

WATERBORNE PAINTS MODIFIED WITH ESSENTIAL OILS AS BIOPROTECTIVE COATINGS FOR RUBBERWOOD

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MATAN N & MATAN N. 2012. Waterborne paints modified with essential oils as bioprotective coatings for rubberwood. Biopreservative waterborne paint was developed for protection of rubberwood by incorporating the paint with 50–500 µg mL⁻¹ of essential oil (cinnamon oil, clove oil, anise oil, citronella oil, orange oil and nutmeg oil) and 0.1% surfactant. Antifungal activities of the paint against moulds (*Aspergillus niger*, *Penicillium chrysogenum* and *Penicillium* sp.) and a decay fungus (*Trametes versicolor*) and anti-termite capability of the paint were investigated. Within the concentration range studied, only cinnamon oil and clove oil at minimum concentrations of 350 and 450 µg mL⁻¹ respectively were capable of providing complete protection against mould growth on rubberwood for up to 12 weeks during storage at 25 °C and 100% relative humidity. In addition, the paints incorporated with 450 µg mL⁻¹ of both oils also showed high resistance to fungal decay and termite attack. Sensory evaluation test revealed that the paint incorporated with clove oil was better in odour and overall liking characteristics than the paint incorporated with cinnamon oil.

Keywords: Antifungal, moulds, decay fungi, termites

MATAN N & MATAN N. 2012. Cat berasaskan air yang diubah suai dengan minyak pati untuk diguna sebagai salut biopelindung kayu getah. Cat biopengawet yang berasaskan air disediakan untuk perlindungan kayu getah dengan mencampurkan cat dengan 50–500 µg mL⁻¹ minyak pati (minyak kayu manis, minyak bunga cengkih, minyak jintan manis, minyak serai wangi, minyak oren dan minyak buah pala) dan 0.1% surfaktan. Aktiviti antikulat cat tersebut terhadap kulapuk (*Aspergillus niger*, *Penicillium chrysogenum* dan *Penicillium* sp.) dan sejenis kulat reput (*Trametes versicolor*) serta keupayaan menentang anai-anai disiasat. Dalam julat kepekatan yang dikaji, hanya minyak kayu manis serta minyak bunga cengkih masing-masing pada kepekatan minimum 350 µg mL⁻¹ dan 450 µg mL⁻¹ didapati berupaya memberi perlindungan sepenuhnya terhadap kulapuk kayu getah sehingga 12 minggu sepanjang penyimpanan pada 25 °C dan kelembapan relatif 100%. Selain itu, cat yang ditambah 450 µg mL⁻¹ minyak kayu manis serta minyak bunga cengkih turut menunjukkan kerintangan tinggi terhadap pereputan kulat serta serangan anai-anai. Ujian penilaian deria menunjukkan bahawa cat yang ditambah minyak bunga cengkih lebih baik dari segi bau serta cita rasa keseluruhan berbanding dengan minyak yang ditambah minyak kayu manis.

INTRODUCTION

Waterborne paints, widely used in wooden toy industries for a number of years are generally toxic free (Tellus Institute 1993). Preservative or biocide treatments are not used for lumber used in the production of wooden toys, even for naturally non-durable wood species such as rubberwood (*Hevea brasiliensis*) (Balsiger et al. 2000). Without any protection, final wooden products are therefore very susceptible to attacks by moulds, decay fungi and insects especially when the products are used in contact with water or are used in hot and humid tropical countries such as Thailand, Malaysia and Indonesia (Khedari et al. 2002, Rahman & Dewsbury 2007). Growth of

moulds and fungi on wooden products has been reported to cause a major health risk concern for many consumers (Clausen 2002, Crook & Burton 2010). Scientific investigation also revealed that inhalation of sufficient numbers of mould spores can trigger symptoms of asthma, rhinitis or bronchitis in people who are immunologically sensitised (Fung & Hughson 2003).

Development of toxic-free or otherwise relatively low-toxic waterborne paints which also possess good antifungal and anti-insect activities should be a way to circumvent many problems mentioned above. Incorporation of food grade essential oils, extracted from herbs or plants

commonly used in cooking, into waterborne paint is worth exploring. Antifungal and anti-insect activities of various essential oils have been reported, e.g. cinnamon oil, clove oil and anise oil possess antifungal activities against moulds on wood (Matan & Matan 2007, Matan & Matan 2008); citronella oil possesses insect-repellant action (Sakulku et al. 2009, Solomon et al. 2011) and antifungal activity (Thanaboripat et al. 2004); nutmeg oil has anti-insect activity (Huang et al. 1997, Iyer 2007) and orange oil shows fungitoxicity against post-harvest pathogens in crop (Sharma & Tripathi 2006). The antifungal properties of essential oils were believed to arise mainly from their components such as phenol (eugenol in clove oil), aldehyde (cinnamaldehyde in cinnamon oil and citral in orange oil), ether (*trans*-anethole in anise oil), alcohol (geraniol in citronella oil) and hydrocarbon (α -pinene in nutmeg oil) (Inouye et al. 2006). This work intended to explore the possibility of using a mixture of essential oils and waterborne paint as a novel biopreservative paint against moulds, decay fungi and termites on rubberwood. A sensory evaluation test of the painted rubberwood specimens was performed in order to evaluate consumer acceptability of the final wooden products.

MATERIALS AND METHODS

Essential oils and rubberwood

Food-grade essential oils (cinnamon oil, clove oil, anise oil, citronella oil, orange oil and nutmeg oil) derived by steam distillation were provided by Thai China Flavours and Fragrances Industry Co. Ltd., Bangkok, Thailand. Rubberwood specimens were prepared from sapwood portion of freshly cut rubberwood lumber obtained from a plantation in Nakhon Si Thammarat province, Thailand. After cutting to size, the specimens were conditioned in a conditioning room at 20 °C and 65% relative humidity (RH) for 2 weeks. The average moisture content of rubberwood specimens before testing was $12 \pm 1\%$ ($n = 10$).

Preparation of paint solution and painting of specimen

Solution of waterborne paint was prepared by dissolving 3 mL of non-toxic waterborne paint

(White color no. 1, Tempera water color paint, complied with the European Standard EN 71-3: 1994) in 10 mL sterile water. The paint solution was amended with Teepol®, a dishwasher detergent containing anionic and non-ionic alkyl polyglycoside at 0.1% (v/v) before incorporating with 50 to 500 $\mu\text{g mL}^{-1}$ of each essential oil (cinnamon oil, clove oil, anise oil, citronella oil, orange oil and nutmeg oil). Vegetable oil (soya bean oil) was used as control treatments to control physical effect of moisture exclusion from the test specimens.

The specimens were coated with the prepared waterborne paints modified with essential oils on all faces by brushing at an application rate of about 200 g m^{-2} . Specimens were then placed in an aseptic beaker and stored in an aseptic biological safety cabinet for 24 hours before further use. This allowed the paint to dry and fix onto the surface of rubberwood.

Cultures and preparation of inoculums

Three strains of moulds (*Aspergillus niger* WU 0701, *Penicillium chrysogenum* WU 0702, *Penicillium* sp. WU 0703) and a white-rot decay fungus (*Trametes versicolor* WU 0704) were identified as representatives for rubberwood degradation by isolation from degraded samples. Codes refer to strains held in the culture collection of the Wood Science and Engineering Research Unit, Walailak University, Nakhon Si Thammarat, Thailand.

Spores of test moulds were obtained from mycelia grown on malt extract agar medium at 30 °C for 14 days and were collected by flooding the surface of the plates with ~5 mL sterile saline solution (NaCl, 8.5 g L^{-1} water) containing Tween 80 (0.1% v/v), a polyethylene sorbitol ester used to evenly disperse the mould spores in the suspension. After the spores were counted using a haemocytometer, the suspension was standardised to concentrations of 10^7 spores mL^{-1} by dilution with sterile water before use. The viability of all strains was checked using quantitative colony counts at 10^7 colony-forming unit (CFU) mL^{-1} .

Mould test on rubberwood

Antifungal efficiency of waterborne paints incorporated with essential oils at various concentrations ranging from 50 to 500 $\mu\text{g mL}^{-1}$ against the test moulds (*A. niger*, *P. chrysogenum*

and *Penicillium* sp.) were examined in accordance with the ASTM standard D4445-03 (ASTM 2003) with some modifications. Five painted specimens (7 mm × 20 mm cross-section by 70 mm long) for each treatment were inoculated with each mould-spore inoculums (10^7 spores mL⁻¹). Specimens were then placed on a glass rod in sterile Petri dishes in which high humidity (~100% RH) was maintained by moistened filter papers placed at the bottom of the dishes. The Petri dishes were incubated at 25 °C and 80% RH in an environmental chamber for 12 weeks. Specimens were then individually rated for mould growth on a scale of 0 to 5, with 0 denoting clean specimens and 5 representing heavy mould growth (0 = clean, 1 = 1–20%, 2 = 21–40%, 3 = 41–60%, 4 = 61–80%, 5 = 81–100%). The relative percentage of stain and mould for each treatment was calculated according to the percentage of stain and mould (based on control) = (A/B) × 100, where A = total score for each mould at each treatment and B = total score for each mould at control (treated with vegetable oil).

Mould morphology on some selected specimens was also examined using scanning electron microscope (SEM). After CO₂ critical point drying, specimens were mounted on stubs and coated with gold for the SEM analysis.

Fungal decay test on rubberwood

Fungal decay resistance of waterborne paints incorporated with essential oils at various concentrations ranging from 50 to 500 µg mL⁻¹ against the white-rot decay fungus *T. versicolor* was examined in accordance with the ASTM standard D1413-05 (ASTM 2005a) with some modifications. Soil block culture bottles were prepared using 120 g sieved oven-dried soil filled in screw-capped bottles. Sterile water was added to achieve 130% of water holding capacity of soil in the test bottles. Rubberwood feeder strips (3 mm × 28 mm × 35 mm) were placed on the surface of the soil. The prepared bottles were then autoclaved at 121 °C for 30 min. After cooling down, the feeder strips placed in the sterilised bottles were inoculated with fungal inoculum sections from freshly grown culture. The bottles were incubated at 25 °C and 80% RH in an environmental chamber for 2 weeks until mycelium of the decay fungus completely colonised the feeder strips.

The painted rubberwood specimens (25 mm × 25 mm × 25 mm) were placed on the feeder

strips in contact with fungal mycelium. The bottles containing painted specimens were then incubated at 25 °C and 80% RH for 12 weeks. After incubation, surface mycelium was removed. The specimens were oven dried at 60 °C for 24 hours and reconditioned at 20 °C and 65% RH in a conditioning room to constant weight. Average percentage weight loss was determined from the conditioned weight before and after exposure to the decay fungus. Five specimens were tested for each treatment group.

Termite test

Sets of five painted rubberwood specimens (10 mm × 10 mm × 10 mm) were subjected to a termite bioassay according to a no-choice test procedure D3345-74 (ASTM 1999). Each specimen was placed in a glass bottle with 50 g sand, 8.5 mL deionised water and 1.0 g subterranean termites (*Coptotermes gestroi*). The bottles were then stored at 28 °C and 75% RH for 4 weeks. A visual rating of termite attack (0 = failure, 4 = heavy, 7 = moderate, 9 = light and 10 = sound) according to the ASTM test method D3345-74 (1999) was recorded for each specimen at the end of the test.

Colour measurement

Colour measurement was performed on rubberwood specimens (7 mm × 20 mm × 70 mm) painted with waterborne paint solution incorporated with 450 µg mL⁻¹ of either cinnamon oil or clove oil. Pure waterborne paint without essential oil was used as control treatment. The measurement was carried out after painting at 4 weeks interval throughout the 12-week storage period in an environmental chamber at 25 °C and 80% RH. The measurement of three replicates per treatment was performed using a tristimulus colour analyser. CIELab colour coordinates were used to determine the degree of lightness (L*), redness–greenness (+ or -a*) and yellowness–blueness (+ or -b*). Total colour difference (ΔE*) between oil treatments and control (without essential oil) at 4-week intervals throughout the 12-week storage period was also calculated using the equation:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Sensory evaluation

Rubberwood specimens (7 mm × 20 mm × 70 mm) painted with waterborne paint solution incorporated with 450 µg mL⁻¹ of either cinnamon oil or clove oil were used for sensory evaluation. The painted specimens were kept in an environmental chamber at 25 °C and 80% RH for up to 12 weeks. Consumer acceptability carried out after 1 day and 12 weeks of storage was studied with 46 untrained panelists (21 females and 25 males) ranging from 18 to 40 years of age. Specimens treated with cinnamon oil and clove oil were randomly coded and presented to each panelist seated separately in booths in a control room. A 9-point hedonic scale ranging from 'like extremely' to 'dislike extremely' was used to determine the degree of acceptance of the specimens in terms of odour, colour and overall liking (Meilgaard et al. 1999). The responses were then converted to numerical values ranging from 1 for 'dislike extremely' to 9 for 'like extremely'.

Statistical analyses

Experimental data of colour and sensory evaluations were tested for normality by applying the Komogorov–Smirnov test while homogeneity of variances was assessed using Levene's test. Data transformation was performed where necessary. Results were expressed as mean ± standard deviation. The data were statistically treated by one-way ANOVA. Duncan's post hoc test with $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Mould test on rubberwood

The inhibitory effect of the waterborne paint incorporated with 50–500 µg mL⁻¹ of essential oils on the three test moulds are presented in Figures 1a–c. Cinnamon oil and clove oil at minimum inhibitory concentrations of 350 and 450 µg mL⁻¹ respectively completely inhibited growth of the three test moulds on the surface of rubberwood for up to 12 weeks. High concentrations of anise oil and citronella oil also strongly inhibited some test moulds. Anise oil at concentrations of 450 and 500 µg mL⁻¹ completely inhibited *A. niger* and *Penicillium* sp. respectively. Zero mould growth

of *Penicillium* sp. was also obtained with the treatment of citronella oil at 500 µg mL⁻¹. Between the three test moulds, *P. chrysogenum* appeared to be the strongest test mould. Orange oil and nutmeg oil at all concentrations examined up to 500 µg mL⁻¹ were unable to inhibit any test moulds with respect to the control treatment of vegetable oil. In another study using agar as medium, orange oil was reported to be fungicidal against *A. niger* and *P. chrysogenum* at concentrations higher than 600 µg mL⁻¹ (Sharma & Tripathi 2006).

Good antifungal effects of cinnamon oil, clove oil and anise oil have been reported to arise mainly from their main components which are cinnamaldehyde, eugenol and *trans*-anethole respectively (Matan & Matan 2007, Matan & Matan 2008, Singh & Chittenden 2010). The minimum inhibitory concentrations of cinnamon (350 µg mL⁻¹) and clove (450 µg mL⁻¹) oils in waterborne paint against the test moulds were much higher than those in methanol (50 and 100 µg mL⁻¹ respectively (Matan et al. 2011). The type of solvent appears to have strong influence on antifungal activity of the solution incorporated with essential oil which is worth exploring in the future.

Antifungal activity of waterborne paint solution incorporated with cinnamon and clove oils at their minimum inhibitory concentrations was also confirmed by microscopic examination of the test specimens. Figure 2a shows the surface of the rubberwood specimen painted with waterborne paint solution incorporated with cinnamon oil at the minimum inhibitory concentration level of 450 µg mL⁻¹ after inoculation with *A. niger* spores and 12 weeks of incubation. The specimen surface was free of conidiophores and the inoculated fungal spores were unable to germinate. This was in contrast to the wood surface of the control treatment with vegetable oil (Figure 2b) where conidia and conidiophores of fully grown *A. niger* were clearly observed.

Fungal decay test on rubberwood

Results of the decay test on rubberwood painted with waterborne paint solution incorporated with cinnamon, clove, anise, citronella, orange and nutmeg oils at various concentrations are presented in Figure 3. Waterborne paint incorporated with cinnamon and clove oils at concentrations ≥ 450 µg mL⁻¹ showed very

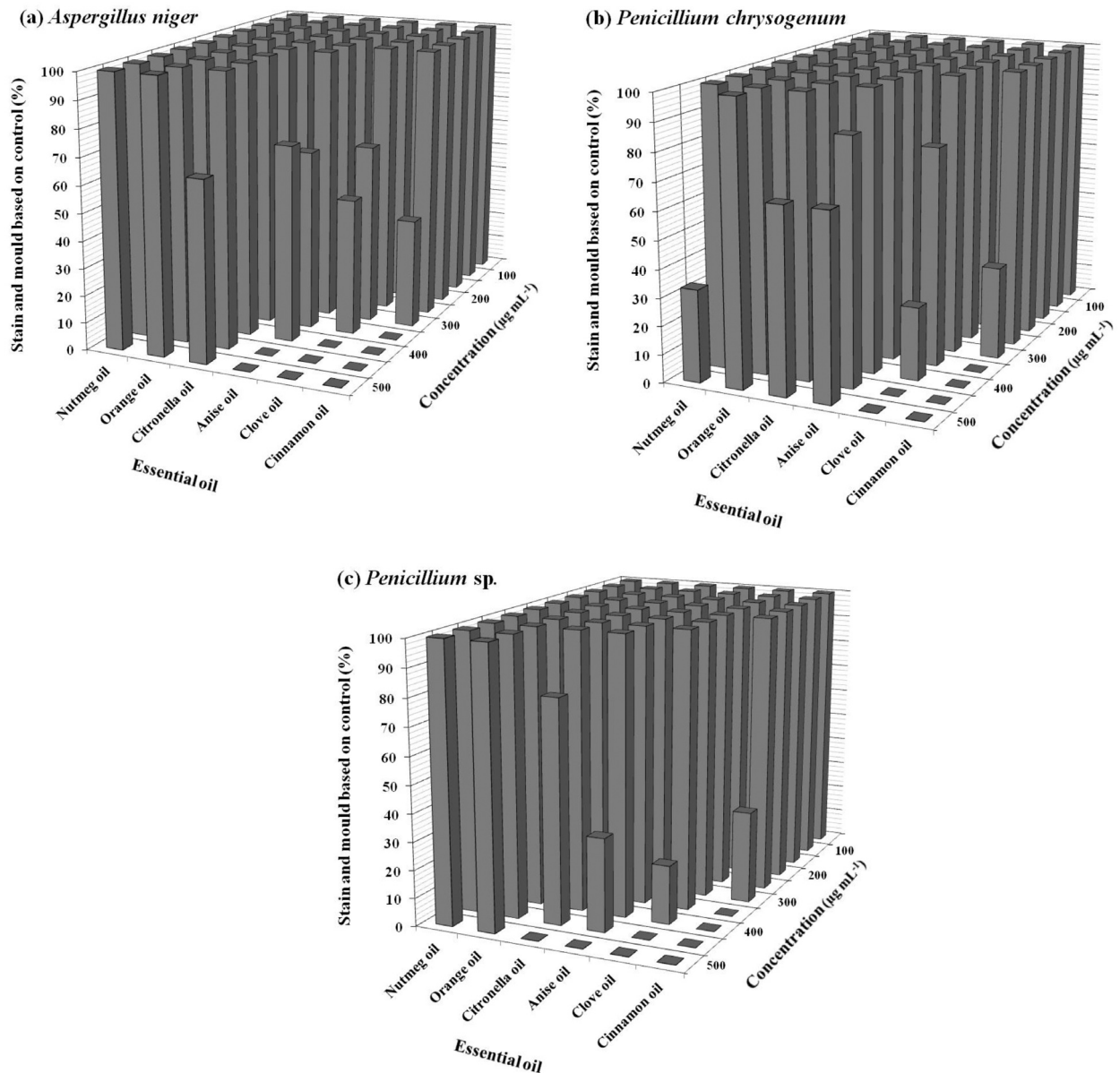


Figure 1 Percentages of stain and mould coverage compared with control of (a) *Aspergillus niger*, (b) *Penicillium chrysogenum* and (c) *Penicillium sp.* on rubberwood specimens painted with waterborne paint incorporated with various essential oils at concentrations between 50 and 500 µg mL⁻¹ after 12 weeks incubation at 25 °C and 100% RH

strong resistance against the attack of *T. versicolor* with almost zero weight loss after 12 weeks of incubation. Moreover, these oils at lower concentrations of 350–400 µg mL⁻¹ and anise oil at concentrations of 400–500 µg mL⁻¹ also displayed low weight loss values of less than 10% which could be classified as highly resistant to fungal decay (ASTM 2005b). Cinnamaldehyde and eugenol, major components in cinnamon oil and clove oil respectively, were reported to exhibit strong antifungal activities against *T. versicolor* in comparison with other constituents

in the oils (Voda et al. 2003, Cheng et al. 2006). Weight loss values (30–47%) of the specimens treated with citronella oil, orange oil and nutmeg oil were similar to those of controls. Obviously, at the concentrations used in this study, these essential oils were not effective to protect against fungal decay of *T. versicolor*.

It should be noted that since paint was applied on the surface of the rubberwood, protection against fungal decay was ensured only for the attack from outside the wood specimens. If the decay fungi are already

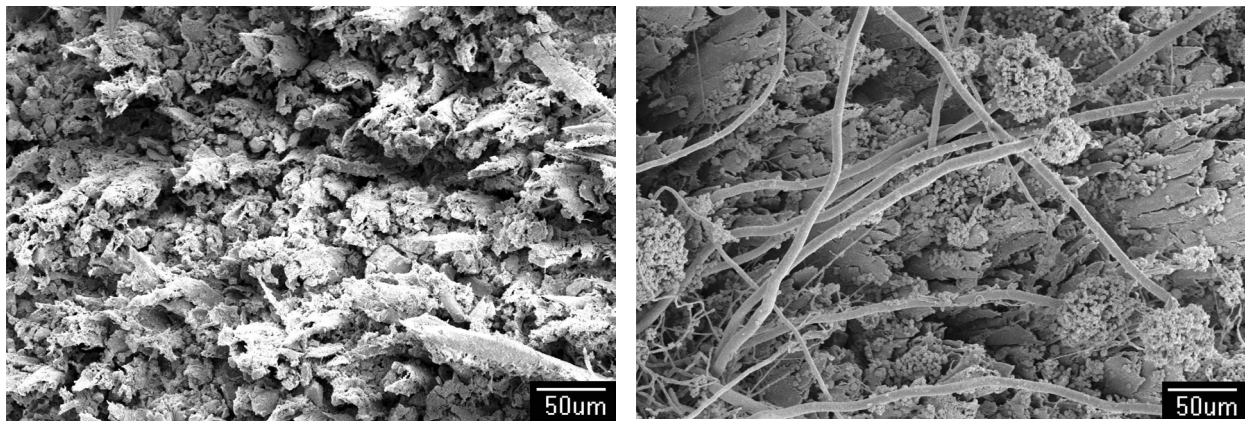


Figure 2 Scanning electron micrographs (SEM) of rubberwood surfaces painted with waterborne paint incorporated with (a) cinnamon oil at 450 µg mL⁻¹ and (b) vegetable oil at the same concentration; the specimens were inoculated with *A. niger* before being incubated at 25 °C and 100% RH for 12 weeks

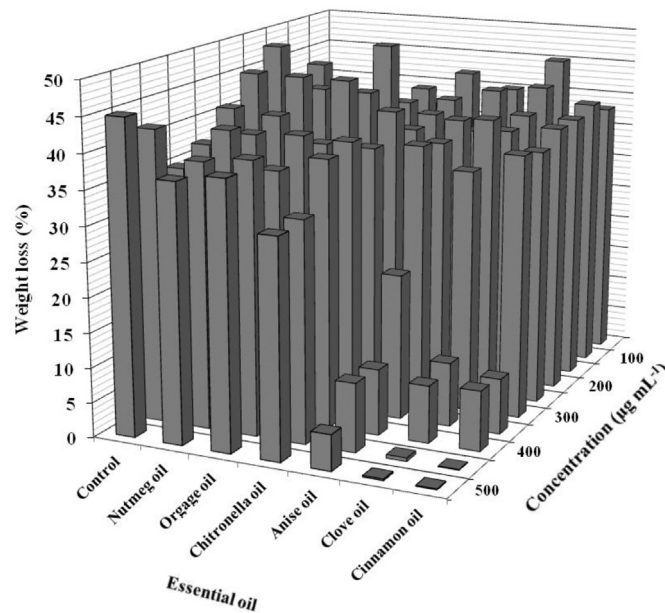


Figure 3 Percentage weight losses of rubberwood specimens painted with waterborne paint incorporated with various essential oils at concentrations between 50 and 500 µg mL⁻¹ compared with control treatments using vegetable oil after exposure to the decay fungus *Trametes versicolor* for 12 weeks

within the lumber, especially within a large size lumber, then the waterborne paint modified with essential oil protection technique may not be effective. Impregnation of essential oil into the lumber may be more suitable (Matan & Matan 2008).

Termite test

Termite attack ratings of rubberwood specimens painted with waterborne-essential oil paint solution are given in Table 1. Treatments of

citronella oil, orange oil, nutmeg oil and the control up to 500 µg mL⁻¹ were unable to protect against termite attack (rating 0). Various attack ratings were observed with cinnamon, clove and anise oils. Waterborne paint solution incorporated with ≥ 450 µg mL⁻¹ of cinnamon oil, ≥ 450 µg mL⁻¹ of clove oil or 500 µg mL⁻¹ of anise oil showed complete anti-termite ability with ratings up to 10 indicating no attack. The paint solution incorporated with cinnamon oil or clove oil at lower concentration of 400 µg mL⁻¹ also provided an acceptable level of protection

Table 1 Average termite attack rating of rubberwood specimens painted with waterborne paint incorporated with various essential oils

Waterborne paint treatment	Termite attack rating									
	Concentration of essential oil ($\mu\text{g mL}^{-1}$)									
	50	100	150	200	250	300	350	400	450	500
Cinnamon oil	0	0	0	0	3.4 ± 0.9	5.2 ± 0.8	6.4 ± 0.7	8.6 ± 0.4	10 ± 0	10 ± 0
Clove oil	0	0	0	0	0	0	5.2 ± 0.4	8.6 ± 0.3	10 ± 0	10 ± 0
Anise oil	0	0	0	0	0	0	0	3.4 ± 1.2	5.1 ± 0.5	9.8 ± 0.2
Citronella oil	0	0	0	0	0	0	0	0	0	1.2 ± 0.3
Orange oil	0	0	0	0	0	0	0	0	0	0
Nutmeg oil	0	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	0

Termite attack rating scale: 0 = failure, 4 = heavy, 7 = moderate, 9 = light and 10 = sound

with attack rating of 8.6. Anti-termite activities of cinnamon oil and clove oil were attributed to their main components, i.e. cinnamaldehyde (Chang & Cheng 2002) and eugenol (Park & Shin 2005, Gupta et al. 2011) respectively.

Colour measurement

Concentration of essential oils at the lowest level sufficiently capable of providing complete protection against mould growth, fungal decay and termite attack (i.e. cinnamon oil or clove oil at $450 \mu\text{g mL}^{-1}$) was chosen for colour and sensory analyses in order to minimise the effects the oils might have on the quality of wood. Examination of specimens just after the treatment using colour analyser revealed that lower L^* (Figure 4a) and higher a^* (Figure 4b) and b^* (Figure 4c) values were observed in specimens painted with waterborne paint solution incorporated with cinnamon oil or clove oil with respect to the control (pure paint without addition of essential oils). The measured values of colour parameters L^* , a^* and b^* of all specimens including control gradually decreased over time during incubation (Figures 4a–c). However, total colour differences (ΔE^*) between treated and control specimens remained unchanged over 8 weeks of incubation ($p < 0.05$) (Figure 4d). After 8 weeks of storage, total colour differences of the treated specimens started to deviate from those of the controls ($p < 0.05$). Further analysis on chemical changes is required to elucidate mechanisms underpinning such behaviour. During storage, the corresponding ΔE^* value of cinnamon

oil treatment was higher than that of clove oil ($p < 0.05$). Larger colour change, as a result of colour components in essential oils, was also observed in films prepared from cuttlefish skin gelatin containing cinnamon oil compared with films containing clove oil (Hoque et al. 2011).

Sensory evaluation

Results of sensory evaluation attributes of rubberwood painted with waterborne paint solution incorporated with cinnamon oil and clove oil after 1 day and 12 weeks storage are summarised in Figure 5. The panelists did not find any colour difference between specimens treated with cinnamon oil or clove oil on the first day and after 12 weeks of incubation ($p \geq 0.05$). Use of cinnamon oil, however, adversely affected the sensory odour and overall liking characteristics of the painted specimens on the first day. The specimen treated with paint incorporated with cinnamon oil possessed very low values of hedonic scale for odour (dislike moderately) and overall liking (dislike slightly). Sensory panelists preferred odour of specimens coated with the paint incorporated with clove oil (hedonic value of 6, like slightly). The hedonic values of odour characteristic of cinnamon oil and clove oil treatments significantly increased ($p < 0.05$) to 7 (like slightly) and 8 (like very much) respectively after 12 weeks storage. Evaporation and/or chemical reactions of some components in essential oils which were responsible for their odour, might occur during storage. However, these components were not

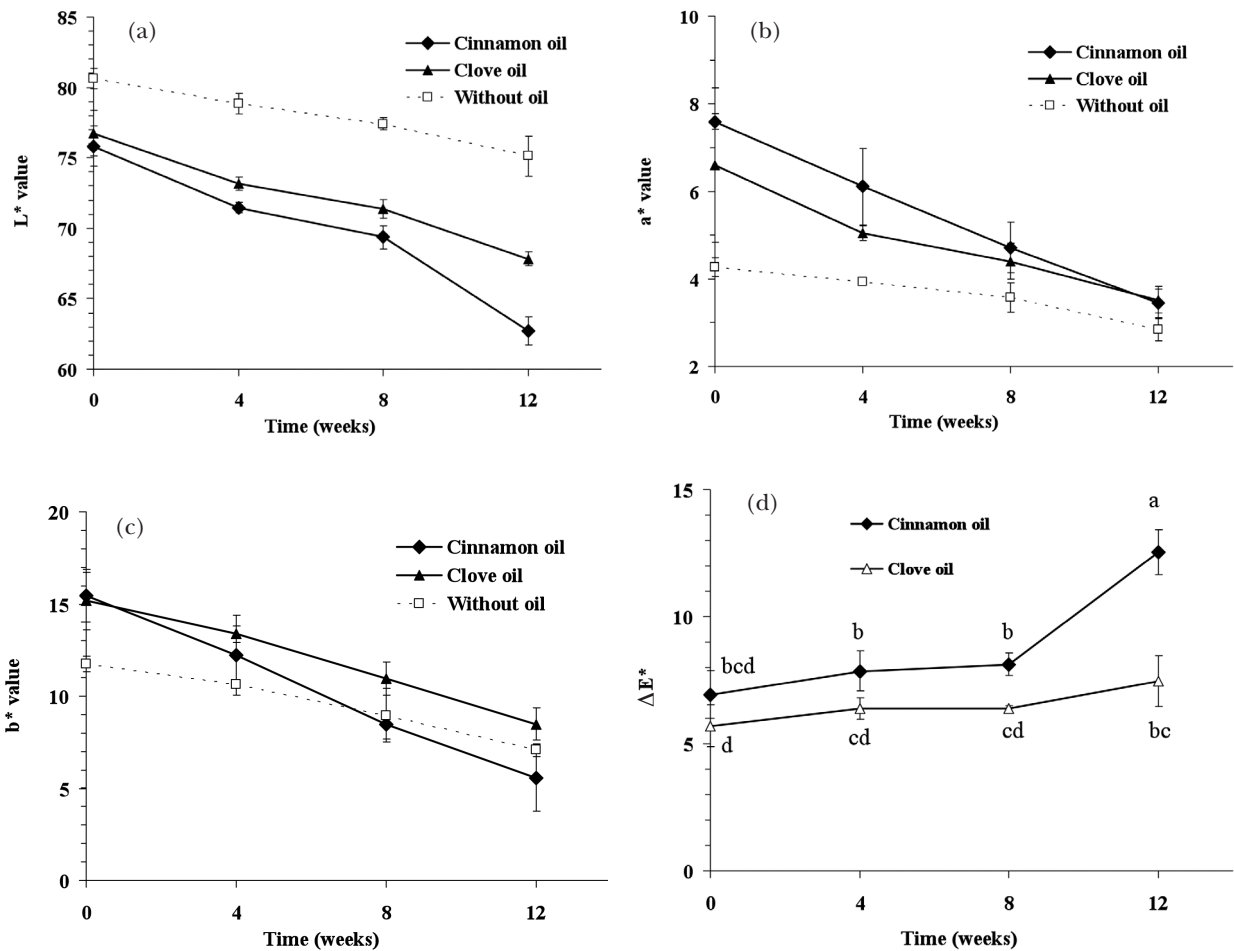


Figure 4 Variation of colour parameters (a) lightness L^* , (b) redness to greenness a^* , (c) yellowness to blueness b^* and (d) total colour difference ΔE^* of rubberwood specimens painted with waterborne paint incorporated with cinnamon oil and clove oil at $450 \mu\text{g mL}^{-1}$ for up to 12 weeks of storage; means with the same letter are not significantly ($p < 0.05$) different according to Duncan's test following ANOVA

responsible for any antifungal activities of the oils since specimens treated with both cinnamon and clove oils were still free from mould coverage and still exhibited very high resistance to attack by decay fungus for up to 12 weeks. Nevertheless, emissions of volatile organic compounds from essential oils during service could produce undesirable odour and could be classified as sources of potential secondary pollutants (Wu et al. 2011).

CONCLUSIONS

Waterborne paint incorporated with cinnamon oil or clove oil at concentration of at least $450 \mu\text{g mL}^{-1}$ was capable of providing complete

protection of rubberwood against all moulds tested (*A. niger*, *P. chrysogenum* and *Penicillium* sp.) for up to 12 weeks at 25°C and 100% RH. Rubberwood painted with these waterborne-essential oil paints also showed high resistance to fungal decay of *T. versicolor* and termite attack. Paint incorporated with clove oil was better rated by sensory panelists in odour and overall liking characteristics than the paint incorporated with cinnamon oil. These findings demonstrated the potential of waterborne paint modified with essential oil as wood preservative in various health-concerned applications such as children's toys, food packaging, kitchenware and indoor wooden structures.

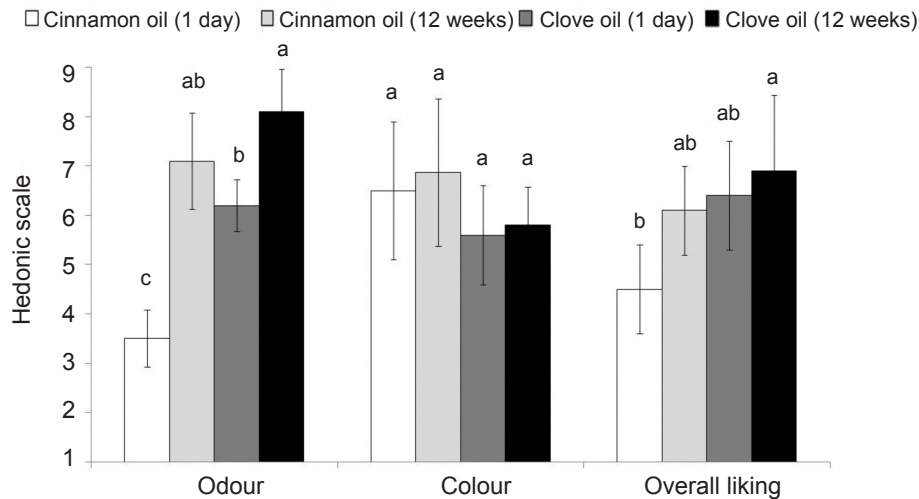


Figure 5 Hedonic scores of odour, colour and overall liking attributes of rubberwood specimens painted with waterborne paint incorporated with $450 \mu\text{g mL}^{-1}$ of either cinnamon or clove oil after 1 day and 12 weeks of storage at 25°C and 80% RH; means with the same letter in each attribute are not significantly ($p < 0.05$) different according to Duncan's test following ANOVA

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