Sweet Basil (Ocimum basilicum) and Mint-Basil (Mentha × piperita f. citrata) leaves' Larvicidal Pellet for Mosquitoes

A Research Paper presented to the Senior High School Department as partial fulfillment of the requirements in Capstone Project, Practical research 2, and Inquiries, Investigations, and immersions

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Abstract

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The growing number of Dengue victims every year on the Philippines with a reported 371,500 cases as of November 2019, according to European Civil Protection and Humanitarian Operations (ECHO), brought about studies that seeks vector control. In this notion, this research with the purpose of creating an alternative solution to the fast propagation of mosquitoes was made. The goal is to make four types of larvicidal pellet made from 100% Sweet Basil, 100% Mint-Basil, 70% Sweet Basil and 30% Mint-Basil, and 70% Mint-Basil and 30% Sweet Basil. Guided with measuring only the duration of dissolution and larvicidal activity, with larvae drawn from clean stagnant water and canals, the dissolution property of the pellets with size ranging from 0.5-0.8 cm had a mean of 15 minutes and 57 seconds. Following Quasi-Experimental approach, each pellet type had three set ups with 20 larvae contained, 10 pellets were dissolved. Mortality rate ranges from 11.67%-20%, 20% from 100% Sweet Basil on 720ppm, 16.67% on 100% Mint Basil, 320ppm, 15% on 70% Sweet Basil and 30% Mint-Basil, 480ppm, and 11.67% on 70% Mint-Basil and 30% Sweet Basil on 320ppm. Determining whether basil type and time interval affects mortality rate was measured through two-way ANOVA which showed that neither of the two nor its interaction affect the mortality rate of the pellets.

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The Researchers

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Chapter I

Introduction

This year 2019, dengue has become a nationwide epidemic in the Philippines. Reporting over 371,500 cases as of November 2019 by the European Civil Protection and Humanitarian Operations (ECHO), Philippines is one of the highest countries in South-East Asia with the largest increase in the number of detected dengue cases. According to the World Health Organization (WHO), on the 42nd week as of 2019, there were about 5,927 cases were reported all over the country. In 2019 there were 371,717 cases including 1,407 deaths were reported which is compared to the 180,072 cases including 927 deaths reported in the same period last year. These tiny, bloodsucking insects can be found all around, especially in dirty, unkempt places around the neighborhood. Diseases such as dengue, malaria, Zika virus, West Nile virus, and Chikungunya virus, are just some of what one can contract from the mosquitoes' notorious bite. While these insects are widely considered as pests for humans, mosquitoes are still vital to the ecosystem, as they contribute to the environment's food chain and pollination; they also increase the diversity of species, and latest medical discoveries found ways to incorporate their saliva for anesthetic properties.

Due to the alarming diseases brought by these mini vampires, mosquito solutions have been widely used in the households to shy away from their bites. Ranging from organic to the chemically-made products, the market is teeming with options to minimize the risk of mosquito-related diseases. The most commonly used solution inside Filipino's homes is the mosquito coil. Burning these coils generate smoke that subdue mosquitoes, but these may possibly have adverse effects to the human body. Other repellants are insect sprays, mosquito patches, insect repellant lotion, anti-mosquito wrist bracelets, and more.

Organic alternatives for insect repellants are available and can be used by either burning, applying through the body, hanging inside the house, or by drinking the decoction. The stem of the ipil-ipil, the leaf of eucalyptus and malunggay, fruit of kupang, and more, are organic plants that can be developed to repel mosquito and lessen the possibility of getting related diseases. One good thing about these organic alternatives is that it is accessible, easy to find, and won't cost a lot.

The researchers are formulating an alternative to eliminate mosquitoes while they are still in their larval stage, to further prevent these insects to grow into full-sized bloodsucking pests. There are ways to eliminate these insects while they are still hatching, particularly by making use of chemicals. The researchers of this study are looking into an organic approach that may save time, resources, and money, while making use of organic plants available in the environment.

Basil are plants common inside the kitchen, particularly for cooking, but research has found that basil essential oil possesses antimicrobial and insecticidal properties, which marks it as a possible component in natural preservatives and organic pesticides. The researchers formulated a more organic, cost-effective, accessible and easy way to repel and eliminate mosquito larvae, by utilizing basil leaves. This study can further help Filipinos keep their homes disease-free and lower the risk of fatalities caused by these bloodsucking insects' bites.

Statement of the Problem

This study aimed to create a larvicidal solution out of Basil (*Ocimum basilicum*) and Mint-Basil (*Mentha* × *piperita f. citrata*) leaves in a form of pellet, and intends to answer the following questions as basis for the whole study.

- 1. How long does it take to dissolve the pellet?
- 2. What is the mortality rate of mosquitoes in the pellet made from;
 - a.100% Sweet Basil
 - b.100% Mint-Basil
 - c.70% Sweet Basil and 30% Mint-Basil
 - d.70% Mint-Basil and 30% Sweet Basil
- 3. Is there a significant difference on the larvicidal strength of pure basil, pure mint-basil, and the mixed proportions of sweet and mint-basil?

Research Hypothesis

The researchers tested the following hypothesis as the research goes and would serve as the source of data for the generation of the findings.

H₀: there is no significant difference on the larvicidal strength of pure sweet basil, pure mint-basil, and the mixed proportion of sweet and mint-basil.

Scope and Delimitations

The rapid and continuous increase on the number of dengue victims every year and the fast propagation of the mosquitoes especially in the tropical countries has been a big problem to the society. For this matter and given the availability of basil in the country, which is known for its natural mosquito killing properties, the researchers intend to create a Larvicidal Pellet that could possibly help to this growing problem. Various sectors of the society, including the Department of Science and Technology (DOST) and the Department of Health (DOH) would be highly benefited from this project.

However, the researchers only utilized the available species of mosquito in the locality drawn from places with clean, stagnant water or canals. Moreover, the experiment only took place for a limited amount of time starting from mid-November 2019 to March 2020, not allowing further tests to be done to support and justify the effectiveness of the Larvicidal Pellet in killing mosquito larvae. Experiments took place at different locations, on the researchers' household at any time available for the generation of raw facts to be used on the findings.

Significance of the Study

The study 'Basil (*Ocimum basilicum*) and Mint-Basil (*Mentha × piperita f. citrata*) leaves' Larvicidal Pellet for Mosquitoes' would be beneficial to the following sectors:

Consumers. A sense of security and a way to eliminate possible dengue-vectors to make them and their family safe.

Community. A safer place to live among families and reduced possibility of mosquito-borne diseases.

Department of Health (DOH). Decrease on mosquito-borne diseases and lesser casualties on both cases and death toll.

Department of Science and Technology (DOST). Provides solution in decreasing the risk of Dengue-Outbreak in the country and gives an alternative way for killing mosquito larvae.

Researcher. The research will serve as a source of topics among young researcher in the future and provides supporting evidences to another future research.

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Chapter II

Review of Related Literature and Studies

This chapter contains the compilation of various past literature and studies used as both basis and support of the current study. Articles and manuscripts contained in this part of the paper are works of different authors combined as one to prove and validate the contents of the study.

Review of Related Literature

As the Department of Health (2008) stressed that because of the big impact of the environment to the health of individuals, measures that involve the protection of the environment has become the centerpiece of public health practices. In this matter, efforts coming from various sectors to ensure the cleanliness of air and safe food supplies and water, well managed sewage and municipal wastes, as well as the control and elimination of vector-borne illnesses are enforced, bringing significant changes in improving public health in the US. This proves that the environment plays an important role in the development, health and diseases of every individual as environment in a broad sense, including all infectious agents is one of the three primary factors that affect human health, with the other being genetic factors and personal behavior (WHO, 1997).

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Dengue is said to be the most important vector-borne viral illness worldwide. Caused mainly by mosquitoes, the number of cases has increased for about 30 folds in the past 50 years based from the Global Alert Response in 2015. In this matter, rainfall plays a vital role as it serves as an excellent breeding ground for mosquitoes. This statement was also supported by Kishor et. al (2018) as he claims that unsheltered containers that are filled with rainwater is an excellent site for larval development for *Aedes* mosquitoes. Another factor is the presence of human households as it increases dengue-carrying mosquito's activity. This was then supported by Banerjee et. al. (2015) as they claimed that household generated wastes and water retentions was said to be playing a great role in the abundance of dengue-carrying mosquitoes.

Basil Leaves are known to be natural insect repellents for its emitted aroma. Characterized as one among volatile plants used for killing and repelling mosquito, it is toxic if used against the larval stage of an insect thus rendering it useful if placed near standing water (Daniels, E., 2018)

Aedes aegypti is the vector of dengue hemorrhagic fever. Vector control is one of the methods to prevent the spread of dengue fever. Principal activity to control this vector can decrease the population as transmitter of disease. One way to control mosquito vector is using basil leaves as bio insecticides. The extract of basil leaves has bio insecticide effect to Aedes aegypti because basil leaf has some active compounds, such as Flavonoid, Saponin, Tanine and essential oils which are toxic to mosquitoes. The extract of basil leaf is effective as Aedes aegypti bio insecticide in mosquito coil. Formulation and methods of administering larvicide and pupicide, that is safe for humans and other animals, capable to kill all instars larval stages and pupae around two hours and fifteen minutes without affecting the surroundings. The formulation and methods of administration thereof eliminating larvae and pupae of Anopheles gambiae and An. stephensi, primary vectors of malaria, and Aedes aegypti and Ae. albopictus, primary vectors of dengue, Zika virus, chikungunya and yellow fever.

In a 2009 study, it is found out that extracts of basil leaves (Ocimum Bacilicum) can kill a wide range of mosquito larvae because of its high toxicity and is rendered usegul for the management of mosquito larvae (Maurya, P., Sharma, P., Moham, L., Batabyal, L., and Srivasta, C.N et. al, 2009).

On the year 2011, literatures listed that basil leaves without any complementary compound can give a 79% efficiency on deterring several species of mosquitoes. The said review also found a 100% deterring rate in mosquitoes under laboratory setting. The review even highlights that basil extracts is the safest to use at 0.07% concentration for the reduction on the risk of exposure to the known carcinogen, methyl eugenol.

One of the most dangerous insects in the world for humanity are mosquitoes. Every year almost over one million people from the population die because of some mosquito diseases which is protozoan in other words malaria, filarial diseases such as dengue, encephalitis and yellow fever. Mosquitoes transmits several diseases and parasites that are very susceptible. Since discovery, there are chemical insecticides that are represented because of mosquito-borne vectors.

Related Studies

In the study conducted by Laraib, I., Raza, F.A., Kiran, A. and Sajid, I. (2018) control bioassay were conducted by mixing acetone to the *Ocimum basilicum* extract. After 24 hours of treatment, 50% mortality was recorded, 83% after 48 hours and 90% mortality after 72 hours. Write the ocimum basilicum leaves extract added to ethanol extract recorded 10% mortality for 24 hours, 43% after 48 hours and 47% after 78 hours.

Negative effects of synthetic insecticides have become the motivation for a quick search for alternatives that is adequate for both the environment and public health. The use of plant extract-based insecticide is currently highly capable from the alternatives of protection against insects. A number of plant extracts containing substances with insecticidal effects also involve a big group of the so-called essential oils (EOs). Some of the EOs were obtained from aromatic plants which include the *Ocimum basillicum* that could become a suitable source of active substances for potential botanical larvicides.

The oviposition preemption and ovicidal potential of five different essential oils, peppermint oil (*Mentha piperita*), basil oil (*Ocimum basillicum*), rosemary oil (*Rosemarinus officinalis*), citronella oil (*Cymbopogon nardus*), and celery seed oil (*Apium graveolens*), were evaluated against female adults of the dengue vector, *Aedes aegypti* L. Several concentration tests were carried out which shown that

adding 100% oil (pure oil) produced a complete oviposition preemption except in *A. graveolens*. Studies in the ovicidal effectiveness of these oils showed that the eggs laid in the water with 100% essential oils did not hatch at all, however when 10% oils were used, only the *r. officinalis* oil resulted in 28% egg hatch. And the *M. peperita. O. basillicum,* and *C. nardus* revealed complete egg mortality at lower concentrations (1%) whereas *A. graveolens* and *R. officinalis* resulted in 71% and 34% egg hatches. When the *O. basillicum* oil is used at 0.1% concentration, it was found to be the only effective oil with 100% egg mortality, whereas the other oils resulted in 16-76% egg mortality and the *A. graveolens* oil having the least mortality caused.

In the study conducted on the National Centre, Sinhar State, Sudan to test the efficacy of hexane extracts of rehan/basil (*Ocimum basilicum*) used as a paper impregnation against the *Anopheles arabiensis pattin* of adult mosquitoes. Results indicate that rehan/basil hexane extract at 10% concentration exhibits repellent property up to two hours and was superior to the standard insecticide.

Organic insecticide made from the extracts of Bitter Melon *(Momordica charantia L.)* and Basil *(Ocimum basillicum)* leaves was applied to dengue mosquito vector and it was observed that the hatchability of eggs to larvae, progress of larvae to pupae and pupae to adult were decreasing. However, there is no effect in the development of pupae to adult.

The toxicity bioassays of Thymus vulagaris, Ocimum basillicum, Origanum dictamnus, Origanum majorana, and Origanum vulgare EOs, along with their

major components (terpenes), thymol, calvacrol, p-cymene, and y-terpinene revealed the most larvicidal effect. Between the terpenes tested, piperitenone expoxide, carvacrol, thymol, and piperitenone yielded highest level the highest level of protection against Ae. albopictus adults. The EOs high number of terpenes was revealed through chemical analysis, mainly, the tested Eos and their major component's biological actions were consistent. The identified EOs and terpenes through current laboratory bioassays were proven effective and may well be used as alternative agents in controlling larvae and repelling adult Ae.Albopictus.

Study conducted by Kumar, et. al., (2017) showed that the essential oil of Basil (*Ocimum basilicum*) leaves is recommended as an efficient repellent and as a moderate larvicide agent against Ae. Aegypti. The discovery of bioactive components of the basil leaves oil. In evaluating the outcome, the creation of a new strategy for mosquito management is needed to be acquired, knowing all aspects with the help of advance way of identifying its properties and components.

The A. arabiensis larvae exposed to the Basil Leaf Powder Extract (LPE) tested concentration at 24 hours resulted in a LC50 of 58mg/L, an LC90 of 143mg/L with an Ld-P line slope 3.04. A20% mortality was observed with the lowest dose of 2mg/L and a 90% mortality resulted from the highest dose 10mg/L. During the 20 min, there was no mortality recorded but in the rest of the concentrations at 10 mins of exposure, mortality was observed.

The bioassay shows that there is a low potential larvicide at LC50 367.6ppm, P value <0.01, and coefficient determination of 0.9980 for the species of M.piperita. The larvae develop from pupas into adult after 60-72 hours so the bioactivity is ineffective. The potential larvicide of the species was observed in periods of 24 and 48 hours (111.9 and 98.66) in reduction of LC50.

The study conducted at the Cebu Doctor's University research laboratory room about the insecticidal potential of Basil plant *Ocimum basillicum*) and Clove *(Syzygium aromaticum)* and its ability on attaining 100% mortality against American cockroaches showed that at 50% concentration, the two leaf extracts achieved a 100% mortality of cockroaches but only differ in terms of time and Basil having 100% mortality significantly faster compared to Clove.

Synthesis State-of-the-Art

Previous studies conducted by Maurya et. al. (2009) along with Daniels (2018), Ramayanti et. al. (2017), Cabilte et. al. (2016), Laraib et. al. (2018), Warikoo, Wahab, and Kumar (2011) as well as Mayang Sari Yeanny (2019) and Cambiado (2018), showed the use of basil leaves as a larvicidal agent among various species of mosquito as a biological control and solution to the fast propagation of mosquito population. Although various methods and approaches in using the plant was administered the use of basil and the larvicidal activities showed are enough support to the current research which utilizes the same organic component.

Given the damage these mosquitoes could bring, producing insecticides for which the study focuses are one of the best solutions to prevent its propagation, but since insecticides can be harmful to both the humans and the environment, organic insecticides drawn from plant-based solutions are what the modern generation seeks for.

The use of volatile plants which includes Basil is one of the key methods that most researches nowadays does. Studies of Maurya et. al. (2009), Daniels (2018), Ramayanti et. al. (2017), Cabicabiltelte et. al. (2016), Laraib et. al. (2018), Warikoo, Wahab, and Kumar (2011), Mayang Sari Yeanny (2019) and Cambiado (2018) followed this principle for which the researchers of the current study also applies. These researchers sought after plant sources that could generate mosquito control solutions.

Gap Bridged by the Study

The study 'Basil (*Ocimum basilicum*) and Mint-Basil (*Mentha × piperita f. citrata*) leaves' Larvicidal Pellet for Mosquitoes' aims to provide an alternative and organic solution for controlling the population of mosquito that are potential disease carriers to ensure that the risk of mosquito-borne diseases is minimized. Furthermore, the insecticide would not be harmful to the environment for purely organic materials are used thus, poisoning and the issue of pollution will not be aggravated.

The present study would also bring in solution to the immunization of some mosquitoes to the insecticides available on the market by introducing a new approach to the formulation of mosquito control.

Conceptual Framework

The conceptual framework shows the relationships among variables involved in the research study to be conducted. The diagram includes the set of dependent variable, dependent variable, and intervening variables which determines the flow of information in the study. It explains briefly the cause and effect relationship among the variables.

The independent variables show the factors that could determine the outcome of the study, the result of tests and experiments, as well as the things that should be considered to be able to conduct the research. It includes the amount of basil extract in the pellet, the amount of pellet per set up and the proportion of the extracts used in making the pellet.

The dependent variable is the set of possible outcomes that could take place in doing the research. Factors involved greatly depend on the Independent variables present as a change and manipulation on the independent variable could highly affect the outcome, dependent variable. The number of mosquito larvae killed, duration before a larva is killed and the pellet's effectiveness are under this factor.

The intervening variables involved the factors that could possibly hinder the relationship of the Independent and Dependent Variable. It includes the environmental factors as well as the external factors that could either prevent the success of the experiment or change a few factors in the study. Under this factor are, source of larvae and the effect of added ingredients to the effectiveness of the pellet.

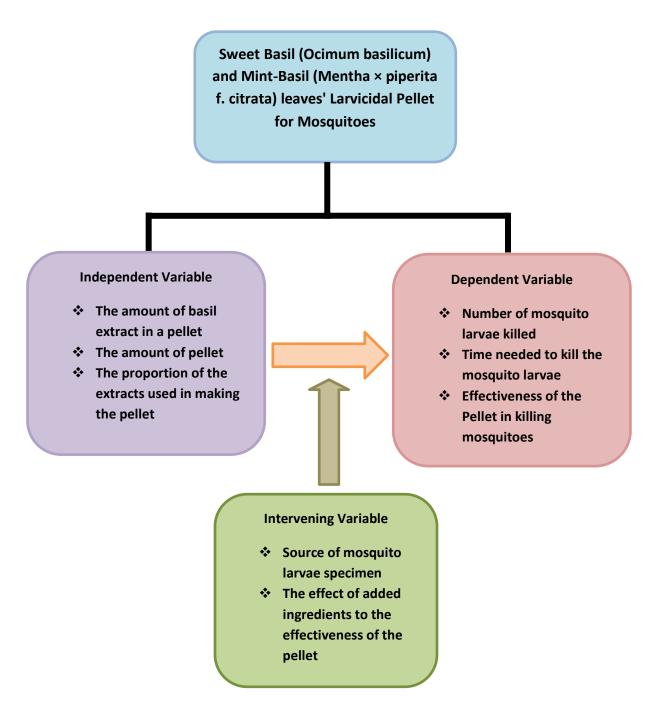


Figure 1: Conceptual Framework

Theoretical Framework

The theoretical framework contains the theories that will be used as basis for the development of the research. The study is anchored to the following; a. Control Theory, b. Seq0uence Theory, c. Ross-Macdonald Model and Theory.

The **Control Theory** was established by Milsum on 1966 and was originally applied to the field of engineering for regulating mechanical systems. The theory states that changes on complex systems can be regulated and controlled by negative feedbacks. Thus, in this study which uses larvicides in order to control vectors, the theory is very essential in supporting its cause.

Howard and Fiske's **Sequence Theory** (1911) states that biological control can only be achieved through the various natural enemies attacking the host and that single best natural enemy would result to a successful biological control. Using natural ingredients and plant-derived chemicals in fighting future vector disease carriers is governed by this theory. The study also targets only one stage of the enemy thus, focusing on it would yield better results.

The **Ross-Macdonald Model and Theory** (1899-1949) describes that the vectoral capacity/daily reproductive rate of mosquito affects the intensity of disease transmission among humans. Through this study, which targets the still harmless developmental stage of mosquitoes would be a great measure to lessen the probability of having high intensity disease transmission. Controlling the subject's propagation would be a necessary step for lower risks.

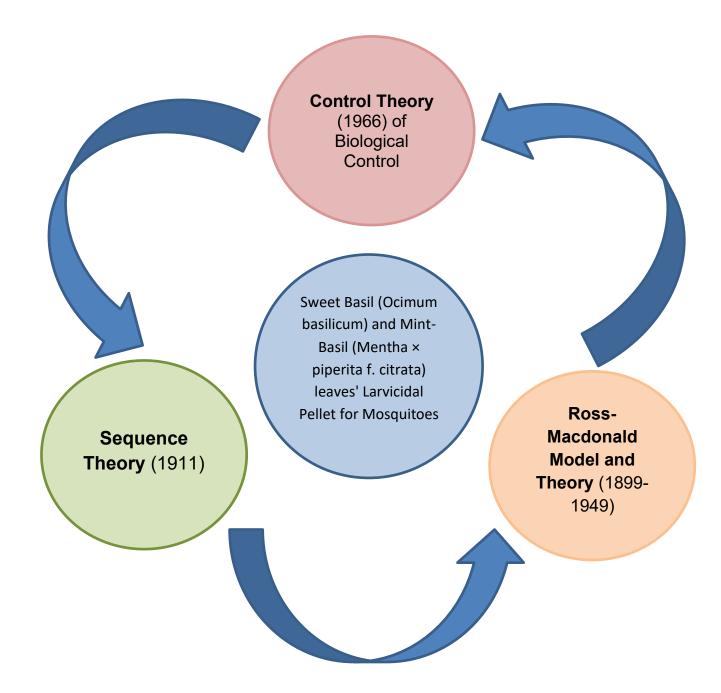


Figure 2: Theoretical Paradigm

Definition of Terms

Basil (*Ocimum basilicum*) – a member of *Ocimum*, family: *Labiatae*, a genus of aromatic plants, especially, *O.basilicum*, sweet basil, and *O. minimum*, bush basil, used as culinary herbs (*O.F. basil from L. basilica*)

Larva (plural: Larvae) – *Biology*, the early form of any animal when it is unlike the parent, or undergoes a metamorphosis. *Entomology*, the first stage of an insect after leaving the egg, preceding the pupa, as a caterpillar, a grub, or a maggot.

Mosquito (*Aedes aegypti*) – any of many two-winged flies of family *Culicidae*, distributed throughout the world, having a narrow abdomen, rigid proboscis and their wings fringed with scales. The female has a needlelike mouthpart for puncturing the skin of animals and man in order to suck the blood.

Pellet – a small round ball or imitation projectiles, as of wax, paper, bread, et. A small shot. A very small pill. A sling stone, also a bullet.

Ovicidal – capable of killing eggs.

Ovicide – an agent that kills egg, an insecticide effective against the egg stage.

Larvicidal

Larvicide – an insecticide or other pesticide used in killing larvae.

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Chapter III

Methods and Procedures

This chapter contains the steps involved in making the study and experiments possible. Plans and procedures that were followed by the researchers as stepping stones to keep the research going and generate all the necessary information in supporting the study.

Research Design

The research under the Quantitative Approach follows under the principle of an Experimental Research Design. Thus, experiments that tests the effect of the pellet made from basil leaves against mosquitoes were conducted. Categorized under Quasi-Experimental Design, the researchers choose to create various set ups giving random sample of mosquitoes to generate data. Guided by the Counter-Balanced Quasi-Experimental Design, different set ups containing 20 mosquito larvae were used to compare with each other's result and weigh the effectiveness of basil leaves.

Sources of Data

In order to produce the necessary data needed to support the study, the researchers through experimentation yielded a set-up composed of 20 mosquito larvae samples. Using the Basil leaves pellet which was dissolved in a cup of water where mosquito larvae was transferred, the researchers observed the

larvicidal activity of the pellets by determining the rate of mortality on the set ups. These data are recorded and serves as a source for statistical figures.

Instrumentation

Primary data generated through observation was used as a means to gather data in the study. Through the help of the senses as well as the various simple tools used in the experiment, the researchers were able to collect the necessary information needed to assemble facts that serves as the foundation of the study. Furthermore, results from tests conducted, given the ability of the researcher's, can only be interpreted through manual processes which was then converted into a sturdier scientific form through the use of statistics.

Data Gathering Procedure

Pre-Experimental

Phase 1: Gathering the Materials

Sweet basil and Mint-Basil plants are gathered along with ethanol and iodine solution that would be used for the extraction. Weighing scale and other measuring devices are used as an aid to ensure that the correct formula for the extraction is met. Clear containers and rods are prepared for building the set up in the experiments.

Phase 2: Making the Pellet

Step 1: Extraction

Two kinds of basil leaves, sweet and mint were prepared each weighing

100 g. The leaves were washed on running water and is drained of excess water before it was cut to pieces. The leaves were soaked on a 200 ml ethanol solution of 70% concentration for 24 hours and then filtered. Residues was then removed. After 3 days a drop of iodine solution is used to determine the presence of excess ethanol, its absence opening the start of pellet formation.

Step 2: Forming the Pellet

The extracts from the two different kinds of basil is poured on a container filled with sucrose. The sucrose would serve as the filler of the pellet along with hydroxypropyl methylcellulose (Hypromellose), as a binding agent to hold components of the pellet. Mixed ingredients are then shaped through Agitation- a process that creates spherical material via the gradual input of liquid into a material and continuous rolling or tumbling motion.

For the formation of the pellet the following formulas were followed:

Pellet 1: 100% Sweet Basil

- o 80 g sucrose
- o 31 ml sweet basil extract
- o 7.5 ml Hypromellose

Pellet 2: 100% Mint-Basil

- o 80 g sucrose
- o 15 ml mint-basil extract

o 7.5 ml Hypromellose

Pellet 3: 70% Sweet Basil and 30% Mint-Basil

- o 200 g sucrose
- o 21.7 ml sweet basil extract
- o 9.3 ml mint-basil extract
- 7.5 ml Hypromellose

Pellet 4: 70% Mint-Basil and 30% Sweet Basil

- o 80 g sucrose
- o 10.5 ml mint-basil extract
- o 4.5 ml sweet basil extract
- o 7.5 Hypromellose

These formulas would produce more or less 351 (Pellet 1), 324 (Pellet 2), 616 (Pellet 3), 362 (Pellet 4) pieces of pellet with size ranging from 0.5-0.8 cm. Each pellet composition being approximately:

Pellet 1: 100% Sweet Basil

- o 0.23 g sucrose
- o 0.09 ml sweet basil extract
- 0.02 ml Hypromellose

Pellet 2: 100% Mint-Basil

o 0.25 g sucrose

- o 0.04 ml mint-basil extract
- o 0.02 ml Hypromellose

Pellet 3: 70% Sweet Basil and 30% Mint-Basil

- o 0.32 g sucrose
- 0.04 ml sweet basil extract
- o 0.02 ml mint-basil extract
- o 0.01 ml Hypromellose

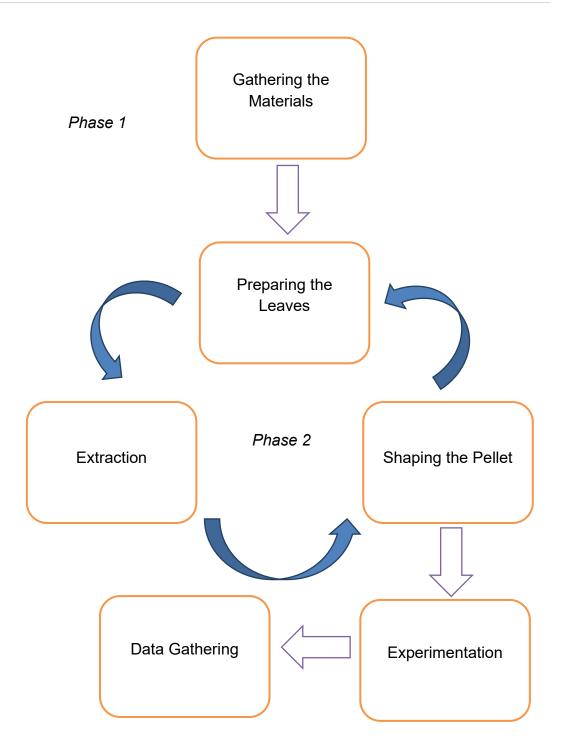
Pellet 4: 70% Mint-Basil and 30% Sweet Basil

- o 0.22 g sucrose
- 0.01 ml sweet basil extract
- o 0.03 ml mint-basil extract
- 0.02 ml Hypromellose

Experimental

Phase 3: Conducting the experiments and Gathering Necessary Information

Larvae drawn from clean stagnant water and canals are transferred into a clear container. Twelve (12) set ups were made each containing 20 larvae. Each pellet type having three (3) trials as per counter-balance strategy. Ten (10) pellets were added and dissolved over time on the container containing larvae. Observation occurs and recording of data takes place.



Phase 3



Statistical Treatment

To evaluate the data from the various set up of the Ovicidal-Larvicidal trap, the following statistical tools are to be used for the summation, comparison, and the measurement of the effectiveness of basil against mosquito ovules and larvae.

Two-Way ANOVA

A statistical tool used to compare two groups of samples to determine whether they are significantly different from each other. Two-Way ANOVA is used to compare two independent variables, pellet type and time interval and its relationship to one dependent variable.

Chapter IV

Analysis and Interpretation

This chapter contains data gathered from experiments conducted following the study. Listed variables, its connections and results, as well as the interpretations of data are presented in this part of the research.

Duration of Dissolution

Four types of pellet were created during the process of pellet production, each with the proportion, 100% Sweet Basil, 100% Mint-Basil, 70% Sweet basil and 30 % Mint-Basil, and 70% Mint-Basil and 30% Sweet Basil.

Two pellets were drawn from each pellet type. These pellets were put in a clear plastic container with 125 ml of water to dissolve. The time for dissolution were as follows:

Set up	1	2	3	4	5	6	7	8
Time of								
Pellet	27.05	15.20	19.11	15.58	15.58	15.20	17.08	13.32
Dissolution								

Table 1: Dissolution Duration Data

The duration for dissolving the pellets are 27 minutes and 5 seconds for set up 1, 15 minutes and 20 seconds for set up 2 and 6, 19 minutes and 11 seconds for set up 3, 15 minutes and 58 seconds for set up 4 and 5, 17 minutes and 8 seconds for set up 7 and 13 minutes and 32 seconds for set up 8. Pellets

in set ups 1 and 2 are 100% Sweet Basil, 70% Sweet Basil and 30% Mint-Basil for set ups 3 and 4, 70% Mint-Basil and 30% Sweet Basil in set ups 5 and 6 and 100% Mint-Basil in set ups 7 and 8. The mean being 15 minutes and 57 seconds.

Mortality Rate

Twelve set ups were made in order to test the mortality rate of four types of pellet: 100% Sweet basil, 1005 Mint-Basil, 70% Sweet Basil and 30% Mint-Basil, and 70% Mint-basil and 30% Mint-Basil. Each having 3 Trials with 10 pieces of pellets dissolved and a total of 20 mosquito larvae. The set ups were observed for a 24 hours and 48 hours interval to determine the number of mosquito larvae killed. Having a concentration of 720ppm for 100% Sweet Basil, 320ppm for both 100% Mint-Basil and 70% Mint-Basil and 30% Sweet Basil, 480ppm for 70% Sweet Basil and 30% Mint-Basil, the following data were gathered:

			Tri	al				
Pellet Type	1		2		3		Total	Percentage
	Mosquito es Killed	Percent age	Mosquito es Killed	Percent age	Mosquito es Killed	Percent age	-	
100% Sweet Basil	5	25%	1	5%	6	30%	12	20%
100% Mint-	0	0%	6	30%	4	20%	10	16.67%

 Table 2: Number of Killed Mosquitoes per Set up and the Percentage

Basil								
70% Sweet basil and 30% Mint- Basil	2	10%	3	15%	4	20%	9	15%
70% Mint- Basil and 30% Sweet Basil	2	10%	1	5%	4	20%	7	11.67%

A total of 12 with a percentage of 20 mosquito larvae were killed using 100% Sweet Basil, 5 with a percentage of 25 on Trial 1, 1 with a percentage of 5 on Trial 2 and 6 with a percentage of 30 on Trial 3. On set ups that used 100% Mint-Basil, 10 with a percentage 16.67 larvae were killed, in Trial 1 no larvae were killed while there are 6 with a percentage 30 and 4 with a percentage 20 in Trial 1 and 2. In 70% Sweet Basil and 30% Mint-Basil there were 9 with a percentage of 15 killed larvae, 2 with a percentage of 10in Trial 1, 3 2 with a percentage of 15 in Trail 2, and 4 with a percentage of 20 in Trial 3. Lastly, the 70% Mint-Basil and 30% Sweet Basil a number of 2 with a percentage of 10 larvae were killed in Trial 1, in Trial 2 only 1 larva with a percentage of 5 were killed, and in Trial 3 there are 4 with a percentage of 20, a total of 7 larvae with a percentage of 11.67 were killed. Percentage were as follows:

On the 24 hours interval of gathering data, the number of mosquito larvae killed were counted for two days, by the basis of 24 hours and 48 hours observation, mosquito larvae killed were as follows:

Trial Pellet Hours Total Percent 2 3 1 Туре Interval age Mosquito Percent Mosquito Percent Percent Mosquito es Killed es Killed es Killed age age age 100% 24 3 7 15% 0 0% 4 20% 11.67% Sweet hours Basil 48 +2 +5% +10 +1 +2 +10 +5 +8.33% % % hours 100% 24 0 0% 0 0% 4 20% 4 6.67% Minthours Basil 48 +0% +30 +0% +10% +0 +6 +0 +6 hours % 70% 24 0% 5% 0 1 3 15% 4 6.67% Sweet hours basil 48 +2 +10 +2 +10 +1 +5% +5 +8.33% and

 Table 3: Number of Mosquito Larvae Killed Per 24 Hour Interval

30% Mint- Basil	hours		%		%				
70% Mint-	24 hours	1	5%	1	5%	2	10%	4	6.67%
Basil and 30% Sweet Basil	48 hours	+1	+5%	+0	+0%	+2	+10 %	+3	+5%

In 100% Sweet Basil 24 hours observation, 3 mosquito larvae were killed and has a percentage of 15% in Trial 1, no mosquito larva in Trial 2, and 4 mosquito larvae with a percentage of 20% in Trial 3, a total of 7 larvae with a total of 11.67. In 48 hours of observation, 2 mosquito larvae with a percentage of 10 were added to the first trial, 1 mosquito larva with a percentage of 5 in Trial 2 and 2 mosquito larvae with a percentage of 10 in Trial 3, an overall total 12 mosquito larvae with a percentage of 20. On the 24 hours observation of 100% Mint-Basil no larva was killed in Trial 1 and 2, while there are 4 mosquito larvae with a percentage of 20 were killed, a total of 4 with a percentage of 6.67 larvae killed. 48 hours observation, no mosquito larva was added in Trial 1, 6 with a percentage of 30 in Trial 2 and none was added in Trial 3, an overall total of 10 with a percentage of 16.67 mosquito larvae. 70% Sweet Basil and 30% Mint-Basil's 24 hours observation resulted to zero larva killed, 1 with a percentage of 5 in Trial 2, 3 with a percentage of 15 in Trial 3, a total of 4 with a percentage of 6.67 larvae. During the 48 hours observation, 2 mosquito larvae with a percentage of 10 were added in Trial 1, 2 with a percentage of 10 in Trial 2, and 1 with a percentage of 5 in Trial 3, an overall total of 9 mosquito larvae with a percentage of 15. 70% Mint-Basil and 30% Sweet Basil showed a total of 4 mosquito larvae with a percentage of 5 in Trial 1, 1 with a percentage of 5 in Trial 1, 1 with a percentage of 5 in Trial 2, and 2 mosquito larvae with a percentage of 5 in Trial 1, 1 with a percentage of 5 in Trial 2, and 2 with a percentage of 10 in Trial 3. In 48 hours of observation, 1 mosquito larva with a percentage of 5 was added in Trial 1, none was added in Trial 2, and 2 with a percentage of 10 in Trial 3, an overall total of 7 with a percentage of 11.67 mosquito larvae.

These results are lower compared to studies conducted by Laraib, I., Raza, F.A., Kiran, A. and Sajid, I. on 2018 that had a 43% mortality rate during its 48 hour observation period, a number 23 digits higher than the results the current study obtained and a 10% rate during 24 hours using ethanol, the same compound used in making the extracts for the pellet. This result proves that the results presented by Kumar, et. al., on 2017 were true as to using *Ocimum basilicum* as only a moderate larvicidal agent. Although the study showed the same mortality rate as the study conducted on A. arabiensis using Basil Leaf Powder Extract with 20% mortality rate, this value was the lowest digit obtained with 2 mg/L. A study on Mint-Basil shows a low potential larvicide at LC₅₀ 367.6ppm supporting the low mortality obtained by 100% Mint-Basil though its value is close to the highest value obtained 20% of 100% Sweet Basil. Here are the images for the larvae used in conducting the experiments:

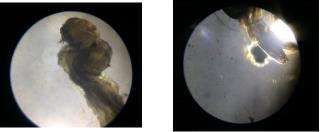


Figure 4: Larvae 1-Head Figure 5: Larvae 1-Tail

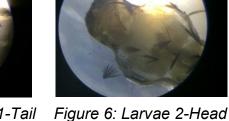




Figure 7: Larvae 2-Tail Figure 8: Larvae 3-Head Figure 9: Larvae 3-Tail

Mortality Difference of Sweet Basil and Mint-Basil

Given all the data gathered during the experiments, the researchers were able to compute the mean and standard deviation of both samples under testing; 100% Sweet Basil and 100% Mint-Basil pellets. In order to determine if there is a significant difference on the sample's effectiveness, comparative analysis was made through t-test. The digits below were used and considered:

Table 4: Number of killed Mosquitoes per 24 hours interval of Sweet and

Sweet	Basil	Mint-Basil				
24 hours	48 hours	24 hours	48 hours			
3	5	0	0			
0	1	0	6			
4	6	4	4			

Mint-Basil

Table 5: Mean Table

	Time I		
Pellet Type	24 hours 48 hours		Average
Sweet Basil	2.33	4	3.165
Mint-Basil	1.33	3.33	2.33
Average	1.83	3.67	2.75

The null hypothesis, there is no significant difference on the larvicidal strength of the four basil types having p value of 0.698981, 0.070323, and 0.977154, is accepted having values that are within the critical value the researchers fail to reject the null hypothesis.

Chapter V

Summary, Conclusion and Recommendation

This section contains the summary of the findings, the conclusions to which answers the statement of the problem and the various recommendations for the improvement of the research.

Summary

Larvicidal pellet made from Sweet and Mint-Basil are used to make four types of pellet designed to kill mosquito larvae. The mean of time duration it takes for the pellet to be dissolved is 15 minutes and 57 seconds. In order to test the mortality rate of the pellet 12 Set Ups are used to decipher the effectiveness of every pellet as larvicidal. Mortality rate ranges from 11. 67% - 20%, 20 % from 100% Sweet Basil on 720ppm, 16. 67% on 100 % Mint-Basil, 320ppm, 15% on 70% Sweet Basil and 30 % Mint-Basil, 480ppm, 11.67% on 70% Mint-Basil and 30% Sweet Basil on 320ppm. There is no significant difference between Sweet Basil and Mint-Basil in terms in larvicidal activity, it proves that the alternative hypothesis is rejected and the null hypothesis is accepted.

Conclusion

Based from the findings of the study on testing the larvicidal activity of Sweet Basil and Mint-Basil pellets as well as the time of dissolution of the output, the researchers can conclude that: 1. The length of time that the pellet completely dissolved in 125 ml of water.

a. Size, the bigger the pellet the longer the time it will consume to dissolve in water.

- 2. The death rate of mosquito larvae using the following:
 - Based on the results, 100% Sweet Basil had the greatest number of mosquito larvae killed.
 - Lesser mosquito larvae were killed with the 100% Mint-Basil compared to 100% Sweet Basil.
 - c. The setup with 70% Sweet Basil and 30% Mint-Basil had almost the same result as the 100% Mint-Basil.
 - d. The 70% Mint-Basil and 30% Sweet Basil had the least death of mosquito larvae recorded.

It is still better to use pure Sweet Basil Pellet and pure Mint-Basil Pellet as a larvicidal pellet for it to produce higher mortality rate.

 There is no significant difference on the larvicidal strength of the four pellet types.

Recommendation

The research is beneficial as it met the goal of the study but knowing all the flaws of the research, further improvements are needed. The Researchers recommend to:

- 1. Produce new pellets that are made of pure Basil that has higher amount than the filler.
- 2. Use molding machine to make the production easier and distribute the content equally.
- 3. Alcohol with 95% solution
- 4. is most preferable in extracting the Basil leaves.
- Pellet must undergo some tests so that the composition of each pellet will be identified.
- 6. Comparative study of larvicidal activity of Sweet and Mint-Basil using ethyl, methyl and acetone.

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APENDIX A

Letter to the Senior High School Coordinator

February 1, 2020

Mr. Jeffrey O. Albania

SHS COORDINATOR

Dear Sir,

Warmest Greetings!

We are the 12-STEM A students who are presently conducting study entitled "BASIL (OCIMUM BASILICUM) AND MINT-BASIL (MENTHA × PIPERITA F. CITRATA) LEAVES IN A FORM OF PELLET" as a fulfillment in our subject in PRACTICAL RESEARCH 1, INQUIRY, INVESTIGATION AND IMMERSION, AND RESEARCH/ CAPSTONE PROJECT. We ask permission to conduct our study and experiment in order to generate the data needed to finished our study.

Your approval to conduct this study will be greatly appreciated. Thank you in advance for your interest and assistance with this research.

Sincerely yours,

THE RESEARCHERS

APENDIX B

Documentation



EXTRACTION









PELLETIZATION



EXPERIMENTATION

Anova: Two-Factor With Replication

SUMMARY	24 hours	48 hours	Total
Sweet basil			
Count	3	3	6
Sum	7	12	19
	2.33333		3.16666
Average	3	4	7
	4.33333		5.36666
Variance	3	7	7
Mint Basil			
Count	3	3	6
Sum	4	10	14
	1.33333	3.33333	2.33333
Average	3	3	3
	5.33333	9.33333	7.06666
Variance	3	3	7
SB & MB			
Count	3	3	6
Sum	4	9	13
	1.33333	Ū	2.16666
Average	3	3	7
C	2.33333		2.16666
Variance	3	1	7
MB&SB			
Count	3	3	6
Sum	4	7	11
	1.33333	2.33333	1.83333
Average	3	3	3
_	0.33333	2.33333	1.36666
Variance	3	3	7
Total			
Count	12	12	
Sum	19	38	
	1.58333	3.16666	
Average	3	7	
5		3.96969	
Variance	2.44697	7	

ANOVA						
Source of						
Variation	SS	df	MS	F	P-value	F crit
	5.79166		1.93055	0.48263	0.69898	3.23887
Sample	7	3	6	9	1	2
	15.0416		15.0416	3.76041	0.07032	4.49399
Columns	7	1	7	7	3	8
	0.79166		0.26388	0.06597	0.97715	3.23887
Interaction	7	3	9	2	4	2
Within	64	16	4			
Total	85.625	23				

Curriculum Vitae



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