RESISTANCE OF *DENDROCALAMUS ASPER* (BULUH BETONG) TREATED WITH PLANT EXTRACTS TO MOLD

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ABSTRACT

Bamboo and bamboo-based products are susceptible to biodeteriorating agents including fungi, powder-post beetles and termites. High starch, sugar and protein content of untreated bamboo makes it highly susceptible to biodeterioration and thus treatment is needed prior to application. Copper chromium arsenic (CCA), and combination of borax with boric acid are the conventional treatments used to protect bamboo, but are banned in several countries due to their toxicity and hazardous implications to human and environment. In the current study, alternative plant-based treatments (camphor, Cinnamomum camphor and lemongrass, Cymbopogon citratus) and borax were pressure treated on bamboo Dendrocalamus asper or locally known as 'buluh betung' and its performance to mold (Aspergillus brasiliensis) was evaluated. These treatments were selected based on the initial screening test that showed the inhibitory effect of the camphor and lemongrass extracts to wood deteriorating fungi, including mold. Visual assessment was conducted at the end of the 4 weeks mold test on all bamboo species. Results showed potential of the plant extracts as alternative for bamboo treatment. Further study is on-going to evaluate the performance of these treatments for outdoor applications.

Key words: Plant extracts, bamboo, *Dendrocalamus asper,* durability, mold

INTRODUCTION

Bamboo is enigmatic, resilience and flexible with thousands of uses and benefits and has always been an integral part of 1/3 of the world populations (Global Industry Report, 2019-2025). Bamboo is an amazing plant and we have yet to fully grasp and avails of its full potential. Scientifically, bamboo produces 35% more oxygen and absorbs 40% more carbon dioxide than the trees, thus becomes a carbon-sink itself, control soil erosion, restore degraded land, conserve biodiversity, and contribute to the purification and regulation of the environment. Above all, in each and every bamboo shoots that grows are capable to combat climate change and act as a "green environmental protector". Other than environmental contribution, bamboo products and services can generate national income. Connotation of bamboo as poor man's timber is no longer suitable since bamboo is now becoming a green gold. With climate and soil suitability to plant bamboo in the tropical countries including Malaysia, there is no doubt that it is the most sustainable plant. Income from eco-tourism, furniture and craft from bamboo are among the niche products. On the same note, the use of construction materials from bamboo has led to the need of improvised preservation technique due to its susceptibility to mold and decay. This notation justifies the need for more research in bamboo treatment and preservation. Conventional preservation of bamboo using toxic materials is no longer suitable and an alternative non toxic materials is crucial. This study examines the potential of 2 types of plant extract namely lemon grass extract and camphor extract as non toxic preservation solution to preserve bamboo. Both extract can be found in Malaysia. Comparison was also made to include boron which is apparently a commercial use. No policy issue involved in this study.

LITERATURE REVIEW

Bamboo and bamboo products are susceptible to bio-deteriorating agents including fungi, powder-post beetles and termites. High starch, sugar and protein content of untreated bamboo make it highly susceptible to biodeterioration. Mean value of selected bamboo starch was reported at 8% (Katrina K.K , *et all*.,2017). Li, X (2004) reported starch content of 2%-6%, deoxidized saccharide of 2%, fat of 2%-4% and protein 0.8% -6%. Mold is classified as being saprophytic organism that utilize sugar and carbohydrates in lumen cell of wood, but does not cause decrease in mechanical properties (Tumirah, K *et al*, 2020). Other than starch, sugar and protein, moisture level also affects the deterioration rate by decay fungi on building made of wood and bamboo structures. Relative humidity ranging between 70% and 90% are required for fungal growth on bulding materials (Hoang, C.P, *et al*.,2010). Bamboo has low natural durability since it has only little content of wax, resin and tannin (Tomak, E.D *et al*, 2013), thus treatment is needed. Most conventional preservatives treatment can caused environmental pollution, and a few of them may be harmful to human health. CCA is a common treatment for timber but also can be used to preserve bamboo. Apparently CCA, combination of Borax and Boric acid are conventional treatments used to protect wood and non wood products. CCA was banned in several countries due to its toxicity to the environment and human because its easily leach out due to oxidation. Borax-Boric

Acid are now widely used to treat bamboo poles and bamboo products by soaking. Essentially, exploration into new non toxic preservation technology is crucial. Extractives from durable wood species can be used to treat non-durable wood species including tannins, flavanoids, lignans, stilbens, terpenes and terpinoids. 9% and 12% concentration of Acacia and quebracho bark extract (*Schinopsis spp*.) respectively had shown decay resistancy towards treated scots pine and poplar wood (Tascioglu, C, *et al*., 2013). Significant progress in plant-based research include extract of elme(*Zelkova carpinifolia*), oak (*Quercus castanifolia*) and mulberry (*Morus alba*). Essential oil of Thuya (*Tetraclinis articulate*) showed excellent decay resistance at 5% concentration. Neem (*Azadirachta indica*) leaves extract showed 4-7 times decay resistance as compared to the untreated wood (Xu, G *et al*, 2013).

Camphor leaves extract (CE) from camphor tree (*Cinnamomum camphor* (l.) presl) has strong antifungal compounds including D-camphor, eucalyptol, α-terpineol, linalool, and 4-terineol, but easily volatised due to its alcohol content. Fixing the camphor extract with 10% MUF (CEMUF) had increase the decay resistance as compared to CE alone. Exposure to white rot and brown rot of *P. Chrysosporium* and *G. Trabeum* had showed decrease in mass loss % of 5-6% when treated with CEMUF. Whilst, bamboo treated with CE showed an substantial mass loss of 16% for both white and brown rotters. thermogravimetric analysis, TGA results showed that CEMUF is thermally stable as compared to CE alone (G, Xu *et al*, 2013). Camphor extract can also be extracted from Kapur tree or *Dryobalanops spp*, which can be found in Malaysia. Previous studies on phytochemicals, compound responsible for biocidal action were not quantitatively determined. It was reported that greater bioactive compounds were found in tree bark rather than the leaves. Since stem bark are not readily obtainable, it limits the utilisation of tree bark as wood preservatives (Adedeji, G.A. *et al*., 2013).

Aqueous extracts of lemon grass, *Cymbopogon citratus*(LGE) was used in this study. Lemongrass oil is the essential oil obtained from the aerial parts of *Cymbopogon citratus* Stapf., from the family Poaceae. The plant has been widely recognized for its ethnobotanical and medicinal usefulness. The insecticidal, antimicrobial, and therapeutic properties of its oil and extracts have been reported. Trado-medicinal preparations of the oil have been used both internally for alleviating colds and fever symptoms and externally to treat skin eruptions, wound and bruises. Plant essential oils in general have been recognized as an important natural resource of pesticides and insecticides, larvicides and repellents The repellents are designed as topical preparations or combustible products that are able to protect the user or environment from harmful insects, such as mosquitoes, which transmit diseases through their bite (Isman, M.B., 2016). No reports found on the use of LGE extract for bamboo preservative.

The use of CCA and Boron (combination of Borax-Boric Acid) are normal practice in Malaysia. Boron is normally used with the ratio of 1.5:1.0 with 5-10% concentration. Boron is more favourable than CCA due to its less environmental impact.

In wood, plant extract and metal salts solution are not chemically bond to each other, therefore leached out easily. Plant extract has lower SG than the metal salt, thus move separately in porous wood structure. Study by Sen, S *et al*., (2009), showed that combination of 1% plant extract and 1% mineral salts ($Al_2(SO_4)_3$, $CuSO_4$ and Boron is suffice to increase decay resistance of unleached wood samples. 3% and higher concentration increase the amount of leaching. Sumac leaf extract, velonia extract and pine bark extract were studied. Combination of these plant extract to salt solution of 1% aluminium sulfate, $Al_2(SO_4)_3$ and copper sulfate, $CuSO_4$ showed positive results. The success of a treatment is determine from the penetration levels expressed as absorption and retention in kg m^{-3}. Ovendry mass is taken before and after treatment to determine the weight percent gain (WPG%) of solid content of retention. Spraying or brushing cucurmin and salicylic acid solution on treated samples is a technique to evaluate the absorption level of treatment (Gauss, C *et al*., (2019).

MATERIALS AND METHODS

Preparation of bamboo samples, treatment solution, screening and mold test were discussed in the followings.

PREPARATION OF BAMBOO SAMPLES

Bamboo samples were collected from Bamboo Jungle Adventure Sdn. Bhd. in Sungai Siput, Perak. *Dendrocalamus asper* (buluh betong) of more than 4 years were selected and bamboo poles were cut into three sections – the top (6m above ground), middle (3m above ground) and bottom (less than 3m above ground) section for sampling. For comparisons, skin and without skin bamboo were prepared. After felling, raw bamboo poles were splitted using splitter into strips and undergone the machining process of planing and cross cutting to obtain the dimension of 20 mm (W) X 70mm (L) in accordance to ASTM D 4445 standard. Planing, cross cutting and drying were carried out at the Forest Research Institute Malaysia (FRIM).

PREPARATION OF TREATMENT SOLUTION

Lemongrass extract and camphor crystal were obtained from BFI Sdn. Bhd. Boron (36% w/w boric acid, 54% w/w borax and 10% w/w inert substance) was obtained from Celcure (M) Sdn Bhd. Melamine-urea-formaldehyde (MUF) with low emmission grade used as adhesives was purchased from Aica (M) Sdn Bhd. Four treatment solutions were prepared including 10% camphor extract with 10% MUF (CE); 10% lemongrass extract with 10% MUF (LGE); 10% of borax and boric acid (Borax); and distilled water (DI) as control. Bamboo samples were treated under 600 mmHg vacuum pressure impregnation method for 2 hours at the Wood Mycology Laboratory, FRIM.

SCREENING TEST

Prior to exposure to mold, screening test was carried out to observe the effect of the prepared treatments used toward fungal growth. Three fungi species consisting of two major white-rot fungi decay species, *Lentinus sajor-caju* and *Pycnoporus sanguineus*, and one mold species, *Aspergillus brasiliensis* were selected. Approximately 2 ml of each treatment is pipetted on the surface of the PDA media agar plate and spread evenly using a sterilized u-stick. A fungi plug (approximately 5 mm diameter) from an active selected fungus culture was then taken and placed on the treated agar plate. A total of five replicates were prepared for each treatment and each fungus. Observation was made by measuring the fungal growth for 21 days with 3-day interval.

MOLD TEST

Test was carried out in accordance to ASTM D 4445-10 standard (ASTM 2010) using mold *Aspergillus brasiliensis*. This mold is one of the most common and fastest growing mold on wood and other cellulosic materials such as bamboo. Mold plug (approximately 5 mm diameter) obtained from active culture was inoculated onto PDA malt agar petri dish. Once the mold culture has fully growth on the media agar after one week, four treated bamboo samples (20 x 70 mm) that were already sterilized using propelyne gas for 48 hours, were placed on each petri dish and visually rated for four consecutive weeks. A total of five replicates were prepared for each treatment. Rating from 0 to 5 denotes the percentage of mold covering was recorded.

Table 1: Mold rating in accordance to the In-house Wood Mycology, FRIM method

Rating	Description
0	No visible growth
1	Mold covering up to 10% surfaces providing. Growth is not so intense or coloured as to obscure the sample color over more than 5% of surfaces
2	Mold covering between 10% and 30% of surfaces providing growth is not so intense or coloured as to obscure the sample color on more than 10% of surfaces
3	Mold covering between 30% and 70% of surfaces providing growth is not so intense or coloured as to obscure the sample color on more than 30% of surfaces
4	Mold on greater than 70% of surfaces providing growth is not so intense or coloured as to obscure the sample color over more than 70% of surfaces
5	Mold on 100% of surfaces or with less than 100% coverage and with intense or coloured growth obscuring greater than 70% of the sample color.

RESULTS AND DISCUSSION

Screening test

The biocidal potential of plant extracts was accessed using the screening test via agar-plate. Out of four treatments used (camphor, lemongrass, borax and distilled water) in this study, distilled water (control) showing full growth within 3 weeks of exposure to all the tested fungi (Table 2 and Figure 2). Agar plates treated with borax also failed to inhibit the growth of the mold, *A. brasilliensis* (Figure 2). Mold is a common organism found on humid and damp area, appearing as black or greenish-brown patches. It is easily removed from the wood or bamboo surfaces using bleach diluted with water. However, the mold presence is a main concern as it may cause allergies and other health related problems (Clausen 2000).

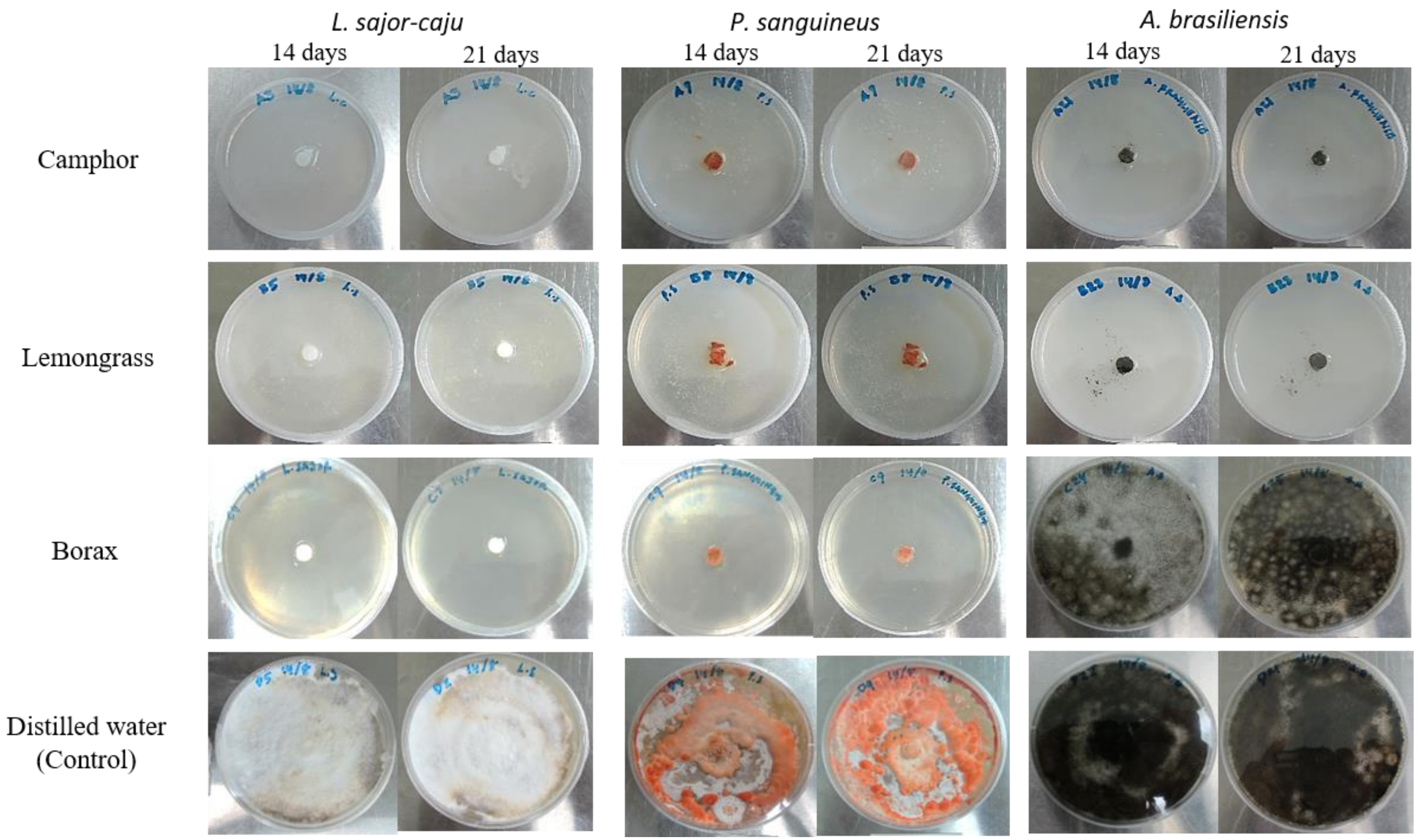
All the agar plates treated with the two plant extracts, camphor and lemongrass was able to inhibit the gowth of all the white rot fungi and mold for up to 21 days after exposure. The screening test showed that camphor and lemongrass are effective to stop fungiand mold from growing while the distilled water really provide a suitable condition for them to grow. For borax treatment, this treatment is not so effective as there is some species of fungi that can still grow such as *Aspergillus brasiliensis.*

Table 2: Average mycelial growth on the white rots (*L. sajor-caju* and *P. sanguineus*) and mold (*A. brasilliensis*) after 21 days of exposure

Fungus & treatment	**Mycelial growth (mm) / Day**							
	3	**5**	**7**	**10**	**12**	**14**	**19**	**21**
Lentinus sajor-caju								
- Camphor	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2
- Lemongrass	4.0	4.6	4.6	4.6	4.6	4.6	4.6	4.6
- Borax	4.2	4.2	4.2	4.2	4.4	4.4	4.4	4.4
- Distilled water	21.8	31.8	40.2	42.0	42.0	42.0	42.0	42.0
Pycnoporus sanguineus								
- Camphor	4.0	4.0	4.2	4.0	4.0	4.0	4.0	4.0
- Lemongrass	4.4	4.6	4.6	4.6	4.6	4.6	4.6	4.6
- Borax	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4
- Distilled water	6.6	12.0	27.0	41.0	41.0	41.0	41.0	41.0
Aspergillus brasiliensis								
- Camphor	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6
- Lemongrass	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4
- Borax	4.0	41.6	41.8	41.8	41.8	41.8	41.8	41.8
- Distilled water	42.2	42.4	42.4	42.4	42.4	42.4	42.4	42.4

*Each value represents the means of 5 replicates.

Figure 1: Mycelial growth of the white rot fungi, *L. sajor-caju*, *P. sanguineus* and mold, *A. brasilliensis* after 14 days and 21 days on different media agr treated with camphor, lemongrass, borax, and distilled water (control)



Mold test

Bamboo *D. asper* treated with camphor and lemongrass were attacked by mold within the first week of the test (Figure 3) with average visual rating within 2.8 to 4.0 (camphor) and 2.8 to 4.5 (lemongrass) (Table 3). Bamboo with skin showed less mold growth compared to those without skin (Figure 4). Borax works best on the bamboo samples with visual rating of only 1.5 to 2.5 at the end of the 4-week test (Figure 2). Control samples i.e. bamboo treated with distilled water showed 100% mold growth after two weeks of test on samples without skin. Other than skin presence, bamboo samples coming from the bottom part of the bamboo showed less severe attacked compared to samples taken from the middle and top parts.

Table 3: Average visual rating on bamboo, *D. asper* (betong) treated with plant extracts (camphor and lemongrass), borax and distilled water after 4 weeks of exposure to mold, *A. brasilliensis*

Position Species / Treatment	Bottom		Mid		Top	
	No skin	**Skin**	**No skin**	**Skin**	**No skin**	**Skin**
Borax						
Week 1	1.0	0.0	2.0	1.0	1.0	0.0
Week 2	3.0	1.0	2.0	1.0	1.0	1.0
Week 3	3.0	1.0	2.0	1.0	2.0	2.0
Week 4	3.0	1.0	2.0	1.0	3.0	2.0
Camphor						
Week 1	4.0	3.3	4.0	3.0	4.0	2.8
Week 2	4.5	3.3	4.5	3.0	4.5	3.0
Week 3	4.5	3.3	4.5	3.0	4.5	3.0
Week 4	5.0	3.3	5.0	3.0	5.0	3.0
Lemongrass						
Week 1	4.8	3.3	4.5	2.8	3.0	3.3
Week 2	5.0	3.3	5.0	3.0	3.5	3.3
Week 3	5.0	3.3	5.0	3.0	3.5	3.3
Week 4	5.0	3.3	5.0	3.0	3.5	3.3
Distilled water						
Week 1	1.8	1.0	3.0	2.0	4.0	3.0
Week 2	4.0	2.0	5.0	2.0	4.0	3.0
Week 3	4.0	2.0	5.0	3.0	5.0	4.0
Week 4	4.3	2.0	5.0	3.0	5.0	4.0

Each value represents the means of 10 replicates
Ratings: 0 (No mold), 1 (<10% mold coverage), 2 (10-30%), 3 (30-70%), 4 (>70%), 5 (100%)

Figure 2: Average visual rating on bamboo, D. asper (betong) treated with plant extracts (camphor and lemongrass), borax and distilled water after 4 weeks of exposure to mold, A. brasilliensis. [Ratings: 0 (No mold), 1 (<10% mold coverage), 2 (10-30%), 3 (30-70%), 4 (>70%), 5 (100%)]

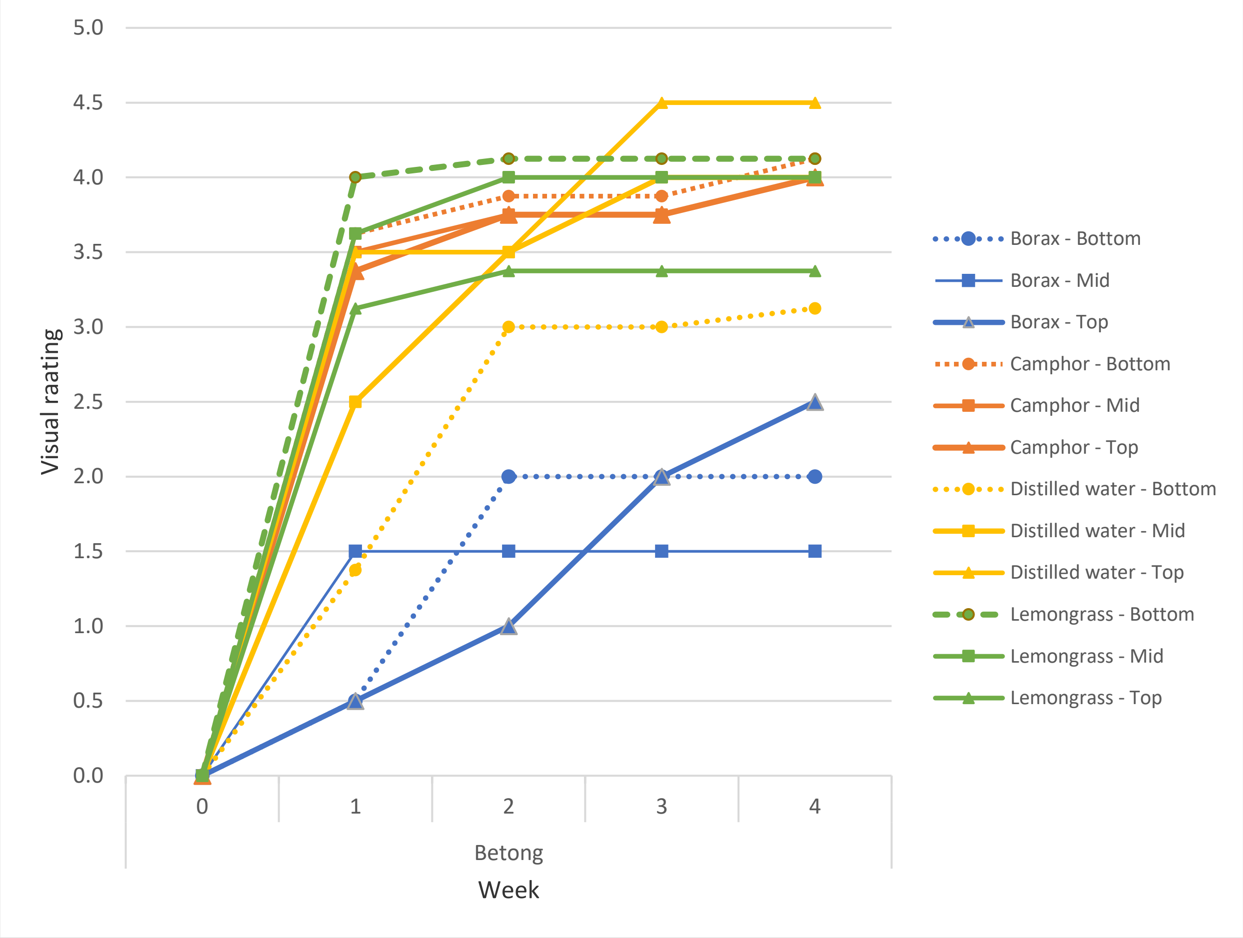
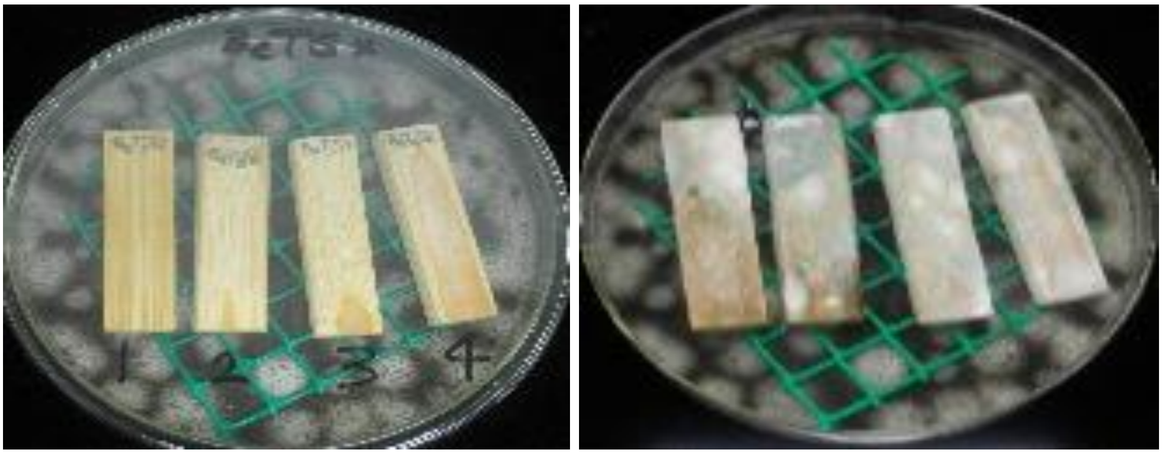


Figure 3: Betong samples with skin (left) and without skin (right) treated with boron showing severe attack by mold on the bamboo samples without skin compared to those with skin



Betong treated with camphor were proned to mold attack at week 1, with all the samples without skin with complete mold growth (rating 5.0) at the end of the 4-week test. This denotes total failure of the camphor treatment, although samples with skin showed some protection (rating 3.0 to 3.3). This result was against the initial screening test which confirmed the durability of camphor towards *A. brasilensis* attack. It was probably due to failure of treatment scheme, in which the treatment solution was not fully inserted into bamboo cells. After 2 hours, vacuum-pressure treatment, it was noticed that samples were floated on the solution.

Bamboo contains 8.3% to 9.23% extractive, 6%-8% starch, 2%-4% fats and 0.8%-6% protein (Katrina K. Knott , *et all*. (2017). Extraction of starch, free sugars and protein should be done from the bamboo vascular bundles before treatment. Samples might not be fully treated because of the existence of non structural contents that hindered the penetration of camphor solution into the bamboo cells. It was also observed the presence of crystallized camphor at the bottom of the treatment flask. This suggested that treatment should be done with supply of heat of at least 60^0C to depolymerize the camphor molecules into smaller molecules.

Similar observation was also made on the samples treated with lemongrass extracts. After the sterilization process using propylene oxide, some solid content of lemongrass solution was observed on the sample surfaces (Figure 4). It was probably due to crystallization of MUF thermoset resin. From this observation, it was suggested that treatment should be done with heat to ensure crystallization of MUF and lemon grass solid happen inside the bamboo cell for full protection against mold.

Figure 4: Betong samples with skin (left) and without skin (right) treated with lemongrass showing the presence of whitish substances due to crystallization of the MUF

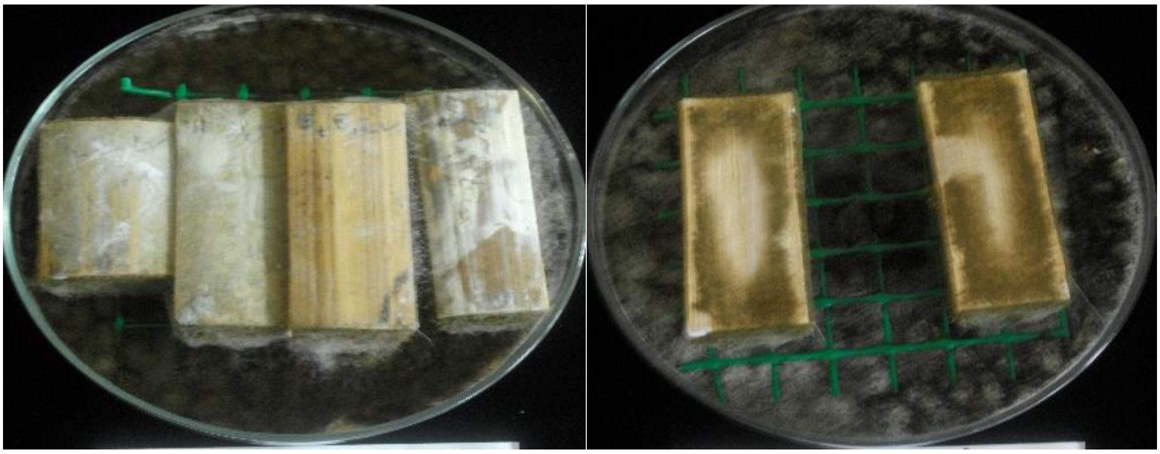


Figure 5: Betong samples without skin (left) and with skin (right) on control samples (distilled water) showing a more severe mold attack compared to samples with skin on week 3 of the test

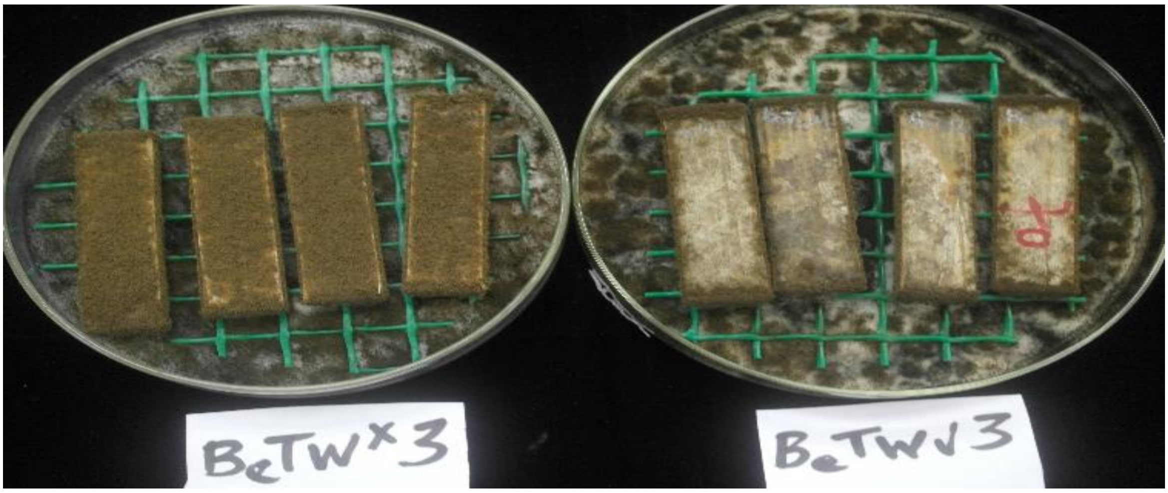
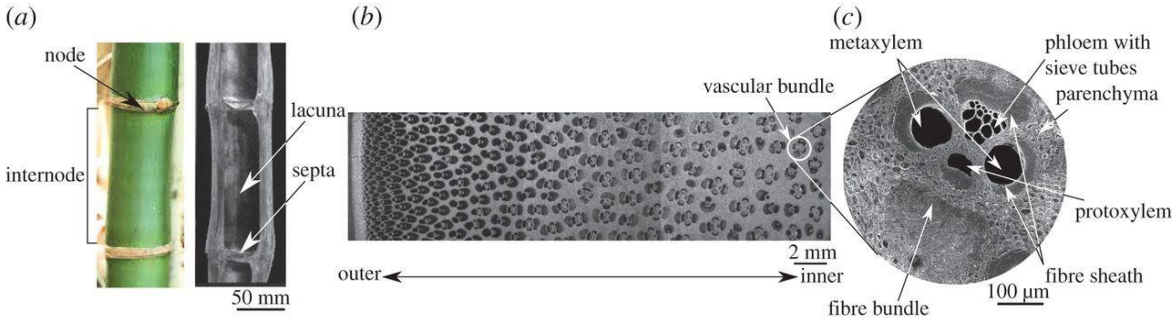


Figure 5 showed result after week 3 for control samples. Mold rating 5.0 was observed which denoted 100% coverage of mold on the bamboo surfaces. Samples without skin on the left showed that mold concentrated on both expose end without skin. The result strengten the hypothesis that bamboo skin had protected the samples from being deteriorated by mold.

At microstructure level as shown in Figure 6, the vascular bundles size of internode become smaller from inner to outer layer as shown in the diagram (Osorio, L *et al*., 2018). Absorption and retention of solution during treatment is higher in inner layer which has bigger vascular bundles, which suggest that inner layer is more protected from mold attack than the outer layer. Removing the outer skin will expose the outer layer to mold attack and exaggerate the situation. This observation explains why bamboo with skin has better protection against mold as compared to bamboo without skin.

Figure 6: Schematic diagram of bamboo microstructure



CONCLUSIONS AND RECOMMENDATIONS

10% Camphor extract, CE + 10% MUF (CEMUF) and 10% Lemon Grass extract, LGE + 10% MUF(LGEMUF) has potential as a safe alternative treatment to boron and CCA. In screening test, CEMUF and LGEMUF are inactive towards *Lentinus sajor-caju*, *Pycnoporus sanguineus*, *Coniophora puteana*, *Gloeophyllum trabeum* and *Aspergillus brasiliensis attack. CEMUF and LGEMUF contain alcohol* and MUF as fixing agent are good combination to avoid mold attack. From observations, there are no differences in mold rating for different bamboo sections. To reduce sampling variables, it is recommended that poles less than 3m above ground is taken to simulate the real practice in the bamboo industry. Above all, bamboo with skin possessed lower mold rating as compared to bamboo without skin, in which further research is needed to re-affirmed this results. It is recommended that bamboo poles need to be processed immediately after felling to avoid attack by biodeteriorating agent because of its high starch, protein and sugar. Drying is immediately needed to keep the bamboo dry. Improper drying experienced in this study due to facilities constraint, had caused the bamboo samples to be attacked by *Minthea rugicollis* or bubuk. Further study need to be done on durability of bamboo towards bubuk attack. Finally, heat is needed during impregnation process. It is best to retain the heat in an enclosed vessel as practiced in the commercial wood preservation.

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