Original Research

Effectiveness of *Cymbopogon citratus* (Lemongrass) oil and *Ocimum basilicum* (Basil) oil as an Organic Anti-Mosquito Agent against *Aedes aegypti*

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ABSTRACT

Mosquitoes are the type of agents that people are eager to eliminate, as they disturb sleep, relaxation time, or any type of activities. This study aimed to investigate the effectiveness of an alternative intervention against the proliferation of mosquitoes. The purpose of this study was to create an organic anti-mosquito agent in the form of oil against Aedes aegypti by extracting oil from *Cymbopogon citratus* (Lemongrass) and *Ocimum basilicum* (Basil). The samples were mixed with water and boiled to extract the oil. The oil extracts in this study were classified into three categories; lemongrass oil, basil oil, and a combination of lemongrass and basil oil that consisted of 50% of each extract. Lemongrass oil showed mortality rates of 51.1% (23/45) for the first hour, 59.5% (25/42) for the second hour, and 74.3%, (26/35) for the third hour. Basil oil showed mortality rates of 43.8% (14/32), 41.7% (15/36), and 28.6% (10/35) in the same time intervals. Lemongrass-basil oil showed mortality rates of 44.4% (20/45), 52.8%, (19/39), and 60%, (18/30) respectively. Results showed that among the three organic anti-mosquito oils that were used, the lemongrass oil and lemongrass-basil oil were effective. This was concluded due to the increased mortality rates of the lemongrass oil and lemongrass-basil oil in each trial as it both reached the 50% mortality rate in killing mosquitoes on the basis of lethal concentration.

Keywords: Mosquito, *Aedes aegypti*, Lemongrass, Basil, Organic oil extracts

INTRODUCTION

One of the most common insects that humans encounter is the mosquito. Mosquitoes are the kind of insect people want to get rid of because they usually ruin your sleep or relaxation time. According to the Illinois Department of Public Health, there is no doubt that mosquitoes are the most dangerous creatures on earth. Every year, millions of people die as a result of their ability to carry and spread diseases to humans. Dengue fever's global incidence has increased 30-fold in the last 50 years, and more countries are reporting their first outbreaks of the disease (CDC, 2021). Mosquitoes and the diseases they spread have been responsible for killing more people than all the wars in history. There are a lot of diseases that mosquitoes can transmit. Some of the most common diseases are dengue, malaria, chikungunya, yellow fever, and Zika fever. The U.S.

Army came up with DEET (N, N-diethyl-3-methylbenzamide) in 1946, which is a man-made insect repellent (EPA, 2022). For years, DEET has been commonly found in insect repellents as an active ingredient. In our generation now, many kinds of insect repellents are already available on the market. People are much safer and better protected from dangerous insects like mosquitoes now that there are insect repellents on the market. However, the use of DEET and other synthetic mosquito repellents can harm not only mosquitoes but also the environment (EPA, 2022). Mosquitoes can also be kept away with things that we normally bring in and out of our homes. Mosquito repellents have progressed into one of the most effective means of preventing disease transmission and the discomfort caused by mosquito bites. Natural products, including essential plant oils, have been used as repellents for at least two millennia in China, Egypt, and ancient India. In recent years, hundreds of studies on plant derivatives with repellent properties have been published. (Zhu et al., 2018)

Organic materials that usually linger in and out of our homes can also be used in repelling mosquitoes. Natural repellents are found to be safe and better for the skin. Plant based repellents are chemical free and have many advantages over the chemical based repellents. Varieties of plant based mosquito repellents are available in the market. . As well as, one can easily prepare mosquito repellent formulations with the natural ingredients available at home. Ingredients like garlic, neem, cloves, camphor, cinnamon, bay leaves, lavender is easily available at home, which are utilized to prepare mosquito repellents which are totally safe and effective and are chemical free. The home-made formulations are found to be effective as well as promote a good safety profile. (Sheikh et al., 2020).

Organic materials that usually linger in and out of our homes can also be used in repelling mosquitoes. One of the commonly known organic repellents is the lemongrass oil. Lemongrass (*Cymbopogon citratus*) and lemongrass oil is usually used in adding flavor and aroma in our food and it has a lot of benefits in terms of medicine. Lemongrass oil contains insecticidal properties that repel insects. Lemongrass essential oil (LEO) contains a significant amount of bioactive compounds such as citral (a geranial and neral mixture), isoneral, isogeranial, geraniol, geranyl acetate, citronellal, citronellol, germacrene-D, and elemol, among other bioactive compounds. These components give LEO pharmacological properties such as antifungal, antibacterial, antiviral, anticancer, and antioxidant properties (Mukarram et al., 2021). Citral is one of the major constituents of the lemongrass oil and it gives the lemony fragrance that helps in repelling mosquitoes.

On the other hand, basil's (*Ocimum basilicum* L.) different varieties are mainly attributed to varying chemical constituents due to variations in temperature, humidity, acidity, and other environmental factors. It contains appreciable quantities of fatty acids, but essential oil obtained from plant leaves is of extreme importance due to diverse applications ranging from flavoring agents to therapeutic medicines. It is known to have strong synergistic and analgesic effects along with antiplatelet, antithrombotic, antihypertensive, phytoremediatory, antihepatotoxic, anticolic, cytotoxic, anxiolytic or sedative, antiosteoporotic, anticardiovascular, antistress, immunomodulatory,

radio protective, antimalarial, insecticidal, antidiabetic, antiulcer, anti-inflammatory, antifungal, antibacterial, antiviral, anticancer, and antioxidant properties. (Nadeem et. al., 2020).

METHODOLOGY

Research Design

This study used experimental research design to determine the effectiveness of *C. citratus* (lemongrass) and *O. basilicum* (basil) as organic anti-mosquito agents against *Aedes aegypti* in a test cage. The concentration of the *C. citratus* and *O. basilicum* was controlled. Experimental research design uses two variables. By using this research design, it ensures that the accuracy of the factors presented is maximized. The dependent variable relies on the independent variables presented in this study (Bell, 2017).

The independent variables are the *C. citratus* and *O. basilicum*; the dependent variable are the mosquitoes. The dependent variables rely on the effectiveness of the two independent variables mentioned above.

Setting of the Study

In this study, the plant extracts that were used in this study were collected in Maharlika Village, Taguig City and the mosquitoes were bred in dirty stagnant water. Consequently, the experiment was conducted in the laboratory of Emilio Aguinaldo College - Manila for an estimated time frame of one (1) month.

Figure 1. Schematic diagram of the study.

Preparation of Materials

For the efficiency of the organic anti-mosquito agent, specific materials are needed to perform the experiment. For this study, the researchers needed mosquitoes that are used to be the subject for the testing of the organic anti-mosquito agents. The organic anti-mosquito agents that are used for this study were the following: lemongrass oil, basil oil, and lemongrass-basil oil.

Collection and Identification of Plant Materials

The plant materials such as *C. citratus* and *O. basilicum* that are used for this experiment was obtained from Maharlika Village, Taguig City. The plants that are used in this study are identified and authenticated by the University of the Philippines Institute of Biology in Diliman, Quezon City.

Preparation of Mosquitoes used for the experiment

The mosquitoes that are used for this experiment were bred in dirty stagnant water. The mosquito that was used in this study was identified by the Museum of Natural History in University of The Philippines Los Baños, Laguna. The number of mosquitoes that were used for the whole experiment was approximately five hundred eighty-five (585) mosquitoes since for each test, approximately forty-five 45 mosquitoes are used and there are five (5) anti-mosquito agents that were used in this study, including the negative and positive control group and the test for each agent are repeated three (3) times.

Handling of Mosquitoes

After the breeding of mosquitoes, the mosquitoes have been put in a clean and empty gallon bottle. The top of the bottle was sealed by a clean cloth so that the mosquitoes can still have

proper ventilation of air. The food of the mosquitoes was a sugar solution and they were fed every 4 days.

Preparation of Mosquito Cage

A size of 45 x 50 x 45 cm test cage was used for the experiment. The frame of the test cage was made from wood. All of the sides of the test cage were covered with observable mosquito nets to be able to see the inside. The wood and mosquito nets that have been used in this study were acquired from the hardware stores. One side of the test cage was made accessible to be the entrance for the mosquitoes and the organic mosquito diffuser.

Preparation of Organic Oil for Diffuser

The samples were chopped into small pieces and then weighed. After weighing, the samples of *C. citratus* and *O. basilicum* were placed in the RB flask. Then, researchers added the distilled water. The level of the distilled water must be at the half of the RB flask. The researchers mixed the sample by shaking the RB flask carefully. After securing the RB flask in the electric heating mantle, the researchers arranged the remaining parts of the Clevenger apparatus. Then, the experiment started by turning on the heater to 90-100 degree Celsius until it starts to boil. After it boils, the heat is lowered down to 40-50 degree Celsius. The set-up is left for 1-2 hours. When it starts to boil, the oil and water vapor slowly evaporates and moves upward and reaches to the condenser and both of the condense gets collected to the glass pipe. The oil was collected using a vial by loosening the knob slowly.

Table 1. Plants that are used as organic anti-mosquito agent

Table 2. Components of organic anti-mosquito oil for the diffuser that was used in the experiment.

Data Collection

The collections of data are done by recording the number of mosquitoes who died on the process during the observation time. The duration of observation for the testing of each organic antimosquito agent including the control test was 60 minutes. Three (3) repetition of this process was done to complete the data.

Data analysis

The data that has been collected were evaluated in mortality rate of each organic anti-mosquito agent to determine if there is a difference between the control group and the treated groups, and also a difference within the data collected from the treated groups. The results from this analysis were determined if the organic anti-mosquito agents were effective against the mosquitoes. Furthermore, this was used to determine if each of the organic antimosquito agent's effectiveness were different from each other.

Risk Assessment

The work makes use of a pathogenic insect vector that could represent a hazard to human well-being. Due to possible occurrence, the researchers adhere in accordance with the Guidelines for biosafety and biosecurity in mosquito rearing facilities under the guidance of competent scientists. The mosquitoes must be collected, handled, and disposed of properly. The researchers must wear proper clothes, gloves, laboratory coats/gowns, at all times to prevent unnecessary injuries (FAO/IAEA, 2021).

RESULTS AND DISCUSSION

The purpose of this study was to determine if the organic anti-mosquito agent made from the oils of C. *citratus* and *O. basilicum* are effective against

A. aegypti and its differences in terms of their effectiveness in different duration and number of mosquitoes that die inside the cage. However due to a lack of time, only a single exposure of the experimentation was done.

Table 3. Results in 1 hour of lemongrass oil, basil oil, and lemongrass-basil oil of the experimentation together with the control groups.

Table 3 interprets the data gathered in the first hour of the experiment. For lemongrass oil, the population started with 45 mosquitoes. 23 of them died (see appendix 4.1.), and it has a 51.1% mortality rate. For basil oil, the population started with 32 mosquitoes. 14 of them died (see appendix 4.4.), and it has a 43.8% mortality rate. For the combined oil of lemongrass and basil, the population started with 45 mosquitoes. 20 of them died (see appendix 4.7.), and it has a 44.4% mortality rate.

The lemongrass oil reached 51.1% killing rate, since it has geranyl acetate and citral that exhibits insecticidal activity (Plata-Rueda et. al., 2020). It also reached the 50% killing rate which means that it is effective in killing mosquitoes based on the definition of lethal concentration wherein half of the population dies after a certain period of exposure (CCPS, 2023). Basil oil reached 43.8% killing rate because it has compounds such as linalool, estragole, 1,8- cineloe, α-pinene, β-pinene, farnesene, germacrene, hexadecenoic acid, menthol pulegone, ocimene, D-camphor etc., which may be the inherent character for its toxic nature to the mosquitoes(Naveen et. al., 2020) However, basil oil does not reach the 50% mark for the LC50. For the combined oil of lemongrass and basil, it reached 44.4% killing rate in spite of having both of the beneficiary components of lemongrass and basil.

Table 4. Results in 2 hours of lemongrass oil, basil oil, and lemongrass-basil oil of the experimentation together with the control groups.

Table 4 interprets the data gathered within the two hours of the experiment. For lemongrass oil, the population started with 42 mosquitoes. 25 of them died (see appendix 4.2.), and it has a 59.5% mortality rate. For basil oil, the population started with 36 mosquitoes. 15 of them died (see appendix 4.5.), and it has a 41.7% mortality rate. For the combined oil of lemongrass and basil, the population started with 39 mosquitoes. 19 of them died (see appendix 4.8.), and it has a 52.8% mortality rate.

It has been demonstrated that lemongrass oil effectively kills mosquitoes based on the increased mortality rate of mosquitoes found in lemongrass (see Table 2.). Due to the presence of active components such flavonoids, saponins, tanines, and essential oils that are harmful to mosquitoes, basil leaf extract has a bio insecticide impact on *A. aegypti* (France et al., 2020). However, despite having these active components, the basil oil still cannot kill half the population of mosquitoes in this period of time. The mortality rate for the Lemongrass-Basil combination is 52.8%. The killing rate in lemongrass-basil oil increased in this period of time because of the different active components that the lemongrass and basil has.

The killing rate in lemongrass-basil oil increased in this period of time because of the different active components that the lemongrass and basil has. Both the lemongrass oil and lemongrass-basil oil reached the 50% killing rate which means that it is effective

in killing mosquitoes based on the definition of lethal concentration wherein half of the population dies after a certain period of exposure (CCPS, 2023).

Table 5. Results in 3 hours of lemongrass oil, basil oil, and lemongrass-basil oil of the experimentation together with the control groups.

Table 5 interprets the data gathered within the three hours of the experiment. For lemongrass oil, the population started with 35 mosquitoes. 26 of them died (see appendix 4.3.), and it has a 74.3% mortality rate. For basil oil, the population started with 35 mosquitoes. 10 of them died (see appendix 4.6.), and it has a mortality rate. For the combined oil of lemongrass and basil, the population started with 30 mosquitoes. 18 of them died (see appendix 4.9.), and it has a 60% mortality rate.

The killing rate for lemongrass oil and the combination of lemongrass and basil oil continues to increase as the time duration also increases. In the 3 hours of experimentation, the average of lemongrass oil is 74.3%. It is because of the different active components that the lemongrass has that helps to kill the mosquitoes (Plata-Rueda et al., 2020). While, 60% average in the lemongrass-basil oil. Both the lemongrass oil and lemongrass-basil oil reached the 50% killing rate which means that it is effective in killing mosquitoes based on the definition of lethal concentration wherein half of the population dies after a certain period of exposure (CCPS, 2023). The basil oil, on the other hand, continues to decrease as the time duration also increases. In this period of time, it has a mortality rate of 28.6%, it is very low compared to the average in the other time duration. Seeing that despite having different active components it is not enough to kill half the population of mosquitoes.

CONCLUSION

Among the different organic antimosquito agents that were used in the experiment in the study, lemongrass oil and lemongrass-basil oil are the only effective among the three (3) plant extracts that were tested. - As the time duration increases, only the lemongrass and the combination of lemongrass and basil are the only effective in killing the mosquitoes. - since the setting of the mosquito is most likely to manifest in the house, using this different organic anti-mosquito is an advantage as it is made in plant extract.

RECOMMENDATIONS

1. Seek for a more efficient and easy way of placing the mosquitoes inside the cages.

2. Increase the number of trial tests done for each organic anti-mosquito agent to have more accurate and precise results.

3. Change the solvent used for making the oils from the different plants to extract more of the active component of the herbs.

4. Different herbs and other combinations of them shall be used as an organic antimosquito agent.

5. Have the same number of mosquitoes per organic anti-mosquito agent to have more accurate and precise results.

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