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THE FIFTH INTERNATIONAL SYMPOSIUM
INDONESIAN WOOD RESEARCH SOCIETY (IWoRS)

UTILIZATION OF RENEWABLE NATURAL RESOURCES
Towards Welfare and Environmental Sustainability

Balikpapan, East Kalimantan, INDONESIA
November 7 - 9, 2013

Organized by :

UTILIZATION OF RENEWABLE NATURAL RESOURCES TOWARDS WELFARE AND ENVIRONMENTAL SUSTAINABILITY

PROCEEDINGS OF THE FIFTH INTERNATIONAL SYMPOSIUM OF INDONESIAN WOOD RESEARCH SOCIETY (IWoRS)

November 7-9, 2013
Novotel Hotel, Balikpapan, East Kalimantan
INDONESIA

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PROVINCIAL GOVERNMENT OF EAST KALIMANTAN
GOVERNMENT OF BALIKPAPAN MUNICIPALITY

BALIKPAPAN, EAST KALIMANTAN, INDONESIA
MEI, 2015

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PREFACE

Firstly, we would like to express our highest gratitude to Allah SWT for everything given us by which the Proceeding of the Fifth International Symposium of Indonesian Wood Research Society (IWoRS) held in Novotel Hotel, Balikpapan, East Kalimantan INDONESIA on November 7-9, 2013 could be completed and published. Secondly, we would like to apologize deeply for delayed publishing these proceedings makes all of you waiting for it, to be uncomfortable.

The theme of the symposium is "Utilization of Renewable Natural Resources towards Welfare and Environmental Sustainability". The theme is suitable and acceptable for research discussion on the symposium in view of the abundance of natural resources in Indonesia implies the needs for wise and sustainable utilization toward people welfare. On the other side, the utilization concepts have to consider the nature carrying capacity and environmental sustainability. Regarding the renewable resources in the forestry, agriculture, crop estate and other fields, their sustainable utilization may be established by ensuring the natural availability of the resources. Many efforts have been done including in technical and policy aspects. However, strong needs for better efforts are still remained. Therefore utilization of wood and non-wood forest products becomes in urgent. Therefore, the objective of the symposium is s a media and forum for sharing knowledge and experience for wood and forest-related researchers in Indonesia and also the researcher from any countries in the world.

In this opportunity, we would also like to extend our sincere gratitude and appreciation to everyone and all parties for their generous support, and for collaboration to the Board of IWoRS period 2013-2015 Coordination of Private University in Kalimantan (KOPERTIS) Region XI, Association of Indonesia Private University (APTISI) Region XI-B, the Provincial Government of East Kalimantan, and Government of Balikpapan Municipality, and also thanks to others sponsors like PT. Kaltim Prima Coal, KONI of East Kalimantan, BKSDA of East Kalimantan, and PT. Martha Tilaar Group.

In addition, we would like to have critical suggestions and to apologize for the delays and any wrong less related to the proceedings. We do hope it can give much usefulness for any readers.

Balikpapan, Mei 2015

Editors

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POSTER PRESENTATION

INTRODUCTION OF NATURAL PRODUCTS CHEMISTRY OBTAINING FROM COOPERATIVE RESEARCHES USING INDONESIAN PLANTS

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Substituted for fossil resources the inflection of the natural energies such as solar light and force velocity has been developed for reducing carbon dioxide positively. Especially the beneficial use of plant resources biomass attracts attention. Before many convenient materials have been synthesized from the fossil resources by the techniques of synthetic chemistry with a rapid progress of technology we have obtained the thing which is necessary for life from the plant materials. Our life becomes convenient for the sake of mass reproducible cheep fossil resources, on the other hand excess carbon dioxide emission introduces the global heating and environmental pollution. To improve such situation an application of plant resources are nowadays entering the stage of attention again.

Tropical woody species have long been viewed as important sources of natural remedies in traditional medicine. They produce a diverse range of secondary metabolites such as flavonoids, terpenoids, and tannins in general are considered to have a variety of biological roles including as plant chemical defenses against pathogens and herbivores (from bacteria and fungi to insects and mammals). Secondary metabolites derived from plant are reported to demonstrate potentially significant pharmaceutical activity such as antiviral, antimicrobial, antioxidant and enzyme inhibiting. Therefore, investigating extractives isolated from tropical woody species offers a valuable opportunity for the utilization of forest products.

We have searched several kinds of bioactivities relating to beauty and health science and identified the effective compounds for a decade by using Indonesian woody plants and medicinal plants extractives. Recently we have obtained the extremely interesting results of bioactivities such as anti-carries, anti-acne, anti-inflammation, inhibitory activities of melanin biosynthesis so on through our cooperative research of Mulawarman university and Bogor Agricultural University with United Graduate School of Agriculture of Gifu University (UGSAS-GU).

In order to discover novel compounds indicating bioactivity from the biodiversity with protecting the Convention on Biological Diversity, we need to keep going the cooperative research ensuring a mutually beneficial result for both of Indonesian research groups and UGSAS-GU.

MOLECULAR TARGET OF TRITERPENOID WITH ANTICANCER ACTIVITY ISOLATED FROM MEDICINAL MUSHROOM, *Ganoderma lingzhi*

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Ganorderma lingzhi, known as “ling zhi” in China and “reishi” in Japan, is a wood-rotting fungus generally found growing on tree stumps. The anticancer activities of *G. lingzhi* include inhibition of tumor growth, angiogenesis and metastasis, and immune enhancement. Among these, the cytotoxic effects of *Ganoderma* triterpenoids and the immunoregulatory activities of *Ganoderma* polysaccharides have been of particular interest. Over one hundred oxygenated triterpenoids have been isolated from *G. lingzhi*. These compounds display a wide range of biological activities resulting in cytotoxicity and antitumor activity and the inhibition of histamine release angiotensin converting enzyme release, and cholesterol synthesis.

While screening mushrooms, we discovered that ethanol extracts of *G. lingzhi* showed the strongest 5α-reductase inhibitory activity among 19 species of mushrooms. Furthermore, treatment with the fruit body of *G. lingzhi* itself, or its ethanol extracts, significantly inhibited testosterone induced growth of the ventral prostate in rats. Our group previously isolated a series of triterpenoids from *G. lingzhi*. These compounds suppressed the proliferation of androgen-dependent and androgen-independent prostate cancer cell lines and estrogen-dependent MCF-7 cells, and inhibited osteoclastic differentiation. Among these triterpenoids, we found that only ganoderic acid DM (**1**, Fig. 1) had multiple functions and inhibited proliferation of prostate cancer cells and differentiation of osteoclasts. Although **1** affects different signaling pathways in different cell lines and has multiple functions, we have identified its target proteins, which explain and clarify the universal mechanism of its medicinal efficacy.

Here we show the important clues about the mechanisms of multi-medicinal action of *Ganoderma* triterpenoids, particularly the anticancer activity of ganoderic acid DM. We examined structure–activity relationships between **1** and its analogs to identify the functional groups required for inhibiting cell proliferation in a prostate cancer cell line, PC-3 cells. We found that the carbonyl group at C-3 was essential for cytotoxic effects of **1** and its analogs.

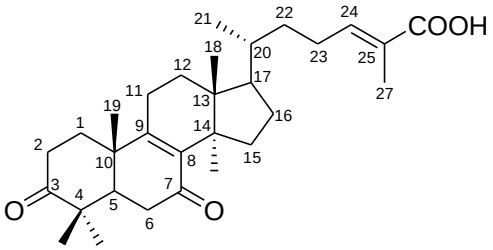
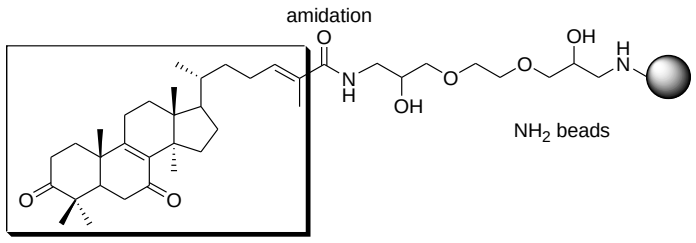


Figure 1. Structure of ganoderic acid DM (1)



Ganoderic acid DM (**1**)

Figure 2. Diagrams for ganoderic acid DM (1) fixation to the magnetic beads by reaction and amidation of the carboxylic group in the side chain of 1.

Since **1** is effective for treatment of various cancer types, it must have a common molecular target and is involved in different signal pathways in different cancer types. To identify the primary target protein of **1**, we used a technique involving ferrite glycidyl methacrylate (FG) beads to isolate the specific binding protein of **1**. Keeping the essential C-3 carbonyl group free, compound **1** was covalently conjugated to the beads at C-26 and incubated with protein fractions of PC-3 cells (Fig. 2). After extensive washing, the bound proteins were eluted and subjected to SDS gel electrophoresis and silver staining. No specific bands were detected from the membrane protein fraction (F2), nuclear protein fraction (F3), or cytoskeletal fraction (F4; data not shown). Representative SDS gel images for control and **1**-treated cytosol fractions (F1) showed a protein band at approximately 50 kD. This band was dependent on the treatment concentration of **1**, and contained α,β-tubulin, as identified by LC-MS/MS. In these experiments, α-tubulin showed a total score of 1253, 61% sequence coverage and 45 matched peptides. Concomitantly, β-tubulin had a total score of 880, 45% sequence coverage and 27 matched peptides. Tubulin is a member of a small family of globular proteins, and the most abundant of these are α-tubulin and β-tubulin. Both of these proteins have a molecular weight of approximately 55 kD, and microtubules are assembled from dimers of α- and β-tubulin. Our results show that **1** specifically interacts with both α- and β-tubulin subunits, thereby effecting microtubule function.

Cancer is characterized by uncontrolled cell proliferation and inappropriate cell survival, as well as defects in cellular morphogenesis that leads to tissue disruption, invasion, and migration. Microtubules play important roles in these cellular processes and comprise one of the oldest, most clearly validated, and efficacious targets for tumor chemotherapy. The formation of microtubules is a dynamic process that involves polymerization of heterodimers formed by α,β-tubulin, and degradation of linear polymers. Drugs that bind to tubulin can block this dynamic equilibrium, either by inhibiting polymerization or by stabilizing the microtubule structure. Both actions abolish microtubule function. To elucidate the mechanism through which **1** acts on tubulin protein, we developed a tubulin polymerization experiment. We used paclitaxel and vinblastine as positive controls, as these agents stabilize the microtubule polymer and protect it from disassembly and suppress microtubule dynamics and reduce microtubule polymer mass, respectively. As we expected, paclitaxel or vinblastine caused increased assembly, or inhibited tubulin polymerization, respectively, at 30 μM. The concentration dependency of tubulin protein on **1** was reflected by increasing effects of 50–100 μM treatments of **1** on microtubule assembly. Unlike other tubulin-targeting drugs (vinblastine) that inhibit microtubule assembly, paclitaxel stabilizes the microtubule polymer and protects it from disassembly. This blocks progression of mitosis, prolongs activation of the mitotic checkpoint, and triggers apoptosis or reversion to the G-phase of the cell cycle without cell division. In support of our results, compound **1** have been reported to cause G1 cell cycle arrest and apoptosis in human breast cancer cells.

CO-EXPRESSION ANALYSIS OF GENES ASSOCIATED WITH CAMBIAL CELL DIFFERENTIATION DURING WOOD FORMATION

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Abstract

Wood is common name of xylem of trees, an important source of fixed carbon used for woody materials and industrial purposes such as timber, pulp, furniture, fibers, and also as energy source or for other products (films, adhesives, etc). During wood formation cambial daughter cells develop and specialize to xylem cells. The mechanism of wood formation based on the observation of cell structures and cell wall components is well understood, but the genetic control of cambial activity and differentiation is still little known. A recent study in the model tree poplar showed evidences for an involvement of *KNOX* genes in controlling differentiation of cambial daughter cells. High resolution transcript analyses of the poplar cambium showed several *KNOX* genes with strong cambial expression (Hertzberg et al. 2001; Schrader et al. 2004). Furthermore, the current understanding of the regulation of differentiation in vascular development was greatly enhanced by the study of the poplar *KNOX* gene *ARBORKNOX1* (*ARK1*) and *ARBORKNOX2* (*ARK2*), which are close homologues of the *Arabidopsis STM* and *BREVIPEDICELLUS(BP/KNAT1)*, respectively. *ARK1* was shown to be expressed in the cambium and over-expression of *ARK1* leads to inhibition of differentiation of vascular cells (Groover et al. 2006).
A co-expression analysis of publicly available microarray data was performed in order to identify genes which act downstream of Arabidopsis *KNAT1* and *STM*, using *Arabidopsis* Co-expression Tool (ACT; www.arabidopsis.leedsac.uk/ACT). Genes, which are positively regulated by *KNAT1* or *STM* should be co-expressed with both of them. From 100 genes co-expressed with either *STM* or *KNAT1*, 69 genes (69%) were identical. In other words, those 69 genes are co-expressed with *STM* and also *KNAT1*. This astonishingly high overlap underlines the redundant function of *STM* and *KNAT1*. Of 69 overlapping genes seven genes were selected based on their association with cambial cell and secondary cell wall formation and their ranking of co-expression.
Quantitative expression analysis in wild-type, *stm-GK*, *knat1^{bp-9}* and the double mutant was subsequently performed for the selected genes. Down-regulation of *STM* and *KNAT1* was always followed by a not significant trend of down-regulation of cellulose synthases (*IRX1, IRX3* and *IRX5*), *cobra-like 4* (*IXR6*), *pectin methylesterase61 (PME61)*, and *fasciclin-like arabinogalactan 11*(*FLA11*) in the single mutants. In the double mutant the down-regulation for all those genes was greater than 10 times and highly significant (Table 5). Only the *lipid transfer protein 4* (*LTP4*) behaved in an opposite manner and was upregulated in the double mutant. Thus, *STM* and *KNAT1* are upstream of *IRX1, IRX3, IXR6*, *PME61* and *FLA11*. To address the potential involvement of *STM* and *KNAT1* in lignin deposition during secondary cell wall formation, key-genes of lignin biosynthesis previously identified by Mele et al (2003) were tested for their expression in the mutants. Interestingly, the expression of *At4CL1, PAL1, CAD1*, and *PRX* in *stm-GK*, *knat1^{bp-9}*, and *stm-GK;knat1^{bp-9}* was not different from wild type (Col-0). In contrast to cellulose biosynthesis, this may indicate that *STM* and *KNAT1* are not directly involved in lignin biosynthesis.

Key words: Co-expression analysis, KNOX genes, Cambial cell differentiation, Secondary cell wall formation.

Introduction

The cambial cells divide periclinally to produce xylem and phloem. Daughter cells of the cambium differentiate to the outer side into phloem and to the inner side into xylem to produce radial files of cells that meet at the cambial zone. Xylem of trees, commonly referred to as wood, is an important source of fixed carbon used for woody materials and industrial purposes such as timber, pulp, furniture, fibers, and also as energy source or for other products (films, adhesives, etc).

During secondary growth, cambial daughter cells develop and specialize to xylem cells. Xylem cells undergo progressive stages of differentiation; (1) elongation/ enlargement, (2) secondary cell wall deposition, and (3) programmed cell death before being mature xylem (Turner et al. 2008). The hallmark of mature xylem is secondary cell wall deposition. Secondary cell wall formation contributes to a large extent to the biomass of wooden tissues. The major compounds of secondary cell walls are cellulose, hemicelluloses and lignin. The wood of economically important poplar

trees typically consists of 45 % of cellulose, 25 % hemicelluloses and 20 % of lignin (Timell et al. 1969; McDougall et al. 1993).

The mechanism of wood formation based on the observation of cell structures and cell wall components is well understood, but the genetic control of cambial activity and differentiation is still little known. A recent study in the model tree poplar showed evidences for an involvement of *KNOX* genes in controlling differentiation of cambial daughter cells. High resolution transcript analyses of the poplar cambium showed several *KNOX* genes with strong cambial expression (Hertzberg et al. 2001; Schrader et al. 2004). Furthermore, the current understanding of the regulation of differentiation in vascular development was greatly enhanced by the study of the poplar *KNOX* gene *ARBORKNOX1* (*ARK1*) and *ARBORKNOX2* (*ARK2*), which are close homologues of the *Arabidopsis STM* and *BREVIPEDICELLUS(BP/KNAT1)*, respectively. *ARK1* was shown to be expressed in the cambium and over-expression of *ARK1* leads to inhibition of differentiation of vascular cells (Groover et al. 2006).

Based on phylogenetic analyses of amino acid and nucleotide sequences, there are eight members of *KNOX* genes divided into two sub families in *Arabidopsis* (Scofield and Murray, 2006). The subfamily *KNOX* I comprises *STM, KNAT1(BRIVIPEDICULOUS/BP), KNAT2* and *KNAT6* and the subfamily *KNOX* II comprises *KNAT3, KNAT4, KNAT5* and *KNA7*.

A well-characterized member of the class I *KNOX* genes is *SHOOT MERISTEMLESS* (*STM*), which is expressed in the centre of the shoot apical meristem (SAM) but not in the newly formed leaf primordia and in the incipient leaf (Long et al. 1996). Loss-of-function mutations in *STM* lead to premature differentiation of meristematic cells and eventually to cessation of the SAM (Long et al. 1996); but its simultaneous over-expression together with the homeodomain transcription factor *WUSCHEL* induces meristem formation at ectopic places (Lenhard et al. 2002). These findings indicate that *STM* is a critical regulator of differentiation, whose expression is required to keep cells in an undifferentiated state. The other characterized members of the class I *KNOX* genes fulfill partly redundant functions to *STM* and are generally suggested to be involved in preventing differentiation of the tissue where they are expressed (Scofield and Murray, 2006). In contrast to class I *KNOX* genes, the members of class II *KNOX* genes are only scarcely described and functional data is mostly lacking.

Materials and Methods

To identify genes co-expressed with *STM* and *KNAT1* the *Arabidopsis* Co-expression Tool (ACT) was employed, a internet based tool for microarray experiment analysis, that is freely available at www.Arabidopsis.leeds.ac.uk/ACT (Manfield et al. 2006). For *STM* and *KNAT1*, the 100 best matches of co-expressed genes were extracted from a database of more than 300 microarray chips. Subsequently, overlapping gene models between genes coexpressed with *STM* and *KNAT1* were identified and selected according to their putative role in secondary growth. To verify co-expression experimentally qRT-PCR was performed. Primers were designed from selected genes by using The Universal ProbeLibrary Design Center (http://www.roche-applied-science.com/sis/rtpcr/upl/ezhome.html).

To study the relationship between *Arabidopsis STM* and *KNAT1* genes and lignin biosynthesis, an expression study using genes associated with lignin biosynthesis was performed. Primers were designed against these genes according to Mele et al (2003). qRT-PCR was carried using the ROCHE qRTPCR SYBR green kit (Roche, Grenzach-Wyhlen, Germany) and reactions were run on a LightCycler®480 (Roche, Grenzach-Wyhlen, Germany) according to the protocol: preincubation (95ºC for 5 minutes) amplification (95ºC for 10 second, 61ºC for 10 second, 72ºC for 10 second), Melting curve (95ºC for 5 second, 65ºC for 60 second, 67ºC – Acqu. 5), and cooling (40ºC).

Data were analyzed using LightCycler®480 Software Release1.5.0 (Roche Grenzach-Wyhlen, Germany). Values for crossing points (Cp) were obtained directly from the software and subsequently transformed to absolute concentration values using following formula:

$$Cp = Slope \cdot Log\,[x] + Y\ intercept$$

$$X = 10^{\left[\frac{Cp - Y\ intercept}{Slope}\right]}$$

Note:

(Cp) Crossing point, (X) concentration of amplified cDNA at time point 0, (slope and Y intercept) slope and intercept obtained from running standard curves generated by template dilution.

The absolute concentration values then were normalized to the expression of *ACTIN2* by dividing the absolute expression value of the gene of interest by the absolute expression value of *ACTIN2* in the corresponding samples. All experiments were performed by using three biological and three technical replicates unless otherwise stated.

To determine slope (efficiency) and intercept, standard curves from dilution series were calculated. The initial first strand cDNA (1 µg of total RNA) was diluted 5x, corresponding to standard 1. Subsequently a series of 5x dilutions starting from standard 1 was made. For the standard curve dilutions from 5^0 to 5^{-7} were used. This procedure was performed for all primer pairs employed.

Results and Discussion

If *KNAT1* and *STM* act as transcription factors, the target genes should be co-expressed with them. In order to identify genes which act downstream of *KNAT1* and *STM* co-expression analysis of publicly available microarray data was performed, using *Arabidopsis* Co-expression Tool (ACT; www.arabidopsis.leedsac.uk/ACT). Genes, which are positively regulated by *KNAT1* or *STM* should be co-expressed with both of them, since they have overlapping function in secondary growth. From 100 genes co-expressed with either *STM* or *KNAT1*, 69 genes (69%) were identical. In other words, those 69 genes are co-expressed with *STM* and also *KNAT1*. This astonishingly high overlap underlines the redundant function of *STM* and *KNAT1*. Of 69 overlapping genes seven genes (Table 1) were selected based on their association with secondary cell wall formation and their ranking of co-expression. Quantitative expression analysis in wild-type, *stm-GK*, *knat1^{bp-9}* and the double mutant was subsequently performed for the selected genes.

Table 1. *STM* and *KNAT1* co-expressed genes selected based on their association with secondary cell wall formation.

No.	**Locus**	**Name of Protein**	**Function (Putative)**
1.	AT3G59010	Pectin methylesterase, PME61	Cell wall modification
2.	AT5g59310	Lipid transfer protein 4, LTP4	Unknown
3.	AT5G3170	Fasciclin-like arabinogalactan 11, FLA11	Unknown
4.	AT4G18780	Cellulose synthase, CesA8 (IRX1)	Cellulose biosynthesis
5.	AT5G17420	Cellulose synthase, CesA7 (IRX3)	Cellulose biosynthesis
6.	AT5G44030	Cellulose synthase, CesA4 (IRX5)	Cellulose biosynthesis
7.	AT5G15630	Cobra like 4 (COBL4), IRX6	Arrangement of cellulose microfibrils

Down-regulation of *STM* and *KNAT1* was always followed by a not significant trend of down-regulation of cellulose synthases (*IRX1, IRX3* and *IRX5*), *cobra-like 4* (*IXR6*), *pectin methylesterase61 (PME61)*, and *fasciclin-like arabinogalactan 11*(*FLA11*) in the single mutants (Figure 1). In the double mutant the down-regulation for all those genes was greater than 10 times and highly significant (Table 3). Only the *lipid transfer protein 4* (*LTP4*) behaved in an opposite manner and was upregulated in the double mutant. Thus, *STM* and *KNAT1* are upstream of *IRX1, IRX3, IXR6*, *PME61* and *FLA11*.

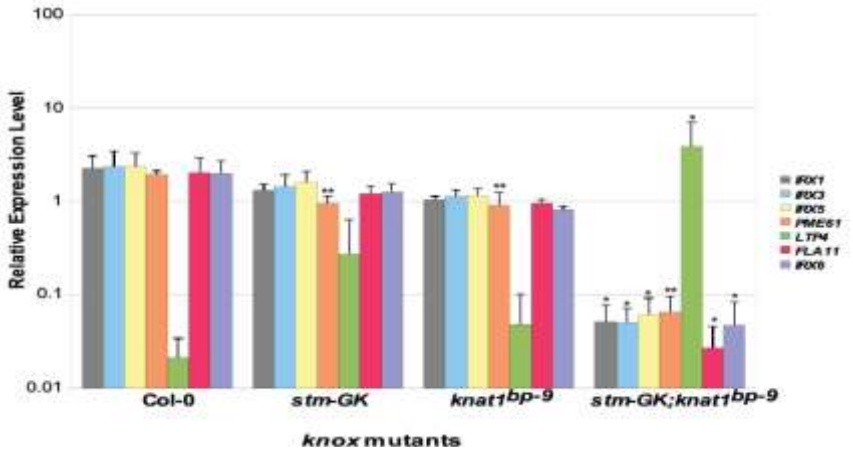

Figure 1. *STM* and *KNAT1* are involved in cellulose biosynthesis. qRT-PCR analysis of co-expressed genes in *stm-GK*, *knat1^{bp-9}* and *stm-GK;knat1^{bp-9}*. Data were analyzed from 3 biological and 3 technical replicates and normalized to the expression of *ACTIN2*. (**) Significant p≤ 0.01, t-test, compared to wild-type, (*) significant 0.01< p ≤ 0.05.

To address the potential involvement of *STM* and *KNAT1* in lignin deposition during secondary cell wall formation, key-genes of lignin biosynthesis previously identified by Mele et al (2003) were tested (Table 2) for their expression in the mutants. Those genes have been shown to be misregulated in *knat1*$^{bp-9}$ five day old seedlings in a microarray experiment employing 2 replicates (Mele et al. 2003).

Table 2. Selected key-genes of lignin biosynthesis as reported by Mele et al (2003). These genes were differentially regulated in two week old *knat1*$^{bp-9}$ seedlings (Mele et al. 2003).

No.	Abreviation	Locus	Name of Protein	Function
1.	At4CL1	AT1G51680	4-Coumarate-CoA ligase1	Lignin biosynthesis
2.	PAL1	AT2G37040	Phenylalanine ammonia-lyase 1	Lignin biosynthesis
3.	CAD1	AT4G39330	Cinnamyl-alcohol dehydrogenase 1	Lignin biosynthesis
4.	PRX	AT3G21770	Peroxidase	Lignin biosynthesis

Interestingly, the expression of *At4CL1, PAL1, CAD1*, and *PRX* in *stm-GK*, *knat1*$^{bp-9}$, and *stm-GK;knat1*$^{bp-9}$ was not different from wild type (Col-0) (Figure 2). In contrast to cellulose biosynthesis, this may indicate that *STM* and *KNAT1* are not directly involved in lignin biosynthesis.

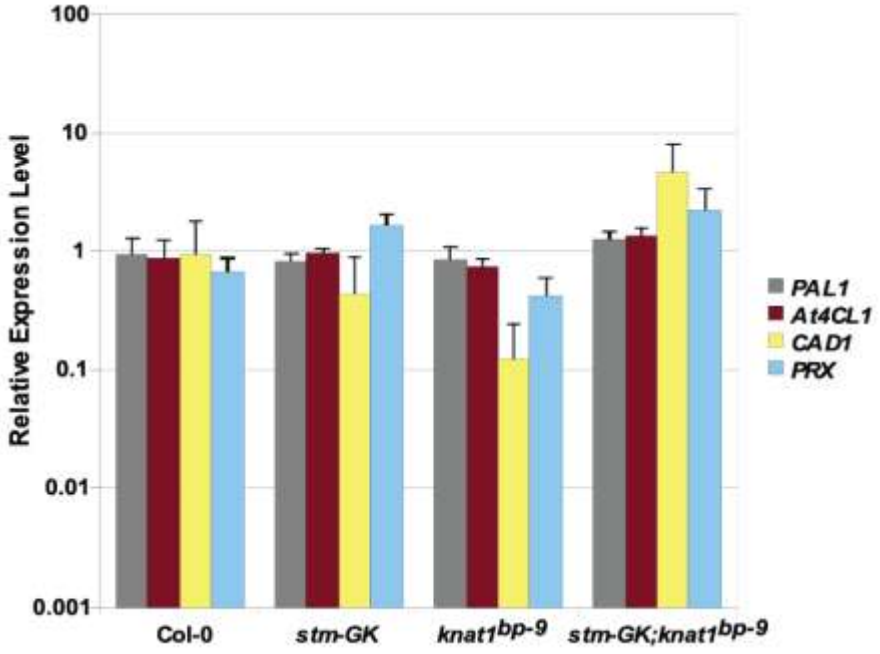

Figure 2. *STM* and *KNAT1* were not required for the expression of key-genes of lignin biosynthesis. Data were analyzed from 3 biological and 3 technical replicates and normalized to the expression of *ACTIN2*. (**) Significant p≤ 0.01, t-test, compared to wild-type, (*) significant 0.01< p ≤ 0.05.

In order to identify more downstream targets of *STM* and *KNAT1*, further candidates of the list of co-expressed genes were tested. Since *STM* and *KNAT1* show genetic redundancy this analysis was restricted to quantitative expression in double mutant hypocotyls compared to wild-type.

Almost all genes selected from listed co-expressed genes by *STM* and *KNAT1* were significantly downregulated in the double mutant compared to the wild-type (Table 3) except for *Lipid transferase protein4* (*LTP4*) and *BELL* (*BELLINGER*). The expression of *ATHB-8* which has been previously identified to be involved in vascular meristem differentiation was significantly reduced by almost 3 times. This supports the previous findings employing GUS reporter constructs (Figure 31), that *ATHB-8* is a downstream target of *STM*/*KNAT1*. Other genes which have been previously reported to be involved in xylem fiber identity (*SND1* and *NST1*, Zhong et al. 2006; Mitsuda et al. 2007; Zhong et al. 2007) and xylem vessel identity (*SND2*) were also downstream targets of *STM/KNAT1* since their expression was significantly reduced in the double mutant. Besides genes associated with cellulose biosynthesis (*IRX1, IRX3, IRX5, IRX6*) and pectin formation (*PME61*) (Figure 1), also hemicelluloses biosynthesis seemed to be a target of combined *STM/KNAT1* action, as seen in the down-regulation of the galacturonosyltransferase *IRX8* (Table 3). In respect to lignin

biosynthesis, the abundance of both the laccase (*IRX12*) and the transcript for the chitinase- like protein *CTL2* were strongly decreased. However, these genes might have opposite functions since a mutation in *CTL2* leads to increased lignification (Hossain et al. 2010). Furthermore, one gene associated with auxin signaling (*IAA27*) was significantly downregulated.

Table 3. The expression of coexpressed-downstream target gene candidates in the double mutant *stm-GK;knat1^{bp-9}*. Data were analyzed from 4 biological and 2 technical replica and normalized to the expression of *ACTIN2*. Negative ratios correspond to a decrease of expression compared to wild-type (Col-0), positive ratio to an increase. (*) Calculated based on t-test. (N.D) Not detectable, (N.A) not applicable.

No.	Locus	Gene	Relative Expression Ratio	p Value (*)
1.	AT3G59010	*PME61*	- 44 x	0.0212
2.	AT5g59310	*LTP4*	+ 47 x	0.0319
3.	AT5G3170	*FLA11*	- 39 x	0.0243
4.	AT4G18780	*CesA8* (*IRX1*)	- 30 x	0.0009
5.	AT5G17420	*CesA7* (*IRX3*)	- 186 x	0.0317
6.	AT5G44030	*CesA4*(*IRX5*)	- 76 x	0.0296
7.	AT5G15630	*COBL4*(*IRX6*)	- 42 x	0.0248
8.	AT4G32880	*ATHB-8*	- 3 x	0.0150
9.	AT1G32770	*SND1*	N.D.	N.A.
10.	AT4G28500	*SND2*	- 107 x	0.0111
11.	AT2G46770	*NST1*	- 278 x	0.0164
12.	AT5G60450	*ARF4*	- 3 x	0.0507
13.	AT4G29080	*IAA27*	- 57 x	0.0005
14.	AT5G54690	*Galacturonosyltransferase (IRX8)*	- 723 x	0.0009
15.	AT2G38080	*Laccase4* (*IRX12*)	- 404 x	0.0116
16.	AT3G16920	*CTL2 (chitinase like)*	- 100 x	0.0033
17.	AT3G42950	*GH28(polygalacturonase)*	- 2 x	0.0899
18.	AT3G10340	*PAL4*	- 3 x	0.0167
19.	AT5G02030	*BELL*	+ 1 x	0.1011

Conclussion

From the co-expression analysis, there is strong indication that *STM* and *KNAT1* are required for differentiation of cambial daughter cells and secondary cell wall formation. STM and KNAT1 activate iaa27 that associated with auxin signaling, activate ATHB-8 for vascular identity, NST1 and SND1 for xylem fiber identity and SND2 for xylem vessel identity. These transcription factors also regulate cellulose biosynthesis, pectin biosynthesis, and Hemicellulose biosynthesis.

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THE EFFECT OF METHYL JASMONATE HORMONE TO STIMULATE THE FORMATION OF TRAUMATIC RESIN DUCTS IN PINES (*Pinus merkusii* Jungh et de Vriese) FROM KPH LAWU DS

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Abstract

Resin is a non timber forest products that can be harvested from pines periodically. Most used methods in harvesting resin are mechanical wounding. Aplication of Methyl Jasmonate hormone to enlarge and increase traumatic resin ducts has been developed in other types plants, such as in rubber wood. In this research, we tried to apply Methyl Jasmonate in Pinus merkusii. The aim of this research is to know the effect of Methyl Jasmonate on the formation of traumatic resin dust in pines.

This research used 2 factors are time duration and concentration of Methyl Jasmonate. This research applied a completely randomized design (CRD) to analyze the effect of time duration factors (1 month and 2 month) and concentration factors (0%; 0,1%; 0,3% and 0,5%) on the formation of traumatic resin ducts. Some parameters were observed including 1) number of traumatic resic ducts, 2) Width (dimension) of traumatic resic ducts and 3) trakeid proportion.

The result showed that concentration level of Methyl Jasmonate factors has affected the number of resin ducts (2,13 ductss/mm2). The average width of resin ducts after the treatment is 0,037 mm2. Trakeid proportion on pines had decreased after applicated methy jasmonate. Decreasing trakeid proportion were caused by increasing the number and width of traumatic resin ductss on pines. Concentration level 0,1-0,3% is relatively effetive to stimulate the resin ducts formation because could obtain about 2 ducts/mm2. The time duration factors give no effect to the number of traumatic resin ducts and trakeid proportion.

Introduction

Most conifers will exude resin if wounded. Others will exude resin spontaneously from branches and cones. Several genera of conifers produce resin in copious quantities, which are then harvested and put to a wide variety of uses. These have made resin one of the most important non-wood productss from conifers. The resin harvested from various species of *Pinus,* the oldest and most important of the non-wood productss from conifers.

In Indonesia, resin is one of most important non timber forest products. Resin can be harvested from pines periodically. Distillation of pine resin yields two productss: turpentine and gum rosin. Gum rosin is the major products obtained from pine resin. It is the involatile residue that remains after the distillation of turpentine. Gum rosin is a brittle, transparent, glassy solid insoluble in water but soluble in a number of organic solvents (Coppen and Hone 1995).

Formation of traumatic resin ducts in norway spruce is elicited by stem boring insects and microbial pathogens as a defense response that can also be induced by mechanical wounding or by wounding and fungal inoculation of trees (Alfaro, 1995; Tomlin et al., 1998, 2000; Franceschi et al., 2000 in Martin *et al* 2002). Because wounding of trees can cause massive bleeding and volatilization of oleoresin and disruption of the tissues that are possibly involved in resin formation, it was important to develop a noninvasive method for traumatic resin ducts inductsion to enable a detailed chemical and biochemical analysis of the traumatic resin response (Martin *et al*, 2002)

Most used methods in harvesting resin from pinus merkusii in perum perhutani are mechanical wounding. Mechanical wounding is done by tapping the tree stem and removal woody tissue (sap wood), then pine will exude resin. Using this methods which involve removal woody tissue, causes damage to pines. The risk of damage to pines is heightened if excessive wood tissue is removed (Coppen, 1995). So to reduce excessive removed wood tissue, we try to applied Methyl Jasmonate hormone (MeJa) to increase formation traumatic resin ducts to obtain more pine resin.

Aplication of Methyl Jasmonate hormone to enlarge and increase traumatic resin ducts has been developed in other types plants, such as in rubber wood, also in norway spruce. In this research, we tried to apply Methyl Jasmonate

in Pinus merkusii. The aim of this research is to know the effect of Methyl Jasmonate on the formation of traumatic resin dust in pine (*Pinus merkusii*).

Material and Methods

Field sampling

Wood samples for this study were collected from 11years old pine tree plantation from Perum Perhutani Unit I, Central Java. Indonesia. More spesific location was on petak 26F, BKPH Jogorogo, KPH Lawu DS. From petak 26F location, forty five (40) pine tree were chosen as tree samples with classification as follow :

- Twenty (20) pine tree used for 1 month duration
- Twenty (20) pine tree for 2 month duration, and

Figure 1. Petak 26F, Located on BKPH Jogorogo, KPH LawuDS, Perum Perhutani

Figure 2 . Mixing methyl jasmonate at 3 concentraion

Research Tools and Materials

a. 40 Pine wood block (*Pinus merkusii)* from pine tree plantation.
b. Lanoline, Methyl jasmonat 3 concentration : 0,1%; 0,3% and 0,5%
c. Silol (C_5H_{10}), *Canada balsam,* aquadest and glacial acetic acid
d. Cutter, loupe, sliding microtome, glass preparates, pipette, volumetric flash,.
e. Test tube, object glass, hot plate, preparates box
f. Microscope fluorescence BX 51 software *Image Pro Plus V 4.5*.

Figure 3. Applicated methyl jasmonate on pines

Samples preparation

Small pine wood blocks were cut from pine tree which had applied with methyl jasmonate hormone for 1 month and 2 month. Then the pine wood blocks were cut to a rectangular prism (about 1 x 1 cm cross section, and 1 cm height) with cutter. Transverse section of 20 µm thickness from each pine wood block were cut with a sliding microtome. The transverse section were stained (with safranin), dehydrated and then observed under the microscope fluorescence Olympus BX51 and photographed at 40x magnify. From the photograph, we mounted the number of traumatic resin ducts, and measured width traumatic resin ducts also percentage of trakeid proportion using video image analyzer (*Image pro plus 4.5*).

This study was conductsed using completely randomized design with two factors; time duration and concentration of methyl jasmonate. Two classification of time duration are 1 month and 2 month. Three level concentration of MeJa are 0.1%; 0.3% and 0.5%. The results were anayized using SPSS statistical program.

Result and Discussion

1. Frequency of Traumatic Resin Ducts (TRD)

Result research about the number of traumatic resin ducts on pine which applied three level concentraton methyl jasmonate hormone are shown on the table below :

Table 1. The number of traumatic resin ducts on pine (*Pinus merkusii*) (unit/2 mm^2)

Time Duration	Concentration MeJa				Average
	K0	K1	K3	K5	
1	0.4	3.2	2.4	1.4	1.85
2	1	3	3.2	2.4	2.4
Average	0.7	3.1	2.8	1.9	

Explanation :
K0 : Control
K1 : Concentration methyl jasmonate level 0.1%
K3 : Concentration methyl jasmonate level 0.3%
K5 : Concentration methyl jasmonate level 0.5%

Table 1 showed that both pine tree with 1 month duration and 2 month duration only produced 0.7 traumatic resin ducts on K-0, whereas pines which applied MeJa had produced 1.9-3.1 traumatic resin ducts. These data indicate that if we did not give treatment with methyl jasmonate so the formation of traumatic resin ducts on pine tree were done slowly. This data also indicate that applied methyl jasmonate hormone on pine had stimulated formation of traumatic resin ducts significantly. Martin *et al* (2002) state that methyl jasmonate induces formation traumatic resin ductss and terpenoid accumulation in developing xylem of norway spruce stems.

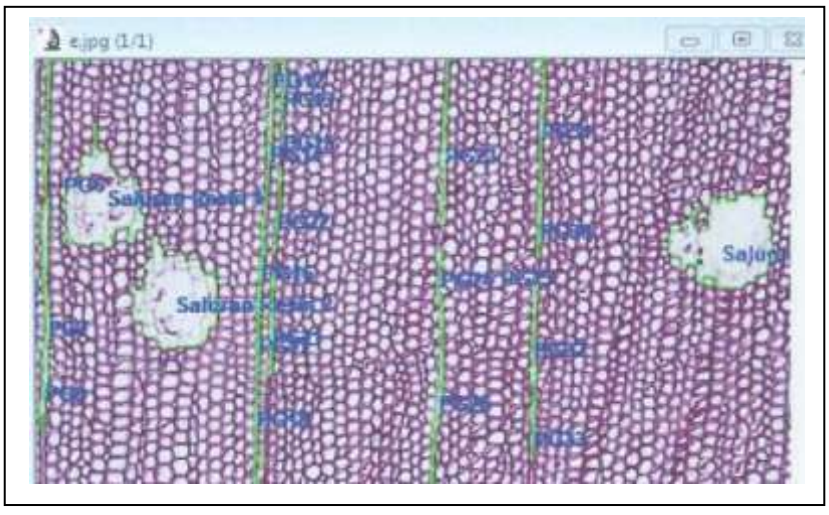
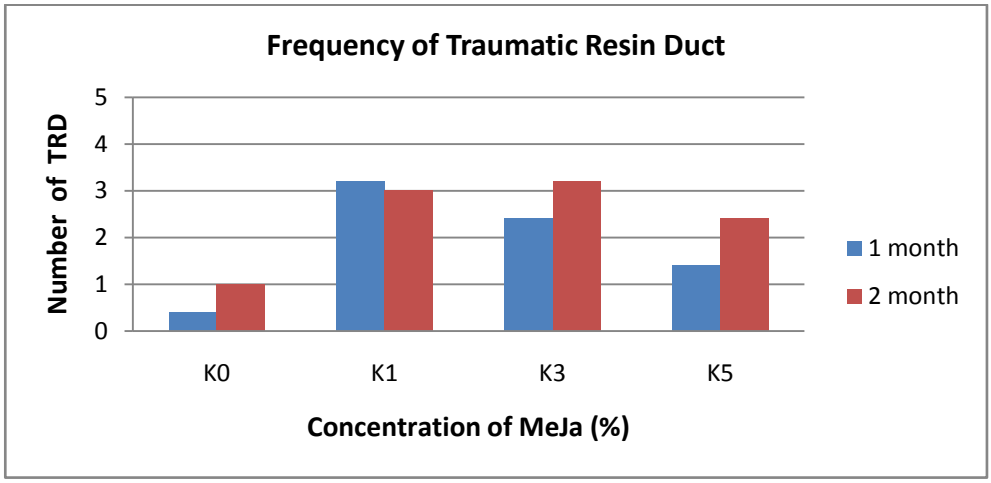


Figure 4. Number of traumatic resin ducts on Pine (*Pinus merkusii*) at transverse section

Graph 1. below showed that pine without MeJa treatment (K0) has lower traumatic resin ducts compared to all pine with MeJa (K1,K3 and K5). Statistical analysis indicate that concentration of MeJa has very significant affected on the number of traumatic resin ducts. Application methyl jasmonate on pine with K1 and K3 concentration showed increasing the number of traumatic resin ducts. Tian (2003) state that aplication MeJa was proven to improve resin productsion on rubber wood. Agree with Tian (2003), Hudgins and Franceschi (2004) reported that increasing MeJa concentration on

Pseudotsuga menziesii had produced traumatic resin ducts as 8 /mm^2, moreover on *Sequoiadendron giganteum* could produced traumatic resin ducts as 10 /mm^2. Meanwhile, Hao and Wu (2000) also reported that application MeJa on rubber wood (*Hevea brasiiliensis*) could improved formation of traumatic resin ducts about 3-7 /mm^2.



Graph 1. The number of traumatic resin ducts on pine at diiferent level concentration of MeJa

Application MeJa to stimulate traumatic resin ducts formation on pine were effective until K3 (concentration 0.3%), because aplication MeJa at K5 concentration showed decreasing formation of traumatic resin ducts. Therefore application MeJa with concentration more than 0.3% was not suggested based on this data, because the number of traumatic resin ducts at K5 concentration has lower compared to K1 and K3 concentration. Hudgins and Franceschi (2004) state that application MeJa to encourage traumatic resin ducts was proven for reprograming of stem cambial zone for traumatic resin ducts formation on conifer. This data also agree with Hao and Wu (2000) who state that concentration MeJa at level 0.1% has produced more number of traumatic resin ducts formation in *Hevea brasiiliensis.*

2. Width of traumatic resin ducts on Pine

Result research about width of traumatic resin ducts on pine which applied three level concentration of methyl jasmonate (MeJa) hormone are shown on the table below :

Tabel 2. Width of traumatic resin ducts on pine

Time Duration (month)	Concentration MeJa (%)				Average
	K0	K1	K3	K5	
1	0.005	0.047	0.037	0.023	0.028
2	0.025	0.04	0.075	0.043	0.046
Average	0.015	0.044	0.056	0.033	

The width of traumatic resin ducts on pine are shown on table 2. On concentration K0, both pine 1 month duration and 2 month duration showed the lowest width traumatic resin ducts. These case indicate that application of methyl jasmonate on pine tree could push wider the dimension of traumatic resin ducts on pine. This data also indicate that applied methyl jasmonate hormone on pine tree has encouraged the bigger traumatic resin ducts dimension. Hudgins and Franceschi (2004) state that observations of stem surfaces indicated that resin exudation was considerably greater with 100 mm Methyl Jasmonate than lower Methyl Jasmonate concentrations and ethylene, which although not quantified correlates with the cross-sectional area of traumatic resin ductss formed.

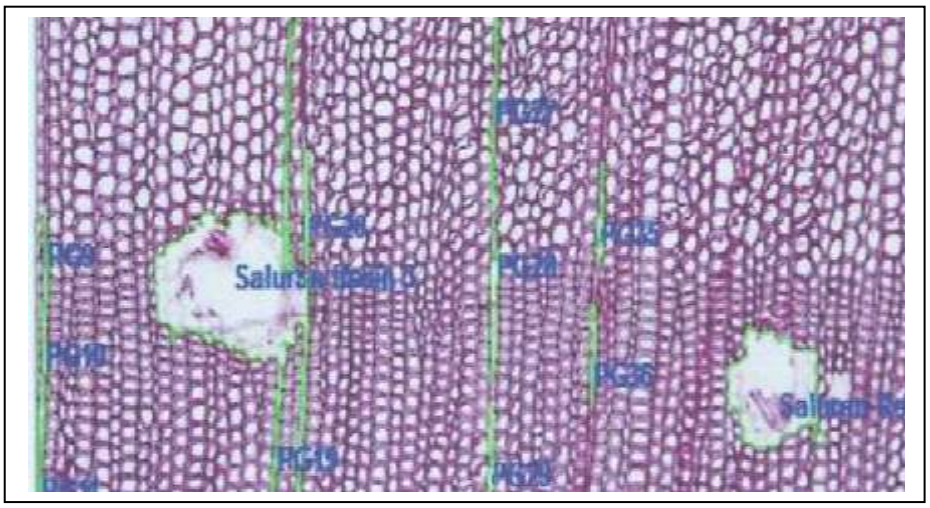
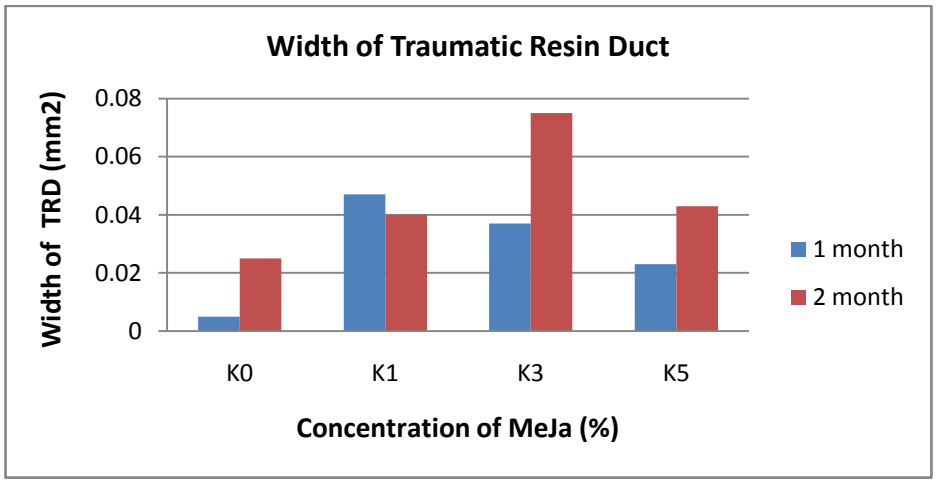


Figure 5. Width of traumatic resin ducts on Pine (*Pinus merkusii*) at transverse section

Graph 2. showed that traumatic resin ducts on pine tree with no MeJa treatment (K0) has smaller size and dimension compare to all the pine tree with MeJa (K1, K3 and K5). Statistical analysis indicate that concentration of MeJa has significantly affected on the width of traumatic resin ducts. Application methyl jasmonate on pine with concentration K1 and K3 showed increasing the width of traumatic resin ducts. Hudgins and Franceschi (2004) state that increasing concentration MeJa could improve the width of traumatic resin ducts as 0.00586 mm^2 on *Pseudotsuga menziesii*, and 0,00612mm^2 in *Sequoiadendron giganteum*. These datas also indicate that adding concentration of MeJa could push the bigger dimension of traumatic resin ducts until K3, but adding concentration MeJa more than K3 were not significant affected.



Graph 2. The width of traumatic resin ducts on pine at diiferent level concentration of MeJa

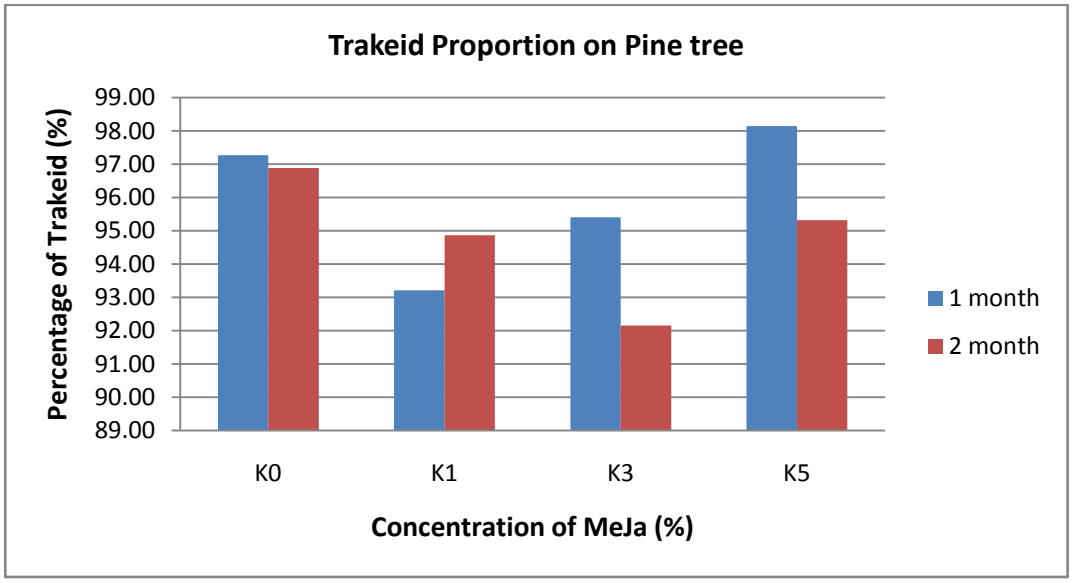
3. Percentage of trakeid proportion

Result research about Percentage of trakeid proportion on pine tree which applied three level methyl jasmonate (MeJa) hormone are shown on the table below :

Tabel 3. Percentage of trakeid proportion on pine tree

Time Duration (month)	Concentration MeJa				Average
	K0	K1	K3	K5	
1	97.27	93.21	95.40	98.14	96.0045
2	96.88	94.86	92.15	95.32	94.8045
Average	97.073	94.035	93.779	96.731	

The percentage of trakeid proportion on pine are shown on table 3. On concentration level K0, both pine tree 1 month duration and 2 month duration showed the highest trakeid proportion compare to the pine tree which applied MeJa (K1, K3 and K5). This case indicate that application of methyl jasmonate on pine tree could decreasing percentage of trakeid proportion on pine. Actually, the decreasing trakeid proportion on pine were caused by increasing the number of traumatic resin ducts. Graph 1 and 2, showed that applied methyl jasmonate hormone on pine, not only increasing the number traumatic resin ducts but also the dimension of traumatic resin ductss have more wider than normal resin ducts. Hudgins and Franceschi (2004) state that observations of stem surfaces indicated that resin exudation was considerably greater with 100 mm Methyl Jasmonate than lower Methyl Jasmonate concentrations and ethylene, which although not quantified correlates with the cross-sectional area of traumatic resin ducts formed.



Graph 3. Trakeid proportion on pine at diiferent level concentration of MeJa

Graph 3. showed that trakeid proportion on pine tree with no MeJa treatment (K0) has highest trakeid proportion compare to the pine with MeJa (K1 and K3). Statistical analysis indicate that concentration level of MeJa has affected significantly on the trakeid proportion. Application methyl jasmonate on pine with concentration K1 and K3 showed decreasing on trakeid proportion, but at concentration level K5, showed increasing the trakeid proportion on pine tree. Increasing trakeid proportion at concentration level K5 were caused by decreasing the number of traumatic resin ducts and more smaller the dimension of traumatic resin ducts.

Conclusion

Methyl jasmonate hormone could stimulate traumatic resin ducts formation on pine (*Pinus merkusii* Jungh et de Vriese). Concentrations of methyl jasmonate were very significant affected on the number traumatic resin ducts and significant affected on the width traumatic resin ducts. Trakeid proportion on pines had decreased after applicated methy jasmonate. Decreasing trakeid proportion were caused by increasing the number and width of traumatic resin ducts on pines. Application of MeJa on pine to stimulate traumatic resin ducts formation were effective only at K1 and K3 (concentration 0.1 and 0.3%). Otherwise, at K5 (concentration 0.5%), showed decreasing the number of traumatic resin ducts. Concentration methyl jasmonate has also significant affected on trakeid proportion.

The duration treatment was not affected signicantly on traumatic resin ducts formation, neither the number nor the width of traumatic resin ducts. The time duration was not affected signicantly on trakeid proportion.

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PROCEEDING OF THE FIFTH INTERNATIONAL SYMPOSIUM OF INDONESIAN WOOD RESEARCH SOCIETY

PROPERTIES OF HEAT TREATED TEAK WOOD FROM COMMUNITY FOREST

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ABSTRACT

Wood product demand in Indonesia has been increasing parallel to the population growth, while wood supply shows the reverse trend. The Indonesian forests potency for log production has been decreasing in area and its productivity. For that reason community forests have emerged as an alternatives log sources. Unfortunately the quality of logs harvested from the community forests are lower quality than logs obtained from natural and plantation forests. For instance, a comparison of teak log quality from plantation with teak log quality of community forests results that the last is inferior to the first. Therefore the logs coming from community forests need to be improved. One of many wood technologies available and suitable for log quality improvement is heat treatment. This research's objective is to know the best quality improvement in teak logs which is harvested from community forests.

In order to achieve the objective of the research, a CRD with factorial experiment was employed. The two factors involved were heating method and heating time with three levels for each factor. The heating method factor consisted of oven, steaming and boiling method, while the heating time factor consisted of 1, 2 and 3 hour heating time. The chosen replication was three times. The log quality improvement was determined by physical parameters namely, color change, surface roughness, wettability and equilibrium moisture content (EMC).

The research results showed that the interaction between heating method and heating time influenced very significantly on wood color change, moisture content and surface roughness. Highest red and yellow color element was produced by steam 2 hours. Lowest red color element was produced by oven 1 hour while lowest yellow color element was produced by oven 3 hours. The highest surface roughness was observed in boiling 1 hour. The highest equilibrium moisture content was produced by boiling 3 hours.

Keywords: heating treatment, color change, surface roughness, wettability, teak wood

INTRODUCTION

Indonesian community forests have emerged as alternatives of log sources recently. Inventory data in 2003 showed that the community forests potency was 1,560,299ha and capable to produce 39,564,003cum (Pandit, 2004). It has been noted for several years that the rural people usually do not follow strictly the teak silviculture system. They cut the teak trees when they need money and no longer pay attention to the silviculture consideration. For that reason the teak log quality coming from this type of community forests are inferior compared to those obtained from Perhutani. In this situation the buyers need some wood technologies for improving the teak log quality. Wood scientists have been doing some intensive research on this low quality of wood. They have come with many technologies so-called wood modification. One of those available technologies is heat treatment. Heat treatment is considered as a wide range application technology and can be used in the community area by the rural people. For that reason, this heat treatment was chosen for log quality improvement. Heat treatment is capable to improve wood properties such as wood defects reduction (bowing, rupture, resin deposits) and strength gain, durability, wood working and machining properties. This wood quality improvement might be caused by wood anatomy changes due to heat treatment. It was proven that heat treament can reduce wood moisture equilibrium (EMC), reduction of volatile organic compound (VOC) emission, increase wood stabilisation, fungi infection reduction and of course darkening wood colour (Esteves *et al*., 2007). Besides, the heat treatment will increase wood weathering durability, wood color uniformity but reduction of wood wettability (Awoyani and Jones, 2010).

Heat treatment is affected by the heating temperature and heating time significantly. Some degree of wood degradation has been observed when wood heating temperature is increased. However the heating time exerts more effect than the heating temperature (Esteves *et al*., 2007). Several technique of heat treatment has been developed such as hydrothermal, steaming or steam injection, oven, radio frequency and others. Each heat treatment technique has advantages and disadvantages. Each technique requires a certain and specific tools for its application and heating process and the ultimate effect. Generally the heat treatment employs relative high heating temperature and high steam

pressure. Finnaly, heat treatment effect is influenced by the wood characteristic itself. For that reason each type of wood will give a certain and specific response to the specific heat treatment applied. Spruce wood heat treatment by oven was done by Pavlo dan Niemz (2003) dalam Kocaefe *et al.* (2008). The research result showed that wood properties improvement were detected in terms increase wood stabilisation and uniform dark colour, but a decreased in mechanical strength. The heat treatment caused some degree of hemicellulose degradation that promoted the increase of wood stabilisation. Heat treatment at 220^0C for 1 hour can reduce wood swelling thickness amoun to 16.5% and wood specific gravity decrease at about 7.91% and MOR reduction of 2.3%. When the heating time is prolonged to 2 hours then the reduction of MOR is more severe but the wood stabilisation becomes better. Heat treatment by steaming european wood namely black locust (*Robinia pseudoacacia*), oak (*Quercus robur*) and tropical wood namely merbau (*Intsia bijuga*), sapupuira (*Hymenolobium petraeum*) was conducted by Varga dan van de Zee (2008). The treatment used four levels of heating temperature of 92°C, 108°C, 115°C, 122°C combined with three levels of heating time of 3; 7,5 and 20 hours. The results showed that adhesion properties is increased parallel with increasing of heating temperature especially for supupuira wood.

Wood adhesion quality is influenced by so many factors. Those factors are classified into three groups namely wood factors, adhesive factors and processing factors. Wood wettability and surface smoothness are the significant wood factors in adhesion. These two wood properties might be influenced by heat treatment (Awoyani dan Jones, 2010). Heat treatment might alter wood wettability in such a way depneding the medium of heating such as steam or boilling water. Heat treatment by oven (radiant heating) or radio frequency might affect differently to the wood wettability (Prayitno, 1999). In mechanical adhesion theorem, adhesive liquid will penetrate to the wood and solidify in the wood in such a way that interlocking of the adhesive with the wood occurred (Brown *et al.*, 1952). For that reason the clean pathway of adhesive flow into the wood is needed.

This research is aimed at determination of wood properties change caused by the heat treatment. The treatment is formed by two factors namely heating medium and heating time. The heat treatment used three type of heating medium namely oven, steaming and boilling. The heating time is 1,2 and 3 hours. For that reason the total treatment is 9 and those treatment is employed in three replicates.

METODOLOGY

A. Materials

The research materials consisted of teak-wood lumber (*Tectona sp*.). The teak log was obtained from the community forest in GunungKidul District, Yogyakarta Province. The log diameter ranged from 13-23cm. The research tools consisted of circular saw, planner, grinder, electronic balances, oven, steam production, gas stove, dessicator, filter paper, thermometer, surface roughness tester, spectrophotometer, moisture meter.

B.Methods

Quarter sawn teak wood lumber is obtained from teaklogs and then cut into 1 cm thick x 4 cm width x 20 cm long. The samples were then subjected to heat treatment according to the combination of heating medium and heating time. Three heating medium were oven, steaming and boiling in water, while the heating time is 1,2 and 3 hours. The total treatment were 9 factor combination and conducted in three replication. After heat treatment the sampels were subjected to conditioning process in the laboratory of wood composite for at least a week.

1. **Color Test**

Color test was conducted on the tangential surface of the wood samples by using *Spectrophotometer NF 333* (*Nippon Denshoku Ind. Co Ltd*.). Three points of color measurements were conducted. Three color elements scale of CIELAB were recorded, namely L* (lightness), a* (red-green scale), dan b* (yellow-blue scale). The wood color was determined with the average values of three measurement points of each sample. The wood color change ΔE*ab was calculated by $(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$, where ΔL*, Δa*, and Δb* were the color element scale value difference before and after heat treatment.

2. **Moisture Content**

The moisture content of the samples was determined after conditioning period. The moisture measurement was conducted by moisture meter.

3. **Wettability**

Wood wettability determination was done by following the *Corrected Water Absorption Height* (CWAH) method. The CWAH measurement used wood particles passed 40 mesh and retained at 60 mesh sieves. The wettability was calculated by using formula:

$$CWAH = h1 \times FK = h1 \times \frac{d^2 \pi h2}{4 w s}$$

where :

CWAH : the corrected water absorbtion
FK : correction factor
h1 : water adsorption height (mm)
h2 : wood particle height (cm)
d : glass tube inner diameter (cm)
π : 22/7= 3,14
w : ovendried weight wood particle (gr)
s : water specific volume (cm3/gr)

4. Surface Roughness

Surface roughness was detected by using the surface roughness tester. The surface roughness variables detected were average (Ra). The instrument was kalibrated every 100 measurement by standard roughness plate (Ra ranges from 3,02 µm - 0,48 µm) and c*ut-off* length 2,54 mm. The surface roughness measurement was conducted after the samples subjected to planner. The instrument for this surface roughness determintation was *Surface Roughness Tester SRG – 4000 Phase II.*

RESULTS AND DISCUSSION

The data of wood sample color elements scale following CIELAB system are presented in the Table 1. They consisted of lightness or brightness differences before and after heat treatment (ΔL*), the differences of red-green scale or redness (Δa*), the differences of yellow-blue scale or yellowness (Δb*) and the wood color change (ΔE) that is the resultant wood color change after heat treatment.

Tabel 1. Average of Brightness, Redness, Yellowness and Wood Color Change of Teak wood

Parameter	Heating Method	Heating Time			Average
		1 hour	2 hour	3 hour	
Lightness ΔL*	Oven	5.30	2.39	2.57	3.42
	Steam	16.54	17.22	22.48	18.75
	Boiling	2.36	0.75	1.65	1.59
Red-green Δa*	Oven	0.73	1.01	1.17	0.97
	Steam	0.91	4.95	1.22	2.36
	Boiling	2.25	3.34	2.85	2.81
Yellow-blue Δb*	Oven	1.37	1.62	0.58	1.19
	Steam	1.62	9.94	8.15	6.57
	Boiling	4.92	7.56	7.87	6.78
Wood Color Change ΔE*ab	Oven	5.54	3.38	2.96	6.22
	Steam	17.24	20.54	24.00	22.67
	Boiling	6.05	9.28	8.64	15.55

Table 1 shows that steam treatment can increase significantly the lightness (brightness) of teak wood samples (18.75), compared to the other color elements scale CIELAB system such as the red-green and yellow-blue scale. It is observed also that wood color change is contributed significantly by steam treatment (22.67). Heating method by oven produced the small lightness or more darker (3.42) than steam one. The darkest color was resulted by boiling method (1.59). Steaming wood has been studied by some researchers by using varied steam temperature or steam pressure and the steaming time from several hours up to several days. Steaming has been observed that affect the wood lightness significantly when heating time is much longer such as daily period (Tolvaj *et al.* 2012). Research on wood steaming and oven treatment comparison was conducted by Todaro *et al*. (2010) on Turkey oak. The results showed that the steaming

is more effective in lightness change compared to the oven method. This research result proves that the effect is parallel with the data presented in Table 1.

In terms of redness scale values, the teak wood after treatment tended to show the red scale than the green side. This was due to the initial color of teak wood itself. The wood color change appeared to be less contributed by this red scale than the yellow coordinate. Table 1 showed that heating method of steaming and boiling is more effective than oven. The average of redness produced by oven method was only 0.97, while the steaming and boiling method showed higher values of 2.36 and 2.81 respectively.

In term of yellow color element scale, the same trend was observed that the highest yellowness was produced by boiling method and then followed by the steam treatment and finally by oven method. The average of yellowness scale of three heating methods were 1.19; 6.57 and 6.78 consecutively.

In terms of wood color change, three heating methods showed quite differerent values of color element change. The highest effect was still produced by steaming method and then followed by boiling method and finally by oven method. When heating wood by oven, the effect was observed to be slowing down from 1 hour to 3 hour heating time. However, the other two heating methods namely steaming and boiling, a different effect was observed. The longer heating time, the greater color change values. The variation color change by oven was 5.54; 3.38 and 2.96 for 1, 2 and 3 hour of heating time. Variation of color change caused by steam was 17.24; 20.54 and 24.00, while boiling method produced 6.05; 9.28 and 8.64 respectively. Esteves *et al*., 2007) mentioned that heating wood causing darkening wood materials and this effect observed the same in the color variation results. The darkening color of wood has been observed in kiln dryer during wood drying before the wood adhesion processing (Prayitno, 1999). In this case of wood drying, the extractives migration from the inner portion to the wood surface was observed and finally making a browning effect on the wood surface. Some wood extractives observed in browning effect was carbohydrate, small weight of nitrogen compound that capable of producing brown reaction products (Sundqvist, 2004). Amadori-Maillard reaction between lignin and hydrolysis carbohydrate support the brown and darkening wood color (McDonald *et. al,* 1997 dan 2000 dalam Sundqvist, 2004). Figure 1 shows a visual variation of all color elements of the teak wood after heat treatment from lightness (A), redness (B), yellowness (C) and wood color change (D).

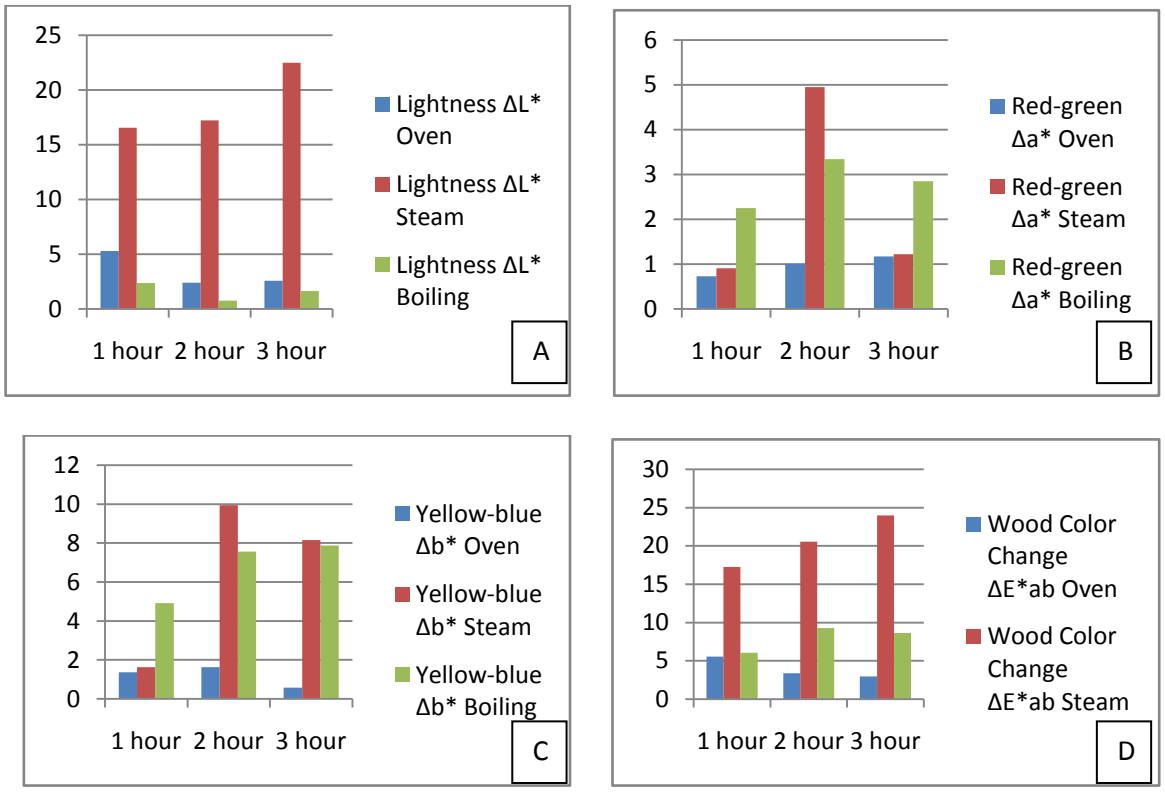

Figure 1. Overall Comparison Color Elemenst of Teak Wood after Heat Treatment

Analysis of varians of the color element data is presented in Table 2. It was shown that the interaction between heating method and heating time was observed only in the color elements (hue), namely redness and yellowness. This result proves that the color change is influenced by the combination of heating method and heating time and can not be subjected to only single factor (Tolvaj *et al,* 2012*)*. On the other hand the brightness and the wood color change were not influenced by the the factors combination but affected significantly by heating method.

Table 2. ANOVA of Teak Wood Color Elements

Variable	F calculated, Significance levels		
	Interaction	Heating Method	Heating Time
Brightness ΔL*	0.186^{ns}	0.000**	0.347^{ns}
Redness Δa*	0.000**	0.000**	0.000**
Yellowness Δb*	0.002**	0.000**	0.000**
Wood Color Change ΔE	0.156^{ns}	0.000**	0.324^{ns}

Remarks: **) highly significant; *) significant; ns) non significant.

The physical wood properties are affected by heating treatment. Esteves *et al*. (2007) mentioned that heat treatment on wood can reduce equilibrium moisture content and increase the wood stability. Heat treatment could increase the hydrophobicity or reduce the hygroscopicity of the wood due to alteration of wood chemistry. The heat treatment therefore could reduce the wood wettability due to increased hydrophobicity. Korkut *et al.*(2008) mentioned in their research that heat treatment reduced the wood surface roughness. As mentioned above in order to know the variation effect of heat treatment on teak wood obtained from community forests the research was conducted. Table 3 summarize the physical properties of teak wood after heat treatment.

Table 3. Average of Equilibrium Moisture Content, Surface Roughness and Wettability of Teak Wood after Heat Treatment

Parameter	Heating Method	Heating Time			Average
		1 hour	2 hour	3 hour	
Equilibrium Moisture Content (EMC) (%)	Oven	2.24	1.75	2.54	2.18
	Steam	0.49	2.69	1.21	1.46
	Boiling	1.48	3.72	3.78	2.99
Surface Roughness (Ra,um)	Oven	0.94	2.23	1.24	1.47
	Steam	1.32	1.06	1,14	1.17
	Boiling	3.31	1.88	2.20	2.46
Wettability (CWAH,mm)	Oven	215.20	233.61	235.35	228.05
	Steam	188.92	196.63	199.87	195.14
	Boiling	209.72	197.31	208.52	205.18

Kallander and Landel (2007) conducting research of heat treatment on equilibrium moisture content and several physical characteristics of wood. The heat treatment was found to reduce EMC of wood. Table 3 shows that lowest EMC of teak wood is produced by steam treatment followed by oven and boiling method. Variation of EMC due to heating time factors does not consistently. Analysis of varians of EMC of teakwood presented in Table 4 shows that this EMC is influenced significantly by the interaction effect. This means that the EMC is specific wood parameter that is not affected by single factor of the research. For that reason EMS is dependent on the combination of heating method and heating time. Sayar and Tarmian (2013) explained that this alteration of EMC might be due to reduction of vapor diffusion coefficient in the wood or among wood cells. Heating treatment at 160-220 ^{o}C in douglas fir could reduce EMC in the range of 5,34 – 42.63%.

Table 4. ANOVA of teak wood physical properties after heat treatment

Variable	F calculated, Significance levels		
	Interaction	Heating Method	Heating Time
Moisture Content (%)	0,000**	0,000**	0,000**
Surface Roughness (Ra,um)	0,000**	0,000**	0,101ns
Wettability(CWAH,mm)	0,514ns	0,001**	0,393ns

In terms of surface roughness, Table 3 shows that the lowest roughness is produced by steam treatment followed by oven and the last is boiling method. The three heat treatment effectively reduce the surface roughness of teak wood. Korkut *et al*. (2008) concluded from their research that surface roughness decreased with increasing temperature and treatment cycles. Heating time factor seems does not exert an effect on the surface roughness. According to the analysis of variance of the surface roughness data, the intercation of the two factors involved in the research, namley heating method and heating time influenced very significantly to the average of surface roughness. This result is similar to the effect on the equilibrium moisture content. This means that this surface roughness is not dependable only to one factor but also the other factor. Oven method produced roughness values of 0,94; 2.23 and 1.24 due to heating time. On the other hand the steam method produced roughness of 1.32; 1.06 and 1.14 respectively, while the boiling method produced values of 3.31; 1.88 and 2.20 consecutively.

Wettability of the wood is a measure of the readiness of the wood to be wetted by a liquid. Generally water is used to measure the wood wettability since the wood is hygroscopic materials. When wood is wettable than water molecules are easily attaced to the wood molecules. On the contrary, the wood that is not wettable is detected by very difficult water molecules to attach to the wood molecules (Prayitno, 1999). This condition of unwettable wood might be caused by heating treatment which convert surface of the wood to the inactive condition Forbes (1998). Wood wettability is determined by following the CWAH procedure. Table 3 shows that the oven heating method produced highest average of CWAH followed by boiling and the least one is produced by steaming method. From the above data this steam method has been showing a significant factor influencing the teakwood properties. Steaming method has shown to produce a higher value of lightness, clear hue (red ness and yellowness), less EMC and less surface roughness, and finally low wettability or high hydrophobicity. Figure 2 shows the variation of EMC, surface roughness and wettability of teak wood after heat treatment.

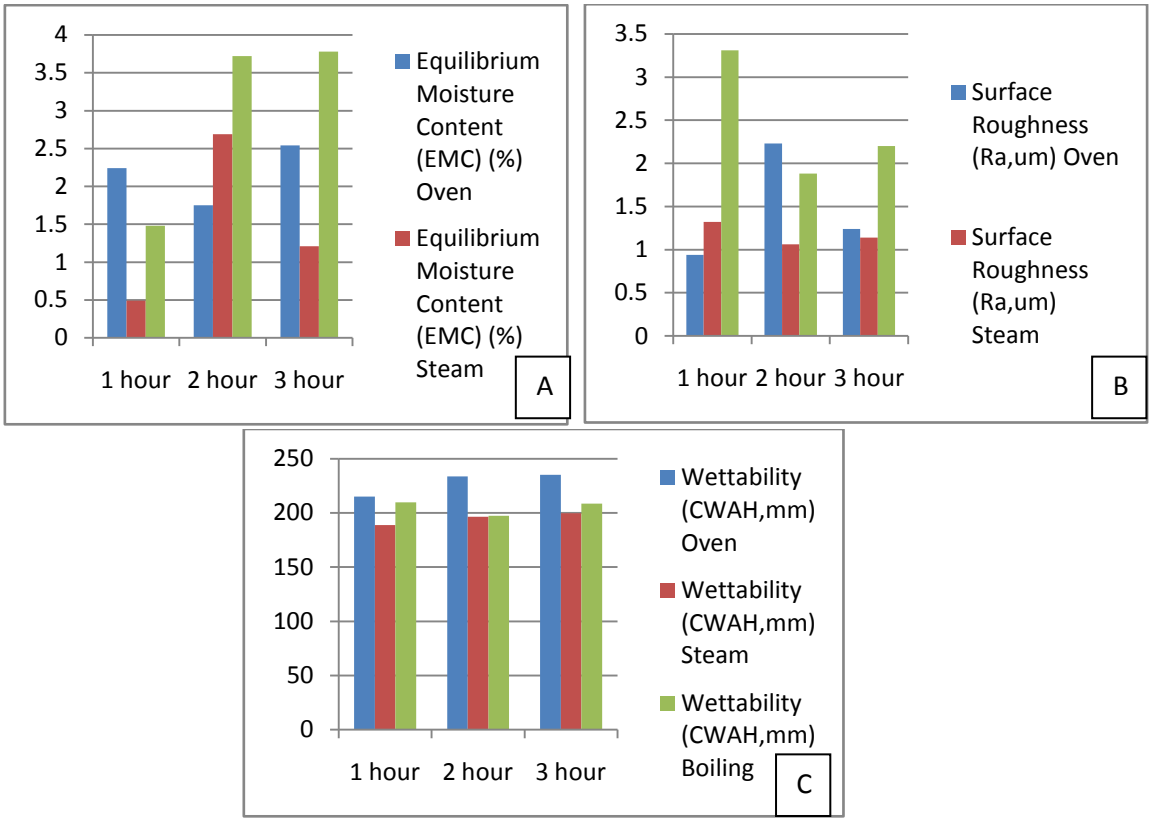

Figure 2. The variation of EMC (A), Surface Roughness (B) and Wettability (C)

CONCLUSION

Based on the data analysis and its discussion, the some conclusion can be drawn as follow.

1. Interaction between heating method and heating time influenced very significantly to red and yellow scale of CIELAB color system. This wood color is dependable not only to heating method but to the heating time as well. The interaction of the two factors affect very significantly to EMC and surface roughness of the teak wood. For that reason these four teak wood properties vary according to the combination effect. Combination of red and yellow color that is matching to the natural teak wood color is produced by steaming and boiling at 2 hours heating time. (redness of 4.95 and 3.34 and yellowness of 9.94 and 7.56 for steaming and boiling respectively). The least EMC steaming and boiling are 0.49 and 1.48 for 1 hour heating time, while the smoothness of the surface produced by oven method 1 hour heating time (0.94) and 1.06 produced by steaming 2 hour.
2. Heating method factor affecting very significantly to the brightness, wood color change and the wood wettability. The steaming method has produced the highest values of lightness and wood color change (18.75 and 22.67 respectively), while the highest wettability is produced by oven method (228.05).

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APPLICATION OF CARBON DIOXIDE INJECTION TECHNOLOGY IN BAMBOO CEMENT BOARD PRODUCTION

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ABSTRACT

The objective of this study was to evaluate the effect of the incorporation of injection of CO2 in liquid phase at 30 minutes of curing time period to the physical and mechanical properties of cement board made from bamboo culm particles of *Gigantochloa atter*, *Dendrocalamus asper* and *Schizostachyium brachycla*. Mixtures of bamboo culm particles, cement, and water on the ratio by weight of 1 : 2.5 : 1.25 were casted in iron plate mold of 25 x 25 x 1 cm^3, pressed and then hold for 24 hours to obtain the targeted density of cement board samples of 1 g/cm3. Samples were cut in accordance with the size of physical and mechanical testing of and then injected by CO_2 in liquid phase at 30 minutes of curing time period. Samples of cement board were tested for physical and mechanical properties according to Japanese Industrial Standard (JIS) A 5417-1992. Results showed that the physical properties consisted of moisture content of the boards ranging from 3.15% to 3.62%, density from 0.68 g/cm^3 to 0.80 g/cm^3, water absorption for 24 hours from 45.11% to 57.60%, linear swelling for 24 hours from 0.13% to 0.27 and thickness swelling for 24 hours from 0.65% to 0.87%. Mechanical properties consisted of internal bond ranging from 0.18 kg/cm^2 to 0.74 kg/cm^2, MOE from 1339.03 kg/cm^2 to 5030.50 kg/cm^2 and MOR from 40.12 kg/cm^2 to 79.59 kg/cm^2. Only cement board made from mixture of *Gigantochloa atter* particles, Portland cement and water met physical properties requirement of JIS A 5417-1992 and no cement board satisfied mechanical properties requirement of JIS A 5417-1992

Key words: bamboo, Carbon dioxide injection, Cement Board.

Introduction

The use of petroleum-based resins such as urea and phenol formaldehyde for conventional lignocellulosic-based composite boards for years has shown the increase of cost due to the gradual increasing of petroleum cost. Also, emission of formaldehyde-based resins in panels has been known to cause eye, nose, and throat irritation as well as coughing and breathing difficulties. The alternative to replace the petroleum-based binders is quite possible since many studies showed that the inorganic binders or ceramic materials such as Portland cement can be used as a potential matrix for lignocellulosic-based composite boards manufacturing.

The ability of Portland cement to bind the lignocellulosic materials is caused by existence of certain chemical elements in Portland cement that can harden at certain temperature. The Portland cement binder provides a durable surface as well as one that can be easily embossed and colored with a range of processing methods to provide a variety of products that are easily machined with conventional wood-working tools (Erakhrumen, A. A. et al., 2008).

The use of lignocellulosics materials as aggregate such as wood has been widely used to produce cement boards for non-structural purposes such as walls and ceilings. However, since the growing concern of resource reduction of wood, many researchers to seek and develop new materials relying on the other renewable sources. Bamboo is one of the most important raw material to substitute wood due to its abundant availability, fast growing, and easy to be planted(Muin, M., et al., 2006). As a cheap and fast-grown resource with superior physical and mechanical properties compared to most wood species, bamboo offers great potential as an alternative to wood.

Use of bamboo as raw material of cement board by using conventional technology has been researched by Suhasman and Bakri (2012). However, some characteristics of the cement board have not fulfilled the requirement. Increased research during the recent years has considerably contributed to the understanding of bamboo as well as to improved processing technologies for broader uses (Li. X., 2008). Taking advantage of the wide distribution, renewability, and recyclability of bamboo, more markets will be developed for these low-cost renewable materials.

Manufacturing of cement board composite is principally similar to conventional board composite. The variations only commonly take place on the pressing time and method to be applied because different type of the binder.

Manufacturing process of cement board composite is accomplished by mixing lignocellulosic material, Portland cement, water and others supplementary materials and then pressed for consolidation. Pressing is hold until 24 hours to harden and densificate the board. Although densification and hardening condition of the board has been developed for 24 hours, fully strength of the board will be achieved at 28 days curing process.

Hardening process will be hampered and curing process will take longer time if the lignocellulosic materials contain large amount of hydrolysable hemiselluloce and extractive substances. The alkali medium produced by cement dissolves the extractives and hemiselluloce which, in turn, reacts as retardant to cement (Moslemi, A.A., 1989). The effort to shorten the setting time was accomplished by the addition of the accelerators (Simatupang, M.H. *et al*., 1991).

Relatively new concept developed to speed up hardening process on cement board composite is to apply carbon dioxide injection in to the cement board. This method was patented by British Patent in 1979 and Japanese Patent in 1986. Injection of CO_2 gas in to the cement board results in calcium carbonate ($CaCO_3$) to increase early strength of cement board. This allows to remove the cement board from the mold just in several minutes.

Conblock heated by CO_2 during pressing process increased the conblock strength (Berger, R.L. *et al*., 1972). Injection of CO_2 gas in to the cement board shortened the pressing time (Simatupang, M.H. and R.L. Geimer, 1990). Hardening process reduced form 8 hours to 5 minutes by CO_2 injection in to the board to speed up the production (Lahtinen, P.K., 1991). CO_2 injection in to the cement board reduced inhibitory effect of several wood species to the cement hydration (Moslemi, A.A. *et al*., 1993). Use of CO_2 injection in supercritical gas phase to reduce curing time the cement board manufacturing increase some physical and mechanical properties of the board (Hermawan (2001a).

Material and Method

Material

All of bamboo species used in this research was taken from Regency of Maros, province of South Sulawesi. 1 to 2 years old of bambu parring (*Gigantochloa atter*), bambu betung (*Dendrocalamus asper*), and bambu tallang (*Schizostachyium brachycladum)* culms were grinded in mill refiner to obtain particles which passed through a 10-mesh screen and remained on a 40-mesh screen for measurement of hydration temperature. Particles used for core layer of cement board manufacturing were particles which passed a 10-mesh screen and remained on 20-mesh screen and for face and back layers were which passed a 20-mesh screen and remained on 40-mesh screen. Type I commercial Portland cement (available on the local market in Makassar) was used as matrix or binder.*)*

Measurement of Hydration Temperature

Bamboo particles mixed with Portland cement and water on the ratio of 1: 13,3:6,65 then stirred to get homogenous paste (Hermawan 2001b). Paste poured in plastic glass and put into airtight styrene foam container. A Thermometer in glass tube (contained barco oil) was put into styrene foam container through a hollow at container cap. Hydration temperature recorded at each 1 hour interval period for 24 hours during the hydration process.

Board Manufacturing and CO2 Injection

Cement board manufactured by mixing bamboo particles, Portland cement and water on the ratio of 1 : 2.5 : 1,25. Targeted density of the cement board was 1 g/cm^3 and targeted thickness was 1 cm. Smoother particles which passed a 10-mesh screen and remained on 20-mesh screen were used for face and back layers, while coarser particles which passed a 10-mesh screen and remained on 20-mesh screen were used for core layer for cement board manufacturing. Particles size ratio of face : core : back was 15% :70 % : 15 %.

Bamboo particles were immersed in water for 48 hours to remove the extractives and dried in room temperature until reached moisture content of 30%. Bamboo particles, Portland cement and water stirred until homogenous mixture obtained. Mixture poured into iron plate of 25 cm x 25 cm x 1 cm that was covered by plastic sheet and pressed for 24 hours during the setting time. Mixture was converted to solid cement board after setting time completed.

Injection of CO_2 in liquid phase into the cement board carried out for curing for 30 minutes. Sample of cement board was put into injection tube and CO_2 flowed into the injection tube. Liquid phase of CO_2 reached by setting the tube temperature at 15^o and tube pressure at 50 kg/cm^2 for 30 minutes. Cement board was removed from the tube and put

into desicator for 15 minutes. Cement board was weighed and put into oven for the next curing at 80° Celsius for 10 hours.

Testing

Samples of cement board were tested for physical and mechanical properties according to Japanese Industrial Standard (JIS) A JIS A 5417:1992. Testing for physical properties included density, moisture content, water absorption, thickness swelling and linear expansion. While testing for mechanical properties included modulus of rupture, modulus of elasticity and internal bond.

Result and Discussion

Hydration Temperature

Result on the hydration temperature during the hydration process of mixture of bamboo particles, cement and water can is shown in hydration curve on Figure 1. Figure 1 shows that maximum hydration temperature of mixture of *Schizostachyium brachycladum* particles, Portland cement and water was 39° C, mixture of *Dendrocalamus asper* particles, Portland cement and water was 38° C and mixture of *Gigantochloa atter* particles, Portland cement and water was 34° C. Based on Kamil Classification (1970), maximum hydration temperature of all of the mixtures was categorized as low. It can be observed from the curve of hydration in Figure 1. that each bamboo species reacted differently with Portland cement. Although time required to reach maximum hydration temperature by mixture of *Dendrocalamus asper* particles, Portland cement and water was shorter (3 hours) than mixture of *Gigantochloa atter* particles, Portland cement and water (7 hours) and mixture of *Schizostachyium brachycladum* particles, Portland cement and water (9 hours), but maximum hydration temperature of mixture of *Schizostachyium brachycladum* particles, Portland cement and water was higher (39° C) than other mixtures.

Low level of maximum hydration temperature of the all of mixtures can be affected by the high content of hemicelluloce and extractives presented in bamboo particles. The compatibility of cement and lignocellulosic materials on the hydration process is influenced by hemicelluloce and extractive content of lignocellulocic materials. Hydration process will be disturbed if cement is mixed with materials containing high content of hemiceluloce and extractives due to the declining exothermic reaction when hydration temperature is released. Hemicelluloce content, calculated from the difference between hollocelluloce and alpha celluloce content, of *Gigantochloa atter* was higher (29.37%) than those of *Dendrocalamus asper* (28.65%) and *Schizostachyium brachycladum* (27.66%) and also extractive content (soluble in alcohol benzene) of *Gigantochloa atter* was higher (4.93%) than those of *Dendrocalamus asper* (4.10%) and *Schizostachyium brachycladum* (3.43%) (Loiwatu, M. dan Manuhuwa, E., 2008 dan Baharuddin, 2013)

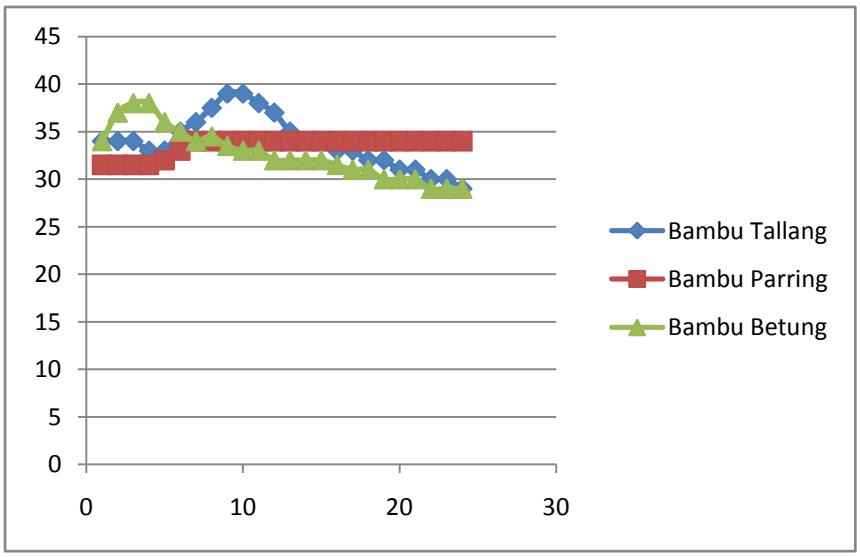

Figure 1. Curve of Hydration Temperature

Physical Properties

Study on the physical properties of the cement boards is shown on Table 1. It was observed from the Table 1. that cement board made from mixture of *Gigantochloa atter* particles, Portland cement and water had most excellent physical properties. Table 1 shows that moisture content of the cement boards for mixture A (*Schizostachyium brachycladum* particles, Portland cement and water) , B (*Gigantochloa atter* particles, Portland cement and water) and C (*Dendrocalamus asper* particles, Portland cement and water) were respectively 3.25%, 3.15%. and 3.62%, while the density were respectively 0.74 g/cm^3, 0.80 g/cm^3 and 0.68 g/cm^3. Difference of cement board density can be affected by the density variation of culm of each bamboo species. In this study, higher density of bamboo species resulted in higher density of the cement board. Culm density of *Schizostachyium brachycladum* was 0.69 g/cm^3, *Gigantochloa atter* was 0.79 g/cm^3 and *Dendrocalamus asper* was 0.62 g/cm^3. Study on cement board density finds that all the cement boards did not achieve the targeted density of 1 g/cm^3. It was supposed to raise the weight of cement boards to complete the targeted density of 1 g/cm^3 by the presence of $CaCO_3$ resulted from reaction of CO_2 injection and $Ca(OH)_2$, but it did not arise while requirement of cement board density in JIS A 5417:1992 is greater than 0.8 g/cm^3. Longer time of CO_2 injection (longer than 30 minutes) might be needed to raise the amount of $CaCO_3$ so that the weight of cement boards become higher. By operating curing temperature at 80^o for 10 hours in this study also lowered the production of calcium silicate hydrate because remaining water was evaporated due to the high temperature. Maximum amount of calcium silicate hydrate cannot be attained and this also did not bring about the desired weight of the cement boards. In conventional process, although setting time of the hydration process of the mixture was completed for 24 hours, remaining water held in the cement boards can be still used for hydration process to produce maximum amount of calcium silicate hydrate during the next 28 days curing in room temperature. Small difference between density of bamboo culm and density of cement board described that density of cement board was mostly influenced by density of bamboo culm, density of cement board may not significantly be influenced by the presence of hydration products such as calcium silicate hydrate and calcium carbonate.

Table 1. Physical Properties of the Bamboo Cement Boards

Cement Board	MC (%)	D (g/cm^3)	WA (%) 24 hours	LE (%) 24 hours	TS (%) 24 hours
Mixture A	3.25	0.74	49.20	0.19	0.87
Mixture B	3.15	0.80	45.11	0.13	0.65
Mixture C	3.62	0.68	57.60	0.27	1.35

Notes :
Mixture A : *Schizostachyium brachycladum* particles, Portland cement and water
Mixtures B : *Gigantochloa atter* particles, Portland cement and water
Mixture C : *Dendrocalamus asper* particles, Portland cement and water

Table 1. Shows also that water absorption, linear expansion and thickness swelling varied between the cement boards. Water absorption of the cement boards can be directly affected by the percentage of cavity volume in the bamboo particles. The percentage of cavity volume in the bamboo particles can be estimated from the bamboo culm density showing that the highest to the lowest percentage of cavity volume were respectively in *Dendrocalamus asper, Schizostachyium brachycladum* and *Gigantochloa atter* particles and this study showed that the highest to the lowest water absorption respectively occured in cement board of mixture of *Dendrocalamus asper* particles, Portland cement and water (57.60%), *Schizostachyium brachycladum* particles, Portland cement and water (49.20%) and *Gigantochloa atter* particles, Portland cement and water and (45.11%). This result noticed that particles of the 3 bamboo species were very higroscopic material because of their high ability to absorp water. Matoke et al. (2012) confirmed that water absortion of bamboo is a disadvantege for composite. This study also found that density of the cement boards was inversely to the water absorption. Although JIS A 5417:1992 does not include water absorption for cement board requirement, it can be used as good prediction to the linear expansion and thickness swelling behavior. As a hygroscopic material, bamboo culm particles absorp water to fill the cell lumen and cell wall. When the cell wall absorb water, cell wall expand depending on the amount of water absorbed. As shown in Table 1., linear expansion and thickness swelling of the cement boards was likely to follow the water absorption trend. Although percentage of of water absorption of the cement boards can be rated as high enough, percentage of linear expansion of cement boards were extremely low and percentage of thickness swelling of the cement boards as well. While rrequirement of thickness swelling of cement board

in JIS A 5417:1992 is lower than 8.3%, it can be observed that cement boards made from all of the 3 mixtures are dimensionally stable. The presence of $CaCO_3$, as a result of reaction CO_2 and $Ca(OH)_2$ filled the tiny cavity in the cell wall or even covered the bamboo particles, together with other hydration products may have controlled the cement boards from swelling.

Mechanical Properties

Study on mechanical properties of the cement boards is shown on Table 2. Contrarily to the physical properties, it was observed from the Table 2. That the most excellent mechanical properties of cement board was found in the mixture of *Schizostachyium brachycladum* particles, Portland cement and water.

Table 2. Mechanical Properties of the Bamboo Cement Boards

Cement Board	IB (kg/cm^2)	MOE (kg/cm^2)	MOR (kg/cm^2)
Mixture A	0.74	5030.50	79.59
Mixture B	0.41	2338.82	49.75
Mixture C	0.18	1339.03	40.12

Notes :
Mixture A : *Schizostachyium brachycladum* particles, Portland cement and water
Mixtures B : *Gigantochloa atter* particles, Portland cement and water
Mixture C : *Dendrocalamus asper* particles, Portland cement and water

Table 2 shows that internal bonding (IB), modulus of elasticity (MOE) and modulus of rupture (MOR) of cement board made from mixture of *Schizostachyium brachycladum* particles, Portland cement and water was superior than cement boards made from other mixtures. In many cement board products, density of cement board can be used as a good indicator to the mechanical properties. The higher the density of the cement board, the better the mechanical properties. As previously stated in this study, higher density of bamboo species resulted in higher density of the cement board. It can be theoretically simply explained that higher density of bamboo particles contains smaller volume on the equal weight of different bamboo particles , so if it is assumed that calcium silicate hydrate produced on the hydration process is equal amount to the all cement boards of different mixture so that the amount of calcium silicate hydrate will be much more to cover the particles or fill the cell cavity and cell wall on the higher density of the cement boards in order to better improve the mechanical properties. However, it did not arise on the cement board of mixture of *Gigantochloa atter* particles, Portland cement and water although this study showed the highest density of cement board was on mixture *Gigantochloa atter* particles, Portland cement and water. Contrarily to the cement board of mixture *Gigantochloa atter* particles, Portland cement and water, although cement board of mixture *of Schizostachyium brachycladum* particles, Portland cement and water had lower density, it showed better mechanical properties than other mixtures. At least, only one main reason to explain this. As shown on the hydration temperature curve in Figure 1., the highest maximum hydration temperature was mixture of *Schizostachyium brachycladum* particles, Portland cement and water specifying that bonding ability of this mixture was better than other mixtures. This better bonding ability was influenced by the lower content of hemicellulce and extractives of *Schizostachyium brachycladum* particles. It might be predicted that the amount of calcium silicate hydrate after hydration process and $CaCO_3$ after injection of CO_2 was higher in cement board of mixture *Schizostachyium brachycladum* particles, Portland cement and water than other mixtures. The higher the calcium silicate hydrate and the calcium carbonate in the cement board, the better the mechanical properties.

It was observed from the mechanical properties that application of CO_2 injection to the cement board in this study has not mostly satisfied the JIS A 5417:1992. As it was desired that higher amount of $CaCO_3$ might be produced after injection of CO_2, in reality it was not. Production of $CaCO_3$ from CO_2 injection depends on the amount of $Ca(OH)_2$ that is resulted from hydration process of water and cement. If the hydration process is hampered by many factors including the presence of hemicelluloce and extractives, less $Ca(OH)_2$ will be produced. Whereas JIS A 5417-1992 requires MOR of ≥ 63 kg/cm2 and MOE of ≥ 24.000, only cement board made from mixture of *Schizostachyium brachycladum* particles, Portland cement and water satisfied MOE requirement.

Conclusion

Physical and mechanical properties of cement board made from different mixture of bamboo particles, cement and water and application of CO_2 injection were various depending on bamboo species. Only cement board made from mixture of *Gigantochloa atter* particles, Portland cement and water satisfied physical properties requirement of JIS A 5417-1992 and no cement board satisfied mechanical properties requirement of JIS A 5417-1992. This indicates that application of CO_2 injection in liquid phase in this study has not effectively controlled desirable physical and mechanical properties of the bamboo cement board.

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GYPSUM BOARD AND CEMENT BOARD AS AN ACOUSTIC MATERIAL FOR BUILDING

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Abstract

In the design of the building, the architect must think of acoustic requirements with the same serious attention and concern poured in thinking about other requirements. Acoustics includes a very broad range, and touch nearly all facets of human life. Thus it is obvious, the acoustics are architectural elements in control of the environment both inside and outside spaces (Doelle and Prasetio, 1993). In the last half-century, architects and designers are all looking for a new acoustic product which are versatility and economical.

The use of solid wood and composite wood materials as acoustics has long been known. Generally of wood used as acoustic panels and placed as floor (including the floor floating), walls, and ceiling. Gypsum board which is a wood processed has been used as an acoustic materials to made a space become soft and comfortable. Gypsum board is very strategic for noise insulation in the room was dominant uses a glass and the concrete wall. Meanwhile, cement board which is also a product of wood composite made from wood particles or other lignocelluloses materials with cement as its glue adhesive; can provide solutions for architecture acoustics issue.

Wood as a material acoustic has long been used, for example, as wall panel, floor, ceiling, until a musical instrument (traditional and modern). Thus, the gypsum board and cement board can be used as acoustic material. The aim of this research to see the comparison of noise absorption between gypsum boards and cement board as acoustic materials.

Keywords: acoustics, gypsum board, cement board, noise absorption

INTRODUCTION

The word of sound has two definitions: (1) physical, sound are pressure deviation, shifting of particles in an elastic medium such as air (referred as objective sound); (2) the physiologically, sound is a sensation of hearing caused by deviation of physically (referred as subjective sound). More precisely, sound are the sensation of hearing passing ears and arises because air pressure deviations. This distortion is usually caused by some object that vibrates, for example string guitar picked or a tuning fork is struck

Meanwhile, there is no distinct definition about acoustics, but it can be said acoustic to be closely associated with sounds and noise. The acoustic more emphasis on environment controlling of sound, and making it comfortable for aural. More details, acoustics related to artificial environment which created for superior than natural conditions, such as concerts or radio studio with sound control will generate an acoustic environment that is not available in natural (Baron, 1993). Controlling sound in architectural had two goals: (1) providing the state of being most favored production, propagation, and acceptance of the sound of the desired in a room used for the various purposes of hearing, or in the open air; (2) counteraction and reduction of noisy (unwanted sounds) and vibrations in sufficient amounts (Doelle and Prasetio, 1993).

Wood (solid wood) and wood composite can be used as material absorbs acoustic because of its ability in a number of sounds in a given space. Wood as an acoustic material very flexible usage in a given space, which can be used as a component of ceiling, wall, floor, or any other components accordance with the needs of the building (Cremer and Muller, 1982). Bucur (1995) explained that the efficiency of sound absorption and reflection from the wooden material depends on structure, surface treatment, mounting, geometry, etc. For example, plywood or particle board can absorb noise in the low frequency region (< 500 Hz), while the porous wood (fiber board) can absorb sound in the frequency of medium to high (2000 - 8000 Hz).

MATERIAL AND METHOD

Material and Instrument

Material used is a gypsum board undersized 120 x 240 x 0.9 cm^3 and cement board undersized 122x 244 x 1.5 cm^3. An instrument used for sound absorption testing is the sound level meter, unit of sound sources, computers/laptops, software tool for sound frequencies, and a stopwatch.

Research Method

1. Making of Objects Test

Gypsum boards and cement board made into a box with three sizes namely 36 x 12 x 12 cm^3; 24 x 12 x 12 cm^3; and 12 x 12 x 12 cm^3; henceforward box of gypsum board and cement board are referred to as test objects.

2. Acoustics Testing

Acoustic testing on the object test is the sound absorption testing by using software of sound tool. Software is connected to the sound source unit that has been programmed on a PC/laptop. Then the sound source unit (speakers) connected with sound receiver (microphone in sound level meter) that is placed in the objects test; and read on the magnitude of sound was accepted at the measurement frequency to get noise reduction index and transmission loss (Figure 1).

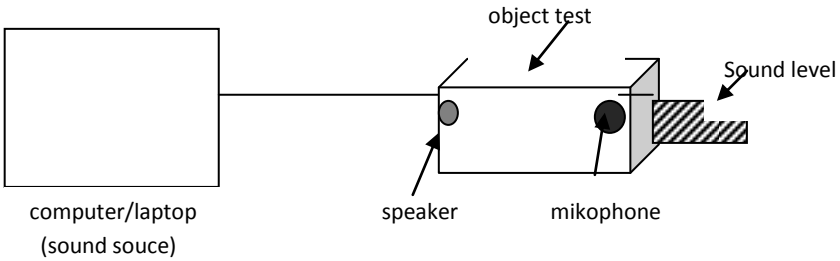

Figure1. The testing scheme on object test

The measurement of frequency at this testing is 125 Hz, 250 Hz, 500 Hz, 1000 Hz, 2000 Hz, 4000 Hz and 8000 Hz; as an important measure in acoustics. In addition, was also performed by measurements of reverberation time on each measurement of frequency to obtain an absorption coefficient. Each frequency measurements will be performed five times repeats on each object test. Before the measurement of sound absorption of the object test, sound absorption measurements done without any objects test.

3. Calculation of Sound Absorption

- Noise reduction index

Noise reduction index is a reduction of sound strength on acoustic board obtained based on ratios among the difference of the sound of (sound source is subtracted sound reflected) with the value of a sound from the sound source, namely:

$$NRI = \{(Ee - Er)/Ee\} \times 100 \quad (1)$$

dimana: NRI = *noise reduction index*
Ee = source of sound energy (dB)
Er = reflected sounds energy (dB)

- Transmission loss

Transmission loss is the transmission medium to inhibit sounds and different on every frequency. This research was lost in transmission obtained based on the results of observations, but can also be calculated by an equation:

$$TL_f = 18 \log M + 12 \log f - 25 \text{ dB} \quad (2)$$

dimana: M = wall mass (kg/m^2)
f = frekuency, Hz

- Absorption coefficient

Absorption coefficient is a measure of the absorption of power per unit area of a surface. Absorption is the comparison between the energy which is not reflected back and overall sound energy coming. Meanwhile, an absorption coefficient is the ability of an ingredient to quell the coming sound, calculated in percent or fractions. Calculation of absorption coefficients determined by the reverberation time from the ingredients being tested, namely:

$$\alpha = (0{,}16\ V)/(T_R\ S) \qquad (3)$$

dimana: α = absorption coefficient
V= volume of room, m^3
T_R = reverberation time
S = energy of sound absorption (dB)

RESULT AND DISCUSSION

Wood as acoustic material has long been used, such as panel of wall, floor, ceiling, until a musical instrument (traditional and modern). On the acoustical research of gypsum board and cement board that was tested is noise reduction index, transmission loss and coefficient of absorption sound.

1. Noise Reduction Index

The result showed that a reduction of sound strength on a cement board higher than gypsum board. Noise reduction index on a gypsum board between 0.14 - 0.23; whereas on a cement board between at 0.24 - 0.30. The research also shows that the bigger of wooden box; hence noise reduction index also higher. Meanwhile, when viewed from the data obtained, there is no difference in noise reduction index on each tested frequency, it is explained that the magnitude of the frequency does not affect the noise reduction index (Table 1 and Figure 2).

Table 1. Noise reduction index on gypsum board and cement board.

Board box (cm^3)	Noise Reduction Index						
	125	250	500	1000	2000	4000	8000
Gypsum							
12x12x12	0,18	0,18	0,15	0,14	0,14	0,14	0,14
21x12x12	0,18	0,19	0,18	0,19	0,19	0,19	0,18
36x12x12	0,22	0,22	0,23	0,21	0,22	0,22	0,22
Cement							
12x12x12	0,24	0,24	0,24	0,24	0,24	0,24	0,24
24x12x12	0,24	0,25	0,25	0,27	0,27	0,25	0,25
36x12x12	0,28	0,28	0,29	0,30	0,30	0,30	0,30

Noise reduction index on gypsum board are lower than cement board; this happens because the cement board is a clone board that uses cement as its adhesive, while gypsum board contains elements of paper. In this case, the pores on the cement board more closer, which in effect results the noise reduction index, is also steeper than gypsum board. In other words, the existence of elements of cement as adhesive on cement board have resulted in better soundproofed properties, even though gypsum board had considered also as material for acoustics.

No occurrence of a significant change in noise reduction index for different frequencies, showed that noise reduction index fixed for all sound frequencies. Meanwhile when viewed the data, there is an increase of noise reduction index seen reduction with the greater volume of the box either on gypsum board or cement board (Table 1 and Figure 2); shows noise caused by the sound can be reduced. This has been explained by Rochmah (1983), that the bigger faintly room is getting better; because the traveled distance of sound pressure

range is long enough, so that it will dampen the sound during the journey. In addition, when the faintly room is getting bigger, resonance does not occur on the wall because it has been occur in the air.

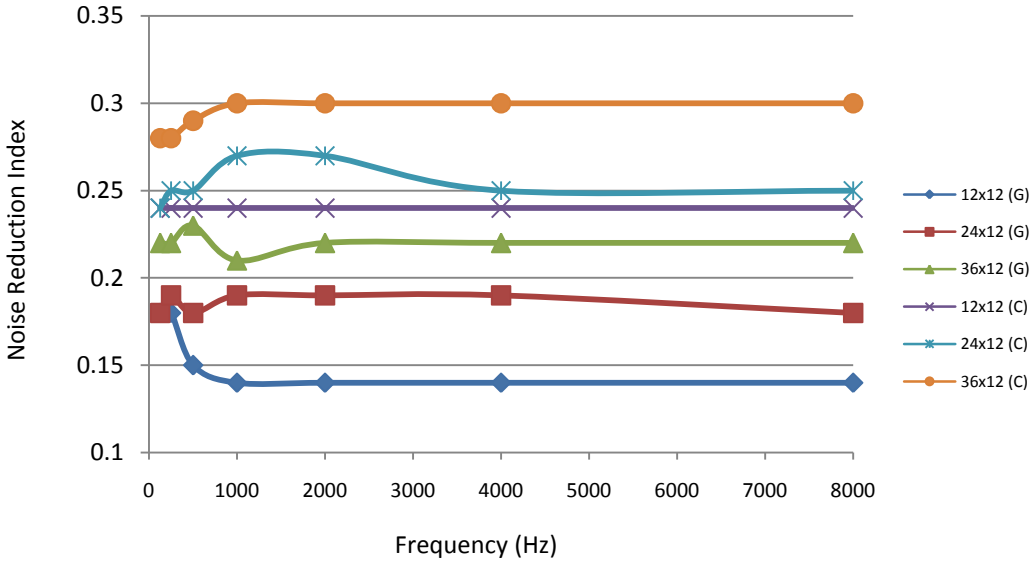

Figure 2. Noise reduction index on gypsum board (G) and cement board (C).

2. Transmission Loss

The result showed that transmission loss on a box of gypsum boards sized 12x12x12 cm^3 between 15.6 - 27,6 dB; box of 24x12x12 cm^3 between 16,1 - 28,6 dB; and box of 36x12x12 cm^3 between 16.7 - 29,4 dB. Meanwhile, transmission lose on a box of cement boards of sized 12x12x12 cm^3 between 11,2 - 20,1 dB; box of 24x12x12 cm^3 between 12,6 - 21,6 dB; and box of 36x12x12 cm^3 between 14.1 - 23,7 dB (Table 2). From the obtained data showed that the lowest of transmission loss occurred at the frequency of 125 Hz and the highest occurred at the frequency of 8,000 Hz. It showed that the higher of sound frequency, so it also greater transmission loss happened.

Table 2. Transmission loss on gypsum board and cement board based on research.

Board box (cm^3)	Transmission Loss (dB)						
	125	250	500	1000	2000	4000	8000
Gypsum							
12x12x12	15,6	17,1	19,6	21,7	24,5	26,8	27,6
24x12x12	16,1	17,4	20,1	22,9	25,1	27,5	28,6
36x12x12	16,7	18,1	20,4	23,8	25,6	27,8	29,4
Cement							
12x12x12	11,2	12,9	14,4	16,7	17,6	19,4	20,1
24x12x12	12,6	13,7	16,6	17,8	18,7	20,0	21,6
36x12x12	14,1	15,1	16,8	18,6	20,2	22,1	23,7

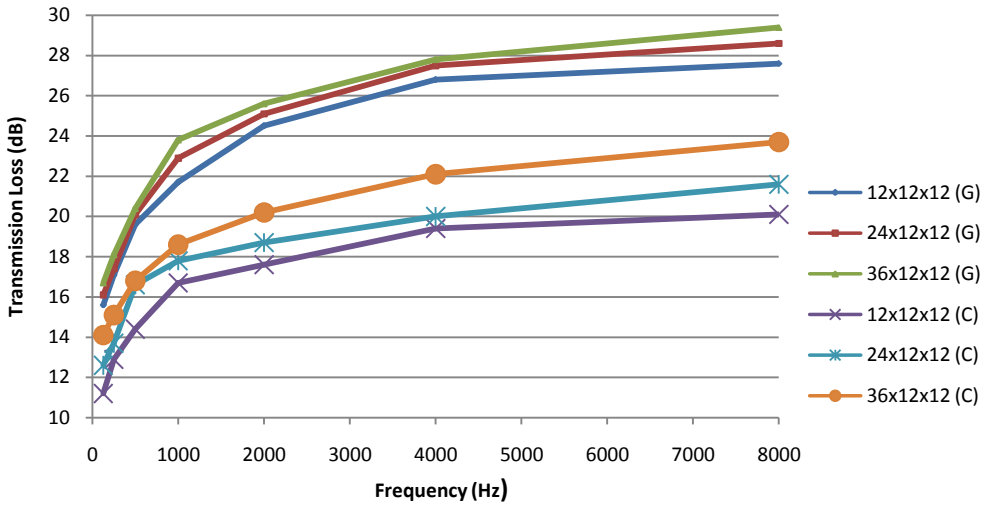

Figure 3. Transmission loss on box of gypsum board (G) and box of cement board (C).

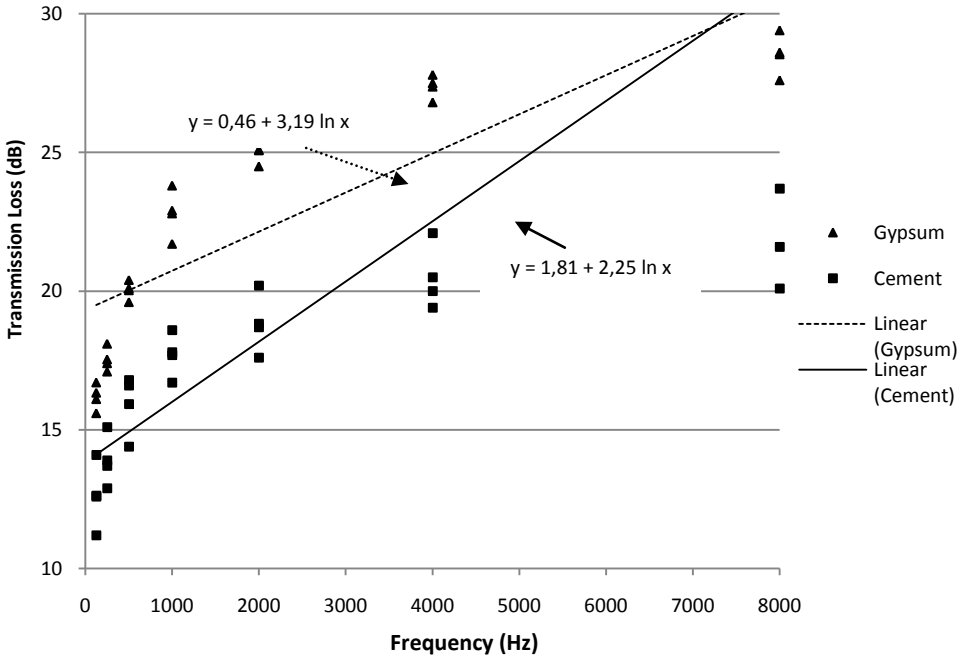

Figure 4. Graphic of relationship between frequency (Hz) and transmission loss on box of gypsum board and box of cement board.

According to Mangunwijaya (1981) and Bucur (1995), the occurrence of a transmission loss of sound by the air molecules in pores resulting in friction one with another, and sound energy is converted into heat energy. A transmission loss on box of gypsum board and box of cement board were different, pointed out that box of cement board has a little pores if compared with a box of gypsum boards (Figure 3). As has been previously mentioned on the noise reduction index; hence this happened because the pores on the cement board closer, which resulted that a transmission loss on these boxes becomes more diminished.

Sound transmission loss in the box of gypsum board and the box of cement board that shows increasing with the increasing of sound frequency; it also shows that there is a positive linear relationship between the sound frequencies and transmission loss (Figure 4). The linear equation had y = 0.46 + 3.19 ln x

for box of a gypsum board and y = 1.81 + 2.25 ln x for box of a cement board; where y is transmission loss (dB) and x is the frequency (Hz).

Table 3. Transmission loss on gypsum board and cement board based on equation (2) (in Material and Method)

Board box (cm^3)	Transmission Loss (dB)						
	125	250	500	1000	2000	4000	8000
Gypsum							
12x12x12	15,6	19,3	22,9	26,5	30,2	33,8	37,4
21x12x12	16,0	19,6	23,1	26,8	30,4	34,0	37,6
36x12x12	16,4	20,0	23,6	27,3	30,9	34,5	38,1
Cement							
12x12x12	23,0	26,6	30,2	33,8	37,4	41,0	44,6
24x12x12	23,2	26,7	30,4	33,9	37,5	41,2	44,9
36x12x12	23,5	27,1	30,7	35,0	37,8	42,0	46,1

Meanwhile, the results of calculation using the equation (2) (see Material and Method), shows a different result with the observations in this research. Based on equation (2) is obtained that the box of cement board greater loss of sound transmission compared to the box of gypsum board (Table 3). This happens because the wall mass in the equation is calculated. Masses of gypsum board wall box (7.30 - 8.02 kg/m^2) smaller than the box of cement board wall (18.45 – 18.70 kg/m^2). As mentioned earlier, the thickness of the gypsum boards box only 0.9 cm, and the thickness of the cement board box 1.5 cm. Thus it is clear that the mass of the wall box from gypsum board is smaller than a box of cement board, so the equation cannot be used to get the magnitude of sound transmission loss for the box of cement board.

3. Absorption Coefficient of Sound

The results showed that absorption coefficient of sound on the box of the gypsum board sized 12x12x12 cm^3 between 0.23 - 0.39; box of 24x12x12 cm^3 between 0.25 – 0.41; and box of 36x12x12 cm^3 between 0.30 - 0.48. Meanwhile, absorption coefficient of sound on the box of cement board sized 12x12x12 cm^3 between 0.17 - 0.31; box of 24x12x12 cm^3 between 0.22 - 0.33; and box of 36x12x12 cm^3 between 0.25 - 0.36 (Table 4). From obtained data, shows that the lowest absorption coefficient of sound occurs at a frequency of 8000 Hz and the highest occurs at a frequency of 125 Hz; except for box of gypsum board sized 36x12x12 cm^3 is the highest sound absorption coefficient occurs at a frequency of 250 Hz. Generally, this indicates that the higher of sound frequency, so the sound absorption coefficient will lower.

Table 4. Absorption coefficient of sound on gypsum board and cement board.

Board box (cm^3)	Frequency (Hz)						
	125	250	500	1000	2000	4000	8000
Gypsum							
12x12x12	0,39	0,34	0,30	0,26	0,25	0,23	0,23
21x12x12	0,41	0,38	0,34	0,34	0,29	0,26	0,25
36x12x12	0,46	0,48	0,40	0,38	0,36	0,33	0,30
Cement							
12x12x12	0,31	0,28	0,26	0,24	0,21	0,18	0,17
24x12x12	0,33	0,30	0,29	0,28	0,27	0,25	0,22
36x12x12	0,36	0,32	0,30	0,30	0,28	0,26	0,25

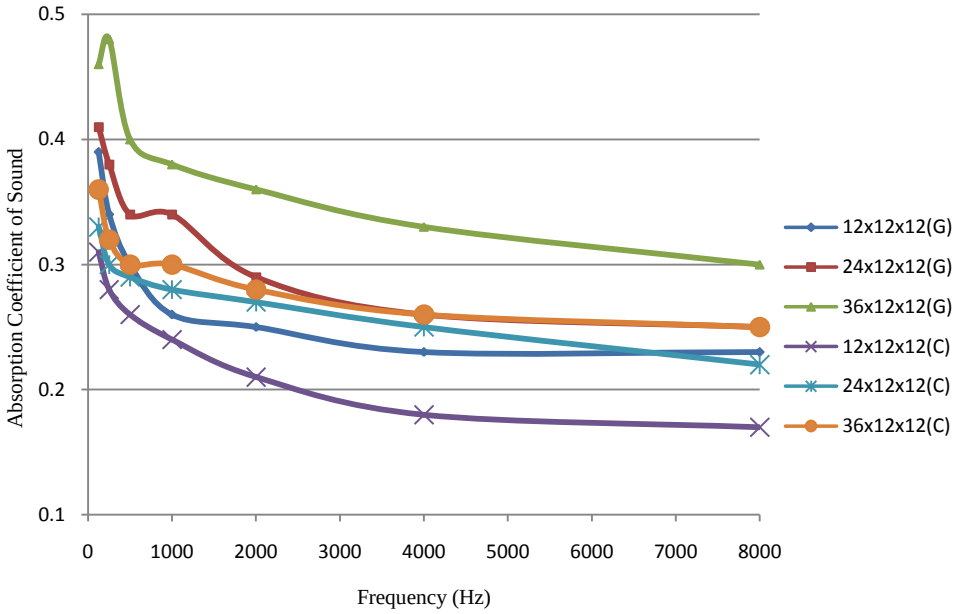

Figure 5. Absorption coefficient of sound on box of gypsum board (G) and box of cement board (C).

According to Satwiko (2003), sound absorption is the ability of a material to quell the sound which comes. Absorption coefficient of sound calculated in percent, or a fractional value between $0 \leq \alpha \leq 1$. From this research is it appears that box of gypsum board has a greater sound absorption coefficient compared with a box of cement board, though no real difference. From this research, it turns out that the sound absorption coefficient of gypsum board and cement board are good enough, so both these materials can be used as acoustic material. The results also indicated that the size of a box less impact on absorptions coefficients of sound, it is probably caused size of the box less/not too different (Figure 5). Nevertheless, the existence of a raw standard not found for sound absorption coefficient for the particular material. Generally the required standard in the sound absorption is designation for a room or building, for example, acoustics needed for a classroom are different with studio room, or between the worship houses with the auditorium building.

The sound absorption coefficient on the box of gypsum board from and the box of cement board that showed decreasing with increasing of sound frequency; it also shows that there is a negative linear relationship between the sound frequency of the with the absorption coefficient of (Figure 6). The linear equation obtained was y = 0.61 - 0.04 ln x for gypsum board and y = 0.46 - 0.03 ln x for the box of cement board; where y is the sound absorption coefficient and x is frequency (Hz).

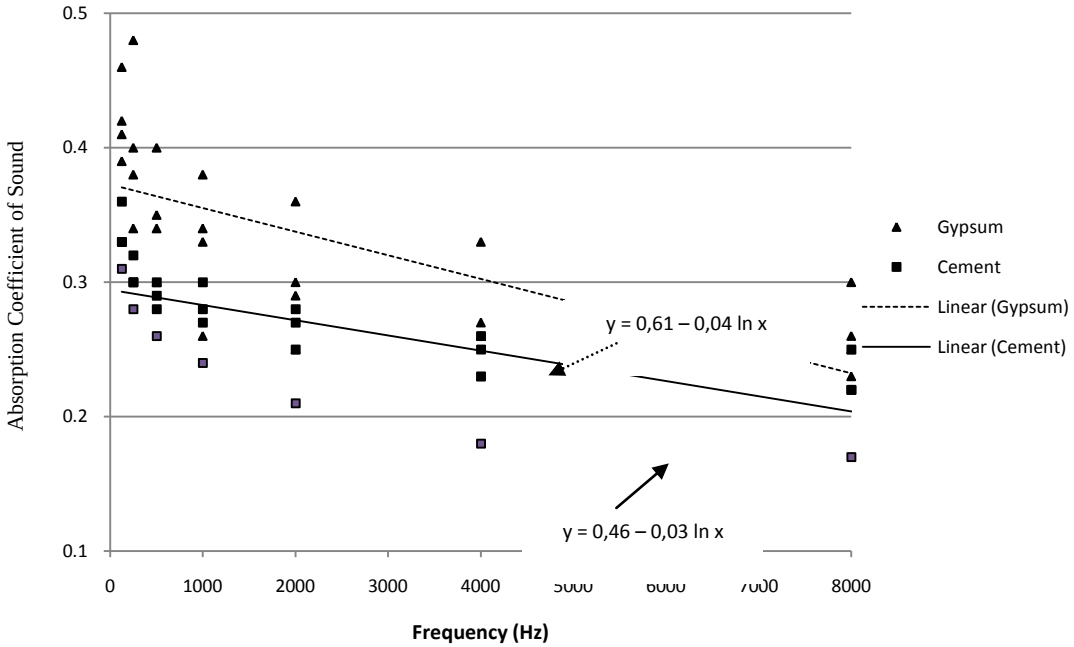

Figure 5. Graphic of relationship between frequency (Hz) and absorption coefficient of sound on box of gypsum board and cement board.

CONCLUSION

No occurrence of a significant change in noise reduction index for different frequency showed that noise reduction index fixed for all sound frequencies. Meanwhile, when viewed that there was increased of noise reduction with the greater volume of box either on gypsum board and cement board; showing that the larger volumes of the box then noise caused by sound can be reduced.

Transmission loss of sound in the box of gypsum board and the box of cement board that shows increasing with the increasing of sound frequency; it also shows that there is a positive linear relationship between the sound frequency with the transmission loss. Meanwhile, the opposite happened on a absorption coefficient of sound. The sound absorption coefficient on the box of gypsum board and the box of cement board that showed decreasing with increasing of sound frequency; and it also shows that there is a negative linear relationship between the sound frequency of the with a absorption coefficient of sound.

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THE FLEXURAL STRENGTH AND BEHAVIOR OF CROSS LAMINATED TIMBER FLOOR

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Abstract

The six cross laminated timber floor specimens was made from hardwood fast growing species, *Albizia falcata*. The cross laminated timber floor was made from three layers of wood plank with specimens variations full and discontinues in middle layer. PvAc glue adhesive was used to laminate between layers. The overall floor dimension was 54 mm x 540 mm x 1260 mm. These fabricated cross laminated timber floor was tested under third point static loading to observe the rigidity and flexural strength of the floor. The rigidity factor was analyzed and presented. The failure mode was flexural and ductile failure. The ultimate load was higher than the proportional load, which is provided a sufficient safety factor. Prediction and suggestion of maximum uniform service live load based on allowable displacement was presented.
Keywords: cross laminated timber, flexural strength, rigidity factor, ductility

1. Introduction

Cross laminated timber become a new element of new building system that was developed in the Europe and North American construction. Some mid-rise building until ten stories can be built in UK, Sweden and Australia. The new building system was made from CLT panels as load bearing wall, floor and roof panel. The panels was light, easy and quick in construction and do not need large foundation. This will save cost of the building, Karacabeyli, 2013. CLT panels commonly made from several odd wood layers stacked crosswise 90° and binding by adhesive. In this study Pv.Ac was used to bind the layers. The floor specimen was made from hardwood fast growing species.

The specimen was made from 3 layers of wood plank with full and discontinuous mid-plank variation, stacked crosswise 90° and glued together as in Fig.1 and 2. The thickness of the wood plank was 18mm, and 180 mm in width, the overall dimension of this CLT floor was 54 mm x 540 mm x 1260 mm. The six cross laminated timber (CLT) floor was tested and analyzed under destructive static two line loading test. Each type of floor or variation has 3 specimens. The static test result was load-displacement curve which is convert to moment-displacement curve. Observation for static loading test was done at proportional and ultime load.

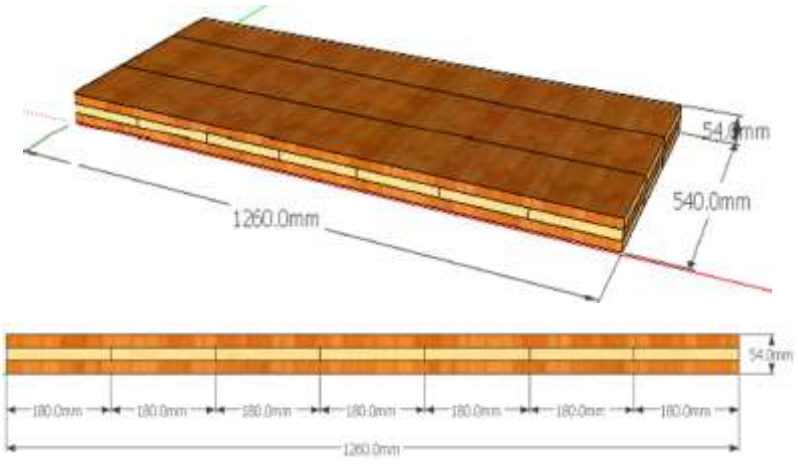

Figure 1. CLT floor specimen and longitudinal cross section with full mid-layer

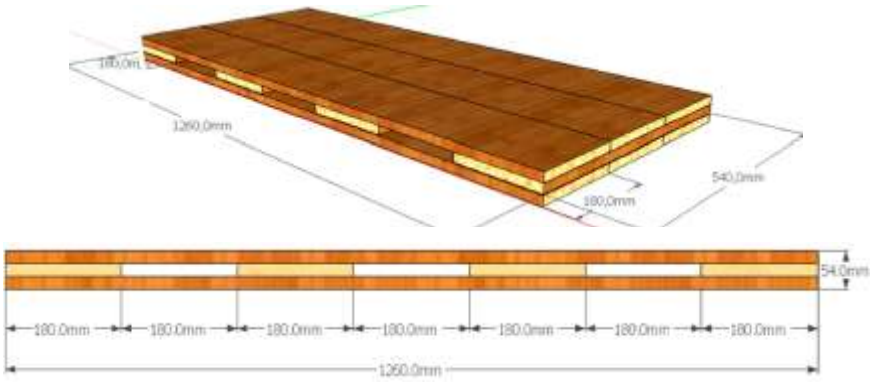

Figure 2. CLT floor specimen and longitudinal cross section with discontinous mid-layer

2. Materials and method

The specimen was made from *albasia (Albizia falcata sp.) wood plank* with a cross section dimension of 18 mm x 180 mm. The properties were tested through some small clear specimens based on the ASTM D143. The specific gravity was 0.31 and the modulus elasticity was 5162 MPa. The MOR was 35 MPa. The CLT floor was fastened by PvAc glue, the shear strength of the glue was tested with different two contact plane, one the two contact planes paralel to the grain and secondly the grain of contact planes was perpendicular each other, the shear strength result was tabulated in Table 1. The average moisture content was in between 14-16%.

Table 1. Shear strength of the PvAc glue, Natalia, 2012.

paralel			perpendicular		
No	F_v (MPa)	Fv_{avr} (MPa)	No	F_v (MPa)	Fv_{avr} (MPa)
S1	3,56	3,84	T1	2,36	2,35
S2	4,50		T2	2,08	
S3	5,24		T3	2,17	
S4	2,04		T4	2,79	

The construction of the CLT floor layer by layer was illustrated e.g. for discontinuous mid-layer as in Figure 3. The weight of the CLT floor was only 20 kg/m^2 for full mid-layer and 18 kg/m^2 for discontinuous mid-layer.

Figure 3. The construction of CLT floor.

Testing Methods

This research based on the experimental study. The specimen was tested under third point loading test regarding to ASTM D198-05a as illustrated in Figure 4 and Figure 5. The central span displacement was measured using LVDT. The clear span for testing was 1050 mm.

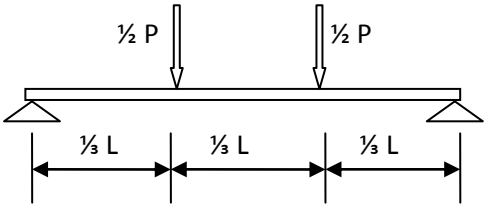

Figure 4. The schematic of floor on the third point loading test, ASTM D198-05a

Figure 5. CLT floor under third point loading test

The calculation of central point displacement, Δ, due to the third point loading and by neglected the shear deformation, Gere, 2001 was:

$$\Delta_p = \frac{23 \cdot P_p \cdot L^3}{1296 \cdot (EI_{xe})} \quad (1)$$

The effective rigidity (EI_{xe}) of floor from static third point loading test can be calculated by equations (2).

$$(EI_{xe}) = \frac{23 \cdot P_p \cdot L^3}{1296 \cdot \Delta_p} \quad (2)$$

where (EI_{xe}) = effective floor rigidity (N.mm^2)
P_p = total load (N/mm^2)
L = clear span (mm)
Δ_p = displacement at proportional load (mm)

3. Results

The result was plotted as load vs. displacement curves as in Figure 6 and Figure 7, and the load at allowable displacement (P_a), proportional load (P_p) and ultimate load (P_u) was observed. The allowable displacement was taken as 1/300 L.

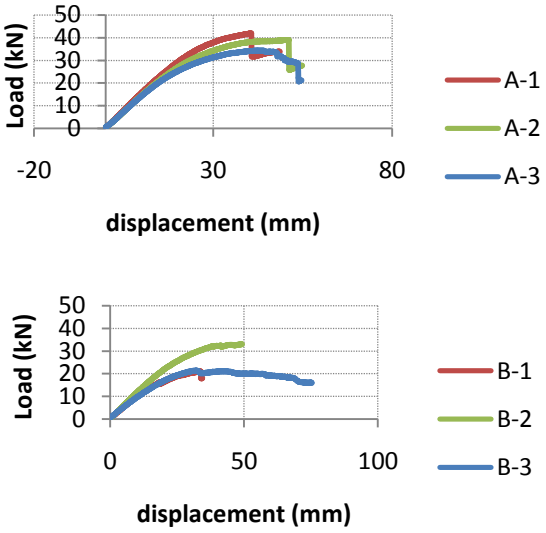

Figure 6. Load vs. displacement curve, Natalia, 2012.

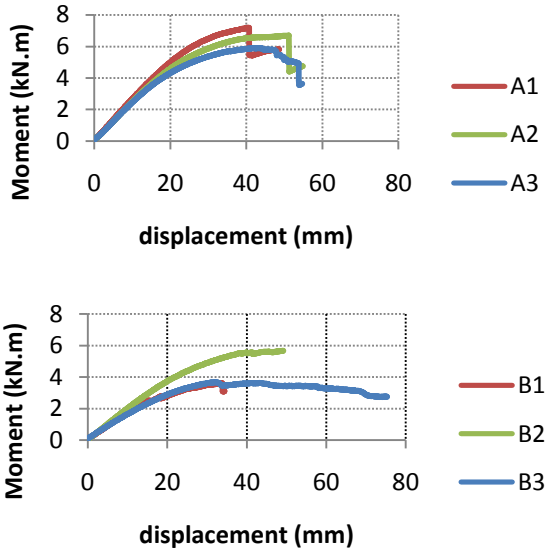

Figure 7. Moment vs. displacement curve, Natalia, 2012.

Figure 6 is the curve for full mid-layer and Figure 7 is for discontinuous mid-layer, the load, moment, displacement at each load condition and ductility was tabulated as in Table 2. The ductility value shows that the CLT floor was not failed in brittle fashion.

Table 2. Load, moment, displacement and ductility

No	P_a (kN)	M_a (kN.m)	Δ_a (mm)	P_p (kN)	M_p (kN.m)	Δ_p (mm)	P_u (kN)	M_u (kN.m)	Δ_u (mm)	μ_u (mm)
A1	5.39	0.94	3.5	24.79	4.34	18.64	41.06	7.19	40.44	2.17
A2	4.91	0.86	3.5	16.77	2.94	12.81	38.20	6.68	51.16	3.99
A3	4.93	0.86	3.5	18.71	3.27	14.71	33.64	5.89	42.40	2.88
B1	3.61	0.63	3.5	12.55	2.20	12.88	20.63	3.61	33.72	2.62
B2	4.03	0.71	3.5	14.81	2.59	14.21	32.31	5.65	49.24	3.47
B3	3.77	0.66	3.5	12.55	2.20	14.94	20.94	3.66	32.08	2.15

Failure Modes

The failure mode of all the floor specimen occurred at ultimate load mainly in tension due to bending, as in Figure 8 and 9.

Figure 8. Tension failure at the bottom of CLT floor

Some part of the glue was separated, this is because when the test was done the age of gluing was less than one week for specimen B2 and B3.

Figure 9. Tension failure on the middle span of CLT floor (left) and failed in the PvAc glue (right).

4. Analysis and Discussions

Based on the elastic condition, the rigidity of the floor was analyzed.

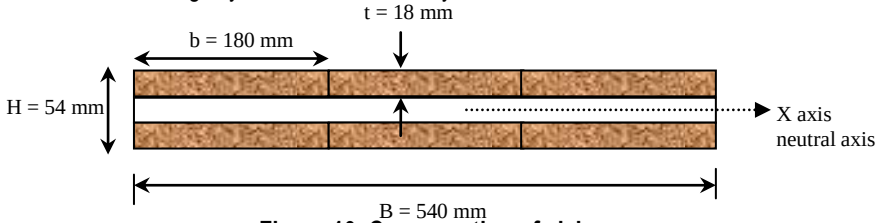

Figure 10. Cross section of slab

The second moment of area I_{xe} was calculated based on the first and third layer, the mid-layer was neglected. Because the layers are not a solid section, the k factor as rigidity correction factor was introduced in the equation (3) and (4).

$$I_{xe} = 6(\frac{1}{12} b \cdot t^3 + k \cdot b \cdot t(t)^2) \quad (3)$$

Then,

$$I_{xe} = \frac{1}{2} b \cdot t^3 (1+12k) \quad (4)$$

Table 3. Rigidity correction factor and actual ultimate bending stress

No	b (mm)	t (mm)	k	Ix (mm⁴)	L (mm)	P_p (N)	Δ_p (mm)	E (MPa)	P_u (N)	M_u (N.mm)	S (mm³)	f_{bu} (MPa)
A1	180	18	0.76	5311786	1050	24789	18.64	5162	41062	7185850	193156	36.5
A2	180	18	0.74	5185814	1050	16773	12.81	5162	38196	6684213	188575	34.8
A3	180	18	0.72	5059843	1050	18708	14.71	5162	33639	5886738	183994	31.4
B1	180	18	0.53	3863117	1050	12554	12.88	5162	20629	3610075	140477	25.2
B2	180	18	0.58	4178045	1050	14813	14.21	5162	32311	5654355	151929	36.5
B3	180	18	0.45	3359232	1050	12554	14.94	5162	20939	3664269	122154	29.5

The k factor was found by substitute equation (2) using experimental data and equation (4).

Equivalent uniform load (q) conversion was done base on the allowable displacement at serviceability with Δ_i = 1/300 L, this is because the deformation or stiffness of the floor was critical. I_{xe} was calculated from equation (4) using k factor from table 3, and q can be found from the equation (5).

$$\Delta_i = \frac{5 \cdot q \cdot L^4}{384 \cdot (EI_{xe})} \quad (5)$$

The result for both types of CLT floor was shown in the chart in Figure 11. The CNLT floor with full mid-layer and 2.0 m in length can carry more than 2.0 kPa, but for CLT floor with discontinuous layer only can carry 1.0 kPa. The uniform load that can carry for other length may be obtained using the chart in Figure 11.

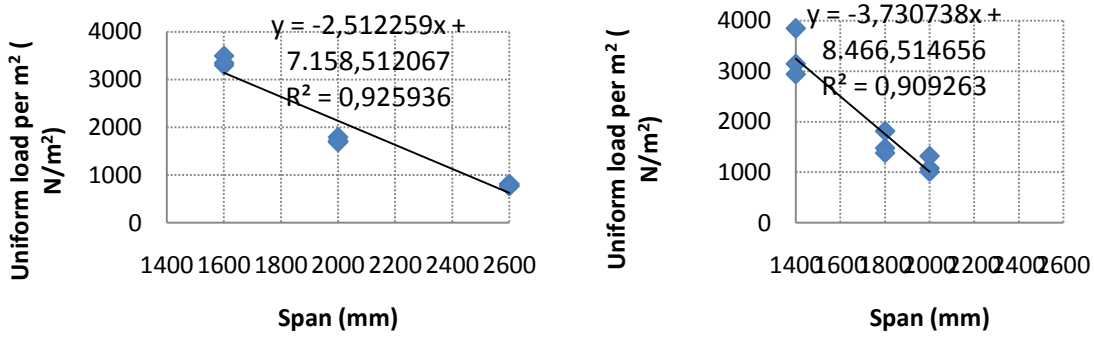

Figure 11. Equivalent uniform load for CLT floor, Natalia, 2012.

5. Conclusions

- The CLT floor using *albasia* wood plank was suitable for housing floor, the load and span can be determine using the equivalent uniform load chart.
- The k factor for CLT with discontinuous mid-layer was significantly lower than the full mid-layer.
- The ductility factor of this CLT floor guarantee that the floor doesn't failed in brittle fashion.
- The weight or mass of the floor was very light and can be used to minimize the earthquake inertial load.

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QUINONES DISTRIBUTION in JUVENILE TEAK WOOD

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Abstract

Quinones and their derivatives are the main causes on the natural termite resistance in teak wood. By using different termite test methods, the previous paper in this series reported on the termite resistance of teak trees of juvenile ages (8-, and 22-year-old trees). In this study, the radial distribution of quinones (tectoquinone, lapachol, desoxylapachol and its isomer) and other components in the different extracting solvents (*n*-hexane, ethyl acetate, and methanol) were analyzed by means of gas chromatography. Appreciable tree to tree variations are observed in extractive component contents even in the same stand. Each solvent gave different tendencies to analysis of variance of component contents. Significant differences in desoxylapachol or its isomer, and squalene content were found among the outer heartwood of 8-, and 22-year old trees, as well as between the inner and outer parts of the heartwood. The highest correlation degree between extractive content and its components was measured in the tectoquinone content (r=-0.68). By using paper disc method, only modest correlations were observed between the mass loss and the content of isodesoxylapachol (r=-0.60) in the sapwood region whereas no significant corellations were measured in the heartwood region.
Keywords : *Tectona grandis,* antitermitic activities, extractive, tectoquinone, *Reticultermes speratus*

Introduction

Teak (*Tectona grandis L. f.*) is a fancy hardwood prized for its workability and high natural durability. Teak grows naturally throughout southeastern Asia and widely planted in all tropical regions. In Indonesia, large teak community forests have been established and managed for fast growth with trees harvested in a rotation period of less than 30 years. The wood from these trees is usually consists larger proportion of sapwood and juvenile wood. This results in a reduced market value, although the technical data with regard to wood quality of young stage trees is still limited. Unfortunately, most studies focused on heartwood with little consideration given to sapwood, although several studies of fast-grown teak trees have shown that a high sapwood fraction is present. One report by Bhat and Florence (2003) demonstrated the lower durability of juvenile teak wood against fungi.

In teak, natural durability is ascribed to the presence of toxic extractives mainly quinone (Sandermann and Simatupang 1966; Rudman and Gay 1961). Difference in natural durability may be related to the concentrations of toxic extractives. In a preliminary result (Lukmandaru 2013), samples of young teak wood trees (8- and 22-year-old trees) were compared to mature wood (51-year-old trees) for antitermitic activities evaluation. That experiment exhibited the wide variation in antitermitic properties on the basis of tree age and radial direction. Further, the results also demonstrated the differences between wood block (natural condition) and wood extracts (paper disc/in vitro) method in termite tests.

In this report, the radial distribution of quinones of teak was investigated on the corresponding samples of those trees to estimate the effect of extractives on the relative antitermitic activities of the wood. This research used three different solvents on the basis of polarity for extracting the wood by cold extraction. The other purposes of this study were to relate the amount of the major compounds to the extractive content, as well as to relate the amount of the active compounds to previous data on anttermitic properties (paper disc method).

Materials and Methods

Preparation of samples

Nine Javanese teak trees were collected previously (Lukmandaru 2013) for this study. The members of the 8-year group (trees 1 to 5) and 22-year-old group (trees 6 to 9) were felled from farm forest (Jogja Province). A 5 cm thick disc was removed at approximately breast height from the trees which were free of signs of incipient decay and colour variations. Each disc was divided into five parts: outer sapwood (OS), inner sapwood (IS), outer heartwood (OH), and inner heartwood (IH). With the limited amount of suitable material available, the IH zone in the 8-year-old discs was excluded. Sections from two opposite radii were converted into wood meal by drilling and were then combined to form a

single sample in order to minimize variation between radii, if any. The wood meal samples were then ground to 20-40 mesh size for chemical analyses and determination of the content of the extractives.

GC and GC-MS analysis

Wood meal samples (one gram oven dry weight) were extracted at room temperature with 10 ml *n*-hexane (C_6H_6) and retained for one week. The extracts of *n*-hexane (C_6H_6), EtOAc, and MeOH (concentration of 100 mg/mL) were analyzed using a Hitachi G-3500 GC equipped with FID and NB-1 capillary 30-m column. Operation temperature was 120−300ºC with a heating rate of 4ºC /min and held at 300ºC for 15 min. Injector and detector temperatures were set at 250ºC. Helium was used as the carrier gas, the split ratio was 80:1, and the injected volume was 1.0 µL. For quantification of individual substances, calibrations were made using known amounts of standard tectoquinone (2-methyl antahraquinone). The amounts of components are expressed as mg per 100 g of oven dry weight. Pure sample of squalene and lapachol purchased from Kanto Chem were also was used for confirmation. Chemical analyses of ethyl acetate (EtOAc) and methanol (MeOH) extracts were obtained separately in the same manner as described for the C_6H_6 extract.

The identification of constituent compounds was based on their mass spectra and gas chromatographic retention behavior. GC-MS GC-MS analysis on a Shimadzu QP-5000 with operation conditions being similar to GC analysis. The MS operating parameters were temperature ionization voltage of 70 eV, transfer line temperature at 250ºC, and scan range of 50–500 atomic mass unit. Deoxylapachol or its isomer was identified by comparison of their mass spectra with those from previous studies by Windeisen et al. (2003) and Perry et al. (1991). From the contents of tectoquinone, lapachol, desoxylapachol and its isomer, the total quinone content (TQC) was calculated.

Extractives Content Determination

The remainder of the extract taken for extractive analyses was filtered and the residue was washed three times with 10 ml of solvent. The extract was concentrated in a rotary film evaporator, dried and weighed to determine the extractives content. The extractives content was calculated as a percentage (w/w) of moisture-free wood meal.

Termite resistance test

The natural termite resistance data were taken from the previous report (Lukmandaru 2013). A petri dish containing 20 g moistened and sterilized sea sand was used as a container test. Paper were impregnated with chloroform solution containing each extract of the test fractions. The treatment retention was 5 % (w/w) per disc. The control discs were impregnated with chloroform only and dried with the same manner. Fifty worker *Reticulitermes speratus* Kolbe termites were introduced into the petri dish. The petri dishes were placed in a dark chamber at 27 ^{0}C and 80 % relative humidity. After 10 days the disc were taken out, dried and the weight loss was determined. This procedure was replicated three times for each sample for each sample for a total of 93 observations. Dead termites were counted in the first day and at the end of observation. The mass loss since the start of the experiment was determined.

Statistical analysis

The variation in the extractive and extratcive component contents was analyzed using general linear models procedure by two-way (tree age and radial direction factors) analysis of variance (ANOVA) followed by Duncan's multiple range test (p = 0.05). The relationships between the dependent variables or observed were studied with a Pearson's correlation analysis. All statistical calculations were conducted using SPSS-Win 10.0.

Results and discussion

Distribution of extractives as related to natural durability

The gas chromatogram of heartwood EtOAc extract is shown in Figure 2. The major compounds detected in those chromatograms were lapachol, tectoquinone, desoxylapachol and its isomer (isodesoxylapachol), and squalene. All these compounds have been reported as teak components (Sandermann and Simatupang 1966, Windeisen et al. 2003, Lukmandaru and Takahashi 2009).

The quantification of three extracts was presented in Table 1-3. As expected, the extractive content of all of the tree age groups followed a general pattern of increasing from pith (IH) to the OH, then decreasing towards the OS. The highest amount levels of squalene, deoxylapachol and its isomer were measured in C_6H_6 extracts whereas tectoquinone content was determined in MeOH extracts. It was noted also that lapachol was not detected in C_6H_6 extracts but it was detected in other extracts although in trace amounts. In the sapwood region, particularly, the comparatively higher total

quinone content levels were found in MeOH extracts. The current results also showed wide variation by examining standard deviations, even in trees from the same sites. This meant that teak may not always have a high amount of certain compounds.

Factorial analysis of variance (Table 4) revealed different result among the extracts. For example in dexylapachol content, significant interactions were calculated in both C_6H_6 and EtOAc extract but not in MeOH extracts. Further, with regard to tectoquinone content, radial variation affected signicantly in EtOAc extract, a significant interaction was found in MeOH content while no significant effects of tree age and radial direction in C_6H_6 extracts. Interactions was found in in all extracts, however, in total quinone content. Those differences reflects the specific capacity in each extract to dissolve the main components of teak. In this regard, the most effective solvent should be choosen by considering the most extracting solvent.

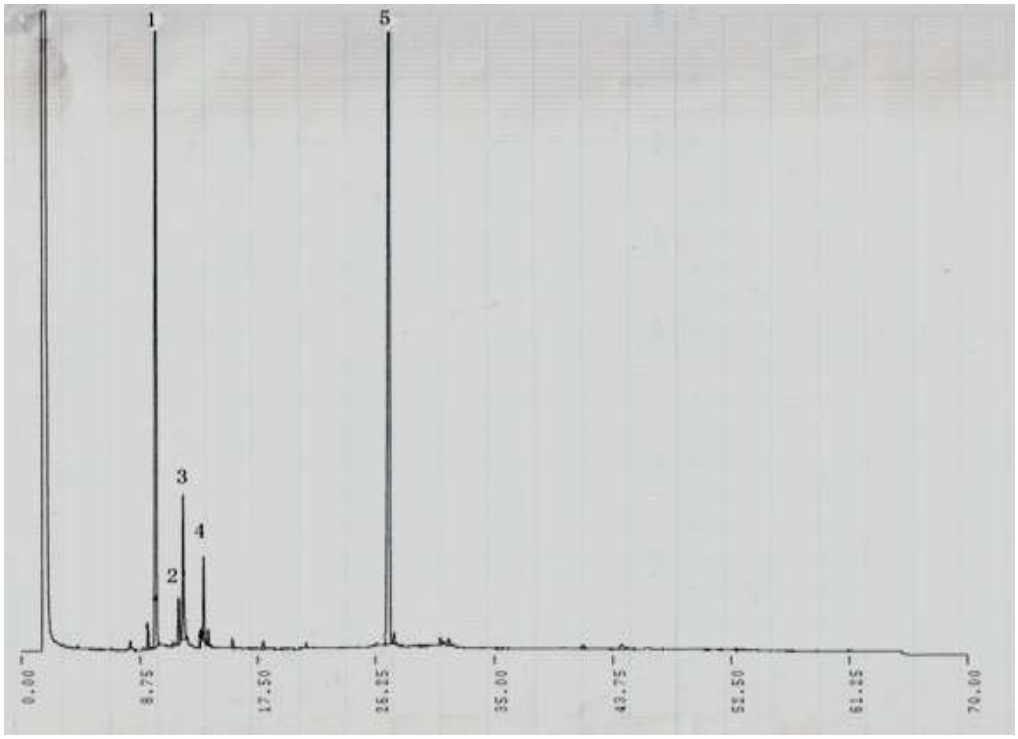

Figure 1. Gas chromatogram of teak from ethyl acetate extract of heartwood. Five major compounds are indicated : peak 1 (R_t 10.1) & 3 (R_t 12.1) = deoxylapachol and its isomer; peak 2 (R_t 11.8) = lapachol; peak 4 (R_t 13.7) = tectoquinone; and peak 5 (R_t 27.4) = squalene.

Table 1. Contents of major components (mg/100 g of oven-dry wood) in the *n*-hexane soluble extracts of teakwood trees aged 8 and 22 (radial position).

Components	Radial position						
	Outer sapwood		Inner sapwood		Outer heartwood		Inner heartwood
	8 y	22 y	8 y	22 y	8 y	22 y	22 y
Desoxylapachol	0 (0)a	10.25 (10.55)b	5.65 (10.63)b	5.37 (6.18)b	9.46 (6.70)b	205.02 (144.70)c	65.85 (83.43)b
Lapachol	0 (0)	0 (0)	0 (0)	0 (0)	8.16 (20.00)	10.37 (12.95)	3.47 (4.14)
Isodesoxylapachol	3.50 (4.56)	0.35 (0.70)	3.66 (2.97)	2.90 (3.10)	11.05 (7.03)	331.15 (539.30)	18.95 (29.97)
Tectoquinone	0 (0)	3.90 (2.61)	2.78 (4.72)	4.15 (3.59)	42.06 (65.05)	19.37 (6.94)	26.87 (19.40)
Squalene	5.51	22.40	16.23	100.45	110.25	473.78	454.72

	(4.84)d	(13.11)e	(7.21)e	(47.95)f	(89.08)f	(346.94)g	(453.15)g
Total quinone content	3.50	14.50	12.10	12.42	70.75	565.92	115.15
	(4.56)h	(12.24)h	(14.25)h	(5.16)h	(92.52)i	(649.97)j	(130.41)i

Table 2. Contents of major components (mg/100 g of oven-dry wood) in the ethyl acetate soluble extracts of teakwood trees aged 8 and 22 (radial position)

Components	Radial position						
	Outer sapwood		Inner sapwood		Outer heartwood		Inner heartwood
	8 y	22 y	8 y	22 y	8 y	22 y	22 y
Desoxylapachol	0 (0)a	0.30 (0.21)b	0.02 (0.04)b	1.00 (0.91)c	1.10 (1.70)c	70.70 (55.70)d	11.10 (12.19)c
Lapachol	0.10 (0.13)	trace	0.70 (0.84)	0.60 (0.39)	3.80 (4.54)	29.60 (51.30)	8.00 (12.85)
Isodesoxylapachol	0.30 (0.28)e	0.40 (0.78)e	0.50 (0.75)e	1.30 (1.62)e	4.60 (3.41)f	77.8 (76.50)g	13.60 (10.82)f
Tectoquinone	0.10 (0.17)h	0.90 (1.16)h	1.20 (2.77)h	3.10 (5.14)h	20.20 (20.63)i	44.8 (43.75)i	20.70 (24.40)i
Squalene	0.60 (0.56)j	1.30 (1.15)j	2.00 (3.09)j	10.90 (8.80)j	14.90 (26.14)j	75.80 (29.12)k	36.20 (24.73)k
Total quinone content	0.50 (0.19)l	1.60 (2.07)l	1.70 (4.08)l	2.50 (5.00)l	30.00 (26.83)m	490.00 (646.89)n	50.00 (57.15)m

Table 3. Contents of major components (mg/100 g of oven-dry wood) in the methanol soluble extracts of teakwood trees aged 8 and 22 (radial position)

Components	Radial position						
	Outer sapwood		Inner sapwood		Outer heartwood		Inner heartwood
	8 y	22 y	8 y	22 y	8 y	22 y	22 y
Desoxylapachol	18.5 (31.05)	7.00 (4.00)	39.20 (74.11)	21.60 (39.41)	2.00 (2.43)	49.90 (98.16)	38.60 (39.27)
Lapachol	0.69 (0. 96)	5.00 (4.81)	1.10 (1.44)	3.80 (6.42)	2.50 (3.64)	41.10 (75.63)	5.40 (7.08)
Isodesoxylapachol	2.80 (2.67)	1.90 (3.21)	3.90 (2.45)	2.80 (3.82)	4.30 (4.04)	25.10 (36.17)	18.60 (20.48)
Tectoquinone	13.10 (20.62)a	10.90 (17.97)a	7.70 (14.79)a	14.20 (15.10)a	17.80 (10.82)a	101.60 (60.69)b	53.80 (17.92)b
Squalene	2.10 (2.75)c	7.30 (3.64)c	5.60 (4.19)c	26.90 (20.65)dc	22.00 (21.95)dc	143.80 (45.23)d	97.20 (44.52)d
Total quinone content	33.30 (37.77)e	25.00 (23.80)e	50.00 (76.42)e	42.50 (59.09)e	25.00 (17.61)e	215.00 (165.43)f	112.50 (60.21)f

Note for Table 1-3 : Mean of 5 trees (8 years old) and 4 trees (22 years old), with the standard deviation in parentheses. The same letters in the same row are not significantly different at $p < 5\%$ by Duncan's test. tr = trace (detected, the value < 0.01 %).

Table 4. Factorial analysis of variance results (probability) for three different extracts (*n*-hexane, ethyl acetate, and methanol).

Components	Source of variation		
	Tree age	Radial direction	Tree age x radial direction
a) *n*-hexane extract			
Desoxylapachol	<0.01**	<0.01**	<0.01**
Lapachol	0.84	0.16	0.96
Isodesoxylapachol	0.12	0.12	0.09
Tectoquinone	0.59	0.09	0.54
Squalene	0.04*	<0.01**	0.13
Total quinone content	0.05	<0.01**	0.02*
b) Ethyl acetate extract			
Desoxylapachol	<0.01**	<0.01**	<0.01**
Lapachol	0.13	0.15	0.11
Isodesoxylapachol	0.01*	<0.01**	<0.01**
Tectoquinone	0.21	<0.01**	0.31
Squalene	<0.01**	<0.01**	<0.01**
Total quinone content	0.06	0.03*	0.04*
c) Methanol extract			
Desoxylapachol	0.74	0.84	0.31
Lapachol	0.12	0.26	0.23
Isodesoxylapachol	0.24	0.15	0.16
Tectoquinone	<0.01**	<0.01**	<0.01**
Squalene	<0.01**	<0.01**	<0.01**
Total quinone content	0.04*	0.05	<0.01**

** Significant at 1 % level, * significant at 5 % level

By ANOVA, in the C_6H_6 soluble extracts, the significant differences between IS and OS were measured in squalene contents while in the EtOAc and MeOH soluble extracts, no significant differences were found. In the C_6H_6 and EtOAc soluble extracts between the OH and IH, significant differences were found in desoxylapachol and its isomer as well as TQC contents whereas no significant differences were determined between IH and OH in any component contents in the MeOH soluble extracts. The tree age factor affected signicantly in desoxylapachol, isodesoxylapachol, squalene, and TQC contents in the heartwood region. Both in the C_6H_6 and EtOAc soluble extracts, the tree age factor affected signicantly desoxylapachol, isodesoxylapachol, squalene, and TQC contents in the heartwood region. In the sapwood region, the same tendency was found also in squalene content in the C_6H_6 extracts and desoxylapachol content in the EtOAc and MeOH extracts. In the MeOH extracts, it is noted that tree age factor affected significantly tectoquinone contents in the heartwood region.

As expected, the interaction in the C_6H_6 and EtOAc extract showed that the highest deoxylapachol and TQC contents were found in the outer heartwood of 22-year-old trees while no significant differences were found between the IH of 22- and OH of 8- year-old trees. Different tendencies between C_6H_6 and EtOAc extracts were seen in squalene isodesoxylachol amounts. In the sapwood region, it is noted that IS of 22-year-old trees gave the highest amounts in squalene content in C_6H_6 and MeOH extracts as well as desoxylapachol content in EtOAc extracts. In the MeOH extracts, with regard to tectoquinone content, significant differences were counted merely between sapwood and heartwood regions as the highest tectoquinone content were measured in the heartwood of 22-year-old trees. Further, on the basis of interactions, it is also revealed that the highest TQC were found in the heartwood of 22-year-old trees.

The OH from 8-, and IH from IH from 30- trees were formed in approximately the same growing seasons (juvenile region, 4–6th ring); this study revealed the significant differences in the squalene (C_6H_6 and EtOAc soluble extracts), tectoquinone and TQC levels (MeOH soluble extracts). Sandermann and Dietrichs (1959) observed that the concentration of tectoquinone is highest in the center of heartwood. Although the highest tectoquinone level was measured in the OH region of both 22-old trees, the ANOVA revealed that there was not a statistically significant difference with those in the OH and IH.

Previous communication (Lukmandaru 2013) exhibited the less antitermitic activity in the hearwood parts compared to sapwood in all extracts. Further, it was showed that the OH was more resistant compared to the IH in C_6H_6 and EtOAc soluble extracts. As would be expected, the sapwood values were significantly lower than heartwood for desoxylapachol, isodesoxylapachol, tectoquinone, squalene, and TQC contents. Levels of deoxylapachol, and TQC in the OH were significantly higher than in the IH of 22-year-old groups. On the basis of radial direction and tree age, no statistical differences were observed with regard to termite mortality rates in the MeOH extracts. This was unexpected results as there was a significantly higher tectoquinone content in the heartwood of 22-year-old tree samples. It is thought that MeOH extracted more extractives than other solvents so that more compounds, especially non-quinones, affected the behaviour of mortality rates in the previous experiment.

As lapachol, desoxylapachol, and tectoquinone have been reported to be active against termites (Lukmandaru 2012; Sandermann and Simatupang 1966; Rudman and Gay 1961), this finding confirms that teak from community forest trees begin producing toxic constituents at the young tree stage. The low amounts of toxic compounds in the sapwood and inner heartwood corresponds reasonably well with Da Costa et al. (1959), Lukmandaru and Takahashi (2008), and Rudman et al. (1967), who reported that the wood regions near pith and sapwood were much less resistant to termite attack than the outer heartwood by using wood blocks method. Rudman et al. (1958) concluded that, although tectoquinone exhibited strong antitermitic properties, this compound is not the sole cause of termite resistance. The considerable amounts of desoxylapachol and its isomer identified in this study suggests that these compounds, along with tectoquinone, play an important role in generating resistance to termites. Da Costa et al. (1958) reported that termite antifeedancy of the outer heartwood increases significantly with the age of the tree. That phenomenon may be related to differences in the amount of desoxylapachol and its isomers between the younger trees (8 and 22 years).

Relationship between extractive compounds and total extractive contents

The Pearson correlations between the extractive content and various extractive compounds are presented in Table 5. Correlations of a comparatively high degree were observed between the EtOAc extrative content and squalene (r=0.79) whereas the highest degree of quinone compounds were observed between EtOAc extractive content and tectoquinone content levels (r=0.68). This results suggest squalene and some quinones were more dissolved in EtOAc so that it could describe its extractive content. The preliminary work (Lukmandaru 2013) revealed that extractive content moderately correlated with antitermitic properties. Thulasidas et al. (2007) demonstrated that the quinones present in teak wood even in minor amount is more significant than its extractive content level against some fungi. As this present results confirmed that no strong correlation was found between quinones and antitermite propertes, it might partially explain the weak relation between extractive content and antifungal or antitermite properties.

Table 5. Pearson's correlation coefficients between extractive content and extractive component contents.

Compound	Extractive content		
	n-hexane	Ethyl acetate	Methanol
Desoxylapachol	0.52**	0.67**	0.37*
Lapachol	0.48**	0.44 **	0.07
Isodesoxylapachol	0.41*	0.55**	0.41*
Tectoquinone	0.55**	0.68**	0.44**
Squalene	0.68**	0.79**	0.48**
Total quinone content	0.49**	0.54**	0.49**

** Significant at 1 % level, * significant at 5 % level

Relationship between extractive compounds and antitermitic properties

Correlation analysis between natural termite resistance parameters and main compounds is shown in Table 6. The highest degree of correlations were determined between mass loss and squalene content in C_6H_6 extract (r=-0.62) or MeOH extract (r=-0.57) in the sapwood region whereas in the EtOAc extracts were measured between mass loss and deoxylapachol content (r=-0.60) as well as between mortality rates and isodesoxylapachol content (r=-0.58) in the sapwood region. Those correlations meant the wood is more resistant against the termites when the content of squalene or desoxylapachol was higher in the sapwood areas. A negative correlation between mortality rates and isodesoxylapachol seemed to be odd as it is interpreted the more isodesoxylapachol content, the less mortality rates of termites will be. The explanation might be the low quantity of isodesoxylapachol in the sapwood did not directly affect its

toxicity but along with the non-structural carbohydrates, it affected the formation other toxic quinones in the sapwood, as suggested by Haupt et al (2003) and Niamke et al. (2011).

As the corellations were observed merely in the sapwood region, it is assumed that the less complexity of extractive composition in that area make it easier to predict its natural termite resistance properties than in the heartwood. Although squalene, a triterpene, was never mentioned to an active compound against termites, this finding suggested this compound could be a hydrophobic barrier, particularly in the sapwood parts. It is generally known that subterranean termites requires more humidity to survive compared to dry-wood termites. Thus, it is necessary to explore the role of squalene in the future work. On the other hand, tectoquinone, as the principal component against termites (Sandermann and Simatupang 1966), did not show any significant correlations with antitermitic properties.

Previous investigation (Lukmandaru and Takahashi 2009) in the form of wood blocks resulted negatively moderate correlation between mass loss and tectoquinone (r=-0.49) or isodesoxylapachol (r=-0.47). Thus, it is concluded that both in the natural condition form (wood blocks) and extracts form (in vitro) is still difficult to predict natural termite resistance by choosing one parameter, particularly in the juvenile stages. Multivariate regressions would be helpful to describe any possibilities of sinergistic or antagonistic relationship among the extractive components of teak wood. In other species, Taylor et al. (2006) found that variations in extractive components could not sufficientlt explain the variation in fungal and termite resistance of *Thuja plicata* and *Chamaecyparis nootkanensis* wood.

Table 6. Pearson's correlation coefficients between natural termite resistance parameters in wood blocks and extractive component contents.

Components	Antitermitic properties					
	Mass loss			Mortality rate		
	Total	Sapwood	Heartwood	Total	Sapwood	Heartwood
a) *n*-hexane extract						
Desoxylapachol	-0,37*	-0.39	-0.19	-0.37*	-0.46	-0.18
Lapachol	-0.34	-	-0.32	-0.30	-	-0.16
Isodesoxylapachol	-0.21	0.31	-0.13	-0.20	0.20	-0.09
Tectoquinone	-0.41*	-0.45	-0.31	-0.40*	-0.34	-0.24
Squalene	-0.51*	-0.62**	-0.39	-0.43*	-0.33	-0.18
Total quinone content	-0.32	-0.36	-0.20	-0.31	-0.41	-0.15
b) Ethyl acetate extract						
Desoxylapachol	-0.33	-0.60**	-0.18	-0.28	-0.52*	-0.08
Lapachol	-0.19	-0.12	-0.13	-0.18	0.16	-0.07
Isodesoxylapachol	-0.34	-0.48*	-0.23	-0.35	-0.58**	-0.23
Tectoquinone	-0.49**	-0.38	-0.44	-0.57**	-0.36	-0.52
Squalene	-0.56**	-0.58*	-0.40	-0.47**	-0.45	-0.15
Total quinone content	-0.27	-0.22	-0.18	-0.26	-0.23	-0.14
c) Methanol extract						
Desoxylapachol	-0.08	-0.05	-0.13	-0.26	-0.10	-0.27
Lapachol	-0.13	-0.27	0.12	0.00	0.08	0.26
Isodesoxylapachol	-0.28	-0.07	-0.06	-0.32	-0. 03	-0.15
Tectoquinone	-0.37*	-0.14	0.04	-0.35	-0.26	0.04
Squalene	-0.55**	-0.57*	-0.20	-0.46*	-0.33	-0.09
Total quinone content	-0.30	-0.13	-0.02	-0.33	-0.01	-0.05

** Significant at 1 % level, * significant at 5 % level

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Conclusions

Tree age and radial position affected the presence and amount of quinone components detected in teak extracts. In addition, the extracting solvents also influenced the results of which *n*-hexane gave the highest amount of some quinones. This study demonstrated that teak at the young tree stage begin producing toxic constituents such as tectoquinone, deoxylapachol and isodesoxylapachol even in the sapwood. Considerable variation was observed in the extractive component contents of wood samples taken from the same site. On the basis of significant interactions, the highest deoxylapachol and total quinone contents were found in the outer heartwood of 22-year-old trees. In the sapwood region, the highest amounts in squalene and desoxylapachol were observed in the inner sapwood of 22-year-old trees. The toxic quinone component contents were positively correlated with total extractive content, with the highest correlation degree being observed in the tectoquinone content. The amount desoxylapachol was moderately correlated with antifeedant properties in the sapwood. Variation in the individual active quinone contents as well as total quinone components, however, could not explain satisfactorily the variation in termite resistance.

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ENZYMATIC SACCHARIFICATION AND ETHANOL PRODUCTION OF XYLEMS FROM INDONESIAN BOTANICAL GARDEN TREES

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Abstract

Saccharification and fermentation processes were applied for xylems derived from 49 trees of Cibodas, 32 trees of Purwodadi, and 19 trees of Bali Botanical Gardens in Indonesia. These xylems were subjected to two different treatments of ethanol production, i.e. saccharification followed by fermentation, and simultaneous saccharification and fermentation (SSF). The sugar released was determined using Nelson-Somogyi method. The results showed that the trees whose yields of enzymatic hydrolysis of cellulose were higher than that of the reference sengon *(Paraserianthes falcataria)* were *Gymnostoma sumatranum*, *Adenanthera microsperma* and *Casuarina junghuhhniana* from Cibodas Botanical Garden; *Firmiana malayana* and *Pterocarpus indicus* from Purwodadi Botanical Garden; and *Alstonia scholaris, Flacourtia rukam, Sapindus rarak* and *Ficus padana* from Bali Botanical Garden. Sugar released after 48 h enzymatic saccharification for *G. sumatranum*, *A. microsperma*, *C. junghuhhniana, F. Malayana, P. indicus, A. scholaris, F. rukam, S. rarak* and *F. padana* were 42.5, 37.1, 28.6, 36.9, 34.0, 38.0, 36.9, 34.9, and 30.3 mg/100 mg xylem, respectively, and for ethanol production were 12.1, 14.1, 13.8, 14.7, 15.3, 11.2, 12.1, 11.1, and 10.0 mg per 100 mg xylem in the same order.

Keywords: saccharification, fermentation, enzymatic hydrolysis, sugar released, ethanol production

Introduction

Tropical countries are the most productive places in producing tree biomass, producing an enormous amount of plants. These places experience abundant rain and no winter climate at any time during the year, generating various kinds of trees as natural resources. Many valuable tree plants have been found in Indonesian forests. In addition to native trees, many tropical trees were also collected from all over the world and planted in Indonesian Botanical Gardens, i.e. Cibodas, Purwodadi and Bali.

Recently, raw materials for bioethanol production are still dominated by food plants (generation I), such as corn, sugar-cane, cassava, etc. This condition will cause conflict of interest as it competes with food supply, causing increasing price of foods. This situation becomes worse as environmental issue such as global warming has been predicted to threaten world food supply. Furthermore, the availability of these raw materials is not guaranteed. Generally, even the total amount of starch and sucrose production of the world (1.5×10^9 tonnes/year) that can be converted into bioethanol can only fulfil at the most of 8×10^{11} L/year while the demand for world fuel is 1.2×10^{12} L/year (Hayashi, 2009). Therefore, alternative sources of raw materials other than food crops are needed, which is the non-food lignocellulosic materials (generation II).

Wood-derived lignocellulosic biomass is a good solution as an alternative raw material for bioethanol that should be developed. Wood has several advantages such as having high cellulosic content (≥80% holocellulose). In spite of that, wood can be planted in marginal land, unlike agriculture crops (Kaida et al., 2009a). Mixing of 85% wood-based bioethanol (E85) with fossil fuel can reduce 65% carbon emission while mixing of starch-based bioethanol with fossil fuel can only reduce it for 17~23% (Watanabe, 2008). Bioethanol raw material from wood does not need spacious storage unlike other lignocellulosic materials. Wood can also be cultivated as Industrial Timber Plantation to more ensure material supply. Cellulose produced from this industrial forest can reach up to 5.0×10^9 tonnes/year that can be converted into 2.6×10^{12} L of bioethanol (Hayashi, 2009). Wood plantation on Industrial Timber Plantation area suits Kyoto Protocol since these planted trees can increase carbon stock on earth which eventually will reduce green-house gasses in the atmosphere.

Plant-derived fuel will be one potential solution as an alternative energy source because abundant production of biomass in Indonesia. Therefore, continuity of plant-derived fuel is more guaranteed than that of fossil fuel. Indonesia has various wood species that strengthen its position as world producer for bioethanol in the future by exploring potential wood species.

The main problem on bioethanol production from lignocellulosic biomass, especially wood is the characteristic of plant cell wall which make it difficult for enzymatic hydrolysis. This drawback limits its utilization and cause uneconomical ethanol production from cellulose. Hayashi (2009) mentioned that wood is highly resistant to enzymatic degradation which inhibits its degradation into fermented sugar. Therefore, easy accessibility for both saccharification and fermentation is also included as the most important factors in the conversion of wood into bioethanol.

Research on uncomplicated hydrolysis process and conversion of a wood species to become bioethanol is still uncommon so that big opportunity is widely opened to seek and explore these wood species. Recent studies revealed that sengon xylem consists of soft walls which are easily hydrolysable with commercial cellulase preparations. On the other hand, mangium xylem consists of hard walls which are less hydrolysable than those of sengon, although lignin content is lower for mangium than sengon (Kaida et al., 2009a). Genetically loosened walls increased the level of saccharification from 30% to 60% in the case of sengon and from 10% to 15% in the case of mangium (Kaida et al., 2009a). It is required to know the variety of saccharification level in the fast-growing tropical trees, in which sengon is the fastest growing tree species in Indonesia. Enzymatic saccharification was employed in this study because the hydrolysis is much more environmentally friendly than other processes such as acid hydrolysis. Therefore, the aim of this study is to screen and to assess the xylems of Indonesian Botanical Garden trees for saccharification in order to be used in bioethanol production.

Materials and Methods

Xylem preparation

Branch of trees from mentioned Botanical Gardens were cut out at the height of 2 to 3 m above the ground. Their barks were peeled and their xylems were dried in an oven at 70°C. The xylem was then milled to a powder using a ball mill at a speed of 15 rps for 30 min. The powder was used as a xylem preparation for saccharification alone or in combination with fermentation.

Wall analysis

Xylem preparation was ground in liquid nitrogen and the resulting fine powder was successively extracted 4 times with water and 24% KOH containing 0.1% $NaBH_4$. The insoluble wall residue (cellulose fraction) was washed twice with water. The amount of cellulose was determined by measuring the acid-insoluble residue: the samples were extracted with acetic/nitric reagent (80% acetic acid/concentrated nitric acid, 10:1) in a boiling water bath for 30 min (Updegraff, 1969) and the resulting insoluble material was washed in water and freeze-dried. Total sugar in each fraction was determined by phenol-sulfuric acid method (Dubois et al., 1956). Lignin content was determined by the Klason method (Chiang and Funaoka, 1990).

Enzymatic hydrolysis

Fifty mg of each xylem preparation was impregnated with water, autoclaved at 120°C for 3 min, and washed once with water by centrifugation. A commercial cellulase preparation (Accelerase, Palo Alto, USA) derived from *Trichoderma viride* was used to digest the xylem. The enzyme preparation contained endocellulases, exocellulases (CBHI and CBHII), xyloglucanase, xylanase, galactanase and polygalacturonase. Enzymatic hydrolysis of the xylem preparation was performed in 1 ml of 50 mM sodium acetate buffer, pH 4.8, containing 0.02% Tween 20 and 0.4 fpu (filter paper units) of a cellulase preparation (2.0 mg). The mixture was incubated at 45°C in a rotary shaker set at 75 rpm. About 100 µl of the supernatant was collected at 48 h of hydrolysis and used for sugar analysis. The sugar released was estimated as reducing sugar by the Nelson-Somogyi method (Somogyi, 1952). Furthermore, free sugars released were directly analyzed according to their alditol acetates using gas chromatography (Hayashi, 1989).

Ethanol production

The mixtures from previous enzymatic hydrolysis were inoculated with a seed culture of *Saccharomyces cerevisiae* (SH1089) and yeast nutrients (4 mg $(NH_4)_2HPO_4$, 0.2 mg $MgSO_4/7H_2O$ and 8 mg yeast extract). The mixtures were incubated at 37°C in a rotary shaker set at 100 rpm. About 100 µl of the supernatant was collected after 48 h fermentation and used for ethanol analysis. The ethanol formed was measured by gas chromatography on a

Supelcowax-10 column (0.53 mm i.d. × 15 m; Supelco, Bellefonte, PA, USA) at 50°C using an Agilent gas chromatograph. Butanol was used as an internal standard.

Simultaneous saccharification and fermentation

Mixtures containing each type of xylem preparation with 1 ml of 50 mM sodium acetate buffer, pH 4.8, 0.02% Tween, 0.4 fpu of a cellulase preparation and a seed culture of *Saccharomyces cerevisiae* (SH1089) with yeast nutrients (4 mg $(NH_4)_2HPO_4$, 0.2 mg $MgSO_4/7H_2O$, and 8 mg yeast extract) were prepared. The mixtures were incubated at 37°C in a rotary shaker set at 100 rpm. About 100 µl of the supernatant was collected after 48 h of hydrolysis and used for ethanol analysis. The ethanol formed was measured by gas chromatography on a Supelcowax-10 column (0.53 mm i.d. × 15 m; Supelco, Bellefonte, PA, USA) at 50°C using an Agilent gas chromatograph. Butanol was used as an internal standard.

Results and Discussion

Cibodas Botanical Garden trees

The results showed that the amounts of celluloses varied between 24.3% (*Pterospermum javanicum*) and 60.0% (*Araucaria glauca*). Hemicelluloses varied between 3.3% (*Agathis borneensis*) and 18.5% (*Firmiana malayana*). Lignin contents varied between 22.2% (*Casuarina junghuhniana*) and 39.6% (*Michelia montana*).

The levels of enzymatic saccharification varied among xylems from the 49 wood species. At 48 h, the xylem of *G. sumatranum* released 42.5 mg of sugars while poplar xylem released only 30.3 mg (Kaida et al., 2009b). The higher incidence of cellulose hydrolysis was also observed in *A. microsperma* (37.1 mg) which was higher than in poplar. *Araucaria glauca* has the highest cellulose content (60%) while its sugar released was only 17.1 mg. Therefore, the levels of enzymatic saccharification were regardless of the cellulose content. The ethanol production was higher in the xylem of *G. sumatranum* (12.1 mg), *A. microsperma* (14.1 mg), and *C. junghuhhniana* (13.8 mg) compared to other wood species in Cibodas Botanical Garden.

Purwodadi Botanical Garden trees

The results showed that the amounts of celluloses varied between 8.0% (*Alstonia scholaris*) and 54.8% (*Acacia catechu*). Hemicelluloses varied between 5.6% (*Lagerstroemia speciosa*) and 26.9% (*Pterocymbium javanicum*). Lignin contents varied between 23.3% (*A. catechu*) and 36.9% (*Syzygium polyanthum*).

The levels of enzymatic saccarification varied among xylems from the 32 trees. At 48 h, the highest level of saccharification was obtained from the xylem of *F. malayana*, which had released 36.9 mg of sugars/100 mg xylem, while sengon only released 29 mg of sugars/100 mg xylem (Kaida et al., 2009a). The higher incidence of cellulose hydrolysis was also observed in *P. indicus* (34.0 mg) which was higher than in sengon. Ethanol production in the xylem of *F. malayana* and *P. indicus* were 14.7 mg and 15.3 mg/100 mg xylem, respectively, while sengon was 12 mg/100 mg xylem.

A. catechu has the highest cellulose content while its sugars released was only 28.4 mg/100 mg xylem. Therefore, the levels of enzymatic saccharification was regardless of the cellulose content. The amount of hemicellulose in xylems varied between 5.6 to 26.9%, in which xyloglucan content varied between 0 to 0.1425%. *L. speciosa* has the lowest hemicellulose content while its sugar released was only 12.0 mg/100 mg xylem. Therefore, the levels of enzymatic saccharification was regardless of the hemicellulose content. Although xyloglucanase activity improved the total hydrolysis of lignocelluloses (Benko, 2008), there was no correlation between ethanol production and xyloglucan content. Lignin is known to be a recalcitrant compound in cellusose hydrolysis (Chen and Dixon, 2007). The data shows the correlation between the high level of enzymatic saccharification and the low lignin content, but it does not happen all the time. *A. catechu* also has the lowest lignin content but its sugar released was only 28.4 mg/100 mg xylem. Therefore, the levels of enzymatic saccharification were regardless of the lignin content.

Bali Botanical Garden trees

The results showed that the amounts of cellulose varied between 35.9% (*Mimusops elengi*) and 51.2% (*Tabernaemontana macrocarpa*). Hemicelluloses varied between 5.7% (*Podocarpus neriifolius*) and 22.4% (*Flacourtia rukam*). Lignin contents varied between 23.2% (*Toona sureni*) and 39.1% (*Calophyllum soulattri*).

The levels of enzymatic saccharification varied among xylems from the 19 wood species. At 48 h, the highest level of saccharification was obtained from the xylem of *A. scholaris* which released 38.0 mg sugars per 100 mg xylem while sengon only released 29.0 mg sugars per 100 mg xylem (Kaida et al., 2009a). Higher levels of cellulose hydrolysis when compared to that of sengon were also observed in *F. rukam* (36.9 mg), *S. rarak* (34.9 mg) and *F. padana* (30.3 mg). The ethanol production in the xylems of *A. scholaris, F. rukam, S. rarak* and *F. padana* were 11.2 mg, 12.1 mg, 11.1, and 10.0 mg per 100 mg xylem, respectively.

T. macrocarpa has the highest cellulose content while its sugar-released was only 24.8 mg per 100 mg xylem. From this condition, it can be said that the level of sugar released was not directly related to cellulose content. The amount of hemicellulose in xylems varied between 5.7 to 22.4%, in which xyloglucan contents varied between 0 to 0.0877%. *P. neriifolius* has the lowest hemicellulose content yet its sugar-released was only 26.6 mg per 100 mg xylem. This result showed that lower level of hemicellulose can not ensure that higher sugar released could be obtained. Although xyloglucanase activity improved the total hydrolysis of lignocelluloses (Benko, 2008), there was no correlation between ethanol production and xyloglucan content. Lignin is known to be a recalcitrant compound in celullose hydrolysis (Chen and Dixon, 2007). The data shown the correlation between the high yield of enzymatic saccharification and the low lignin content, but this condition was not a must. *T. sureni* has the lowest lignin content (23.2%) but its sugar-released was only 23.0 mg per 100 mg xylem. Therefore, the yield of enzymatic saccharification was also not directly related to lignin content.

A study on the relationship between chemical components of wood and their sugar released for ethanol production has been reported by Dwianto et al. (2011). The content of cellulose in wood was not exactly related to its sugar released. This trend was also occurred for the relationship between hemicellulose and sugar released. However, lignin content in woods gave an expected trend where the less lignin content, the higher the sugar released. The complexity of wood structure and high lignin content were estimated to be the major cause that inhibits contact between cellulose and the enzyme in sacharification process. It might occur that even though the wood has high level of cellulose, the composition of its other chemical components such as hemicellulose and lignin may act as inhibitors in the conversion of its cellulose into reducing sugar that will be further converted into ethanol.

Conclusion

Both saccharification and fermentation occurred at the greatest rate in *G. sumatranum* xylem. The authors have concluded that *G. Sumatranum*, *A. microsperma*, and *C. junghuhhniana* were suitable tree species for bioethanol production in Cibodas Botanical Garden. *G. Sumatranum*, *A. microsperma*, and *C. junghuhhniana* xylem's levels of saccharification and ethanol production were higher than those of poplar xylem.

The tree species for bioethanol production from Purwodadi Botanical Garden are *F. malayana* and *P. indicus* as their yields from saccharification and ethanol production were higher than those from sengon xylem.

Saccharification of xylem occurred at the greatest rate in *A. scholaris* while the highest fermentation yield was obtained from the xylem of *F. rukam*. The best tree species for bioethanol production from Bali Botanical Garden were *A. scholaris, F. rukam, S. rarak* and *F. padana* as their yields from saccharification and ethanol production were close to those from sengon xylem. Genetic improvements would be expected to transform these species into more hydrolysable wood for cellulase preparation.

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A REVIEW: SCREENING OF POTENCY AKAR KUNING STEM (*FIBRAUREA TINCTORIA* LOUR) AS ANTIMALARIAL COMBINATION THERAPY

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ABSTRACT

Malaria is one of the indicators development target of Millenium Development Goals (MDGs), and still a health problem in many countries around the world. Data 2010 showed 80% malaria mortality occur in 14 countries. In Indonesia, 80% are endemic malaria and 45% of the population living in endemic areas. Many factors to cause malaria eradication failure, such as discovered a new species of malaria parasites, that is *P. knowlesi* and development of Plasmodium resistant to antimalarial drugs. In order to prevent resistance, the rational antimalarial combination therapy is highly recommended and a discovery of a new antimalarial drug is strongly needed. Now, antimalarial combinations are Artemeter-Lumefantrin, Artesunate+Amodiaquine, Artesunate+Mefloquine, Artesunate+ Sulfadoxine-Pyrimethamine. Studies of new combination of antimalarial from traditional plant become a challange for researcher. On several studies reported that Pasak Bumi root (*Eurycoma longifolia* Jack) contain quassinoid that responsible for antimalarial activity. Quassinoid active substance in Pasak Bumi root are *eurycomanine, eurycomanone,* dan *eurycomalactone*. *Eurycomalactone* inhibit protein synthesize, that avoid the development stage of Plasmodium in merozoites to tropozoit thereby inhibiting parasitemia. Pasak bumi root extract 60mg/kg combine with artemisin 1,7 mg/kg decrease parasitemia 80%. Furthermore, studies of Akar Kuning stem (*Fibrauerea tinctoria* Lour) reported that it has secondary metabolites; alkaloid and terpenoid/steroid. Alkaloid known to inhibit parasite growth through intraseluler colin transport, whereas terpenoid inhibit protein synthesize. This review article aims to determine Akar Kuning stem potency as antimalarial combination therapy. The combination artemisinin and alkaloid and terpenoid/steroid in Akar Kuning Stem will be a potent antimalarial combination therapy that expected decrease parasitemia > 80%.
Keywords: Antimalarial, Akar Kuning Stem (*Fibrauerea tinctoria* Lour), Alkaloid, Terpenoid, Screening.

INTRODUCTION

Malaria is a major global public health problem and is responsible for death of over 1 million people annually, with more than 90% of cases found in sub-Saharan Africa (Poupli et al, 2007). Four species of malaria parasite cause disease in humans (*Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale*) but *P. falciparum* causes most problems as a result of its prevalence, virulence and drug resistance. The pathogenicity of this parasite is thought to result from its rapid rate of asexual reproduction in the host and its ability to sequester in small blood vessels (Winstanley, 2000; Sharma, 2012). Most cases of endemic *P. falciparum* malaria remain uncomplicated, however some develop a number of severe complications including cerebral malaria, severe anemia and plancental malaria (Rasti et al, 2004). The increasing resistance of malaria parasites to available antimalarial drugs represents a major impediment for disease treatment and control (Bedolla et al, 2013).

The current WHO drug policy allows change of antimalarial drug if 15% of the population shows in vivo resistance to it. Resistance to antimalarial drugs has beeen a particular problem with *P. falciparum* in which widespread resistance to chloroquine, sulphadoxine-pyrimethamine and mefloquine has been observed (Srikanth et al, 2012).

History shows that plants have been an important source of medicines againts malaria with two of the major drugs used in malaria treatment, quinine and more recently artemisinin both having derived from traditional medicine and from plants. These two drugs are now the mainstay of the treatment of severe malaria worldwide and the artemisinin derivatives in combination with a second antimalarial drug now at the heart of the World Health Organization strategy to control malaria globally. Traditional medicines are a potential rich source of new drugs againts malaria and other infectious disease and given the remarkable contribution this has made to the development of potent antimalarial drugs over the last five hundred years it is clearly an approach that must be continued (Pouplin et al, 2007).Akar Kuning stem (*Fibrauerea tinctoria* Lour) reported that it has secondary metabolites; alkaloid and terpenoid/steroid. Alkaloid known to inhibit parasite growth through intraseluler colin transport, whereas terpenoid inhibit protein synthesize.

Antimalarial Resistance

The development of resistance by the parasite againts first line and second line antimalarial drugs has under scored the importance to develop new drug targets and pharmacophores to treat the disease. With the availability of increased support for malaria research, a variety of drug targets and candidate moleculs are now available for further development (Padmanaban et al, 2007).

Approach to Antimalarial Chemotherapy

Approaches to antimalarial drug discovery and development such as optimization of therapy with existing agents, develop analogs of existing agents, natural products, compounds active againts other diseases, drug resistance reversers, and compound active againts new targets (Rosenthal, 2003).

Optimization of therapy with existing agents

A first approach is to optimize therapy with existing agents. New dosing regimens or formulations may optimize activity. Combination therapies, including newer agents (e.g. artemisinin derivatives, atovaquone) and new combinations of old agents (e.g. amodiaquine/sulfadoxine/pyrimethamine, chlorproguanil/dapsone), are under study as first-linne therapies for Africa and other areas with widespread drug resistance. The use of combination antimalarial therapy offers two important potential advantages. First, the combination should improve antimalarial efficacy, providing additive or synergistic antiparasitic activity. Second, and most important , the use of combination therapy should slow the progression of parasite resistance to the new agents.

Development of analogs of existing agents

Another approach to antimalarial chemotherapy is to improve upon existing antimalarials by chemical modification of these compounds. This approach does not require knowledge of the mechanism of action or the biological target of the parent compound.

Natural products

Plant-derived compounds offer a third approach to chemotherapy. Importantly, this approach can benefit from knowledge of medicinal plants among natives of malarious regions, where the appreciation of the use of plant products to treat febrile illnesses has grown over many generations. Natural products are the sources of the two most important drugs currently available to treat severe falciparum malaria, quinine and derivatives of artemisinin. Extensive evaluations of natural products as potential new therapies for many human diseases are underway. It is important that such trials include the evaluation of the antimalarial activity of plant extracts and potential drugs purified from these extracts. As with both the quinolines and artemisinins, it is likely that antimalarial naturalnatural products will be the parent compounds for the semi-synthetic or fully synthetic production of new drug.

Compounds active againts other diseases

A fourth approach to antimalarial chemotherapy is to identify agents that are developed as treatments for other diseases. These compounds might act againts orthologs of their targets in other systems or by different mechanism againts malaria parasites.

Drug resistance reversers

Many drugs have been shown to reverse the resistance of *P. falciparum* to chloroquine *in vitro*, most notably the antihypertensive verapamil and the antidepressant desipramine.

Compounds active againts new target

The most innovative approach to chemotherapy is the identification of new targets and subsequent discovery of compounds that act on these targets. New targets for antimalarial therapy will be considered based on their lovations within the malaria parasites. The target location such as cytosol, parasite membrane, food vacuole, mitochondrion, and apicoplast.

Akar Kuning Stem (*Fibraurea tinctoria* Lour)
Taxonomy

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Magnoliidae
Order	Ranunculales
Family	Menispermaceae
Genus	Fibraurea Lour
Species	*Fibraurea tinctoria*

Picture 1. *Fibraurea tinctoria*Stem

Phytochemical Screening

*Fibraureatinctoria*Lourstem numerous inEast Kalimantan, local people call itas"Akar Kuning". Researchinethnobotany, thestem of the plantis usedtocurejaundice(Ilona, 2003;Kulip, 1997).Phytochemicalscreening studiesonethanolextract ofFibraureatinctoriastem secondarymetabolitesareof alkaloids, flavonoids, polyphenolsandterpenoids/steroidcapabledampenincreasedin vitrolipidperoxidationstrongerthantocopherolacetatethroughthe ironthiocyanatetestandtiobarbiturat. Acuteoraltoxicity testwith asingledose ofup to2g/ kginmale and femalemicewithlongobservationfor oneweekdid not showthe deathandcytotoxictestsonArtemia salinalarvaehaveLD50values>1000 ppm(Fikriah, 2006).

Four of the secondary metabolites is only terpenoids / steroid that can not reduce DPPH radicals were sprayed on the TLC. This means that secondary metabolite alkaloids, flavonoids and polyphenols have antioxidant activity. Rf value secondary metabolites that have antioxidant activity can be seen in Table 1. Research on plant extracts or secondary metabolites with antioxidant activity in vitro by DPPH radical reduction test, if known to have activity DPPH radical reduction can also hepatoprotective effects in vivo.

Table 1. Identification ofSecondaryMetabolitesandRfvalueonTLC (Fikriah, 2006)

No.	Secondary Metabolite	Rf Value	DPPH Positive
1	Alkaloid	0.08; 0.30	0.08; 0.30
2	Flavonoid	0.17; 0.20; 0.23; 0.39; 0.48; 0.51; 0.59; 0.70; 0.74; 0.88	0.39; 0.51
3	Polyphenol	0.04	0.04
4	Triterpenoid/Steroid	0.31;0.77	-

DISCUSSION

Fibraureatinctoriastem secondarymetabolitesareof alkaloids, flavonoids, polyphenolsandterpenoids/steroid. Alkaloid known to inhibit parasite growth through intracelular choline transport, whereas terpenoid inhibit protein synthesis.Alkaloids, flavonoids and polyphenols have antioxidant activity (Hayati, 2012).

In the search for new antimalarial agents, parasite transporters are of great interest as drug targets and as potential selective drug delivery routes in blood-stage parasite. Parasite is known to take up choline from external medium for the novo synthesis of phosphatidylcholine (PC). Cholinephosphate cytidylyltransferase is a regulatory step in this pathway, and choline transport (which regulate the supply of precursor) is a rate-limiting step. Phospholipid metabolism is an ideal target for new chemotherapy because of its vital importance to the parasite. Phospholipid metabolism is absent from normal mature human erythrocytes, but after malarial infection, the erythrocyte phospholipid content increases by as much as 500%. PC and phosphatidylethanolamine (PE) are the major phospolipids of of the

infected erythrocyte. Alkaloid activity to inhibit parasite growth through intracelular choline transport shows great promise for the selective targeting of new agents for chemotherapy of malaria (Biagini et al, 2004; Mamoun et al, 2009).

Malarial parasites obtain the amino acids necessary for protein biosynthesis in three ways such as biosynthesis of amino acids from carbon sources, uptake of preformed free amino acids present in the plasma or host cells, and proteolysis of hemoglobin with the release of amino acids. Nucleic metabolism involving the three major targets: purine metabolism, pyrimidine metabolism and folat metabolism. Protein that mediate the uptake, intracellular trafficking and metabolism of essential nutrient in the *Plamosium* infected erythrocyte have been reported as potential antimalarial drug targets (Na-Bangchang, 2009).Terpenoid inhibition activity to protein synthesis might be a viable compound for the development of vovel antimalarial drugs.

An alternative drug design strategy could be based on the sensitivity of *Plasmodium* sp to oxidative stress. Malaria parasites (*Plasmodium* sp) are exposed to oxidative stress as a result of their metabolic processes and their hosts responses to infection, both in vertebrate hosts and vector mosquitoes. Host erythrocyte hemoglobin digestion and heme production within food vacuoles and the synthesis and folding of proteins within the endoplasmic reticulum, as well as the produstion of the needed energy in the mitochondria are the main sources of oxidative stress. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in all cells and play important roles in cell physiology, such as life cycle regulation, development, migration, induction of signalling pathways, activation of second messangers, and triggering of antioxidant responses. Reactive species are common products of metabolic enzymatic reactions of cells. Their accumulation is prevented by the production of antioxidant molecules, which maintain the inner cellular environment in a homeostatic (redox balance). The loss of the redox balance, either by an increase of oxidant molecules (ROS and RNS) or by decreased antioxidant system activities, causes a state of oxidative stress.The search for new molecules of the parasite, but that do not affect the host, offers opportunities for a renovated antimalarial (Bedolla et al, 2013).

Antimalarial drug discovery approaches currently being deployed largely include optimization of therapy with available drugs including combination therapy.On several studies reported that Pasak Bumi root (*Eurycoma longifolia* Jack) contain quassinoid that responsible for antimalarial activity. Quassinoid active substance in Pasak Bumi root are *eurycomanine, eurycomanone,* dan *eurycomalactone*. *Eurycomalactone* inhibit protein synthesize, that avoid the development stage of Plasmodium in merozoites to tropozoit thereby inhibiting parasitemia. Pasak bumi root extract 60mg/kg combine with artemisin 1,7 mg/kg decrease parasitemia 80%. Furthermore, studies of Akar Kuning stem (*Fibraurea tinctoria* Lour) reported that it has secondary metabolites; alkaloid and terpenoid/steroid. Alkaloid known to inhibit parasite growth through intraseluler colin transport, whereas terpenoid inhibit protein synthesis.Alkaloids, flavonoids and polyphenols have antioxidant activity. This review article aims to determine Akar Kuning stem potency as antimalarial combination therapy. The combination artemisinin and alkaloid, terpenoid/steroid, flavonoid, and polyphenols in Akar Kuning Stem will be a potent antimalarial combination therapy that expected decrease parasitemia > 80%.

CONCLUSION

Fibraureatinctoriastem secondarymetabolitesareof alkaloids, flavonoids, polyphenolsandterpenoids/steroid. Alkaloid known to inhibit parasite growth through intracelular choline transport, whereas terpenoid inhibit protein synthesis. Alkaloids, flavonoids and polyphenols have antioxidant activity. These are shows great promise for the selective targeting of new agents for chemotherapy of malaria Antimalarial drug discovery approaches currently being deployed largely include optimization of therapy with available drugs including combination therapy.The combination artemisinin and alkaloid, terpenoid/steroid, flavonoid, and polyphenols in Akar Kuning Stem will be a potent antimalarial combination therapy that expected decrease parasitemia > 80%.

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INSECTICIDAL ACTIVITY OF *Melaleuca leucadendron* OIL AGAINST GREENHOUSE WHITEFLY *Trialeurodes vaporariorum*

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Abstract

The greenhouse whitefly, *Trialeurodes vaporariorum,* is increasing pest problem on horticultural crops such as tomato, melon, strawberry, raspberry, pepper, cucumber, lettuce, citrus, bean and cotton. During the last decades, a worldwide spread of the pest insects *T. vaporariorum* has led to local devastation of vegetable and ornamental crops, resulting in large economic losses. *T. vaporariorum* dwells on the undersurface of plant foliage, not easily reached by conventional spraying equipment. These problems have highlighted the need for the development of selective *T. vaporariorum* control alternatives with fumigant action in greenhouses. Plant essential oil can be used as an alternative source for *T. vaporariorum* control. *M. leucadendron* is one of the essential oil suppliers including in Myrtaceae family, this oil has chemical and biological properties due to its components. In this study, insecticidal activity of *M. leucadendron* and their main components has been elucidated. Samples of *M. leucadendron* leaf were collected from Gundih, Central Java, Indonesia. Oil samples were obtained by water-steam distillation from fresh leaves of *M. leucadendron.* This essential oil and it major compounds were used in this study. Fumigant assay was used in this study to against whitefly of *T. vaporariorum.* The result showed that *M. leucadendron* oil has potency to be used as pesticide against greenhouse whitefly *T. vaporariorum*. LC_{50} of treated whitefly was evaluated after 30 min. exposure time indicated that *M. leucadendron* oil (15.28 µl/l), 1,8-cineole (11.52 µl/l), α-terpineol (0.56 µl/l), *d*-limonene (48.48 µl/l) and β-caryophyllene (123.36 µl/l) exhibited fumigant toxicity against *T. vaporariorum.* In this study, the effectiveness of *M. leucadendron* oil as insect control fumigant is likely influenced by main compounds of this oil such as 1,8-cineole and α-terpineol.

Keywords: *Melaleuca leucadendron*, essential oil, insecticidal, *Trialeurodes vaporariorum*

Introduction

Trialeurodes vaporariorum westwood, knowns as greenhouse whitefly, is a cosmopolitan pest originating from China. This insect is an extremely polyphagous pest. It is a primary insect pest of many fruit, vegetable and ornamental crops, frequently being found in greenhouse and other protected horticultural environments. It was registered for the first time as a pest in 1870, in greenhouse in the USA. Later it became the main pest of greenhouse in the world. In Japan whitefly was registered for the first time in 1977 (Choi *et al.*, 2003; Zabel *et al.,* 2001). This insect is now well established in the greenhouse ecosystem and is an economically important pest of various greenhouse vegetables. It is a major pest of tomato pepper, cucumber, bean, string beans, lettuce, tobacco, watermelon, melon, begonia, and many other ornamental crops.

This pest is commonly controlled by synthetic insecticides. Insect pest management is nowadays a worldwide ecological challenge mainly due to environmental pollution caused by extensive use of synthetic chemical pesticides. The use of pesticides has some detrimental consequences on environment, such as groundwater pollution, soil erosion, excessive water use, and the development of weeds and diseases resistant to chemical control (Boulogne *et al*., 2012; Lichtfouse *et al.,* 2009). It also has negative impact on health with human poisonings and their related illnesses. The increase of the risk of developing insect resistance and the high cost–benefit ratio of synthetic pesticides pushed research towards investigating alternative insecticides. Thus, there is a need to develop less hazardous alternatives to synthetic pesticides. Plant extracts have long been a subject of research in an effort to develop alternatives to conventional insecticides causing minimal effects on the environment and non-target organisms. Plant essential oils have

been suggested as alternative sources for insect control products because some are selective, biodegrade to nontoxic products, and have few effects on non target organisms and the environment (Isman, 2000; Mohamed *et al*., 2010).

However, there have been no attempts to study the toxicity of *M. leucadendron* oil on economically important greenhouses pests. Greenhouses, like storehouses and beehives, are closed spaces where essential oil flavors can be applied as fumigants. The aim of this study was to evaluate the fumigant potential of *M. leucadendron* oil and its major compounds to control greenhouse pest of whitefly *T. vaporariorum*.

Materials and Methods

Essential oil and Chemicals

M. leucadendron leaf oil from Gundih, Central Java, Indonesia and its major compounds (commercial compounds) were used in this study. Major compounds used in this study are commercial chemicals of 1,8-cineole purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan), β-caryophyllene was purchased from Tokyo Kasei (Tokyo, Japan), α-terpineol and *d*-limonene purchased from Nacalai Tesque, Inc (Kyoto, Japan).

Biological Material

The adults of whitefly *T. vaporariorum* were collected from tomato greenhouse in Vegetable Crops Science Laboratory, Faculty of Agriculture, Kochi University, Japan. Whiteflies were collected from tomato greenhouse under temperature condition 38 ± 1ºC.

Fumigant Assay

The square (2 cm x 2 cm) of filter paper (Whatman No. 1) was attached to a 125 ml glass bottle. Filter papers were impregnated with *M. leucadendron* oil and 4 major compounds of its oil (1,8-cineole, α-terpineol, *d*-limonene, and β-caryophyllene) in different oil concentrations. Concentrations were converted to give equivalent fumigant concentrations µl/125 ml air (concentration x 8 µl/l air). Ten adults of whiteflies from fresh tomato leaves were placed in glass bottle by taking from tomato leaves using vacuum suction and were sealed with Parafilm. Three replicates of each concentration and control (without oil) were carried out. Mortality was recorded every 30 minutes for 6 hour. The whiteflies were considered dead if they were not fly and move.

Statistical Analysis

Percentages of mortality were calculated using Abbott's correction formula (Abbott, 1925). Lethal concentrations of 50% (LC_{50}) of treated whitefly in 30 minute (0.5 hour) exposure time were estimated using Probit analysis (Finney, 1971) by SPSS statistical program.

Results and Discussion

The insecticidal activity of *M. leucadendron* oil, 1,8-cineole, α-terpineol, *d*-limonene, and β-caryophyllene against whitefly *T. vaporariorum* were summarized in Table 1 and Figure 1. The results showed that all samples have toxic effect on *T. vaporariorum*. In general, higher mortality was observed as the concentrations of essential oils and exposure time increased.

The mortality rates of *T. vaporariorum* adult with treatment of *M. leucadendron* oil in 6 hour in many concentrations obtained 100%. In concentration 3.2 µl/l , 4.8 µl/l , 6.4 µl/l , 8.0 µl/l , and 16.0 µl/l all the adults (100%) died at exposure time 6h, 4h, 3h, 2h, 1,5 h and 1,5 h, respectively. For α-terpineol, in the lowest concentration of 1.2 µl/l, all whiteflies adult (100%) were dead at exposure time less than 0.5 h (< 30 min.). The 100% adult whiteflies mortality in exposure time 1 h for 1,8 cineole and *d*-limonene were obtained at concentration 16 µl/l and 48 µl/l, respectively. The β-caryophyllene showed the lowest effect as fumigant with 100 % adult whiteflies mortality in exposure time 2,5 h at concentration 128 µl/l. Compared with α-terpineol this essential oil has less toxin, compared with 1,8-cineole has similar fumigant activity, and compared with *d*-limonene and β-caryophyllene, *M. leucadendron* oil has higher fumigant activity. The results show, the highest effect was obtained from α-terpineol, followed by 1,8-cineole, *M. leucadendron* oil, *d*-limonene, and β-caryophyllene, respectively.

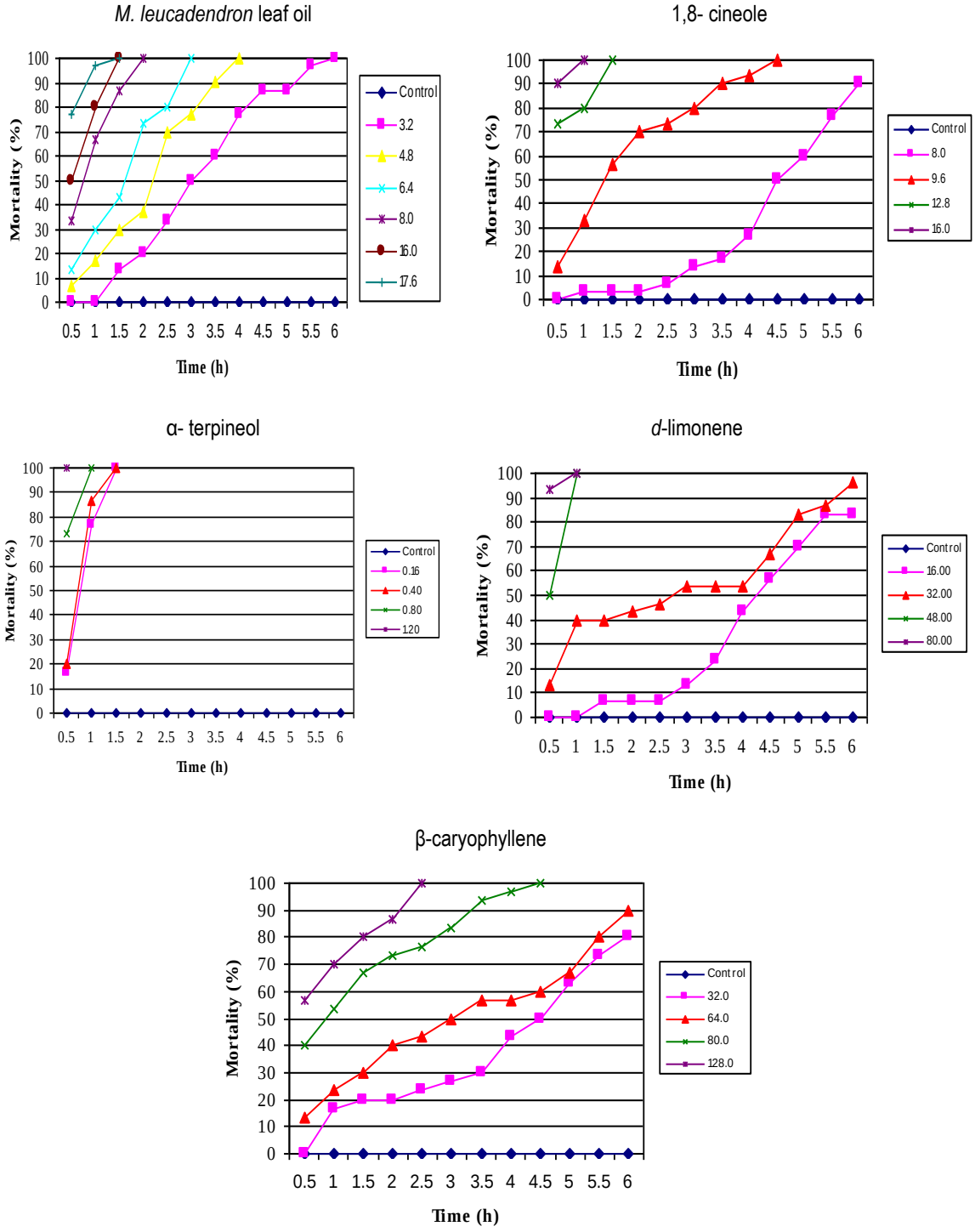

Figure 1. Percent mortality of whitefly *T. vaporariorum* (concentration in µl/l air).

Two major compounds of α-terpineol and 1,8-cineole in this study were very effective as fumigant, The effect of *M. leucadendron* oil as fumiagant is probably due to the synergic effect of these two major compounds. Previous study also showed that 1,8-cineole and α-terpineol have insecticidal activities (Enan *et al*., 2001; Prates, 1998; Sfara, 2009).

Lethal concentrations of 50% (LC_{50}) of treated whitefly were evaluated after 0.5h (30 min.) exposure time. The LC_{50} values are shown in Table 2 and Figure 2. Results indicated that *M. leucadendron* oil and its major compounds exhibited fumigant toxicity against *T. vaporariorum*. Comparison of LC_{50} shows that α-terpineol (0.56 µl/l) and 1,8-cineole (11.52 µl/l) are relatively more toxic against *T. vaporariorum* than *M. leucadendron* oil. However, LC_{50} of *M. leucadendron* oil (15.28 µl/l) is more toxic than *d*-limonene (48.48 µl/l) and β-caryophyllene (123.36 µl/l).

Table 1. Percent mortality of whitefly *T. vaporariorum* in 6 h exposure time

Sample	Conc. (µl/l air)	Mean value of mortality (%)											
		0.5 h (30 min)	1 h	1.5 h	2 h	2.5 h	3 h	3.5 h	4 h	4.5 h	5 h	5.5 h	6 h
Control	-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
M. leucadendron oil	3.2	0.00 ± 0.00	0.00 ± 0.00	13.33 ± 11.55	20.00 ± 10.00	33.33 ± 15.28	50.00 ± 17.35	60.00 ± 17.32	76.67 ± 15.28	86.67 ± 15.28	86.67 ± 15.28	96.67 ± 5.77	100.00 ± 0.00
	4.8	6.67 ± 5.77	16.67 ± 5.77	30.00 ± 10.00	36.67 ± 5.77	70.00 ± 10.00	76.67 ± 5.77	90.00 ± 0.00	100.00 ± 0.00				
	6.4	13.33 ± 5.77	30.00 ± 17.32	43.33 ± 15.28	73.33 ± 11.55	80.00 ± 10.00	100.00 ± 0.00						
	8.0	33.33 ± 5.77	66.67 ± 11.55	86.67 ± 11.55	100.00 ± 0.00								
	16.0	50.00 ± 8.16	80.00 ± 8.16	100.00 ± 0.00									
	17.6	76.67 ± 5.77	96.67 ± 5.77	100.00 ± 0.00									
1,8-cineole	8.0	0.00 ± 0.00	3.33 ± 5.77	3.33 ± 5.77	3.33 ± 5.77	6.67 ± 11.55	13.33 ± 15.28	16.67 ± 11.55	26.67 ± 20.82	50.00 ± 17.32	60.00 ± 20.00	76.67 ± 5.77	90.00 ± 10.00
	9.6	13.33 ± 11.55	33.33 ± 23.09	56.67 ± 11.55	70.00 ± 10.00	73.33 ± 5.77	80.00 ± 10.00	90.00 ± 10.00	93.33 ±11.55	100.00 ± 0.00			
	12.8	73.33 ± 5.77	80.00 ± 0.00	100.00 ± 0.00									
	16.0	90.00 ± 10.00	100.00 ± 0.00										
α-terpineol	0.16	16.67 ± 5.77	76.67 ± 5.77	100.00 ± 0.00									
	0.40	20.00 ± 0.00	86.67 ± 5.77	100.00 ± 0.00									
	0.80	73.33 ± 11.56	100.00 ± 0.00										
	1.20	100.00 ± 0.00											
d-limonene	16	0.00 ± 0.00	0.00 ± 0.00	6.67 ± 5.77	6.67 ± 5.77	6.67 ± 5.77	13.33 ± 5.77	23.33 ± 5.77	43.33 ± 5.77	56.67 ± 11.55	70.00 ± 10.00	83.33 ± 5.77	83.33 ± 5.77
	32	13.33 ± 5.77	40.00 ± 10.00	40.00 ± 10.00	43.33 ± 5.77	46.67 ± 5.77	53.33 ± 5.77	53.33 ± 5.77	53.33 ± 5.77	66.67 ± 11.55	83.33 ± 5.77	86.67 ± 5.77	96.67 ± 5.77
	48	50.00 ± 10.00	100.00 ± 0.00										
	80	93.33 ± 5.77	100.00 ± 0.00										
β-caryophyllene	32	0.00 ± 0.00	16.67 ± 5.77	20.00 ± 10.00	20.00 ± 10.00	23.33 ± 11.55	26.67 ± 15.28	30.00 ± 10.00	43.33 ± 5.77	50.00 ± 0.00	63.33 ± 5.77	73.33 ± 15.28	80.00 ± 20.00
	64	13.33 ± 5.77	23.33 ± 5.77	30.00 ± 10.00	40.00 ± 10.00	43.33 ± 11.55	50.00 ± 10.00	56.67 ± 15.28	56.67 ± 15.28	60.00 ± 17.32	66.67 ± 5.77	80.00 ± 10.00	90.00 ± 17.32
	80	40.00 ± 10.00	53.33 ± 11.55	66.67 ± 15.28	73.33 ± 11.55	76.67 ± 15.28	83.33 ± 11.55	93.33 ± 11.55	96.67 ± 5.77	100.00 ± 0.00			
	128	56.67 ± 5.77	70.00 ± 10.00	80.00 ± 10.00	86.67 ± 5.77	100.00 ± 0.00							

Table 2. LC_{50} of whitefly *T. vaporariorum* in 30 minutes (0.5 h) exposure time.

Sample	LC_{50} (µl/l)
M. leucadendron oil	15.28 a
1,8-cineole	11.52 a
α-terpineol	0.56 b
d-limonene	48.48 c
β-caryophyllene	123.36 d

Number wit different letters indicate significant difference among samples (P<0.05)

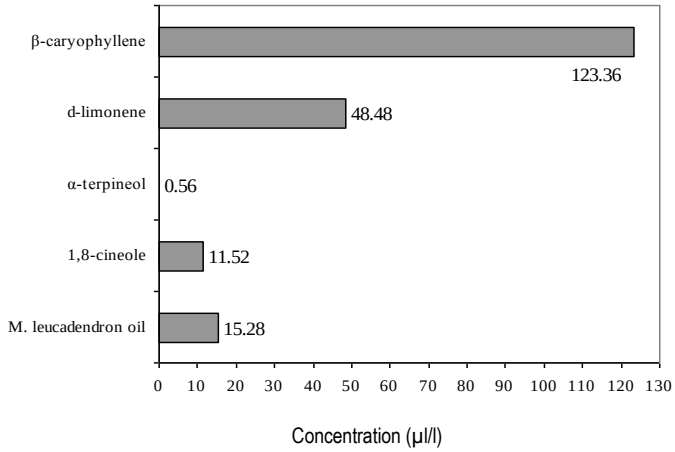

Figure 2. LC_{50} of whitefly *T. vaporariorum* in 0.5h exposure time

Insecticidal activity of *M. leucadendron* oil in this study probably stems from high amounts of 1,8 cineole and α-terpineol in this essential oil, although others constituents of this essential oil also have activity (e.g.: *d*-limonene and β-caryophyllene).

Previous studies showed strong positive correlation between the fumigant activity of essential oils and their corresponding 1,8-cineole concentration that essential oils which contain 1,8 cineole has insecticidal activity (Alzogaray et al., 2011; Ukeh *et al*., 2011). The previous study also reported that α-terpineol has insecticidal activity against several insects and mites (Chu and Jiang, 2011). This study showed that *M. leucadendron* oil had potent fumigant activity against greenhouse whitefly *T. vaporariorum*.

Conclusion

M. leucadendron leaf oil may have potential to be used as an ecologically safe pesticide against greenhouse whitefly *T. vaporariorum*. Effectiveness of *M. leucadendron* oil as insect control fumigant is likely influenced by major compounds of this oils such 1,8-cineole and α-terpineol. However, for practical use of this oil as novel fumigants, further studies need to be conducted to evaluate the cost, efficacy, and effect in crops of this essential oil on wide range of pests in commercial greenhouses.

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POTENTIAL OF *Acanthus ilicifolius* EXTRACT TO REDUCE DISEASES ON PRAWN

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ABSTRACT

Achantus ilicifolius is a mangrove plant that is often used by coastal society as a traditional medicine. In the future, this plant may be useful as a source of pharmaceutical products. The research was done to investigate the leaf extract to reduce vibriosis diseases and to inhibit the growth of *Vibrio harveyi* on prawn. The dried leaf was macerated using methanol, and then was evaporated to make crude extract. A part of the crude extract was fractionated by using column method with silica gel and with some solvents which were n-hexane, ethyl acetate, n-buthanol, ethanol and methanol. Prawn was infected with *Vibrio harveyi* until clinical symptoms arised, and was given the extract of immersion. Leaf extract could reduce mortality and symptoms of disease on prawn. N-butanol fraction was more effective to reduce the disease on prawn, but n-hexane was fastest to heal wounds and ulcers.
Keywords: Acanthus ilicifolius; Leaf extract; Penaeus monodon; vibriosis

INTRODUCTION

Conversion of mangrove forests into farm land, agriculture, plantation, oil and gas mine, and settlement would have a negative impact on regeneration of stock of fish and shrimp. It could lead to ecological functions obliteration of mangrove forests as the area is where fish and other organisms search for foods, protection, and spawning. Exploration of bioactive components from natural resources is an alternative in tackling diseases on prawn. To be able to control and to prevent disease is one success in prawn cultivation. Aquatic environment is a complex ecosystem that makes distinction between health, performance and disease. Until recently, the disease outbreak is still major problem on tiger shrimp culture in East Kalimantan by causing failure of production and financial losses.

Jeruju (*Acanthus ilicifolius*) is a mangrove plant often found in aquaculture coastal area of East Kalimantan, Delta of Mahakam in particular. The plant, normally, lives in the area, which is rather low in its salinity (± 15-20 ‰), forming bush around mangrove palms (Saptiani *et al*., 2012[a]). *A. ilicifolius* is a valuable medicinal plant that is widespread in tropical Asia and Africa, through Malaya to Polynesia (Khajure and Rathod, 2010). The leaves of *A. ilicifolius* are used to treat rheumatism, neuralgia and poison arrow wounds (Malaysia). It is widely believed among mangrove dwellers that chewing the leaves will protect against snakebite (Singh *et al*., 2009). In Bontang, tea brewed from the leaves relieves pain and purifies blood.

The mangrove plant *A. ilicifolius* plays mayor role in the defense and potential source of metabolites against the skin infection diseases (Govindasamy and Arulpriya, 2013). *A. ilicifolius* extract decreases protein exudation and leukocyte migration in the peritoneal fluid, thereby indicating its effectiveness toward inhibiting peritoneal inflammation (Kumar *et al*., 2008). The extract *A. ilicifolius* plant is potential to inhibit the growth of *V. harveyi in vitro* (Saptiani *et al*., 2012[b]). The plant is reported to contain phytochemicals including alkaloid and wide range of glucosides (Singh *et al*., 2009).

The purpose of this study is to assess the potential of *A. ilicifolius* extract to reduce diseases on prawn as an antibacterial agent and as an agent that could enhance the durability of shrimp against *V. harveyi*. The result of this study is expected could be used as a basis for further research; moreover, it is also an alternative as an effort to prevent and to control *Vibriosis* disease naturally by utilizing the vegetation around the grow-out pond.

MATERIALS AND METHODS

The leaves of *A. ilicifolius* were collected from the shrimp culture area on Muara Badak District, Kutai Kartanegara Regency of East Kalimantan. Collected leaves were cut into small pieces and shade dried at room temperature for 15 days. The extraction and fraction of the leaves were carried out by using different solvents in soxhlet apparatus. The dried leaves were macerated by using methanol, and then evaporated to make crude extract. A part of crude extract was fractionated by using column method with silica gel and with some solvents which were n-hexane, ethyl acetate, n-buthanol. The extraction and fraction were concentrated using vacuum distillation.

The prawns used in the study were 3-4 gram in weight and 1,5 months old and came from Basuki Hatchery in Muara Badak District, Kutai Kartanegara Regency of East Kalimantan. Before bred, the healthy prawn was not given antibiotics, chemical substances and other medicine. Next, the healthiest and motile larvae in PL 8 stadia from the breeding were chosen and cultivated in grow-out pond until they grew around 3-4 gram in weight. The healthy prawns were selected, put in a plastic bag, given oxygen and brought to laboratory. In the laboratory, the prawns were put in aquarium to be adapted for 24 hours, and next, screening test was performed by immersing the prawns by using 200 ppm formalin for 15 minutes, and then used as animal research.

The treatment contained 4 active materials with 2 different type doses. They were crude extract (300 and 500 ppm doses), n-hexane (200 and 400 ppm doses) ethyl acetat (200 and 500 ppm doses), n-butanol (100 and 300 ppm doses), and control (PBS 0,85 %), with 3 replications of each. The prawns were put in 30 different aquariums (15 prawns in each), and acclimated for 3 days. The prawns challenged with 10^5 cfu/ml dose of *Vibrio harveyi* as much as 0,1 ml and were conducted intra-muscularly on dorsal part, and then on the 4th day were treated with the extract. The extract of *A. ilicifolius* leaves treatment was performed by dipping method for 20 minutes.

The inhabitation observation or bactericide was performed with bacterial content test (Total Plate Count/TPC) by taking hepatopancreas sample to be isolated, cultivated on TSA and TCBSA media, and incubated at 33 ^{0}C for 24 hours. Then, the amount of colony growth was counted. The inhabitation observation and inspection covered clinical symptom, phonological anatomy, prevalence of attacks and viability.

RESULT AND DISCUSSION

The observation of clinical symptom after the challenged of *Vibrio harveyi* showed that the prawns were decrease in feed consumption, became lethargic and the body color changed more black. On the 3th day after the challenged of *Vibrio harveyi*, the prawns were lethargic. The symptoms included some appearance changes of reddish black, red patches on the legs and tail, haemorhagi on the body, deformity and molting death. The prawn showed that the challenged of *V. harveyi* caused specific clinical symptoms and death. In the beginning of extract treatment showed that the body color changed more blue. The prawns color changing indicated the occurrence of body reaction toward the extract of *A.ilicifolius* leaves treatment, and it was caused by the enlargement of prawns' cuticle. The color changing was also a sign that the immunity process occurred in order to fight foreign object entering the body since chromatophore is one of the body's defense system in prawn (Saptiani and Hartini, 2008 and Saptiani *et al*., 2012^a). On the 7th day, the prawns were looked getting better, active and getting increase in feed consumption. After the 14th day, the prawns were healthy and the body color became normal.

Similarly with the clinical symptom, the extract treatment showed that the pathology anatomy changing caused by *V. harveyi* infection could be reduced. Pathology anatomy performed on the 14th day showed that the prawns' hepatopancreas on control seemed brownish and mushy, some declined, and its bowel became reddish and hardened. The bacteria in prawns' digestion and organs functioning as digestive system would disrupt the work of the digestive system because the bacteria were able to parse the various polysaccharides and carbohydrates and took nutrients needed, and finally became the cause of prawns' death (Saptiani *et al*., 2012^a). In the treatment of leaf extracts and fractions of *A.ilicifolius*, it changed the pathological anatomy getting better, though initially there were some prawns' hepatopankreas brownish change. This indicated that bioactive of *A.ilicifolius* leaves could reduce diseases of prawn, so as to inhibit infection and to protect prawns from *V. harveyi* attack.

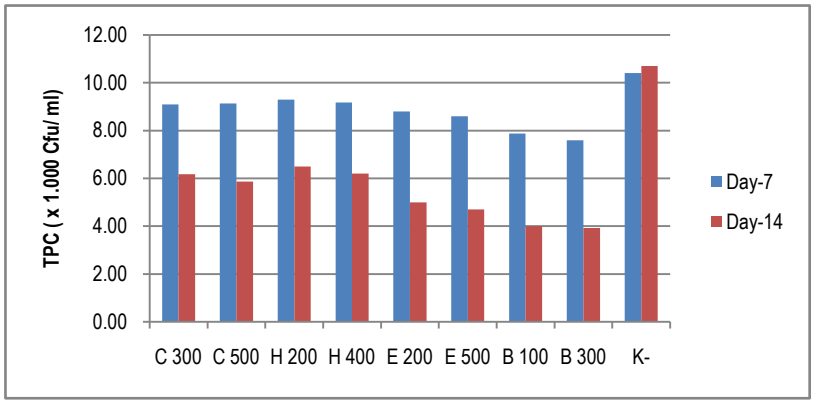

Fig. 1. Bacterial content (TPC) in tiger prawn hepatopancreas given the leaves extract and fractions of *A..ilicifolius*.
Description: C= crude, H= n-heksane, .E= Ethyl acetate, B= n-butanol, K-= control

The observation and calculation of *V. harveyi* content (TPC= Total Plate Count) in prawns' hepatopankreas organs, on the 7th day after the challenged of the *V. harveyi*, showed that the treatment of n-butanol of 300 ppm and 100 ppm were the lowest in its TPC (7,6-7,87 x 1.000 cfu/ml). In the meantime, TPC in the other treatment was ethyl acetate fraction of 500 ppm and 200 ppm (8,6-8,8 x 1.000 cfu/ml), 300 and 500 ppm crude (9,1-9,13 x 1.000 cfu/ml), fraction of n-heksane 200 and 400 ppm (9,17-9,30x 1.000 cfu/ml), and its TPC on the control was 10,40 cfu/ml. After the 14th day, its TPC slowed down except for negative control, as showed on Fig. 1. According to Saptiani *et al*. (2013), the extract and the fraction of *A. ilicifolius* leaves are potential to inhibit the growth of *V. harveyi in vitro*. *A. ilicifolius* has bioactive compounds that are potential as an anti-bacterial ingredient (Manilal *et al*., 2009).

The prawns survival rate on the 7th day that were given the extract and fraction of *A.ilicifolius* was around 91,10- 100,00 %, while the survival rate that was resulted from the control was 75,60%. The complete results could be seen in Fig. 2. The administration of *A. ilicifolius* leaves extract and fraction could enhance the survival of tiger prawn better than the control treatment toward *V. harveyi* attack. It showed that the bioactive of *A. ilicifolius* leaves extract and fraction could inhibit *V. harveyi* growth. The extract of *A.ilicifolius* leaves and root can inhibit bacteria growth, but the leaves extract inhabitation is better than the root (Khajure and Rathod, 2010; Saptiani *et al*., 2012b).

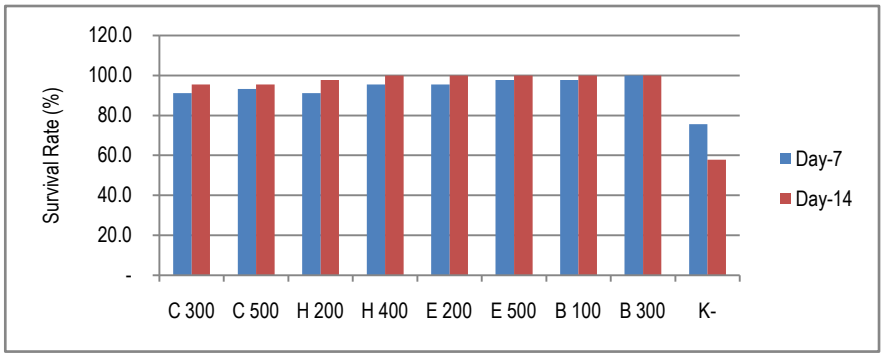

Fig. 2. The survival of tiger prawn given the leaves extract and fractions of *A. ilicifolius*
Description: C= crude, H= n-heksane, .E= Ethyl acetate, B= n-butanol, K-= control

The observation of the prevalence of the *V. harveyi* infection in prawns, administrated with the extract and fraction of *A.ilicifolius* leaves, showed that bioactive in the leaves could decrease the prevalence of infection or *V. harveyi* attack. The average prevalence of *V. harveyi* attack on the *A. ilicifolius* leaves extract and fraction treatment on the 7th day was 60,00-68,90 %, and after the 14th day decreased to 25,60-59,90 %, meanwhile the negative control was 82,1-84,5 %. The prevalence on the 14th day showed that all the treatments decreased. It occurred because challenge test or *V. harveyi* injection could increase the prawns immunity. As reported by Saptiani (2001), test challenge can increase antibody where the antibody is built after the infection, and it will increase more if there is secondary infection. This event will be beneficial to the organism itself, for it will increase resistance to the pathogenic organisms.

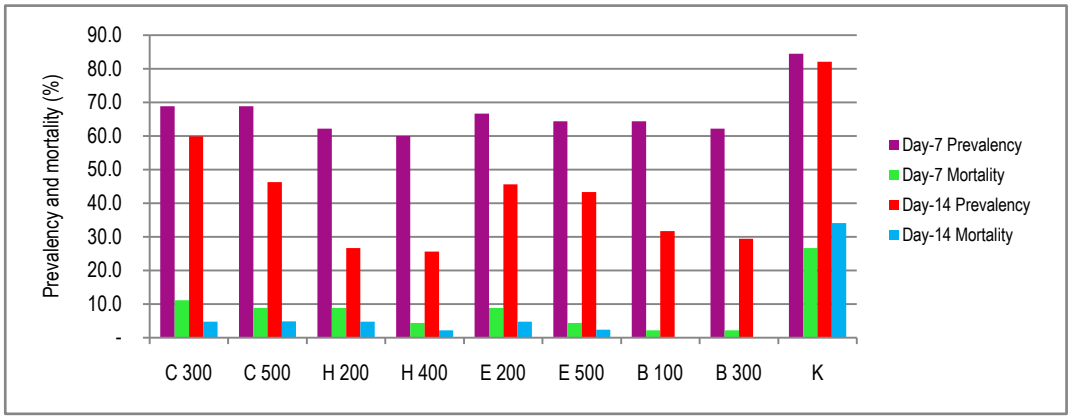

Fig. 3. The prevalence and mortality of tiger prawn given the leaves extract and fractions of *A. ilicifolius*
Description: C= crude, H= n-heksane, .E= Ethyl acetate, B= n-butanol, K= control

The leaves of *A.ilicifolius* extract and fraction could inhibit *V. harveyi* on prawns, primarily on the treatment of n-butanol fraction in all concentrations which could inhibit and reduce *V. harveyi*. This result showed that the bioactive content on *A. ilicifolius* extract and fraction was bactericidal toward *V. harveyi* and reduced diseases on prawns. Therefore it can be used in aquaculture. Sivaram *et al*., (2008), stated that the methanol extracts of herbal pathogenic *Vibrio* can control and improve the immunity system of the Grouper larvae (*Epinephelus tauvina*). Some researchers stated that *A. ilicifolius* mangrove contains bioactive compound that is potential to be used as antibacterial agent (Manilal *et al*., 2009; Khajure and Rathod 2010; Thirunavukkarasu *et al*., 2011). The plant contains chemical compound of glucose, alkaloid, flavonoid, fatty acids, steroids, lignans, and component of phenol and terpenoid (Kanchanapoom *et al*. 2001: Wostmann and Liebezeid 2008).

CONCLUSION

Acanthus ilicifolius leaf extract could reduce prawns diseases caused by *V. harveyi* attack and does not cause any vibrosis symptom. It could reduce mortality and the prevalence of prawns against *V. harveyi* compared to the control treatment. *A. ilicifolius* leaf extract could inhibit the growth of *V. harveyi* and protect prawns from *V. harveyi* attacks. Anatomic pathology of hepatopancreas and any other organs are normal.

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POTENCY USAGE OF PLANTATION FOREST OF *Acacia mangium* and *Acacia crassicarpa* AS SOURCE OF HONEYBEE FORAGE AND ITS PROBLEM

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ABSTRACT

The chosen of *Acacia crassicarpa* and *Acacia mangium* as raw material for pulp and paper industry in Riau was based on various factors. At the plantation area, these two species have higher growth than other species and easier to adapt to marginal soils. **Problems**. However, the existences of this species do not bring the positive benefits to local communities in the concession area. **Purpose**. The purpose of this study was to determine the potency of *A. crassicarpa* and *A. mangium* as a source of bee forage for honeybee farm in Riau province and the problem that can make the possibilities for cultivation efforts. **Results**. Measurement of extra floral nectar secretion of *A. mangium* age of 1 year showed that the volume of nectar secreted is 83250 cc / ha / day with an average water content 33%. Whereas, the age of 2 years reach 112387 cc / ha / day with an average water content 33%. On *A. crasicarpa* age of 1 year showed nectar secretion 42774 cc / ha / day with an average water content 33%. Whereas, the age of 2 years reach 53288 cc / ha / day with an average water content 33%. Placement of honeybee colonies of *A. cerana* in 1 year *A. mangium* showed the honey productivity of 1.8 lt /month, 1.19 lt /months at 2 years, and 1.172 liters/month at 3 years. Whereas, in 1 year *A. crassicarpa* plantations would produce honey 1.6 liters /month, 1.51 lt /month in 2 years, and 1.2 lt/month in 3 years. The problems when placed the honeybee colonies under stands of *A. mangium* and *A. crassicarpa* is scarcity of pollen source so it will impact to the honey productivity.

Key word: *Acacia mangium*, *Acacia crassicarpa*, *Apis cerana*, nectar, honey

I. INTRODUCTION

There are two big pulp and paper industries that had concession area in Riau nearly 800,000 ha by the realization of nearly 600,000 ha (Riau Forestry Service, 2006). Clearing of land for concessions plantation forest cannot be inevitable, because this industry has given a great contribution to the national economy. Contribution of prominent industry and its derivatives, the other contribution is in the form of employment and foreign exchange contribution of exports. Absorption of labor by the pulp industry is estimated around 110,000 people in 2006 (APKI, 2006). On the production side, of the total 6.5 million tones of pulp and its derivates had resulted and nearly 2.6 million tons of which are exported to Japan with estimated foreign exchange value of 3.3 to 3.5 billion dollars (Arisman, 2006).

In the Riau province there are at least 3 species that used as raw material for pulp and paper industry, namely *Acacia mangium* and *Eucalyptus* sp. in the mineral/dry land and *Acacia crassicarpa* in the peat land (Mindawati, 2010). Some reasons for selecting *A. crassicarpa* in peat/wet land and *A. mangium* in mineral/dry land is this species can grow well, have high increment, easy maintenance, high yield production, relatively content of low lignin, and have the high strength (Pasaribu and Tampubolon, 2007).

One of the social problems that arise as the negative effects of forest management by the plantation forest is conflict between the industry and the local community. One study noted between 1997 to 2004 there has been 359 conflicts in the forestry sector. While 34% occurred in the area of conflict conservation (including protected forests and national parks) and 27 % occurred in the area of forest concessions (Wulan *et.al*., 2004). This conflict can be developed more widely, since there are currently approximately 10 million people who live in poor communities around the forest area (Directorate General of Forestry Production, 2006). Environmental problems are also rising from plantation forest, such as increasing of sedimentation in rivers around the area (especially at the opening area, prone to forest fires, and the spread of pests and diseases, and potentially reducing the water table) .

The solution is to utilize the non wood potency that abundant on plantation forest. The potency is extra floral nectar that can be used as local honey bee (*Apis cerana*) forage. Honey that produced by *A. cerana* that comes from *Acacia* nectar can be used to increase the economy of local community that live around the concession area. Vegetation of *A. crassicarpa* and *A. mangium* as a honey bee farm is suspected to be a source of feed honey bees because there are abundant, sustainable, and never stop to secreted the nectar in every day so it is possible to develop beekeeping

business (Purnomo, 2010). The purpose of this study were (1) to determine the potency of *A. crassicarpa* and *A. mangium* as a source of bee forage for honeybee farm in Riau province and (2) the problem that can make the possibilities for cultivation efforts.

II. Potency nectar of *A. crassicarpa* and *A. mangium*

Observation of the nectar secretion in age 1 of *A. crassicarpa* showed that the nectar is secreted in the base of the leaves (Figure 1) produces secretions of 42.774 liters per day / ha, while at the age of 2 years reach 53.288 liters per day / ha. This trend will continue to increase until the end of the harvest (5 years), total to 84.590 liters per day / ha (Table 1). In observation of nectar water content showed a similar trend for each age standing in the amount of 33%.

Table 1. Secretion volume of *A. crassicarpa* plantation in each planted age

A. crassicarpa age	Secretion volume per day/ha (lt)
1	42.774
2	53.288
3	63.682
4	74.136
5	84.590

Figure 1. *Acacia* extra floral secreted on the base of leaf

Mean wahile, measurement of extra floral nectar secretion of *A. mangium* age of 1 year showed that the volume of nectar secreted is 83.250 lt / ha / day with an average water content 33%. Whereas, the age of 2 years reach 112.387 lt / ha / day with an average water content 33%. This trend will show the increasing proportional to the end of the stand (199.8 lt /ha/day)(Table 2).

Table 2. Secretion volume of *A. mangium* plantation in each planted age

***A. mangium* age**	**Secretion volume per day/ha (lt)**
1	83.25
2	112.38
3	141.52
4	170.66
5	199.8

Calculation of extra floral nectar potency of *A. crassicarpa* and *A. mangium* as honeybee forage is influenced by many factors, such as :

(1) total amount of leaf in each stand in every age
(2) competitor; organisms such as insects other than honey bees that also utilize the extra flora nectar. Results showed that only red ants eat this nectar (spend about 10 % of the total nectar)

(3) decreasing of water content of the nectar secretion . The analysis showed that the water content of extra floral nectar is about 33 % . While on placement of *A. cerana* in *A. mangium* and *A. crassicarpa* plantation showed the honey that extracted from the colony only reach 22 % of water content.

(4) the daily consumption of honey bees to be taken into account as much as 10 % of the amount of honey stored in the colony .

III. Productivity of honey bee that placed on *Acacia* plantation

Placement of local honeybee colonies (A. cerana) in 1 year A. mangium Showed the honey productivity of 1.8 lt / month, 1.19 lt / months at 2 years, and 1,172 liters / month at 3 years. Contrast to the tendency of nectar secretion in *A. mangium* that showed increase in every year to the end of the rotation, the pattern of the honey productivity showed decresing trends (reached only 0.45 lt / colony / month in the end of *A. mangium*) (Table 3).

Table 3. Honey productivity of *A. cerana* that placed on *A. mangium* plantation

A. mangium age	Honey productivity per colony (lt)
1	1.80
2	1.19
3	1.17
4	0.76
5	0.45

Whereas, in 1 year A. crassicarpa honey plantations would produce 1.6 liters / month, 1.51 lt / month in 2 years, and 1.2 lt / month in 3 years. The same trend occurred in the honey productivity by *A. cerana* that placed on stands of *A. crassicarpa* which showed the declining up to 0.84 liters / colony on A. crassicarpa age of 5 years (end of the rotation) (Table 4).

Table 4. Honey productivity of *A. cerana* that placed on *A. crassicarpa* plantation

A.crassicarpa age	Honey productivity per colony (lt)
1	1.60
2	1.50
3	1.20
4	1.03
5	0.84

Declining trend in the honey productivity is indirectly proportional to the stand age of *A. mangium*. It might be caused by a variety of factors, such as pollen lack. Based on the analysis of vegetation indicates that the older the age of the stand, there is low population of vegetation in under the *A. mangium.* But in the 1 year of *A. mangium*, in which the sun light can penetrate forest floor, vegetation such as *Mimosa pudica*, *Ageratum conyzoides*, and *Assystasia* sp. that produce pollen are still grow well so the *A. cerana* can sufficient the protein needs from the pollen source. Whereas at the stands age above 3 years which the sun light cannot penetrated to the forest floor vegetation. So the vegetation under the stands will die and effect to loss of pollen source for honey bee. The other cause is the alelopati effect of *A. mangium* will also repressed the vegetation below the *A. mangium* stands.

Whereas, the honey productivity of local honeybee that placed on *A. crassicarpa* showed similar tendency to the honey productivity of *A. cerana* that placed on *A. mangium* (decline opposite to the stand age). The difference is the type of vegetation that available on the under of *A. crassicarpa* stands that dominated by fern (*Neprolephis* sp. and *Stenochlaena palustris*) which are not produced pollen. Kleinschmidt (1982) stated that the healthy honeybee must contain between 40% to 67% of Crude Protein (CP). Honeybee must consumed much pollen that contain at least 18% of protein to get the 40% CP. *A. cerana* that under pollen lack conditions will effected to their activities to get nectar and also to the fecundity of honeybee queen.

According Mourizio (1975), pollen is a protein source for honeybee larvae and development of adult honeybee. In addition, pollen also contain fat, vitamins and minerals. Dietz (1975), larvae of honeybee required a total of 120 up to 150 mg of pollen to reach the adult phase. The proteins that available on the pollen serves as the important matter for the

formation of hypopherengeal glands located on caput of worker honeybee to produce royal jelly (special food only for queen honeybee).

IV. Conclusion

1. Observation of the extra floral nectar secretion at *A. crassicarpa* age of 1 year reached 42.774 liters per day / ha, while at the age of 2 years to reach 53.288 liters per day / ha. This trend will increase until the end of the harvest cycle (5 years), amounting to 84.590 liters per day / ha.
2. Observation of the extra floral nectar secretion at *A. mangium* age of 1 year reached 83.250 liters / ha / day, while at the age of 2 years reach 112.387 liters / ha / day. This trend will continue to increase proportional to the age of the stand (5 years), amounting to 199.8 l liters per day / ha.
3. The problems of beekeeping when placed under the stands of *A. mangium* and *A. crassicarpa* plantation are scarcity of pollen source so it will impact to the honey productivity.

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Study on Land Rehabilitation at Mined Lands of PT Trubaindo Coal Mining, West Kutai, East Kalimantan (2011 - 2012)

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Abstract

In many cases, mined lands is not immediately ready to support plant growths due to bad drainages, soil structure disturbances, high temperature, uncontrollable overland flow, high erosion rate, lack understanding of suitable plant species, planting techniques and its maintenance. The study objective is to find out applicable design - procedure - techniques of mined-out lands revegetation to support and accelerate the ecological recovery of mined-out lands. Land revegetation is conducted by planting 10 trees species (3.000 seedlings) at mined-out lands after reclamation works. Plants species are selected based on previous studies and expected ecological function. Plant height, diameter, and growth performance are used as parameters associated with physical and chemical soil characteristics. The plant growth percentage is 93% with the lowest of *Artocarpus champedens* (84%) and the highest of *Peronema canescens* (86%) consecutively. Moreover, *Terminalia catappa*, *Annona muricata*, and *Muntingia calabura* show the strongest growth performance, while *Shorea balangeran*, *Artocarpus champedens*, *Pterocarpus indicus* are found weak. Soil is found being high compacted but no significant difference of fertility status between upper and lower layers, and also recognized being acid with moderate CEC and very high base saturation. Organic fertilizing reduces soil compactness, increasing soil porosity, CEC and its water content. Fertilizing of 100g NPK (16-16-16) stimulates biggest diameter and height increments of *Shorea balangeran*, *Terminalia catappa*, *Annona muricata*, *Ficus variegata*, *Schima walichii*. Moreover, *Shorea balangeran*, *Entrolobium cyclocarpum*, *Terminalia catappa*, *Annona muricata*, *Ficus variegata*, *Schima walichii*, *Peronema canescens*, and *Muntingia calabura* are still responsive to fertilizer application. Considering low nutrients contents, chemical fertilization are still needed to immediately increase macro nutrients content. Suggested dosage of NPK (16-16-16) fertilizer is 100 gr for *Entrolobium cyclocarpum*, *Peronema canescens*, *Muntingia calabura*, *Terminalia cattapa*, and *Schima walichii*; 150 gr for *Annona muricata*, *Ficus variegata*, *Shorea balangeran*; and 50 gr for *Pterocarpus indicus* and *Artocarpus champedens*. Simultaneously with revegetation works, the phenomenon of soil erosion potential dynamic is depend on influencing factors upon soil erosion occurences that are rainfall erosivity, soil erodibility, landform slope-lengths, vegetation cover, practical soil and water conservation. Vegetation growth cover is the most significant factor related with soil erosion dynamic at revegetated mined-out lands.

Keywords: *mined-out lands*, *lands rehabilitation*, *physical-chemical soil characteristics*, growth *percentage*, *tree species, soil erosion potential*

Introduction

Background

The utilization of natural resources and environmental management have to be conducted properly to minimize its negative impacts into environments, in order being possible to retain quality and sustainability of natural resources for pepoples welfare. PT Trubaindo Coal Mining (TCM) as one of leading coal mining company strongly efforts to monitor and manage all potential - on going - predictable arising negative impacts due to coal mining activities. Coal mining activities causes a significant environmental impacts related over lands slope stability, hydrology, environmental pollution, natural resources decreasing, infiltration rate and capacity, vegetation covers dissapear, topsoils removal, wildlife and its habitat, peoples health, social economic disparities ect. Therefore coal mining activities have to be followed by maximum efforts in line with its standar operating procedures of lands clearing, topsoils management, mining waste treatments, water resources monitoring, mined-out lands restoration - reclamation - revegetation and other important efforts related with coal mining operation.

A part of working area of PT TCM is located at production forest area based on borrow-use pattern permit earned from Ministry of Forestry, and therefore PT TCM has a mandatory obligation to rehabilitate its mined-out lands to be rocovered into productive lands of production forest area in the future. The achievement of mined-out lands rehabilitation needs a proper knowledge and field experiences especially focused in soils and lands dynamic, reclamation-rehabilitation techniques, species site matching based on specific government rules, and also lands preparation, planting techniques and its vegetation maintenances.

PT TCM applies open pit/cast method of mining operation started with general preparation followed by land clearing - topsoils striping and removal - blasting - over burden opening and coal mining. However, considering that coal mining is a kind of non-renewable resources utilization, it must be fully considered to the principles of rational - efficient, and as far as possible to minimize the environmental impacts and disturbances in the spirit of keep an enough stock for next generation. Moreover, it is also well known that application of open pit mining system causes landscape changes and at the same time influencing *bio - geo - physics - chemical characteristics* changing, social-economic-culture and health of peoples. For this reason, it is needed to prepare and anticipate of all countermeasure efforts in relation with sustainable capacity of environmental for supporting peoples life and welfares.

Rehabilitation of Mined-out Lands

As a part of environmental setting and management planning which is effective and efficient, mined-out lands rehabilitation have to be done following the technical method and procedures explained at documents of Environmental Impact Assesment, Monitoring, and Environmental Management Plans. Land clearing have to be followed by field practical works relating to lands rehabilitation efforts. Removed topsoils as about 30cm should be temporarily placed at designed site surrounding mining operation area and later on to be replaced at the top of reclamied sites to support plant growth of lands revegetation.

Topsoils stockpile is protected with dykes in order for not being eroded by surface runoff during rainy days. To make sure that lands rehabilitation after open mining system could be performed, mined-out lands have to followed by backfilling whether in our out pit dump immediately. Moreover, concerning to the coal seam position and its slope, specific method of contour mining, area mining and panel mining are applied by PT TCM. Mined-out lands reclamation - reshaping and recontouring are conducted at each block consecutively and at the end all of block are completed. Both of mining water and rainfall which are flowing as surface runoff and potentially transporting mass mud are controlled and directed into catchpond/settling pond for mud sedimentation.

Open pit system causes decreasing lands surface and new disposal of over-burden and inter-burden. To reduce such mass dumping disposals, these materials are then used to fill former pit. As backfilling is completed generally is directly followed by slope and drainage works to avoid increasing and uncontrollable surface runoff. Finally, this reclamation works is continued by spreading of topsoils and therefore ready for revegetation works. Revegetation works is initiated by land cover crops to minimize potential soil erosion. Legume plants is used to accelerate soil fertilization and followed by tree planting with selected plants of fast growing species. After cover crops and fast growing species plants are growing well, it is possible to conduct enrichment planting by using selected tree species based on some considerations such as lands status, economically productive trees, and also ecpected ecological role for supporting life system.

General Characteristics of Mined-out Lands

As mentioned before, open pit system produces a mass disposal of over-burden and inter-burden which is then used to fill mined-out pits. After backfilling process, most of dumping materials is in condition of wreak or disturbed structure and pores and could be a mass of unstable dumping materials. Moreover, some time also unavoidably mixed with dirty coal and off course almost without any organic material. This condition lead to a bad drainage and low water holding capacity, highly compacted and difficult to be penetrated by plant roots, opening space and high temperature.

Problem Formulation

Regarding to lands rehabilitation through reclamation and revegetation activites, the main problem is that backfilled mined-out lands not fully ready to support plants growth due to bad drainage, disturbed soil structure and also relatively high land temperature. Subsequently, uncontrollable high overland flow followed by high soil erosion rate, fragmented soil materials, soil mixed with fine coals and lack of organic materials are also have to be properly treated before planting. In relation with planting, species site matching (trees, land cover crops), planting techniques and its procedures for maintenance are also still needed to be solved. So far, the results of mined-out lands revegetation has not been satisfy as expected especially in species diversity due to plants species still limited to monoculture species.

Study Objective and Expected Results

The main objective of this research is focused to develop design, procedurs, techniques, and the successfulness of mined-out lands revegetation. Therefore, the results of this reseach is expected to be one of important consideration to implement many efforts in ecological function recovery, economics and social aspects of forest areas post coal mining actvities.

Study Methodology

Study Site and Research Period

The study of mined-out lands reabilitation is conducted at the coal concession of PT TCM Site Adong, West Kutai, East Kalimantan. Panoramic view of the rest forested area is shown at **Photo-01** as representative of surrounding mined-out lands. The green spots of forested area shows a good forest with natural regeneration with high density ranging 3.000 - 5.000 seedlings per hectare with identified species of *Shorea parvifolia*, *Dipterocarpus cornutus*, *Shorea ovalis*, *Shorea ochracea*, and *Shorea superba*.

Photo-01.View of Original Forest Vegetation Surrounding Mined-out Lands of PT TCM, West Kutai, East Kalimantan

Regarding to government acts in forestry, the status of study site area is a non forest area (KBNK) with a massive destruction post mining operation. Administratively, the site is located at West Kutai regency as a part of East Kalimantan Province. Geographically, all of coal consession area of PT TCM is lying at the coordinate of 0°27'44'' - 0°51'41'' South and 115°30'00'' - 115°51' 30Œast.

Research Procedures

The research is performed during arround two years (June 2011 - December 2012) covering of documentation and refferences study, field data collections, data organization, analysis and reporting. Field observation and measurement are performed into diameter, height, and physical field performance of plant growth. The soil chracteristics and soil erosion potential are also studied simultaneously with the efforst of reclamation and revegetation of mined-out lands.

Photo-02. View of Study Area - Reclamation of Mined-out Lands of PT TCM, West Kutai, East Kalimantan

Study Site

The study of mined-out lands rehabilitation is conducted at Block-2 Ex-Pt with ± 6 hectares large in area. Before this study, earthworks was done in this location such as ex-pit back filling, re-contouring, re-shaping and topsoils spreading. The final result of these earthworks is a landscape with 8-15% slope as viewed at **Photo-02**.

Plants Species

The plants used for field planting trial in this study are consisting of ten species that are S01-*Shorea balangeran* (Kahoi), S02-*Artocarpus champedens* (Cempedak), S03-*Entrolobium cyclocarpum* (Sengon Butho), S04-*Terminalia catappa* (Ketapang), S05-*Pterocarpus indicus* (Angsana), S06-*Annona muricata* (Sirsak), S07-*Ficus variegata* (Kondang), S08-*Schima walichii* (Puspa), S09-*Peronema canescens* (Sungkai), serta S10-*Muntingia calabura* (Kersen/Cherry). Plants materials of several species are shown at **Phto-03** of which **e**ach species as much as 25 individual plants.

Photo-03. Several Plant Species for Mined-out Lands Revegetation Work

Repetition of Plants Species

The experimental design for data collection and analysis of field observation and measurement results is done by using completely randomized design. This experimental design is used by considering available location for study, accessibilty and supporting facilities. Moreover, the sum of repetition for each species is 3 (three) following randomization and requirement for statistical analysis reason.

Soil Treatment on Planting Holes

This research is also perform 4 (four) soil treatments of T1, T2, T3 and T4. T1 is regular treatment done by PT TCM in their mined-out lands rehabilitation and therefore is used as benchmark for other treatments of this research (**Table-01**). Planting pattern is 3 m x 3 m and as much as 25 invidual plants are planted at each compartment (PUC) for each species.

Table-01. Soil Treatment Applied at Mined-out Lands Rehabilitation of PT TCM

Treatment	Organic Fertilizer (kg)		Dolomite (kg)		NPK Fertilizer (g)	
	Per-plant	Total	Per-plant	Total	Per-plant	Total
Year 2011	**First Year**					
T2	20	15.000	0,5	375	50	37.500
T3	20	15.000	0,5	375	100	75.000
T4	20	15.000	0,5	375	150	112.500
Total		45.000		1.125		225.000
Year 2012	**Second Year**					
T2	10	7.500	0,5	375	50	37.500
T3	10	7.500	0,5	375	100	75.000
T4	10	7.500	0,5	375	150	112.500
Total				1.125		225.000

Note: Number of Plant/Treatment = Σ Species (10) x Σ Plant/PUC (25) x Repetition (3) = 750

Design of PUC

The layout of PUC is made as long as following the availability of mined-out lands in the field (**Figure-01**). For statistical reason, the placement of PUC completely is randomized which based on repetition (U), soil treatments (T) and its plant species (S) as shown at **Table-02**. Each PUC is quadrangle of 15 m x 15 m in shape or 225 m^2 in area and therefore the study area of mined-out land is 120 x 225 m^2 = 29.700 m^2 or 2,97 hectares large in area.

Table-02. Randomization of PUC at Mined-out Lands Revegetation

No.	PUC	No.	PUC	No.	PUC	No.	PUC	No.	PUC
001	U2T2S06	025	U2T3S01	049	U2T1S06	073	U2T2S09	097	U3T4S09
002	U2T4S01	026	U2T1S04	050	U1T3S09	074	U1T2S05	098	U1T2S04
003	U3T1S03	027	U2T3S04	051	U2T4S02	075	U3T1S10	099	U1T4S06
004	U2T2S04	028	U3T3S05	052	U1T2S09	076	U2T4S10	100	U2T2S07
005	U1T3S02	029	U1T4S08	053	U1T4S07	077	U2T2S03	101	U1T2S06
006	U1T1S10	030	U1T4S05	054	U3T4S01	078	U3T1S05	102	U2T4S04
007	U1T4S04	031	U1T3S03	055	U2T3S10	079	U2T4S09	103	U3T4S07
008	U3T4S02	032	U1T1S01	056	U3T4S03	080	U3T3S10	104	U2T4S08
009	U2T1S01	033	U3T3S02	057	U2T1S03	081	U1T3S10	105	U1T2S07
010	U1T1S02	034	U3T3S06	058	U2T3S05	082	U3T2S03	106	U2T3S09
011	U3T2S09	035	U2T2S02	059	U1T4S09	083	U1T1S08	107	U1T1S06
012	U2T1S07	036	U1T3S05	060	U3T3S09	084	U3T2S04	108	U3T1S08
013	U1T1S03	037	U3T1S09	061	U1T2S01	085	U3T4S04	109	U1T2S08
014	U3T1S02	038	U2T2S01	062	U3T4S06	086	U2T4S05	110	U3T4S08
015	U2T1S10	039	U2T2S05	063	U2T3S08	087	U3T3S08	111	U1T3S04
016	U1T3S08	040	U3T1S01	064	U3T3S04	088	U2T2S10	112	U3T2S06
017	U2T1S05	041	U1T2S02	065	U1T4S03	089	U2T4S06	113	U2T4S07
018	U3T3S01	042	U3T1S07	066	U2T1S08	090	U3T4S10	114	U3T2S08
019	U1T3S01	043	U3T2S01	067	U3T4S05	091	U3T2S07	115	U2T3S03
020	U2T1S02	044	U1T2S03	068	U3T1S06	092	U1T4S02	116	U1T1S07
021	U1T1S05	045	U1T4S10	069	U2T3S06	093	U1T1S09	117	U1T3S07
022	U3T3S03	046	U2T3S02	070	U3T1S04	094	U2T3S07	118	U1T1S09
023	U1T4S01	047	U3T2S02	071	U2T4S03	095	U2T2S08	119	U3T2S05
024	U1T1S04	048	U3T2S10	072	U1T3S06	096	U3T3S07	120	U1T2S10

Measurement of Plant Height and Diameter

The plant diameter and height are used as qunatitative parameter for plant growth percentage. Plant height is measured by scaled wooden stick while diameter measurement is done by using micro-caliper and plastic metline scale. Both of measurement are using centimeter scale.

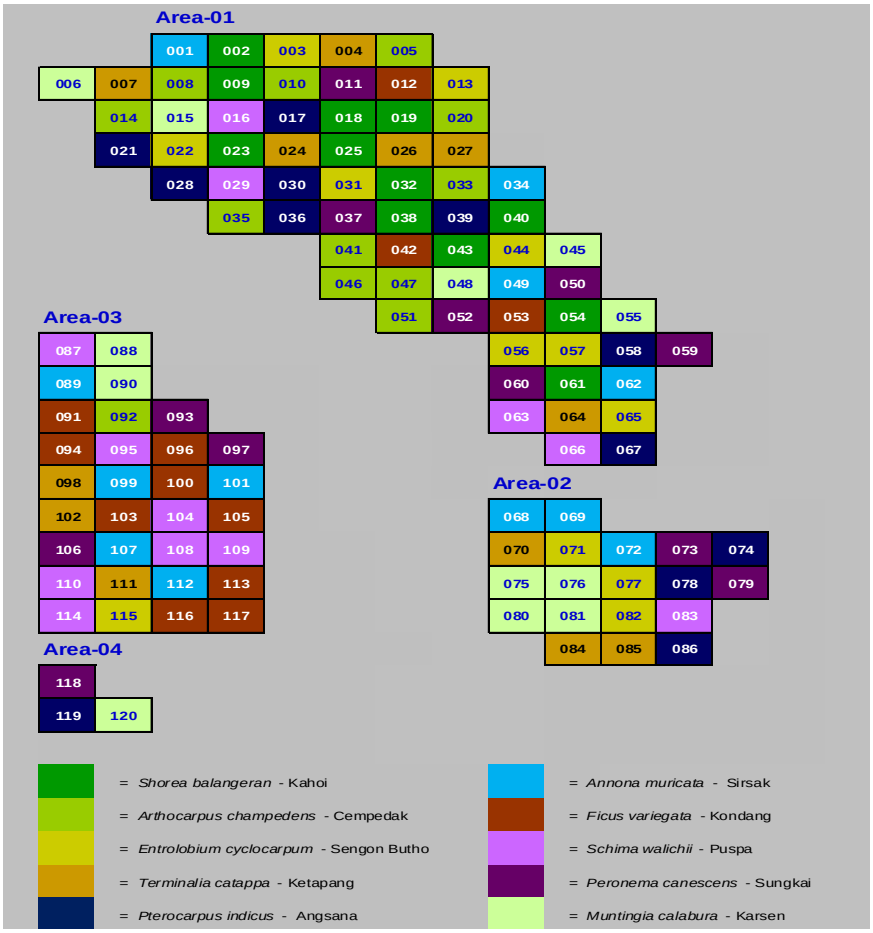


Figure-01.Layout Distribution of Plant Species at Mined-out Lands

Result and Discussions

Description of Study Site

Geographycal Position

The coal mining concession of PT TCM is 23.650 hectares and located at West Kutai, East Kalimantan. Travelling throughout provincial way connecting Samarinda City to study site needs about 7 (seven) hours. Meanwhile, using river transportation in about 16 (sixteen) hours travelling along Mahakam River. Geographically, all of coal consession area of PT TCM is lying at the coordinate of 0°27'44'' - 0°51'41'' South Latitude and 115°30'00'' - 115°51' 30Œast Longitude.

Geology

Geological condition covering study site shows two existing major synclines of North and South areas. Northern area consist of North and East blocks, while in Southern area are Dayak Besar, Nage, Biangan, Perak, and Lati blocks in which prospective mined coal seam is in layer group between 1.700 - 9.300. The slope of coal seam is in 20° - 50° ranging and distributed in several geological formation. Variation of lithology covere coal seam consist of sandstone and silstone, and the rock structure has been indicated as Miocene to Holocene. The rock structure is a repetition of sandstone-siltstone-coal with formation depth more than 4.000 m in form of syncline in the direction to West-South and East-North. Average coal quality indicates a gross calorific value of 6.400-6.600 Kcal/kg in North Block, 6.100-7.600 Kcal/kg in South Block and more than 7.000 Kcal/kg for East Block. In general, the ash content is low with high variation of sulphure. The coal potential is about 33,7 MT (North), 28,t MT (Dayak Besar), and 34,5 MT (Biangan).

Climate

According to Climate Classification System of Schmidt and Ferguson (1951), the study site is under influence of A type (very wet) indicated by Q = 0. Annual rainfall is 3.832 mm with monthly average of 319 mm, highest in April (463 mm) and the lowest in August (171 mm) followed by mean evapotranspiration 1.795 mm/year or about 150 mm/month. The monthly maximum air temperature 34,6°C and minimum 21,8°C with average of 27,8°C. Subsequently, monthly average of relative humidity is ranging 83,0-87.0% with average 85,9%. Monthly wind velocity is 7,2 m/sec. highest in May (12,9 m/sec.) and lowest in July (3,6m/sec.). Using rainfall - evapotranspiration approach, water deficit is in September as much as 89,3 mm but in overall is still surplus of water as much as 1.579 mm/year or in about 131,7 mm/month.

Vegetation

Based on the results of environmental impact assesment document of PT TCM, it is known that the diversity of plant stage, seedling - sapling - pole - tree is relatively high. For seedlings, the dominant species are *Mellastoma sp* (Karamunting), *Macaranga triloba* (Mahang), *Piper aduncum* (Siri-siri), *Macaranga retiniodes*, and *Eurycoma longifolia* (Pasak Bumi). Moreover for sapling stage are *Mellastoma sp* (Karamunting), *Macaranga triloba* (Mahang), *Macaranga gigantea* (Bingkungan), *Ptelocolobium dulce* (Petai hutan), and *Octomeles sumatrana* (Binuang bini). For pole stage are *Macaranga triloba* (Mahang), *Vitex pubescens* (Laban), *Arenga pinata* (Aren), and *Eugenia sp* (Jambu-jambuan). For tree stage is dominated by species of *Peronema cenescens* (Sungkai), *Vitex pubescens* (Laban), *Arthocarpus elasticus* (Terap), *Gluta renghas* (Rengas), and *Trigonopleura malayana*.

Mined-out Lands Rehabilitation

Mined-out lands rehabilitation has to be conducted in relation with environmental management efforts to recover ecological soil function. PT TCM has been keeping the mission to create an added value of rehabilitated mined-out lands by planting several multipurpose trees species which is not only for ecological function but also trying hard to increase economic value by planting local tree species. Land reclamation itself is performed in several steps as topsoils removal-ex-pit backfilling-recontouring-reshaping-topsoils spreading. Subsequently, lands revegatation is performed through lands preparation-planting-maintenance to support plants growth. Since 2006, PT TCM has been a lot of lands rehabilitation works pattern by planting cover crops and primary species such as Gmelina, Rubber, Bananas.

Plants Species - Planting Tecchnique - Maintenance

The plants used for field planting trial in this study are consisting of ten species that are *Shorea balangeran*), *Artocarpus champedens*, *Entrolobium cyclocarpum*, *Terminalia catappa*, *Pterocarpus indicus*, *Annona muricata*, *Ficus variegata*, *Schima walichii*, *Peronema canescens*, *Muntingia calabura*. All of plant materials are prepared by Researcher and also PT TCM. Planting holes, organic fertilizer and dolomite application were done by personels of Rehabilitation Section (RS) of PT TCM. Subsequently, measurement and plant maintenance are also performed by RS.

Plants Growth Percentages

In overall, ten plant species with 300 plant individu for each and therefore as much as 3.000 plant individu were planted in third week of October 2011. Based on field observation, it is obtained that growth percentage of plant is ranging at 66% (Cempedak) to 98% (Kondang). In overall 3.000 plant 84% is survived. From 3.000 planted plants, the survive plants including replantted plants is 2.525 individu (84%) with lowest survival Cempedak (66%) and Sirsak (70%), while Sengon butho, Ketapang, Angsana and Kondang are higher than 90%.

Plant Growth

This study uses ununiform plants material of 1-2 years age with relative tall height and young age of 3 months with small size plants due to limited availability of expected species. Aged plants material are Cempedak, Sengon, Kondang, dan Sungkai; and the small plants material are Ketapang, Angsana, Sirsak, Puspa, and Kersen. Considering the ununiformity assessment of plants increament (height and diameter) is expressed in relative value. Plants species classified fast in growth are Sengon butho, Angsana, Sirsak, Kondang and Kersen wich recorded 1.000% diameter increament compared to initial diameter. Moreover, plants species is fast in height increament are Ketapang, Sirsak and Kersen which is recorded 7-9 times increament higher compared with initial height.

Table-03. Plant Species and Performance at Mined-out Lands Rehabilitation in 19 December 2012

No	Species	Survive	Dead	Diameter (cm)		Height (cm)		Rd (%)	Rh (%)	Performance	(%) Survive
				(10/2011)	(12/2012)	(10/2011)	(12/2012)				
01.	Kahoi	239	61	0,53	1,92	134,12	119,20	262	-11	62	80
02.	Cempedak	199	101	0,40	1,81	86,76	112,00	353	29	62	66
03.	S-butho	290	10	0,44	12,88	90,73	490,42	2.827	441	38	97
04.	Ketapang	272	28	0,47	4,72	16,87	182,28	904	980	39	91
05.	Angsana	274	26	0,28	6,14	65,78	295,67	2.093	349	48	91
06.	Sirsak	209	91	0,14	2,79	14,25	130,55	1.893	816	47	70
07.	Kondang	294	6	0,45	7,28	82,96	288,01	1.518	247	40	98
08.	Puspa	239	61	0,21	1,99	21,70	98,07	848	352	49	80
09.	Sungkai	227	73	0,46	4,04	56,96	168,80	778	196	42	76
10.	Kersen	282	18	0,29	7,36	37,85	333,13	2.438	780	45	80
	Total	**2.525**	**475**								
	Total		**3.000**								

Using physical strong performance expressed in height-diameter ratio limit = 80, all of species can be categorized to be strong. However after 14 months planting, the ratio decrease under 50% except Kahoi and Cempedak. Both of these species is found to grow well. The value of height-diameter ratio limit in 2011 is 212% and 177% consecutively and in 2012 decrease to be 62%. The main cause of this condition is to much longer in nursery with uncomfort situation and hard competition of light for growing. At the end of 2012, all of plants spcies show a prospective growth. The biggest growth diameter and height is owned by Sengon butho.

Initial Soil Condition

Bulk density is ratio of dry soil mass weight to soils volume including soil pores that indicates the soils compaction and therefore more bigger means more compact soils. In general the value of bulk density is ranging between 1,1 - 1,6 g/cc (Hardjowigeno, 2003). Soils is quiet compact indicated by average of bulk density 1,69 g/cc in the range of 1,51 - 1,80 g/cc. The total pores shows that most of soils volume is mineral and organic materials (± 64%) and only 36% can be filled with air and/or water. Normally, about 50% of mineral soils volume is filled with air and/or water and other 50% are mineral and organic materials, and therefore soils compaction must be reduced and increasing soils pores for supporting plants growth. The common way to increase the proportion of mineral and organic materials is by adding soils organic materials due to an enough organic materials in the soils could make a better condition and plants roots can penetrate the soils even in a low available soils water as generally found at mined-out lands. The initial view of mined-out lands before planting works is shown at **Photo-05** and the detail of physical and chemical soils characteristics is figurized in **Table-04**.

Photo-05. View of Mined-out Lands After Lands Reclamations Works and Lands Preparation for Revegetation Works

The laboratory analysis on chemical soils characteristics show that no significant difference of soil fertility status between upper and lower soils layers. Both of these layers are found being acid with N, P, K very low, moderate CEC while base saturation is very high. This condition indicates that a high cations absorbtion lead to fertilizers being not leached easily following water percolation. High base saturation denotes that soils is dominated by base cations (Ca, Mg, K, NH4, Na), which is good for plants growth due to these cations are needed in a large amount to support plants growth. Conversely, an acid soils and a very low N, P, K contents should be improved by adding dolomite and NPK fertilizer application.

Table-04. Physical-Chemical Soisl Characteristics at PUC of Mined-out Lnds Rehabilitation

Description	BD (g/cc)	Total Pores (%)	pH H_2O	Nitrogen (%)	Carbon (%)	P_2O_5	K_2O	CEC	BS	Al Available
Soil Layer	**00 - 30 cm**									
Mean	1,69	36,36	4,92	0,05	0,69	7,57	6,66	16,78	74,5	25,5
Maximum	1,80	43,19	6,60	0,13	4,39	15,30	17,14	31,40	100,0	60,5
Minimum	1,51	32,06	4,30	0,02	0,21	4,96	2,85	9,32	39,4	0,0
Deviation	0,07	2,74	0,54	0,02	0,68	2,34	3,51	5,74	13,8	13,8
Criteria	-	-	**A**	**VL**	**VL**	**VL**	**VL**	**M**	**VH**	**M**
Soil Layer	**>31 - 60 cm**									
Mean	-	-	5,24	0,06	0,82	8,59	7,93	15,93	74,2	25,8
Maximum	-	-	7,40	0,11	3,30	23,98	20,74	27,47	100,0	73,2
Minimum	-	-	4,20	0,02	0,15	3,93	3,11	5,40	26,8	0,0
Deviation	-	-	0,88	0,02	0,77	4,24	4,99	4,73	20,8	20,7
Criteria	-	-	**A**	**VL**	**VL**	**VL**	**VL**	**M**	**VH**	**M**

Note: A = Acid, VL = Very Low, M = Moderate, VH = Very High

Soil Charateristics Improvement

In many crops culture, soils characteristics improvement is performed as soils show a decline function being media for plants growth. In this case, soils fertility based chemical characteristics is used as a refference to improve it's soils characteritics as nutrients source for plants. Similar to chemical characteristics, if it was found an unfavourable physical soils characteristics, it is also need to be improved in order root systems able to absorb nutrients for plants growth. **Table-04** shows that both of chemical and physical soils charateristis status are in bad condition and therefore considered to be improved whether before and after plantings.

Soil Acidity (pH)

There are a lot of soils characteristics being worse due to low soil acidity compared with the favourable range for plants growth as neutral acidity. In neutral range acidity, most of nutrients are easily soluted into water and also easily absorbed by plants. A lot of Aluminium ion is found at acid soils due to Phospor ion fixation and being toxic to plants. And also, micro nutrients are easily soluted at acid soils reaction and will be accumulated. In the condition of soil acidity close to neutral, the maximum avaliable phospor and micro nutrients solubility will decline. The bactery is well developed at pH 5.5 or more and will restricted at lower pH 5.5. The nitrogen fixation bactery and nutrification bactery are only develop at pH 5.5 or more, and therefore addition of dolomite should be applied at acid soils.

Reffering to **Table-04**, both of average pH at upper (4.92) and lower (5.24) soils layer are found to be acid. To increase pH is done by adding dolomite two weeks before planting with the amount based on exchangable Aluminium (e-Al). Dolomite addition as much as 1.5 e-Al is able to nuetralize 85-90% e-Al containing 2-7% organic materials. If soils contain a higher organic materials, dolomite should more be added due to ion H^+ released by organic materials or Fe and Al hydroxide. **Table-04** also shows that organic materials is categorized to be low (0.69%) and therefore added dolomite is 1.5 e-Al or equal to 0.5 kg/plant. In relation with base saturation which is categorized to be high, dolomite application might be not a proper aplication, however it is rather than soils nutrients defficiency but due to high soils acidity. Soil acidity improvement is indicated by increasing pH in both upper (0-30 cm) and lower (>30-60 cm) soil layers whereas the initial acidity status is acid (A) and changed into neutral (N) (**Figure-02**).

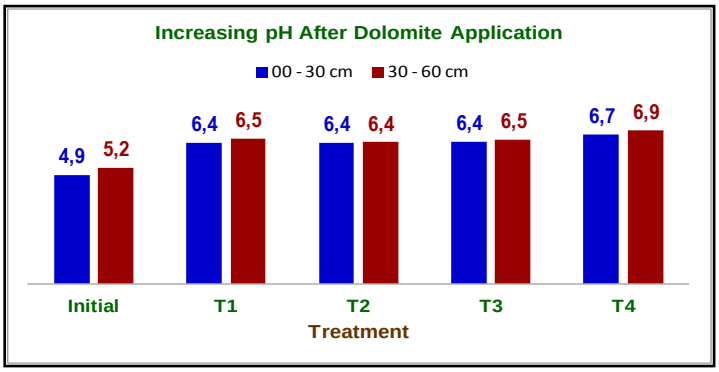


Figure-02.The Increase of Soil pH 14 Months After Dolomite Application

Macro Nutrients Content (N, P, and K)

The content of N, P, and K nutrients are very low and therefore applied with complex fertilizer of NPK (16:16:16) in a dosage of 50 g, 100 g, 150 g per plant. NPK (16:16:16) is applied after plants reach 3 months in age, which is aimed to make a nutrients balance of plant requirements and the need of immediate additional nutrients to support vegetative growth at the initial plant growth. The form of N of which imediately able to be used by plants is anorganic N supplied by chemical fertilizer. Organic decomposition produces humus will increase cation and anion absorption capacity. Moreover, absorbed cations and anions are not easily lost by leaching process and easy to be relased when they are needed by plants. The more available humus leads to a more efficient of soils fertilization.

Table-05. Macro Nutrients Content (N, P, and K) Post Fertilizer Application

No.	Nutrients	Initial	Post Fertilizer Application			
			(T1)	(T2)	(T3)	(T4)
Upper Soil Layer (00-30 cm)						
01.	Nitrogen (%)	0,05	0,04	0,03	0,05	0,04
02.	Phospor (ppm P_2O_5)	7,60	21,70	27,40	30,60	27,60
03.	Kalium (ppm K_2O)	6,70	60,90	49,70	63,70	64,30
Lower Soil Layer (>30-60 cm)						
04.	Nitrogen (%)	0,06	0,05	0,04	0,05	0,06
05.	Phospor (ppm P_2O_5)	8,60	29,80	23,40	30,00	32,50
06.	Kalium (ppm K_2O)	7,90	59,90	45,80	61,30	74,50

In case of N, there is no increase of it's content in one year after soils fertilization of all treatments, even for T1, T2, and T4 the content of N is lower than before soils fertlization. In most of all treatments except T1, the content of P and K increase in line with soils fertilization in a dosage of 50 g, 100 g, 150 g per plants. The P and K contents at T1 are obtained similr with T3. In spite of the fetrilization is done at the soils upper layer of ± 10 cm depth but the influence reach until lower soils layer (**Table-05**).

Soil Bulk Density

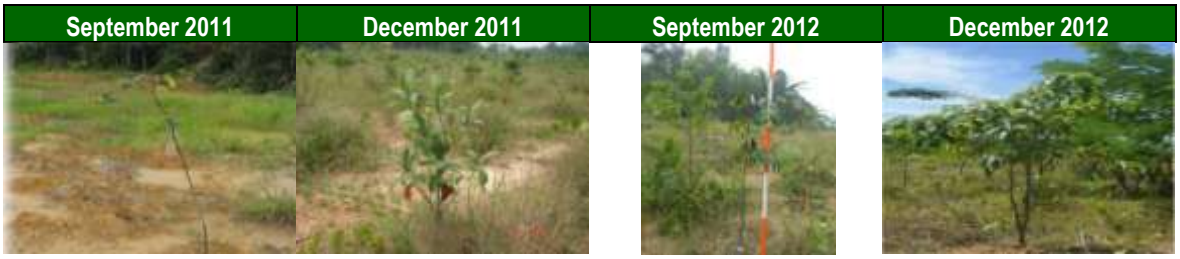
Soils compaction is indicated by bulk density (BD) value, the more bigger BD means more compact soils and therefore roots is likely difficult to penetrate into soils of which also difficult to make a better water drainage. Lands preparation using heavy equipments tends to disturb soils structures and reduce soil pores. Soil pores itself is influenced by organic materials content, soil structres and textures. Soil structures formation is significantly influenced by the existing organic materials bounding soil particles. To reduce the soil compaction, soil improvement is performed by adding organic compos as much as 20 kg at the initial satage before planting and 10 kg at one year after. Soil compaction monitoring shows a decreasing compaction of which indicated by it's bulk density.

Soils Improvement and Plants Growth

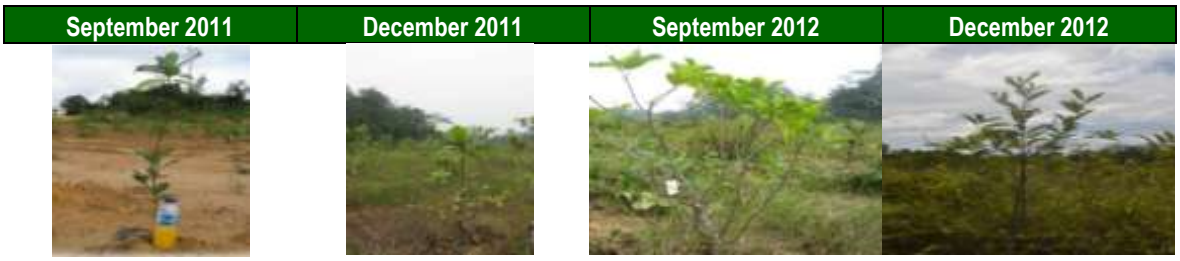
Diameter Increament

The influence of treatments on to biggest diameter increament of each species can be described that Sengon butho, Ketapang, Puspa, Sungkai, and Kersen are at T3, while Kahoi and Kondang at T4. Other species of Cempedak and Angsana at T1 and T2 (**Figure-03**) and the field performance can be seen at **Photo-05**.

S-01 - *Shorea balangeran* (Kahoi)

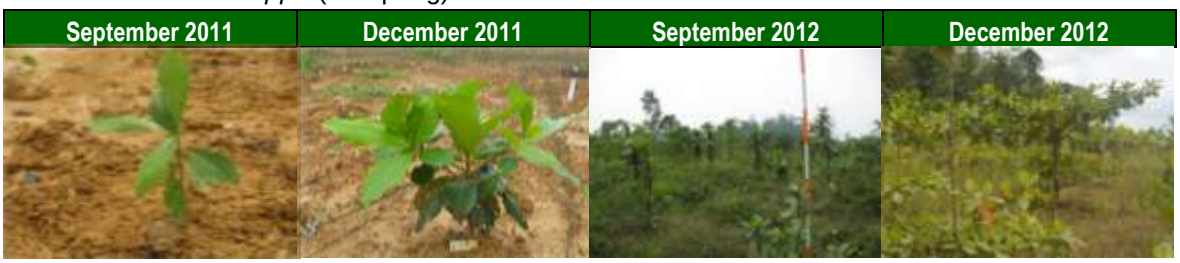


S-02 - *Artocarpus champedens* (Cempedak)



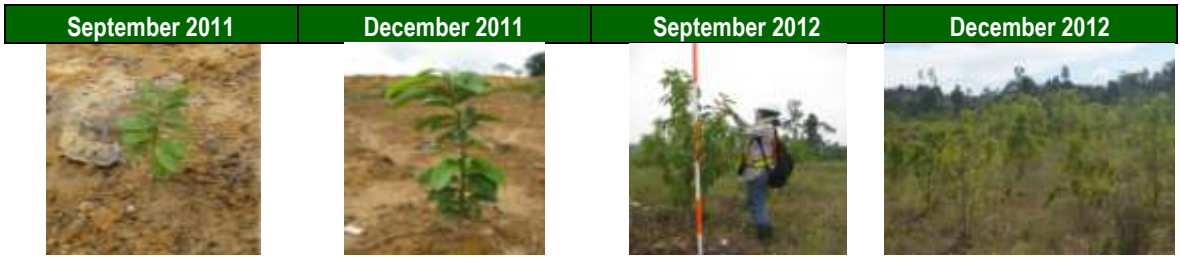
S-03 - *Enterolobium cyclocarpum* (Sengon Butho)

S-04 - *Terminalia catappa* (Ketapang)



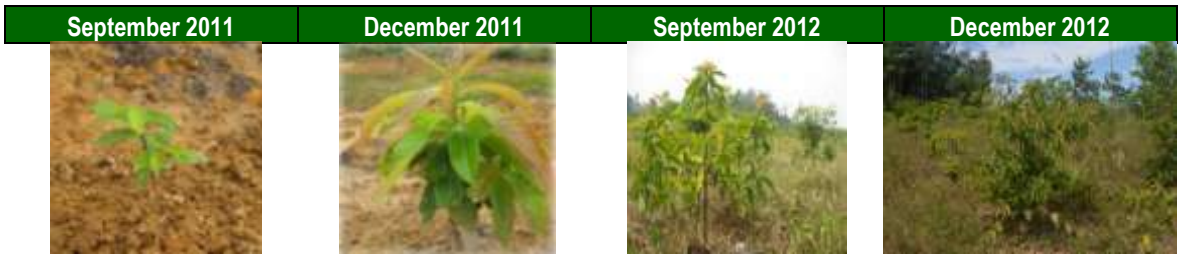
S-05 - *Pterocarpus indicus* (Angsana)

S-06 - *Annona muricata* (Sirsak)



S-07 - *Ficus variegata* (Nyawai)

S-08 - *Schima walichii* (Puspa)



S-09 - *Peronema canescens* (Sungkai)

S-10 - *Muntingia calabura* (Kersen)

Photo-05. View of Plant Height Increament at Study Site of PT TCM, West Kutai, East Kalimantan

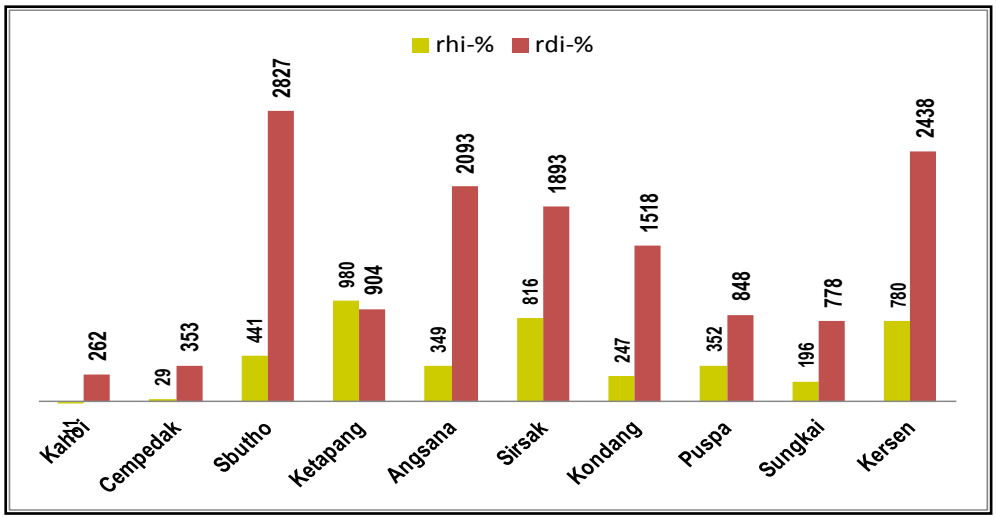


Figure-03.Relative Height (rh) and Diameter (rd) Increament of Plants 14 Months After Soil Fertiliztion

Concerning to the influence of treatments on to height increament, the biggest increament of Sengon butho, Sungkai and Kersen are at T3, Ketapang, Sirsak, Kondang and Puspa at T4, while Cempedak and Angsana at T1. Descriptive analysis of both diameter and height increaments show that most of all species - except Cempedak and Angsana, still responsive to soils fertilization. This might be due to these species needs a large amount of nutrients to grow as also they are classified as fast growing species. Bad quality of plant materials of Kahoi and Cempedak because of too long kept at nursery and being stressed growth before planting in the field. However, at the last monitoring in December 2012, there is a recovery performance on their growth indicated by several new leafs growing at the tops of their branches.

Soil Erosion Potential

Process and Mechanism

In general, soil erosion can be defined as a movement of soil mass from a site to other lower sites transported by flowing water or winds due natural process or human activities. Concerning to this process, they can be classified into geological or accelerated erosion. Normal erosion is a soil mass movement occurred at the natural condition such as eroded soil mass at slopes and/or hills. Rainfall, wind, slopes and undisturbed land surfaces are factors influencing erosion proccesses. In this stage, erosion makes a dynamic equilibrium in which the solum depth being relalively stable. This disturbed equilibrium is mostly caused by human activities tending to accelerate erosion rates. Soil erosion caused by main agent of water covers several procceses of soil detachment - dissaggregtion - dispersion - particles transportation, and eroded soils mass deposition. In the soil erosion occurrences, whether natural or accelerated, there are at least five factors causing the magnitude of soil erosion rate, that are climate, soils, topography, vegetation, and human being.

The climate element has significant influence to soil erosion is rainfall (*precipitation*), while physical soils characteristics closely related with soil erosion are texture, structure, infiltration - rate and capacity, and organic material contents. Soil texture controls water flow in the soils in form of infiltration rate, plants root penetration and water holding capacity. The occurrence of overland flow is highly depends on infiltrtation capacity and soil permeability. Landform topography has a significant role in relation with the velocity and volume of overland flow or surface runnof.

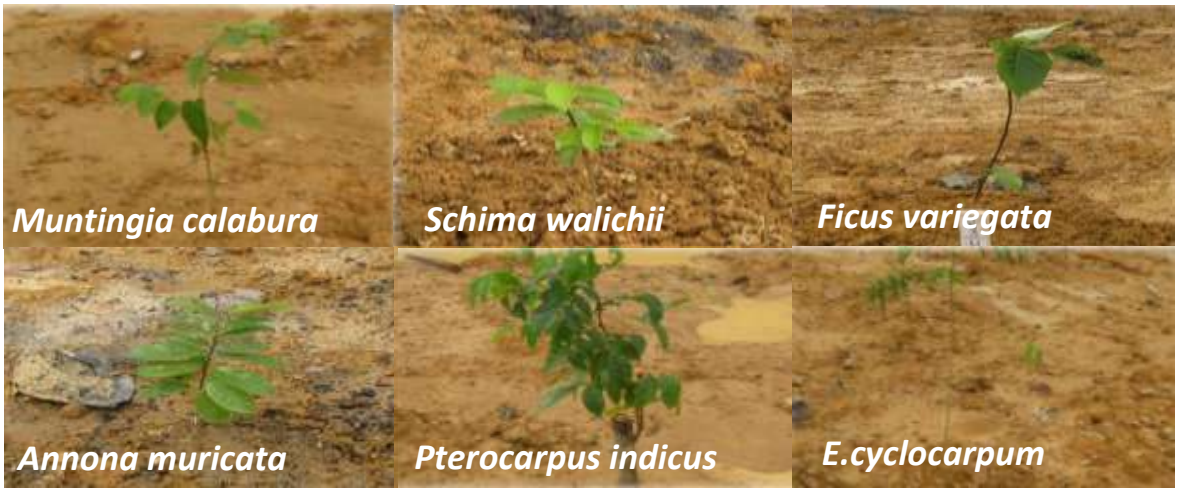
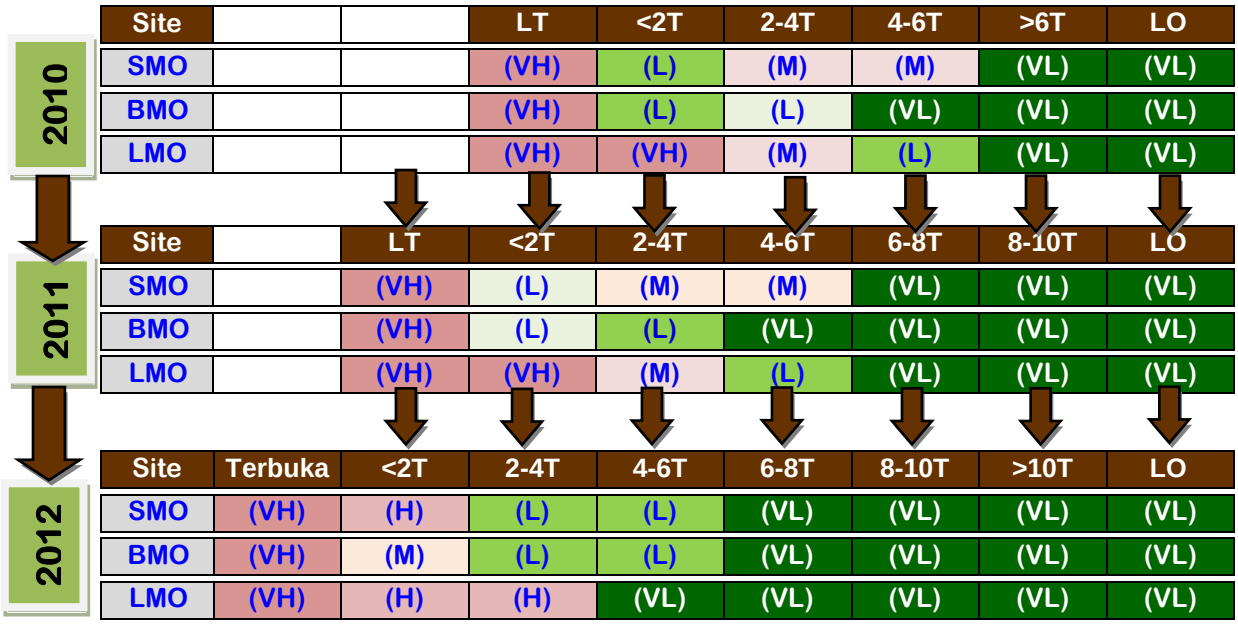


Photo-06. Condition of Mined-out Lands After Rehabilitation Works (Reclamation and Revegetation

Photo-07. Initial Growth of Land Cover and Soil Erosion 3 Months After Revegetation Works

Regarding to topography, the length and slope have an important role to soil erosion. The more steep and more length of slope, the velocity and volume of overland flow will be more bigger and finally increasing the soil erosion hazard. On the other hand, vegetation is opposite to erosive influence forces such as intercepting raindrops energy, reducing velocity and volume of overland flow and also increasing soil stability. In general, it is well known that human activities are most important factors causing a huge mass of soil erosion related with land clearing for settlements, agricultural lands, ect. Landform changes and tillage followed by soil fertilization influence the soil structures. Land clearing creates an initial condition to the occurrence of a large scale soil erosion causing the balance of soil formation and soil erosion. However, human being also plays an important role to conserve soils from erosion disturbance through various soil conservation activities such as revegetation including mined-out lands rehabilitation.



S, B, L - MO: Sambarata, Binungan, Lati Mining Operation, VL = Very Low <15 Ton/ha/Year, L = Low (15-60 Ton/Ha/Year), M = Modeate (60-180 Ton/Ha/Year), T = High (180-480 Ton/Ha/Year), VH = Very High (>480 Ton/Ha/Year).

Figure-04. Schematic Indication of Dynamic Soil Erosion Potential and Erosion Hazardous Index Based on Vegetation Cover Development at Mined-out Lands

Reffering to the physical soil erosion form in the field, soil erosion occurrences are generally classified as splash rosion, overland flow erosion, riil erosion, channel erosion, gully erosion, streambank erosion, and internal or sub-surface erosion. Field observation on mined-out lands revegetation is shown at **Photo-06** and **Photo-07**, as a field recording after mined-out lands reclamation (*backfilling - recontouring - reshaping - topsoils spreading*), land revegetation (land preparation - planting and 3 months development). From the field observation, it is learned that expected soil erosion reduction is land coverage at the initial stage of revegetated lands by growing various land cover crops.

As a specific refference, the research at PT Berau Coal (2010-2012) indicates that soil erosion potential related with vegetation cover development (**Figure-04**) showing that the status of Erosion Hazardous Index (EHI) at the open lands is very high (VH) during 2010-2011 years. In line with vegetation cover until 4-6 years age, the EHI declines to be very low (VL) - low (L) - moderate (M). Reaching the age of > 6 years the EHI is very low (VL). Indeed this estimation is being an indicative parameter and will vary under local specific condition such as rainfall - land slopes - rate of land coverage - vegetation maintenance - practical soil conservation. Several factors supporting the successfulness of revegetation works in line with soil erosion potential reduction, the most possible factor to be managed are slope, land prepartion and the intensity of vegetation maintenance. It also has to be well understood that lands is an synergitical aggregate factors of landform, geology, soil, hydrology, climate, flora-fauna, and its allocation usage. Therefore, land recovery is not only related with soils recovery but also the recovery of other components (micro-climate, hydrology, flora-fauna ect.).

Dynamic of Soil Erosion Potential

The phenomenon of soil erosion dynamic might be analyzed based on principles of phylosopy and functional relationship between soil erosion magnitude and important influencing factors. The development of vegetation cover is considered to be one of the most significant and important factor related with soil erosion at mined-out revegetated lands (**Photo-08**). Basically, lands disturbance e.g. mined-out lands is initially started with soils disturbances such as disrturbed soils structure and pores followed by other soils characteristics and even components forming lands. It is therefore the assessment of soil recovery should be based on pedogenesis - natural procces of soil forming and edaphologic - growth media function of biomass production points of view.

Photo-08. Growth of Plants, Land Cover and Soil Erosion 14 Months After Revegetation and Maintenance Works

Land reclamation followed by revegetation works is an effort to accelerate lands recovery and have to be supported all of needed works. Basic requirement for soils function as plants growth media are being roots system development site for supplying soils nutrition to plants. Lands reclamation works are performed by providing soil materials depth, recontouring proper landslope to guarantee proper overland flow drainage, whereas soils aeration and available soils nutrients are done in line with revegetation works. Soil erodibility is very high at open lands and decline following vegetation growth. To enhance a very low soil erosion potential depends on factors influencing it's successfulness. The most possible factors to be fully managed are slopes forming, land preparation and the intensity of vegetation maintenance works.

Topography has an important role in determining velocity and volume of overland flow. Comparing those factors, landform slope is more important than it's slope length due to flowing water velocity and it's energy to transport materials will increase following a more steep landforms. Threfore, the more slope steep and longer slope length will increase accumulated volume and the velocity of overland flow. It is found that soil erosion potential at revegetated mined-out lands decreasing from open lands - VH following the vegetation growth - H - M - L - VL consecutively. High soil erosion potential at open lands is due to lack of vegetation cover and low infiltration capacity. Direct raindrops on land surfaces causes soil particles dissaggregation - dispersion and possibly transported by overland flow along landslope into lower sites. Basically, soil erosion potential reduction might be done by intercepting direct raindrops and controlling overland flow. In the vegetative approach, planting of land cover crops might be performed while for physical - mechanical approach by constructing drainage networks. The EHI status at revegetated mined-out lands tends to decrease of which susceptibilitically indicating raindrops interception and increasing lands surfaces infiltrating exessive rainfall.

To retain and increase vegetation cover, the most possible way is by managing revegetated mined-out lands intensively. Practically, it might be performed by vegetation maintenances (*replanting dead trees*, *fertilization*, *weeds and phatogen controls, enrichment planting*) to guarantee the existence of revegetated mined-out lands. Subsequently, this also considers some facts that the status of EHI (Very Low) due to interception of direct raindrops and overland flow control by developed vegetation growth favouring a faster infiltration rate and higher infiltration capacity. Indicatively, enhancing EHI status of VL - L - M needs time at least 5 - 6 years after proper revegetation works.

Mined-out Lands Recovery

Lands Reclamation and Revegetation

Land rehabilitation (*reclamation*, *revegetation*) have to be conducted at mined-out lands to recover being a productive lands based on it's status and allocation usage. Performing such lands rehabilitation needs specific knowleges and experiences related with soil development, rehabilitation techniques, species site matching, and vegetation maintenance. Open pit system produces a mass disposal of over-burden and inter-burden which is then used to fill mined-out pits. After backfilling process, most of dumping materials is in condition of wreak or disturbed structure and pores and could be a mass of wreaks in these dumping materials. However, some time also unavoidably mixed with dirty coal and almost any organic material. This condition leads to bad drainage system and low water holding capacity, highly compacted soils followed by a high temperature. The main problem is that backfilled mined-out lands not fully ready to support plants growth and uncontrollable high overland flow followed by high soil erosion rate. In relation with planting, species site matching, planting techniques and its procedures for maintenance are also still needed to be achieved.

Land Recovery

Naturally, lands is an elements aggregate of land form, geology, soils, hydrology, climate, flora-fauna and its specific usage alocation. For this reason, lands recovery is not only concerned with soils recovery itself but also recovery of other elements. Mined-out lands disturbance is generally initiated by soils disturbance as of soil structures, pores and followed by other soil characteristics and also other elements disturbance. For this reson, lands recovery assessment have to be initiated with soils recovery viewed from pedogenesis and edaphological aspects. This means that lands recovery is not only asessed from natural forming process but also the main function as plants growth media and it's productivity.

Reclamation works followed by revegetation is an effort to accelerate land recovery process. As far as soils function works as plant growth media, it will guarantee to the availability of aeration and drainage for roots system to penetrate and providing nutrients needed by plants. In reclamation works, it is done by constructing the depth or thickness of soils materials overlay overburden layers. Increasing soil aeration can be instantly done by applying organic fertilizer, lands cover crops or other soil conditoners. Moreover, soils nutrients need for supporting plant growth can be provided by organic fertilizer application.

Lands recovery acceleration might be achieved if the minimum requirements is fulfilled. As a plant growth media, the thickness of soil materials have to be depth enough to support root system function activities and creating a proper soil drainage condition. Mined-out lands revegetation with annual plants generaly needs 50 cm thikness of materials soils and free mixed other materials. However, it is well known to be quiet difficult due to in the field has been unavoidable mixing with overburden and/or fine coals. Also, bad soil drainage is found indicated by trapped surface water at severals spots of land surfaces.

The existing degraded lands in the field can be observed from it's physical characteristics such as lack of vegetation cover, moslty eroded topsoils layer due erosion caused by surface runoff or overland flows. Facing such existing degraded lands, single or combination betwwen phyisical-mechanical and vegetative approaches migh be applied in rehabilitation works. One of selected refference, the field experience study of observation - monitoring - analysis on the dynamic of soil erosion potential at PT Berau Coal - one of a leading coal mining company (2010-2011) might be refferred in mined-out lands rehabilitation to achieve lands recovery as is shown at **Figure-05**.

Site	Erosion Hazardous Index (EHI)							
	OL	<2Y	2-4Y	4-6Y	6-8Y	8-10Y	>10Y	OrLand
SMO	(VH)	(H)	(L)	(L)	(VL)	(VL)	(VL)	(VL)
BMO	(VH)	(M)	(L)	(L)	(VL)	(VL)	(VL)	(VL)
LMO	(VH)	(H)	(H)	(VL)	(VL)	(VL)	(VL)	(VL)

Note:

S, B, L - MO: Sambarata, Binungan, Lati Mining Operation, VL = Very Low <15 Ton/ha/Year, L = Low (15-60 Ton/Ha/Year), M = Modetate (60-180 Ton/Ha/Year), H = High (180-480 Ton/Ha/Year), VH = Very High (>480 Ton/Ha/Year).

Figure-05.Scheme of Soil Erosion Potential Dynamic Based on Development on Lands Vegetation Cover

As mentioned before, magnitude of soil erosion potential is influenced by main factors of rainfall erosivity, soil erodibility, length and slope, vegetation cover, and the application of soil and water conservation practices. The dynamics of lands vegetation cover is a significant factor in relation with the soil erosion dynamic at mined-out lands. In line with it's vegetation cover development in 4-6 years, KBE status decreases from VH to VL - L - M. When the vegetation reaches > 6 years in age, KBE decreases into VL. These KBE decrease indicate that management of mined-out lands related with it's expected recovery have to be intensively performed at least at the first 5 years after revegetation works. Specifically, lands recovery efforts have to pay much attention on slope construction and lands preparation at reclamation step, and the intensity of plants management covering species selection, planting techniques and plants maintenance.

The initial works of lands rehabilitation based on physical-mechanical approahes combined with vegetative approach are to control surface runoff/overland flows, especially at lands with hard topography. It is aimed in order the force of surface runoff can be properly controlled and the transportability of eroded soil particles being significantly decrease. This can be done by cutting the slope length to reduce surface runoff velocity and as far as possible directing into designed places to undisturb mined-out lands. The surface runoff control actualy depends on it's physical-mechanical function. If surface runoff can be successfully controlled, it will make possible condcuting lands preparation followed by planting at available planting areas of mined-out lands.

Vegetation plants is expected to grow and developing crown trees to intercep direct rainfall force and therefore reducing it's rainfall drops energy, covering lands surfaces and increasing the possibility of rainfall water reached lands surface to infiltrate into the soils, anf finally surface runoff savely flowing over the lands. One of the important thing is that nutrients lost can be avoided and the soils nutrients being retained. If vegetation plants grow well will promote nutrients cycle and leading to recovery at mined-out lands by accumulating organic materials forming topsoils. For the next step if the developed plants still not fully match with in function as expected before, it is possible to change or enrich with other expected plants species. The principles of mined-out lands mentioned above should be performed step by step consecutivelly and needs enough time to succeed. Each step of mined-out lands rehabilitation will be important and being foundation for the next effort step of it's land rehabilitation.

Conclusions

01. As much as 3000 individual plants studied, the survived plants including replanting is 2.788 individu (93%) with the lowest Cempedak (84%) and Sungkai (86%). Best physical performance in the field are Kersen, Ketapang and Sirsak while the worse are Kahoi, Cempedak and Angsana;
02. Soils is found to be very compact with average BD 1,68 g/cc (1,51-1,80 g/cc). Most of soils volume (64%) is mineral and only 36,36% filled with air and water. There is no difference in chemical soils fertility status between upper and lower soil layers and both layers are acid with very low N, P, K contents, moderate CEC and very high base saturation. Soils acidity increases by adding dolomite 0,5 kg/plant and changing status from acid into close to neutral;
03. The application of NPK (16:16:16) with 150 g/plant dosage does not change N status in the soils while 50 g/plant dosage changing P and K status from very low to very high;
04. Increasing nutrients lost at open mined-out lands is due to high volatyle N and it's mobility while for P and K changing can be compensated by organic fertilizer application;
05. Organic fertilizer application 20 kg/plant is due to reduce soils compaction and at the same time increasing soils pores and CEC and water contents. Field observation indicates that organic fertilizer causing a large influence upon plants growth due to a better physical soils characteristics and therefore root system functioning properly;
06. All of soil fertilization treatments by applying NPK (16:16:16) increase P and contetns but not for N in soils. The dosage of 100 g NPK (16:16:16) per plant (T3) increases a maximum plant height and diameter of Sengon butho, Sungkai and Kersen, and for Ketapang and Puspa diameter only. Dosage of 150 g NPK (16:16:16) per plant (T4) similarly increases a maximum plant height and diameter of Sirsak and Kondang, while for Kahoi (diameter) and for Ketapang and Puspa (height). Until 14 months plant age, all plants are still responsive to fertilization application except Cempedak and Angsana;
07. Soil erosion potential at open mined-out lands is very high due to lack of vegetation cover, low infiltration capacity and a high overland flow/surface runoff. Subsequently, at revegetated mined-out lands tends to decline into very low - moderate range, indicating there is an interception raindrops energy and increasing infiltration capacity;
08. To achieve a very low soil erosion potential at revegetated mined-out lands needs an effective time at least in about 5-6 years. The most important factors highly possible to be consistently managed are reclamation works (recountouring, reshaping) and revegetation works (plants maintenance);
09. The phenomenon of soil erosion potential dynamic is depend on influencing factors upon soil erosion occurences that are rainfall erosivity, soil erodibility, landform slope and lenths, vegetation cover, practical soil and water conservation. Development of vegetation growth cover is the most significant factor related with the soil erosion dynamic at revegetated mined-out lands.

Recommendation

01. Additional organic materials application should be continued in the proper amount to reduce soils compactness, increasing soils pores and water contents;
02. Considering a very low contents of N, P, K nutrients and it's increasing need, the dosage of N fertilizer should be added in amount to increase N contents as fast as possible. Meanwhile, the recommended dosage of NPK (16:16:16) for Sengon butho Sungkai, Kersen, Ketapang, and Puspa is 100 g/plant, whereas for Sirsak, Kondang and Kahoi 150 g/plant, and for Angsana and Cempedak is 50 g/plant;
03. Considering that reducing soils erosion rate is performed by intercepting raindrops and controlling overland flow, the practical alternatives based on vegetative approch is by planting land cover crops - fast growing trees - annual plants, while for physical and mechanical based approach is by preparing drainage networks with proper capacities;
04. To realize and retain the intensity of land covers for reducing soils erodibilty requiers an intensive maintenance of revegetated mined-out lands by performing vegetation growth and development management. It will be more effective in line with prior to proper slope and length preparation in mined-out lands reclamation works.

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PROCEEDING OF THE FIFTH INTERNATIONAL SYMPOSIUM OF INDONESIAN WOOD RESEARCH SOCIETY

The Areal Number Density Of Vascular Bundles Strongly Correlates With The Mechanical Properties Of Oil Palm Wood (*Elaeis guineensis* Jacq.)

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Abstract

Currently most oil palm trunks in Thailand are left on the field to rot or are burnt in the field, not utilized as lumber. To promote such value-added uses, the objective of this study was to characterize the levels and variation within oil palm trunks of their key mechanical properties. In addition, the vascular bundle population was assessed, because this structural characteristic affects density and mechanical properties, the key ones being here modulus of rupture (MOR), modulus of elasticity (MOE) and hardness. The 25 years old oil palm trunks were selected from a palm plantation in Surat Thani province, in southern Thailand. The trees were cut down at 500 mm above ground, cut into dices, then sawn into small pieces in radial direction. Vascular bundle populations and basic densities were determined. Oil palm lumber was sawn from the logs between wood dices and their mechanical properties determined. The results indicate the vascular bundle population density gradually decreased towards the central axis of trunks, and the population density positively correlates with basic density and mechanical properties. This is because the main component of a vascular bundle has fibers with thick cell walls. The data obtained may help select or create products that match the properties of oil palm wood (*Elaeis guineensis* Jacq.), or contribute to the sorting of wood raw material based on for example machine vision.

Keyword: oil palm wood, vascular bundle, basic density, mechanical property

Introduction

Oil palm trees (*Elaeis guineensis* Jacq.) are important non-forest agricultural plants that may provide alternative raw material for the wood industries. In Thailand, oil palm plantation areas have expanded from 329,120 hectares in 2003 to 690,560 hectares in 2012, providing palm oil for food and renewable energy. More than 90% of total plantation area in Thailand is in its southern peninsular part, especially in Surat Thani, Krabi and Chumphon provinces (Office of agricultural economics 2012). When the oil palm trees are past their economic life at 25 to 30 years old, the trunks range from 15 to 18 meters in height and 45 to 60 centimeters in diameter. They are felled to make room for replanting, and normally left to rot or burnt down in the field (Katemanee 2006). Presently oil palm trunks are not a raw material for wood industries, due to low density and poor mechanical properties compared to other commercial wood species from agro-forestry, such as rubberwood (Ratnasing and Ioras 2010). An oil palm trunk contains 31.70% to 47.30 % cellulose, 21.20% to 34.40 % hemicelluloses, and 18.40% to 29.60% lignin (Kaida et al. 2009; Yuliansyah et al. 2010; Chin et al. 2010; Verman and Saka 2011). The middle and core parts of trunk are 12.19% to 17.17% starch and have high free sugar in sap (Yamada et al. 2010; Hashim et al. 2011). Especially in Thailand these glucose based reserves in oil palm trunks have been studied for fermentation to produce bio-ethanol and hydrogen renewable energy (Punsuvon et al. 2005; Hniman et al 2011). However, the oil palm wood taken from the bottom peripheral zone of stem can be used in furniture and in non-structural construction components (Ratnasingam and Ioras 2010). Previous studies report that the utilization of oil palm wood as lumber or laminated wood is difficult, because the product quality is affected by variation of physical and mechanical properties within trunk (Ratnasingam et al. 2008; Feng el at. 2011). The oil palm tree is a monocotyledon, and its main structure consists of vascular bundles embedded in parenchyma cells. The vascular bundles are the mechanical support and serve as a conduits for the transportation of water and nutrients in the trunk. Their number density per volume decreases towards the axial center, and increases in the trunk from the ground towards top of the tree (Erwinsyah 2008).Therefore, the objective of this study was to clarify the relation of vascular bundle population density with basic density and some mechanical properties of oil palm wood. The results may help rapidly estimate the mechanical properties of oil palm wood samples, and help with such selective use of oil palm wood that the quality control problems mentioned above are ameliorated.

Materials and methods

Oil palm trees 25 years of age were harvested from a local plantation in Surat Thani province, in southern Thailand. The trees were cut down at 500 mm above ground, and cut into 60 mm thickness dices alternating with 1 m logs, as shown in Fig. 1. The oil palm dices were sawn into long pieces in the radial direction from pith to bark of trunk, 60 mm wide in tangential, and stored in plastic bags to avoid loss of moisture. The vascular bundle population density and the basic density were determined from these pieces. The logs between wood dices were sawn to lumber for mechanical property testing.

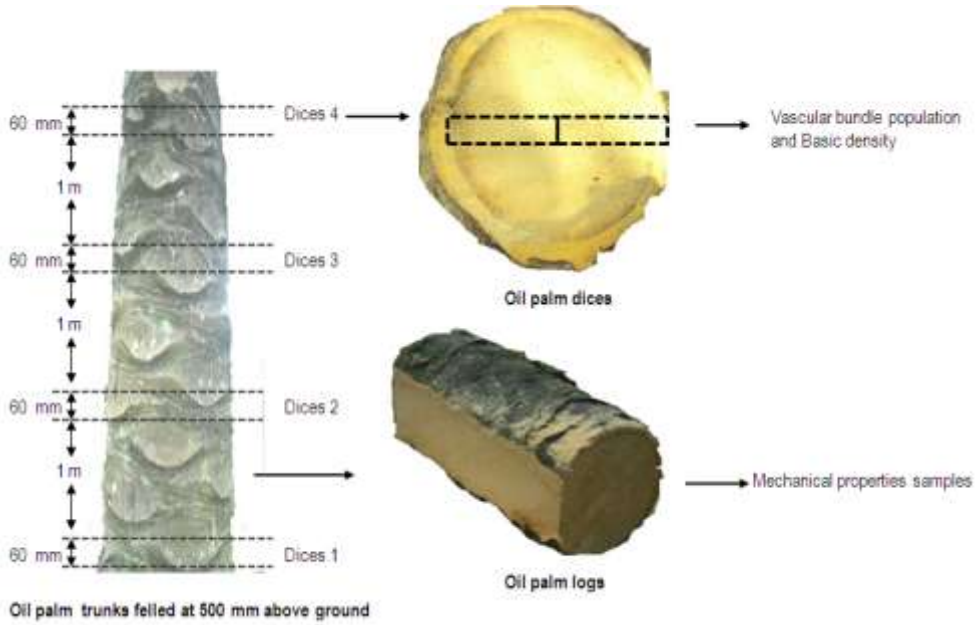

Fig.1 A schematic illustration of conversion of an oil palm trunk to the samples studied for vascular bundle population density, basic density, and mechanical properties.

The green oil palm woods which cut from dices were photographed from trunk axis to bark, using a stereo microscope, and the vascular bundles were counted from the images. Then, the same wood samples were cut to small 20 mm (tangential) x 20 mm (radial) x 25 mm (longitudinal) pieces to determine basic density profile, similarly from axis to bark. These green samples were measured in radial, tangential and longitudinal directions with a digital caliper to the nearest 0.01 mm, weighed to nearest 0.01 g, and oven-dried at 103± 2 °C to constant weight. Finally, the oven-dry weights to nearest 0.01 g were determined after cooling in desiccator. The basic density was calculated from the ratio of the oven-dry weight (g) and volume in green condition (cm^3).

Oil palm logs were sawn into 50 mm thick lumber using a polygon sawing pattern (Baker et al. 2006), to separate the wood to inner, middle, and outer zones, that match the distribution of vascular bundles. The green lumber was air dried to a moisture content of 8-12%, and then planed and cut to 20 mm (tangential) x 20 mm (radial) x 300 mm (longitudinal) samples for static bending (modulus of rupture, MOR and modulus of elasticity, MOE), and to 20 mm (tangential) x 20 (radial) x 60 mm (longitudinal) samples for Janka type hardness test. All samples were conditioned in ambient 20°C temperature and 65% relative humidity until equilibrium moisture content, before the mechanical properties were determined using a universal testing machine. The testing of mechanical properties followed BS 373 standard (BS 373 1957) with slight modifications.

Results and discussion

Microphotography of wood anatomy, using a stereo microscope, showed that the vascular bundles were unevenly distributed across the trunk, and surrounded by parenchyma cells. The population density incresed radially from 23 Vp/cm^2 at the axial center to 115 Vp/cm^2 near bark, and varied by height along the trunk. The graph in Fig. 2 indicates a close relationship between this number density and basic density that increased from 0.11 g/cm^3 at the center to 0.40 g/cm^3 near bark. The vascular bundles are mostly fibers with thick cell walls, and this thickness was 8.08 µm at 2 m height. In addition, the thin walled parenchyma cells have a higher moisture content (Erwinsyah 2008; Sitti Fatimah et

al. 2012). Therefore, the oven-dry weight of oil palm wood at high the percentage of vascular bundles is high after their loosed the water in cell. This is the reason for the strong correlation with basic density.

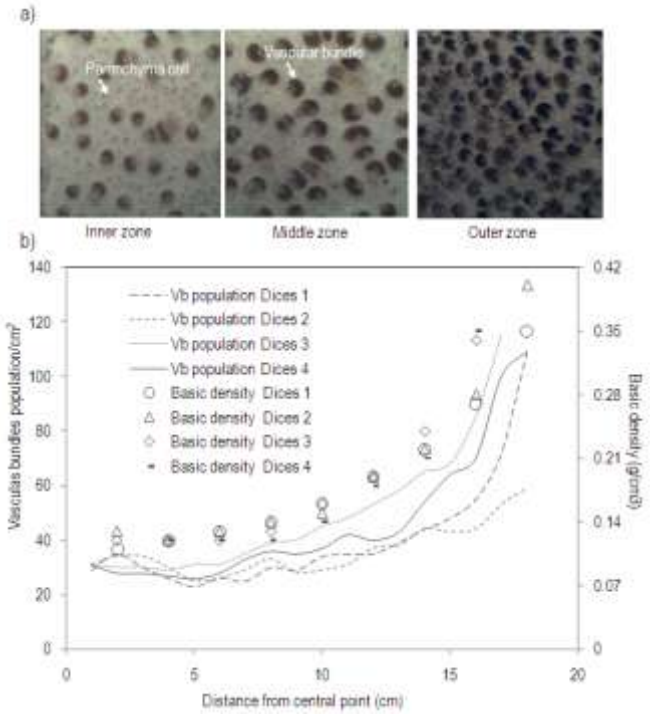

Fig. 2 Microphotographs of inner, middle, and outer zones (a), and the relationship of radial profiles of vascular bundle density and basic density of oil palm wood, at various heights along the trunk (b).

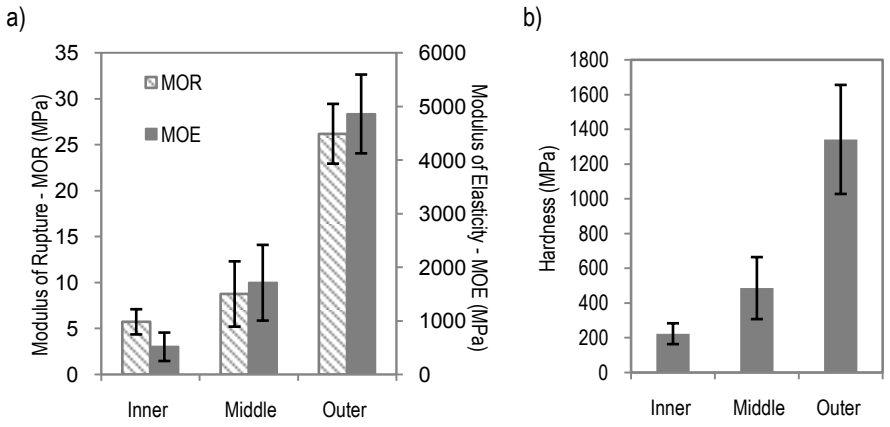

Fig. 3 Modulus of rupture, Modulus of elasticity (a), and Hardness (b) of oil palm wood in inner, middle and outer zones of the trunk.

The results in Fig. 3 showe that the overall mechanical properties of oil palm wood improve outwards from the axis. The average modulus of rupture, modulus of elasticity and hardness of outer zone had the highest values at 26.20 MPa, 4861.49 MPa and 1342.10 kg, respectively. Those properties were, in the same order, decreased to 8.76 MPa, 1711.39 MPa and 486.10 kg for the middle zone, and further to 5.73 MPa, 516.53 MPa and 223.60 kg for the inner zone. The areal number density of vascular bundles in the outer zone ranged from 68 Vp/cm^2 to 115 Vp/cm^2, and in the middle and inner zones from 44 Vp/cm^2 to 58 Vp/cm^2 and from 23 Vp/cm^2 to 40 Vp/cm^2, respectively .

Conclusions

The basic density and mechanical properties (static bending and hardness) of oil palm wood strongly correlated positively with the areal number density of vascular bundles. The data suggest that quick estimation of oil palm wood mechanical characteristics could be partly based on this areal number density. Such characterization could assist in rapid selection, avoiding destructive testing, of the best parts of oil palm trunks for specific applications, for example by sorting based on machine vision.

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THE ADHESIVE BONDABILITY OF ETHYLENE GAS STIMULATED RUBBERWOOD, AN EXPERIMENTAL STUDY

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Abstract

This experimental investigation of bondability of rubberwood used 20-25 years old rubber trees (strain RRIM 600) stimulated with ethylene gas for 6 years, and compared these with untreated control samples of similar age. Both types of rubberwood were collected from Chaibury district in Surat Thani province. The penetration of urea formaldehyde resin into wood, shear strength and area fraction of wood failure at sheared bond interface, along both tangential and radial cut planes of wood, were examined. Shearing was in the axial direction, parallel to wood fibers. There was no significant difference between the two directions in the depth of urea formaldehyde resin penetration, but ethylene gas stimulated rubberwood had significantly ($P<0.01$) deeper resin penetration than untreated rubberwood. The shear strengths of tangential surfaces were significantly higher than of radial surfaces, and the treatment significantly increased the shear strengths relative to control. Wood failure area fractions from tangential shear were significantly higher than from radial shear, and the treatment significantly increased these fractions about three fold relative to control. The results suggest that ethylene gas treatment, beneficial for latex production, also improves the adhesive bonding properties of rubberwood lumber.

Keyword: bondability, urea formaldehyde resin, rubberwood, ethylene gas

1 Introduction

In Thailand, farmers have conventionally harvested latex using a multi-annual tapping system with a half-spiral (S/2) or a one third-spiral (S/3) cut downward tapped at a frequency of two days (d2). In the last decade, however, the continuous decrease in the size of Thai rubber plantations has led to general adoption of more intensive frequencies than d2, namely daily tapping (d1), two days out of three (2d/3), or three days out of four (3d/4), with only one day of tapping rest (Chantuma et al., 2011).

Further, rubber smallholders in southern Thailand have introduced ethylene gas stimulation to increase latex production, land productivity and tapping labor efficiency (Sainoi and Sdoodee, 2012). Normally, ethylene gas stimulation is recommended for over 15-year-old rubber trees. Stimulation delays latex vessel plugging, prolongs duration of latex flow and results in a 20 to 100 percent increase in latex yield. However, the yield stimulation will lead to 2–7 percent reduction in the dry rubber content, even though it has no effect on rubber properties (Nair, 2010). The rubber plantations have become a major source of industrial timber in the rubber-growing countries of South and Southeast Asia. Since the 1980s, rubberwood is internationally accepted as an eco-friendly source of timber for manufacture of household furniture. Rubberwood has excellent physical properties and has become an important raw material also for the manufacture of panel products, such as particle board, block board, and medium-density fiber board, among others (Nair, 2010; Teoh et al., 2011). However, the effects of ethylene gas stimulation on the properties of rubberwood have not been studied. The properties that are important to applications include chemical composition, permeability, glue bonding characteristics, and mechanical properties.

The objective of this study was to investigate the glue bondability of ethylene gas stimulated rubberwood. The characteristics that were determined and compared between treated and untreated wood samples were adhesive penetration, shear strength, and wood failure in two cutting directions, namely radial and tangential directions.

2 Materials and methods

2.1 Rubberwood raw material

Rubberwood RRIM 600 clone is the most widely planted clone in Thailand, and was used for the experiment. Four 20-25 years rubber trees were collected from Chaibury, Surat Thani province, in southern Thailand. Two of the

trees had been ethylene gas stimulated for 6 years, and two were conventionally tapped untreated rubber trees, acting as untreated controls in comparisons.

Rubberwood logs of 1100 mm length were cut from the trees at a height of 0.5 m from the ground, and cut with a circular saw into boards of 40 mm thickness in different cutting directions. After initial air-drying the boards were further dried in a laboratory kiln drier, and planed to the final dimensions of 1000 mm×150 mm×30 mm. Then, these boards were cut into radial and tangential blocks of 100×30×5 mm^3 in order to investigate the penetration of adhesives into tangential and radial surfaces, and potential effects on the shear strength of adhesive joints. Before each experiment the blocks were conditioned at 20±2°C at 65±5% relative humidity until they were at equilibrium or they gave a constant weight.

2.2 Urea formaldehyde adhesive

Urea formaldehyde (UF) adhesive with a solids content of 50 percent, pH 8.64 at 30°C, and 375 cps viscosity at 30°C, was supplied by Dynea Krabi Co, Ltd., Songkhla, Thailand. The adhesive mixes used in the penetration experiments were prepared by addition of 0.05% (by mass) of Safranin as a marker based on solid resin, to enable fluorescence imaging. The glue was applied on wood surfaces at ambient temperature of about 27°C with a brush.

2.3 Bonded samples and determination of the adhesive penetration, shear strength and the area fraction of wood failure

The adhesive was applied at 200 g/m^2 onto one of the two wood specimen surfaces to be bonded. Assembly was always performed with parallel grain directions, and the ply without direct application of adhesive was at the bottom position to improve adhesive penetration into its structure by gravity. Special care was taken in order to have the taper of the cut surface relative to grain as low as possible, in order to have comparable penetration conditions across tested samples. The jointed samples were pressed in a hydraulic press at 130°C and 1.0 MPa for 5 minutes.

For adhesive penetration determination, the jointed samples were cut across the bond line and then the depth of adhesive penetration measured under a fluorescence microscope. The depth of adhesive penetration was determined at 45 positions along the bond line, and was defined as the width of detectable resin normal to the bond line. There was no separate individual characterization for the two plies, only a characterization of their common bonded interface. The average penetration depth (AP) was determined as the mean value of penetration depths from five jointed sample replicates for each treatment. The adhesive penetration area was also observed with a scanning electron microscope (SEM-Quanta).

Prior to the single lap shear tests, specimens were conditioned at 20 ± 2 C and 65 ± 5% relative humidity for one week. The tensile shear tests were conducted on a hydraulic test machine with a measuring scale of 50 kN at a testing speed of 6 mm/min in tensile mode, and the load direction was always parallel to the grain in all tested specimens. The shear area (20 mm × 10 mm) was assessed for the area fraction of wood failure and its thickness, after failure of the joint. Ten replications were performed for each set of parameters.

3 Results and discussion

3.1 The average adhesive penetration depth

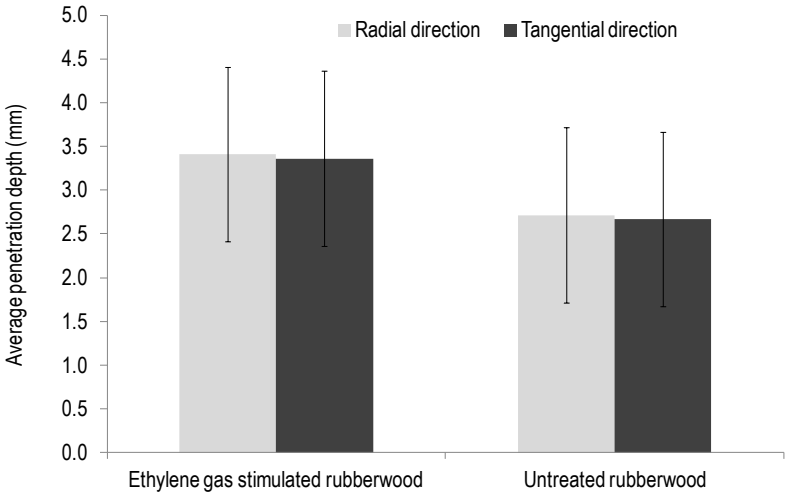


Figure 1. Average penetration depth of urea formaldehyde adhesive in radial and tangential directions of ethylene gas stimulated rubberwood and untreated rubberwood.

Penetration is the ability of an adhesive to enter into the lumen and into cell walls as a flowing fluid. It depends on the characteristics of wood, such as the lumen diameter and the grain slope on wood surface, on properties of the resin or adhesive mix (such as molecular weight distribution, adhesive mix composition, viscosity, amount of adhesive spread, and its rate of curing), and the physical conditions during bonding (such as assembly time, press temperature and pressure, and moisture content of wood) (Gavrilović-Grmuša et al., 2012). The current study was aimed to determine the effects of rubber tree tapping system on adhesive penetration, so the other factors affecting were held constant to the extent possible. The 3.4±1.2 mm depth of UF adhesive penetration in ethylene gas stimulated rubberwood was significantly higher than the 2.7±0.5 mm in untreated rubberwood control samples. The adhesive penetrations in radial and tangential directions were not significantly different at P<0.01 level, in agreement with a prior study where the cutting plane had no influence on the adhesive penetration (Gavrilović-Grmuša et al., 2012).

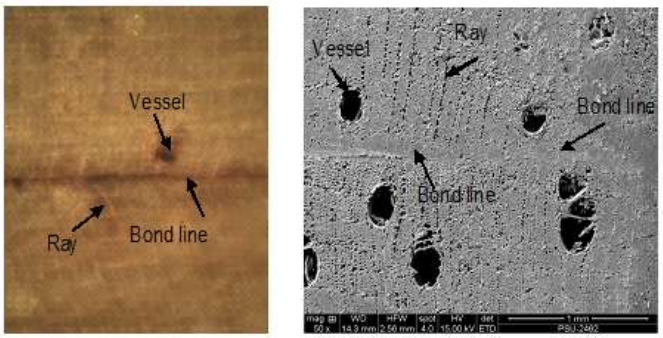


Figure 2. The distribution of UF adhesive penetrating into radial cut surface of ethylene gas stimulated Rubberwood, observed under a fluorescence microscope (left, 30x) and a scanning electron microscope (right).

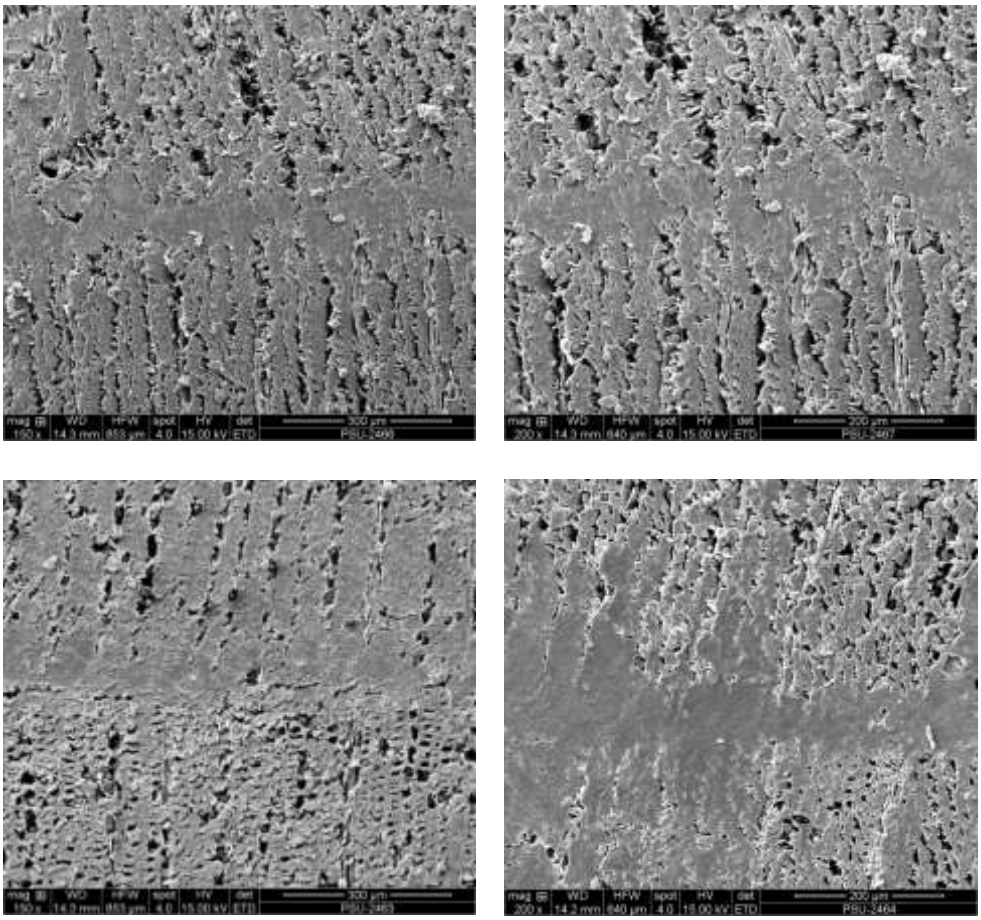
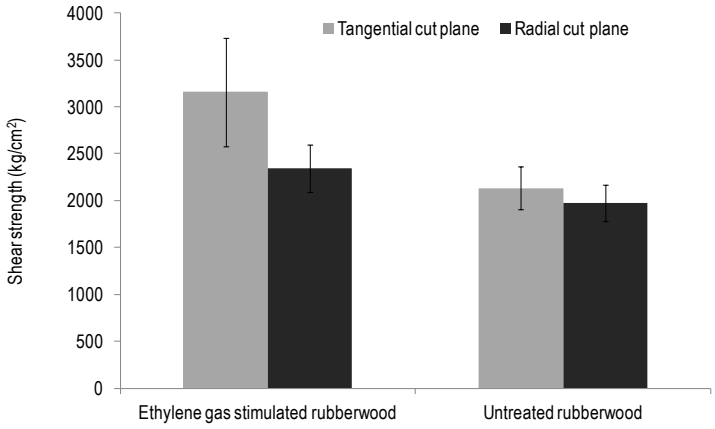


Figure 3. The distribution of UF adhesive penetrating into tangential cut surface of untreated rubberwood (above) and ethylene gas stimulated rubberwood (below), imaged with a scanning electron microscope.

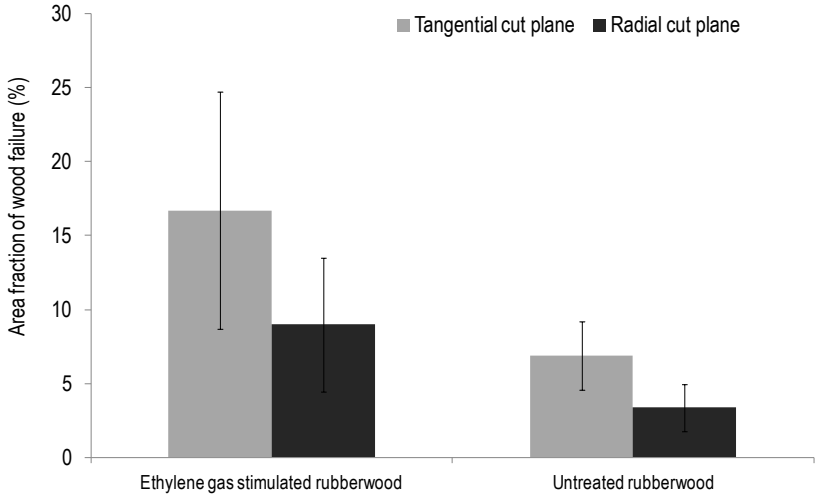
The extent of adhesive penetration into rubberwood was determined by examining the cross section of a bond line with a fluorescence microscope and with a scanning electron microscope (Figures 2 and 3). The penetration of UF adhesive into radial direction was of small extent, so that adhesive was only found immediately by the bond line. The adhesive did partly fill rays and vessels in contact with the bond line. Figure 3 illustrates the somewhat larger penetration in ethylene gas stimulated rubberwood relative to untreated control. However, local variations in the anatomy of wood at the bond line, such as the presence of vessel elements, has a large influence on adhesive penetration and area filled with adhesive (Gavrilović-Grmuša et al., 2012), and this makes small observed differences inconclusive.

However, the porosity and permeability of wood contribute to adhesive penetration, and a previous study reports that ethylene gas stimulated rubberwood has a higher permeability than untreated rubberwood (Cherdchim and Sudchada, 2013), corroborating the slightly increased adhesive penetration observed in the current study.

3.2 Shear strength and wood failure



(a)



(b)

Figure 4. Shear strength (a) and average area fraction of wood failure (b), of the tested adhesive joints.

The shear strength and the average area fraction of wood failure are shown in Figure 4, for the tested adhesive joints. The shear strengths of were significantly higher for ethylene gas stimulated wood than for untreated control samples. Shear failure generally occurred near the bond line and the average thickness of wood failure was close to 0 mm. The fractional area of wood failure was almost three fold for ethylene gas stimulated wood relative to control samples. This also corroborates the observed wider bonding interphase of stimulated wood. In prior research a dominant portion of the wood failure occurred in the fortified interphase and a smaller portion in the “pure” wood without adhesive (Gavrilović-Grmuša et al., 2012). The shear strength appears to depend on penetration depth of adhesive and it was directly proportional to the area fraction of wood failure.

4 Conclusions

Ethylene gas stimulated rubberwood had better bondability than untreated rubberwood, apparently due to higher porosity and permeability that improved adhesive penetration deeper into the wood structure. The observed bond

interphase of stimulated rubberwood was wider than for control samples, and the bond shear strengths and the area fractions of wood failure after shear tests were also higher than for untreated rubberwood. These results suggest that the ethylene gas treatments that are beneficial for latex production are also beneficial for adhesive bonding of rubberwood lumber.

5 Acknowledgements

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Potential Of Boron Rubberwood Preservatives Against Asian Subterranean Termite *Coptotermes gestroi* (ISOPTERA: RHINOTERMITIDAE)

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Abstract

This work evaluated the efficacy of rubberwood preservative using boron for decrease Asian subterranean termite *Coptotermes gestroi* (Isoptera: Rhinotermitidae) attack. Fresh-cut rubberwood samples were divided into two groups, first group treated by 2% boric acid and second group treated by 2% borax under 100 mmHg vacuum for 20 minutes. Five treatments were carried out in the experiment, samples treated with 2% borax and leaching, samples treated with 2% borax and not leaching, samples treated with 2% boric acid and leaching and samples treated with 2% boric acid and not leaching and control. For leaching tests, all samples were stirred in distilled water for 20 minutes and dried at 103 °C for 48 h. Rubberwood samples were then subjected to laboratory termite resistance test using *C. gestroi*. Result present that the relative mass loss of control treatment was highest. Rubberwood samples treated with preservative chemicals and not leaching had lowest mass loss caused by *C. gestroi* infestation. There was not significant different in the percent of mass loss among leaching rubberwood samples treated with 2% boric acid and 2% borax. Borax and Boric acid affected on *C. gestroi* infestation in equally. Properties improvement of rubberwood using borax and boric acid can decrease insect infection.

Key words: leach, moisture content, wood borer, vacuum

Introduction

The Asian subterranean termite, *Coptotermes gestroi* (Wasmann) (Isoptera: Rhinotermitidae) is known to cause tremendous losses to finished and unfinished wooden structures in buildings, and losses in agriculture and forestry crops (Sen-Sarma et al., 1975). *Coptotermes gestroi* is a small sized species of termites, living underground and has spread to many parts of the world. Preservative treatment is required to protect timber in regions with high *C. gestroi* pressure. To avoid environmental pollution and health problems caused by traditional wood preservatives or synthetic pesticides, there is increasing interest in alternative chemical compounds. Boron wood preservatives have been used for many years and there is growing interest in their low mammalian toxicity and environmental acceptability (Laks and Manning, 1994). Wood specimens were treated with boron containing quaternary ammonia compound (DBF) and the same emulsion in surface treatments resistance to termite and fungi (Kartal et al., 2004). Boric acid and borax are most common boron compounds which have found many application areas in the wood preservation industry in order to get the benefit of their biological effectiveness and fire retardancy (Le Van and Tran, 1990). Mai and Militz (2004) stated that decay resistance against *Tyromyces palustris* and *Trametes versicolor* revealed the highest protection by combined treatment of water glass and boron salts (Furuno et al., 1992, 1993; Furuno and Imamura, 1998). The termite resistance of leached specimens was particularly enhanced in the samples treated with boric acid, borax, and potassium borate combined with water glass (Furuno and Imamura, 1998). The objective of this study was to investigate the resistance to *C. gestroi* infestation, of rubberwood treated with borax and boric acid.

Materials and Methods

Non-choice tests followed, with slight modifications, the standard D3345-74 of International standards 2001 by the American Society for Testing and Materials (ASTM). All experiments were done in a laboratory.

Wood preparing

All rubberwood samples were cut to 2.5 × 2.5 × 0.64 cm along trunk axis direction. Five treatments were carried out in the experiment, samples treated with 2% borax and leaching with water, samples treated with 2% borax and not leaching, samples treated with 2% boric acid and leaching and samples treated with 2% boric acid and not leaching and control. All samples were pre-dried in oven and immersed in 2% borax and second group treated by 2% boric acid under 100 mmHg vacuum for 20 minutes. After that, all samples were dried in an oven at 103 ± 5 °C and weighed. For leaching tests, all samples were stirred in distilled water for 20 minutes and dried at 103 °C for 48 h.

Non-choice experimental procedure

The non-choice experiment relates to termiticidal toxicity, when no alternative food source is provided and the diet consists only of treated or only of untreated control wood.
A single treated sample was put on sterilized sand in a 5 × 8 × 5 cm clear plastic box with aeration holes covered with net. *Coptotermes gestroi* workers were collected from rubberwood logs in the field. One gram of *C. gestroi* was released to the plastic box and left for 30 days. Ten replicates were done for each treatment. The samples were weighed before treatment, after treatment and drying, and after 30 days of infestation. The determined masses were used to calculate dry mass absorbed from wood vinegar and relative mass loss due to infestation.

Statistical analysis

One-way ANOVA was used to analyze the loss of mass due to termite infestation, to determine differences between treatment groups. The response variable analyzed was percent loss of mass, and these data were log (x + 1) transformed when necessary to meet the assumptions of statistical analysis, and then back-transformed for presentation in tables and graph.

Results and Discussion

In the non-choice experiments the relative loss of mass decreased with leaching and non- leaching treatment, indicating high termiticidal activities for samples treated with 2% boric acid and borax (ANOVA: $F_{4,20}$ = 75.871, P ≤ 0.001). Results presented that the relative loss of mass of control treatment was higher significantly from other treatment groups (Figure 1). There was no significant difference in the relative loss of mass between treatments with 2% boric acid and borax. Both leaching treatments of boric acid and borax did not differ significantly in the percentage of mass loss (Figure 1).

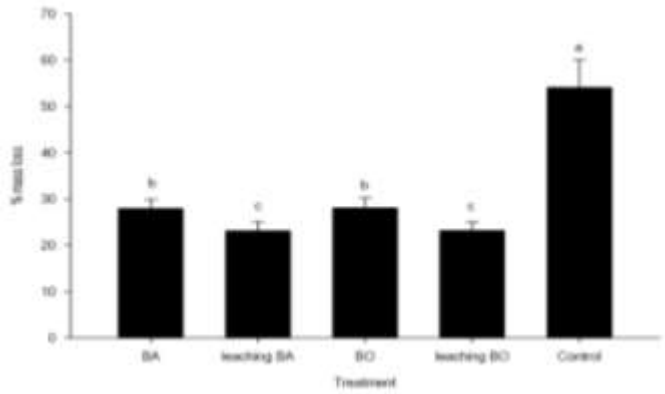

Figure 1. Relative loss of mass (mean ± SD) for each treatment in non-choice experiments. BA is boric acid and BO is borax. Different letters indicate statistically significant differences (Tukey test: P < 0.05; n = 5). Significance is based on log (x + 1)-transformed data, non-transformed data are plotted.

In our study, with the exception of control treatment, the rubberwood treated with either 2% boric acid and borax showed resistance against the subterranean termite *C. gestroi* even if after the leaching process. This result is consistent with the previous researchs indicated treated boron wood was effective against termite and fungi (Mai and Militz, 2004; Yamaguchi, 2005; Kartal et al., 2007). Hence, results of this experiment indicate that borax and boric acid affected on *C. gestroi* infestation in equally. Properties improvement of rubberwood using borax and boric acid can decrease insect infection.

Continuous leaching tests and field trials are needed to determine the amount of depletion of boron as well as the decay and termite resistance of wood treated with boron. The results overall suggest that treatments with 2% boric acid and borax give rubberwood resistance against *C. gestroi* infestation.

Conclusion

The rubberwood treated with either 2% boric acid and borax showed resistance against the subterranean termite *C. gestroi* even if after the leaching process. Properties improvement of rubberwood using 2% borax and 2% boric acid can decrease insect infection.

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ANTITERMITIC ACTIVITY OF THE BARK EXTRACTS OF TEAK

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Abstract

Teak (*Tectona grandis*) wood is recognized to exhibit antitermitic activities. The antitermitic activities of its bark, however, is still unexplored. This research is aimed to evaluate teak bark extracts and their components against *Reticultermes speratus* Kolbe termites species. Materials used in this study were wood powder from teak trees aged 8 years (6 trees) and 22 years (4 trees). The extraction were performed separately by cold extraction (one week) by three solvents: *n*-hexane, ethyl acetate and methanol. No choice antifeedant bioassay test by paper discs was carried out in this research. By analysis of variance, the results showed that methanol soluble extracts (1.04 %) is significantly higher than those other solvents. The extracts of *n*-hexane exhibited the antifeedant activity (4-5 mg) which is measured by the mass loss due to termites. The similar tendency was also found in the mortality rate of termites (20-38 %) although the magnitude was not so high compared to the controls (17 %). Tree age factor did not affect significantly to both extractive contents and antitermitic properties of the bark. By means of gas chromatography, deoxylapachol detected in the teak wood extracts was also found in the bark extracts. This compound, however, did not significantly correlate to the antitermitic properties.

Keyword : *Tectona grandis,* antitermitic activities, bark extractive, *Reticultermes speratus*, cold extraction

Introduction

The bark is a major by-product of wood processing. It has a formidable barrier providing both constitutive and induced defence mechanisms. Therefore, bark is believed to contain chemical components, which enable the tree to resist those hazards. It is expected that more effective uses of bark will develop from the viewpoint of green environments. Unlike antifungal activities, only few studies have been published on the antitermitic effect of bark extracts of the natural inhabiting wood species. Those species were *Picea glehnii* (Shibutani et al. 2004), *Adina recemosa* (Yaga 1977) and some temperate species (Harun and Labosky 1985).

Tectona grandis is a widely distributed hardwood in Indonesia. The wood is used as raw material for the production of furniture and other wooden structures. Previous works have already reported that extractives from the wood of teak inhibited the wood-destroying temites. Of these extractives, quinones that were present in appreciable levels in the wood of teak showed strong inhibition against the attack of termites (Rudman and Gay, 1961; Sandermann and Simatupang, 1966; Lukmandaru and Ogiyama 2005, Lukmandaru 2012). However, studies about as antitermitic effects as well as chemical constituents of teak bark are still limited.

The aim of this study was to find out the antitermitic effect of bark extracts from teak as well as to detect some quinones and related components in the bark. It is assumed that similar quinones in the wood to also play an important role in the antitermitic activity of the bark. The results are discussed with focus on the bark extracts effect by means of the paper disc tests.and to relate it with the present of quinones. The comparison with the results of our parallel study (Lukmandaru 2013) was also discussed.

Material and methods

Bark extracts

All the plant materials were collected at community forests in Gunungkidul Regency, Jogja. The ages of the test trees were 8 (comprised of 6 samples) and 22 (comprised of 4 samples) years. All the bark samples were collected from the stems of the trees. After drying, these dried bark samples were ground to pass through 40 mesh size. The bark meal (1 g) of each sample was subjected to extraction in *n*-hexane (10 ml) at room temperature for one week. The bark was also separately extracted with ethyl acetate (EtOAc) and methanol (MeOH) in the same manner. The resulting solutions were filtered and the filtrate was evaporated to dryness. The yields of the extractives were calculated on the basis of the weights of the dried extracts to the oven-dried weights of the bark samples.

No-choice feeding test

Subterranean termites were collected from an active wild colony of *Reticulitermes speratus*. The colony was maintained in a dark room at 28°C and 80% relative humidity (RH) until use. A petri dish (diameter 9 cm, height 2 cm) containing 20 g moistened and sterilized sea sand was used as a container test. Paper disc (diameter 8 mm; Whatmann International) were impregnated with chloroform solution containing each of the test fractions. The treatment retention was 5 % (w/w) per disc and 3 duplicates were applied for each sample. After drying at 60 ^{0}C for 2 hours, followed by drying in a vacuum dessicator for 24 hours, they were put on a petri dish. The control discs were impregnated with chloroform only and dried with the same manner. Fifty worker *Reticulitermes speratus* Kolbe termites were introduced into the petri dish. The petri dishes were placed in a dark chamber at 27 ^{0}C and 80 % relative humidity. After 10 days the disc were taken out, dried in the same manner and the weight loss was determined. Mortality was calculated based on the surviving number of termites.

Extractive analyses

Bark meal samples were separately extracted by *n*-hexane (C_6H_6), EtOAc, and MeOH as mentioned above. Aliquots of each solution (200 µl) were removed, placed in glass tubes and dried. A 20 µl aliquot of C_6H_6 was added to dilute each extract. One µl of this solution was then injected with a micro syringe into a GLC (Hitachi Model G-3000) under following conditions, detector: FID, column: NB-5 bonded capillary 30 m, column temperature: 180 - 280 ^{0}C (programming 4 ^{0}C/min), carrier gas: Helium. For quantification of individual substances, a commercial tectoquinone (2-methyl anthraquinone) was employed as a standard. The amounts of components were expressed as mg per 100 g of oven dry weight. Pure sample of squalene and lapachol purchased from Kanto Chem were also was used for confirmation. The identification of constituent compounds was based on their mass spectra and gas chromatographic retention behavior. GC-MS (JEOL XS mass spectrometry at 70eV) was used for gas chromatographic separations. Deoxylapachol or its isomer was identified by comparison of their mass spectra with those from previous studies by Windeisen et al. (2003) and Perry et al. (1991).

Statistical Analysis

The results were expressed as mean ± standard deviation (S.D.). Statistically significant differences between groups were measured using two-way analysis of variance (ANOVA) with post hoc comparison by Tukey, $*p < 0.05$ was considered statistically significant. The relationships between the independent variables were studied with a Pearson's correlation analysis. All statistical calculations were conducted using SPSS-Win 10.0.

Results and Discussion

Extractive content

The ANOVA of mean extractive content showed that there was no significant tree age factor (p= 0.72), while the effects of extract (p <0.01) are significant. Comparisons among the extracts revealed that the MeOH soluble extractive content (3.44$\pm$0.70 %) significantly gave the highest values while C_6H_6 soluble extractive content (0.27$\pm$0.07 %) gave the lowest (Fig.1). This result could be explained as methanol dissolves both polar and to some extent non-polar compounds. Theoretically, the polar compounds such as tannins and other polyphenols were abundant in the bark. It was also showed that teak bark contains only small amounts of non-polar compounds. The extractive content levels in this experiment could be higher if the hot-extraction or soxhlett extraction was performed.

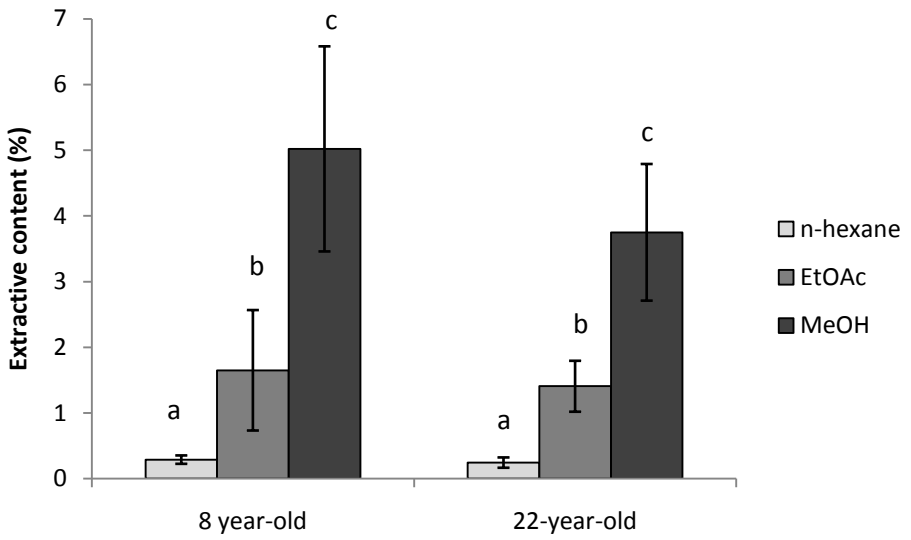

Figure 1. Extractive content (% oven dried mass m/m) of teak bark by tree age and extract. Mean of 6 trees (8- year-old) and 4 trees (22-year-old), with the standard deviation error bar. The same letters on the same graphic are notstatistically different at $P < 0.05$ by Duncan's test.

Antitermitic test

The mass loss due to termites was displayed in Figure 2. By ANOVA, it was calculated that tree age factor was not significant (p= 0.88) but the extract factor was significant (p= 0.01). The mass loss of C_6H_6 extracts levels were significantly lower (5.01±3.34 mg) compared to those of methanol (8.59±4.63 mg) or EtOAc extracts (10.35±2.63 mg). Compared to the control samples (11.35±3.60 mg), the C_6H_6 extracts exhibit a higher antifeedant activity although it was not a huge difference. This finding also indicated the existence of some active components in that extract. The similar tendency was also found with regard to mortality rate of termites. The results showed that tree age (p= 0.28) did not show significant effects, on the other hand, the extract factor (p= 0.04) had significant effects. As shown in Fig. 3, the highest mortality rate levels were obtained in the C_6H_6 and MeOH soluble extracts (24-29 %). The ANOVA of mortality rate between the C_6H_6 and MeOH soluble extracts did not vary significantly. The mortality rate value of the control was about 17 %. Based on these results, the toxicity of teak bark is thought to be weak. In general, by the same samples and method (Lukmandaru 2013), compared to the outer heartwood extracts (mortality rate of 27-65 %, mass loss of 1-3 mg), those of teak barks exhibit lower degree of antitermitic activity. No significant effect of tree age factor to the termite resistance properties is likely due to the low variation in chemical properties between the 8- and 22-year-old barks.

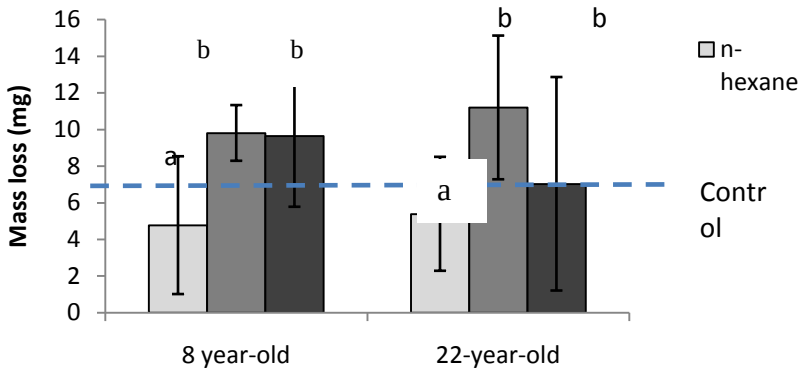

Figure 2. Mass loss due to *Reticulitermes speratus* on 10-day observation of teak bark by tree age and extracts. Mean of 6 trees (8- year-old) and 4 trees (22-year-old), with the standard deviation error bar. The same letters are not statistically different at $P < 0.05$ by Duncan's test.

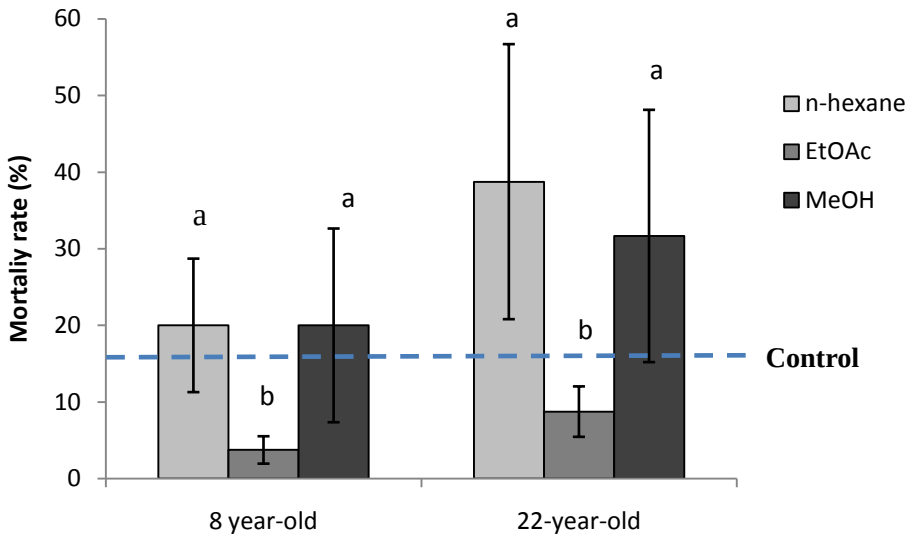

Figure 3. Mortality rate against *Reticulitermes speratus* on 10-day observation of teak bark by tree age and extracts. Mean of 6 trees (8- year-old) and 4 trees (22-year-old), with the standard deviation error bar. The same letters are not statistically different at $P < 0.05$ by Duncan's test.

Table 1. Pearson correlation coefficients (*r*) for the termite resistance parameters and extractive contents.

Extractive content	Mass loss	Mortality rate
n-hexane	-0.76**	-0.24
EtOAc	-0.31	0.05
MeOH	0.02	-0.02

Note : ** Significant at the 1% level; * significant at the 5% level.

Correlation analysis (Tab.1) confirmed a highly significantly negative correlation between the mass loss and C_6H_6 soluble extracts content ($r = -0.76$). This meant that teak bark was more resistant when it contained higher amounts of these extracts.This indicated that non-polar extractives in the bark contained compounds that were distasteful or repellent to termites. However, the relationship between mortality rate and any extractive content levels was not clear in this study. This outcome was reasonable since the bark extracts do not exhibit a strong toxicity. It was observed in our previous findings (Lukmandaru 2013) that lower degree correlations were measured between mortality rate or mass loss levels and extractive content levels in the teak wood extracts.

Analysis of n-hexane extract

As C_6H_6 soluble extracts levels were related to the termite antifeedancy property (Table 1), GC and GC-MS investigation was further performed. This analysis refered to previous work in the teak wood extract (Lukmandaru and Takahashi 2009) which some bioactive quinones (tectoquinone, lapachol, deoxylapachol or its isomer) as well as non-quinone constituents (squalene) were detected. The quantification of those compounds in the C_6H_6 soluble extracts was presented in Table 2.

Table 2. Contents of some components (mg/100 g of oven-dry wood) in the *n*-hexane soluble extract of teak bark aged 8 and 22.

Teak samples	Compounds of *n*-hexane extracts			
8-year-old trees	Deoxylapachol	Lapachol	Tectoquinone	Squalene
1	10.17	0	0	14.37
2	9.95	0	0	74.04
3	168.11	0	0	37.43
4	13.18	0	0	15.43
5	15.25	0	0	17.72
6	49.22	0	0	30.40
Average	44.31	0	0	31.55
22-year old -trees				
1	8.54	0	0	14.14
2	0	0	0	4.53
3	0	0	0	16.27
4	0	0	0	16.52
Average	2.13	0	0	12.86

In a literature review, Sandermann and Simatupang (1966) mentioned that tectoquinone is the principal component responsible for natural durability against termites in the wood. Furthermore, desoxylapachol has been found to exhibit strong antitermitic activity, but lapachol has only weak antitermitic activity. Squalene have never been mentioned as factors in natural durability. The presence of deoxylapachol and squalene in the bark was confirmed, although at very low levels compared to that in the heartwood (Lukmandaru and Takahashi 2009). Both lapachol and tectoquinone were not detected. Therefore, the lower activity against termites in the bark compared to its wood was probably due to the absence of tectoquinone or comparatively low amounts of quinones.

Previous work reported that some of the polyphenols in the bark were tested to have antitermitic properties (Harun and Labosky 1985). Stilbenes from bark of *Picea glehnii* acted as toxicants against termites rather than as mere feeding deterrents (Shibutani et al. 2004). Yaga (1977), found that scopoletin and oily scopolin were the main termicidal substances from the *Adina recemosa* bark. The Pearson's correlation coefficients between total deoxylapachol content and mass loss levels was only 0.32. This weak link means that this compound is not the sole cause of termite resistance in the bark. Thus, further investigation with regard to non-polar compounds (fats, resin, oils, terpenes etc.) in the teak bark would be useful in determining the relationships between termite resistance and extractives of the bark.

Conclusions

Methanol soluble extractive content significantly gave the highest values in the teak bark extracts. No significant tree age factor affected the variation in the extractive content as well as termite resistance properties. As *n*-hexane soluble extracts exhibited the largest antifeedant activity (low mass loss values), it seemed that the repellent or distasteful substances existed extensively in non-polar constituents of bark materials. Further, correlation analysis showed significantly negative correlation (r=-0.76) between the mass loss due to termite attacks and *n*-hexane soluble extract contents. There was no strong antitermicidal activity was observed in all extracts. Deoxlylapachol was detected in the *n*-hexane soluble extracts, however, it explained relatively little the variation in termite antifeedancy of teak bark.

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MICROWAVE-ASSISTED ACID HYDROLYSIS OF SUGARCANE BAGASSE PRETREATED WITH WHITE-ROT FUNGI

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Abstract

Sugarcane bagasse is one of the largest lignocellulosic residue in tropical countries that can be used as an alternative raw material for bioethanol production. Sugarcane bagasse is pretreated and hydrolyzed for reducing sugar production, then the sugars are fermented to obtain bioethanol. The aim of this research was to study the effects of substrate concentrations (1%, 2%, 3%, 4%, 5%), duration of microwave-irradiation (0, 2.5, 5, 7.5, 10, 12.5, 15 minutes), and concentrations of dilute acid solutions (1%, 2%, 3%, 4%, 5%) on the reducing sugar yield and the formation of 5-hydroxymethyl furfural (HMF) during the hydrolysis of sugarcane bagasse pretreated with mixed culture of white rot fungi under microwave irradiation. The sugarcane bagasse was pretreated with 10% (w/v) mixed culture of two white-rot fungi (*Trametes versicolor* and *Phanerochaete chrysosporium*).They together degraded 16.78% of lignin in the sugarcane bagasse after 2 weeks of incubation. Then, the pretreated sugarcane bagasse was hydrolyzed by two kinds of acids, sulfuric and oxalic acids, under 550 watt of microwave power. The results showed that the hydrolysis using sulfuric acid produced more sugars than didoxalic acid, and reducing sugar production was affected significantly by substrate concentrations, duration of microwave-irradiation, and acid concentration. In order to suppress the formation of HMF as byproduct, the hydrolysis of fungi pretreated sugarcane bagasse should be carried out in less than 10 minutes.
Keyword : dilute acid hydrolysis, microwave heating, reducing sugar yield, sugarcane bagasse, white-rot fungi

1. Introduction

Bioethanol production from lignocellulosic biomass has attracted much interest all over the world in recent years because of limited of fossil oil and environmental pollutions. It is desirable to use cheaper and more abundant substrates for large-scale production of bioethanol for its economic feasibility. One of the largest lignocellulosic biomass produced in tropical countries is sugarcane bagasse, the dried residue left after extracting the juice from sugarcane in the sugar production process. Sugarcane bagasse is produced in large quantities by the sugar and alcohol industries in Indonesia, Brazil, India, Cuba, China, México, and Colombia. In general, 1 ton of sugarcane generates 280 kg of bagasse, and 5.4 x 10^8 dry tons of sugarcane are processed annually throughout the world (Cardona et al, 2010). In Indonesia, there are 58 sugar industries which have capacity to grind around 195.662 tons of cane per day (Hermiati et al, 2010).

Sugarcane bagasse contains approximately 50% cellulose and 25% each of hemicellulose and lignin (Pandey et al, 2000). Pretreatment is crucial for lignin removal and make the cellulose available for enzymatic hydrolysis and fermentation. Several methods of lignocellulosic biomass pretreatment have been developed, including physical, chemical, physico-chemical and biological pretreatment. The biological pretreatment is environmental friendly and can be carried out under mild conditions, consumes low energy, and requires simple procedures and equipments (Sun et al, 2011). For biological pretreatment of lignocellulosic, white-rot fungi are the most effective basidiomycetes (Sun and Cheng, 2002). Recent studies have shown that *Aspergillus terreus*, *Trichoderma* spp*, Cyathus stercoreus*, *Lentinus squarrosulus*, *Lentinus edodes*, *Trametes pubescens*, *Pleurotus* spp, *Penicillium camemberti*, *Phanerochaete chrysosporium* grown at 25-35°C for 3-22 days on lignocellulosic materials and resulted in 45-75% and 65-80% holocellulose and lignin degradation, respectively (Mtui, 2009).

The pretreatment process is followed by hydrolizing the cellulosic biomass to obtain reducing sugar and fermentating the sugars into bioethanol. Hydrolysis of pretreated lignocellulosic materials can be carried out with acid hydrolysis or enzymatic hydrolysis. Acids can be used as catalysts for the hydrolysis as they breakdown heterocyclic ether bonds between sugar monomers in the polymeric chain (Velmurugan and Muthukumar, 2011). Commonly, dilute

acids which can be used as catalyst of hydrolysis are H_2SO_4, HCl, HF, or CH_3COOH (Aguilar et al, 2002). Oxalic acid, which is one of organic acid, also plays an important role in the degradation of wood and other lignocellulosic materials. Besides that, oxalic acid can catalyze the hydrolysis of hemicellulose and cellulose directly (Lee et al, 2009).

The conventional heating process in hydrolysis can be replaced by using gamma-ray or electron-beam irradiation, or microwave irradiation (Demirbas, 2005). Microwave irradiation is one of the methods that has been studied because of its high and selective energy transfer efficiency (Sasaki et al, 2011). The objective of this research was to study the effects of substrate concentrations, dilute sulfuric and oxalic acid concentrations, and irradiation time on hydrolysis of sugarcane bagasse pretreated with mixed culture of white-rot fungi (*Trametes versicolor* and *Phanerochaete chrysosporium*) under microwave irradiation for reducing sugar and 5-hydroxymethylfurfural (HMF) production.

2. Materials and Methods

Sugarcane bagasse used in this research was obtained from sugar plant in Magetan, East Java-Indonesia. The bagasse was milled and sieved to get particles of 40-60 mesh. Two species of white-rot fungi, which were *Trametes versicolor* and *Phanerochaete chrysosporium*, were used in this study for biological pretreatment of sugarcane bagasse.

For preparation of inoculum of fungi, both white-rot species were cultured on Potato Dextrose Agar (PDA) slant and incubated for 7 days. JIS-medium broth was prepared by mixing 3 g KH_2PO_4, 2 mg $MgSO_4.7H_2O$, 25 g glucose, 5 g peptone and 10 g malt extract in 1 liter of distilled water. After incubated for 7 days, the mycelium from each slant was molted and diluted with 5 ml of JIS-medium broth. The suspension was then poured into 95 ml JIS broth media and incubated stationery at 27°C for 10 days. After the 10-days incubation, each inoculum was homogenized using warring blender with high speed (2 x 20 sec). The *Trametesversicolor* and *Phanerochaete chrysosporium* inocula were mixed with 1:1 ratio, then they were used for biological pretreatment.

About 200 g (dry weight) of sugarcane bagasse was put in a heat resistant plastic bag. Nutrient media (JIS broth) was added to the bagasse (3:1 w/w), then this medium was sterilized in an autoclve for 20 minutes. After that, 10% (v/w) of working inoculum was added, and the medium was incubated at ± 27°C for 2 weeks. Pretreated sugarcane bagasse was weighed to determine weight loss.Lignin, α-cellulose, and hemicellulose content after biological pretreatment were also determined [9]. The pretreated bagasse was then hydrolized under microwave irradiation with dilute acid catalyst.

A commercial microwave oven (2450 MHz, 1100 W) was used forthe hydrolysis. The output power of the microwave oven is 1100 W. The oven's power could be set to 50% which was assumed equal to 550 W. This single factor experiment involved five levels of substrate concentrations (1%, 2%, 3%, 4%, and 5% w/v), five levels of dilute acid concentrations (0, 0.5%, 1%, 2%, and 3%) and seven levels of duration of microwave-irradiation (0, 2.5, 5, 7.5, 10, 12.5 and 15 minutes). All solutions and the sugarcane bagasse were assumed to have a specific gravity of 1 g/ml. Firstly, certain amount of the wet pretreated sugarcane bagasse was immersed in certain concentration of sulfuric or oxalic acid solution and made up 20 g of total mixture. They were mixed at 200 rpm for 20 minutes, then the mixture was heated under microwave irradiation. After that, it was cooled as soon as possible. The soluble fraction from the hydrolysis process was filtered to determine its reducing sugar (Nelson-Somogyi method) and HMF (SNI 01-3545-2004 based on AOAC Official Method 980.23-1999) contents.

3. Results and Discussion

3.1. Pretreatment of sugarcane bagasse with mixed culture of white rot fungi

The major components of sugarcane bagasse (before biological pretreatment) used in this research were carbohydrate fraction and lignin. The carbohydrate fraction of the raw sugarcane bagasse was 69.65% of the total biomass, consisted of 40.98% α-cellulose and 28.67% hemicellulose. The α-cellulose is a polymer of glucose which can be used as a sugar source for ethanol production. The lignin content of the raw bagasse in this research was 29.27% which is in range of lignin content in sugarcane bagasse previously reported (Cardona et al, 2010).

The biological pretreatment was carried out by mixing two species of white-rot fungi, *P. chrysosporium* and *T. versicolor*, for delignification of sugarcane bagasse. These fungi were selected because of their effective degradation of lignin (Shi et al, 2008; Anita et al, 2011; Taniguchi et al, 2005). The percentage loss of sugarcane bagasse's component was calculated after the mixed culture of white-rot fungi's pretreatment (Figure 1).

The growth of mixed culture fungi resulted in weight loss of sugarcane bagasse. The weight loss of sugarcane bagasse after two weeks of incubation was 12.94%. Taniguchi et al. (2005) reported that*P.chrysosporium* and *T. versicolor* pretreatment decomposed nearly 30% of rice straw after24 days of incubation. In this research the mixed culture of *P. chrysosporium* and *T. versicolor* decomposed less than 30% of the sugarcane bagasse, because of shorter time of incubation and different chemical compositions of the substrate. Shorter incubation time of fungal pretreatment could reduce the weight loss of biomass (Orozco et al, 2007).

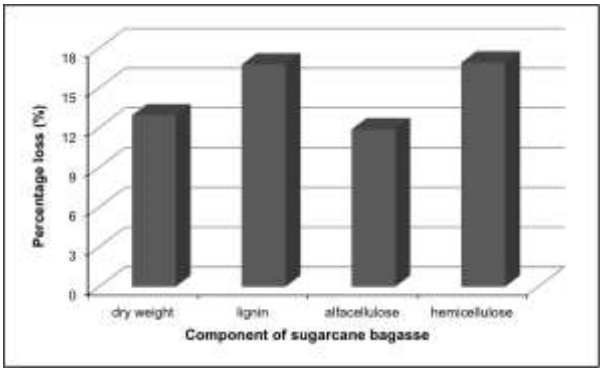


Figure 1. Loss of sugarcane bagasse components after pretreatment with mixed culture of*P. chrysosporium* and *T. versicolor* for 2 weeks

Single culture of *P. chrysosporium* or *T. versicolor* was not able to remove the lignin component in the lignocellulosic biomass selectively (Taniguchi et al, 2005). However, this study reveals that mixed culture of *P. chrysosporium* and *T. versicolor* in showed a promising combination, because they could remove or degrade more lignin than α-cellulose in the biomass. They together degraded 16.78% of lignin, 11.88% of α-cellulose and 19.92% of hemicellulose in sugarcane bagasse after 2 weeks of incubation. Anita et al. (2011) reported that another mixed cultureof fungi, *Trametes versicolor* and *Pleurotus ostreatus*, degraded 17.21% of lignin, 26.94% of α-cellulose, and 48.45% of hemicellulose in sugarcane bagasse after 4 weeks of incubation.

3.2. Hydrolysis under microwave irradiation

Previous research reported that power level of microwave had significant effectson the hydrolysis of lignocellulose. Fajriutami et al.(2012[b]) reported that the higher the power, the more reducing sugar produced in the hydrolysis of sengon pulp. The reducing sugar production from dilute acid hydrolysis of sengon pulp was started after 10 minutes of microwave irradiation at 30% of microwave power and after 5 minutes of microwave irradiation at 50% of microwave power. Therefore, in this research we used 50% of microwave power which was equal to 550 W.

3.2.1. Effects of Pretreated Sugarcane Bagasse (PSB) Concentrations

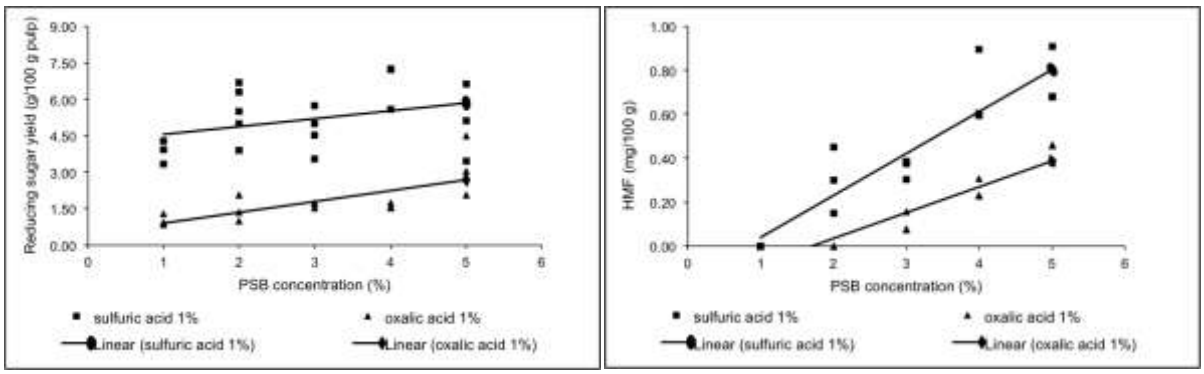
Study on the effect of PSB concentrationson reducing sugar and HMFproductions were conducted by mixing 1%, 2%, 3%, 4% and 5% PSB in acid solution at 1% (v/v) and irradiated for 5 minutes. Figure 2a shows the effect of PSB concentration on reducing sugar production after hydrolysis under microwave irradiation with sulfuric acid and oxalic acid catalysts. The PSB concentration had significant effect based on analysis of variance ($p<0.05$). However, the increase of PSB concentrations up to more than 2% was not efficient, because multiple comparisons analysis showed thatthe significant reducing sugar yield were obtained after increasing PSB concentration from 1% to 2%. The average reducing sugar yield was increased from 3.86 g/100 g pulp to 54.81 g/100 g pulp.

The PSB concentrations also had significant effect on the production of HMF ($p<0.05$). HMF is a by-product formed in acid hydrolysis of lignocellulosic materials that contained polymers of C_6 sugars, such as cellulose . It is the most important inhibitor during fermentation of dilute-acid hydrolyzates.Therefore, lower amount of HMF in the hydrolysatesis desirable [15]. Figure 2b shows that the highest HMF concentration was 0.91 mg/100 g when 5% of PSB was hydrolyzed. At 2% of PSB concentration, the average HMF concentration was 0.30 mg/100 g. Thus, it is recommended to use 2% of PSB concentration, because at higher PSB concentration there was more HMF produced and no significant increase of reducing sugar yield detected.

3.2.2. Effect of Duration of Microwave-Irradiation

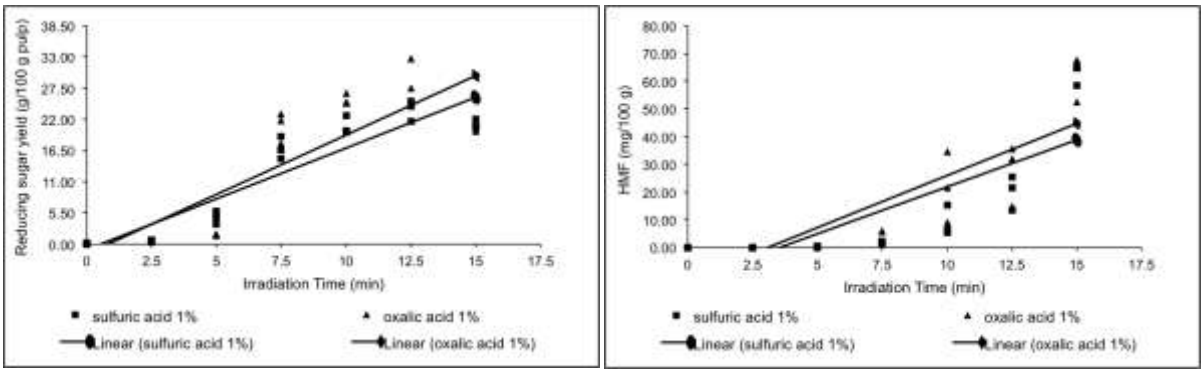
The duration of microwave-irradiation had significant effect on reducing sugar production ($p<0.05$). The reducing sugar production was started after 2.5 minutes irradiation of acid hydrolysis (Figure 3a). The highest reducing sugar production was obtained when PSB was hydrolyzed by dilute sulfuric and oxalic acid for 15 minutes, which were 21.15 g/100 g pulp and 21.91 g/100 g pulp, respectively. These results were in agreement with our previous research (Fajriutami et al, 2012[a,b]) which reported that 15 minutes duration of microwave irradiation resulted in the highest reducing sugar production from dilute acid hydrolysis of oil palm empty fruit bunch pulp and sengon pulp.

Unfortunately, the 15 minutes irradiation resulted in the highest HMF production as well. Figure 3b shows that the HMF production was significantly affected by duration of microwave-irradiation ($p<0.05$). The highest HMF concentration after 15 minutes of irradiation was 62.31-62.66 mg/100 g, either with dilute sulfuric acid or oxalic acid catalyst. Based on multiple comparisons analysis, during the first 7.5 minutes of microwave irradiation the HMF concentration did not increase significantly. The HMF concentration was increased from 5.42 to 64.91 mg/100 g and from 8.99 to 67.43 mg/100 g when PSB was microwave heated for 10 minutes with dilute sulfuric acid and oxalic acid, respectively. Therefore, in order to prevent the production of HMF, the duration of irradiation should not be more than 10 minutes.



(a) (b)

Figure 2. Effects of pretreated sugarcane bagasse (PSB) concentrationson reducing sugar yield (a) and HMF concentration (b) when hydrolysis reaction was catalyzed by 1% dilute acid solution under microwave irradiation (550 W) for 5 minutes.

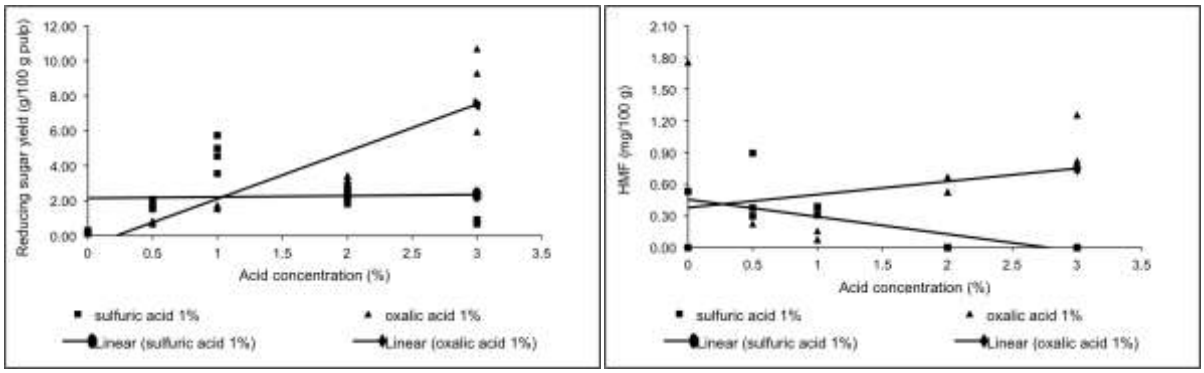


(a) (b)

Figure 3. Effects of duration of microwave-irradiation on reducing sugar yield (a) and HMFconcentration (b) when 3% (w/v) of PSB was hydrolyzed under microwave irradiation (550 W) with 1% acid solution

3.2.3. Effect of Dilute Acid Concentration

The dilute sulfuric and oxalic acid concentrations had significant effect on reducing sugar yield ($p<0.05$). However, the increasing of sulfuric acid concentration to more than 1% did not have any significant effect on the sugar yield. The increasing of oxalic acid concentration to more than 2% also did not have significant effect on the sugar yield. Figure 4a showsthat the reducing sugar yield were 4.70 g/100 g pulp and 1.66 g/100 g pulp when hydrolyzed uder microwave irradiation of 550 W power for 5 minutes with 1% sulfuric acid and 1% oxalic acid, respectively. The reducing sugar yield from dilute sulfuric acid hydrolysis was higher than that from oxalic acid, because at the same acid concentration there was lower concentration of H+ ion formed in oxalic acid solution than that in sulfuric acid solution. Qin et al. (2012) reported that organic acid pretreatment resulted in lower glucan content than sulfuric acid at the same condition. On the other hand, the HMF production during 5 minutes of irradiation was not affected by concentration of dilute acid solution ($p<0.05$). Figure 4b showsthat the average concentration of HMF after microwave irradiation for 5 minutes with dilute sulfuric acid and oxalic acid catalysts were 0.00–0.52 mg/100 g and 0.00–1.04 mg/100 g, respectively.



(a) (b)

Figure 4. Effects of dilute acid concentrations in the microwave- assisted hydrolysis (550 W, 5 minutes) of PSB (3% w/v)on reducing sugar yield (a) and HMF concentration (b)

4. Conclusions

The mixed culture of*P. chrysosporium* and *T. versicolor* could selectively remove or degradelignin during the pretreatment of sugarcane bagasse. This research also showed that the pretreated sugarcane bagasse concentrations and duration of microwave-irradiaton had significant effect on reducing sugar yield and the HMF formation.The dilute acid solution concentrations had significant effect only on reducing sugar yield. It was recommended that the hydrolysis was conducted using 2% (w/v)substrateconcentration and,1% dilute sulfuric acid. To prevent the production of HMF, the duration of irradiation should not be more than 10 minutes at 550 W microwave power.

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