Lantana Camara Leaf Extract: A Prospective Organic Mosquito Larvicide

Llorera, Daisyl Ann L. , Lugo, Lucila O., Macatangay, Maica R., Magpantay, Janssen C., Ogbuji, Cynthia C., Sheshe, Nasiru M.

Abstract

The larvicidal activity of Lantara camara Linn (Verbenaceae) against the larvae of common species of mosquitoes found within the Philippines was investigated in the present study. The researchers collected 50 grams of Lantana camara leaves which were dried and mixed with 250mL of methanol. It was kept for 24 hours with periodically shaking. The procedure had been repeated for three times with the fresh volume of methanol and the final filtrates were collected, concentrated and evaporated to dryness. There were three samples used: 2mg, 3mg and 4mg of the filtrates and each of them was mixed with 1ml of distilled water. The researchers conducted three trials for each treatment and used nine pieces of 250mL beakers filled with 100mL tap water that were added to the corresponding samples. Each beaker contained 5 mosquito larvae, covered with a net to make sure that the larvae would have enough oxygen and assure to grow in its normal habitat. One additional beaker has been set aside and served as the control. Completely randomized design has been utilized by the researchers to control for the effects of extraneous variables. After arranging the treatments randomly, it was then observed for 24 hours. After the 24 hours of observation, researchers found out that the mortality rate of all the methanolic extracts that were prepared from Lantana camara leaves were not significantly effective against the larvae causing only 0-20% mortality on all of the treatments except the 60% result on trial 1 of the treatment 3 with 4mg/mL concentration. The following concentrations did not manage to kill all or majority of the larvae. The larvae had survived and managed to grow into pupa and some turned into mosquitoes.

Keywords: Lantana camara, leaf, exract, larvicide, mosquito, organic

---

**** Paper presented in First Regional Congress in Research Across Disciplines, March 4, 2014 at Lyceum of the Philippines University Cavite***

INTRODUCTION

Mosquitoes are believed to be one of the dangerous insects in the world, as they bring diseases fatal to mankind. In the Philippines, mosquito-borne illnesses such as malaria, dengue, filariasis and chikungunya remain to be a major public health concern. Dengue fever is a mosquito-borne viral infection wherein children are the ones usually affected. The Department of Health has documented around 30,000 cases of dengue all over the Philippines from January to April 2013 alone. Another mosquito-borne disease which is prevalent in the country is malaria. Malaria affects a total of 14 million people in the Philippines. An additional to these is chikungunya which is a new viral infection caused by same species of mosquito that causes dengue, the Aedes aegypti and the Aedes albopictus mosquitoes. Lastly, there is Lymphatic filariasis, more commonly known as Elephantiasis which is a disease caused by an infection with filarial worms which enter the human body through mosquito bites. (Know more about Mosquito-borne diseases, 2013).

Mosquitoes are accountable for over 300 million clinical cases and one million deaths yearly worldwide. Ineffective measures and the complexity of illness life cycles need integrated approaches to control, eliminate, and eventually eradicate diseases (Molecular and population Biology of Mosquitoes..., 2013).

Mosquitoes are tiny blood-sucking insects that depend on standing water to reproduce. Female mosquitoes must feed on blood to lay eggs. They feed by sticking their mouthparts into the skin of an animal and sucks blood rapidly. More often, they carry viruses that can be transmitted to a person while they are feeding (Mosquito-Borne Diseases, 2013).

Environmental conditions like temperature and moisture are where the length of mosquito life cycle between species depends. Nonetheless, the life cycle of all mosquitoes consists of the egg, larval, pupal and adult stages. Male mosquitoes feed on the nectar of the plants only while females extract the blood of the hosts in order to develop and nourish eggs. Nearly all mosquitoes lay their eggs directly into water and others lay their eggs just near but not within the water. Within 24 to 48 hours, the eggs will hatch into larvae. The larvae will soon grow approximately 5mm in length. They breathe through siphon or air tubes. The larger ones can be seen floating just above the surface of waters that are infested. Within a span of seven to 10 days, the larvae will enter the stage of a pupa. Pupae are also visible upon the surface of the breeding site. When a mosquito is fully developed, it will emerge from its pupal case and will become a mosquito. The new adult, at this time, will stand upon the water to dry its wings and prepare for its flight. The female mosquitoes will then seek an animal to suck on and feed. They are capable of flying for miles and lays over 100 eggs at a time. Larvae and pupae ordinarily cannot survive without water so if a water source evaporates while they are still in these stages, they will die. (Mosquito Life Cycle, 2013)

Several approaches were extensively used to control the threat posed by these mosquitoes. Over the years, synthetic insecticides were introduced but although these are effective, the insect tends to develop resistance to such products (Jirakanjanakit *et al.*, 2007). Aside from being costly, the use of these repellents also generates problems such as environmental pollution and has toxic side effect to humans (Sarwar, Ahmad & Toufiq, 2009). This imposes a need for other alternative methods which are efficient, economic and environmentally safe. Botanical derivatives materialize as a possible larvicide for the Aedes aegypti or the common household mosquito (Zhu *et al.*, 2008).

Lantana camara Linn (Verbenaceae) is also known as wide sage or lantana weed. It is a hefty extensive evergreen shrub which can grow up to 3 m in height and has a strong scent. It is a perpetual shrub found growing up to 2000 m altitude in tropical, subtropical and clement parts of the world. All parts of this plant have been used conventionally for numerous illnesses all through the world. The leaves of this plant were used as an antibacterial and antihypertensive agent, roots for the treatment of malaria, rheumatism, and skin rashes (Thamotharan, 2010). Extract from the leaves of *Lantana camara* possessed larvicidal activity while extract from flowers of the plant showed repellent activity against adult mosquitoes (Sathish, 2008).

In this study, the larvicidal activity of *Lantara camara Linn (Verbenaceae)* against the larvae of common species of mosquitoes found within the Philippines will be investigated.

Review of Related Literature and Studies

Preventing or minimizing mosquito-borne diseases transmission depends completely on control of the mosquito vectors or interference of human-vector contact. Efforts to control transmission should target the vector in the habitats of its immature and adult stages (WHO, 2009).

The various problems associated with the efficacy of synthetic chemicals and the growing prevalence of resistance in the mosquito have emphasized the call for the development of new strategies for mosquito control (Zhu *et al*., 2008).

Lantana is a gregarious, upright or half-climbing, more of a hairy strong-smelling shrub. When upright, it is usually one to two meters high. Leaves are egg-shaped, five to nine centimeters long, three inches long, sharp at the tip and curved at the bottom and saw-like in the borders. Flowers are pink, orange, yellow, white, lilac and other shades, according to the diversity and bear in stalked heads which are two to 3.5 centimeters in diameter. Fruit is drupaceous, sweet tasting, purple or black, fleshy ovoid and about five millimeters long. The plant is a gregarious weed in the Philippines, in settled areas in woods and waste places at low and medium altitudes. Certain varieties are urbane as a trimmed hedge either alone or with other vegetation. The phytochemical analysis of the plant detected common secondary metabolites–alkaloids, phenolics, terpenoids and other minor compounds such as phytosterols, saponins, tannins, phycobatannin and steroids. It has mosquito larvicidal activity. Phytochemical screening of leaves and flowers yielded saponin, terpenoids, flavonoids and cardiac glycosides. Phytol, a diterpene, is present in higher concentration in the methanol leaf extract of *Lantana camara*. The larvicidal activity noted was attributed to the phytochemicals and results suggest the shrub may have a potential in the control of vector borne diseases.

Zhu (2008) stated that activities have been focused on plant extracts or phytochemicals as sources of agents to control mosquito. During the last decade, a variety of studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides. Plants are rich source of bioactive chemicals, some of which have medicinal and pesticidal properties (Rahuman *et al.*, 2008; Kamaraj *et al.,* 2009).

Lantana camara has therapeutic value because of the presence of natural agents. Greater part of their activity is due to "bioactive compounds like flavones, isoflavones, flavonoids, anthocyanins, coumarins, lignans, catechins, isocatechins, alkaloids, tannin, saponins and triterpenoids" (Ganjewala, 2009). Terpenoids or terpenes occur in almost every plant and represent the biggest class of secondary metabolites. They contribute to the taste, smell and colors of plants. The main active components of essential oils like terpenoids are highly volatile compounds called monoterpenoids and sesquiterpenoids that contribute to their distinctive scents. Sterols are complex terpenoids which are precursors to essential hormones in plants. Terpenoids are insect growth regulators which are used by many companies. The chemicals disrupt larval development and increase insect mortality. Saponins are soap-like substances that are able to disrupt the cell membranes of insects which make it toxic to them at specific concentrations. D-limonene, a saponin, can destroy the wax coating of the respiratory systems of insects, causing suffocation. Pyrethrin is a chemical which is a neurotoxin that attacks the nervous systems of all insects. In non-fatal dosage, it has an insect repellent effect. Phenolics are another large class of secondary metabolites produced by the plants. They are aromatic compounds that give the plants their distinctive smells and encompass a wide variety of defense-related compounds that include flavonoids, anthocyanins, phytoalexins, tannins, lignin and furanocoumarins. These toxic molecules disrupt the pathogen metabolism or cellular structure of insects but are often pathogen to the specific to their toxicity. The wide varieties of plants produce the toxins that are activated by ultraviolet light. These are some of the phenolics that can kill the pests: Eugenol, Salicylate, Quartering and Methoxyphenol. Alkaloids are plant components used as insecticides because it interferes with insect's nerve impulses. Before artificial pesticide was discovered, salts of nicotine and anabasine were used as insecticides. Their use was limited by their high toxicity to humans. This is another neurotoxin poisonous to insects (Plants that Repel… 2013)

Study of the leaves and flowers extract have given an idea about similar carbohydrates and lipid compositions. The carbohydrate levels were greater in the flowers than the leaves while the lipids are greater in the leaves extract (Ganjewala, 2009).

The methanolic and ethanolic extracts of *Lantana camara* were found to have an advanced larvicidal rate against *Aedes aegypti*, while in the *Culex quinquefasciatus* diversity, the concentration of extracts have to be improved for better larvicidal effect. The highly-complex combinations of these compounds materialize as an efficient environmentally-safe vector and pest-managing agent (*Sathish, 2008)*.

Sathish and Maneemegalai (2008) discovered that several essential oils and their isolates have been assessed as efficient indoor insect repellents. The high rate of mortality may be due to a component in the essential oil known as terpenoids in the methanol extracts of the leaves of *Lantana camara*. A study by Murusegan (2012) indicates that *Lantana camara* leaves yielded 0.8% of essential oil, and thirty six components. The constituents were α- Copaene, Germacrene, α– Cubebene, β – Elemene, α – Guaiene, α – humulene , Aromadendrene, β – Selinene, α – Selinene, Caryophyllene oxide, Nerolidol, Spathulenol and Delta – Cadinene which were the major components of the essential oil of *Lantana camara* in high altitude. The major components found to have insecticidal, insect repellent and attractant are as follows: α- Copaene which strongly attracts to an agricultural pest, α – Cubebene, Delta- cadinene and α- Guaiance which are all very good insecticides and insect repellents. Methanol flower extract showed 100% mortality at a concentration of 2.0mg/mL in 3rd instar larvae and methanol leaf extract showed 100% mortality in 4th instar larvae at 2.0mg/mL concentration, but the required concentration to have maximum mortality rate is at 3.0mg/mL.

Many researchers reported influence of different extraction solvents, techniques on the content of natural antioxidants in extracts. Efficiency of solvents and methods are strongly dependent on plant medium used (Michiels *et al*., 2012). A solvent such as methanol has been commonly used for the extraction of phenolics from fresh product (Alothman *et al*., 2009). The properties of extracting solvents significantly affected the measured total phenolics content and antioxidant capacity (up to 30% variation) in fruits and vegetables (Michiels *et al*., 2012).

Statement of the Problem

To determine the efficacy of *Lantara camara* leaf extracts against the larvae of common species of mosquitoes found within the Philippines:

1. What is the larvicidal potential of *Lantara camara* leaf extract against larvae of common species of mosquitoes found within the Philippines?
2. Which among the treatments: 2.0mg/1mL, 3.0mg/1mL, 4.0mg/1mL concentrations is effective in eliminating the larvae?
3. Is there a significant difference in the mortality among the treatments?

MATERIALS AND METHODS

Research Design

The researchers used the experimental type of research. This utilized the Completely Randomized Design (CRD). With this design, subjects are randomly assigned to treatments. A completely randomized design relies on randomization to control for the effects of extraneous variables. The researchers assumed that, on average, extraneous factors will affect treatment conditions equally, so any significant differences between conditions can fairly be attributed to the independent variable. (Statistics and Probability Dictionary, 2013)

Research Locale

The materials were collected within Brgy. Darasa, Tanauan City Batangas. The experiment was done on the following laboratories; Anatomy Laboratory, Physics Laboratory, Microbiology and Parasitology Laboratory of Lyceum of the Philippines University – Laguna and Analytical Service Laboratory of the Institute of Chemistry located at the University of the Philippines – Los Baños.

Collection of Plant Material

The leaves of *Lantana camara* were collected locally in and around Brgy. Darasa, Tanauan City, Batangas. The plant was authenticated by Prof. Annalee S. Hadsall, Curator of Botanical Herbarium, Museum of Natural History, University of the Philippines Los Baños, Laguna. Following the identification of the plant, 50g of leaves were washed thoroughly using water (Sathish and Maneemegalai, 2008). Afterwards, the washed leaves were dried in shade at room temperature and powdered with the help of mortar and pestle which were aseptically cleaned using 70% alcohol.

The powdered leaves were sieved to get fine powder from which the extracts will be prepared (Sathish and Maneemegalai, 2008).

Collection and Rearing of Common Species of Mosquitoes in the Larval Stage

The larvae were collected along the households within the Barangay, dispersed in a jar half-filled with tap water. The larvae gathered ranges from 1^{st} to 4^{th} instar larvae were all utilized to be able to know the efficacy of the treatments regardless of the stage of larvae. The researchers utilized 50 larvae all throughout the experiment. Five larvae per container is the set-up, consisting of 10 containers. The larvae were placed in the container, allowing them to disperse on their own just like on their original habitat.

Containers

The researchers used 10 pieces of 250mL glass beakers filled with 100mL tap water, maintained in a room temperature. The beakers were sealed with a net to make sure that the larvae would get oxygen and to assure that it is growing in its normal habitat.

METHOD

Extraction

The 50g powdered leaves were placed in a different sealed container where 250mL of methanol will be added. The treatment is kept for 24hrs with periodic shaking. Afterwards, the mixture was filtered using filter paper and the filtrate will be collected. The procedure was repeated for three times using fresh volume of methanol. The final filtrates were concentrated using rotary vacuum evaporator and evaporated to dryness (Sathish and Maneemegalai, 2008).

Treatments

Three samples of 2mg, 3mg and 4mg of the filtrates were set aside. These were mixed with 1mL of distilled water separately. Three samples of each concentration are needed in order to obtain three trials. The nine pieces of 250mL beakers filled with 100mL tap water were added with corresponding treatments. The 10^{th} beaker will not have any treatment diluted and will serve as the control.

Larvicidal Assay

The treatments were placed in the nine beakers prepared and will be observed until results would be gathered. The treatments will be observed only for 24hrs. Dead larvae were removed as soon as possible to prevent decomposition which can cause the death of other larvae.

Any of the following conditions is indicative that the treatments are effective (Mosquito Life Cycle, 2013):

1. If the larva stopped growing into a pupa.
2. If the larva grows into a pupa but did not manage to continue being an adult.
3. If the larva grows to a pupa and mosquito but did not manage to live as an adult.
4. If the larvae stopped from moving as compared to the ones in the control.

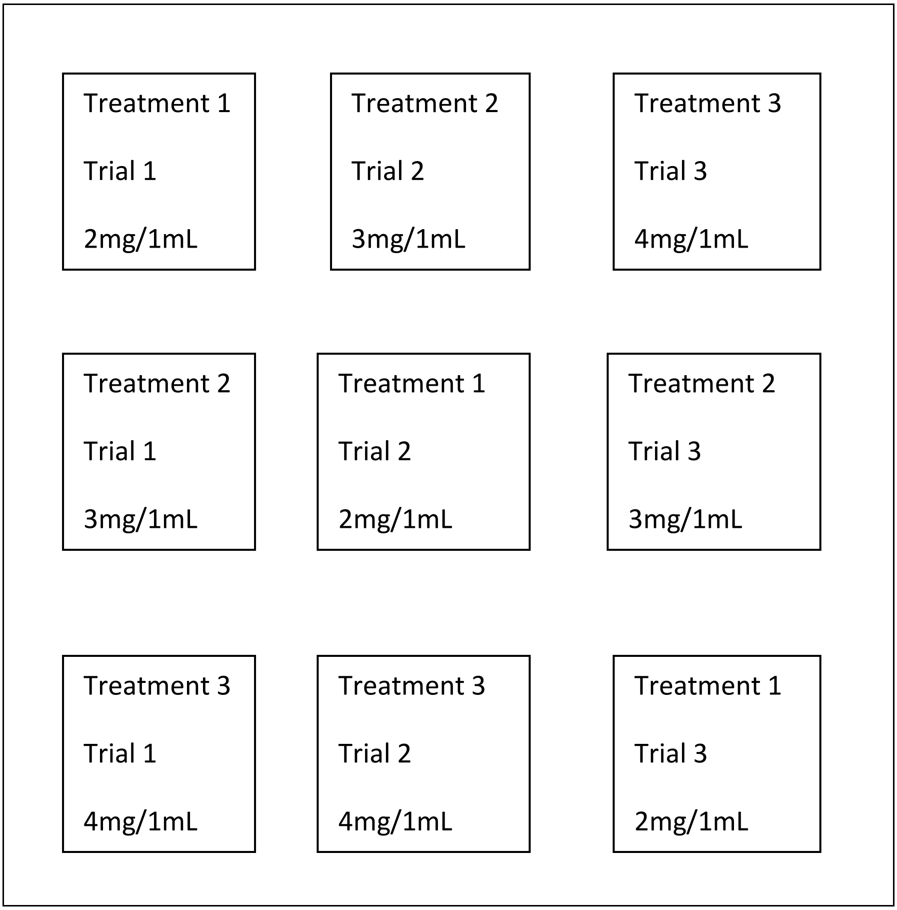

Figure 1. Completely Randomized Design

Statistical Treatment

The researchers utilized the following statistical techniques to ensure valid and reliable analysis and interpretation of data.

1. Percentage of Mortality = $\frac{\text{Number of dead larva}}{\text{Number of larva}} \times 100$
2. Analysis of Variance (ANOVA)

   Anova is a statistical test which analyzes variance. It is helpful in making comparison of two or more means which enable a researcher to draw various results and predictions about two or more sets of data (Anova Formula, 2013).

The decision will be to reject the null hypothesis if the test statistic from the table is greater than the F critical value with k-1 numerator and N-k denominator degrees of freedom.

If the decision is to reject the null, then at least one of the means is different. (Stats: One-Way ANOVA, 2013)

RESULTS AND DISCUSSIONS

The results of the larvicidal tests at different concentrations performed against the common mosquito larvae found in the community are presented in Table 1. The results clearly revealed that all the methanolic extract treatments prepared from the leaves of *Lantana camara* were not found significantly effective against the larvae, causing only 0-20% mortality on all of the treatments except the 60% result on just trial 1 of the treatment 3 with 4mg/mL concentration. It also showed that even with further trials, the following concentrations didn't manage to kill the all or even a majority of the larvae used (see Table 1).

The treatments were even left in place for three days, but to no avail, all the larvae that had survived managed to grow into pupa and some turned into mosquitoes.

Table 1 Percentage of Mortality

Treatment	Trial 1	Trial 2	Trial 3
2mg/1ml	1/5 = 20%	0/5=0%	1/5=20%
3mg/1ml	0/5=0%	0/5=0%	1/5=20%
4mg/1ml	3/5=60%	0/5=0%	0/5=0%

Table 2 Analysis of Variance (ANOVA)

	DF	TSS	MS	F compared to critical value
Treatment	2	0.666	3	3.50 < 6.94
Trial	2	2.666	0.75	0.875 < 6.94
Error	4	4.666	0.875	
Total	8	8		

DF: degrees of freedom TSS: total sum of squares
MS: mean square F: ratio of the variance between groups to the variance within group
6.94: Critical value of F for the 0.05 significance level

Table 2 shows that there is no significant difference among the treatments used (3.50 < 6.94). It also illustrates that there is no significant difference among the trials used (0.875 < 6.94).

Discussions

The present studies revealed that the methanolic leaf extracts of *Lantana camara* were not significantly effective against the mosquito larvae as they caused only 0-60% mortality. The results are contrary to the works published by Sathish and Maneemegalai (2008) to which the methanolic leaf extract showed 100% mortality in the mosquito larvae of Aedes aegypti using a concentration of 2mg/1mL. Even the recommended dose of 3mg/mL yields an ineffective result. The researchers hypothesized various factors which may potentially contribute on why the experiment arrived to such results.

The recent study was not able to identify the specie of the mosquito larvae that was obtained from the community as opposed to the research made by Sathish and Maneemegalai (2008), wherein they have used Aedes aegypti and Culex quinquefasciatus. This can be one factor why the present experiment did not manage to kill or even stop the growth of the larvae. The researchers may have used different larval species which are resistant to the effects of the methanolic leaf extract of lantana camara.

The present results are comparable with the studies reported by other researchers. The same methods were employed, but the only difference is the solvents used which enables them to yield a different result. Kumar (2008) reported that at 1 mg/mL the ethanol extracts of the leaves of *Lantana camara* caused 84% larval mortality while the methanol extracts showed 48% mortality in the fourth instar larvae of A. aegypti. Looking at the percentages of mortality and comparing them, there is a significant difference, wherein the ethanol extract has 36% more percentage of mortality as compared to the methanol which only proves that ethanol can be used as a better alternative to methanol. Ethanol, as compared to other solvents, has the highest total phenolic content extracted wherein phenols have insecticidal properties. This is due to the wide range of phenols that it can dissolve. Furthermore, ethanol is safe to man, not as highly toxic as methanol which is known to produce skin irritations and even blindness when ingested (Durling *et al*., 2007, Alothman *et al*., 2009). Then again, in a study conducted by Rajasekaran & Duraikannan (2012), aqueous extract *Lantana camara* showed 100% mortality after 24hrs of incubation. Conventional methods of extraction from plants use organic solvents such as methanol, which can pose hazards to health and the environment. These solvents are expensive and require extensive measures to reduce hazards to health, safety and the environment. Some solvents, such as methanol even require explosion proof facilities for production. There are also significant costs related to disposal of organic solvents and any waste material from the process. The extraction with the use of water, on the other hand, is cost-effective and environmentally sustainable extracting high-value phytochemicals such as polyphenols and saponins from plants for use in foods, pharmaceuticals, cosmetics and other products (Green Extraction for Phytochemicals, 2012).

As opposed to the comparative study of Sathish and Maneemegalai (2008) wherein they utilized different parts of *Lantana camara,* the present study only used the leaf part which is the abundant part of the plant. In their study, methanolic flower extract yields 100% mortality rate on the concentration of 2mg/mL, achieving the same results with ethanolic extract of the flower. Methanol flower extract contains flavonoids, cardiac glycosides, which is the same with the leaves but has an additional terpenoid which increases the larvicidal potential of the extract against mosquito larvae.

The present study also shows that there is no significant difference among the treatments and the trials done. The concentrations of 2, 3 and 4mg/mL all yields *Lantana camara* methanolic leaf extract as an ineffective larvicide as opposed to the study made by Sathish and Maneemegalai (2008). The extraction solvent and the part of the plant utilized greatly affects the result of the experiment recently done.

CONCLUSIONS

A variety of problems associated with the use of artificial chemicals and the rising incidence of resistance in the mosquito larvae has underscored the need for the development of new approach for mosquito larvae control. Biological pesticides provide a substitute to the synthetic insecticides because of the very low environmental pollution, low toxicity to mankind and other huge benefits that they bring (Zhu *et al*., 2008). Moreover, the rising documentation of negative environmental and health impact of these artificial pesticides and gradually harsh environmental regulation of insecticides have resulted in rekindled interest in the development and use of botanical insect management products for mosquito control and other insect pests.

Throughout the last years, numerous researches on natural plant products against disease-carrying mosquitoes indicate them as possible substitutes to the synthetically-made chemical insecticides. Plants are rich sources of bioactive compounds which are of medicinal and pesticidal properties (Rahuman *et al.*, 2008; Kamaraj *et al.,* 2009). The intricate mixtures of these compounds can be used to develop environmentally-safe pest-managing agents. As a matter of fact, many studies have reported the effectiveness of plant extracts or essential oils, especially *Lantana camara* which is a weed known for its insecticidal prperties, against mosquito larvae (Zhu *et al.*, 2008).

Phytochemical analysis of insecticidal properties of the leaves of the *Lantana camara*, its wide distribution and the trends of growing mosquito-related diseases in the Philippines serves as the basis of the researchers in utilizing it as the subject of the research.

The recent study is guided by the following objectives all throughout the research, to determine the larvicidal potential of *Lantana camara* leaf extract against larvae of common species of mosquitoes found within the Philippines, to assess the effectiveness of the treatments in a dose-dependent manner which are 2.0mg/1mL, 3.0mg/1mL, 4.0mg/1mL and to figure out if there is a significant difference in the mortality among the treatments used. The present study obtained the following results which are written in the paragraphs as follows.

The *Lantana camara* methanolic leaf extract is found to have minimal larvicidal potential against mosquitoes found within the Philippines, specifically Brgy. Darasa, Tanauan City, Batangas. Results show that it has only 0-60% percentage of mortality on the larvae tested for 24hrs, with the 60% occurred only in just the first trial. It is enough for the researchers to render it as ineffective.

Neither of the treatments can be recommended if one is to make a larvicide. Even though the concentration is increased up to 4mg/1mL, it is still ineffective as a larvicide. There is no significant difference between the treatments and the three trials used.

RECOMMENDATIONS

For future researchers, to consider using other parts of the *Lantana camara* such as the flowers, because it has been proven to have higher mortality rate than the leaves that the researchers had used in this experiment. Second, choose other alternative solvents like ethanol because it contains a higher total phenolic content that can further enhance the larvicidal effect compared to methanol. Third, utilize a higher treatment dosage to check the potency of the extract. Fourth, extend the observation of larvae for about 48 to 72 hours after pouring the extract because the efficacy of the extract may take longer hours. Last, to identify the mosquito larval specie and the exact instar they are going to utilize, to know if the *Lantanca camara* has a different effect based on a specific specie and instar.

REFERENCES

Alothman, M., Rajeev, B., Karim, A.A., (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chemistry. Vol.115. Issue 3. 785-788

Anova Formula. (2013). Retrieved October 16, 2013 from http://formulas.tutorvista.com/math/anova-formula.html

Capinera, L., (2008). "Larvicide." Encyclopedia of Entomology. 2nd ed. Dordrecht, The Netherlands: Springer, 2142.

Duraikannan, G., Rajasekaran, A., (2012). “Larvicidal activity of plant extracts on Aedes Aegypti L.”, Asian Pacific Journal of Tropical Biomedicine, pp 1578 S1582

Durling, N. E., Catchpole O.J., Grey J.B., Webby R.F., Mitchell, K.A., Foo, L.Y., Perry, N.B., (2007). “Extraction of phenolics and essential oil from dried sage

(Salvia officinalis) using ethanol–water mixtures", Food Chemistry, Vol. 101, Issue 4, pp 1417-1424.

Ganjewala, D., Sam, S., Hayat, K., (2009). Biochemical compositions and antibacterial activities of *Lantana camara* plants with yellow lavender, red and white flowers. Eurasia. Jour. Bio. Sci, 69-77.

Green Extraction for Phytochemicals. (2012). Retrieved October 15, 2013 from http://www.fmc-gac.com/content/green-extraction-phytochemicals-0

Jirakanjanakit, NP., Rongnoparut, S., Saengtharatip, T., Chareonviriyaphap, S., Duchon CB., & Yoksan. (2007). S. Insecticide susceptible/resistance status in Aedes (Stegomyia) aegypti and Aedes (Stegomyia) albopictus (Diptera: Culicidae) in Thailand during 2003 - 2005. J Econ Entomol, 545-550.

Kamaraj, C., Bagavan, A., Rahuman, AA., Zahir, AA., Elango, G., Pandiyan, G., (2009). Larvicidal potential of medicinal plant extracts against Anopheles subpictus Grassi and Culex tritaeniorhynchus Giles (Diptera: Culicidae). Parasitol Res, 1163-1171.

Kumar, S., (2008). Evaluation of larvicidal effect of *Lantana camara* Linn against mosquito species Aedes aegypti and Culex quinquefasciatus. Adv Biol Res 2, 39-43.

Kantutay. (2013). Retrieved June 3, 2013 from http://www.stuartxchange.com/Kantutay.html

Know more about mosquito-borne diseases. (2013). Retrieved June 3, 2013 from http://pamilyaonguard.abs-cbnnews.com/article/ KNOW_MORE_ABOUT_ MOSQUITO-BORNE_DISEASES.html

Larva 1st-, 2nd- & 3rd- Instars. (2010). Retrieved June 4, 2013 from http://www.flycontrol.novartis.com/species/ housefly/en/larva_instars.shtml

Michiels, J.A., Kevers, C., Pincemail, J., Defraigne, J.O., Dommes, J., (2012). Extraction conditions can greatly influence antioxidant capacity assays in plant food matrices., Food Chemistry. Vol.130. Issue 4., 986-993.

Molecular and Population Biology of Mosquitoes and other Disease Vectors: From Basic Vector Biology to Disease Control. (2013). Retrieved June 3, 2013 from http://events.embo.org/13-mosquito/

Mosquito-Borne Diseases. (2013). Retrieved June 3, 2013 from http://www.cdph.ca.gov/HealthInfo/discond/Pages/MosquitoBorneDisease s.aspx

Mosquito Larvae. (2013). Retrieved June 4, 2013 from

http://www.wumcd.org/mosquito/larvae.html
Mosquito Life Cycle. (2013). Retrieved June 3, 2013 from http://www.orkin.com/other/mosquitoes/mosquito-life-cycle/
Murugesan, S., Rajeshkannan, C., Suresh, D., Sumathi, R., & Manivachakam, P., (2012). Identification of insecticidal properties in common weed – Lantana camara Linn by Gas Chromatography and Mass Spectrum (GC-MS-MS), 2754-2759
Plants That Repel or Kill Pests. (2013). Retrieved October 16, 2013 from http://www.freewebs.com/jumblebox/blogf/plantpest1b.html#sthash.EnyPa puf.
Rahuman, AA., Venketesan, P., (2008). Larvicidal efficacy of five cucurbitaceous plant leaf extracts against mosquito species. Parasitol Res, 133-139
Rédei, G., (2008). "Aedes Eǵypti." Encyclopedia of Genetics, Genomics, Proteomics and Informatics. 3rd ed. Vol. 1. New York: Springer, 39.
Sarwar, M., Ahmad, N., & Toufiq, M. (2009). Host plant resistance relationships in chickpea (Cicer arietinum L.) against gram pod borer (Helicoverpa armigera Hubner). Pak J Bot,3047-3052.
Sathish, KM., Maneemegalai, S., (2008). Evaluation of larvicidal effect of Lantana camara Linn against mosquito species Aedes aegypti and Culex quinquefasciatus. Adv Biol Res, 39-43.
Statistics and Probability Dictionary. (2013). Retrieved June 3, 2013 from http://stattrek.com/experiments/experimental-design.aspx
Thamotharan, G., Sekar, G., Ganesh, T., Senthil.kumar, N., et al. (2010). Antiulcerogenic effect of Lantana camara Leaves on in vivo test models in Rats. Asian Jour. Pharm. Clinical. Res, 57-60.
World Health Organization. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control. (2009). 147: 59
Zhu, J., Zeng, X., O'neal, M., Schultz, G., Tucker, B., Coats, J., et al. (2008). Mosquito larvicidal activity of botanical-based mosquito repellents. J Am Mosq Contr Assoc, 161-168.
1st Instar. (2010). Retrieved June 3, 2013 from http://www.mlmp.org/Resources/LarvalFieldGuide/1stInstar.aspx
4th Instar. (2010). Retrieved June 3, 2010 from http://www.mlmp.org/Resources/LarvalFieldGuide/4thInstar.aspx