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Resistance of Green Treated *Dendrocalamus asper* (Buluh Betong) against *Aspergillus brasiliensis* Attack

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Abstract
Dendrocalamus asper, D.asper (Buluh Betong) were pressure treated with a plant-based extract from (*Cinnamomum camphor* (l.) presl) and *Cymbopogon citratus* Stapf.). The durability of treated D. asper toward mould (*Aspergillus brasiliensis*) attack was evaluated. Camphor leaf extract has strong antifungal compounds, including D-camphor, eucalyptol, α-terpineol, linalool, and 4-terpineol that suppress mould activity. Fixing the camphor extract with 10%, MUF increased mould resistance as compared to treatment with camphor extract alone. Treatments were selected based on the initial screening test that showed the inhibitory effect of mould. Results showed the potential of plant extracts as green bamboo treatment.

Keywords: Plant extracts, Green Treatment, *Dendrocalamus asper, Aspergillus brasiliensis*

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1.0 Introduction

Bamboo is enigmatic, resilient, and flexible, with thousands of uses and benefits, and has always been an integral part of 1/3 of the world's population (Global Industry Report, 2019-2025). Bamboo is a fantastic plant, and we have yet to grasp and avail of its full potential fully. Scientifically, bamboo produces 35% more oxygen and absorbs 40% more carbon dioxide than trees, thus becoming a carbon sink, controlling soil erosion, restoring degraded land, conserving biodiversity, and contributing to the purification and regulation of the environment. Above all, each bamboo shoot that grows can combat climate change and act as a "green environmental protector". Other than environmental contribution, bamboo products and services can generate national income. The 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 tropical countries, including Malaysia, there is no doubt that it is the most sustainable plant. Income from eco-tourism, furniture, and crafts from bamboo are among the niche products. On the same note, using construction materials from bamboo has led to the need for improvised preservation techniques due to its susceptibility to mould and decay. This notation justifies the need for more research in bamboo treatment and preservation. Wooden and non-woden forest products are susceptible to bio-deteriorating agents, including fungi, powder-post beetles and termites. Bamboo and rattan are no exception. Untreated bamboo's high starch, sugar and protein content make it highly susceptible to biodeterioration. Bamboo has low natural durability since it has only a small content of wax, resin, and tannin (Tomak E.D et al., 2013); therefore, it needs treatment. Most conventional preservative treatments can cause 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, a combination of Borax and Boric acid, is a conventional treatment used to protect wood and non-wood products. CCA was banned in several countries due to its toxicity to the environment and humans because it easily leaches out due to oxidation. Borax-boric acid is now widely used to treat bamboo poles and bamboo products by soaking. Essentially, exploration into new preservation technology is crucial.

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Plant-based extract in nanosized preservatives is an innovative method over the conventional ones. Ultra small-sized nano-preservatives penetrate deeper into bamboo poles and, therefore, less leaching to the ground. Nanosized biocide encapsulation is an emerging technology nowadays, and research is ongoing. (Tumirah K., et al, 2016).

This particular study explored the potential of green treatment as bamboo preservatives against fungi attacks. The objective of the study is to determine the durability of green-treated betong bamboo against *Aspergillus brasiliensis* attack. These include extracts of camphor leaves, lemongrass, and another plant-based extract from local timber species in Malaysia. Conventional bamboo preservation using toxic materials is no longer suitable, and alternative non-toxic materials are crucial. This study examines the potential of 2 types of plant extract, namely lemon grass extract and camphor extract, as non-toxic preservation solutions to preserve bamboo. Both extracts can be found in Malaysia. A comparison was also made to include boron, a commercial use. No policy issue is involved in this study.

2.0 Literature Review

Bamboo and bamboo products are susceptible to bio-deteriorating agents, including fungi, powder-post beetles, and termites. Untreated bamboo's high starch, sugar, and protein content make it highly susceptible to biodeterioration. A mean value of selected bamboo starch was reported at 8% (Katrina K.K, et al.,2017). Li, X (2004) reported starch content of 2%-6%, deoxidized saccharide of 2%, fat of 2%-4%, and protein of 0.8% -6%. Mould is classified as a saprophytic organism that utilizes sugar and carbohydrates in the lumen cell of wood but does not cause a decrease in mechanical properties (Tumirah K. et al., 2020). Other than starch, sugar, and protein, moisture level also affects the deterioration rate by decaying fungi in a building made of wood and bamboo structures. Relative humidity ranging between 70% and 90% is required for fungal growth on building materials (Hoang, C.P, et al.,2010). Bamboo has low natural durability since it has only a small content of wax, resin, and tannin (Tomak, E.D et al., 2013). Thus, treatment is needed. Most conventional preservative treatments can cause environmental pollution, and a few of them may be harmful to human health. Copper chromium arsenic, CCA, is a common treatment for timber but also can be used to preserve bamboo. CCA and a 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 humans because it easily leaches out due to oxidation. Borax-boric acid is now widely used to treat bamboo poles and bamboo products by soaking. Essentially, an exploration into new non-toxic preservation technology is crucial. Extractives from durable wood species, including tannins, flavonoids, lignans, stilbenes, terpenes, and terpenoids, can be used to treat non-durable wood species. 9% and 12% concentrations of Acacia and quebracho bark extract (*Schinopsis spp*.), respectively, had shown decay resistance towards treated scots pine and poplar wood (Tascioglu, C. et al., 2013). Significant progress in plant-based research includes the extract of elm (*Zelkova carpinifolia*), oak (*Quercus castanifolia*), and mulberry (*Morus alba*). The essential oil of Thuya (*Tetraclinis articulate*) showed excellent decay resistance at 5% concentration. Neem (*Azadirachta indica*) leaf 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) increased the decay resistance as compared to CE alone. Exposure to white rot and brown rot of *P. Chrysosporium* and *G. trabeum* showed a decrease in mass loss % of 5-6% when treated with CEMUF. Bamboo treated with CE showed a substantial mass loss of 16% for both white and brown rotters. In the thermogravimetric analysis, the 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 the Kapur tree or *Dryobalanops spp*, which can be found in Malaysia. Previous studies on phytochemicals and compounds 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 is not readily obtainable, it limits the utilization of tree bark as a wood preservative (Adedeji, G.A. et al., 2013).

Aqueous extracts of lemon grass (LGE) and Cymbopogon citratus were used in this study. Lemon grass 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, wounds, 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 can protect the user or environment from harmful insects, such as mosquitoes, which transmit diseases through their bite (Isman, M.B., 2016). To date, no reports have been found on using LGE extract as a bamboo preservative.

The use of CCA and boron (a combination of borax and boric acid) is a regular practice in Malaysia. Boron usually is used with a ratio of 1.5:1.0 with 5-10% concentration. Boron is more favourable than CCA due to its less environmental impact.

Plant extract and metal salts solution are not chemically bonded to each other, therefore leaching out easily. The plant extract has lower specific gravity than the metal salt, thus moving separately in the porous wood structure. A study by Sen S. et al. (2009) showed that a combination of 1% plant extract and 1% mineral salts ($Al_2(SO_4)^3$, CuSO4, and Boron is sufficient to increase the decay resistance of unleached wood samples. 3% and higher concentrations increase the amount of leaching. Sumac leaf extract, velonia extract, and pine bark extract were studied. A combination of these plant extracts with a 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 determined from the penetration levels expressed as absorption and retention in kg m-3. Oven-dried mass is taken before and after treatment to determine the weight percent gain (WPG%) of the solid content of retention. Spraying or brushing curcumin and salicylic acid solution on treated samples is a technique to evaluate the absorption level of treatment (Gauss C. *et al*., (2019).

3.0 Methodology

3.1 Preparation of Bamboo Samples

Bamboo samples were collected from Bamboo Jungle Adventure Sdn. Bhd. in Sungai Siput, Perak. D. asper of more than 4 years were selected, and bamboo poles were cut into three sections, including the top (6m above ground), middle (3m above ground), and bottom (less than 3m above ground) section for sampling. For comparisons, skin and un-skin bamboo were prepared. After felling, raw bamboo poles were split using a splitter into strips and underwent the machining process of planning and cross-cutting to obtain the dimension of 20 mm (W) X 70mm (L), following the ASTM D 4445 standard. Planning, cross-cutting, and drying were carried out at the Forest Research Institute Malaysia (FRIM).

3.2 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 emission grade was used as an adhesive and 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 the 600 mmHg vacuum pressure impregnation method for 2 hours at the Wood Mycology Laboratory, Forest Research Institute Malaysia (FRIM).

3.3 Screening Test

Before exposure to mould, a screening test was carried out to observe the effect of the prepared treatments used on 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 (A. 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. Fungi plug (approximately 5 mm diameter) from an actively 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. The observation was made by measuring the fungal growth for 21 days with a 3-day interval.

3.4 Mold Test

The test was carried out in accordance with ASTM D 4445-10 standard (ASTM 2010) using mold species of *A.brasiliensis*. This mould is one of the most common and fastest-growing moulds on wood and other cellulosic materials, such as bamboo. A Mold plug (approximately 5 mm diameter) obtained from active culture was inoculated onto a PDA malt agar petri dish. Once the mould culture had fully grown on the media agar after one week, four treated bamboo samples (20 x 70 mm) that had already been sterilized using propylene 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 mould covering recorded.

Table 1: Mold rating

Rating	Description
0	No visible growth
1	Mould covers up to 10% of surfaces provided. Growth is not so intense or coloured as to obscure the sample color over more than 5% of surfaces
2	Mould 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	Mould 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	Mould 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	Mould on 100% of surfaces or with less than 100% coverage and with intense or coloured growth obscuring greater than 70% of the sample colour.

(Source: In-house Wood Mycology, FRIM method)

4.0 Findings

4.1 Screening test

The biocidal potential of plant extracts was accessed using the screening test via an agar plate. Out of four treatments used (camphor, lemongrass, borax, and distilled water) in this study, distilled water (control) showed full growth within three 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 *A. brasilliensis* (Figure 2). Mold is a common organism found in humid and damp areas, appearing as black or greenish-brown patches. It is easily removed from wood or bamboo surfaces using bleach diluted with water. However, mould presence is a main concern as it may cause allergies and other health-related problems (Clausen, C.A., 2000).

All the agar plates treated with the two plant extracts, camphor and lemongrass, were able to inhibit the growth of all the white rot fungi and mould for up to 21 days after exposure, as shown in Table 1. The screening test showed that camphor and lemongrass are effective in stopping fungi and mould from growing, while the distilled water provides a suitable condition for them to grow. For borax treatment, this treatment is not as effective as some species of fungi that can still grow, such as *A. brasiliensis*.

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

Fungus & treatment	Mycelial growth (mm) / Day						
	3	5	7	10	12	14	19
Lentinus sajor-caju							
- Camphor	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
- Borax	4.2	4.2	4.2	4.2	4.4	4.4	4.4
- Distilled water	21.8	31.8	40.2	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
- Lemongrass	4.4	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
- Distilled water	6.6	12.0	27.0	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
- Lemongrass	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
- Distilled water	42.2	42.4	42.4	42.4	42.4	42.4	42.4

*Each value represents the means of 5 replicates.

4.2 Mold test

Bamboo treated with camphor and lemongrass was attacked by mould within the first week of the test (Table 3), with average visual ratings within 2.8 to 4.0 (camphor) and 2.8 to 4.5 (lemon grass). Bamboo with skin showed less mould growth compared to those without skin. Borax works best on the bamboo samples with a visual rating of only 1.5 to 2.5 at the end of the 4-week test. Control samples, i.e. bamboo treated with distilled water, showed 100% mould growth after two weeks of testing on samples without skin. Other than skin presence, bamboo samples from the bottom part of the bamboo showed less severe attacks 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 four weeks of exposure to mould, *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 mould), 1 (<10% mould coverage), 2 (10-30%), 3 (30-70%), 4 (>70%), 5 (100%)

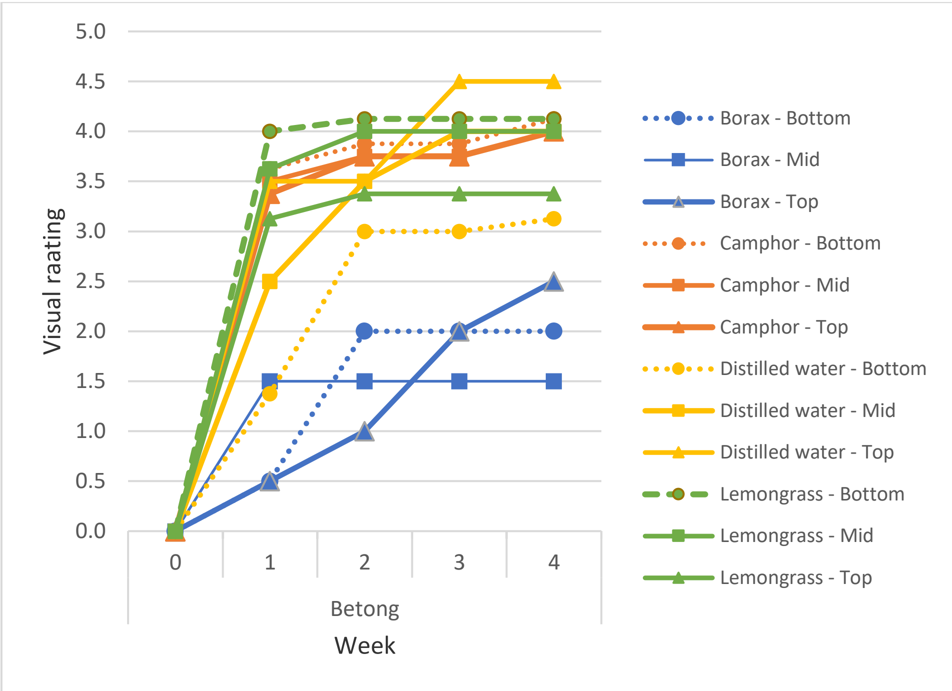

Fig. 1: Average visual rating on *D. asper*, treated with plant extracts (camphor and lemon grass), borax and distilled water after four weeks of exposure to mould, *A. brasilliensis*. [Ratings: 0 (No mould), 1 (<10% mould coverage), 2 (10-30%), 3 (30-70%), 4 (>70%), 5 (100%)]

5.0 Discussion

Fig. 2: Schematic diagram of bamboo microstructure
(Source: Osorio, L. et al., 2018)

Betong treated with camphor was prone to mould attack at week 1, with all samples without skin showing complete mould 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 contradicted the initial screening test, which confirmed the durability of camphor towards *A. brasiliensis* attack. It was probably due to the failure of the treatment scheme, in which the treatment solution was not fully inserted into bamboo cells due to high molecular volume.

At the microstructure level, as shown in Figure 2, the vascular bundle's internode size becomes smaller from the inner to the outer layer, as shown in the diagram (Osorio L. et al., 2018). Absorption and retention of solution during treatment are higher in the inner layer, which has bigger vascular bundles, which suggests that the inner layer has more protection against mould attack than the outer layer. Removing the outer skin will expose the outer layer to mould attack and exaggerate the attack. This observation explains why bamboo with skin has better protection against mold as compared to bamboo without skin.

Bamboo contains 8.3% to 9.23% extractive, 6%-8% starch, 2%-4% fats, and 0.8%-6% protein (Katrina K. Knott et al. (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 that there is a presence of crystallized camphor at the bottom of the treatment flask. This suggested that treatment should be done with the supply of heat of at least 60^0C to depolymerize the larger camphor molecules into smaller molecules.

A similar observation was also made on the samples treated with lemon grass extracts. After the sterilization process using propylene oxide, some solid content of lemon grass solution was observed on the bamboo surfaces. It was due to the crystallization of MUF thermoset resin. From this observation, it was suggested that treatment must be done with heat to ensure a smaller molecular weight of treatment solution was inserted in the lumen and crevices of *D.asper*, thus crystallizing in the inner part of the bamboo to provide higher protection against mould.

The success of camphor and lemon grass extract in this study has shown the potential of using extracts from other types of plants. Factors that impact the antifungal activities of plant extract are molecular weight. Lower molecular weight antimicrobial agents, which are aromatic and non-aromatic compounds, are produced by plants and need further studies. These compounds include phenols, terpenoids, alkaloids, lectins, and polypeptides, which contain antifungal properties that provide protection to bamboo products.

6.0 Conclusion & Recommendations

10% Camphor extract, CE + 10% MUF (CEMUF), and 10% Lemon Grass extract, LGE + 10% MUF(LGEMUF) have the potential as a safe alternative treatment to boron and CCA. CEMUF, LGEMUF, and MUF are good combinations to avoid mould attacks. From observations, there are no differences in mould rating for different bamboo sections. To reduce sampling variables, it is recommended that poles less than 3m above ground are taken to simulate the real practice in the bamboo industry. Above all, bamboo with skin possessed a lower mold rating as compared to bamboo without skin, in which further research is needed to confirm current results. It is recommended that bamboo poles need to be processed immediately after felling to avoid attack by the bio-deteriorating agent because of its high starch, protein, and sugar. Drying is immediately needed. Improper drying experienced in this study due to facilities constraints had caused the bamboo samples to be attacked by *Dinoderus minitus* and *Minthea rugicollis*, or beetle both locally known as bubuk. Further study needs to be done on the durability of bamboo towards these species due to the pronounced attack. Finally, heat is needed during the impregnation process. It is best to retain the heat in an enclosed vessel, as practiced in the commercial wood preservation industry. A new conceptual framework considering toxicity, metabolism and maintenance energy is proposed for research on microbial degradation by Lin Gao and Ji-Dong Gu. (2021). This framework corresponds to microbial physiology, biochemistry, and toxicology to establish a foundation and a common ground for further scientific research advancement on biodegradation. The challenges faced in degradation research are about the understanding and also implementation of the most effective scientific methods to test the research hypothesis to obtain the most significant and convincing results. Hopefully, this framework can be applied to bamboo biodeterioration research in the future. Information on the molecular weight, toxicity, screening test scheme, and research framework involving the bio-agent and treatment are limited. Therefore, thorough study needs to be carried out in the future.

The environmentally friendly wood preservative has recently emerged as a common acceptable alternative for the wood and bamboo preservation industry. This becomes more important since the demand for environmentally friendly wood preservatives has been shown to increase by environmental organizations, consumers, and authorities. Furthermore, the durability and stiffness properties of green-treated bamboo exposed to other types of bio-agents, including termites, beetles and marine borers, are crucial. Durability and stiffness of green-treated bamboo will prolong the utilization of engineered bamboo products in construction. Longer utilization of engineered bamboo products means the product acts as carbon sequestration, contributing to net zero carbon in the environment. Green treated bamboo products are also recyclable and contribute to the circular economy in achieving sustainable development goals or SDG's.

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Paper Contribution to Related Field of Study

The results of this research provide preliminary information on the potential of using plant extract as a green treatment to protect bamboo from mold attack.

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