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Research Paper

Oregano (*Plectranthus amboinicus*) and Lemongrass (*Cybopogon*) as Primary Ingredients for Liquid Mosquito (*Culicidae*) Larvicide

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Abstract— Mosquito-Borne diseases or diseases that spread from host to host are a big problem around the world. This study intends to find out if processed Oregano (*Plectranthus amboinicus*) and Lemongrass (*Cymbopogon*) natural extract can be used as an inexpensive and environmentally friendly mosquito larvicidal solution. Based in some studies Oregano (*Plectranthus amboinicus*), and Lemongrass (*Cymbopogon*) contains natural chemical that can kill larvae in their early stage. The researchers used two (2) set-ups with four (4) containers including one (1) control group which will be the basis of the experiment, all the containers are containing twenty (20) live mosquito larvae from the Institute of Weed Science, Entomology and Plant Pathology (IWEP) of University of the Philippines, Los Baños, Laguna. The researchers added different concentrations and ratios of the solution in each container in this experiment. As the experiment conducted, the results show that less than fifty percent (50%) of the specimen was eliminated in the whole set-up, The experiment revealed that Oregano and Lemongrass larvicide solution is not enough to reach what this study aims for. Also, experiment results indicate that Oregano and Lemongrass solution is not enough to kill the larvae of mosquitoes. The researchers conclude that the Oregano (*Plectranthus amboinicus*), and Lemongrass (*Cymbopogon*) larvicide solution is not effective as an alternative mosquito (*Culicidae*) larvicide solution.

Keywords— Mosquito Larvae, Lemongrass Solution, Oregano Solution, Liquid Mosquito Larvicide, STEM SHS Philippines

1. Introduction

When you think of an organism who kill the most people a year, you think of a Lion, Sharks, and Snakes, but the animal who killed the most humans (except we humans ourselves) are tiny insects known as mosquito, mosquitoes kill at least 725,000 people every year whereas snakes kill an estimated 50,000 and sharks a mere 10 persons. Mosquitoes are one of the vectors of deadliest diseases among humans. Deadly diseases that are spread to people by mosquitoes include Zika virus, West Nile virus, Chikungunya virus, Dengue, Malaria, and many more minor diseases. Every year, malaria alone, transmitted by the *Anopheles mosquito*, kills 400,000 people (children), and incapacitates another 200 million for days. Other mosquito-borne diseases include dengue, which causes 50 to 100 million cases per year worldwide, yellow fever, which has a high mortality rate, or Japanese encephalitis, which causes more than 10,000 deaths per year, mostly in Asia. Not to forget Zika virus, with its recently described devastating and long-term neurological effects in babies born to infected mothers [1].

Depending on situation, people are using mosquito repellents that work by blocking mosquito's sense of smell and prevent

them on finding their target, or larvicides that can kill mosquito larvae and hatchlings before it matures and transform into a mosquito. However, some chemicals that are used in repellent and larvicides have a potential hazard for other organisms and the environment. The researchers would like to study the natural extract of the given herbal plant Oregano (*Plectranthus amboinicus)* and Lemongrass (*Cymbopogon*) that are commonly found in the backyard of homes if it can be a natural larvicide and in what concentration of it has the highest mortality rate on mosquito larvae.

According to Djouaka et al., (2016), the WHO Global report on insecticide resistance in malaria vectors: 2010–2016 showed that resistance to the 4 commonly used insecticide classes – pyrethroids, organochlorines, carbamates, and organophosphates – is widespread in all major malaria vectors across the WHO regions of Africa, the Americas, South-East Asia, the Eastern Mediterranean and the Western Pacific [2]. Over a period, mosquito will develop a resistance into the chemical we are using or the decreased susceptibility of a pest population to a pesticide, it is a big concern for the effect of insecticide along with its toxicity hazard to another organism and environment.

Extracts from plants may be alternative sources of mosquito egg and larval control agents, since they constitute a rich source of bioactive compounds that are biodegradable into non-toxic products and potentially suitable for use in control of mosquito larvae.

In fact, many researchers from A comparative Study of the Larvicidal Activity of Lemongrass (*Cymbopogan citratus*) from Different Methods of Extraction (2019), have reported on effectiveness of plant extracts or essential oils against mosquito larvae, such as *Cymbopogan citratus* (lemongrass). Lemongrass is extremely popular and used for medicinal, food and insect repellent products. The lemongrass oils are used in cosmetics, soaps, perfume, dyes, and odorizes along with thousands of other products. Lemongrasses are extremely safe and are listed on the EPA'S GRAS list (Generally Regarded as Safe), unlike other insecticides containing chemical compounds like DEET (N,NDiethlymetatoluamide) used as an active chemical ingredient in insect repellent [3].

Using mosquito larvicide is a better option to control the population of mosquitos than using repellent. Compared to repellent, Larvicide is applied to wet places where mosquitoes can lay their egg, killing its larvae (wriggles) before they mature into a mosquito reproduce, and become a vector for diseases.

There are diverse ways of preventing a mosquito outbreak, people throughout the years have been using variety of processes like using repellent and such. But the best way to prevent its spread is to stop it before it starts. Killing mosquito eggs before it turns into an adult will not only prevent the outbreak but also the producing of its future generations. Larvicide is a type of insecticide that is used to control the reproduction of mosquitoes, effective on both outside and inside the household. There are two (2) forms of larvicide, the liquid form of larvicide, and the one that is solid but come in different forms. The liquid form larvicide is the one that is directly applied into the water, it is the common one used when the community officials decided to spray larvicide all around the community. The liquid form of larvicide comes in two (2) forms; the one that needs to be ingested by the larvae to work and the other one works immediately when encounters the larvae. The type of larvicide that come in solid form are usually dunk, tablets, bits, granules, and briquettes. It is also applied to the water where the mosquito lay eggs, [4].

Aside from its different forms it also has diverse types according to their purposes. The Bacterial Larvicides, Insect Growth Regulators, and Oils and Films. The Bacterial Larvicide are the one that are made from natural substances. Bacterial larvicide also have three (3) distinct kinds first is bacterium *Bacillus thuringiensis* subspecies *israelensis (Bti),* it is found in soils and has been proven to be toxic to mosquito larvae, blackflies, and fungus gnats for it used as mosquito control for 30 years. Second is bacterium *Saccharopolyspora spinosa*, it is also found in soil, toxic to mosquitoes, ants, fruit flies, and other insects, the commercial larvicide called Spinosad is derived from this bacterium. Lastly, is the bacterium *Lysinibacillus sphaericus*, also known as *Bacillus sphaericus*, also found in soil, and it is known to work to some species of mosquito but does not work in *Aedes aegypti* (also known as the Yellow Fever Mosquito). Insect Growth Regulators are the larvicide used to kill the larvae before it can develop into an adult mosquito. *Methopreneexternal* and *pyriproxyfenexternal* are examples of Insect Growth Regulator. While the Oils and Films are used by licensed mosquito control professions. The mineral oils and film, when applied to water, spreads a thin layer that drowns the larvae and pupae. It is the only effective method on killing the mosquito pupae [5].

People have been using synthetic insecticides throughout the years that make some vectors species become resistant to it. Added to that the synthetic chemicals used as insecticides had an effect aside from the mosquito, it can also damage the environmental quality that is not supposed to be insecticide's target such as humans. Study shows that there is an active toxic agent that came from plant extracts. In ancient times this kind of mosquito control strategy was already in used. These non-toxic agents are cheap and eco-friendly and show that there is active toxic agent that came from plant extracts. In ancient times this kind of mosquito control strategy was already in use. These non-toxic agents are cheap and eco-friendly and show results that they only affect the specific targets, which are distinct species of mosquito.

Once mosquito larvae become adults, it can transmit various kinds of diseases. Some are fatal, and others can be treated by a vaccine. There are many diseases that humans can get from mosquitoes. Sometimes, not only human beings are being affected. In some cases, even animals are affected by the

diseases spread by the mosquitoes. Some diseases can be treated with vaccine, but some are deadly.

Dengue virus is transmitted by *Aedes aegypti*, according to (Williams et al, 2009), the virus may range inapparent infections to dengue fever, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). (WHO, 2020) *Aedes aegypti* breeds in a collection of water in containers, but not on swamps, puddles, or any large bodies of water [6].

Human Malaria is an infectious disease caused by *Anopheles* mosquito which affected people from tropical and subtropical countries [7]. Eyear more than 200 million people are reported to be infected. Malaria is caused by parasites called *Plasmodium* parasites.

Chikungunya is a disease transmitted by *Aedes aegypti* and was first isolated in Tanzania during 1953. During 2006, it was reported that 1,400,000 were infected during the Indian outbreak, [8]. Chikungunya is a virus that was transmitted by infected mosquitoes, including *Aedes aegypti* and *Aedes albopictus*. Philippines is one of the Asian countries that was affected by chikungunya.

Yellow fever is a flavivirus. disease transmitted by the infected *Aedes aegypti* mosquitoes. It occurred in the tropical areas of Africa and Central and South Africa. The term "yellow" refers to the jaundice that affects some patients [9].
The Eastern Equine Encephalitis Virus (EEEV) is transmitted to humans and horses by the bite of an infected mosquito. It is one of the most severe illnesses transmitted by a mosquito in the United States with a mortality rate of 33% and survivors develop a significant brain damage. The primary vector of the virus is called *Culiseta melanura*, also known as black-tailed mosquito (VDH, 2018). According to Deresiewicz M.D, et al (1997) the mortality rate was 36% and 35% of the survivors are moderately to severely disabled. There were abnormal findings in the patients which also included the focal lesions in the basal ganglia, thalami, and brain stem [10].

The St. Louis encephalitis (SLE) was spread to human through the bite of an infected mosquito, *Culex tarsalis, Culex quinquefaciatus, Culex pipiens* (Somboonwit M.D FACP, 2020) and was found in across America. According to Purdue University (n.d.), the transmission is via *Culex* mosquito females and there is no known human to human transmission.

La crosse encephalitis is a viral disease caused by an infected mosquito. Most cases happen in the upper Midwestern, mid-Atlantic, and Southeastern states (CDC, 2019). The *Aedes triseriatus* mosquito is the vector of this disease, [11] and the other two recently found new species of mosquitoes, *Aedes albopictus* and *Aedes japonicus*.

West Nile Virus is a virus in which transmission is between birds, mosquitoes, humans and other mammals. WNV can cause neurological disease and even death to people. WNV was first found in a woman during 1937 at West Nile district in Uganda. *Culex* mosquitoes are the considered main vectors of this virus, particularly *Culex pipiens.*

Zika virus is transmitted from *Aedes* mosquitoes, Aedes *aegypti*, which was first discovered in Uganda in 1947 in monkeys. The outbreaks are reported in Africa, America, Asia, and Pacific. Infection may start Guillain-Barre syndrome, neuropathy, and myelitis. Infection during pregnancy may also cause abnormalities in the baby and complications such as stillbirth, miscarriage, and premature birth [12].

The study aims to answer, will oregano and lemongrass solution be an effective mosquito larvicide? Specifically, it aims to answer the following questions:
1. Which of the following composition will have the highest mortality rate?
1.1) 40% of 30 mL Oregano extract, 60% of 30 mL Lemongrass extract.
1.2) 50% of 30 mL Oregano extract, 50% of 30 mL Lemongrass extract.
1.3) 40% of 30 mL Lemongrass extract, 60% of 30 mL Oregano extract.
2. How much amount of the larvicide solution will it take before all larvae in the set-up container(s) dies?
3. How long will it take for the different larvicide solutions made of lemongrass and oregano to take effect?

2. Methods

Researchers used experimental research design to know if the extract from Oregano and Lemongrass is effective as a larvicidal solution. The elements that remained constant throughout the research or the control group are the water level wherein the larvae has hatched and experimented with, dropper, the mosquito larvae, and the amount of solution that was used in the experiment. Also, the independent variable is the ratio of the two (Oregano, and Lemongrass) extract. Meanwhile, the dependent variables are the Mortality rate and duration of effectivity. The materials that were used by researchers for the larvicide are blender, kettle, container, droppers, strainer, syringe, and plastic cup(s). While the ingredients used for the larvicide are Oregano and Lemongrass which are divided into three (3) different solutions. The three (3) different solutions are 40% of 30 mL Oregano extract and 60% of 30 mL Lemongrass extract, 50% of 30 mL Oregano extract and 50% of 30 mL Lemongrass extract, and a 40% of 30 mL Lemongrass extract and 60% of 30 mL Oregano extract.

Table 1. Research Design Table

Control Group	**Experimental Group (Set 1)**		
	Set-up A	**Set-up B**	**Set-up C**
	60% Oregano extract and 40% Lemongrass extract larvicidal solution	50% Oregano extract and 50% Lemongrass extract larvicidal solution	40% Oregano extract and 60% Lemongrass extract larvicidal solution

Water level (60mL)	20 pieces of mosquito larvae	20 pieces of mosquito larvae	20 pieces of mosquito larvae
	Water level (60mL)	Water level (60mL)	Water level (60mL)
	Experimental Group (Set 2)		
	Set-up A	**Set-up B**	**Set-up C**
	60% Oregano extract and 40% Lemongrass extract larvicidal solution	50% Oregano extract and 50% Lemongrass extract larvicidal solution	40% Oregano extract and 60% Lemongrass extract larvicidal solution
	20 pieces of mosquito larvae	20 pieces of mosquito larvae	20 pieces of mosquito larvae
	Water level (60mL)	Water level (60mL)	Water level (60mL)

Table 1 shows that there were two experimental groups consisting of three set ups. For set A, the researcher facilitated 60% Oregano extract and 40% Lemongrass extract larvicidal solution. Set B used 50% Oregano extract and 50% Lemongrass extract larvicidal solution. While 40% Oregano extract and 60% Lemongrass extract larvicidal solution or set up C. Each set ups used 20 pieces of mosquito larvae with 20 mL of water for each set.

The researchers prepared the materials for getting the extract of oregano. First, is to wash your ingredients which are the Oregano and Lemongrass then let it dry for a minute. Second, put the oregano into the blender and grind it until it turns to liquid. After you liquefied the Oregano use a strainer to separate the small pieces of leaves to the extract and set aside.

In getting the Lemongrass extract the experimenter will use different ways compared to getting the Oregano extract. First, is to slice the Lemongrass into small pieces and slightly crush it before putting in the kettle/casserole with five (5) cups of water then put it on fire for one (1) to (2) hours. Pour the extract on a clean container and let it cool. Prepare the paper cup that will serve as the container for the mosquito larvae. Use the dropper to safely separate the larvae from others and set aside. In every container there will be twenty (20) pieces of mosquito larvae. Next, prepare the first vessel that will contain 60% Lemongrass oil and 40% Oregano extract. In the second vessel mix 50% of Lemongrass oil and 50% of Oregano extract. For the third vessel blend 40% of Lemongrass oil and 60% of Oregano extract. Once done preparing the three (3) types of solution the experimenter can now proceed with the testing part of the experiment.

The researchers used the percent mortality rate by which the researchers can obtained by dividing the number of dead larvae by the sample size of larvae and multiply by 100 percent.

$$\% \text{ Mortality Rate} = (M \div n) \times 100$$

Wherein:
n= sample size of the larvae
M= number of the dead larvae

3. Result and Discussion

This chapter shows the investigation and observation of Lemongrass and Oregano as alternative larvicide solutions. A total of two (2) set-ups. In set-up one (1) there are four (4) containers including the two (2) control groups per set-up which is sixty (60) mL of water. The second set has four containers consisting of experimental and control groups. The corresponding analysis and interpretation of the data is also included in this chapter.

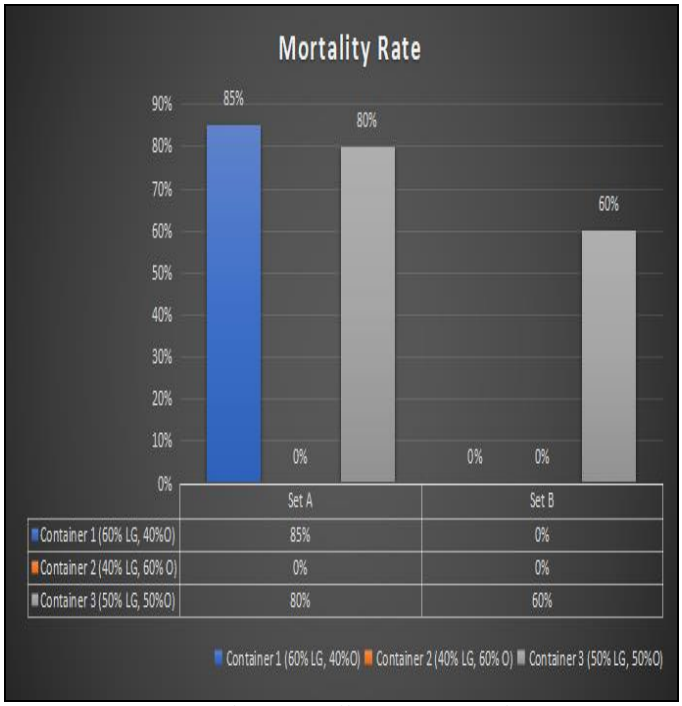

Figure 1: Mortality Rate of three (3) different containers of both set A and set B.

Figure 1 shows that the experiment consists of two (2) experimental groups (Experimental Group Set 1 & Experimental Group Set 2) both group sets will be conducted with three (3) set-ups (Set-up A, Set-up B, Set-up C) to get a precise and accurate result. The experiment also has a control group where the researchers will not apply any solution and will be the standard to which comparisons are made on this experiment. All of it contains the same amount of mosquito larvae (20) alive mosquito larvae and the same volume of water (60 ml), the only thing that is subjected to change is the ratio of concentration of the solution (Oregano and Lemongrass Extract). As shown on the graph in Set A container one (1) eighty-five percent (85%) mortality rate, container two (2) has zero percent (0%) mortality rate, and container three (3) has eighty percent (80%) mortality rate. Meanwhile, in set B container one (1) the mortality rate was zero percent (0%), container two (2) had zero percent (0%) mortality rate unlike container three (3) which has sixty percent (60%) mortality rate.

The study of Chandra, G., Chowdhury, N., & Ghosh, A., (2012) stated that active toxic agents that are present in plants have been used since ancient times to control mosquito breakout. Non-toxic agents that are cheap and eco-friendly. Since there are synthetic chemicals in the commercials larvicide that can also damage other living organisms near it and the environment, people have been studying the use of natural active agents that can be found in plants [13].

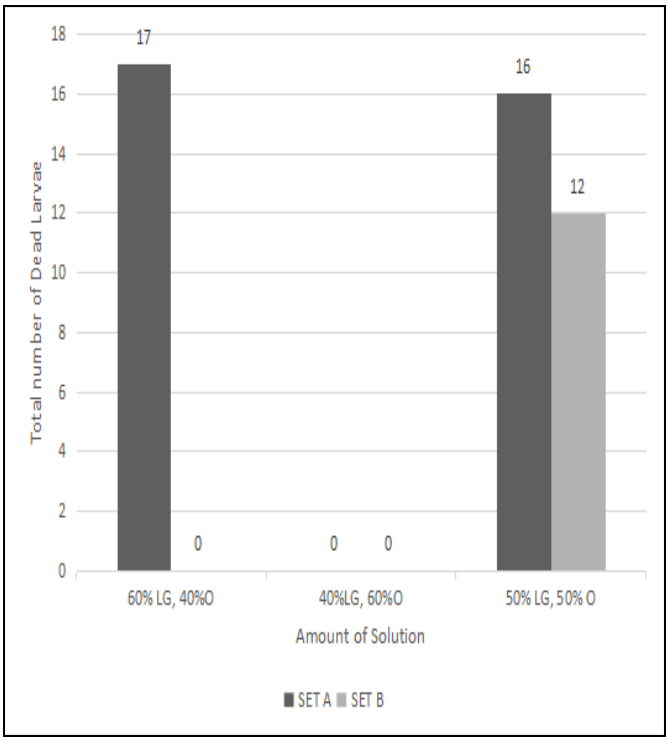

Figure 2: Amount of solution taken before the larvae die in different containers of set A and set B.

Figure 3 shows that in Experimental Group Set one (1) the result varied in every Set-up, in set-up A where the solution concentration is sixty percent (60%) Lemongrass and forty percent (40%) Oregano extract and has a total number of twenty (20) larvae, where seventeen (17) pieces of larvae or eighty-five percent (85%) of larvae died. In Set-up B where the concentration of the solution is forty percent (40%) Lemongrass and Sixty percent (60%) Oregano extract and has a total number of twenty (20) larvae, where there is no death recorded or zero (0%) of larvae died. In Set-up C where the concentration of the solution is fifty percent (50%) Lemongrass and fifty percent (50%) Oregano extract and has a total of twenty (20) larvae, where 16 or eighty percent (80%) of larvae died. In the 2nd Set of Experimental Group where the experiment will be repeated the results are: In Set-up A where the solution concentration is sixty percent (60%) Lemongrass and forty percent (40%) Oregano extract and has a total number of twenty (20) larvae, all of them remain alive or zero percent (0%) of larvae died. In Set-up B where the concentration of the solution is forty percent (40%) Lemongrass and Sixty percent (60%) Oregano extract and has a total number of twenty (20) larvae, all of them remain alive or zero percent (0%) of larvae died. In Set-up C where the concentration of the solution is fifty percent (50%) Lemongrass and fifty percent (50%) Oregano extract and has a total of twenty (20) larvae, where 17 or eighty-five percent (85%) of larvae died and three (3) remain alive.

Looking back to the study of Shah G, Shri R, Panchal V et al. (2011), they stated that Lemongrass (*Cymbopogon*) has been used in the past year as insecticide/ pesticide and repellents against mosquitoes [14]. According to the study, they used different kinds of mosquito species and produced the result that 95% of the species was repelled due to the strong odor that the Lemongrass has. As stated by Arumugam, G., Swamy, M. & Sinniah, U. (2016), Oregano has a distinct odor that can be used as pesticide/insecticides and repellents; aside from that it also has excessive number of bioactive compounds that are commonly the main components for antibacterial and anti-fungal effects [15].

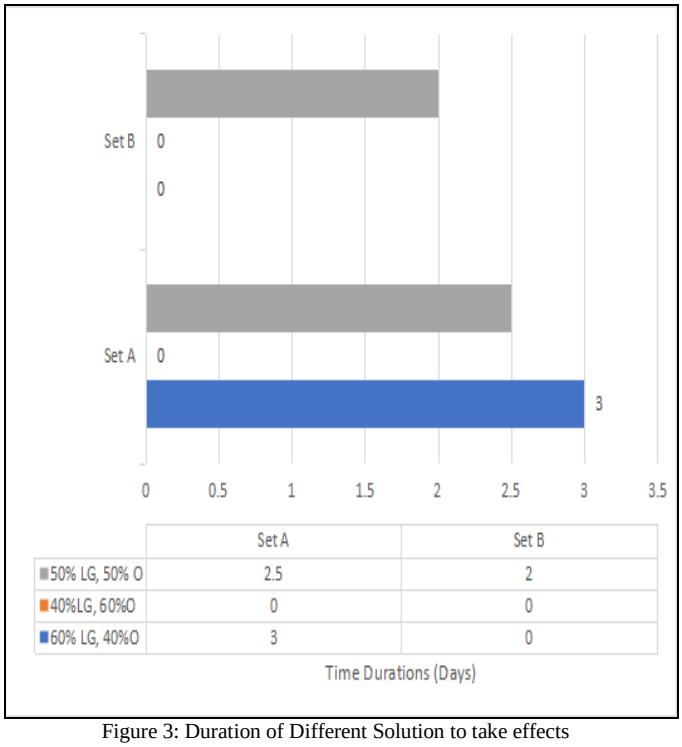

Figure 3: Duration of Different Solution to take effects

In Experimental Group Set A, as shown on the graph the solution concentration of the Lemongrass is sixty percent (60%) and (40%) Oregano extract where seventeen (17) out of twenty (20) larvae died and the remaining three (3) survived in a span of three (3) days. In Set-up B the solution concentration of Lemongrass is forty percent (40%) and sixty percent (60%) of Oregano extract where there is no death recorded in 3 days. In Set-up C where the concentration of the solution is fifty percent (50%) Lemongrass and fifty percent (50%) Oregano extract takes effects on 16 out of 20 larvae died and the other 4 larvae remain alive in course of 3 days.

According to the article of New Tech Bio's article Lemongrass is widely as medicinal plant, and insect repellent; and as stated in the study of Shah G, Shri R, Panchal V et al. (2011), it repelled 95% of distinct species of mosquito due to its strong odor.

In addition, at the first ten (10) to thirty (30) minutes the specimen stops moving and can make them mistakenly dead, but after one (1) to two (2) hours they will start moving again. Moreover, the researchers ensure that the specimen is regularly given a food and are placed in a cool place to make sure that there is no other cause of their death.

4. Conclusion and Recommendation

The researchers used three (3) different solutions 40% of 30 mL Oregano extract, 60% of 30 mL Lemongrass extract, 50% of 30 mL Oregano extract, 50% of 30 mL Lemongrass extract, 40% of 30 mL Lemongrass extract, 60% of 30 mL Oregano extract.

It shows that in set-up A that the solution concentration of the Lemongrass is sixty percent (60%) and (40%) Oregano extract were seventeen (17) out of twenty (20) larvae died and the remaining three (3) survived in three (3) days.

The experiment revealed that Oregano and Lemongrass larvicide solution is not enough to reach what this study aims for. Also, experiment results indicates that Oregano and Lemongrass solution is not enough to kill the larvae of mosquitoes.

Therefore, the researchers conclude that the oregano *(Plectranthus amboinicus)* and lemongrass (*Cymbopogon)* larvicide solution is not effective as an alternative mosquito (*Culicidae)* larvicide solution.
The following recommendations are presented.

Recommendations for future researchers.

1. To use a different species of mosquito because the researchers only used *Aedes aegypti* mosquito eggs in the experiment.
2. Improve the solution by adding more ingredients that can help to add to the effectiveness of the solution.
3. Explore using the solution on several types of insects especially for pests to test if the solution will also be effective aside mosquitoes.
4. Do multiple trials to test the effectiveness of the solution.

Recommendations for people who will use the solution.

1. Using the solution may not kill all the mosquito larvae because the researchers only used *Aedes aegypti* in the experiment.
2. The effectiveness will only be effective if the solution will cover the mosquito larvae.
3. The continuous feeding of larvae may weaken the effectiveness of the solution, so it is recommended to use more.

Conflict of Interest
The authors hereby declare no conflict of interest in this research.

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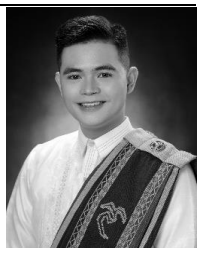

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