TITLE:

INSCTICIDAL ACTIONS OF CRUDE EXTRACTS FROM OREGANO, MAYANA, AND ONION LEAVES AGAINST MOSQUITO LARVAE SPECIES

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APPROVAL SHEET

This research entitled "**Insecticidal Actions of Crude Extracts from Oregano, Mayana, and Onion Leaves against Mosquito Larvae,**" prepared and submitted by **Wely Jesch Sabalilag, Jundy Ray Subosa, and Rey-Mar Macate,** in partial fulfillment of the requirement in the Science Research II, has been examined and is recommended for acceptance and approval.

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Chapter 1

Introduction

A. Background of the Study

During these rainy times, many people complain of being disturbed by those aggressive mosquitoes and being ripped of in buying those expensive insecticides. The people also complain that most insecticides affect their health due to inhaling the chemicals from the insecticides. The term "insecticide" is a misnomer, or it leads people to believe that they only kill pests (Doshefsky, 1994). Insecticides were composed of deadly chemicals being used to destroy not only pests, but also the environment and humans (Doshefsky, 1994). As years gone by, studies revealed that plants have insecticidal properties, which can be a good substitute for inorganic pesticides.

Inorganic pesticides have been the first way of terminating pests but due to some studies it shows that some plants have the capability of terminating pests; thus people may turn off their dependency on inorganic pesticides, which always have some side effects. As mentioned by Magsuci and Ponteres (1998), oregano and marigold extracts have insecticidal effects against mosquitoes and other insects; therefore organic pesticides could replace inorganic insecticides.

Despite the use of an estimated 2.27 million metric tons of pesticides applied worldwide, as well as the use of biological and other nonchemical control, pests such as mosquitoes still continue to bring dreadful disease like malaria, dengue, etc. to sickly children and unhealthy adults. In addition, the toxicity and biological effectiveness of those newer pesticides or insecticides have increased at least tenfold. However, highly resistant pests have not shown concurrent decline (Werner, 1993).

Mosquitoes are always the people's night enemy. People were fond of testing different insecticides if there's no effect in mosquitoes. Due to the use of different insecticides, mosquitoes have increased their resistance against insecticides. And these inorganic insecticides have caused lung problems when individuals are being exposed for hours with these insecticides (Grolier, 2000).

To prevent this thing to happen, we have to treat those mosquitoes in their larvae stage. One of the most effective techniques in killing mosquitoes is by killing them in their larvae stage because in this stage they're still weak and vulnerable to insecticides (Grolier, 2000).

There are studies about the use of insecticides from crude extracts of plants, but his study will test the effectiveness of

mayana, which has not been tested as an insecticide against mosquito larvae.

B. Statement of the Problem

Will the crude extracts from oregano, mayana, and onion leaves possess insecticidal properties against mosquito larvae?

C. Hypotheses of the Study

The crude extracts from oregano, mayana and onion leaves possess insecticidal properties against mosquitoes.

D. Objectives of the Study

This study aims to determine and compare the insecticidal properties of oregano, mayana, and onion extracts against mosquito larvae after a three-hour exposure period.

E. Significance of the Study

This study will help every human being, especially those poor families who could not afford a mosquito killer. Many of these poor families burn leaves or rubber to get rid of these mosquitoes, not knowing that it could affect their health. People buy some inorganic pesticides but some of these are low-quality insecticides.

This study will help the people to eliminate mosquitoes using cheap materials with less harmful side effects on humans and environment.

This study will also help our people to make new products out of crude extracts from selected plants, so they can save their money for their basic necessities from buying low-quality insecticides. Furthermore, this study will help in developing an organic fertilizer with insecticidal properties using modern technologies.

F. Scope and Limitations of the Study

We conducted our study and plant collection both in Leganes and Oton. We conducted our plant extraction in the Science Research laboratory of Philippine Science High School. It took about one or two months to conduct this study. In our study, there had been treatments that were tested: oregano, mayana, and onion. There were three replicates in our experiments.

G. Definition of Terms

Insecticides - naturally or chemically processed substances, which is used to prevent insects that harm humans and environments

Crude - existing in a natural state and unaltered by cooking and processing

Mosquitoes - one of the most harmful insects that penetrate on a human's skin using its proboscis and the carrier of different diseases like malaria, dengue, etc.

Chapter 2

Review of Related Literature

I. Insecticides/Pesticides

Pest Control is any of a wide range of environmental interventions that have as their objective a reduction in the incidence of insect pests, plant pathogens, and weed populations, to enable maximum productions of high quality food and other crops. Specific control techniques include chemical, physical, and biological mechanisms. Ninety percent of the world is dependent for food supplies on just fifteen major crops and seven animal species. Despite all the control efforts used, pests and insects annually destroy about thirty-five percent of crops and threatening millions of people worldwide. Despite many areas of the world facing serious food shortage and dreadful diseases from insects, industrial and other development adds more problems to the situation rather than to minimize the problem(Encarta Encyclopedia, 2000).

A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling or migrating any pests. Pests can be insects, mice, or other animals, unwanted plants (weeds), fungi, or microorganisms, like bacteria and viruses. Though often is understood to refer only to insecticide, the term also applies to herbicides, fungicides, and other various substances used to control pests (www.epa.gov/pesticides, 1996).

Botanical pesticides were at first crude dusts made from dried and ground leaves, flowers, stems, and

roots, all of which contain some active pesticidal gents such as alkaloid(nicotine, strychnine, ryahodine); esters(pyrethins, rotenoids); glycocides; coumarins; and essential oil extracts such as terpenoids. Botanicals were prepared by diluting concentrated water or oil extracts were applied as a spray (Grolier International Encyclopedia, 1997).

II. Mosquitoes

A. Mosquitoes

Mosquito is a common name for any of about 2000 species of two-winged insects. They are found from the tropics to the Arctic Circle and from lowlands to the peaks of high mountains. Mosquitoes have long, slender wings and are unusual among flies in having small scales over most of the wing veins. The body is narrow. The long antennae have numerous whorls of hair, short in the female and long and bushy in the male. In one large group of mosquitoes, the mouthparts of the female are long, adapted for piercing and for sucking blood. The male, which feeds on nectar and water, has rudimentary mouthparts. Females of this group prefer the blood of warm-blooded animals. When they bite, they inject some of their salivary fluid into the wound, causing swelling and irritation. Many inject infectious microorganisms and thus transmit such diseases as malaria, yellow fever, dengue, and filariasis.

0 Female mosquitoes lay their eggs only in water; some species lay their eggs in running water, others in

woodland pools, marshes, swamps, estuaries, or in containers such as rain barrels. The larvae are known as wrigglers because of their wriggling motion in the water. A large number of mosquito eggs and larvae are destroyed by small fish. Mosquitoes may be controlled by eliminating their breeding places or by spraying these places with oil or insecticides.

The typical species is most abundant in warm regions. The common house mosquito of the United States is a carrier of encephalitis. Other species are responsible for the spread of yellow fever and dengue. Several species of a related group transmit malaria. This group is characterized by palpi, or sense organs, on the mouthparts that are as long as the sucking tube in both the male and the female. The wings of these mosquitoes are spotted with white and dark areas. These mosquitoes rest with the head and sucking tube parallel to the surface on which they are resting and with the body bent at an angle to this surface; other mosquitoes, including the common house mosquito, rest with the body parallel and with the head at an angle to the surface.

Another species, the Asian tiger mosquito, has caused health experts concern since it was first detected in the United States in 1985. Probably arriving in shipments of used tire casings, this fierce biter can spread a type of encephalitis, dengue fever, and other diseases. Hardy and resistant to pesticides, it may be difficult to control (Encarta Encyclopedia, 2001).

While there are several options available to reduce the number of mosquitoes breeding on your premises, this may not result in a reduction in mosquito numbers or biting activity. To put it very simply, our most common species of mosquito can migrate several miles from where it developed. Therefore, you could eliminate all mosquitoes breeding on your property and still be bothered by mosquitoes migrating into your backyard from adjacent areas.

Adulticiding is one option available fro mosquito control. However, it does have its limitations. Coarse sprays of products such as Sevin, Dursban, Malathion, or Resmethrin applied to shrubbery and other dense, low growing vegetation can reduce adult mosquitoes for a few days. This is fine if the application is made the day before a backyard picnic or family gathering but has little benefit in reducing adult mosquitoes for an extended period of time. Hand held foggers that adapt to lawn mowers can also provide temporary relief from adult mosquitoes but will also have no lasting effect. The best option for mosquito control is to target your control efforts at the larval stage. Any area or object that can hold water for a period of one week should be filled, drained or discarded. This include area such as stagnant puddles, pools, or ditches, and objects such as cans, buckets, old tires, clogged rain gutters, bird baths, and child wading pools. A number of insecticides are also available for application to a body of water to control mosquito larvae. However, before applying any

insecticide to a body of water you need to ask the following questions. First, can the body of water be effectively drained or filled so that no application is needed. Second, are there enough mosquito larvae present in the water to warrant an application. And third, are there fish or other aquatic wildlife present in the body of water that might be damaged or killed if an application is made. These are critical questions that need to be answered. Contrary to popular opinion, not all bodies of water serve as abundant mosquito breeding areas. For example, ponds that have steed banks, are relatively free of organic matter, and have little or no vegetation extending to the edge of the pond that will typically produce very few mosquitoes. In contrast, shallow marshy areas can serve as a breeding area of tremendous numbers of mosquitoes. Whether mosquitoes are developing on your property or in adjacent areas, the use of mosquito repellents can provide protection against the biting activity. Products that contain the active ingredient N, N-diethyl-metatoluamide (DEET) are the products of choice and should provide protection for several hours (Homeculture and Homepest News, 1999).

B. Information about Mosquitoes

1. Mosquito control agencies reduce mosquito populations in various ways, including water management, biological control agents, and insecticides , which can be effective in controlling mosquito larvae (larvicides) or mosquito adults (adulticides).

Mosquito populations can increase rapidly, and, depending on flooding and general weather conditions, mosquito control agencies cannot always keep up with mosquito problems in all areas. Very often, residents can help significantly by controlling mosquitoes around their homes and properties.

2. Stages of Mosquitoes

All mosquitoes have four stages of development-egg, larva, pupa, and adult-and spend their larval and pupal stages in water. The females of some mosquito species deposit eggs on moist surfaces, such as mud or fallen leaves, that may be near water but dry. Later, rain or high tides reflood these surfaces and stimulate the eggs to hatch into larvae. The females of other species deposit their eggs directly on the surface of still water in such places as ditches, street catch basins, tire tracks, streams that are drying up, and fields or excavations that hold water for some time. This water is often stagnant and close to the home in discarded tires, ornamental pools, unused wading and swimming pools, tin cans, bird baths, plant saucers, and even gutters and flat roofs. The eggs deposited on such waters soon hatch into larvae. In the hot summer months, larvae grow rapidly, become pupae, and emerge one week later as flying adult mosquitoes. A few important spring species have only one generation per year. However, most species have many generations per year, and their rapid increase in numbers becomes a problem.

Most mosquito species survive the interwinter, or overwinter, in the egg stage, awaiting the spring thaw, when waters warm and the eggs hatch. A few important species spend the winter as adult, mated females, resting in protected, cool locations, such as cellars, sewers, crawl spaces, and well pits. With warm spring days, these females seek a blood meal and begin the cycle again. Only a few species can overwinter as larvae.

3. Mosquito Control

Several commercially available insecticides can be effective in controlling larval and adult mosquitoes. The public considers these chemicals sufficiently safe for use. Select a product whose label states that the material is effective against mosquito larvae or adults. For safe and effective use, follow the instructions for applying the material. The label lists those insects that the Environmental Protection Agency (EPA) agrees are effectively controlled by the product. Read the label. For use against adult mosquitoes, some liquid insecticides can be mixed according to direction and sprayed lightly on building foundations, hedges, low shrubbery, ground covers, and grasses. Do not overapply liquid insecticides - excess spray dips from the sprayed surfaces to the ground, here it is ineffective. The purpose of such sprays is to leave a fine deposit of insecticide on surfaces where mosquitoes rest. Such sprays are not effective for more than one or two days. Some insecticides are available as

premixed products or aerosol cans. These devices spray the insecticide as very small aerosol droplets that remain floating in the air and hit the flying mosquitoes. Apply the sprays upwind, so the droplets drift through the area here mosquito control is desired. Rather than applying too much of these aerosols initially, it is more practical to apply them briefly but periodically, thereby eliminating those mosquitoes that recently flew into the area(New Jersey Mosquito Homepage, 1996).

Chapter 3

Methodology

A. Materials

B. A.1 Plant Extraction

Quantity	Equipment
	Oregano Leaves
	Mayana Leaves
	Onion Leaves
	Fine Cloth
	Blender
100 ml	Distilled Water
3	Funnel
	Freezer
3	Beaker
	Scissors
1	Stirring Rod

A.2 Collection of Test Organisms

Quantity	Equipment
12	Plastic cups
	Spoon

A.3 Testing of Plant Extracts

Quantity	Equipment
	Plant Extracts
	Mosquito larvae
120	Dropper

B. General Procedure

B.1 Collection of Test Organisms

The mosquito larvae were collected from stagnant water specifically, in the areas of Leganes, where the mosquitoes breed. The mosquito larvae were kept in a container. The mosquito larvae were divided

into three groups and each group was composed of eight larvae. Each set-up was treated with extracts of the three plants.

B.2 Collection of Different Plants

Oregano leaves were collected from Oton, Iloilo; Onion leaves, from Arevalo, Iloilo, and the Mayana leaves, from Leganes, Iloilo.

B.3 Crude Extraction of Plant

The plants were washed with running water and air-dried. Oregano, Mayana and Onion leaves were cut into small pieces, then each plant was placed in a beaker.

After being cut, one beaker of each plant material was mixed with 100 ml of distilled water and mashed using a blender. The mixture was poured through a fine cloth fitted to a flask. Plant residue was separated. Accumulated extracts were kept in an Erlenmeyer flask covered with a cork to prevent from contamination.

B.4 Testing of Mosquito larvaes

The mosquito larvaes were kept in a cup filled with 100 ml of tap water. Each set of mosquito were treated by dropping one ml of corresponding plant extracts assigned to the set. After testing with one ml of plant extracts, another set of mosquito larvaes were treated with 5 ml of plant extracts. Every three hours, each setup was inspected to determine if there are dead mosquitoes, and percent mortality was then determined using the formula:

Percent mortality = $\frac{\text{No. of dead mosquito larvae X 100}}{\text{No. of mosquito larvae used}}$

Note: There were three replicates in each setup.

B.5 Statistical Analysis

One way Analysis of Variance was used to determine the significance between treatments/extracts.

Table 1. Average percent mortality of mosquito larvae treated with 1ml and 5 ml of crude extracts in a 3-hour exposure period.

	Average Percent Mortality	
Plant Extracts	1ml	5ml
Onion	13.33 %	26.67 %
Oregano	7.8 %	21.1 %
Mayana	17.8 %	30 %
Control	0.0 %	0.0 %

Table 2. Table showing the significant or insignificant difference between non-treated mosquito larvae and onion, mayana, and oregano extracts.

Control	Onion Extracts	Oregano Extracts	Mayana Extracts
	Not Significant	Not Significant	Significant

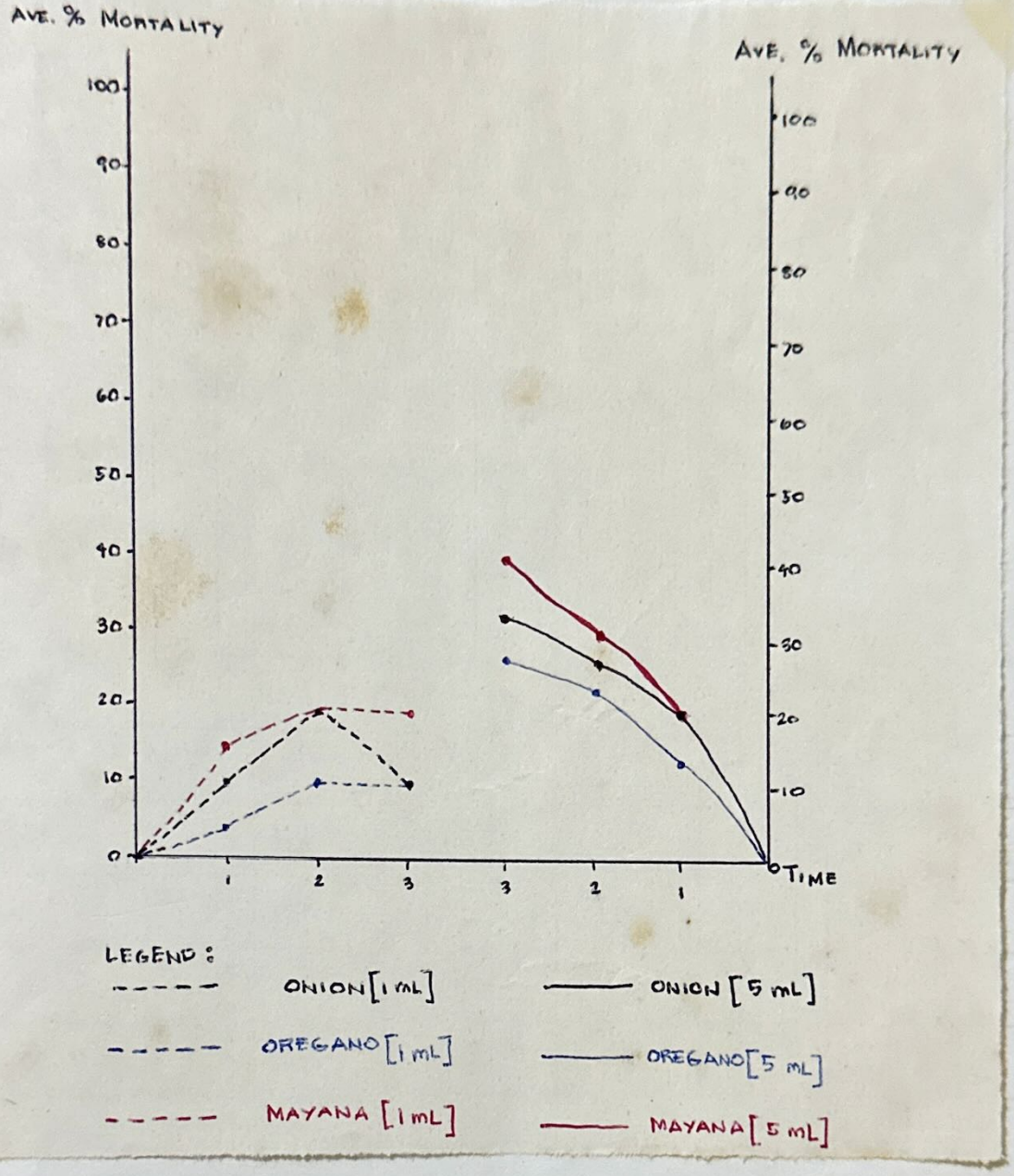

Figure 1. Graph of percent mortality of mosquito larvae treated with 1ml and 5ml of crude extracts in 3-hour interval.

Table 6. Average percent mortality of mosquito larvae treated with 10 ml of crude extracts in a 3-hour exposure period.

Plant Extracts	Average Percent Mortality
	10 ml of extracts
Onion	31.1%
Oregano	37.8%
Mayana	45.0%
Control	0.0$

Table 7. Actual F-computed between non-treated mosquito larvae and 10 ml of onion, mayana, and oregano extracts.

Control	Onion Extracts	Oregano Extracts	Mayana Extracts
	Not Significant	Not Significant	Not Significant

Chapter 4

Results and Discussion

The study entitled "Insecticidal Actions of Crude Extracts from Oregano, Mayana and Onion Leaves against Mosquito Larvae (Aides sp.)" aims to find out if the oregano, onion and mayana leaves extracts possess insecticidal properties against mosquito larvae. We had found out that dead mosquito larvae show pale skin, and transparent white body.

Table 1 shows the mean results of the experiments. Results showed the plant extracts were effective against mosquito larvae. In 1 ml of Oregano, Mayana and Onion Extracts, test organism showed a highest mortality rate on the 2nd hour. In 5ml mayana extracts test organism showed a highest mortality rate on the 3rd hour. In the control var., no test org. were found dead, so it means that the plant extracts were really effective.

Table 2 shows that there is a significant difference between non-treated larvae and larvae that are treated with

1 ml mayana extracts. It also shows that 5ml of mayana extracts have a significant difference on non-treated. However, no significant difference has been found between non-treated mosquito and onion extracts and between non-treated mosquito and oregano extracts. This was concluded using one-way ANOVA for each pair using the degree freedom of 0.05, which is equal to an $F_{tabular}$ 5.14. The F computed of 1 ml and 5 ml of Onion, Oregano and non-treated are 3.002, 1.499, 3.57, 4.33 respectively, which is smaller than $F_{tabular}$ w/c means that there is no significant difference between them.

Figure 1 shows the Ave. percent mortality for each treatment. 1 ml and 5 ml of mayana extracts has the highest mortality rate followed by the Onion extracts, respectively. The Oregano extract has the lowest percent mortality.

Chapter V

Summary of Results

1. It was observed that onion extracts and oregano extracts have no significant difference to the effect on non-treated mosquito larvae after 3-hr exposure period.
2. After 3 hours, the oregano extract has a notable effect against mosquito larvae but it was not as strong as the onion and mayana extracts.
3. The oregano extracts has minimal effect on mosquito larvae while mayana extracts has the maximum effect against mosquito larvae.

Conclusions:

By the given data and information, the researchers, therefore conclude that the mayana extract has a significant effect on mosquito larvae, and its effect is very strong than that of onion and oregano extracts.

The onion and oregano extracts also have an effect on mosquito larvae but there is no significant effect against mosquito larvae. The oregano extract has the lowest effect on mosquito larvae.

TABLE 3. Actual F-computed between non-treated mosquito larvae and 1ml and 5 ml of onion, mayana, and oregano extracts(1 ml/5 ml).

	Onion Extracts	Oregano Extracts	Mayana Extracts
Control	3.0002/1.499	3.57/4.33	11.37/9.01

TABLE 5. Mean no. of dead mosquito larvae per 1 ml/5 ml of onion extracts.

	No. of dead mosquito larvae per 1ml/5ml of onion extracts			
Time	T1	T2	T3	Mean
1	1/1	1/3	1/2	1/2
2	1/2	2/3	3/3	2/2.67
3	1/2	1/4	1/4	1/3.33

TABLE 6. Mean no. of dead mosquito larvae per 1 ml/5 ml of oregano extracts.

	No. of dead mosquito larvae per 1ml/5ml of onion extracts			
Time	T1	T2	T3	Mean
1	0/1	0/1	1/2	0.33/1.33
2	1/2	1/2	1/3	1/2.33
3	1/3	1/2	1/3	1/2.67

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