

**Determination of Knock-down Time and Level of Toxicity of Panyawan
(*Tinospora rumphii* L.) Leaf Extract against American Cockroach
(*Periplaneta americana*)**

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Abstract

This paper aims to determine the insecticidal activity of Panyawan (*Tinospora rumphii*) leaf extract against American cockroach (*Periplaneta americana*). A quantitative experimental research design was employed in the study. Cockroach strains were collected through trapping and hand catch methods. Panyawan leaf was collected and extracted to achieve seven different concentrations. The concentrations and controls were tested against the strains through the standard contact glass jar bioassay. Results revealed that the shortest knock-down time was achieved by the positive control at 1.08 minutes while the Extracts of Panyawan leaf reported a 1.42 to 20.25 minutes knock-down time. Probit Regression reported that the lethal concentration 50 of Panyawan leaf extract was 15.836%. One-Way ANOVA revealed that there is a significant difference in the knock-down times of cockroaches when exposed to different Panyawan leaf extract concentrations, negative and positive controls ($p < 0.01$). Homogeneous subsets derived from Post Hoc Test using Tukey's Honest Significant Difference reported that there is no significant difference between positive control and Extract 100% ($p > 0.01$).

Keywords: Panyawan (*Tinospora rumphii*), Knock-down time, Experimental, Davao City

Chapter 1

INTRODUCTION

Background of the Study

According to the World Health Organization (2018), cockroaches have been present for about 360 million years. These insects are harmful pests because they spread filth and destroy fabrics, food, and book-bindings. They expel portions of their partially consumed food at intervals and drop excretions. They also release a nauseous secretion both from their mouths and from the gland openings on the body which gives an enduring, aggressive smell to areas or food visited by them. Cockroaches are not typically the most important cause of a disease, but they may play an additional role in some allergic diseases as well as the spread of some infections. For instance, previous researches found out that cockroach sensitization is an important risk factor for the development of asthma, diarrhea, leprosy, and poliomyelitis (Do, Zhao, & Gao, 2016; Ifeanyi & Omowumi Odunayo, 2015; Paredes & Morales, 2016; Shahraki, Parhizkar, & Nejad, 2013).

Several species of *Enterobacteriaceae* including *Escherichia* spp., *Klebsiella* spp. and *Salmonella* spp. can be harbored in the cuticle of the cockroaches while 63% of the parasites taken from the pest were comprised of hookworm, a human intestinal parasite (Jalil et al., 2012; Oyeyemi, Agbaje, & Okelue, 2016). According to the findings of Tatang, Tsila, and Pone (2017), of the 844 adult cockroaches collected, the medical parasites carried by the pests were *Ascaris* (33.76%), *Trichuris* (11.97%), *Capillaria* (6.16%), *Toxocara* (4.86%), and

Eimeria (2.73%). The Global Burden Disease (2017) found out that these parasites caused more than one million deaths in the year 2017.

The American cockroach, *Periplaneta americana*, is the largest of the common domestic cockroaches in the Philippines. Control of this species has been very difficult to achieve. To keep it under control, different synthetic insecticides have been used. However, the huge extent and continuous application of commercial insecticides resulted in many problems. Among them are toxic residues in food and humans, pollution of the environment, toxicity to non-target organisms, high cost, development of insect resistance strain and workers safety. At present, resistance has become a significant problem that sometimes causes failure of control operations in many countries (Khan et al., 2015). Hence, there is a need to find alternative biodegradable insecticides derived from plant products that are easy to utilize, effective, and safe to environment as well as human health.

The abundance of plants in the Philippines and the huge potential to produce extracts using simple and affordable means is a very significant basis for conducting researches of the effects of natural products from plants against cockroaches. Panyawan, *Tinospora rumphii*, is a climbing vine plant commonly grown and matured in the wild places in the Philippines. Various researches revealed that the Panyawan plant possessed pesticidal and antimicrobial activities towards several pests. For instance, Gutierrez (2015) found out that *T. rumphii* is effective as grain protectant against adult maize weevil, *Sitophilus zeamais* M. pest. Moreover, Aminul, Islam, and Mohammad (2011) stated that the methanolic and ethanolic extracts of the plant have antimicrobial, cytotoxicity and antioxidant

activity. Further phytochemical analysis revealed that the plant leaf contains alkaloids, saponins, flavonoids and tannins that are known to possess medicinal and pesticidal properties (Ahmad, Jantan, & Bukhari, 2016). It provides an alternative to costly drugs and potentially toxic chemical pesticides.

Much of the work regarding insecticide has been done on German cockroach; however, only little data is available concerning American cockroach. Therefore, the present work was designed to evaluate the insecticidal efficacy of Panyawan (*Tinospora rumphii*) leaf extract on *Periplaneta americana*, and the susceptibility of the cockroaches to this formulated insecticide through laboratory bioassay using contact glass jar bioassay. The findings of the study will be valuable for the end users to choose better choices for the management of these pests.

Statement of the Problem

This study aims to determine the insecticidal activity of Panyawan (*Tinospora rumphii*) leaf extract against American cockroach (*Periplaneta americana*). Specifically, this study attempts to the answer the following questions:

1. What is the knock-down time of the cockroaches exposed to Panyawan leaf extract in terms of the following concentrations:
 - 1.1 0.1%
 - 1.2 5%
 - 1.3 10%
 - 1.4 25%
 - 1.5 50%
 - 1.6 75%

1.7 100%

2. What is the level of toxicity of the Panyawan leaf extract on the cockroaches in terms of:

2.1 Lethal Concentration 50 (LC₅₀)

3. Is there a significant difference in the knock-down times of cockroaches when exposed to different Panyawan leaf extract concentrations, negative and positive controls?

Hypothesis

The null hypothesis was formulated and tested at 0.01 level of significance stating:

H1: There is no significant difference in the knock-down times of cockroaches when exposed to different Panyawan leaf extract concentrations, negative and positive controls.

Conceptual Framework

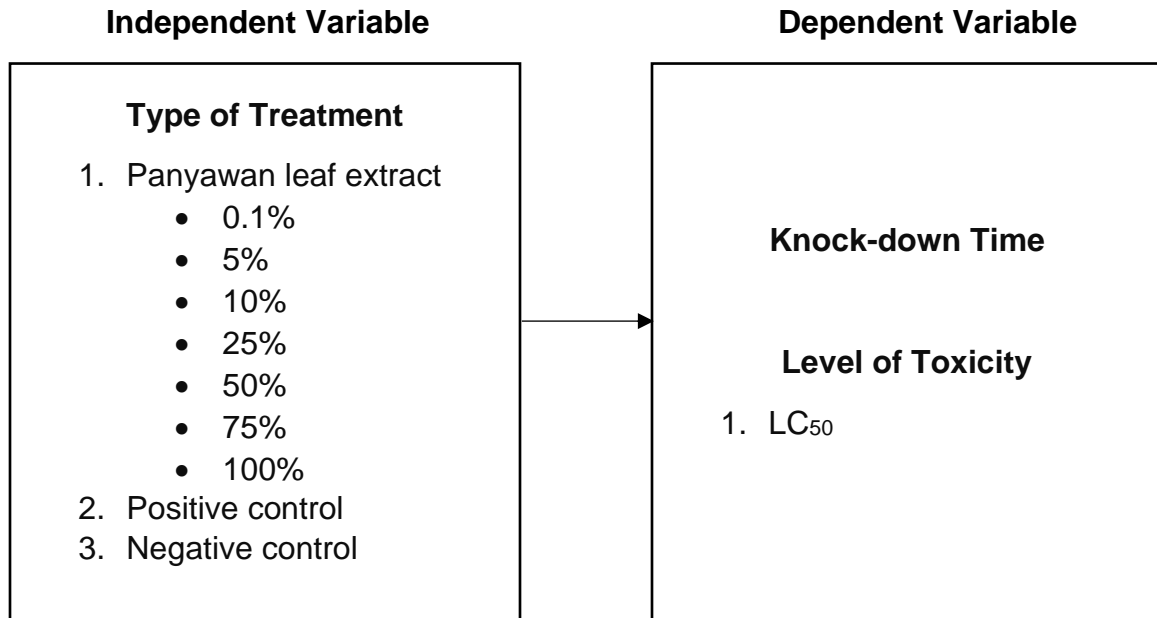


Figure 1. Research paradigm of the study showing the type of treatment will directly affect the knock-down time and level of toxicity

Presented in Figure 1 is the research paradigm of the study showing the type of treatment will directly affect the knock-down time and level of toxicity. As shown in the table, the independent variable is the type of treatment (Panyawan leaf extract), positive control and negative control while the dependent variables are the mortality rate and level of toxicity.

Significance of the Study

This study is important to the following:

Household. The findings of the study will find an alternative insecticide that is cheaper and environment-friendly against the cockroaches. The households in the community can use the product to control the proliferation of the pests.

Students. The study will add to the body of scientific knowledge and hence, will allow the students to explore scientific developments about Panyawan leaf.

Future Researchers. The proposed study will benefit and help the future researchers. The ideas presented may be used as reference data in conducting new researches or in testing the validity of other related findings. This can also open in the development of the study.

Definition of Terms

The following terms were defined operationally for ease and clarity:

Contact glass jar bioassay. This refers to the standard measure of the World Health Organization in testing the efficacy of insecticide against the cockroach strains.

Knock-down time. This refers to the time of mortality of the test subjects during the experiment.

Lethal Concentration 50. This refers to the minimum concentration required to kill 50% of the test subjects.

Toxicity. This refers to the degree to which a chemical substance can harm humans or animals.

Review Related of Literature

American Cockroach (Periplaneta americana)

As stated in the Columbia Encyclopedia 6th ed. (2015), the Cockroaches, *Periplaneta americana*, is a scavenging insect that resembles a beetle, it is usually measures about 13- 16 mm long and weighs under 1.1 oz (30 grams) having long antennae and six long, spiny legs that allow them to run quickly across almost any surface and typically has a broad, flattened body. Under favorable conditions they live for one year throughout the year an adult females produce between six and 14 oothecae in one lifetime. After carrying the egg case on the tip of her abdomen for hours to a couple of days, the female deposits it in a hidden location.

According to Brimner (2016), several tropical kinds of cockroaches have been established worldwide as household pests; house pests are one of the most common problems in our homes. And one of this house pests are the cockroaches. Cockroaches are considered to be the most irritating household pests since it is one of the unhygienic creatures that causes great destruction and contamination of food supplies.

According to the study of Solilan (2015), to support the claims above, they prefer to live in warm, dark, wet areas, like sewers and basements. They often enter structures through drains and pipes.

According to Healthline (2017), in connection with the study of Solilan, moderate to high numbers of cockroaches' can affect rural businesses like restaurants since they can spoil food with feces and urine and can transmit diseases and parasites to humans and livestock (eg salmonella). Although American cockroaches can be found in homes, they are also common in large commercial buildings, grocery stores and hospitals.

As cited by Mella (2010), while other cockroaches have been known to spread some serious diseases to humans such as Salmonella Typhi, which causes Typhoid, Poliomyelitis, which causes Polio, and they can also cause Dysentery

which is one of the common disease that came from cockroach, a disease in the Philippines that causes severe diarrhea that may include bleeding, it has an estimation of more than 500 million of people who are infected with *E histolytica* worldwide. Between 40 000 and 100 000 will die each year, placing this infection second to malaria in mortality caused by protozoan parasites. Dysentery is not one of the seasonal diseases it only happens if the environment is lack of cleanliness.

An article published online by New Zimbabwe (2018), to prove the claims above, a two patients' from Masholand in Mt. Davis experiences a severe diarrhea and vomiting during observation and died after two days. Four days after, the doctor said that the two patients died caused by dysentery.

According to Sander (2014), cockroaches can also be seen in cropping areas and they target most major crops including cereals, legumes, pulses, sorghum and maize. High-protein vegetable crops including peas, beans and chickpeas are also at risk along with intensive vegetable crops such as zucchini, tomatoes, eggplants, capsicums and melons. Summer and winter cereal crops are vulnerable at several stages of development including at sowing, at flowering and during the doughy, milky or pudding stages through to pre-harvest mature crops.

Crop damage is often unnoticed until it is severe. Sometimes cockroach's damage is misdiagnosed as snail or slug damage, or the effect of moisture stress or disease.

According to Somerton (2011), signs of cockroach activity include chewed stems, damage to seed heads or debris at the base of the plant. In cereal crops such as wheat, cockroaches chew the growing stems of the plant to feed on sap, stopping development of the head or causing the stem to collapse.

Cockroaches can drop seed heads by chewing through the top node at flowering and also attack the maturing heads. This can cause losses of up to 50% at pre-harvest stage. In Australia direct and indirect costs of serious plagues can exceed A\$100 million nationally. Individual restaurant owners can suffer partial or complete business loss.

According to Stratford (2010), in connection with Somerton, cockroaches are well recognized by people because of its reputation of being dirty and for invading households. Cockroaches can also cause damage in crop paddocks immediately after sowing by digging into loose soil to find larger seeds such as maize, sunflower, wheat, oats, barley, pulses, pumpkin and marrow. They also eat the newly sprouted seedlings before and after they emerge from the soil. The impact of cockroaches' is not as great on plants beyond the seedling stage, at least not until seeds or grains begin to mature. Plants such as wheat are then damaged by cockroaches gnawing at the nodes on the stems causing developing seed heads to fall. In maturing crops of wheat, oats, barley, pulses, sorghum and maize, losses of up to 30% have been reported. Heavy losses can also occur in vineyards and vegetable crops from eating and fouling of produce.

On the word of Matte (2016), cockroaches can ruin an organization's reputation. If clients and customers spot evidence of insect infestation in the premises you manage, they are likely want not to do business with you.

Insecticide Study

According to Ware, G.W et al (2004) Insecticides are agents of chemicals or biological origin that control insects. That may result from killing the insect or else preventing it from having a large population. Insecticides may be natural or manmade and are applied to target pests in a myriad of formulations and delivery systems. The science of biotechnology has, in recent years, even incorporated bacterial genes coding for insecticidal proteins into various crop plants that deal death to unsuspecting pests that feed on them.

According to Whitacre, D. et al (2004) Some 10,000 species of the more than 1 million species of insects are crop-eating, and of these, approximately 700 species worldwide cause most of the insect damage to man's crops, in the field and in storage. and Humans have been on earth for more than 3 million years, while insects have existed for at least 250 million years. We can assume that among the first approaches used by our primitive ancestors to reduce insect annoyance was hugging smoky fires or spreading mud and dust over their skin to repel biting and

tickling insects. Today, such approaches would be classified as repellents, a category of insecticides.

As stated on the Encyclopaedia Britannica (2018) Insecticides can be classified in a several ways, on the basis of their chemistry, their toxicological action, or their mode of penetration. In the latter scheme, they are classified according to whether they take effect upon ingestion (stomach poisons), inhalation (fumigants), or upon penetration of the body covering (contact poisons). Most synthetic organic insecticides penetrate by all three of these pathways, however, and hence are better distinguished from each other by their basic chemistry.

According to Peter J.V (2000)Organic insecticide poisoning continues to be a major health problem not only in the developing communities but also in the Western population. The insecticides commonly used are the organophosphates, organocarbamates, organochlorides and pyrethroids. Patients with organic insecticide poisoning present with a spectrum of manifestations ranging from gastrointestinal symptoms of nausea, vomiting and diarrhoea to severe neurological manifestations of fasciculations, seizures and neuromuscular weakness and paralysis or cardiac manifestations of arrhythmias and conduction disturbances. A strong clinical suspicion is necessary to make an early diagnosis of insecticide poisoning. Treatment is primarily supportive and includes decontamination, protection of airways and cardiac and respiratory monitoring. Specific therapy for organophosphates and organocarbamates includes the use of anticholinergics. The use of oximes, especially high dose, is controversial and may be associated with a higher mortality rate. Low-dose oximes given early in the course of the illness may be beneficial.

According to Salvisberg, W. (2004) Spontaneous discharges of a tarsal motor nerve preparation of the jumping leg of *Locusta migratoria* were recorded extracellularly under the influence of various insecticides applied to the in situ metathoracic ganglion of the insect. Insecticides from different chemical classes were found to exhibit rather specific electrophysiological symptoms: cholinesterase inhibitors produced groups of action potentials with highly

increased frequencies, and led to the appearance of new units which were not present in untreated control insects. They also caused transitory phases of conduction blocks, the length of which increased as intoxication proceeded. The DDT analog tested could be characterized by pronounced repetitive discharges, whereas the neuroactivity of pyrethroids was typically associated with a very regular spike pattern in continuous phases of extremely high frequencies. Also chlordimeform, a formamidine insecticide, was found to increase the spike frequency, but to a much lesser extent than that observed for the other insecticides tested.

Deng, L., et al (2007). The effects of an organophosphorous insecticide, methamidophos, on the pest control potential of the spider *Hylyphantes graminicola* (Sundevall) (Araneae: Linyphiidae) were investigated in the laboratory with the fruit flies (*Drosophila melanogaster* Meigen). The influence of methamidophos on predation by *H. graminicola* was very obvious in female spiders, which preyed on fewer prey in the 8 h after exposure to the insecticide but subsequently recovered. On the other hand, the predation rates in male spiders were not affected by the insecticide within 24 h of treatment. However, a 10% lethal dose (LD10) of methamidophos resulted in an enhanced predation rate per day for male spiders, whereas a 50% lethal dose reduced the predation rate. In addition, it was shown that the functional response of *H. graminicola* to the fruit fly was a type II response, and the type of functional response of insecticide-treated females changed from type II to type I, with no change in the response of male spiders. The attack rate of males treated with the LD10 dosage of insecticide was significantly higher than the controls, which suggests that the insecticide stimulates the performance of spiders. Prey utilization of males treated with low doses of insecticide was lower than the control, which indicates that the insecticide did not result in these spiders eating more prey, but killing more.

According to Kunkel, D. (1998) An insect repelling composition includes a mineral oil based carrier and, as an active ingredient, an insect repelling amount of cactus extract made from the leaves and stem of the Prickly Pear cactus. A pest

repellant formulation can be prepared by combining an oil based carrier with an insect repelling amount of cactus extract and applying the repellant to the skin, hair or fur of a mammal.

According to Isman, M. (2006) Botanical insecticides have long been touted as attractive alternatives to synthetic chemical insecticides for pest management because botanicals reputedly pose little threat to the environment or to human health. The body of scientific literature documenting bioactivity of plant derivatives to arthropod pests continues to expand, yet only a handful of botanicals are currently used in agriculture in the industrialized world, and there are few prospects for commercial development of new botanical products. Pyrethrum and neem are well established commercially, pesticides based on plant essential oils have recently entered the marketplace, and the use of rotenone appears to be waning. A number of plant substances have been considered for use as insect antifeedants or repellents, but apart from some natural mosquito repellents, little commercial success has ensued for plant substances that modify arthropod behavior. Several factors appear to limit the success of botanicals, most notably regulatory barriers and the availability of competing products (newer synthetics, fermentation products, microbials) that are cost-effective and relatively safe compared with their predecessors. In the context of agricultural pest management, botanical insecticides are best suited for use in organic food production in industrialized countries but can play a much greater role in the production and postharvest protection of food in developing countries.

According to Gutierrez P. (2017) The ethanolic stem extract of *T. rumphii* contains the following phytochemicals: alkaloids, flavonoids, steroids and tannins. These phytochemicals are known to possess pesticidal activities against insects and other pests. The grain protectant efficacy of *T. rumphii* against corn weevils was manifested by the percentage of mortality of weevils, number of corn seed holes; weight loss and percentage corn germination. Results revealed that the corn weevils treated with the highest concentration (18%) of *T. rumphii* manifest the highest percentage of weevils' mortality and significantly higher ($p= 0.000$) as

compared to the negative control and the other treatment concentrations. In addition, the mortality of corn weevils treated with the plant extract is concentration-dependent. The average number of corn seed holes treated the *T. rumphii* extract is statistically lower as compared to the negative control. Moreover, the highest concentration (18%) of the plant extract showed the lowest average number of holes. This implies that *T. rumphii* is responsible for the inhibition of the weevils from infesting the corn seeds. Furthermore, result revealed significant decrease ($p= 0.000$) on the average weight loss of corn seeds previously treated with the plant extract.

According to Schulz, R. (2004). Recently, much attention has been focused on insecticides as a group of chemicals combining high toxicity to invertebrates and fishes with low application rates, which complicates detection in the field. Assessment of these chemicals is greatly facilitated by the description and understanding of exposure, resulting biological effects, and risk mitigation strategies in natural surface waters under field conditions due to normal farming practice. More than 60 reports of insecticide-compound detection in surface waters due to agricultural nonpoint-source pollution have been published in the open literature during the past 20 years, about one-third of them having been undertaken in the past 3.5 years. Recent reports tend to concentrate on specific routes of pesticide entry, such as runoff, but there are very few studies on spray drift-borne contamination. Reported aqueous-phase insecticide concentrations are negatively correlated with the catchment size and all concentrations of >10 micro g/L (19 out of 133) were found in smaller-scale catchments (<100 km²). Field studies on effects of insecticide contamination often lack appropriate exposure characterization. About 15 of the 42 effect studies reviewed here revealed a clear relationship between quantified, non-experimental exposure and observed effects in situ, on abundance, drift, community structure, or dynamics. Azinphos-methyl, chlorpyrifos, and endosulfan were frequently detected at levels above those reported to reveal effects in the field; however, knowledge about effects of insecticides in the field is still sparse.

Panyawan (Tinospora rumphii L.)

(Panyawan is a native plant from tropical and subtropical rain forest in India, Myanmar, Sri Lanka, and Philippines. It belongs to the family of Menispermaceae. It has long history of medicinal use in India. In Ayurveda (Hindu traditional medicine), panyawan is considered one of the most divine herbs. It is use to promote longevity, prevent diseases and to treat various illness from arthritis to cancer. In the Philippines, panyawan extract combined with oil is use to treat rheumatism and arthritis, abdominal pains, scabies, and skin ulcers. According to the research conducted in the Indian Medicine Research Laboratory, panyawan can modulate Glut-4 and the predominant protein that influences glucose metabolism. Thus, it is good for people who have diabetes. However, this medicinal plant is generally for topical application and moderate intake only. Pregnant and lactating women should avoid taking this (Anonymous, 2015).

According to Hook F. & Thomson (2018), Panyawan is a climbing, dioecious vine achieving a tallness of 4 to 10 meters. Stems are up to 1 centimeter thick and somewhat fleshy, with dissipated bulges. Leaves are slender, applaud, 6 to 12 centimeters in length, and 7 to 12 centimeters wide, with pointed and truncate or fairly heart-molded based, smooth and sparkling. Petioles are 3.5 to 6 centimeters in length. Racemes are lone or in sets emerging from axils of fallen leaves, light green, thin, 10 to 20 centimeters in length. Blossoms are light green and short pedicelled. Natural product is 8 millimeters in length, in long groups.

Acording to Estoconing, Descallar, & Pillones (2014), Panyawan Plant (*Tinospora crispa* Linn) is a small herb which grows in temperate and tropical parts of Asia. More specifically, the plant is widely found on tropical and subtropical Philippines, Vietnam, Thailand, Malaysia, Indonesia, India and China and is attributed to its ethno-medical uses since ancient times by traditional healers and physicians (Mohammed et.al. 2012). The aqueous stem extract is known to be effective in ameliorating inflammation and thus supports the folkloric use of the stem in the treatment of various inflammation disorders. As the plant is readily

available in the rural areas, it is a good alternative to costly synthetic drugs. The plant is claimed to be effective for the treatment of various inflammatory disorders like rheumatism and arthritis when prepared as a poultice with coconut oil (Hipol R.,2012).In this study, the presence of secondary metabolites, cytotoxicity, and the effectiveness of Panyawan plant (*Tinospora crispa*) as an anti- inflammatory agent was being investigated.

The result for phytochemical screening showed that Alkaloids and Steroid are the secondary metabolites that are present which determines that Panyawan Plant (*Tinospora crispa*) leaf extract contains secondary metabolites which may have significant effect on human health or can be considered as a therapeutic agent. For cytotoxicity, it showed that the highest percent mortality is at 5 ppm for all the concentrations. The result for LC50 shows that the ethanolic extract concentration exhibit bioactivity which is 4.03ppm. The results of this study verify the findings of Peteros (2010), that the rate of mortality increases as the concentration increases. And for Anti-Inflammatory property, it showed that there is significant difference on five treatments: Indomethacin, croton oil, 25%, 50% and 100% extract concentration of the Panyawan Plant extract. The result showed that the F computed (4.68) is greater than the F critical (3.48) and the p-value is 0.022 which is less than 0.05 level of significance. This means that Panyawan plant (*Tinospora crispa*) leaf extract has anti-inflammatory property.

Chapter 2

METHODOLOGY

Research Design

In this study, a quantitative experimental research design was utilized. According to Creswell (2013), this design is a systematic and scientific approach to research in which the researchers manipulate one or more variables, and controls and measures any change in other variables. Hence, it measures the causal relationship of the independent variable which is the type of treatment and dependent variables which are the knockdown time and level of toxicity. It is this light that this design was used because the researchers want to know if the Panyawan (*Tinospora rumphii*) leaf extract has insecticidal activity against American cockroach (*Periplaneta americana*).

Setting

The experiment was conducted at the University of Immaculate Conception – Bonifacio Campus, De Jesus Street, Poblacion District, Davao City. University of Immaculate Conception is a Roman Catholic higher education institution in Davao City, Philippines administered by the Religious of the Virgin Mary. The researchers conducted the study in this area because of the availability of the materials and machine that were used in the experiment. Specifically, the study was conducted at the Physics Laboratory of the school. The setting of the study is shown through a site map in Figure 2.

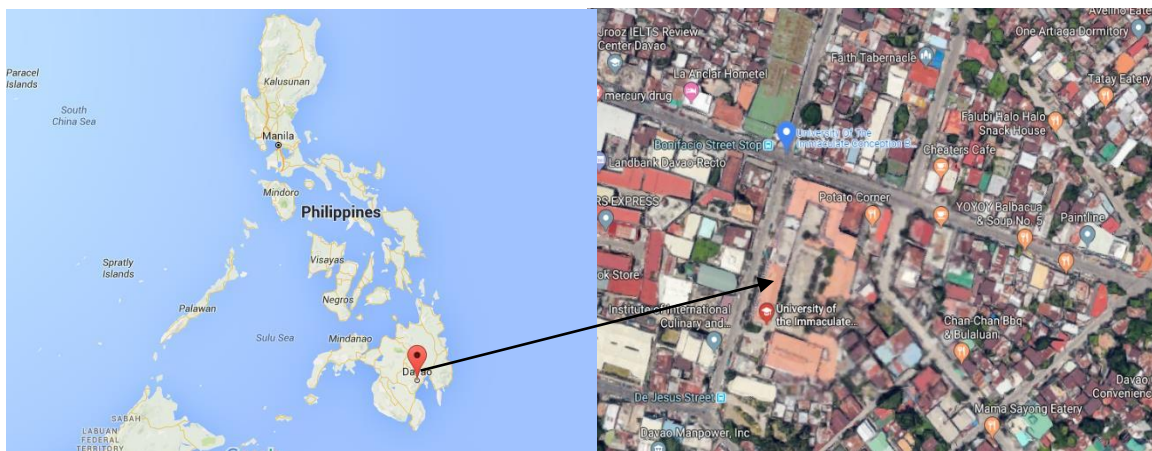


Figure 2. Site Map of University of Immaculate Conception
<https://www.google.com/maps>

Procedure

A letter of permission to conduct the study was given to the Principal of University of Immaculate Conception – Senior High School. After securing the approval, the researchers proceeded to the actual experimentation of the Panyawan (*Tinospora rumphii*) leaf.

Cockroach strains

A total of 27 male *Periplaneta americana* specimens were collected from different parts of the household of the researchers. The collections were made by trapping and hand catch methods. Traps were made by glass bottles containing bread and butter. The upper internal edge of the bottles, were coated with a fine layer of butter to prevent the escape of cockroaches. These traps were externally covered with dark paper. The samples were collected weekly and sent to the Physics Laboratory of the school. Cockroach colonies were reared in an insectary, maintained in big glass containers at 27°C and a relative humidity of 77%. They were provided with rodent diet, a cotton plugged water vial and a cardboard as a harborage.

Collection of Plant Materials

The Panyawan (*Tinospora rumphii*) plants were taken from an authorized plant shop at Bankerohan Public Market, Building Number 2, Marfori Street, Barangay 5-A, Davao City, Philippines. These plants were specified and verified by Maria Theresa C. Baslot, a Botanist. Leaves were separated from the stem and roots of the collected *Tinospora rumphii* plant. The leaves were then washed with distilled water to remove any debris. These were air dried at room temperature for several days until the leaves became brittle and moisture-free. The plant materials were inspected daily until optimum dryness was achieved.

Preparation of Extract

Collected dried leaves were ground using a house blender and the extracted material was then subjected to the extraction process. The extraction process followed the procedures written in the book of Guevara (2005) entitled, "A Guidebook to Plant Screening: Phytochemical and Biological". The ground dried plant material was weighed at 500 g, placed in an Erlenmeyer flask and treated with 650 mL of 95% ethanol to completely submerge the material. The flask was stoppered and the material was kept soaked for 24 to 48 hours. The mixture was then filtered using Whatmans filter paper No. 1 and the filtrate was concentrated by rotary evaporator at temperatures below 50°C to about 10 mL. The concentration of leaf extract was recorded as grams of dried plant material per mL of the extract obtained. The container was properly labeled with the name of the plant, the concentration of the plant extract and the date of extraction. The extract was stored with a tight stopper in the cold, at temperatures between 0°-5°C.

Contact Glass Jar Bioassay

Tests were conducted on adult male strains of cockroach using the World Health Organization standard method (WHO, 1970). Each of the nine treatments was prepared in acetone and 2.5 mL was pipetted into a 0.4-L glass jar (inner surface area = 302.6 cm²). Nine jars containing different treatments were rolled horizontally over a flat surface until all of the acetone had evaporated, so that the treatment was deposited evenly over the inner surface of the jar. Negative control group received acetone only. Insecticidal activity of each treatment was evaluated by adding one adult cockroach per glass jar. Knock-down time was determined by subtracting the time of mortality and time the treatment was given. Mortality criterion was as follows: if a cockroach was unable to return back to its normal position within 2–3 minutes after being touched with forceps, it was considered dead and counted in mortality data. To calculate the lethal toxicity concentration, another set of bioassays was carried out but this time, ten cockroaches were added per glass jar. The duration of contact was 1 hour and cockroaches were then transferred to holding cups after the contact. The readings of mortality were taken during this time and expressed as percentage mortality. The transferred cockroaches were still provided with rodent diet, a cotton plugged water vial and a cardboard as a harborage. Three replicates were set up for the treated and controls.

Ethical Considerations

The researchers secured the Plant Ethics Checklist before conducting the study involving plant subjects. The form was filled-up to guide the researchers on

the ethical way of utilizing plant subjects that were used in the experiment of this research.

Data Analysis

The following statistical tools were employed in the study using the SPSS Software 23.0 version:

Mean. This was used to determine the mortality rate of *Periplaneta americana* after application of nine treatments.

One-Way Analysis of Variance (ANOVA). This was used to know if there is significant difference in the mortality rate of cockroaches when exposed to different Panyawan leaf extract concentrations, negative control and positive control. To confirm where the differences occurred between groups, Homogeneous Subsets derived from Tukey's Honest Significant Difference were used.

Probit Regression. This was used to determine the Lethal Concentration-50 (LC₅₀) of Panyawan leaf extract against *Periplaneta americana*.

Scope and Limitation of the Study

This study focused on the insecticidal activity of Panyawan (*Tinospora rumphii*) leaf extract against American cockroach (*Periplaneta americana*). This study used only seven concentrations of the leaf extract to confirm the insecticidal activity of Panyawan (*Tinospora rumphii*). The study was conducted from November 2018 to February 2019.

Chapter 3

RESULTS AND DISCUSSION

Knock-down times of the cockroaches exposed to different Panyawan leaf extract concentrations, negative control and positive control**Table 1.** Knock-down time of the cockroaches (*Periplaneta americana*) after exposure to the test substance Panyawan leaf extract in various concentrations (100%, 75%, 50%, 25%, 10%, 1%, and 0.1%), negative control and positive control

Treatment	R	Knock-down Time	Average Knock-down Time
Positive control	1	1 minute	1.08 minutes
	2	1.25 minutes	
	3	1 minute	
Extract 100%	1	1.25 minutes	1.42 minutes
	2	1.75 minutes	
	3	1.25 minutes	
Extract 75%	1	3.50 minutes	3.33 minutes
	2	3.25 minutes	
	3	3.25 minutes	
Extract 50%	1	3.75 minutes	3.83 minutes
	2	3.75 minutes	
	3	4 minutes	
Extract 25%	1	5 minutes	5.08 minutes
	2	5 minutes	
	3	5.25 minutes	
Extract 10%	1	8 minutes	8.17 minutes
	2	8.25 minutes	
	3	8.25 minutes	
Extract 5%	1	13 minutes	13.25 minutes
	2	13.50 minutes	
	3	13.25 minutes	
Extract 0.1%	1	20 minutes	20.25 minutes
	2	20.50 minutes	
	3	20.25 minutes	
Negative control	1	15.50 minutes	15.42 minutes
	2	15.25 minutes	
	3	15.50 minutes	

Presented in Table 1 are the knockdown times of the cockroaches exposed to different Panyawan leaf extract concentrations, negative control and positive control. Three replicates were done in the study to ensure reliable results. As

shown in the table, the treatment Extract 0.1% had the longest average knock-down time at 20.25 minutes. This was followed by negative control, Extract 5%, Extract 10%, and Extract 25% at 15.42 minutes, 13.25 minutes, 8.17 minutes, and 5.08 minutes, respectively. Average knock-down times of cockroaches treated with Extract 50%, Extract 75%, and Extract 100% were 3.83 minutes, 3.33 minutes, and 1.42 minutes, respectively. The positive control had the shortest average knock-down time among all treatments at 1.08 minutes.

Level of toxicity of the Panyawan leaf extract on the cockroaches in terms of LC₅₀ using Probit Regression

Table 2. Lethal Concentration 50 of Panyawan leaf extract

Leaf Extract	LC₅₀
Panyawan (<i>Tinospora rumphii</i>)	15.836%

An LC₅₀ value is the concentration of a material in air that will kill 50% of the test subjects when administered as a single exposure. This value gives the relative acute toxicity of an inhalable material and is related to the lowest concentration reported to have killed the test subjects. Using Probit Regression to analyze the data, it was found out in Table 2 that the leaf extract has a lethal concentration 50 of 15.836%. This means that if a cockroach would receive a Panyawan Leaf Extract with a concentration of 15.836%, there is a 50% chance the pest will die and if this survives, it will not be in good shape.

Test of significant difference in the knock-down times of cockroaches when exposed to various Panyawan leaf extract concentrations

Table 3. One-Way ANOVA in the knock-down times of cockroaches when exposed to different Panyawan leaf extract concentrations, negative and positive controls

	Sum of Squares	Df	Mean Square	F	Sig.	Decision	Interpretation
Between Groups	1114.45	8	139.31	3761.266	0.00	Reject	Significant
Within Groups	0.67	18	0.04				
Total	1115.12	26					

Alpha: 0.01

Table 3 shows the One-Way Analysis of Variance (ANOVA) in the knock-down times of cockroaches when exposed to different Panyawan leaf extract concentrations, negative and positive controls. The F-value was 3761.266. This means that the the variability of group means is large relative to the within group variability. Because the p-value of 0.00 was lesser than 0.01 alpha level of significance, the null hypothesis is rejected; therefore, there is a significant difference in the knock-down times of cockroaches when exposed to different Panyawan leaf extract concentrations, negative and positive controls. This means that the knock-down times of cockroaches was statistically dependent on the type of treatment used. To know where the significant difference has occurred, Homogeneous subsets derived from Post Hoc Test using Tukey's Honest Significant Difference were utilized.

Table 4. Homogeneous subsets derived from Post Hoc Test using Tukey's Honest Significant Difference

Treatment	N	Subset for alpha = 0.01						
		1	2	3	4	5	6	7
Positive control	3	1.08						
Extract 100%	3	1.42						
Extract 75%	3		3.33					
Extract 50%	3		3.83					
Extract 25%	3			5.08				
Extract 10%	3				8.17			
Extract 5%	3					13.25		
Negative control	3						15.42	
Extract 0.1%	3							20.25
Sig.		.490	.092	1.000	1.000	1.000	1.000	1.000

Table 4 presents the homogeneous subsets derived from Post Hoc Test using Tukey's Honest Significant Difference. As shown in the table, the treatments positive control and Extract 100% are in subset 1 while Extract 75% and Extract 50% are in subset 2. Extract 25%, Extract 10%, Extract 5%, negative control and Extract 0.1% are in subsets 3, 4, 5, 6, and 7 respectively. Within a subset, there is no significant difference while between subsets, there is a significant difference. This means that there is no significant difference between positive control and Extract 100% as well as between Extract 75% and Extract 50%. However, both of these two groups are significantly different from the Extract 25%, Extract 10%, Extract 5%, negative control and Extract 0.1%. The following treatments are arranged in decreasing effectivity in killing the cockroaches based on the average knock-down times: 1) Positive Control and Extract 100%, 2) Extract 75% and Extract 50%, 3) Extract 25%, 4) Extract 10%, 5) Extract 5%, 6) Negative Control, and 7) Extract 0.1%.

Chapter 4

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The present study aimed to determine the insecticidal activity of Panyawan (*Tinospora rumphii*) leaf extract against American cockroach (*Periplaneta americana*). Specifically, the study attempts to know the knock-down times of the cockroaches exposed to different Panyawan leaf extract concentrations, negative control and positive control, to determine the level of toxicity of the Panyawan leaf extract on the cockroaches in terms of LC_{50} using Probit Regression, and to determine if there is a significant difference in the knock-down times of cockroaches when exposed to various Panyawan leaf extract concentrations. The null hypothesis was then formulated and tested at 0.01 alpha level of significance.

A quantitative experimental research design was used in the study. The experiment was conducted at the University of Immaculate Conception – Bonifacio Campus, De Jesus Street, Poblacion District, Davao City. The cockroach strains were collected from different parts of the household of the researchers. Panyawan leaves were collected and extracted to obtain seven different concentrations. Efficacy of the concentrations were measured through contact glass jar bioassay. After a series of experimentation, the data were tabulated and analyzed using the SPSS 23.0 version.

The findings of the study were summarized and arranged based on the formulated research questions:

1. The Extract 0.1% had the longest average knock-down time at 20.25 minutes, followed by negative control, Extract 5%, Extract 10%, and Extract 25% at 15.42 minutes, 13.25 minutes, 8.17 minutes, and 5.08 minutes, respectively. Average knock-down times of cockroaches treated with Extract 50%, Extract 75%, and Extract 100% were 3.83 minutes, 3.33 minutes, and 1.42 minutes, respectively. The positive control had the shortest average knock-down time among all treatments at 1.08 minutes.
2. Probit regression was used to calculate the Lethal Concentration 50. The LC_{50} of the leaf extract was 15.836%.
3. One-Way Analysis of Variance (ANOVA) was used to determine if there is a significant difference in the knock-down times of cockroaches when exposed to various Panyawan leaf extract concentrations. Because the p-value of 0.00 was lesser than 0.01 alpha level of significance, the null hypothesis is rejected; therefore, there is a significant difference in the knock-down times of cockroaches when exposed to different Panyawan leaf extract concentrations, negative and positive controls. Homogeneous subsets derived from Post Hoc Test using Tukey's Honest Significant Difference reported that there is no significant difference between positive control and Extract 100% as well as between Extract 75% and Extract 50%; however, both these two groups are significantly different from the Extract 25%, Extract 10%, Extract 5%, negative control and Extract 0.1%

Conclusion

Based on the findings of the study, the following conclusions were drawn:

1. The shortest knock-down time was achieved by positive control while the Extracts of Panyawan leaf reported a 1.42 to 20.25 minutes knock-down time.
2. The concentration of Panyawan leaf extract required to kill 50% of the cockroaches was 15.386%.
3. There is a significant difference in the knock-down times of cockroaches when exposed to different Panyawan leaf extract concentrations, negative and positive controls. There is no significant difference in the knock-down times of cockroaches when exposed to positive control and 100% Panyawan leaf extract.

Recommendations

Based on the findings and conclusion of the study, the following recommendations were formulated:

1. Panyawan leaf extract concentrations of 5% to 100% were found to kill the cockroaches faster than the negative control. Therefore, the households can utilize the extract as an alternative to much expensive commercial pesticide.
2. The lethal toxicity of Panyawan leaf extract was 15.386%. Therefore, less concentrations are required to control the proliferation of the pests.
3. Future researches should be done to determine other factors that could affect the mortality of the cockroaches.

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