduction

Chemicals have been used as wood preservatives because they prevent the attack of termites and fungi. However, since these chemicals can harm the environment and humans, their use is limited (Kartal *et al.*, 2004; Verma *et al*, 2009). This study introduces a natural preservative that is not harmful to humans and also environmentally friendly.

This study explores natural preservatives from Ebony Wood, with Lignocellulosic bio-oil biomass that is also called pyroligneous acid or wood vinegar, which is a product with a potential source of high-value chemicals by processing high-temperature carbonization of lignocellulosic bio-oil biomass without oxygen (Lee *et al.*, 2011; Oramahi and Yoshimura, 2013). Due to its complex structure and chemical composition, lignocellulosic bio-oil biomass has the potential to be a source of activity that anti-microbial, anti-fungal and termite (Yatagai *et al.*, 2002; Kartal *et al.*, 2004; Hwang *et al*, 2005; Jung, 2007; Mazela, 2007;). Lignocellulosic bio-oil biomass is derived from a variety of woods that have been used as natural wood preservatives against the attack of biological organisms (Kartal *et al.*, 2004; Lee *et al.*, 2011; Sunarta *et al.* 2011; Temiz *et al.*, 2013).

Oramahi and Yoshimura (2013) have demonstrated that lignocellulosic bio-oil biomass obtained from Laban Wood shows a thermicidal activity against *Reticulitermes speratus* and *Coptotermes formosanus* in vitro. Termicide activity is evaluated by 'choice' and 'no choice' tests. These tests can show that lignocellulosic bio-oil biomass act as an anti-fungal and anti-termite agent. Lignocellulosic bio-oil biomass derived from Giant Cane (*Arundo donax* L.) is effective as wood preservatives (Temiz *et al.*, 2013). Furthermore, Sunarta *et al.*, 2011, mention that bio-oil from palm fruits also has the potential as wood preservatives. They claim that bio-oil indicates termicidal activity against the attack to *Coptotermes* spp and inhibits the growth of blue fungus stains on Pine Wood (*Pinus merkusii*) and Sengon Wood (*Paraserianthes falcataria*).

The purpose of this study is to identify and produce lignocellulosic bio-oil biomass and also evaluate lignocellulosic bio-oil biomass as a wood preservative to prevent subterranean termites attack (*Coptotermes curvignathus* Holmgren) on Red Jabon Wood.

Material and methods

The research is conducted at the Bogor Forest Products Research and Development Center of Laboratory from November to December 2016. The materials used are: Red Jabon Wood (*Anthocepalus cadamba* Miq), which is 10 years old and has a diameter of 147 cm and 12 m of height from Siweri Village, Balaesang Sub-District, Donggala, to be preserved; A bundle of Ebony Wood from sawmill and Ebony Wood furniture for natural preservatives (liquid smoke) that is used as a solvent. The wood preservative chemicals used are BAE (Boric Acid Equivalent) in the form of borax mixture ($Na_2B_4O_7.10H2O$) and borate acid (H_3BO_3) with different solvent concentrations, oil paint that is used to cover the ends of wood, sand and Subterranean Termites (*Coptotermes curvignathus* Holmgren) are used to test a sample of Red Jabon Wood that has been preserved. The tools used are: chain saw, caliper, planner machine, digital scales, meter, preservative, brush, stirrer, pipette, fine cloth, measuring cup, test bottle, calculator, stopwatch, electric oven, desiccator, GC-MS and writing supplies. Tests on Subterranean Termites conducted on 30 wood samples of 2.5 cm × 2.5 cm × 0.5 cm. As much as 10 samples used for the control, 5 samples without treatment and 5 samples of Ebony Wood liquid smoke treatment. After that, the wood samples are dried for 2 weeks then weighed. After that the samples are put in the oven until the weight is stable. Then, preservative solution is prepared by mixing liquid smoke as a solvent with 4 different concentrations of BAE that consist of 2% concentration (E1), 3% concentration (E2), 4% concentration (E3) and 5% concentration (E4).

Collection and identification of subterranean termites

The termites used are taken from a subterranean termite colony in Cikampek (Plot of Wood Experiment Against Termites) Research and Development Center of Forest Products in Cikampek. The termites were identified using a microscope using a Termite Identification Book (Tho, 2000). Identification includes the size of termites' heads, mandibles, and bodies.

Identification and pyrolysis lignoselulosa biomass bio-oil

Lignosellulosa biomas bio-oil comes from Ebony Wood (*Diospyros celebica* Bakh) and carbonization process under the temperature between 400ºC-450ºC. Lignosellulosa biomas bio-oil is analyzed with Gas Chromatography-Mass Spectrometry (Mun and Chang, 2010; Oramahi and Diba, 2013) with Shimadzu model QP-2010 (Shimadzu Manufacturing Co. Ltd, Kyoto, Japan). The tool is owned by Bogor Forest Products Research and Development Center.

Chemical preservatives

Preservative used is BAE (*Boric Acid Equivalent*), a mixture of borax ($Na_2B_4O_7.10H_2O$) and Boric Acid (H_3BO_3) with different solution concentrations, and also the liquid smoke of Ebony Wood as a solvent. The concentration of BAE preservative solution used is 2%, 3%, 4% and 5%. The preservatives used have permethrin active ingredients of 384.96 grams/l (packaging labels) with a total of solution weight is 1000 g/l.

Wood preservation

The wood used to test the attack of Subterranean Termite is Red Jabon Wood. Prior to the process of preservation by cold soaking method, the sample wood is expected dry until the weight is constant (B1) and then weighed.

The samples are placed on a measuring cup and given a ballast (stone). Then, the measuring cup is filled with a preservative solution with a certain concentration. After that, the measuring cup is allowed to stand for 24 hours. The immersion process of the preservative for 24 hours can be seen in Figure 1.

This preservation process uses five treatments that are: control with solvent from Ebony Wood wooden liquid acid (K), combination of Ebony Wood smoke liquid + BAE 2% (E1). Combination of Ebony Wood smoke + BAE 3% (E2), combination of Eboni smoke liquid + BAE 4% (E3), combination of Ebony Wood smoke liquid + BAE 5% (E4). After this preservation process, the sample is weighed (B2). The retention value of Red Jabon Wood was measured using Diba *et. al* (2009) method as follows:

$$R\ (\%) = \quad X$$

Where:

R: Retention (g/cm^3)

B_1: sample weight before preserved (g)

B_2: sample weight after being fed (g)

V: the volume of preserved wood (cm^3)

C: concentration of preservative solution (%)

Sample wood feeding

The wood samples that have been preserved, treated, and have a constant weight (W1) are tested by placing the subterranean termites on the testing bottles that have been mixed with the soil. Termites used in this study were 200 Subterranean Termites (*Coptotermes curvignathus* Holmgren) consisting of 90% termite workers and 10% of soldier termites that were being laid for four weeks in each test bottle containing sand. At the end of the test, the wood sample is weighted and termites mortality is written in percentage. The value of wood weight reduction becomes the standard of wood preservation classification based on SNI standard (Anonymous, 2014). The samples placed in a cool and dark room as viewed in Fig. 2.

Every week, the activity of Subterranean Termites in the test bottles is observed without disrupting their activity. After four weeks, wood samples were dismantled, cleaned and the number of living termites counted to determine their mortality level.

Termite mortality (%) was calculated by the following equation:

TM = X 100 %

Where:

TM: Termites Mortality (%)

D: The number of dead Termites

200: The number of Termites in the beginning of the test

After wood samples are fed to the termites for four weeks, the wood samples were cleaned and put into the oven at 60 ± 2°C for 48 hours, then weighed to obtain the final weight (W2). Furthermore, the weight of sample wood is calculated.

The degree of damage indicates the intensity of the termite attack on the sample wood, calculated as the percentage of sample wood weight loss after feeding to the weight loss of sample wood before feeding with the following equation (Oramahi *et al.* 2014):

$$WL (\%) = (W_1 - W_2) / W_1 X 100$$

Where:

WL: Weight loss (%)

W_1: Weight of wood sample before exposure to termite

W_2: Weight of wood sample after exposure to termite.

Statistical analysis

This research uses a completely randomized design with 3 factors and 5 replicates consist of the concentration factor of preservatives (2%, 3%, 4%, 5%), pyrolysis temperature (400°C) and soaking time (24 hours).

Diversity test of ANOVA bi-directional was done to determine the effect of different real factors. Furthermore, further analysis using Tukey HSD (Honestly Significant Difference) was conducted to see the difference in the mean value of each treatment. All data were analyzed using Statistical Product and Service Solution software (SPSS 16.0).

Result and discussion

Lignocellulosic bio-oil biomass component of Ebony Wood

The chromatogram GC-MS compounds contained in lignocellulosic bio-oil biomass on Ebony Wood with 400°C pyrolysis temperature have 40 chemical components as shown in Fig. 3. The results of GC-MS analysis show that the amount of the relative percentages of the compounds identified by GC-MS in bio-oil Lignocellulose biomass pyrolisis in temperature 400°C summed up in Table 1.

Table 1. Identification of GC-MS Total Relative Percentages of Compounds in Lignocellulosic bio-oil biomass of Ebony Wood with Pyrolysis Temperature 400°C.

No.	R. Time	Lignocellulosic bio-oil biomass	Concentration (%)
1	3.645	Formamide (CAS) Methanamide	3.49
2	3.792	Acetaldehyde (CAS) Ethanal	4.21
3	4.125	2,3-Butanedione (CAS) Diacetyl	3.41
4	13.545	Phenol, 2-methoxy-(CAS) Guaiacol	3.31
5	14.886	1-Octene (CAS) Caprylene	2.42
6	15.275	2,3-Dihydro-benzofuran	2.40
7	16.023	Ethanone, 1-(2-hydroxy-5-methylphenyl (CAS) 1-Hydroxy-2-acetyl-4-methyl	3.01
8	16.369	Phenol, 2,6-dimethoxy-(CAS) 2,6-Dimethoxyphenol	3.51
9	17.207	4-Methoxy-3-(methoxymethyl)phenol	4.21
10	18.342	Hexanoic acid, 1-methylethyl ester (CAS) isopropyl hexanoate	12.86
11	18.477	Phenol,2,6-dimethoxy-4-(2-propenyl) (CAS) 4-Allyl-2,6-dimethoxyphenol	16.42
12	19.137	Benzaldehyde, 4-hydroxy-3,5-dimethoxy (CAS) Syringaldehyde	2.47
13	19.289	Phenol,2,6-dimethoxy-4-(2 propenyl) (CAS) 4-Allyl-2,6-dimethoxyphenol	4.59
14	20.258	1H-Indene-4-carboxylic acid, 2,3-dihydro-1,1-dimethyl-,ethyl ester (CAS) ET	3.64
15	20.692	Benzene, 1,1'- (1,3,5-hexatriene-1,6diyl)bis- (CAS) Diphenylhexatriene	4.04

Overall, the main component of lignocellulosic biomass bio-oil Ebony Wood wood with Formamide (CAS) Methanamide ; Acetaldehyde (CAS) Ethanal; 2,3-Butanedione (CAS) Diacety ;, Phenol, 2-methoxy- (CAS) Guaiacol; 1-Octene (CAS) Caprylene; 2,3-Dihydro-benzofuran; Ethanone, 1- (2-hydroxy-5-methylphenyl (CAS) 1-Hydroxy-2-acetyl-4-methyl ;; 4-Methoxy-3- (methoxymethyl) phenol; Hexanoic acid; 1-methylethyl ester (CAS) isopropyl Hexanoate; Pentanoic acid, 4-oxo, ethyl ester (CAS) Ethyl levulinate; Phenol; 2,6-dimethoxy-4- (2-propenyl) (CAS) 4-Allyl-2,6-dimethoxyphenol; Benzaldehyde, 4-hydroxy -3,5-dimethoxy (CAS) Syringaldehyde, Phenol, 2,6-dimethoxy-4- (2 propenyl (CAS) 4-Allyl-2,6-dimethoxyphenol; 1H-Indene-4-carboxylic acid, 2,3- Dihydro-1,1-dimethyl-, ethyl ester (CAS) ET; Benzene, 1,1'- (1,3,5-hexatriene-1,6diyl) bis- (CAS) Diphenylhexatriene.

Table 2. The Mean Value of Red Jabon wood's retention at different concentrations and temperature pyrolisis of Lignosellulosa bio-oil Biomass Ebony Wood.

Carbonization Temperature	Concentration (%)	Retention (g/cm^3)*
400°C	2	55.77 a
	3	73.846 b
	4	95.128 c
	5	126.604 d

*Means (n = 5)±SD. Number followed by different letter (a-d) are significantly.

Different at the level of $P < 0.05$ according to Tukey's test.

Table 3. The Mean Value of Sample Wood Weight Loss Without Treatment.

Sample No	Initial weight (g)	Final weight (g)	Wood Weight loss (%)
1	1,60	1,40	12,54
2	1,89	1,64	13,26
3	1,80	1,56	12,90
4	1,94	1,68	13,29
5	1,62	1,41	12,80
Mean		12,96	

This result is in line with the research of Temiz *et al.* (2013), which reports the liquid smoke of Giant canes (*Arundo donax* L.) has high thermicidal activity against *Reticulitermes plavipes*. Compounds such lignocellulosic components are acid, ketone, furan, benzen, phenol, sugar, guaiakol and multifunnosa compounds. Yatagai *et al.* (2002) also reported that acetic acid has the potential as a wood preservative to prevent dry wooden termite attack and blue stain fungus attack.

Table 4. The Mean Value of Sample Wood Weight Loss with Ebony Wood Liquid Smoke.

Sample No	Initial Weight (g)	Final Weight (g)	Wood Weight loss (%)
1	1,88	1,69	9,99
2	1,84	1,68	8,79
3	2,05	1,86	9,09
4	1,94	1,75	9,72
5	1,89	1,71	9,37
Mean		9,39	

Wood Preservation

Wood Retention: The mean value of Red Jabon wood's retention at different concentrations and temperature pyrolisis of Lignosellulosa bio-oil Biomass Ebony Wood is presented in Table 2.

Statistically, there is a significant difference in the retention of Red Jabon wood samples. The higher the concentration, the higher the retention value. In general, the addition of a preservative concentration even with the same length of immersion will result in greater retention values. This is consistent with the assertion that high concentrations will be faster and more entering into the wood than lower concentrations. In this study, the average retention value of Jabon Merah wood ranged from 55.77 to 126.604 g/cm3. The highest wood retention at 5% concentration is 126,604 g / cm3 and the lowest wood retention at 2% concentration is 55,77 g/cm3.

Fig. 1. Immersion of Preservatives For 24 Hours In Wood Samples.

Fig. 2. Testing Wood Samples on Subterranean termites Attack for Four Weeks.

Wood Weight Loss: A four-week test of controlled wood samples clearly detected a termite attack on the wood surface. The value of weight reduction of control log sample can be seen in Table 3.

The mean value of weight loss of controlled samples is 12.96%. This shows that the classification of wood resistance based on the percentage of weight loss due to termite attack of 12.96% classifies resistant level IV, which shows inability to withstand.

This result means that Red Jabon wood is a wood that unresistant to subterranean termites attack.

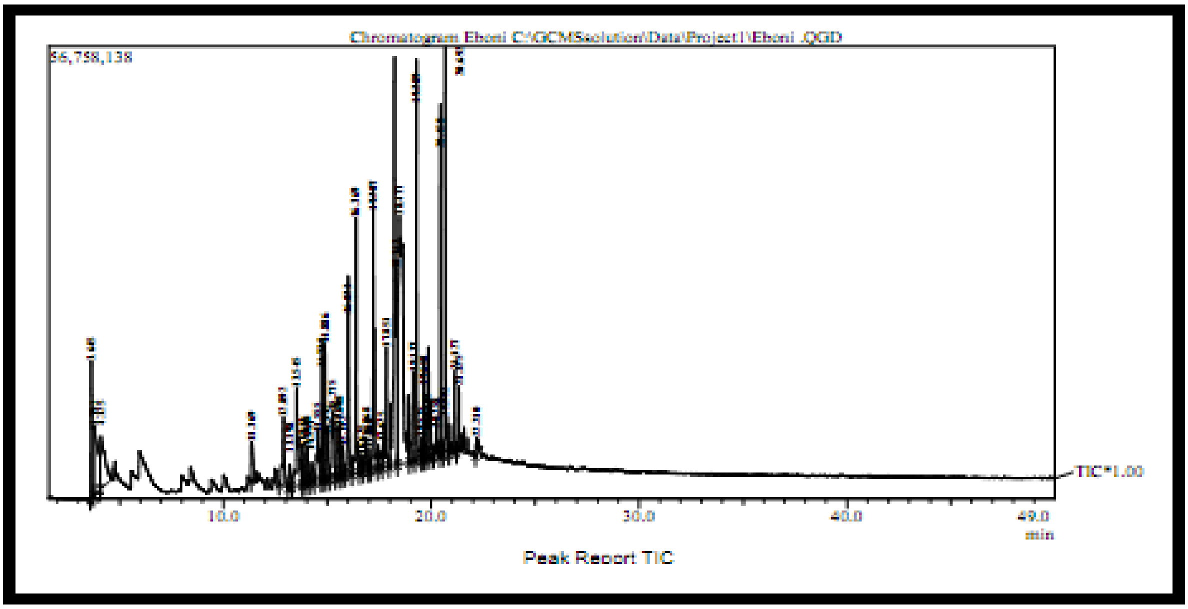

Fig. 3. The chromatogram GC-MS compounds contained in lignocellulosic bio-oil biomass on Ebony Wood with 400°C.

A four-week test of controlled wood samples using Ebony Wood liquid smoke shows that Subterranean Termite attacks on wood surfaces is existed, only fewer attacks than controlled wood samples. The value of weight loss of wood control sample by using Ebony Wood smoke liquid can be seen in Table 4.

The mean value of weight loss of wood sample treated with Ebony Wood smoke liquid is 9,39 %. This shows the classification of wood resistance based on the percentage of heavy degradation caused by Subterranean Termites attack, then 9.39% is classified in resistant level III or medium level. This means that the Red Jabon wood level that has been preserved by using Ebony Wood liquid smoke increased to class III.

During a four-week test on a wood sample with a combination of Ebony Wood liquid smoke and BAE chemical preservatives at different concentrations; E1 = 2%, E2 = 3%, E3 = 4% and E4 = 5%, Subterranean Termite attack on the surface of the sample is almost invisible, but the the weight loss of wood is decreased as the concentration of preservatives is increased. For more details, the mean value of the weight loss of wood samples with the combination of Ebony Wood liquid smoke and chemical preservatives of BAE at concentrations of 2%, 3%, 4% and 5% can be seen in Fig. 4.

It is seen from Figure 4 that the higher the concentration of preservatives, the lower the weight, and the lower the value. In this study, the lowest value at a concentration of 5% (E4) of 7.40%, the highest at 2% concentration (E1) of 8.92%. Therefore, the weight loss of wood is ranged from 7,40 - 8,2%.

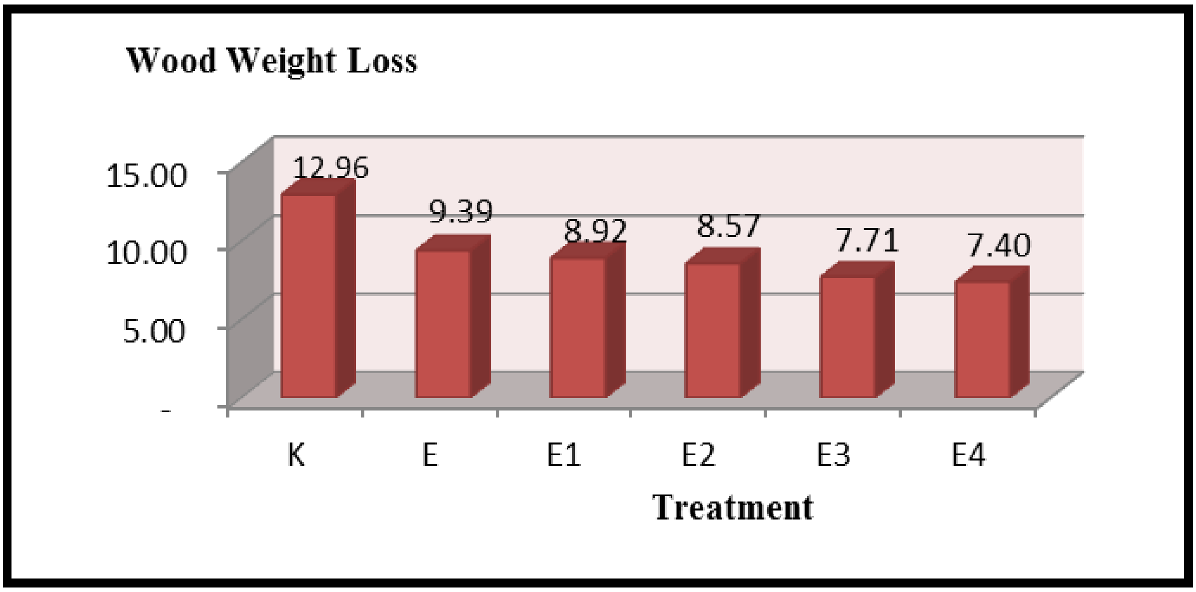

Fig. 4. The Mean Value of Reduced Weight of Red Jabon Wood from Subterranean Termite Attack at Different Level of Concentrations .

In line with Oramahi's research *et al.* (2014) reported that there were significant differences in the weight of Acacia wood that was preserved using liquid smoke (biomass bio-oil lignocellulose) from Laban wood ranging from 5.65 to 10.89%. Diba *et al.* (2009) demonstrates that the thermicidal activity of liquid smoke (biomass bio-oil lignocellulose) from wood waste with an average weight loss ranges from 6-20%.

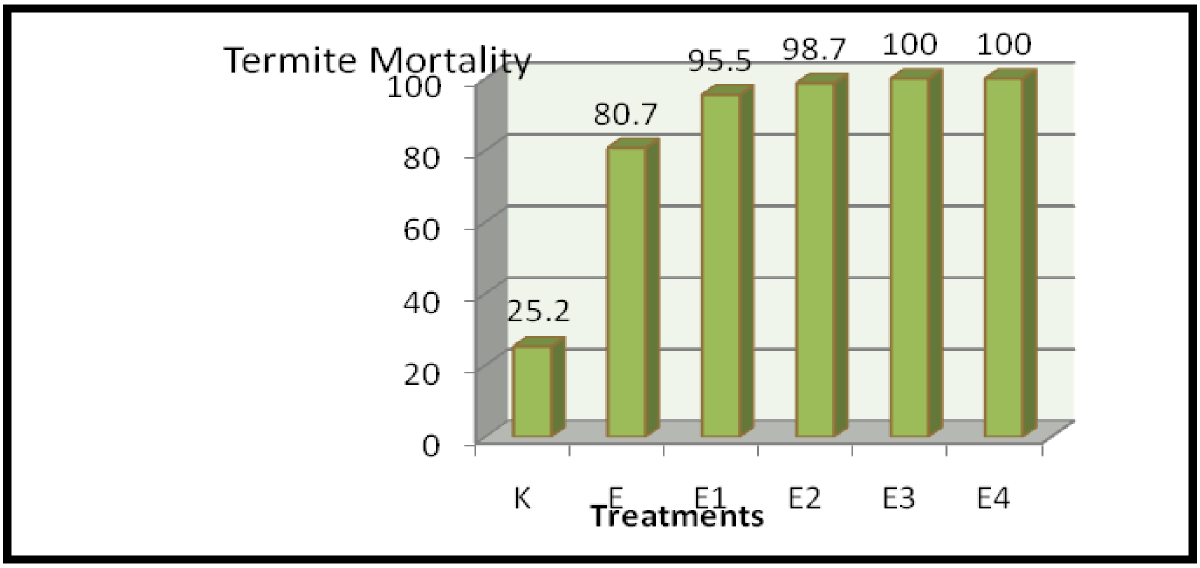

Fig. 5. The Mean Average Value of Subterranean Termite Mortality In Red Jabon sample at Different Level of Concentrations.

This study has similar results to previous studies, although liquid smoke comes from different wood materials. Chemical components present in liquid smoke (biomass bio-oil lignocellulose) may be responsible for preventing termite attack on wood.

Termites Mortality:

A four-week test of Subterranean Termite attack on wood samples of various treatments can be seen in Fig. 5 below.

Statistically, the result shows that there are significant differences in termite mortality in various treatments. The higher the concentration of preservatives, the higher the percentage of termite mortality. This shows that in combination of Ebony Wood liquid smoke with BAE at 4% concentration has the ability to destroy termites.

Conclusion

Biochemical extract of lignocellulosic bio-oil biomass from Ebony Wood is effective as a wood preservative against *Coptotermes curvignathus* Holmgren. GC-MS analysis of lignocellulosic biomass of lignocellulose from Ebony wood shows that the predominant component of lignoselusa biomass bio-oil was Phenol 2,6-dimethoxy-4- (2-propenyl) (CAS) 4-Allyl-2,6-dimethoxyphenol; Hexanoic acid, 1-methylethyl ester (CAS) isopropyl hexanoate; Pentanoic acid, 4-oxo, ethyl ester (CAS) Ethyl levulinate; Acetaldehyde (CAS) Ethanal; 4-Methoxy-3- (methoxymethyl) phenol.

The highest wood retention value at 5% concentration was 126,604 g/cm3 and the lowest wood retention at 2% concentration was 55,77 g / cm3. To reduce the weight of wood the lowest value at the concentration of 5% (E4) is 7.40% and the highest at 2% concentration (E1) is 8.92%. For the lowest termite mortality rate at concentration 2% (E1) = 95,5% and highest value at concentration 4% and 5% (E3 and E4) = 100%.

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