Efficacy of tannin extracts isolated from acacia bark waste against the subterranean termite *Coptotermes gestroi* Wasmann

by

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Abstract

Efficacy extracts of acacia bark waste from *Acacia crassicarpa* and *A.mearnsii* against *Coptotermes gestroi* was examined using no-choice and three-choice feeding tests. The results showed that *A.mearnsii* extract has higher tannin than *A.crassicarpa* and age of the tree is assumed to contribute on tannin yield. In the no-choice test, the number of surviving termites and percent mass loss of treated paper were observed every two days fortwo weeks. Mortality rates shows no significant difference with concentration of *A.mearnsii* and a slight difference for *A.crassicarpa* extracts. In the no-choice tests, both Acacia species showed significant differences in percent mass loss, while slight differences in toxic effects wereonly obtained with *A.crassicarpa* extract. The condensed tannin and hydrolyzable tannin, ethyl acetate extract (EA) and water-soluble extract (WS) were examined in a three-choice feeding test to evaluate termite feeding deterrent activity. Each sample was appliedto paper discs at two doses. Based on the, percent mass lossresults the higher dose was more deterrent. Both 70% acetone extracts from *A.crassicarpa* and *A.mearnsii* are potentially useful as a termite control agent. They act as feeding a deterrent, whereas both EA and WS from *A.mearnsii* provided higher feeding deterrence than *A.crassicarpa*.

Key words : Termite-feeding-deterent, Tannin, *A.crassicarpa, A.mearnsii, C.gestroi*

Introduction

As an industrial byproduct, bark has not been utilized properly. In small portions, bark waste has been used as pulp chips, glue-laminated timber and on an industrial scale they are used as fuel for co-generation or as firewood. Tannins are present at high concentration in bark and there is rising interest in the development of tannin utilization rather than leaving it as a waste product of the pulp industry. Many studies on tannin utilization as a wood preservative have been reported, either as extracts or chemical tannin-modification, for use as a decay inhibitor for *Trametes versicolor* or for termicidal resistance against *Coptotermes formosanus Shiraki* (Yamaguchi et al., 2001; Ohmura et al., 2000). In addition, Ohara et al., 1994 reported antitermite activity from *Acacia mearnsii* bark extract condensed tannin showing 100% termite mortality in 18 days.

In the initial stage of study, this experiment aimed to discover a use for acacia bark waste as a wood preservative, particularly on anti-termite activity towards the subterranean termite *C.gestroi*

Wasmann. The utilization of natural extracts as a wood preservative could be indicated from its function as a repellent, feeding deterent or toxicity toward the pest target. Thus, this study was carried out to examine the toxicity and feeding deterrence using *C.gestroi* because it is considered the most destructive subterranean termite species in South East Asia.

Materials and Methods

Tannin extraction

Bark powder of acacia extract, both *A.mearnsii* and *A.crassicarpa* was soaked in 70% acetone at room temperature for 4 days. The filtrate extracts were evaporated to remove solvent and freeze-dried. The freeze-dried extract samples were extracted with ethyl acetate and water (4:1 v/v) in a 1 L vacuum funnel flask. The ethyl acetate and water layers were concentrated by rotary evaporator and the resulting ethyl acetate extract (EA) and water-soluble extract (WS) were tested.

Termite assay

The subterranean termite, *C.gestroi* were collected from a laboratory colony maintained in R&D unit for Biomaterial, LIPI, Cibinong, Indonesia.

A termite toxicity bioassay was conducted in this study using 70% acetone extract at various concentration (1%, 2%, 5% and 10% w/v), respectively. About 60µl of each concentration was impregnated into a paper disc (thickness = 2mm,) with 70% acetone as solvent, dried at 60^{o}C for 1h and then put in petri dishes (Ø = 60mm, high = 15mm). Fifty workers and five soldierswere introduced into petri dishes. Three replicantions were carried out in covered petri dishes in a dark room. To keep the humidity, water was constantly added during the test period usingplaster of paris. Termite mortality was observed every two days for 14 days and mass loss of the paper disc determined at the end the test.

A three-choice feeding test was performed using the EA and WS extract with 1% (w/v), respectively. A 60µl dose of each concentration was impregnated into paper discs using two doses dose #1 (one dose) and dose #2 (two times) as treatment and then dried at 60^{o}C for 1h to evaporate solvent. For control, 70% acetone was used as solvent to impregnate the paper discs and dried in the same manner for the treatment discs. Feeding deterent bioassay was undertaken with fifty worker and five soldier of *C.gestroi* for 3 days in a dark room. Three replicates were conducted for control and each treatment. To measure the feeding deterent activity mass loss of paper disc determined at the end of the test period.

Difference in mass loss by treatment, concentration and period were determined using one-way analysis of variance (ANOVA) and means were separated using Tukey's HSD.

Results and Discussion

Results of extract yield percentage are shown in Table 1. *A.crassicarpa* yields 1/7 the amount ofextract as did thebark waste sample (WS). Extract yields from 70% acetone for *A.mearnsii* and *A.crassicarpa* were 34.79% and 19.14%. Compared to other acacia species, this bark has lower extract content for 70% acetone, while tannin yields from whole bark of *A.mangium*, *A.auriculiformis*, *Rhizophora apiculata*, *Larix leptolepis* were 37.9%, 28.6%, 20.2% and 11.0%. It was confirmed that *A.mearnsii* bark is as good source for tannin. In another study, Ohara et al., 1994 extracted from the inner bark of *A.mearnsii* with 70% acetone and obtained an extract yield of52.7% and 38% for extractive and phenolics, respectively. The factors which affected the quantity of tannin content are tree age, bark part and location. Bark samples of *A.mearnsii* were obtained from Chile that were harvested around 10 years of age. That is probably the reason why tannin content was higher than *A.crassicarpa*.

Table 1. Yield of extract resulted from bark waste of *A.crassicarpa* and *A.mearnsii.*

Name of sample	***A.crassicarpa***	***A.mearnsii***
	(%)[1]	
70% acetone	6.96	34.40
EA	11.66	33.66
WS	94.29	64.11

[1] % of oven-dried bark waste sample

Table 2 shows the mean percent mass loss calculated at the end of period test for both Acacia bark wastes at various concentrations. The mean percent mass loss of the control or solvent was 71.03±1.38 (mean±Sdev). It was confirmed that 70% acetone used as a solvent did not significantly affect termite feeding activity. This bioassay conducted to evalute termite feeding deterrent activity, both of acacia bark waste extracts showed there was a significant difference between concentration and percent mass loss for 1%, 2% and 10%, whereas acacia type did not have any effect. Both of the tree types influenced paper discs consumption by termites, specially at lowconcentrations namely, 1% and 2%. Further investigation, indicated that small concentrations of extract clearly showed how concentration effects feeding deterrence. The total amount of 70% acetone extract impregnated into paper discs for the various concentrations (1%, 2%, 5%,and 10% w/v) were 0.6mg, 1.2mg, 3.0mg and 6.0mg, respectively. Concentration showed a difference in termite consumption rate, extractive compound or tannin content present in acacia effects a reduction in feeding activity.

Table 2. Percent mass loss for the 70% acetone bark waste extract from the no-choice test.

Species	Mass loss (%)[i]				
	0 % (Control)	Concentration (w/v,%)			
		1%[cd]	2%[bc]	5%[b]	10%[a]
A.crassicarpa[b]	71.03±1.38	31.4±24.1	20.1±10.0	18.0±9.5	4.0±3.2
A.mearnsii[b]		30.9±4.9	26.6±9.3	2.9±4.3	2.0±2.4

[i]) Each figure is the mean±standard deviation

Mean (±SD) of 3 replicates foe each species. Percentages values followed by the same letter are not significantly different in the same group at the 0.05 level of probability.

Figure 2 shows the number of alive termites calculated every two days during the test period. For the control, after two weeks the number of live termites was about 27 termites or only 50% survivorship. This result suggests two possibilities. One is the influence of the solvents, and another is that paper discs are not better than wood as a food source for termites because it contains only cellulose. In the *A.crassicarpa* treatment, the number of live termites is inversely proportional to concentration, wherein increasing concentration decreased the number of surviving termites. Meanwhile, *A.mearnsii* results show a mortality relationship directly proportional to concentration. Therefore, *A.crassicarpa* extract has an effect on termite mortality.

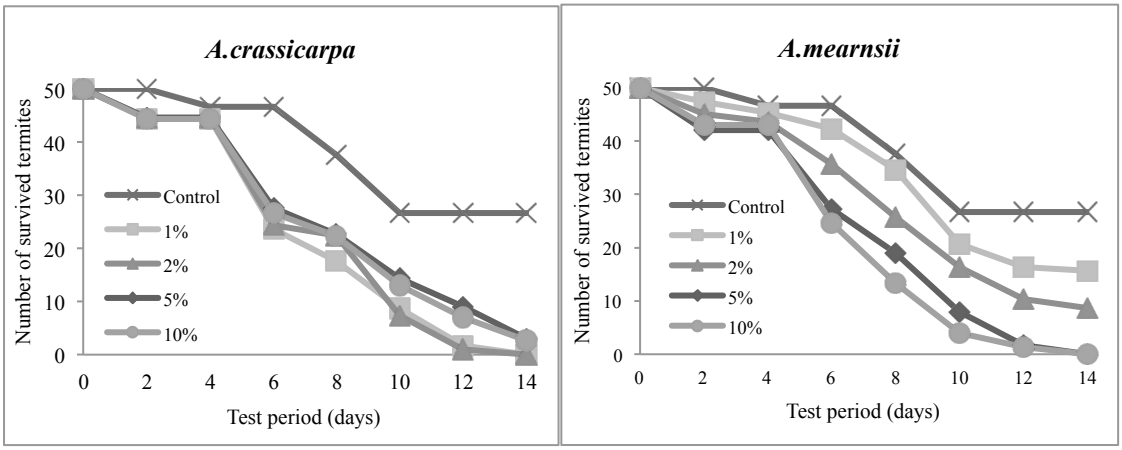

Figure 2. Number of surviving termites from the no-choice test.

Accordingly, it was confirmed that acacia extract did not show toxicity towards *C.gestroi* but acts as a feeding-deterrent as shown by the reduced paper disc consumption by termites.

To evaluate feeding deterrent activity from condensed and hydrolyzable tannin, three-choice tests were conducted using *C.gestroi*. The results from the EA and WS extracts from *A.crassicarpa* and *A.mearnsii* are shown in Table 3. At two different concentrations the mass loss of dose #1 is higher than dose #2 but there is no significant difference as determined by the statistic analysis. Percentage mass loss for *A.crassicarpa* shows that EA was higher than WS at dose #2, it means that WS shows

a higher feeding deterrent activity than EA. On the other hand, there were significant differences between the two extracts (EA and WS). In this study, both EA and WS from *A.mearnsii* showed remarkable activity by decreasing termite consumption. Condensed tannin and hydrolyzable tannin which were contained in the bark effected termite consumption because it caused inactivation of enzyme in termite digestion system (Ohara et al., 1994; Tascioglu et al., 2012).

Table 3. Percent mass loss of EA and WS extract bark waste evaluated in a three-choice test.

Species	Extract	Mass loss (%, **Mean± Sdev)**		
		Control	Dose #1[b]	Dose #2[b]
A.crassicarpa	EA[b]	31.4±4.8	17.6±2.0	14.0±1.6
	WS[b]	26.4±5.6	20.8±2.2	6.3±1.3
A.mearnsii	EA[a]	37.7±1.3	22.0±0.7	33.0±6.1
	WS[ab]	29.6±6.6	13.8±1.8	10.5±1.0

Mean (±SD) of 3 replicates foe each species. Percentages values followed by the same letter are not significantly different in the same group at the 0.05 level of probability.

In fact, many factors effect termite activity from natural extractives such as compound type, quantity and also extract retention activity. Our previous work on these extracts reported that EA showed a similar tannin dimmer and only has a difference on the C5 functional group. Two potential tannin dimmers of *A.crassicarpa* were identified as 5-hydroxyfisetinidol-(4α-8)-galocatechin and 5-hydroxy-robinetinidol-(4α-8)-galocatechin while in *A.mearnsii* we identified fisetinidol-(4α-8)-catechin and Robinetinidol-(4α-8)-catechin. Quercetin that has a hydroxyl group in the C-5 A-ring showed higher antifeedant activity than fisetin. This indicates that the C-5 and C-7 hydroxyl groups in the A-ring are important for anti-termite activity.

Further study is needed to evaluate this phenomenon using pure compounds isolated from acacia bark waste, either from *A.crassicarpa* or *A.mearnsii.* Finally, that information can enhance tannin utilization for establishment a use for this waste product of the pulp industry.

Conclusion

In summary, both 70% acetone extract from *A.crassicarpa* and *A.mearnsii* are potentially useful as a termite control agent. They act as a feeding deterrent, whereas both EA and WS from *A.mearnsii* contained a higher concentration than *A.crassicarpa*

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