

Efficacy of Crude Extracts from Indigenous Plant Species against Philippine Termites

by

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Abstract

The toxicity of extracts from four (4) selected Philippines plant species which include tubangbakod (*Jatropha curcas*) Bayati (*Anamirta cocculus*), cashew nut shell (*Anacardium occidentale*) and river red gum (*Eucalyptus camaldulensis*) was evaluated against subterranean termites, Los Baños termites (*Microcerotermes losbañosensis*), Philippine milk termites (*Coptotermes gestroi*) and drywood termites (*Cryptotermes dudleyi*) by topical application method. Likewise yield of extracts from the plants was also determined. Results showed the volume of extracts obtained from the four (4) plant species varied. Likewise, toxicity of the extracts varied according to test insect, concentration and source of plant extract (plant species). Crude extracts of *Jatropha* methyl ester (JME), CNSL and *E. camaldulensis* were highly toxic to Los Baños termites while JME and cashew nut shell liquid (CNSL) were highly toxic to drywood termites and JME was highly toxic to Philippine milk termites (PMT). *Anamirta cocculus* was not toxic to the test insects. Identification of compounds that contributed to toxicity has to be further investigated. Likewise, formulated products from the extracts showed high potential as a promising insecticide.

Keywords: Natural products, plant crude extract Philippine termites, *Jatropha curcas*, Cashew nut shell liquid, *Eucalyptus tereticornis*, *Microcerotermes*, *Coptotermes*, *Cryptotermes*

Introduction

The use of preservatives for control of insect and fungal attack in wood and non-wood forest products has raised health and environmental concerns. Although the risk to workers and the environment can be reduced to acceptable levels through proper handling and use there is still a need to address those concerns to abate possible apprehension of manufacturers and exporters on the use of wood preservatives used in treating raw materials and finished products.

At present, new environment-friendly wood protection systems based on “green” technologies are considered necessary which create a major challenge for manufacturers of wood preservatives worldwide. Thus, the effort to develop eco-friendly wood preservatives from natural sources over the past decade.

This project involves selected Philippine plant species, River Red Gum (*E. camaldulensis*), Bayati (*A. cocculus*), Cashew nut shell (*A. occidentale*) and Tubang-bakod (*J. curcas*) which contain secondary metabolites in the leaves, bark, wood, flowers, fruits and/or seeds reported to be antagonistic to some insects. Through the use of appropriate extraction methods these substances can be made available and applied to wood or bamboo to prevent or control insect and fungal attack. The results presented were obtained from an on-going project funded by the Department of Science and Technology – Grants in Aide (DOST-GIA) of the Philippine Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD), Los Baños, Laguna, Philippines.

Objectives

1. To determine the yield of the crude extracts from selected indigenous plants materials.
2. To evaluate the efficacy of the crude extracts against Philippine termites.

Materials and Methods

a. Collection and Preparation of Raw Materials

The raw materials from Bayati and cashew nut shells were collected from Quezon Province and Bataan, respectively. These raw materials were sorted, cleaned, and sliced/chopped into required particle size prior to extraction. *Jatropha* crude oil and methyl ester from seeds were solicited from the project on "Pilot production and testing of biodiesel from *J. curcas* of the Industrial Technology Development Institute (ITDI-DOST), Philippines. Leaves of *E. camaldulensis* were collected from San Fernando, Pampanga.

b. Extraction of compounds from five plant species

Jatropha

The solicited crude volatile oil was obtained from seeds of *Jatropha*, which were extracted using a mechanical expeller, filtered, and then stored in covered bottles for characterization (2010 ITDI).

Jatropha methyl ester was also obtained from ITDI. The *Jatropha* crude oil was subjected to the trans-esterification process that involved degumming with phosphoric acid; saponification of free fatty acid with lye, methanol and a catalyst was added to produce the methyl ester (ITDI 2010).

Bayatistems

The crude extract of finely chopped bayati was processed by the cold extraction method. A 100g sample was soaked in water for three days in 700 ml of water inside a refrigerated cabinet (8°C). After soaking, the solution was filtered using a Buchner funnel and the filtrate evaporated in a boiling water bath. The final dried samples were stored in labeled bottles.

Cashew Nut Shells

The cashew nut shells were cleaned and extracted using an expeller developed by FPRDI to produce crude CNSL.

***E. camaldulensis* Leaves**

Leaves were chopped into small pieces using a Moulinex cutting machine. The materials underwent steam distillation using a clavenger apparatus to produce essential oils. The steam-distilled essential oils were collected and used in the bioassay against the test insects.

c. Test on the Toxicity of Extracts Against Termites and Powder-Post Beetles

Preparation of crude extracts

The crude extracts were subjected to a toxicity test against wood destroying organisms. The toxicity of various plant extracts were evaluated against test insects using a manually operated topical applicator. The extracts were prepared at various concentrations (10%, 20%, 30%, and 40% v/v). These concentrations gave an initial indication of the efficacy of the extracts and its fractions.

Preparation of Test Insects

Workers of wood-destroying insects included the subterranean termites (Los Baños termites, *Microcerotermes losbañosensis* and Philippine milk termites, *Coptotermes gestroi*) and drywood termites (*Cryptotermes dudleyi*) were collected, kept in trays lined with filter paper and conditioned prior to testing. All test insects were conditioned for 24 hrs prior to toxicity test.

Procedure for Testing

Twenty healthy and active insects were used per treatment and replicated three times. One to two µL of extract were applied on the thoracic region of the test insects, after which they were placed in a petri dish lined with filter paper. The insects were observed after 24 hrs and the mortality recorded. Insects that did not move when touched gently with a soft brush were considered dead.

$$\text{Corrected Mortality} = \frac{\% \text{ test mortality} - \% \text{ solvent mortality}}{100 - \% \text{ solvent mortality}} \times 100$$

Assessment of Toxicity of the Extracts

The degree of toxicity of the treatments to test insects was arbitrarily classified as follows:

% Insect Mortality	Classification of Toxicity
0	Not Toxic (NT)
1 - 25	Slightly toxic (ST)
26 - 50	Moderately Toxic (MT)
51 - 75	Toxic (T)
76 - 100	Highly Toxic (HT)

Results and Discussion

a. Yield of Extracts

Jatropha seeds had an average yield of 25.0% extracted oil (Almanzor et al 2010) (Table 1). Conversion of oil into methyl ester through the double stage trans-esterification process yielded 83.0%-89.0% methyl ester. About 1500ml of water extract from bayati stems yielded 70-80% and CNSL had an average crude extract yield of 38% to 40%. Chopped leaves of *E. camaldulensis* had an average yield of essential oils of 2% to 4%.

Table 1. Percentage Yield of Plant Extracts.

Plant Material	Common Name	Percent Yield
		Water or Crude Extract
<i>J. curcas</i>	Tubang-bakod	25.0
<i>A. cocculus</i>	Bayati	70.0 – 80.0
<i>A. occidentale</i>	Cashew	38.0 – 40.0
<i>E. camaldulensis</i>	River Red Gum	2.0 - 2.5

b. Toxicity of extracts against Philippinetermites

Toxicity of J. curcas crude extract

Results of the initial screening showed that *J. curcas* crude extract was slightly toxic to the Los Baños termites *M. losbañosensis* (Table 2). *Jatropha* crude extract regardless of concentrations provided 3.3% to 18.3% kill of *M. losbañosensis*. The standard, deltamethrin,

was highly toxic and 100% mortality of test insects was noted after 24 hrs. The standard chemical was highly toxic while crude extract was only slightly toxic to termites. The untreated group had 0% mortality.

The 10% to 30% crude extracts were not toxic to milk termites, *C. gestroi* as shown by 0% mortality of test insects. The highest concentration, 40%, was slightly toxic and provided 6.7% mortality. The standard chemical eliminated 100% of *C. gestroi*. The 10% and 20% crude *J. curcas* extract was not toxic to drywood termites (*C. dudleyi*) while the higher concentrations of 30% and 40% were slightly toxic with 3.3% and 5.0% mortality, respectively. The toxicity of higher concentrations of *J. curcas* was significantly lower than the 100% kill provided by standard chemical.

Treatment	% Mortality		
	Subterranean Termites		Drywood Termites
	<i>M. losbañosensis</i>	<i>C. gestroi</i>	<i>C. dudleyi</i>
1. 10% JcCE	3.3 ST	0 NT	0 NT
2. 20% JcCE	3.3 ST	0 NT	0 NT
3. 30% JcCE	5.0 ST	0 NT	3.3 ST
4. 40% JcCE	18.3 ST	6.7 ST	5.0 NT
5. Deltam - Std	100 HT	100 HT	100 HT
6. Control	0	0	0

Legend: % Insect Mortality(Efficacy of Treatment): 76–100% (Highly toxic); 51–75% (Toxic); 26–50% (Moderately toxic); 1 – 25% (Slightly toxic) and 0% (Not toxic)

Based on the preliminary results, *Jatropha* crude extract was non-toxic to slightly toxic to *M. losbañosensis*, *C. gestroi* and *C. dudleyi*. The zero mortality in the untreated group suggests that the population used was ideal for testing.

Toxicity test of J. curcas methyl ester

The 10% to 40% *Jatropha* methyl ester extract was as toxic as the standard chemical that provided 100% kill of *M. losbañosensis* (Table 3). All test concentrations except at 10% *Jatropha* methyl ester were highly toxic to *C. gestroi* and *C. dudleyi* and the level of toxicity was comparable to the standard chemical. The 20% to 40% *Jatropha* methyl ester caused 65.0% to 85.0% to *C. gestroi* and 91.7% to 96.7% mortality to *C. dudleyi*. The lowest concentration of 10% methyl ester was slightly toxic to both test insects (8.3% to 16.7% kill). No termite mortality was recorded in the untreated group.

Treatment	% Mortality		
	Subterranean Termites		Drywood Termites
	<i>M. losbañosensis</i>	<i>C. gestroi</i>	<i>C. dudleyi</i>
1. 10% Jc-ME	78.3 HT	16.7 ST	8.3 ST
2. 20% Jc-ME	100 HT	65.0 HT	91.7 HT
3. 30% Jc-ME	100 HT	83.3 HT	96.7 HT
4. 40% Jc-ME	100 HT	85.0 HT	95.0 HT
5. MeOH	25 ST	20.0 ST	1.7 ST
6. Deltam - Std	100 HT	100 HT	100 HT
7. Control	0	0	0

Legend: % Insect Mortality(Efficacy of Treatment): 76–100% (Highly toxic); 51–75% (Toxic);

26–50% (Moderately toxic); 1 – 25%(Slightly toxic) and 0% (Not toxic)

Toxicity test of *A. cocculus* crude extract

The crude extract of *A. cocculus* was not toxic to slightly toxic to three species of Philippine termites (Table 4). The crude extract regardless of concentration was slightly toxic to *M. losbañosensis* and mortality of test insects ranged from 3.3% to 13.3%. For *C. gestroi* and *C. dudleyi*, the crude extract was not toxic to slightly toxic as shown by 0% kill in the former and 3.3% to the latter species of test insects. The standard chemical was highly toxic and caused 100% kill regardless test insect. There was no mortality of test insects in the untreated group.

Table 4. Toxicity of *A. cocculus* crude extract to Philippine termites by topical application method.

Treatment	% Mortality		
	Subterranean Termites		Drywood Termites
	<i>M. losbañosensis</i>	<i>C. gestroi</i>	<i>C. dudleyi</i>
1. 10% AcCE	3.3 ST	0 NT	0 NT
2. 20% AcCE	10.0 ST	0 NT	0 NT
3. 30% AcCE	13.3 ST	0 NT	0 NT
4. 40% AcCE	13.3 ST	0 NT	3.3 ST
5. Deltam - Std	100 HT	100 HT	100 HT
6. Control	0	0	0

Legend: % Insect Mortality (Efficacy of Treatment): 76–100% (Highly toxic); 51–75% (Toxic); 26–50% (Moderately toxic); 1–25% (Slightly toxic) and 0% (Not toxic)

Toxicity test of CNSL crude extract against subterranean termites and drywood termites

Table 5 summarizes the results of initial toxicity of CNSL crude extract to Philippine termites. CNSL crude extract regardless of test concentration was as highly toxic as standard chemical with 100% mortality. Likewise, the 20% and 30% CNSL crude extract was highly toxic to *C. dudleyi* providing 95.2% to 100% kill. On the other hand, the crude extract was slightly toxic to *C. gestroi* with mortality ranging from 3.3% to 11.7%. The 40% CNSL crude extract encountered a problem in application because it was too viscous and difficult to apply. No mortality of test insects was noted in untreated group.

Table 5. Toxicity of CNSL crude extract to Philippine termites by topical application method.			
Treatment	% Mortality		
	Subterranean Termites		Drywood Termites
	<i>M. losbañosensis</i>	<i>C. gestroi</i>	<i>C. dudleyi</i>
1. 10% CNSLCE	79.5 HT	3.3 ST	32.9 MT
2. 20% CNSLCE	100 HT	6.7 ST	100 MT
3. 30% CNSLCE	100 HT	11.7 ST	95.2 HT
4. Deltam - Std	100 HT	100 HT	100 HT
5. Control	0	-	0

Legend: % Insect Mortality(Efficacy of Treatment): 76–100% (Highly toxic); 51–75% 1 (Toxic);26–50% (Moderately toxic); 1 – 25%(Slightly toxic) and 0% (Not toxic)

Toxicity test of *E. camaldulensis* crude extract

Only the 40% concentration of the crude extract of *E. camaldulensis* was highly toxic to *M. losbañosensis*. It eliminated 98.3% of the test insects and as highly toxic as the 100% kill caused by the standard chemical (Table 6). Lower concentrations of 10% to 30% were still toxic to termites but caused lower mortality at 66.7% to 73.3% compared to standard chemical with 100% kill. Generally, the crude extract of *E. camaldulensis* was slightly toxic to *C. gestroi* and *C. dudleyi* with mortalities that ranged from 11.7% to 36% and 3.3 to 28.3%, respectively. All untreated test insects remained active at the end of the test.

Table 6. Toxicity of <i>E. camaldulensis</i> crude extract to Philippine termites by topical application method.			
Treatment	% Mortality		
	Subterranean Termites		Drywood Termites
	<i>M. losbañosensis</i>	<i>C. gestroi</i>	<i>C. dudleyi</i>
1. 10% Ec-CE	66.7 T	11.7 ST	3.3ST
2. 20% Ec-CE	73.3 T	16.7 ST	5.0ST
3. 30% Ec-CE	73.3 T	36.0 MT	15.0ST
4. 40% Ec-CE	98.3 HT	60.0 T	28.3 MT
5. Deltam - Std	100 HT	100 HT	100 HT
6. Control	0	0	0

Legend: % Insect Mortality(Efficacy of Treatment): 76–100% (Highly toxic); 51–75% (Toxic); 26–50% (Moderately toxic); 1 – 25%(Slightly toxic) and 0% (Not toxic)

Based on the results of the screening the toxicity of extracts and fractionated forms from five selected plants, the degree of toxicity varied according to plant species, and concentration. Subterranean termites *M. losbañosensis* were more susceptible than Philippine milk termites, *C. gestroi* and drywood termite *C. dudleyi*. Out of the 6 extracts from 5 species of plants 3 of the indigenous materials were found highly toxic to *M. losbañosensis*, 2 extracts to drywood termites compared to only 1 treatment offered high toxicity to *C. gestroi* (Table 7).

Table 7. Toxicity test of the various crude extracts of selected indigenous plant materials against Philippine termites.	
	Test Insects

Treatment	<i>M. losbañosensis</i>	<i>C. gestroi</i>	<i>C. dudleyi</i>
1. <i>Jatropha</i> crude	ST	NT	ST
2. <i>Jatropha</i> Methyl Ester	HT(10-40%)	HT(20-40%)	HT(20-40%)
3. <i>A. cocculus</i> crude	ST	NT	NT
4. CNSL crude	HT (10-30%)	ST	HT(30%)
5. <i>E. camaldulensis</i> crude	HT(40%)	ST-T	ST-MT
Legend:			
% Mortality	Level of Toxicity		
0	Not Toxic (NT)		
1 - 25	Slightly Toxic (ST)		
26 - 50	Moderately Toxic (MT)		
51 - 75	Toxic (T)		
76 -100	Highly Toxic (HT)		

Conclusions

The volume of crude extracts obtained from the four (4) plant species varied. The toxicity of plant extracts from selected plant species varied according to test insect, concentration and source of plant extract (plant species). Out of the five (5) treatments, *Jatropha* methyl ester was highly toxic to Philippine termites regardless of test species. CNSL were highly toxic to *M. losbañosensis* and *C. dudleyi* but slightly toxic to *C. gestroi*. *E. camaldulensis* was highly toxic to *M. losbananosensis* but slightly toxic to *C. gestroi* and *C. dudleyi*. The crude extractsof *Jatropha* and *A. cocculus* were slightly to not toxic to Philippine termites.

The high toxicity of plant extracts indicates an insecticidal potential in preventing and controlling the attack of Philippine termites. However, follow-up screening is being conducted to verify the results of this toxicity of plant materials to Philippine termites.

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