Termiticidal Potential of Sappan (*Caesalpinia sappan*) Seeds

Liwayway H. Acero, Ma. Eliza P. Cruz, and Fedeliz S. Tuy

***Abstract*—Termites (*Coptotermes formosanus*) has been *known as* household destructive insect in rural and urban areas. It feed on wooden scaffoldings and infrastructures of houses and buildings. *Cesalpina sappan* grows in tropical countries, and is known as one of herbal plant use in rural areas. The heartwood is utilized as firewood. Researches on its medicinal use, was already explored, but no study on its potential as control to termites is conducted. This research was undertaken to determine what concentration of *Sappan* seed ethanol extract (SSEE) will kill termites. Houses and buildings in rural and urban areas infested with termites will be benefited with the result of this study. It is most beneficial in places where *Sappan* seeds are just thrown away, which can be used as potential source of insecticide from plants. Experimental research method with four treatments and 80 experimental animals was used. SSEE was macerated in 95% ethanol. Eighty termites were assigned in four groups/treatments, with 20 termites per treatment. They were exposed to different concentrations as; T-0% SSEE, T_1, 10% SSEE, T_2, 20% SSEE and T_3, 30% SSEE. Gathered data was analyzed using Analysis of Variance (ANOVA) and Fisher Least Significant Difference test (LSD). Findings of the study revealed that highest percentage of mortality after 1 hour observation, was obtained from treatment 3 (30%percent SSEE). Based on this result *Sappan* seeds ethanol extract has a significant potential in the control of termites specifically at 30% concentration.**

***Index Terms*—C*aesalpinia sappan*, wood termites.**

I. Introduction

Termites are highly destructive insects; a termite can cause significant economic damages from paper fabrics to wood constructions. It also constitutes a serious menace to agriculture and forestry [1]. Although, termites are part of a long "Superorder" that includes cockroaches, they are classified separately in a group called "Isoptera" and a new family called "Termicidae". In another hand these pest insects are a large and diverse group consisting of over 2500 species worldwide [2]. Subterranean termites are insects that feed on wood, frequently becoming pests of homes. There are two types of termites commonly encountered by homeowners: the worker and the swarmer. Worker termites are creamy colored, 3-4 mm long, and typically only seen when a mud foraging tube is broken, or infested wood is broken open. Swarmers/soldiers are the reproductive cast of the termite colony. They are approximately 4 mm long and dark brown or black in color. They may or may not have wings, as the swarmers lose their wings shortly after emergence [3].

The use of plant extracts as drugs in agricultural production and home pesticides may have arisen from their potency in treatment of ailments, destruction or reduction of insect pests and wading off of rodents. A vast number of chemical compounds from plant extracts can offer many potential uses or alternatives in pest and agricultural management [4]. Plants are considered to be a chemical factory of inexhaustible resources [5]. The discovery of these plant based pesticides has led to increased use of plant extracts in place of synthetic pesticides and has also shifted attention to the use of natural products in recent years [6].

Sappan (*Caesalpinia sappan*) is a small to medium-sized, shrubby tree, 4-8(-10) m tall; trunk up to 14 cm in diameter; bark with distinct ridges and many prickles, greyish brown; young twigs and hairy buds. Seeds are ellipsoid, flattened, about 18-20mm x 10-12mm in size, brown. It is locally known as *Sibukaw* tree in the Philippines and can be found mostly in the province of Negros Oriental and usually found in the shaded places along rivers. It is mostly used as a native medicine of the Visayan people [7]. Phytochemical screening yielded flavonoids, phenolic compounds, tannins, saponin, protein, oxalic acid, carbonate, oil and fat. The pods contain 40% tannin. Tannin is found in the leaves, 19%, bark and fruit walls, 44% [8].

Indigenous plant base environment friendly insecticide are now gaining popular than commercial insecticide which is known to have damaging effect in the environment. This research intends to tap beneficial potential of Sappan seeds that is eco-friendly termite control agents. The output of this research will be disseminated to partner communities of San Beda College where there is abundance of Sappan in the locality. This study determined the following: mortality every 10 minutes observation for 60 minute: and concentrations of Sappan seed ethanol extract (SSEE) that will give significant result.

II. Methodology

A. Materials

1) Preparation of Sappan Seeds Ethanol Extract (SSEE)

Materials used were; Sappan seeds, 95% ethanol, hammer, kitchen knife, chopping board, mortar and pestle, cheesecloth, Whatman paper no.1, and glass containers for maceration (Fig. 1).

2) For administration of SSEE/exposure technique

For administration of SSEE, the following materials were used: Eighty subterranean termites (*Coptotermes formosanus),*

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The authors are with the Department of Natural Sciences College of Arts and Sciences, San Beda University Manila Philippines (e-mail: lilyacero1@yahoo.com, lhiscruz@yahoo.com, fidelistuy@yahoo.com).

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laboratory gown, gloves, masks and surgical caps, holding tray, clean cloth, filter papers, and disposable syringe (Fig. 2).

3) Cages of experimental animals

This study employed experimental research method, using eighty soldier termites in adult stage. Six pairs of petri dishes with circular filter papers in the bottom of each sterilized petri dish were used. Each Petri dish contained 10 termites thus each treatment was contained in 2 pairs of petri dishes (Table I).

TABLE I: EXPERIMENTAL LAYOUT

T- (0% SSEE)	T_1 (10% SSEE)	T_2 (20% SSEE)	T3 (30% SSEE)
Petri dish A			
T-S1	T1S1	T2S1	T3S1
T-S2	T1S2	T2S2	T3S2
T-S3	T1S3	T2S3	T3S3
T-S4	T1S4	T2S4	T3S4
T-S5	T1S5	T2S5	T3S5
T-S6	T1S6	T2S6	T3S6
T-S7	T1S7	T2S7	T3S7
T-S8	T1S8	T2S8	T3S8
T-S9	T1S9	T2S9	T3S9
Petri dish B			
T-S10	T1S10	T2S10	T3S10
T-S11	T1S11	T2S11	T3S11
T-S12	T1S12	T2S12	T3S12
T-S13	T1S13	T2S13	T3S13
T-S14	T1S14	T2S14	T3S14
T-S15	T1S15	T2S15	T3S15
T-S16	T1S16	T2S16	T3S16
T-S17	T1S17	T2S17	T3S17
T-S18	T1S18	T2S18	T3S18
T-S19	T1S19	T2S19	T3S19
T-S20	T1S20	T2S20	T3S20

B. Methodology

1) Sample size and sampling technique

Eighty termites were used and were assigned equally in four groups. First group T- no SSEE, only filter paper moistened with distilled water in the petri dish, T_1, were exposed to 10% SSEE, T_2, termites were exposed to 20% SSEE and T_3 exposed to 30% SSEE.

2) Care and management of termites

Termites in each treatment were placed in two pairs of sterilized petri dishes. Each treatment/group has 20 samples as shown in the experimental layout in table 1. Extra care was done when termites were transferred from their colony to the petri dishes.

3) Preparation of Sappan Seeds Ethanolic Extract (SSEE)

The method was patterned from several studies with some modification. Sappan premature seeds were sundried for 5 days [8]. Sappan pods were hammered to expose the seeds. Seeds were chopped using kitchen knife and pounded with the use of mortar and pestle [9]. The pounded Sappan seeds were soaked in 95% ethanol for 3 days with frequent agitation The ratio of ground Sappan seeds to solvent (95% Ethanol) is 1:1.5 w/v (500gm of seeds in 750 ml ethanol) (Fig. 3) [10]. The mixture was filtered with the use of cheese cloth and Whatman paper no. 1 and was subjected to rotary evaporation to remove the ethanol (Fig. 4).

4) Application of SSEE/exposure technique

The different concentrations of Sappan Seed Ethanol Extract (SSEE) per treatment was; T- 0% SSEE, T_1-10%, T_2-20% and T_3-30% (Fig. 5). Force-feeding tests were conducted following the procedure adopted by several termiticidal studies using herbal extracts with slight modification [11]-[15] and [16]. Six pairs of sterilized petri dishes (dia. 5.5 cm) were used. Each filter paper in the bottom was infused with 1 ml. of the respective extracts concentrations to the extent that it was fully absorbed (Fig. 6). Infusion was carried out using a syringe. For each concentration a new syringe was used. Distilled water was used for control. Ten termites were subsequently introduced in each petri dishes after which the termites were touched at the abdomen using glass rod to determine its mobility. Each treatment has 20 termites (soldiers). Data for the mortality of the termite was recorded every 10 minutes for one hour [17], [18].

The ANOVA was used to interpret and analyze data, since the experiment involves 4 treatments/groups with 20 samples per group. Fisher Least significant differences test was used to determine significant differences among the treatments.

5) Data gathered

a) *Mortality every 10 minutes of observation*

b) *Behavior of live termites*

Fig. 1. Materials used in preparation of SSEE.

Fig. 2. Materials used in administration of SSEE.

Fig. 3. Maceration of SPEE.

Fig. 4. SSEE in rotary evaporator.

Fig. 5. SSEE in different concentrations.

Fig. 6. Impregnation of SSEE.

Fig. 7. Colony of termites .

III. Results and Discussion

A. Number of Termites, per Treatment on the Start of the Study

Table II shows the number of live termites (soldier) per treatment. To ensure uniformity and avoid bias in the result on the controlled variables, twenty live termites were obtained from the same colony of termites from infested wood in Tarlac, Philippines (Fig. 7).

TABLE II: Number of Termites per Treatment on the Start of the Study

Treatment	-	1	2	3
Number of termites	20	20	20	20
Mean	20	20	20	20

B. Percentage of Mortality per Treatment Every Ten Minutes Observation Period

Table III. A displays the mortality per treatment every 10 minutes of observation. Figure 8 displays the percentage of mortality per treatment. Highest mortality was observed in treatment 3 with 30 % SSEE (90), followed by T_2 with 20% SSEE (80%), treatment 1 with 10 % SSEE (65%). Termites in negative control (T-) were all alive after 1 hour observation. The result showed significant differences among the four treatments as shown in Analysis of Variance (ANOVA- Table III-B). Significant differences exist between pairs of means with different superscripts. - as revealed by Fisher LSD. The result further implies that 10% to 30% of SSEE caused death/mortality of the termites in an hour but much faster time in T_3. The mortality of the termites may have resulted from the biocidal effects of the plants which contains active components [19].

TABLE III. A: Mortality per Treatment Every Ten Minutes Observation Period

Treatment	-	I	2	3
First 10 minutes	0	4	9	9
Second 10 minutes	0	2	1	2
Third 10 minutes	0	2	2	2
Fourth 10 minutes	0	1	1	1
Fifth 10 minutes	0	2	1	1
Sixth 10 minutes	0	2	2	3
Total	0	11	16	18
Mean	0^{a}	2.16^{bef}	2.66^{ceg}	3^{dfg}
% of mortality	0	65	80	90

TABLE III.B: ANOVA

SV	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	62.7	5	12.5	5.02*	0.006	2.90
Columns	32.8	3	10.9	4.37*	0.021	3.28
Error	37.4	15	2.5			
Total	132.9	23				

Legend: * significant at 5% level of significance

Crude plant extracts causes toxicity [20] and feeding inhibition [21]. Studies on the effects of higher doses of flavonoids in insects alter normal body functions. The presence of these phytochemical alters some biochemical functions of organisms [22].The effects of flavonoids on the trans hydrogenation, NADH oxidase, and succinate dehydrogenase reactions suggest that compounds of this nature may prove valuable in the control of insect populations by affecting mitochondrial enzyme components [23]. Extracts of *C. sappan* showed broad spectrum activity against both gram-positive and gram-negative bacteria and fungi attributed to the identified alkaloids and tannins [24]. A study on C sappan identified the compound, diterpenoids and flavones [25]. A new cassane-type diterpene, named Phangininoxy A (1) and one known Phanginin A (2) were isolated from the exact of seeds of *Caesalpinia sappan* Linn.[26]. The compound **3** (phanginin D) is one of the main active components of the seed of *C. sappan* activating caspases-3 which contribute to apoptotic cell death [27].

C. Behavior of Termites for One Hour and Ten Minutes Observation.

The behavior of live termites every 10 minutes is shown in Table IV. On the first 10 minutes, experimental animals are lethargic /weak and evaded the SSEE area (Fig.9).The pungent odor and the tannin content of SSEE had caused insects to evade the area where SSEE is impregnated [28]. On the 2nd 10 minutes, the insects wobbled. On the third 3rd 10 minutes, remaining termites were totally weak and lie on their back, but still moving if touch by glass rod in their abdomen. Termites in negative control (T-) were all active. Dipentene is found in Sappan seeds are also found in the following products: Pesticides, Dipentene is a known skin and eye irritant. Ingestion of dipentene can irritate the gastro-intestinal tract [29]. It is a colorless liquid with a lemon-like odor. It is used as a solvent, in rubber compounding and reclamation, and to make paints, enamels, lacquers and perfumes. It is also used as an active ingredient in pesticides and insecticides. It may cause a skin allergy. If allergy develops, very low futurefuture exposure can cause itching and a skin rash [30].

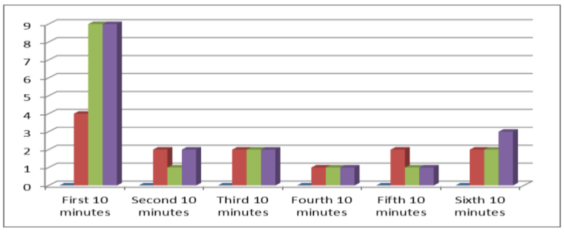

Fig. 8. Percentage of mortality per treatment.

TABLE IV: BEHAVIOR OF TERMITES FOR ONE HOUR OBSERVATION

Treatment	-	I	2	3
First 10 minutes	active	weak	weak	weak
Second 10 minutes	active	woobles	woobles	woobles
Third 10t minutes	active	Lie on their back	Lie on their back	Lie on their back
Fourth 10 minutes	active	Lie on their back	Lie on their back	Lie on their back
Fifth 10 minutes	active	Lie on their back	Lie on their back	Lie on their back
Sixth 10 minutes	active	Lie on their back	Lie on their back	Lie on their back

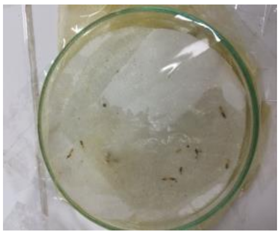

Fig. 9. Termites evade the SSEE area.

IV. CONCLUSION

The result of this study expressed that Sappan seeds ethanol extract is a potential source of termiticidal agent and it is more potent as the percentage of concentration of SSEE increased. With this significant result, Sappan seeds particularly the premature one, can be explored as source of insecticide, specifically in areas where Sappan seeds are abundant and where termites cause severe damage to properties.

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Liwayway H. Acero is a member of Asia Pacific Chemistry, Biology, Environment, Engineering Society, editorial member for Global Science and Technology Forum and Palawan Scientist. She is one of the Technical panels of the International Journal of Food Engineering and Technology. Board of Director BIOTA U-Belt Chapter. Educational background: DST Biology in 2009 from the University of the Philippines-Open University in Los Banos Laguna. She got her doctor of education from Palawan State University on March 2003. She conducted her dissertation at Okayama University Graduate School Education in Japan on March to June 2000 as research fellow. She received her Master of Science degree in agricultural education-Plant Science (Agronomy) from the Western Philippines University in Aborlan, Palawan, Philippines on April 1993. She got her Bachelor of Science degree in Agriculture (**cum laude**), major in Animal science and minor in Plant Science (agronomy) from the Western Philippines University in Aborlan, Palawan Philippines on April 1986.

She is a professor and the chairperson of the Department of Natural Sciences, College of Arts & Science in San Beda University, Mendiola, Manila, Philippines. She is a recipient of numerous academic awards and scholarships as; Professorial Research Chair in 2017-San Beda College Alumni Foundation, Professor Brand Awardee for International Research presentation in 2015-San Beda College, College of Arts and Sciences, Faculty Association, Professor Brand Awardee for Community Involvement in 2015 San Beda College, College of Arts and Sciences, Faculty Association, Best Professor in Sciences-Students Choice Award in 2013-San Beda College, Department of Marketing. Research fellow in Okayama University Japan-Graduate School of Education in 2000. Science High School scholarship from high school (1978) to College (1986) from Western Philippines University-Aborlan Palawan Philippines.

Prior to her employment in San Beda College in Manila, she had served as professor for 20 years in Western Philippines University in Puerto Princesa City, Palawan, Philippines. She handled several administrative works aside from teaching. She served as assistant dean of Western Philippines University, Puerto-Princesa Campus, Director for Instruction, Department Chairperson of the Education, Department chairperson of the Agribusiness Department & chairperson for the thesis committee.

She has 18 research publications. Sixteen are international publications and can be found in the data-base system. Four are indexed by EBSCO and proquest and twelve are indexed by google scholar and google.

Maria Eliza P. Cruz is an assistant professor at the Department of Natural Sciences of the College of Arts and Sciences of San Beda University Manila. She is also an associate professorial lecturer at the Institute of Education- Graduate Studies of the Far Eastern University and the School of Advanced Studies of The National Teachers College-both schools being her Alma Mater in her Undergraduate (BS Biology, 1998) and Graduate Studies (MAEd- Science Education, 2009 and Ed.D.- Major in educational leadership, 2013) respectively.

She is the 2013 recipient of the Tanglaw Academic Excellence Award for the doctor of education- Major in educational leadership program of NTC, the 2013 Training Grantee of the La Main A La Pate Foundation and the French Embassy to Manila to the 4th Inquiry- Based Science Education Training- Workshop held at Sevres, France June of 2013 and the 2016 recipient of the FEU Publication Award for her Scopus- indexed publication of a full paper for Jurnal Teknologi, a reputable journal in Malaysia. In 2014, she acted as Consultant in the Text Translation (French- English- Filipino) of Marie Curie's Lessons in Physics alongside experts from SEAMEO-INNOTECH and UP- NISMED. Currently, she is the Associate Editor of the FEU- IE Graduate Research Journal and a Journal Reviewer for the Global Science and Technology Forum based in Singapore.

Dr. Cruz' research interests are philosophy, science and education.

Fedeliz S. Tuy is currently the executive vice president of the Philippine Federation of Chemistry Societies (PFCS), the internal vice president of the Philippine Association of Chemistry Teachers, Inc (PACT), member of the following professional organizations: Organic Chemistry Teachers Association (OCTA),

She acquired her doctor of education major in educational management in 2006, masters in education major in educational management in 1992, bachelor of science major in chemistry in 1982, all from Far Eastern University in the Philippines.