Sappan (*Caesalpinia sappan*) Seeds in the Control of Cockroach (*Periplenata americana*)

Liwayway H. Acero, Maria Eliza P. Cruz, and Fedeliz S. Tuy
Department of Natural Sciences College of Arts and Sciences, San Beda University, Manila, Philippines
Email: {lilyacero1, lhiscruz, fidelistuy}@yahoo.com

***Abstract*—*Cesalpina sappan* has been known in tropical countries, as one of herbal plant use in rural areas. The heartwood is utilized as firewood. There are studies on its medicinal use, but its potential as control to cockroach is not yet explored**. T**his research was undertaken to determine what concentration of seed ethanol extract (SSEE) will control cockroach. Result of this study is beneficial to rural and urban areas where cockroaches are abundant. 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. Eghty cockroaches were assigned in four groups/treatments, with 20 cockroaches per treatment. They were exposed to different concentrations as; T- 0% SSEE, T_1, 25% SSEE, T_2, 50% SSEE, and T_3, 75% 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 48 hours observation, was obtained from treatment three (75 %percent SSEE). Based on this result *Sappan* seeds ethanol extract has a significant potential in the control of cockroaches specifically at higher concentration.**

Index Terms*—*Caesalpinia sappan, Periplenata americana

I. Introduction

According to World Health Organization (WHO) as [1], more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases.

In the Philippines, traditional medicine (or folklore medicine) has been widely practiced even during the pre-Spanish period owing to the rich experiences of our "arbularios" in utilizing the abundance of natural resources for medicinal purposes. Most Filipinos, especially those in the rural areas belong to the marginalized groups. Thus, medicinal plants are more popular than commercially prepared drugs, among impoverished sectors of society. These are relatively cheaper, more available, and have been confirmed by traditional herbalists ("arbularyos" or medicine men) of their potency [2].

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 buds hairy, brownish.. Seeds are ellipsoid, flattened, about 18-20mm x 10-12mm in size , brown. *Sibukaw* tree is a native tree in Philippines and can be found mostly in the province of Negros Oriental and usually found in the shaded places along rivers. The *sibukaw* tree is mostly used as a native medicine of the Visayan people [3]. 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% [4].

Cockroaches are known as vectors of pathogens that causes diseases. One of the known diseases that triggered by this pest is asthma. Cockroaches and rodents are present in the homes of many urban residents in the United States [5] and tropical countries like Philippines as well. Besides causing annoyance and stress, they are sources of allergens, and vectors of pathogens [6]. Cockroaches can trigger asthma symptoms sensitized individuals, and they may increase the risk of allergic sensitization [7]. The output of this research will be disseminated to partner communities of San Beda College where there is abundance of Sappan in the locality. The use of commercial insecticide is known to have damaging effect in the environment, thus the use of indigenous insecticide is encourage. This study determine the following; mortality every 6 hours observation for two days and concentrations of Sappan seed ethanol extract 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 cockroaches, laboratory gown, gloves,

Manuscript received February 16, 2019; revised August 12, 2019.

doi: 10.18178/ijpmbs.8.4.123-127

masks and surgical caps, holding tray, clean cloth, litmus papers, cat feed and disposable syringe (Fig. 2).

Figure 1. Materials used in preparation of SSEE.

Figure 2. Materials used in administration of SSEE.

3) Cages of experimental animals

This study employed experimental research method, using eighty male cockroaches in adult stage. Improvised cages with holes were provided.

B. Methodology

1) Sample Size and Sampling Technique

Eighty cockroaches were used and was assigned equally in four groups. First group T- no SSEE in their cages, (T_1), was exposed to 25% SSEE; T_2, was exposed to 50% SSEE and T_3, was exposed to 75% SSEE.

2) Care and management of cockroaches

Cockroaches were caged individually in transparent plastic containers with holes. Cat feed was provided as food in the entire duration of the study. Each treatment/group has 20 samples as shown in the experimental layout in Table I.

TABLE I. EXPERIMENTAL LAYOUT

T- (0% SSEE)	T_1 (25% SSEE)	T_2 (50% SSEE)	T_3 (75 % SSEE)
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
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

3) Preparation of Sappan Seeds Ethanol Extract (SSEE)

The method was patterned from several studies with 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 grinded 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 using cheese cloth and Whatman paper no. 1 and was subjected to rotary evaporation to remove the ethanol (Fig. 4).

Figure 3. Maceration of SPEE.

Figure 4. SSEE in rotary evaporator.

4) Application of SSEE/Exposure Technique

The different concentrations of Sappan Seed Ethanol Extract (SSEE) per treatment was; T_1-25%, T_2-50% and T_3-75% (Fig. 5). The litmus paper soaked in 1 ml extract was placed at the bottom of small improvised cage (Fig. 6). The cage was turned over during exposure, so that the insect was in contact with the extract [11].Each treatment has twenty samples and the cages were kept under observation for 48 hours in the dark environment. The normal, dead and moribund animal was counted from each cage and was recorded every 6 hours observation period. The moribund animals are those whose color who had not turned totally black like the dead but weak limb movement [12].

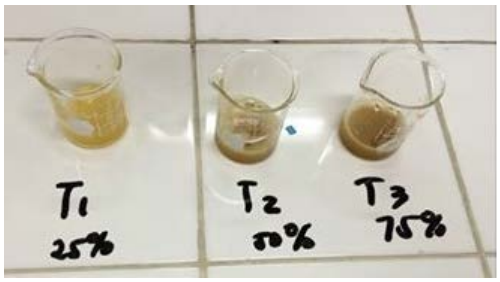

Figure 5. SSEE in different concentrations.

Figure 6. Sample SSEE inside the improvised cage.

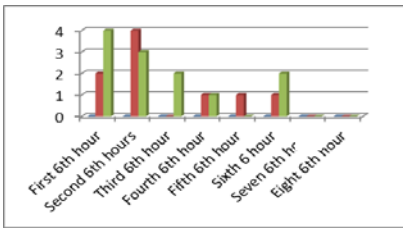

Figure 7. Percentage of mortality per treatment.

The parametric test was used to interpret and analyze data, since the experiment involves 4 treatments/groups with 20 samples per group. To determine significant difference among the treatments, analysis of variance (ANOVA) was used. Fisher Least significant difference (LSD) was employed to determine what treatment is significant over the other.

5) Data gathered.

a. Mortality every 6 hours of observation

b. Behavior of live cockroaches for 2 days observation.

III. Results and Discussion

A. *Number of Cockroach per Sample, per Treatment on the Start of the Study*

Table II shows the number of live cockroaches per treatment. To ensure uniformity and avoid bias in the result on the controlled variables, twenty live cockroaches were obtained from the same source.

TABLE II. Number of Cockroaches per Treatment on the Start of the Study

Treatment	1	2	3
Number of cockroaches	20	20	20
Mean	20	20	20

B. *Percentage of Mortality per Treatment Every Six Hours Observation Period*

Table III shows the mortality per treatment every 6 hours of observation. Fig. 7 displays the percentage of mortality per treatment. Highest mortality was observed in treatment 3 with 75 % SSEE (100 % mortality after 30 hours), followed by T_2 with 50 % SSEE (60% mortality at 48 hours), treatment 1 with 25 % SSEE (45% mortality at 48 hours. Cockroaches in negative control (T-) were all alive after 48 hours.The result revealed that cockroaches with 75% SSEE had the highest percentage of mortality. Analysis of variance (ANOVA-Table IV) resulted to significant difference among treatments with different concentrations of SSEE every 6 hours of observation in favor of T_3 (75% SSEE). Fisher Least significant difference test revealed significant difference between the pair of means of; T-vs.T_1, T- vs T_2, T- vs T_3, T_1 vs.T_3 and T_2 vs T_3 as indicated by different superscripts in the mean. However no significant differences exist between the comparisons of treatment 1 and treatment 2 as indicated in the similar superscripts in the means. The result further implies that 75% of SSEE will cause death/mortality of the cockroaches in much faster time (30 hours).

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 [13]. The effects of flavonoids on the transhydrogenation, 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 [14]. 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 [15]. A study on C sappan identified the compound, diterpenoids and flavones [16}. 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.[17]. 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 [18].

TABLE III. Mortality per Treatment Every Six Hours Observation Period

Treatment	-	I	2	3
First 6th hour	0	2	4	6
Second 6th hours	0	4	3	8
Third 6th hour	0	0	2	3
Fourth 6th hour	0	1	1	2
Fifth 6th hour	0	1	0	1
Sixth 6 hour	0	1	2	0
Seven 6th hour	0	0	0	0
Eight 6th hour	0	0	0	0
Total	0	9	12	20
Mean	0^{a}	1.125^{be}	1.5^{ce}	2.5^{d}
% of mortality	0	45	60	100

TABLE IV. Analysis of Variance (ANOVA)

SV	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	53.21875	7	7.60	4.03*	0.006	2.489
Columns	25.59375	3	8.53	4.52*	0.0137	3.073
Error	39.65625	21	1.88			
Total	118.4688	31				

Legend: * significant at 5% level of significance

C. Behavior of Cockroaches for Two Days Observation

Table IV posited the behavior of cockroaches every six hours. On the first 6th hour, experimental animals are lethargic /weak and evaded the SSEE area of the cage .The pungent odor and the tannin content of SSEE had caused insects to evade the area where SSEE is located [19]. On the second 6th hour, quartile of insects died (sum of T_1, T_2 and T_3) as shown in Table III. The remaining insects exhibited trembling. On the third 6th hour, remaining cockroaches were totally weak and paralyzed/moribund. The antennae are still moving but the entire body is paralyze. Cockroaches 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 [20].

TABLE V. BEHAVIOR OF COCKROACHES FOR TWO DAYS OBSERVATION

Treatment	1	I	2	3
First 6th hour	active	weak	weak	weak
Second 6th hours	active	Trembling	Trembling	Trembling
Third 6th hour	active	Paralysis/ moribund	Paralysis/ moribund	Paralysis/ moribund
Fourth 6th hour	active	Paralysis/ moribund	Paralysis/ moribund	Paralysis/ moribund
Fifth 6th hour	active	Paralysis/ moribund	Paralysis/ moribund	Paralysis/ moribund
Sixth 6 hour	active	Paralysis/ moribund	Paralysis/ moribund	morbid
Seven 6th hour	active	Paralysis/ moribund	Paralysis/ moribund	morbid
Eight 6th hour	active	Paralysis/ moribund	Paralysis/ moribund	morbid

IV. CONCLUSION

With the increasing cost of insecticides in the market, herbal insecticides can be used to maximize the benefits that can be derived from indigenous plants and at the same time environment friendly. The result of this study expressed that the percentage of mortality of cockroaches increased as the concentration of SSEE increased.

ACKNOWLEDGMENT

This work was supported in part by the Office of Research and Innovation, San Beda University, Manila Philippines.

The authors acknowledges, the San Beda University Administrators of the College of Arts and Sciences, San Beda College Manila; Dr. Christian Bryan Bustamante-Dean, Dr. Moses Aaron Angeles -Vice-Dean, for their encouragement. Other administrators of San Beda University Manila; Dr. Nomar Alviar-Vice President for Research and Innovation. Dr. Divina Edralin- Director for Research and Innovation for the financial support and insight. Science laboratory personnel Mr. Danilo Seguban (head), Ms. Rhoda Reyes and Mrs. Benjelyn Inocencio for laboratory support.

REFERENCES

[1] H. Ammara, S. Rahman, F. Deeba, and S. Mahmud, "Antimicrobial activity of some plant extracts having hepatoprotective effects," *Journal of Medicinal Plants Resources*, vol. 3, no. 1, pp. 20-23, 2009.

[2] F. Natividad, J. Lacdan, R. Limpin, I. Balubal, and R. Navarro. (2001). Ethnopharmacologic documentation of selected philippine ethnolinguistic groups: The mangyan (Tadyawan) people of mindoro island. [Online]. Available: http://www.tkdlph.com/index.php/files/9/ethno-research/42/mindoro%20Island%20-%20

[3] K. Mariappan, S. Ramesh, S. Kumar, and K. Surendar, "Caesalpinia sappan L.: Comprehensive review on seed source variation and storability," *Plant Gene and Trait*, vol. 5, no. 2, pp. 11-21, 2014.

[4] T. Chang, S. Chao, and Y. Ding, "Melanogenesis inhibition by homoiso flavavone sappanone A from *Caesalpinia sappan*," *International Journal of Molecular Science*, vol. 13, no. 8, pp. 10359-10367, 2012.

[5] A. Pomés, S. Wunschmann, J. Hindley, L.Vailes, and M. Chapman, "Cockroach allergens: Function, structure and allergen city," *Protein Peptide Let*., vol. 14, pp. 960-969, 2007.

[6] O. Özdemir, "Cockroach allergy, respiratory allergic diseases and its immunotherapy," *International Journal of Immunology. Immunotherapy*, vol. 1, no. 1, pp. 1-5, 2014.

[7] X. Bonnefoy, H. Kampen, and K. Sweeney, *Public Health Significance of Urban Pests*, Copenhagen, Denmark: World Health Organization, 2008.

[8] V. Tychopoulos and J. Tyman, "Long-chain phenol- the termal and oxidative deterioration of phenolic lipids from the cashew (*Anacardiumoccidentale*) nut shell," *Journal of Science, Food, and Agriculture*, vol. 52, no. 1, pp. 71-83, 1990.

[9] I. Ahmad and T. Suliyat, "Development of fipronil gel bait against German cockroaches, blattella germanica (Dictyoptera: Blattellidae)," *Journal of Entomology*, vol. 8, no. 3, pp. 288-294, 2011.

[10] P. Gedam and P. Sampathkumaran, "Cashew nut shell liquid: Extraction, chemistry and applications," *Progress in Organic Coatings*, vol. 14, no. 1, pp. 15-157, 1986.

[11] G. Anand, M. Ravinanthan, R. Basaviah, and A. Shetty, "In vitro antimicrobial and cytotoxic effects of *Anacardium occidentale* and *Mangifera indica* in oral care," *Journal of Pharmacology and Bioallied Sciences*, vol. 7, no. 1, pp. 69-74, 2015.

[12] A. C. Udebuani, P. C. Abara, K. O. Obasi, and S.U Okuh, "Studies on the insecticidal properties of *Chromolaena odorata* (Asteraceae) against adult stage of Periplaneta Americana," *Journal of Entomology and Zoology Studies*, vol. 3, no. 1, pp. 318-321, 2015.

[13] C. Catherine, I. Jude, and I. Ngozi, "Profile of *Chromolaena odorata*," *International Journal of Scientific and Research Publication*, vol. 3, no. 1, pp. 1-2, 2009.

[14] L. H. Acero, "Dried siam weed (*Chromolaena odorota*) as RiceWeevils (*Sitophilus oryza*) eradicant," *International Journal of Chemical Engineering and Applications*, vol. 5, no. 5, pp. 363-365, 2014.

[15] S. Wu, M. Otero, F. Unger, F. Goldring, A. Phrutivorapongkul, C. Chiari, A. Kolb, H. Viernstein, and S. Toegel, "Anti-inflammatory activity of an ethanolic Caesalpinia sappan extract in human chondrocytes and macrophages," *Journal of Ethnopharmacology*, vol. 138, no. 2, pp. 364-372, 2011.

[16] P. Chen and S. Yang, "Flavonol galactoside caffeiateester and homoisoflavones from *Caesalpinia millettii*," *Chemical and Pharmaceutical Bulletin*, vol. 55, no. 1, pp. 655-657, 2007.

[17] M. Nguyen, S. Awale, T. Tezuka, Q. Tran, and S. Kadota, "Neosappanone A, a xanthine oxidase (XO) inhibitory dimeric methanodibenzoxocinone with a new carbon skeleton from Caesalpinia sappan," *Tetrahedron Lett*., vol. 45, no. 1, pp. 8519-8522, 2004.

[18] M. Tran, M. Nguyen, H. Nguyen, T. Nguyen, and T. Phuoung, "Cytotoxic constitutuents from the seeds of *Vietnamese Cesalpina sappan*," *Phtamaceutical Biology*, vol. 53, no. 10, pp. 1549-1554, 2015.

[19] L. H. Acero, "Fresh siam weed (*Chromolaena odorota*) leaf extract in the control of housefly (*Musca domestica*)," *International Journal of Food Engineering*, vol. 3, no. 1, pp. 56-61, 2014.

[20] Y. Xu, J. Zhang, C. Tang, and Y. Ye, "A new diterpenoid from the seeds of *Caesalpinia sappan* Linn," *Records of Natural Products*, vol. 7, no. 2, pp. 124-128, 2013.

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.

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. She is a Professor in Chemistry, Earth Science, and Biology in the Department of Natural Sciences and the Associate for Dissemination & Publication in Research and Innovation Office of San Beda University Manila, Philippines. She had served as Associate Vice Dean for College of Arts and Sciences of San Beda University, Manila 2012-2016. She served as the Assistant Prefect of Student Activities in 2011-2012 She was an assistant professor in Chemistry at Far Eastern University from 1982-1996.