Insecticidal potential of Sappan (*Cesalpinia sappan*) seeds ethanol extract against rice weevil (*Sitophilus oryzae*)

Liwayway H. Acero
San Beda University Mendiola Manila
Correspondence: lilyacero1@yahoo.com
https://doi.org/10.69721/TPS.J.2019.11.1.07

ABSTRACT

Insects are known not only as pests at home but in agricultural farms as well. There are insects that even destroy the quality of farm harvest and decrease its palatability and marketability. One of the very well- known insect pests is the rice weevil (*Sitophilus oryzae*) which damage the quality of rice grain and lowers its market value. Indigenous plant base environment friendly insecticides are now gaining popularity than commercial insecticides. There are many herbal plants in the Philippines which can be used as potential source of insecticide. One of these is Sappan (*Cesalpinia sappan*) which grows well along riverbanks. The aim of this study was to determine which of the following concentrations (T- control, 0%; T_1, 15%; T_2, 30% and T_3, 45%) of Sappan seed ethanol extract (SSEE) will give the highest mortality in 1.5 h of observation. Experimental research method with four treatments and twenty samples of rice weevils were used to gather pertinent data for this study. Sappan seeds were sundried, chopped, macerated in 95% ethanol for 3 days and subjected to rotary evaporation. Data on mortality for 1.5 h with 15 minutes intervals was analyzed using Analysis of Variance and Fisher Least Significant Difference (LSD) Test as post hoc Test. Results revealed significant difference on the means of four treatments. It is imperative that rice weevils in T_3 (45% of SSEE) had highest mortality in 1.5 h exposure. It implied that SSEE is a potential source of insecticide particularly for rice weevils.

Keywords: rice, herbal, pesticide, tannin

INTRODUCTION

Insect pest like weevil destroys rice grains in storage areas causing decrease in quality and palatability. The use of insecticide from herbal plants in lieu of synthetic insecticide is becoming popular in the market because it will not harm the environment and will maximize the use of herbal plants in the locality. This study explored the potential use of Sappan seeds against rice weevil.

In the Philippines, rice is the main energy source for Filipinos. Most farmers grow rice to supply the staple food for the entire populace. Philippines being tropical, favor the existence of insect pests not only in their farm but even on their post-harvest facilities like rice granary. Last August 2018, the Philippine government through its National Food Authority imported rice from Thailand and Vietnam. The quality of the said rice was at stake since it was infested with rice weevil (Macatuno and Jaucian 2018). The rice weevil, (*Sitophilus oryzae)* is a small (2.5 to 4 mm), dark brown insect belonging to order Coleptera and family Curculionide. It has chewing mouthparts at the end of its snout or prolonged head. The adult rice weevil is a dull reddish-brown with round or irregularly shaped pits on the thorax and four light spots on the wing covers. Adult rice weevils live for four to five months and each female lays 300 to 400 eggs during this period. The female uses her strong mandibles to chew a hole in the grain kernel where she deposits a single egg and seals the hole with a gelatinous fluid. Damage to grain caused by this weevil includes reductions in nutritional value, germination, weight and commercial value (Gentry et al. 1991).

One of the herbal trees is Sappan (*Cesalpinia* sappan) locally known as "sibukaw" in the Philippines. This shade loving tree is usually found along the river banks. It is a small to medium-sized shrubby tree, 4-10 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 18-20 mm x 10-12 mm in size, brown. It is commonly used as a native medicine of the Visayan people (Mariappan et al. 2014).

There are studies using the heartwood and leaves of Sappan as medicinal and anthelmintic agent. Mehrothra and Sharma (1984) stated that "Sappan" is considered a valuable astringent, alterative tonic, emmenagogue, blood purifier and anticoagulant. It strengthens the bones and teeth and is also used in boils and eruptions. The use of Sappan leaves as anthelmintic agent showed that, the leaves can serve as a good natural source of potent antioxidants and anthelminthiasis medicines (Harjit et al. 2016). Phytochemical screening revealed the presence of 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% (Chang et al. 2012).

No published study on the use of its seeds as insecticide, hence this study. This study was conducted mainly to investigate the insecticidal potential of Sappan seeds against rice weevil. The result of this study is beneficial, to partner communities of San Beda University-Institutional Community Involvement Center, where rice farming and selling is the main source of livelihood. This study aimed to determine the mortality of rice weevils in different concentrations of Sappan seed ethanol extract (SSEE) every 15 minutes observation, for 1.5 h; describe the behavior of rice weevils in different concentration of SSEE every 15 minutes observation; and determine the significant difference on the mortality of rice weevils in different concentrations of SSEE every 15 minutes observation.

METHODS

This study utilized experimental methods, with four treatments and 20 sample insects per treatment. Each treatment was duplicated. T- (control) no SSEE; T_1, 15% SSEE, T_2, 30% SSEE, and T_3, 45% SSEE.

Preparation of Sappan Seed Ethanol Extract (SSEE).

The method of SSEE preparation was patterned from several studies. Sappan premature seeds were sundried for 5 days (Chang et al. 2012). As reported by Elkhalifa et al. (2005) "pods were generally significantly better than bark in their tannin`s contents and the premature pods were always better than the mature ones." The study of Bourmita et al. (2013), guided the researcher in turning the seeds into smaller parts. Sappan pods were hammered to expose the seeds. Seeds (Figure 1) were chopped using kitchen knife and pounded with the use of mortar and pestle. The study of Tychopoulos and Tyman (1990) served as guide in maceration of ground Sappan seeds to solvent (95% ethanol), which is 1:1.5 weight in grams per volume in milliliter (500 gm of seeds in 750 ml ethanol). The pounded Sappan seeds were soaked in 95% ethanol for 3 days with frequent agitation. 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 (Figure 2).

Figure 1. Pods (left) and seeds (right) of *Cesalpinia sappan* used as insecticide for rice weevils.

Figure 2. Sappan seed ethanol extract (SSEE) in rotary evaporator.

Number of Rice Weevils, per Treatment

To ensure uniformity and avoid bias in the result on the controlled variables, 80 live rice weevils of almost the same size were obtained from the same infested sack of rice from the rice granary of a rice trader in Palanan, Makati City Philippines.

Application of SSEE/Exposure Technique

Rice weevils were exposed to SSEE following the procedures adopted by several insecticidal studies using herbal extracts with slight modifications, (Smith 1979; Gedam and Sampathkumaran 1986; Ahmad and Suliyat 2011; Abbas et al. 2013; Sattar et al. 2014; Edori and Ekpete 2015). Four pairs of sterilized petri dishes (5.5 cm diameter) were used. SSEE was diluted in distilled water, in different concentrations as follows; T1, 15% SSEE, T2, 30% SSEE, and T3, 45% SSEE. The concentration was patterned from the study of Oyedokun et al. (2011) with slight modification. Each filter paper in the bottom of petri dish was applied with concentration of SSEE except for the control treatment. Infusion was carried out using a syringe. Distilled water was used for control (T-). Ten live rice weevils were subsequently introduced in each petri dish. After which the insects were touched at the abdomen using glass rod to determine its mobility. Mortality in this study is described as non-motility of rice weevils even if touch by glass rod on their abdominal part. Dead rice weevils after 15 minutes of observation were transferred in an empty petri dish, as evident of 15 remaining rice weevils in Figure 3 (sample of observation in T3 after 30 minutes of observation). Data for the mortality of the rice weevils was recorded every 15 minutes for one and half hour (Aihetasham et al. 2018; Edori and Ekpete 2015).

Data Gathering Procedure

Mortality per treatment and behavior of insects were observed every 15 minutes interval for 1.5 h of observation. Behavior of insects was described based on the study of Edori and Ekpete (2015). Insects were touched by glass rod in the abdominal region. Non motility denotes mortality. Paralyzes denotes movement of the limbs when touch in the abdominal region. Weakening means slow movement in the petri dish.

Data Analysis

Data on mortality was analyzed using single factor Analysis of Variance (ANOVA) and Fisher Least Significant Difference Test as post hoc test (Gomez and Gomez 1984).

RESULTS

Mortality of Rice Weevils per Treatment for 1.5 h Observation

Mortality in this study is described as non-motility of rice weevils even if touched by glass rod on their abdominal part. Table 1 posited the mortality per treatment every 15 minutes of observation. Highest mortality was observed in treatment 3, 100% mortality, followed by T_2, 70% mortality and T_1, with 65% mortality. No mortality was observed in T- (control). Table 2 (Fisher Least Significant Test) revealed significant differences among the four treatments. Significant differences exist between pairs of means with different superscripts as revealed by Fisher LSD (T- vs T_1, T- vs. T2 and T- vs. T3). The result further implies that 15% to 45% of SSEE caused death/mortality of the rice weevils in 90 min or 1.5 h but much faster time in T_3. Result revealed 45 %SSEE is more potent than the other treatments.

Table 1. Number of dead rice weevils exposed to different concentrations of SSEE every 15 minutes of observation, Mean values with similar superscript are not significantly different.

Time (minutes)	Treatments			
	- (n=20) 0% SSEE	I (n=20) 15% SSEE	2 (n=20) 30% SSEE	3 (n=20) 45% SSEE
15	0	4	6	6
30	0	4	3	5
45	0	3	2	4
60	0	1	2	5
75	0	1	1	0
90	0	0	0	0
Total	0	13	14	20
Mean	0_a	2.166^b	2.33^b	3.83^b

Table 2. Fisher Least Significant Difference Test (Post hoc Test) comparing significant difference among treatment means.

Paired Means	Difference on Paired Means	LSD Value	Analysis
$T-^a$ vs. $T1^b$	2.166	2.033	significant
$T-^a$ vs. $T2^b$	2.330	2.033	significant
$T-^a$ vs. $T3^b$	3.830	2.033	significant
$T1^b$ vs. $T2^b$	0.165	2.033	Not significant
$T1^b$ vs. $T3^b$	1.664	2.033	Not significant
$T2^b$ vs. $T3^b$	1.500	2.033	Not significant

Behavior of Rice Weevils Every 15 Minutes of Observation

Table 3 summarizes the behavior of rice weevils per treatment. On the first 15 minutes, experimental insects are lethargic/weak. Insects moved toward the edge of petri dish (Figure 3). After 30 minutes, insects lie on their back and the legs are still moving. After 45 minutes, remaining insects were totally weak, paralyzed, but still moving if touched by glass rod in their abdomen. All paralyzed insects in T-, T1, T2 and T3 died after 1.5 h of observation. Dead rice weevil is in bent/crooked position (Figure 4). Rice weevils in negative control (T-) were all active.

Table 3. Behavior of rice weevils exposed to different concentrations of Sappan seeds ethanol extract, for 1.5 hours observation.

Time (min)	Treatments			
	- (0% SSEE)	1 (15% SSEE)	2 (30% SSEE)	3 (45% SSEE)
15	20 were found to be active	16 were found to be weak	14 were found to be weak	14 were found to be weak
30	20 were found to active	16 lay on their back	17 lay on their back	15 lay on their back
45	20 were found to be active	17 were paralyzed	18 were paralyzed	16 were paralyzed
60	20 were found to be active	19 were paralyzed	18 were paralyzed	15 was paralyzed
75	20 were found to be active	19 were paralyzed	19 were paralyzed	All insects died
90	20 were active	20 were paralyzed	20 were paralyzed	All insects died

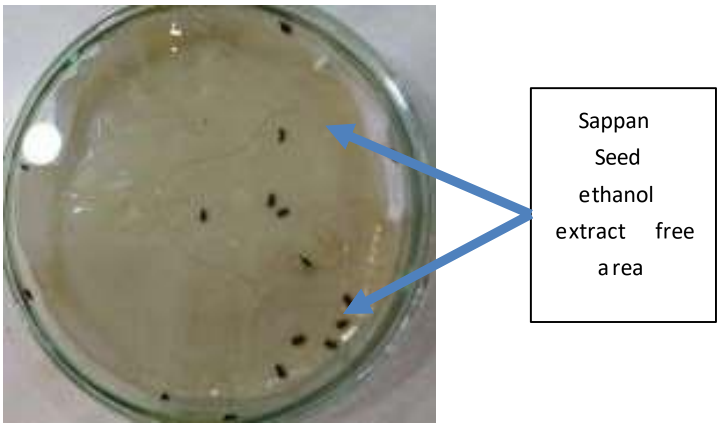

Figure 3. Rice weevils moved to the edge of petri dish (Sappan seed ethanol extract free area) after 15 minutes of exposure to SSEE.

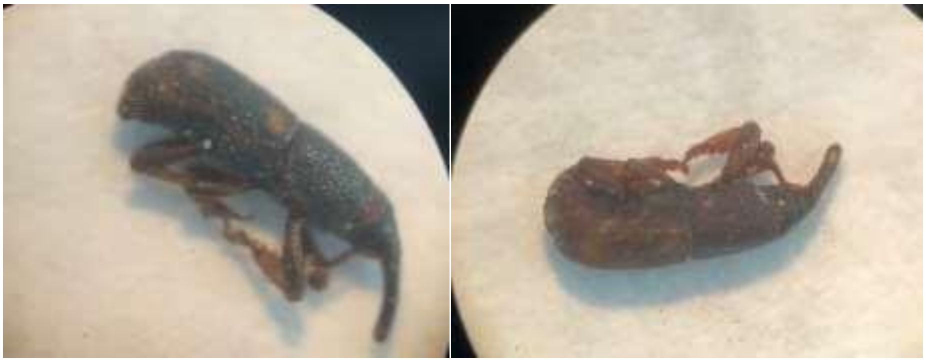

Figure 4. Dead rice weevil (magnified 20 x) in crooked position after 90 minutes of exposure to Sappan seed ethanol extract (SSEE).

DISCUSSION

Mortality of Rice Weevils per Treatment for 1.5 h Observation

Several studies can attest to the mortality of rice weevils in this study. The mortality of the rice weevils may have resulted from the biocidal effects of the plants which contains active components, tannin in particular (Al-Saady 2001). Crude plant extracts cause toxicity (Hiremath and Ahn 1997) and feeding inhibition of insects (Wheeler and Isman 2001). Wu et al. (2011) identified the compound, diterpenoids and flavones in *C. sappan*. Nguyen et al. (2004) isolated a new cassane-type diterpene, named Phangininoxy A (1) and one known Phanginin A (2) from the exact of seeds of *Caesalpinia sappan* Linn. Catherine et al. (2009) stated that effects of higher doses of flavonoids in insects alter normal body functions. The presence of these phytochemical alters some biochemical functions of organisms. 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. Furthermore, Tran et al. (2015) found out that 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. These studies can attest, to the result of this study, that the premature seeds of Sappan can cause mortality of insects.

Behavior of Rice Weevils Every 15 Minutes of Observation

It can be gleaned from Table 3 that rice weevils were affected by the biochemical components of SSEE. Acero (2017) reported that the insects will tend to evade areas with pungent odor and with tannin content. Edori and Ekpite (2015) reported that “Tannin enters the epidermal tissues of insects and causes off-feeding of insects thereby affecting its body movements." The conspicuous/abrupt change in their locomotion is characterized by weakness as early as 15 minutes of exposure to SSEE. After 30 minutes of exposure, paralysis was observed in treatments with SSEE. Touching the abdominal regions of the rice weevils, with glass rod indicates that they are still alive, by movement of the legs even if the insects lie on their back. Paralysis of the entire body except the limbs indicated that the physiologic functions were already disturbed. The paralyzed insects later on die after 1.5 h of observation. Tannin present in Sappan seeds is characterized by astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. Tannins are astringent (mouth puckering) bitter polyphenols and act as feeding deterrents to many insect pests (War et al. 2012).

Another phytochemical component of Sappan seed is dipentene. It is found in pesticides. It is a colorless liquid with a lemon-like odor, and exposure of insects to dipentene causes allergic contact dermatitis (Rycroft 1980). Dipentene is a known skin and eye irritant. Ingestion of dipentene in insects can irritate the gastro-intestinal tract (Xu et al. 2013). Dipentene also called D-Limonene**.** Dipentene in human has different effect as reported by Kim et al. (2013), "*d*-Limonene has been designated as a chemical with low toxicity based upon lethal dose (LD50) and repeated-dose toxicity studies when administered orally to animals. In experimental animals and humans, oxidation products or metabolites of *d*-limonene were shown to act as skin irritants. Painting infested rice sacks with SSEE to eradicate rice weevils is suggested.

With the highest mortality in T_3, it is imperative that Sappan seeds can be used as insecticide (rice weevil) at 45% to attain significant result. Studies on the use of Sappan seeds in different concentrations shall be explored on other insect pests.

ACKNOWLEDGEMENTS

The author acknowledges the insights and valuable suggestions and comments of the two anonymous reviewers.

REFERENCES

Abbas M, Shahid M, Iqbal F, Anjum S, Sharif S, Ahmed T and Pirzada T. 2013. Antitermitic activity and phytochemical analysis of fifteen medicinal plant seeds. Journal of Medicinal Plant Research, 22(1): 1608-1617.

Acero LH. 2017. Fresh siam (*Chromolaena odorota*) weed leaf extract in the control of housefly (*Musca domestica*). International Journal of Food Engineering, 3(1): 56-60. DOI: 10.18178/ijfe.3.1.56-61

Ahmad I and Suliyat T. 2011. Development of fipronil gel bait against german cockroaches, *Blattella ermanica* (Dictyoptera: Blattellidae): laboratory and field performance in Bandung, Indonesia. Journal of Entomology, 8(3): 288-294.

Aihetasham A, Umer M, Akhta MS, Din MI, and Rasib KZ. 2018. Bioactivity of medicinal plants *Mentha arvensis* and *Peganum harmala* extracts against *Heterotermes indicola (Wasmann) (Isoptera)*. International Journal of Bioscience, 7(5): 116-126.

Al-Saady TA. 2001. The effect of some plants extracts on the survival and production of adult of *Callosobrachus maculatus* (Forbicius) (Coleoptera: Bruchidae). MS Agriculture. Basrah University, P.O. Box 49-Basrah Iraq. 85pp.

Bourmita Y, Cheriti A, Didi M, El Hadj O, Mahmoudi M and Belboukhari N. 2013. Anti-termitic activity of aqueous extracts from Saharan toxic plants against *Anacanthotermes ochraceus*. Journal of Entomology, 10 (1): 207-213.

Catherine C, Jude I and Ngozi I. 2009. Profile of *Chromolaena odorata*. International Journal of Scientific and Research Publication, 3(1): 1-2. DOI: 10.3923/pjn.2009.521. 524

Chang T, Chao S and Ding Y. 2012. Melanogenesis inhibition by homoiso flavavone sappanone A from *Caesalpinia sappan*. International Journal of Molecular Science, 13(8): 10359–10367.

Edori OS and Ekpete OA. 2015. Phytochemical screening of aqueous extract of *Icacina trichantha* roots and its effect on mortality of wood termites. World Journal of Pharmaceutical Research, 4(10): 213-224.

Elkhalifa KF, Suliman I and Assubki H. 2005. Variations in tannin's contents of *Acacia nilotica* (L.) Willd. ex Del. in the Sudan. Pakistan Journal of Biological Sciences, 8(1): 1021-1024.

Gedam P and Sampathkumaran P. 1986. Cashew nut shell liquid: extraction, chemistry and applications. Progress in Organic Coatings, 14 (1): 15-157.

Gomez K and Gomez A. 1984. Statistical Procedures for Agricultural Research. John Wiley and Sons INC. USA. 680pp.

Gentry JW, Harris KL and Gentry JW Jr.1991. Microanalytical Entomology Food Sanitation Control. Lithographic Altamonte Springs, Florida. 361pp.

Harjit K, Amini MH and Suttee A. 2016. Evaluation of antioxidant and anthelmintic properties of *Caesalpinia sappan* L. Leaves. International Journal of Pharmacognosy and Phytochemical Research, 8(2): 362-368.

Hiremath IG and Ahn YJ. 1997. Parthenium as a source of pesticide. Conference paper, First International Conference on Parthenium management. Dharwad, India, 6(1): 86-89.

Kim YW, Kim MJ, Chung BY, Bang DY, Lim SL, Choi SM, Lim DS, Cho MC, Yoon K, Kim HS, Kim KB, Kim YS, Kwack SJ and Lee BM. 2013. Safety evaluation and risk assessment of *d*-Limonene. Journal of Toxicology and Environmental Health, Part B, 16(1): 17-38. DOI: 10.1080/10937404.2013.769418

Macatuno A and Jaucian MB. 2018. NFA: 330,000 bags of imported rice infested with weevils. https://newsinfo.inquirer.net/1024529/nfa-330000bags-of-imported-rice-infested-with-weevils#ixzz5g8HyLZzy. Accessed on 21 February 2019.

Mariappan K, Ramesh S, Kumar S and Surendar K. 2014. *Caesalpinia sappan* L.: Comprehensive review on seed source variation and storability. Plant Gene and Trait, 5(2): 11-21. DOI: 10.5376/pgt.2014.05.0002.

Mehrothra S and Sharma HP. 1984. Pharmacognostic studies on "Sappan" (*Caesalpinia sappan* Linn.*)* and its market samples. Proceedings of the Indian Academy of Sciences - Section A Part 3 Mathematical sciences, 93(2): 135-150.

Nguyen M, Awale S, Tezuka T, Tran Q and Kadota S. 2004. Neosappanone A, a xanthine oxidase (XO) inhibitory dimeric methanodibenzoxocinone with a new carbon skeleton from *Caesalpinia sappan*. Tetrahedron Letters, 45(1): 8519-8522.

Oyedokun AV, Anikwe JC, Kelana FA, Mokwunye IU and Azeez OM. 2011. Pesticidal efficacy of three tropical herbal plants' leaf extracts against *Macrotermes bellicosus*, an emerging pest of cocoa, *Theobroma cacao L.* Journal of Biopesticides, 4(2): 131-137.

Rycroft RJ. 1980. Allergic contact dermatitis from dipentene in honing oil. Contact Dermatitis, 6(5): 325-329.

Sattar A, Naeem M and Haq E. 2014. Efficacy of plant extracts against subterranean termites i.e., *Microtermes obesi* and *Odontotermes lokandi* (Blattodea: Termitidae). Journal of Biodiversity Bioprospecting Development, 1(2): 122. DOI: 10.4172/2376-0214.1000122

Smith VK. 1979. Improved techniques designed for screening candidate termiticides on soil in the laboratory. Journal of Economical Entomology, 72(1): 877-879.

Tran M, Nguyen I, Nguyen H, Nguyen T and Phuoung T. 2015. Cytotoxic constituents from the seeds of Vietnamese *Cesalpina sappan*. Pharmaceutical Biology, 53(10): 1549-1554.

Tychopoulos V and Tyman JHP. 1990. Long-chain phenol- the termal and oxidative deterioration of phenolic lipids from the cashew (*Anacardium occidentale*) nut shell. Journal of Science, Food, and Agriculture*,* 52(1): 71-83.

Wheeler DA and Isman M. 2001. Antifeedant and toxic activity off *Trichilia americana* et extract against the larvae of *Spodoptera litura*. Entomologia Experimentalis Applicata, 98(1): 9-16.

War AB, Paulraj MG, Amad T, Buhroo AA, Husain B, Ignacimuthu S and Sharma HC. 2012. Mechanisms of plant defense against insect herbivores. Plant Signaling and Behavior, 7(10): 1306-1320. DOI: 10.4161/psb.21663

Wu S, Otero M, Unger F, Goldring F, Phrutivorapongkul A, Chiari C, Kolb A, Viernstein H and Toegel S. 2011. Anti-inflammatory activity of an ethanolic *Caesalpinia sappan* extract in human chondrocytes and macrophages. Journal of Ethnopharmacology, 138(2): 364-72. DOI: 10.1016/j.jep.2011.09.011

Xu Y, Zhang J, Tang C and Ye Y. 2013. A new diterpenoid from the seeds of *Caesalpinia sappan* Linn. Records of Natural Products, 7(2): 124-128.

ARTICLE INFO

Received: 06 June 2018
Revised: 15 January 2019
Accepted: 24 February 2019
Available online: June 1, 2019