Reduction in Toxicity of Tectoquinone Against *Reticulitermes speratus* Kolbe Termites

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Abstract

This study investigated the cause of low toxicity against *Reticulitermes speratus* Kolbe termite species of teakwood (*Tectona grandis* L.f.) under natural conditions. Anti-termite test was conducted to evaluate the effectivity of four major components (tectoquinone, deoxylapachol, tecomaquinone, and squalene) of ethanol-benzene extracts in the teak heartwood. Tectoquinone was far superior to other components and exhibited both strong toxicity and antifeedancy. The strength reduction of tectoquinone bioactivity is assumed to be due to interaction with other major components. As squalene was found in considerable amounts or 1.8 to 13.1 times as high as the tectoquinone concentration in woods, termite feeding was set to the mixtures of tectoquinone and squalene in various ratios (1:1, 1:5, 1:10, and 1:20). It was revealed that squalene addition could decrease the termite mortality from 15% to 44% from its initial value (tectoquinone only). On the other hand, the mixtures only slightly reduced mass loss due to termite attacks.

Keywords: teak, tectoquinone, antifeedant, squalene, anti-termite.

Introduction

Teak wood is valued for its high natural durability due the presence of toxic components in the heartwood. Quinones and its derivatives are bioactive components against insects and fungi in teak heartwood (Haupt *et al*. 2003; Thulasidas *et al*. 2007; Niamké *et al*. 2011; 2013). Among them, in an isolated state, tectoquinone or 2-methylanthraquinone exhibits the highest level of toxicity against *Reticulitermes speratus* Kolbe termites (Lukmandaru and Ogiyama 2005; Lukmandaru 2012). Under natural conditions, however, teak heartwood only deters termites (Rudman *et al*. 1967; Lukmandaru and Takahashi, 2008; Lukmandaru 2011). Therefore, the exact causes of reduction in termiticidal properties should be explored.

Along with tectoquinone, several components i.e., squalene, desoxylapachol and its isomer, tectol, lapachol, 2-hydroxymethylanthraquinone, natural rubber or caoutchouc, sugar and sugar derivatives are found in varied amounts in teak extractives in which squalene shows significant concentrations (Yamamoto *et al*. 1998; Windeisen *et al*. 2003; Lukmandaru 2015). It is hypothesized that interaction between the primary compounds would affect the efficacy against termites. Thus, the main objectives of this work were to measure the content of main components in the heartwood, to evaluate the anti-termite activity of isolated compounds, as well as to test the toxicity levels of tectoquinone mixed with squalene.

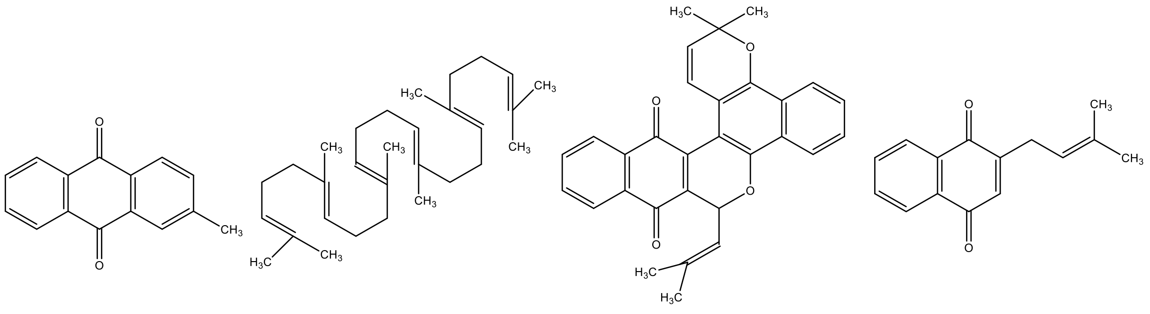
Material and Methods

Gas chromatography spectra and mass spectra were obtained using GC (Hitachi Model G-3 500) and GC-MS (JEOL XS mass spectrometry at 70 eV). The ^{13}C (in 400 MHz) and ^{1}H NMR (in 100 MHz) spectra were obtained using JEOL JNX-400 spectrometer.

Sample Preparation and Extraction

Tectoquinone and squalene standard components were purchased from Kanto Chemical Co. (Japan). Tecomaquinone and deoxylapachol were isolated in a previous study (Lukmandaru 2013). In brief, teak heartwood meals (1 kg oven dried) were extracted by refluxing with *n*-hexane for 6 h. The obtained extract then was fractionated into neutral and acidic fractions by usual way. The neutral fraction (10 g) was then separated by column chromatography on a silica gel successively eluted with *n*-hexane, benzene, and ethyl acetate (EtOAc). Then, deoxylapachol was isolated by repeating column chromatography of the *n*-hexane fraction. By similar fashion, tecomaquinone was isolated from the EtOAc fraction. The GC-MS chromatogram of deoxylapachol was in agreement with that in the studies of Perry *et al*. (1991), and Lukmandaru and Takahashi (2009). Tecomaquinone was identified by comparing the NMR spectral data with previously published data (Lemos *et al*. 1999).

The outer heartwood samples from 8 individual trees were collected to study the component variation. The trees were planted in Perhutani Plantation, Randublatung, Central Java with a selected diameter range of 30-40 cm (class age IV). The wood meals were obtained by drilling from two opposite radii and were then combined to form a single sample. The wood meal (2 g) was subjected to Soxhlet extractions with ethanol-benzene (1:2, v/v) for 6 h. The extracts were dried on a rotary evaporator and the extractive contents were determined. The chemical structures of tested components are displayed in Figure 1.



(a) (b) (c) (d)

Figure 1. Chemical structures of tectoquinone (a), squalene (b), tecomaquinone (c), and deoxylapachol (d).

Component Analysis

The extract (100 mg/mL) was injected to a GC/MS analysis. The setting conditions of column were: NB-1 bonded capillary 30 m, detector: FID, injection: splitless at 250°C. Carrier gas: He. Column temperature program: 120-300°C (programing 4°C min^{-1}), detector and injector temperature at 250°C, held at 300°C for 15 min. MS conditions: temperature ionization voltage of 70 eV, transfer line at 250°C, acquisition mass ranging from 50–500 atomic mass unit. The content of the extractives was determined by comparison with using known amounts of reference samples and was expressed as percentages of oven-dried wood mass.

Termites

Reticulitermes speratus termites (workers) were collected from a laboratory colony maintained in Yamagata University, Japan. The colonies were maintained on wood pieces in a controlled room for 3 months before the initiation of the test.

Anti-termite Test

The no-choice test was conducted according to a method in a previous study (Lukmandaru and Ogiyama 2005). A test container was made from a Petri dish (diameter 9 cm, height 2 cm) containing 20 g moistened and sterilized sea sand in the bottom. Paper disc (diameter 8 mm; Whatmann International) were permeated with chloroform solution containing each of the test compounds (Fig. 2). The treatment retention of the single compound was 0.1%; 0.5 %, and 1.0% (w/w) per disc and 3 duplicates were applied for each sample. The retention of tectoquinone and squalene mixture was 2% whereas the ratios of tectoquinone and squalene were 1:1, 1:5, 1:10, and 1:20. The paper discs were dried at 60 ^{0}C for 2 h. In a control Petri dish, only chloroform was used. Fifty worker *Reticulitermes speratus* Kolbe termites were then added to each Petri dish. The petri dishes were covered in a dark chamber at 27°C and 80% relative humidity for the entire test period (10 days). The numbers of surviving and dead termites were counted. Finally, the discs were taken out, dried in the same manner, and the weight loss was weighted.

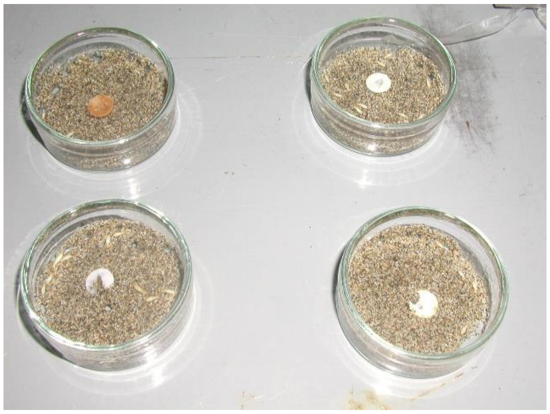


Figure 2. No-choice test against *Reculitermes speratus* termites using paper discs.

Statistical Analysis

The effect of different mixture concentration of components was analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (*p* = 0.05). The termite mortality rates (percentages) were transformed by the arcsine function for analysis. All statistical calculations were performed using SPSS-Win 16.0.

Results and Discussion

Component Variation

Ethanol-benzene mixture was used to extract most of the quinones in the teak heartwood. The extractive content ranged in 6-12%. The detected compounds by means GC/MS are displayed in the chromatogram (Figure 3). Some quinones (deoxylapachol and its isomer; tectoquinone, lapachol, tectol) as well as non-quinones (squalene, palmitic acid) were identified. The other 2 peaks were unknown compounds (Un1 and Un2). The compounds detected here were mostly similar to the earlier reports (Windeisen *et al*. 2003; Lukmandaru and Takahashi 2009; Lukmandaru 2015). The quantification of the extractives based on dry-wood showed squalene to be the most abundant component judging by the average values (Table 1) followed by tectoquinone and tectol. On the other hand, palmitic acid and an unknown compound (Un2) were found in minor amounts. Although tecomaquinone has been isolated by column chromatography, the green compound tecomaquinone was not detected by GC-MS. This might be due to the column in the GC used in this experiment did not able to detect this compound. Unfortunately, the spot test by thin layer chromatography was not performed to confirm this finding.

It has been suggested that the bioactivity reduction of tectoquinone might be due to its interaction with other main compounds in the wood. Therefore, the amount of each compound was counted relative to tectoquinone content (Table 1). On the basis of 8 individual trees, it was found that the amount of tectoquinone was consistently higher than palmitic acid and UN 2 but lower compared to squalene (1.8 to 13.1 times). Compared to other quinones, the ratio values were varied depending on the individual samples. In term of natural durability against fungi, the ratio between tectoquinone and deoxylapachol could be a good indicator for predicting the resistance of teakwood against wood destroying fungi (Haupt *et al*. 2003). Tectol, unfortunately, was not isolated in this experiment. This dimeric naphtoquinone compound has never been mentioned in regards to teakwood durability (Windeisen *et al*. 2003).

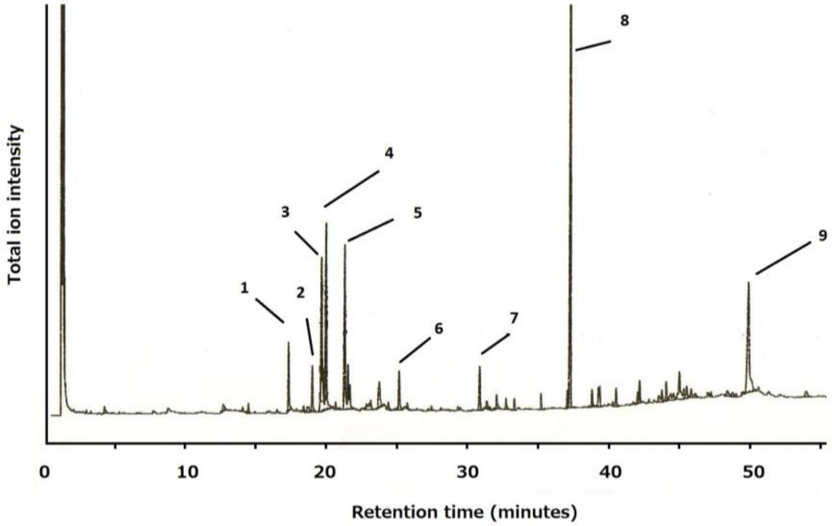


Figure 3. Gas chromatogram of ethanol-benzene extracts from teak heartwood. The detected components are peak 1 and 4 = desoxylapachol or its isomer; peak 2 = palmitic acid; peak 3 = lapachol; peak 5 = tectoquinone; peak 6 = unidentified compound 1; peak 7 = unidentified compound 2, peak 8 = squalene; and peak 9 = tectol.

Table 1. Tree characteristics and between-tree variation in the content of major compounds of ethanol-benzene extracts from teak heartwood expressed as percent of dry-wood meal weight.

Tree number	Dbh (cm)	EC (%)	DE(%)	PA (%)	LP(%)	ID (%)	TQ (%)	Un1 (%)	Un2 (%)	SQ (%)	TO (%)
1	39	6.44	0.06	0.01	0.03	0.22	0.12	0.12	0.08	1.52	0.23
2	33	9.57	0.06	0.05	0.14	0.21	0.59	0.10	0.09	1.13	0.55
3	35	9.25	0.35	0.01	0.73	0.75	0.34	0.42	0.06	1.34	1.00
4	37	7.95	0.15	0.06	0.09	0.34	0.10	0.10	0.05	0.49	0.27
5	38	7.02	0.36	0.07	0.06	0.20	0.08	0.40	0.02	0.96	0.33
6	30	11.32	0.15	0.70	0.11	0.46	0.84	0.19	0.00	1.54	0.95
7	32	8.58	0.98	0.13	0.48	0.67	0.31	0.33	0.12	1.79	1.01
8	35	11.72	0.05	0.01	0.03	0.05	0.18	0.00	0.02	0.52	0.15
Average		8.98	0.27	0.13	0.21	0.36	0.32	0.21	0.06	1.16	0.56

Remarks : Dbh : diameter breast height, EC = extractive content, DE = deoxylapachol, PA = palmitic acid, LP = lapachol, IDE = isodeoxylqapachol, TQ = tectoquinoine, Un1 = unknown 1, Un2 = unknown 2, SQ = squalene, TO = tectol

Anti-termite Test of Single Components

In a literature review, tectoquinone and lapachol were effective antifeedant and toxic compounds against *Cryptotermes brevis* termites (Sandermann and Simatupang 1966). In another work, tectoquinone and deoxylapachol or its isomer were moderately correlated with the attacks of *Reticulitermes speratus* (Lukmandaru and Takahashi 2009). In this study, the anti-termite test was conducted on compounds where the quantity was similar to or higher than tectoquinone amounts, i.e., deoxylapachol, and squalene, in addition to tecomaquinone. The concentration interval was adjusted by its range in the natural condition (Table 1). The results of three concentrations (0.1%, 0.5%, and 1.0%, based on paper disc weight) are presented in Figure 3.

In general, the higher concentration caused a lower mass loss and higher mortality rate. As expected, tectoquinone showed the highest toxicity effect (mortality rates: 33-98%) and lowest mass loss (0.79-1.79 mg). Beside tectoquinone, no other compounds showed a strong termicidal activity. However, moderate levels of antifeedant activity or feeding deterrent effect) were exhibited by deoxylapachol, squalene, and tecomaquinone, particularly for the concentration of 1.0%. It was indicated by mass loss rate (control mass loss = 6.19 mg as the values were 3.27 mg; 3.92 mg, and 4.28 mg, respectively. For comparison, however, tectoquinone was not shown to be toxic and was only deterred *Nasutitermes exitiosus* (Rudman and Gay 1961). This condition probably was caused by a differential feeding preference among termite species. In an earlier report (Lukmandaru and Ogiyama 2005), tecomaquinone in high dosage (5 %, w/w) exhibited a strong antifeedant activity (mass loss of 0.04 mg) against the same species (*Reticulitermes speratus*) but in a low level of termicidal activity. Thus, this result confirms the effectivity of high concentrations as a deterrent.

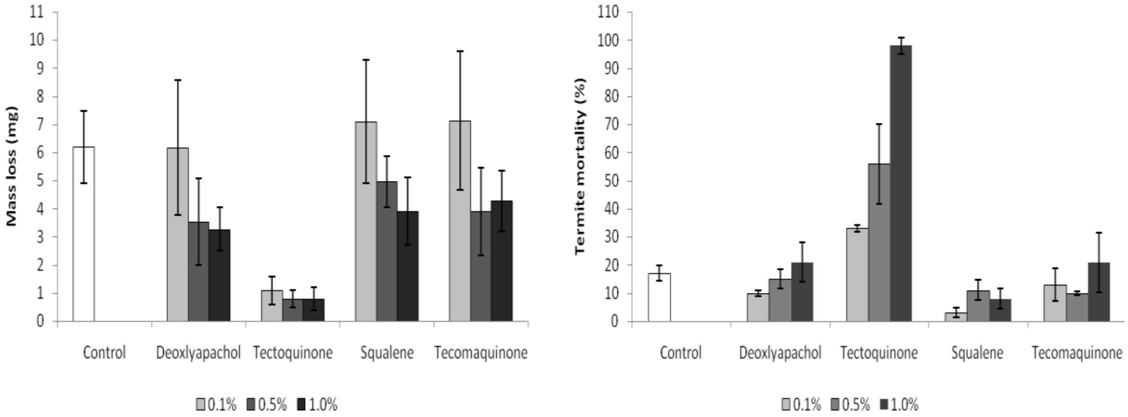


Figure 4. Mass loss (a) and mortality rate (b) against *Reticulitermes speratus* on 10-day observation of teakwood components by treatment retention (based on paper disc weight). Mean of 3 replications (with the standard deviation error bar.

Anti-termite Test for Tectoquinone-squalene Mixtures

As tectoquinone exhibited the highest termite mortality rate, the interaction with other compounds warrants future study. In this experiment, squalene, which had a concentration that was consistently higher than tectoquinone in the wood (Table 1), was mixed with tectoquinone. It is assumed that squalene in a high amount would reduce the effectivity of tectoquinone. The total concentration of mixture was determined for 2% of mass weight in different ratios i.e. 1:1, 5:1, 10:1, and 20:1 or equivalent to tectoquinone concentration of 1.0%, 0.4%, 0.2%, and 0.1%, respectively. The ratios range was set to variation found in Table 1 and the results are summarized in Figure 4.

The ANOVA revealed that there was a statistically significant difference for component factor (p<0.01) for both mass loss and termite mortality levels. By Duncan's test, it was found that the mass loss was less affected (mass loss = 1.2 mg) even until squalene concentration was 20 times as high as tectoquinone concentration and compared to tectoquinone alone in 1% concentration (mass loss = 1.09 mg). This finding suggests that squalene partly affects the antifeedancy against termites. On the other hand, the 1:5 ratio had a mass loss of 2.69 mg. Furthermore it was also found that the addition of squalene in the mixture 1:1 did not follow the trend of mass loss in a linear pattern. This mixture proved to more effective than tectoquine alone.This was an unexpected result as different ratios gave lower mass loss values. The cause of this result as yet unknown, but may be related to the effectivity of squalene to dissolve tectoquinone in the mixture or distribution of tectoquinoe in the paper disc.

With regard to toxicity, tectoquinone alone (in 1% concentration) significantly showed the highest value (mortality rate = 85%). After mixing tectoquinone with squalene, a decrease in termite mortality was observed although not linearly related. The average values for squalene/tectoquinone ratio of 1:1, 5:1, 10:1, and 20:1 were 72%, 65%, 49% and 47,5%, respectively. The highest termite mortality reduction was equal to 44% which showed by 20:1 ratio. Furthermore no significant difference was noted in Duncan's test between termite mortality level of 20:1 dan 10:1 ratios. The ratio of 1:1 or equal to 1% tectoquinone and 1% squalene concentrations showed termite mortality of 72% or 15% reduction from the initial value.

The high content of squalene is theorized to affect the natural durability of teakwood in the form of a hydrophobic barrier (Windeisen *et al.* 2003). In this experiment, it was demonstrated that squalene could reduce termite mortality considerably but less for feeding deterrent. However, other components in a significant amount that could not be detected by GC-MS may also contribute towards anti-termitic properties. Previously, it was postulated that natural rubber or caoutchouc in teak might improve the decay resistance by a synergetic effect with bioactive extracts (Yamamoto *et al.* 1998). The other components might be non-structural sugars that have been associated with decay resistance in teak (Niamké *et al.* 2011). Therefore, further research is needed to explore the role of other the other inactive components in teak and their ability to affect the natural durability.

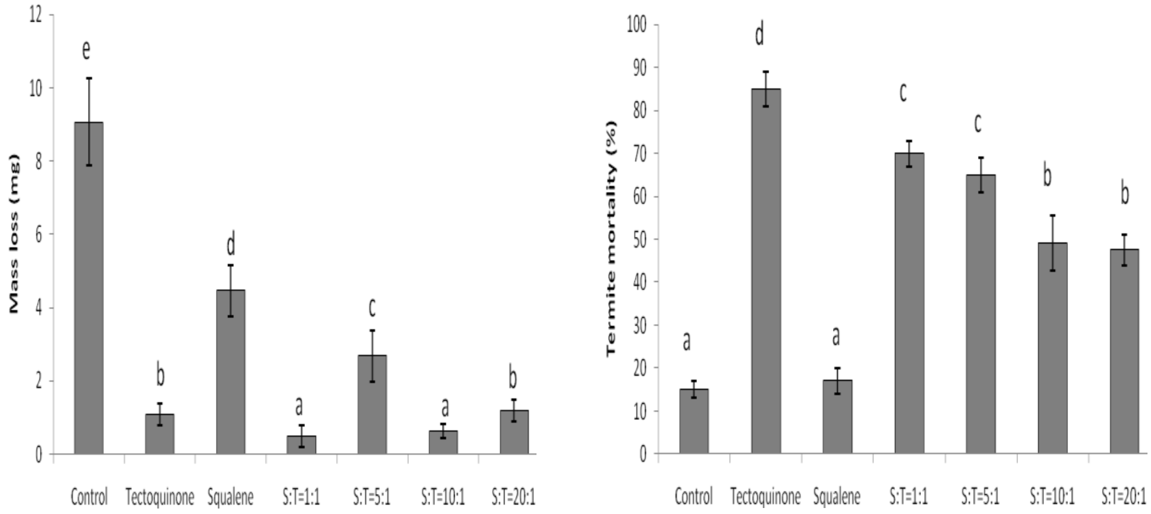


Figure 5. Mass loss (a) and mortality rate (b) against *Reticulitermes speratus* on 10-day observation of squalene and tectoquinone mixtures. Treatment retention (based on paper disc weight) of only squalene or tectoquinone was 1%. Treatment retention of squalene and tectoquinone mixtures was 2%. Mean of 3 replications (with the standard deviation error bar. The same letters on the same graphic are not statistically different at p < 0.05 by Duncan's test.

Conclusions

Under natural condition, teak wood is not toxic but merely deters the *Reticulitermes speratus* Kolbe termites. Therefore, the anti-termite activity of main extractive components of teak (tectoquinone, tecomaquinone, deoxylapachol, and squalene) was evaluated by no-choice tests. The activities of these compounds were evaluated in several concentrations (0.1%, 0.5%, and 1.0%, based on paper disc weight). It was revealed that only tectoquinone showed strong toxicity whereas the other compounds merely showed a moderate level of deterrent effects or antifeedant properties. As the most abundant constituent in the etanol-benzene extracts of teakwood, the effect of squalene to tectoquinone efficacy was also evaluated. Various mixture concentrations have been set in the proportions present in teak (squalene/tectoquinone = 1:1, 1:5, 1:10, and :20). It was observed that squalene reduced the termite mortality of tectoquinone 15% to 44% from its initial value (single tectoquinone compound). The mass loss that indicated the repellency, however, was less affected by squalene addition.

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