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Study of Natural Extracts as Replacers of Borax in the Treatment of Rubber Wood

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Abstract – This research focused on the feasibility of using natural extracts as replacers of borax in the treatment of rubberwood. The main consideration for such replacers is that they must not show any adverse effect on the human body and the environment, while indicating sufficient prevention of attacks from fungi and termites. Neem extract, wood vinegar, and vinegar were tested for minimal inhibition concentration of fungal growth using the Broth dilution method. After that, these solutions were compressed into the rubberwood at 80 psi for 1 hour, dried at 80˚C for 5 days to reduce the moisture content to 8.59±0.21% d.b. (dry basis), and test for inhibition of fungal growth. After experimentation, results showed that neem extract, wood vinegar and vinegar solution at 25% concentration by volume are capable of inhibiting the growth of fungi. Moreover, after 1 month of mold test on rubberwood, the highest inhibition of fungal growth was around 80%, which is at a satisfactory level. Furthermore, after economic consideration, it is feasible that operator and entrepreneur use these environmental friendly, natural substances as replacers of Borax.

Keyword*: *Drying, Rubberwood, Treatment of Rubberwood, Neem Extract, Wood Vinegar

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1. Introduction

Thailand is one of the leading producer of rubberwood in the world, but after wide spread planting and dwindling of forests, the government closed forest concessions in 1989 [1]. Nowadays, the industry remains popular and produces many commercial products, such as furniture, home stead, and children toys [2]. However, the processed products from rubberwood face problems of destruction from fungi and termites, thus, shortening their lifetime [3]. Consequently, nearly all operators and entrepreneurs in Thailand use Borax as a treatment chemical for the preservation of rubberwood.

Borax is a powerful antioxidant that penetrates into the wood to protect the wood from fungi and insect attacks. A typical enamel process includes spray painting and compression. However, the most popular method is compression of chemicals in solution form into the wood product using high pressure. This allows for the spread of chemicals into timber within a short time, while offering the best protection [4]. The current wood treatment process includes placing 3.5-8 m^3 of rubberwood into a high pressure vessel, using vacuum pressure to remove the air inside, and compress the rubberwood with chemical solutions at 150-200 psi for 1.5-2 h. There are many chemicals that canbe used, but some of the most common ones include Timbor® (50-60 Baht/kg), Boric and Borax (29-40 Baht/kg), and Cellbor® (36-42 Baht/kg) [5].

However, in 2009, EU placed Borax in a watch list of chemicals posting potential dangers to the environment and possible harmful effects on children playing with Borax treated wooden toys. This led to in prohibition of the use of Borax in high-end children toys products from Thailand, while also disallowing the imports of other Borax treated products from Thailand such as furniture items. According to research, whenever children touch, play and inhale the chemical coating (Borax) from rubberwood, it will affect them by reducing their growth rate [6]. In addition, long exposure to Borax by consumers and lengthy accumulation of this chemical in surrounding areas will have toxic effects on the environment [7].

In recent years, many researchers have studied the effects of various compounds on microbial activities. This includes peppermint oil and eucalyptus Matan et al. (2009) anise oil, lime oil, and tangerine oil Matan and Matan (2008), cinnamon and clove oil Matan and Matan (2007), and wood vinegar [16, 17, 20]. Neem extracts have also been studied as a natural pesticide by Shmutterer [18, 19] and Von der Heyde et al. [21]. Moreover, Madua and Sanyano [12] found that wood vinegar and vinegar can increase durability of rubberwood and prohibit the growth of fungus, however, they did not condcuct a standardized test against insect attacks. Furthermore, Kartal et al. [8]. found that the seeds of neem which has Azadiractin which can eliminate and prevent mold insect pesticide by Sanguanpong [9] and Schauer et al. [22]. Moreover, Azadirachtin of neem [23, 24]. Therefore, the purpose of this research is to study the effect of natural substances, such as wood vinegar, vinegar and neem extract as a replacer for chemical substance (Borax). These natural extracts have the potential to improve the quality of rubberwood by avoiding the usage of chemicals, and applications in an industrial operation should be investigated.

2. Materials

2.1 Natural substances and chemicals

Neem extracts, wood vinegar and vinegar were purchased from Kasedsomboon Co., Ltd., Chiang Mai, Thailand. Borax and boric acid of commercial grade were purchased from Ajax Finechem Pyt. Ltd., Auckland, New Zealand.

2.2 Cultures

Two strains of molds (*Penicillium sp.* and *Aspergillus niger.*) were obtained from the Department of Microbiology, Faculty of Science, Prince of Songkla University.

2.3 Rubberwood

Fifty pieces of rubberwood (20 mm x 20 mm x 70 mm) were obtained from Plan Creation Co., Ltd. Trang, Thailand.

3. Experimental

3.1 Preparation of fungal strains

Two surface fungi on rubberwood *Penicillium sp.* and *Aspergillus niger* were grown on potato dextrose agar (PDA) from Department of Microbiology, Prince of Songkla University. Spores of remaining test fungi were collected by flooding the surface of plates with sterile saline solution (NaCl, 8.0 g/L_{water}) and 5 ml of Tween80 (0.1%v/v). A haemacytometer was used to count number of spores, and number of microbes was standardized to concentrations of 10^7 spores/ml by dilution with sterile water.

3.2 Minimal inhibitory concentration (MIC)

Determination of anti-fungal effect and minimal inhibitory concentrations (MIC) for neem extract, wood vinegar and vinegar was performed by the Broth dilution method. Each natural substance was prepared at concentrations of 10%, 15%, 20%, 25%, and 30% v/v, respectively, added to 1 ml of fungal strains (10^7 spores/ml), and mixed with 5 ml of nutrient broth solution. After that, the solution was incubated at 25ºC for 5 days [10]. The lowest concentration showing no visible growth was regarded as the minimal inhibitory concentration (MIC).

3.3 Moisture content after treatment process and amount of absorbed solution

The moisture content (% dry basis) of rubberwood sample prior to treatment was calculated according to the ASTM test method D1413-05b. method [11]. The initial weight of a piece of rubberwood (20 mm x 20 mm x 70 mm) was recorded as W_1. After treatment, the wood sample was dried in a convective oven at 100°C until it reached constant weight (W_2). The % dry basis was determined according to Eq. (1) as follows:

$$\%\text{Dry basis} = \frac{H_2O}{Solid} \times 100 = \frac{(W_1 - W_2)}{W_2} \times 100 \qquad (1)$$

For the treatment process, a 3 L high pressure chamber was used. Wood samples were placed in the chamber and treated with natural extracts at 80 psi for 1 hour [12]. The actual amount of extracts compressed into the rubberwood samples was determined by drying the treated samples at 80°C for 5 days [13] and weighing the sample. The difference between the final weight and the pre-treatment weight represents the amount of natural extracts compressed into the wood.

3.4 Mold test on rubberwood

Mold test on rubberwood was conducted by placing treated samples in a controlled environment (100% relative humidity) at 25°C for up to 12 weeks. Samples treated with neem extract, wood vinegar, and vinegar at the minimal inhibitory concentration (MIC) were designated as 1*, 2*, and 3*, respectively. The sample treated with 1% boron solution (borax/boric acid) was marked as 4*, while the untreated sample (control) was designated as 5*. A drop of fungal solution (10^7 spores/ml) was then placed at the center of the sample using a pipette. The capability to inhibit the growth of fungi in rubberwood was calculated by Eq. (2)

$$\frac{A}{B} \times 100 \qquad (2)$$

where,
A is total amount of fungi in each concentration.
B is total amount of fungi of control.

A scale from 0 to 5 was used to indicate the rate fungal growth (0 represents no fungal growth, 1=20%, 2=40%, 3=60%, 4=80%, and 5=100%) according to methods by Matan and Matan (2008). The inhibition rate between sample treated with borax and those treated with the natural substances were compared to test the effectiveness of the latter.

3.5 Termite test on rubberwood

Termite test on each of the samples was conducted according to ASTM test method D3345-74 [15]. Each block was placed in a lidded test dish filled with 50 g of sand mixed with 8.5 ml of distilled water. One gram of subterranean termite (*Coptermes gestroi*) was placed inside the test dish. After that, the test dish was incubated at 25°C and examined after 4 weeks for evidence of tunneling and termite mortality. After 4 weeks, a visual rating of attack was recorded for each block. The effectiveness in resisting termite attacks between natural extract treated rubberwood and borax treated rubberwood were compared.

3.6 Statistical analysis

All measurements were made using at least 3 samples and the mean values with standard deviations were reported. The experimental data were analyzed using ANOVA. Duncan's multiple range test was used to establish multiple comparisons of the mean values; mean values were considered at 95% confidence level ($p = 0.05$). The statistical program SPSS (Version 12) was used to perform all statistical calculations.

4. Result and Discussion

4.1 Determiation of minimal inhibitory concentration (MIC)

As shown in Figure 1, the MIC for the inhibition of *Aspergillus niger*. for neem extract, wood vinegar and vinegar was 25% by volume. On the other hand, the MIC for inhibiting the growth of *Penicilium* sp. was 20% for neem extract and wood vinegar and 15% for vinegar, respectively. Therefore, the 25% concentration was chosen to treat the rubberwood for mold and termite test.

From previous research, Matan and Matan (2008) found that the MIC for anise oil, lime oil, and tangerine oil for both of the above fungi ranged from 4% (40 µL/ml) to 18% (180 µL/ml). In another research, Matan et al. (2009) found that the MIC for peppermint oil, eucalyptus, eucalyptus oil, and menthol also ranged from 10% (100 µL/ml) to 30% (300 µL/ml). Even though some of these compounds showed lower MIC's, the cost of these extracts are from 5-10 times higher.

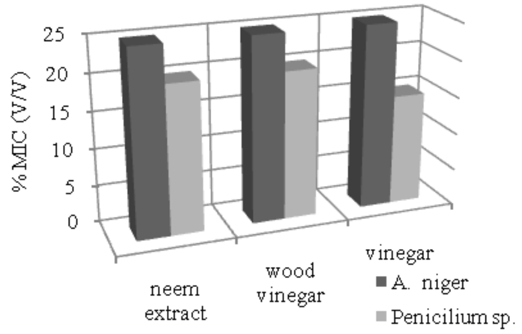

Fig.1. Minimal inhibitory concentrations for the inhibiting the growth of fungi *Aspergillus niger* and *Penicillium* sp.

4.2 Moisture content and amount of compounds absorbed

From Table 1, the average moisture content for the wood samples was about 7.20±0.02% dry basis. However, after treatment for 1 hour at 80 psi and drying for 5 days, the average moisture content increased to about 8.59±0.21% dry basis. This result indicated that some solution was compressed into the rubberwood from the treatment process. From calculations, it was found that neem extract (3.18±0.28%) and vinegar (3.31±0.27%) had significantly higher amounts of compounds absorbed into wood samples as compared to wood vinegar (2.62±0.22) and borax (2.51±0.52) at $p < 0.05$. This variation has implications on the amount of chemicals needed in the industrial treatment process and how economical each of the compounds will be compared to borax.

Moreover, Figure 2 shows that moisture content of the wood samples was lower than the required 12% dry basis (ASTM-D143) after 4 days of drying. Therefore, the wood samples could now be used for mold and termite test accordingly.

Table.1. Moisture content of sample and amount of compounds absorbed.

Solution Compressed	% Dry Basis		% Compound Absorbed
	Before Treatment	After Treatment	
Neem Extract (1*)	7.21±0.06[a]	8.30±0.19[a]	3.18±0.28[b]
Wood Vinegar (2*)	7.18±0.05[a]	8.63±0.48[b]	2.62±0.22[a]
Vinegar (3*)	7.22±0.04[a]	8.75±0.51[b]	3.31±0.27[b]
Borax (4*)	7.19±0.05[a]	8.95±0.36[b]	2.51±0.52[a]

Values in the same column with different superscripts mean that the values are significantly different ($p < 0.05$).

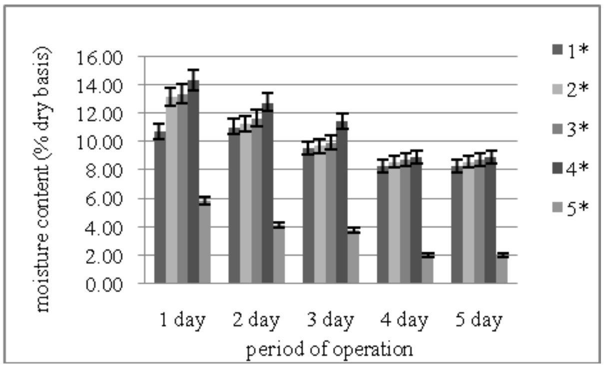

Fig. 2. Moisture content of treated wood samples after drying at 80°C for 5 days.

4.4 Efficacy of natural extracts on the inhibition of fungi in rubberwood

From Figure 3, the growth incidence of *Penicilium sp.* at 100% relative humidity for a 1 month period was highest for the untreated (control) sample (35%) and lowest for borax (7%), as expected. However, for the natural extracts, vinegar showed the lowest amount of fungal growth at 20.0% followed by neem extract at 21.4%, and wood vinegar at 24.1%, respectively. In other words, vinegar, neem extract, and wood vinegar had inhibition efficiencies of 80%, 79%, and 76%, respectively. Even though these extracts had lower inhibition efficiency compared to borax (93%), they do not pose a threat to the environment and are save for human beings.

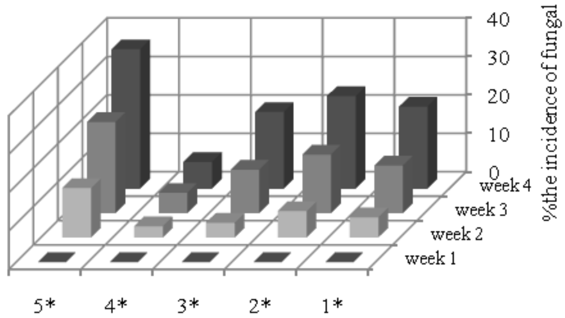

Fig.3. Incidence of fungi *Penicilium* sp. in treated rubberwood samples at 100% relative humidity as compared to the untreated (control) sample.

For the case of *Aspergilllus niger*, neem extract exhibited the lowest fungal growth of 27.8%, followed by wood vinegar and vinegar at 29.4% and 31.2%, respectively (see Figure 4). When considering the inhibition efficiency, neem extract, wood vinegar, and vinegar has values of 72%, 71%, and 69%, respectively. Borax, once again, showed the highest inhibition efficiency of 90%. However, this method was based on 100% relative humidity which greatly induced the growth of fungi.

Generally, finished wood products are not exposed to this type of relative humidity for such a prolong period of time. Consequently, an additional experiment was conducted by placing the wood samples in ambient environment (approximately 25°C and varying relative humidity from 10-50%). Results of this study (not shown) confirmed that after a one month period, no trace of fungal growth were found for both *Penicilium sp.* and *Aspergilllus niger* in samples treated with neem extract, wood vinegar, and vinegar. Both wood vinegar and vinegar are comprised mainly of acetic acid which has corrosive effect and a souring taste, thus enabling the solution to elimate pathogens such as fungal viruses and bacteria. Neem extracts have Azadirachtin, which has general effects of preventing repelling, and inhibiting the growth of insects, while also eliminating fungal growth in soil and leaves of plants. Therefore, these natural extracts have the potential for usage in the industrial treatment process with their inhibitory capability.

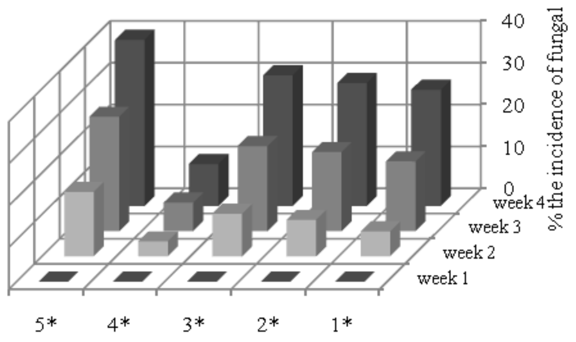

Fig.4. Incidence of fungi *Aspergilllus niger* in treated rubberwood samples at 100% relative humidity as compared to the untreated (control) sample.

4.5 Termite test on rubberwood

Termite mortality and visual attack ratings for 1 month by control relative humidity at 100% are given in Table 2. Neem extract, wood vinegar and borax at their MICS showed complete anti-termiteability with a rating of 10, indicating no attack. Vinegar, on the other hand, showed an acceptable attack rating of 9.5,while the untreated (control) sample had an unacceptable attak rating of 0. In addition, the mortality rating of termites was found to be complete (100%) for neem extract, wood vinegar, and borax. Only wood viengar showed "heavy" mortality, while the control sample had a "slight" mortality rating. Therefore, considering this result, neem extract and wood vinegar showed better inhibition of termite attacks as compared to vinegar, and has the potential to replace borax for prevention of termite attacks.

Table .2. Average termite attack and mortality rating.

Treatment Solution	Termite bioassay	
	Attack rating*	Mortality rating**
Neem extract, 25%	10	Complete
Wood vinegar, 25%	10	Complete
Vinegar, 25%	9.5	Heavy
Borax, 1%	10	Complete
Control	10	Slight

*Termite attack rating scale: 0=failure; 4=heavy; 7=moderate; 9=light; 10=sound.
**Mortality rating: 0-33%=slight; 33-66%=moderate; 67-99%=heavy; and 100%=complete.

4.6 Economic Analysis

Economic comparison between the three natural extracts and borax is shown in Table 3. While vinegar showed the lowest cost at 5 Baht/ft3, it still showed the least effectiveness in prevention of termite attacks. ON the other hand, both wood vinegar and neem extract cost more than 15 times that of the cost of borax at only 2 Baht/ft^3. However, taking into account the fact that they are safer for the environmental and human health, these natural compounds should be considered as replacement for borax in future industrial process.

Table.3. Prices per ft^3 of rubberwood treated.

Compounds	**Solution (L)**	**Price (Baht/L)**	**Conc. (%v/v)**	**ft^3**	**Price per ft^3 of wood (Baht)**
Vinegar	1000	12	25	250	5.00
Wood vinegar	1000	80	25	250	35.00
Neem extracts	1000	70	25	250	30.00
Borax and Boric	1000	29	1	250	2.03

5. Conclusion

From experiments conducted in this research, it was found that neem extract, wood vinegar, and vinegar have MIC of 25% by volume. Considering this MIC, all three compounds were able to inhibit fungal growth by as much as 80% in 100% relative humidity environment, while showing full inhibition in ambient conditions after 1 month of exposure. From the termite test, it was also found that the mortality rate of termite was fully "complete" for neem extract and wood vinegar, while vinegar showed only a "heavy" rate of mortality. Therefore, despite the higher cost for wood vinegar and neem extract, there application in the rubbewood treatment process should be conisdered.

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