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Effectiveness of Pyroligneous Acids from Vapour Released in Charcoal Industry Against Biodegradable Agent under Laboratory Condition

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Abstract: Lignocellulosic biomass natural and abundantly available resource has been steadily gaining attention from relevant industries as feedstock for the production of chemicals, fuels and biocompatible materials due to increased concern in economic and environmental issues. The potential of being converted into usable chemicals which pyroligneous acids is one of the chemicals from the distillation of smoke generated during charcoal making. It was found to be potential use as bio-preservative due to the complexity of its chemical compounds. In Northern Malaysia, there are 336 charcoal making kilns, with a total production of around 3, 500 tonnes of charcoal and sales of about RM3 million (USD808, 300) per month. Thus, chemicals recovery from the vapours released during charcoal making could lead to a flourishing industry. This study focuses on development of pyroligneous acid as bio-preservative against wood biodegradable agents. Pyroligneous acids derived from *Rhizophora* and *Bambusa* at 300-400°C. The chemical compounds were then analysed using Fourier Transform Infrared (FT-IR). For the efficacy of pyroligneous acid test, rubberwood test block was immersed in pyroligneous acid for 24 h at room temperature. Treated rubberwood test block were later tested against mold (*Penicillium* sp.), white rot fungus (*Pycnoporous sanguineus*) and subterranean termites (*Coptotermes curvignathus*) according to ASTM standard methods. Results showed that pyroligneous acids treated test blocks were effective against mold for the surface coverage area and white rot decay in the weight loss but not effective against termiticidal activity. It was concluded that pyroligneous acid could be used as fungicide but not as insecticide.

Key word: Pyroligneous acid, *Rhizophora,* mold fungi, decay fungi, termites

INTRODUCTION

Lignocellulosic biomass is a naturally, abundant and renewable resource that is not only essential to the functioning of industrial societies but also critical to the development of a sustainable global economy. With increasing economic and environmental concerns on the use of petrochemicals, lignocellulosic biomass would be relied upon as feedstock for the production of chemicals, fuels and biocompatible materials (Hu, 2008).

Lignocellulosic biomass which constitutes a huge and renewable resource can be converted to usable chemical and fuel feedstocks. One of chemicals that can be derived is pyroligneous acid, a crude condensate produced from the distillation of smoke generated in the process of making charcoal (Loo *et al*., 2008).

With the development of the carbonizing industry, in addition to wood, other biomaterials, such as bamboo, corn cobs, pine cone, fruit stone (such as apricot kernel), fruit shell (such as coconut and walnut shells) and weed could be used as raw materials to be pyrolyzed into pyroligneous acids. These alternatives being not only similar to wood but also contain various types of organic substances, from which many compounds show biological activities, provides a great indication on the potential development of such products (Lee *et al*., 2010).

Chemically, pyroligneous acid comprises of water (10-20%), a mixture of carboxylic acids with acetic acid being the most prevalent at approximately 10%, several aldehydes and alcohols and pyrolytic lignin (Lee *et al*., 2010; Ninomiya *et al*., 2004).

Currently, pyroligneous acid is mainly used as fertilizer additives, deodorization agents and a natural aid

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for amongst others include detoxification, mild pain relief and to sterilize and promote healing of minor wounds. Meanwhile, unused pyroligneous acid which are just disposed could lead to pollution of local environment.

Pyroligneous acids have a potential source of valuable chemicals that can be developed as a new wood preservative against biological degradable (Lee *et al*., 2010). Mohan *et al*. (2008) stated that the pyroligneous acid can be used as preservatives for timber in wet condition while Jung (2007) stated it could inhibit the growth of pathogenic fungus, *Alternaria mali* which is known to be the agent of Alternaria blotch of apple plants.

Pyroligneous acid could also be used as a fungicide for wood preservative with Bruce and Highley (1991) stating that it is useful for controlling the wood decay Basidiomycetes by *Trichoderma* spp. Lee *et al*. (2010); Sameshima *et al*. (2002); Yatagai *et al*. (2002) correlated the termiticidal activity with phenolic and acetic acid contents in the pyroligneous acid produced from charcoal production. Nakai *et al*. (2007) found that pyroligneous acid from pyrolysis of sugi and acacia wood increased the resistance of wood against brown-rot fungi. Femi-Ola *et al*. (2008) stated that many researchers are now focused towards the alternative non toxic and biological methods of controlling termites. Therefore, the production of these chemical products from pyroligneous acid could become economically viable and profitable.

In Northern Malaysia, there are 336 charcoal making kilns, with a total production of around 3, 500 tonnes of charcoal and sales of about RM3 million (USD808, 300) a month, making recovery of chemicals from the vapours released during charcoal manufacturing a flourishing industry.

This study focuses on the efficacy of pyroligneous acid produced from vapour released when bamboo and mangrove charcoal production against biological agent under laboratory condition using rubberwood as test block. Fourier Transform Infrared (FT-IR) spectroscopy was utilised to analyse the chemical compounds of the pyroligneous acids.

MATERIALS AND METHODS

This project, implemented from 2008 to 2010 used two types of pyroligneous acids *Rhizophora* sp. and *Bambusa* sp. Both types of pyroligneous acids collected from the charcoal industry in Northern Malaysia, has clear reddish brown colour, similar to the pleasing hue of black tea, beer or wine. The samples were stored in the dark at below 20°C before use.

Pyroligneous acids treated wood: Five pieces of rubberwood (*Hevea brasiliensis*) blocks (20 mm in width, 70 mm in length and 7 mm thick) were prepared for molds test, while ten pieces of rubberwood (25 mm in width, 25 mm in length and 9 mm in thick) were prepared for decay fungi and termites tests. All the test samples were immersed in the pyroligneous acid at room temperature for 24 h in water bath, after which the rubberwood test blocks were taken out, the excess solution wiped off and allowed to dry to constant weight under room temperature. The pyroligneous acid retention was calculated based on the gain in weight of the untreated wood.

Fourier transform infrared (FT-IR) spectroscopy test: In this study, the chemical components of different types of pyroligneous acids were analyzed using Fourier transform infrared (FT-IR) spectroscopy. FT-IR spectroscopy has been used for determination of molecular structures, identification of compounds in biological samples and investigation of complex polymer. FT-IR spectra were recorded in the wavenumber range from 600-4000 cm^{-1} with PerkinElmer Spectrum. A resolution of 4 cm^{-1} and 4 scans/sample was used.

Biological durability evaluation test

Mold tests: Mold resistance was tested according to standard ASTM D 4445: Standard Test Method for Fungicides for Controlling Sapstain and Mold on Unseasoned lumber (Laboratory Method) (ASTM, 2003). The rubberwood test block was placed on a U-shaped glass rod (3 mm in diameter) together with a control test block (no treatment). The glass rod was then placed on top of wet papers inside the sterilized Petri dish. Mold (*Penicillium* sp.) was applied onto the wet paper and the petri dish was sealed with cellophane tape to prevent contamination through contact with surrounding atmosphere then incubated for four weeks. After four weeks, the number of days for the mold to start to grow on the test block along with coverage area by mold on test block were evaluated.

Decay fungi tests: Decay resistance was tested according to ASTM method (1978) D 2017-71 using cultures of common white rot fungus *Pycnoporous sanguineus* (ASTM, 1978). The rubberwood test blocks were conditioned to constant weight and steam-sterilized at 100°C, weighed and exposed to *Pycnoporous sanguineus*. After eight weeks of incubation at 27°C and 70% RH, the surface fungus mycelium was removed, the specimens were dried at 60°C and weight loss was determined as percentage of total rubberwood test block mass.

Termite tests: Termite resistance on the rubberwood test blocks were tested according to standard ASTM D 3345-74 (1980) using the subterranean termites, *Coptotermes curvignathus* (ASTM, 1980). Test blocks were placed in the center of a cylindrical plastic container (50 mm in diameter and 38 mm in height) with 1 g of subterranean termites. The test blocks were set upon 1 g of washed sand and covered with a wet 42.5 mm Whatman filter paper circle as a food source and to maintain humidity. The containers were maintained at 25°C and 80% RH for four weeks. Termite activity in each bottle was observed and rated after week 1 and week 4 of the testing period. At the end of the testing period, the percentage of weight loss due to termite attack and termites mortality rate was calculated.

RESULTS

Retention of pyroligneous acid in treated rubberwood: Table 1 show the weight gain of rubberwood test blocks immersed in pyroligneous acid for 24 h at room temperature. The pyroligneous acid content for mangrove and bamboo pyroligneous acid treated wood was 2.01 and 1.77%, respectively.

FT-IR analysis: Table 2 show the possible chemical compounds based on the functional group analysed using FT-IR spectroscopy. The broad band of the hydroxyl stretching group with wave number of 3600-3200 cm^{-1} from FT-IR spectrum of both mangrove and bamboo pyroligneous acids indicate that the presence of water impurities and other polymeric O-H in the pyroligneous acids (Lee *et al*., 2010; Islam *et al*., 2003). The spectrum also showed that the band of C-H stretching with wave number of 3000-2800 cm^{-1} indicates the presence of alkanes groups in the pyroligneous (Islam *et al*., 2003; Tsai *et al*., 2007).

The C = C stretching vibrations from the FT-IR spectrum indicate presence of alkenes and aromatics compounds in the pyroligneous acids (Beis *et al*., 2002; Acikgoz *et al*., 2004). The peaks between 1600 and 1500 cm^{-1} represent the -NO stretching vibration, indicating presence of nitrogenous compounds. The overlapping peaks between 1300 and 950 cm^{-1} were due to the presence of primary, secondary and tertiary alcohols, phenols, ethers and esters showing the C-O stretching and O-H deformation vibration (Qian *et al*., 2007). For wave number below 1500 cm^{-1}, all the bands were very complex and had their origins in a variety of vibrational modes. The pyroligneous acids were acidic as the present of oxygenated functional groups of O-H while C-O and aromatic compounds shows in the FT-IR results had the potential as a chemical feedstock.

Biological durability

Mold tests: The number of days that mold started to grow on test block after exposure to the mold *Penicillium* sp.

Table 1: Pyroligneous acid content in different types of pyroligneous acid treated wood

Type of pyroligneous acid treated wood	Gain in weight (%)
Untreated	0.00
Mangrove pyroligneous acid	2.01
Bamboo pyroligneous acid	1.77

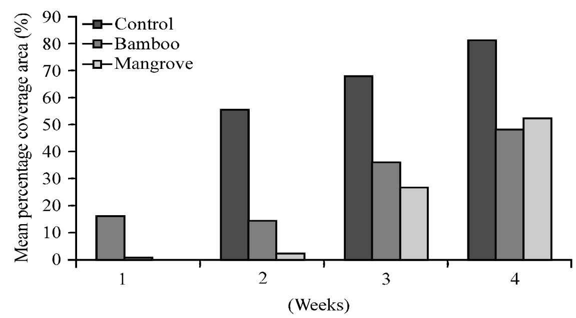

Fig. 1: Mean percentage of mold growth on different types of treatment

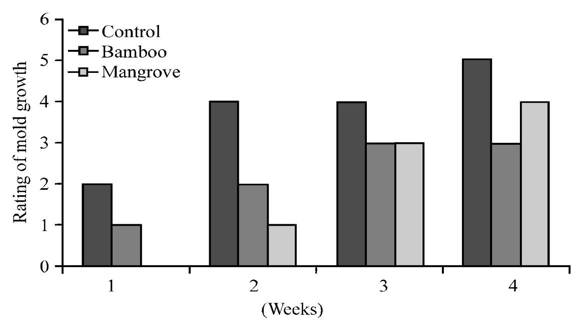

Fig. 2: Rating of mold growth on different types of treatment

are presented in Table 3. All the rubberwood test blocks treated with pyroligneous acid discouraged the growth of mold. Results of the dip treatment showed that both bamboo and mangrove pyroligneous acid completely inhibit all mold tests for 5 to 8 days and for 12 to 18 days, respectively while control specimens showed 80% mold coverage at the initial 1 to 2 day rating.

Figure 1 shows the percentage of coverage area by mold on the surface of rubberwood test blocks after exposure to mold for four weeks under laboratory condition. At moisture contents greater than 20%, mold establishment can occur on unseasoned wood in 24-48 h if temperatures permit and rapid drying of the wood does not occur. Both bamboo and mangrove pyroligneous acid showed approximately 50% surface coverage with mold growth after four weeks exposure to the testing.

According to the American Society for Testing and Material (ASTM D-4445-03), the growth of the fungi were estimated visually using a scale of 0 to 5, with 5 being the maximum intensity covered by fungi and molds. Figure 2

Table 2: FT-IR analysis for functional group of different types of pyroligneous acid treated wood

Types of treatment	Wave numbers, cm^{-1}	Functional group	Class of compound
Mangrove pyroligneous acids	3600-3200	O-H	Polymeric, water impurities
	3500-3200	N-H	Amines compound
	3000-2800	C-H	Alkanes
	1300-950	C-O, O-H	Alcohols, Phenol, Esters, Ethers
Bamboo pyroligneous acids	3600-3200	O-H	Polymeric, water impurities
	3000-2800	C-H	Alkanes
	1600-1500	-NO	Nitrogenous compound
	1600-1400	C=C	Aromatic compound
	1300-950	C-O, O-H	Alcohols, Phenol, Esters, Ethers

Table 3: Days of molds start to grow on different type of pyroligneous acid treated wood

Type of pyroligneous acid treated wood	Days
Untreated	1-2
Bamboo pyroligneous acid	5-8
Mangrove pyroligneous acid	12-18

Table 4: Mean weight loss and resistance class of untreated, bamboo pyroligneous acid and mangrove pyroligneous acid dip-treated test blocks after exposure to *P. sanguineus* for 8 weeks

Type of treatment	Mean weight loss (%)	Resistance class
Untreated	24.23	Moderately resistant
Bamboo pyroligneous acid	8.56	Highly resistant
Mangrove pyroligneous acid	13.71	Resistant

Table 5: Average termite attack and mortality rating for rubberwood test blocks treated with different types of pyroligneous acids

	Termite bioassay	
Treatment	Attack rating[a]	Mortality rating[b] (%)
Untreated	7	52.68
Bamboo pyroligneous acid	7	59.98
Mangrove pyroligneous acid	7	65.65

[a]Termite attack rating scale: 0, failure; 4, heavy; 7, moderate attack; 9, light attack and 10, sound. [b]Mortality rating: 0-33%, slight; 34-66%, moderate; 67-99%, heavy and 100%, complete

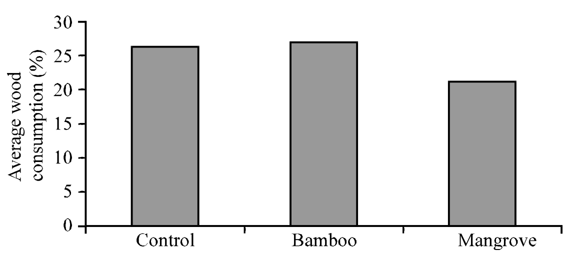

Fig. 3: Consumption by subterranean termites, *Coptotermes curvignathus* of wood treated with different types of pyroligneous acids

shows that rubberwood without treatment scored a 5 whereas, rubberwood dip treated with mangrove and bamboo pyroligneous acid scored 4 and 3, respectively indicating low mold inhibition.

Decay fungi tests: After eight weeks of being exposure to fungus *Pycnoporous sanguineus*, all untreated blocks and dip-treated blocks, be it bamboo or mangrove pyroligneous acid showed fungus colonization visibly on the surface of the board. The bamboo pyroligneous acid dip-treated blocks showed little fungus colonization visually on the board surface. The mean weight loss values and resistance class of untreated, bamboo pyroligneous acid and mangrove pyroligneous acid dip-treated blocks are shown in Table 4.

The results in Table 4 indicate that there are significant difference between the mean weight loss value of the three types of test blocks with the mean weight loss value for untreated blocks being the highest at 24.23% while the mean weight loss for the mangrove pyroligneous acid and bamboo pyroligneous acid dip-treated test blocks were 13.71 and 8.56%, respectively. The bamboo pyroligneous acid dip-treated test blocks were clearly the most fungus resistant due to the blocks' low mean weight loss value. The resistant classes were based on weight loss of test blocks according to Standard ASTM D-2017-71 (ASTM, 1978). Untreated test blocks showed moderate resistance to fungus, while test blocks treated with bamboo pyroligneous acid showed the highest resistance against the white rot fungi. mangrove pyroligneous acid treated blocks on the other hand showed only resistance to white rot decay.

It was reported that the antimicrobial activity of pyroligneous acid is attributed to the presence of compounds like phenolic compounds, carbonyls and organic acids (Lee *et al*., 2010; Loo *et al*., 2008; Vitt *et al*., 2001; Suzuki *et al*., 1997). They suggested that the phenolic compounds of 4-ethyl-2-methoxyphenol and 4-propyl-2-methoxyphenol contained inside pyroligneous acid might have some preservation effects asost phenolic compounds have disinfectant properties which may explain why lignin-rich fractions are more effective preservatives than the whole bio-oil.

The mean weight loss values for untreated and treated test blocks showed that the pyroligneous acids extracted from bamboo and mangrove trees have successfully reduced the degradation of the rubberwood caused by the white rot fungus.

Termite tests: Wood consumption as mean percentage weight loss, of treated and untreated rubberwood test blocks caused by subterranean termites, *Coptotermes curvignathus* is shown in Fig. 3 while mortality and visual attack ratings are provided in Table 5.

All the treated and untreated test blocks were attacked by termite as reflected by the weight loss values. The summary of ANOVA showed that there was no significant difference in mean weight loss value between treatments at $p>0.66$. From the result shown, all the treated and untreated test blocks were rated as termite-susceptible due to the high mean weight loss value of approximately 25%. Pyroligneous acids pyrolised from bamboo and mangrove trees showed no resistance against subterranean termite attack. Kartal *et al.* (2004) found that pyrolysis liquids from sugi and acacia wood showed increased resistance against brown-rot fungi however, they showed no resistance against subterranean termite attack.

CONCLUSIONS

Pyroligneous acids pyrolised from bamboo and mangrove trees completely inhibit both mold and decay fungi test. The bamboo and mangrove pyroligneous acids acts as a good fungicides, however, it is not recommended for insecticide it exhibit termiticidal activity.

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