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Effectiveness of *Carica papaya Linnaeus* (Papaya) and *Azadirachta indica A. Jussieu* (Neem) Crushed Leaves as Potential Larvicides for Mosquitoes

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ABSTRACT

Mosquito borne diseases are widespread in our environment nowadays. Control measures of the increasing number of mosquito vectors should be done. Larvicides are one control measures that could help in fighting against the rising number of mosquitoes and mosquito borne diseases. This project was involved in discovering the effectiveness of *Carica papaya Linnaeus* (Papaya) and *Azadirachta indica A. Jussieu* (Neem) crushed leaves as potential larvicides for mosquito. The goal was to determine the number of live mosquito larvae within 8 weeks in controlled and treatment set-ups, determine the effects of the expression pure extracts of papaya, neem and mixed extracts to mosquito larvae, and know the genera of the mosquito larvae. The methods used for this study were counting the live mosquito larvaes in the experiment replicates, expression method to acquire *Carica papaya Linnaeus* (papaya), *Azadirachta indica A. Jussieu* (neem) crude extracts, and use of microscope to identify the genera of the mosquito larvae. Crushed papaya and neem leaves became food source for mosquito larvae. However, crude extract of papaya may be potential larvicides because mosquito larvaes exposed to it died.

Keywords: Mosquito larvae, effectiveness, *Carica papaya*, neem, larvicides

INTRODUCTION

Mosquitoes are insects that can transmit many diseases throughout the world and infect by the millions. They can be vectors for serious and life-threatening diseases such as dengue fever, malaria, filariasis and chikungunya. They have been declared by the World Health Organization as "public enemy number one". According to the Department of Health Regional Office X 2014 Annual Disease Surveillance report, 31% of dengue cases from Northern Mindanao are coming from Cagayan de Oro City. Barangay Nazareth is one of the top 15 most number of dengue cases in a barangay in Cagayan de Oro from January to December 2014 according to DOH-Regional Office X. The cases in Barangay Nazareth from January to December 2014 is 77% higher compared to the previous year.

Research efforts have been done to find ways in controlling the spread of mosquito vector borne diseases. Larvicides are among the methods used in preventing the increase of mosquito numbers.

Commercial insecticides are readily available in the market at a price. They are also created through synthetic and not so eco-friendly substances. According to Indian Journal of Medical Research, one of the most effective alternatives for insect control is to explore the floral biodervisity or plant life. Plants are natural insecticides and larvicides.

Larvicides are agents commonly used to eliminate juvenile or immature mosquitoes for control measure purposes. Commercialized larvicides are readily available in the market, however, local and indigenous materials may be an alternative. Budgetary constraints are often the reasons for not buying larvicides. Commercial larvicides may range from Php 800 to Php 3,500. This research will look into alternate larvicides which are locally available and with no price cost.

Monzon, *et al.* (1994) had looked into local Philippine plants as larvicides namely sugar apple "atis", olive gum eucalyptus "bagras", "lansones", "neem" and "San Fransico" croton. The identification of local plants as larvicides can be advantageous to Filipinos because of its easy availability and cost effectiveness.

A study by Ghosh *et al.* (2012), plant extracts as potential mosquito larvicides show promising results. Phytochemicals are the main ingredients in plants that make it as larvicides. Monson *et al.* (1994) made a study in the Philippines in order to test local available plants as potential larvicides. *Azadirachta indica A. Jussieu* (neem) is one of the research instrument used, which ranked 2nd in the potency as larvicides. *Carica papaya Linnaeus* (papaya) is also used as a research instrument for mosquito as potential larvicides in the research study of Sesanti *et al.* (2014) in Jayapura, Papua

Indonesia.

Papaya is very abundant in the Philippines. It is one of the agricultural products of the Philippines that are experted to other countries. Also, papaya is very common in Filipino communities. In Barangay Nazareth, Cagayan de Oro, papaya is ordinarily found in the backyards of residences. It is one of the healthiest fruits in the world. It is rich in fiber and vitamin C. It also helps in weight loss by promoting the feeling of fullness and controlling cravings. Papaya leaves are also being used in certain countries for its heath benefits. A study done by Koverndan *et al.* (2012), wherein Papaya leaves extract can be used a traditional medicine treatment for malaria. There are health benefits from drinking Papaya leaf juice which is a treatment for dengue, typhoid and relief from menstrual pain. Often times, Papaya leaf extract are also used as treatment for wounds and sores, and also can be effective against dandruff.

Neem leaves are also beneficial to the body. Neem leaves boosts immune system and promotes healthy respiratory and digestive system. Maii *et al.* (2011) studied that neem leaves can be potential insect repellents. Other health benefits of the plant neem include treatments for malaria, leprosy, eye disorders, bloody nose, intestinal worms, stomach upset, loss of appetite, skin ulcers, diseases of the heart and blood vessels (cardiovascular disease), fever, diabetes, gum disease (gingivitis), and liver problems.

The study on larvicides could bring about an added choice for controlling mosquitoes especially in the community. Prevention of diseases is one of the major role of community health nurses. Having a cheap and accessible method in combating vector borne diseases could aid in a healthier community.

OBJECTIVES OF THE STUDY

This study aimed to determine the effect of *Carica papaya Linnaeus* (papaya) and *Azadirachta indica A. Jussieu* (neem) crushed leaves as potential larvicides for mosquito.

Specifically, this study sought to: 1) Determine the number of live mosquito larvae within 8 weeks in: T0: Control group, 250mL water, T1: Department of Science and Technology (DOST) Ovitrap pellets in 250 mL water, T2: 5 grams of crushed *Carica papaya Linnaeus* (papaya) leaves and 250 mL water, T3: 10 grams of crushed *Carica papaya Linnaeus* (papaya) leaves and 250 mL water, T4: 5 grams of crushed *Azadirachta indica A. Jussieu* (neem) leaves and 250 mL water, T5: 10 grams of crushed *Azadirachta indica A. Jussieu* (neem) leaves and 250 mL water; 2) determine the effects of the expression pure extracts of *Carica papaya Linnaeus* (papaya), *Azadirachta indica A. Jussieu* (neem) and mixed extracts to mosquito larvae; 3) know the genera of mosquito larvae.

MATERIALS AND METHODS

Research Design

This study was a controlled experimental research design to explore the effects *Carica papaya Linnaeus* (papaya) and *Azadirachta indica A. Jussieu* (neem) crushed leaves as larvicides for mosquito. This study further employed a triangulation approach wherein quantitative methods of experimental research were used. The results of the treatment samples were compared to the control samples.

Research Setting

The research was conducted at the residence of Credo B. Rubio in 33rd street Barangay Nazareth, Cagayan de Oro. Nazareth is one of Cagayan de Oro City's barangays with clustering of dengue cases as of November 2014.

Research Instruments

The standard Ovitrap cups and Ovi-paddle from the Department of Science and Technology were used together with Ovitrap pellets in collecting the mosquito larvae.

Data Gathering Acquisition of Ovitraps cups and Ovi-Paddle

The Department of Science and Technology Ovitraps cups and Ovi-Paddles were essential in the experiment because they were used to create the treatment replicates of the whole study (Plate 1). The Ovitraps cups and Ovi-Paddles stocks were donated coming from the Department of Health Regional Office X Provincial Health Office of Bukidnon.

Plate 1. Acquisition of Ovitraps cups and Ovi-Paddle.

Gathering of papaya and neem leaves

The *Carica papaya Linnaeus* (Papaya) and *Azadirachta indica A. Jussieu* (Neem) leaves were collected from the residence of Credo Rubio and neighbors at 33rd Street Nazareth, Cagayan de Oro City (Plate 2).

Plate 2. Gathered papaya and neem leaves.

Crushing of the papaya and neem leaves

The crushing of *Carica papaya Linnaeus* (papaya) and *Azadirachta indica A. Jussieu* (neem) leaves with the use of mortar and pestle was the main method used to create the treatment and replicates of the study. After which, the by products were placed inside labeled glass jars in preparation for the next method (Plate 3).

Plate 3. Crushing of the leaves.

Preparation of the Ovitrap cups, paddles and replicate boxes

Six Ovitrap cups and Ovi-paddles were set-up for each area. A total of 36 ovitrap cups and 36 ovi-paddles were used for the experiment proper (Plate 4). All of the cups were placed with Ovi-paddle and arranged in a box (Plate 5). To prevent spillage, barbeque sticks were added for support and stability.

Plate 4. Preparation of the Ovitrap cups, paddles and replicate boxes.

Plate 5. Preparation of the Ovitrap cups, paddles and replicate boxes

Preparing the Control and Treatment replicates set-up

One cup was filled with only clear water as the control variable. Another cup was filled with water and Ovitrap pellets as the Treatment 1. Two other cups were filled with water and with crushed papaya leaves, one teaspoonful and two teaspoons. Crushed neem leaves were also filled with water with same quantities as the papaya leaves in two separate cups (Plate 6).

Plate 6. Preparing the Control and Treatment replicates set-up

Placement of replicate boxes in designated area

The replicate boxes were placed in selected areas of the research setting wherein the researchers chose the conditions of the areas (Plate 7). The conditions are shady areas away from exposure to direct rain, near canals and garbage areas, inside outdoor comfort room, and near plant life.

Additional data were gathered by utilizing the expression extract method to acquire *Carica papaya Linnaeus* (Papaya) and *Azadirachta indica A. Jussieu* (neem) crushed leaves oil by first chopping the papaya and neem, then pounded on mortar

and pestle. Next, was placing the by-product on thin gauze then squeezing it by hand to acquire crude extract. The crude extracts produced were then placed in separate reagent bottles and labeled. Three separate bottles were labeled Papaya leaves extract, Neem tree leaves extract and Mixed Extract.

Plate 7. Filling of Ovitraps with water and placement in designate areas

Five mosquito larvae from the control group were added in each of 9 petri dishes. The Replicates were added with 0.5 ml of extracted papaya, neem, and mixed (both papaya and neem) plant oil extracts. The reaction to the extracts were timed and recorded.

Statistical Techniques

Data gathered were processed using MS Excel. The descriptive analysis of the data was made using central tendencies measurements such as average, percentage and mean.

RESULTS AND DISCUSSION

Objective 1. Determine a weekly tally for 8 weeks of mosquito larvae using the following treatments.

1.1. T0: Control group, 250mL water
1.2. T1: Department of Science and Technology (DOST) Ovitrap pellets in 250 mL water
1.3. T2: 5 grams of crushed *Carica papaya Linnaeus* (papaya) leaves and 250 mL water
1.4. T3: 10 grams of crushed *Carica papaya Linnaeus* (papaya) leaves and 250 mL water
1.5. T4: 5 grams of crushed *Azadirachta indica A. Jussieu* (neem) leaves and 250 mL water

1.6. T5: 10 grams of crushed *Azadirachta indica A. Jussieu* (neem) leaves and 250 mL water

Table 1. Number of Alive Mosquito Larvae in different Larvicidal Treatment Repicates December 20, 2014 to February 14, 2015

	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 6	WEEK 7	WEEK 8
T0	52	40	38	55	60	68	48	65
T1	21	35	37	42	41	32	34	45
T2	18	14	7	20	30	30	30	28
T3	13	11	5	8	14	4	10	13
T4	21	12	15	14	4	18	25	28
T5	31	8	3	11	3	10	9	10
TOTAL	156	119	105	149	152	162	155	188

Table 1 shows the number of alive mosquito larvae in different Larvicidal Treatment Replicates. As shown on the table, total of the weekly average of 1, 186 mosquito larvae were tallied for 8 weeks from December 20, 2014 to February 14, 2015. The most number of mosquito larvae was recorded during week 8 (February 7-February 14, 2015).

T0 had the highest number of live mosquito larvae in every weekly tally and followed by T1. The mosquito larvae thrived in these experimental set-ups. As cited in the study of Soleimani-Ahmadi *et al.* (2014) stated in Asian Pacific Journal of Tropical Biomedicine that the most common habitats for mosquitoes are clear water bodies with sandy substrates and still water. The control group indicated small, thin but very fast moving mosquito larvae. T1 had brownish colored mosquito larvae and were bigger compared to the control group. T2 and T3 had very dark brownish green colored mosquito larvae and were slow moving. T4 and T5 ha slim and long light brownish colored mosquito larvae and moving fast. This confirms the study of Mwangangi *et al.* (2007) that there is an influence of biological and physicochemical characteristics of larval habitats on the body size of mosquitoes. A study conducted by Oyewole *et al.* (2009), that the type of water can affect the physiochemical characterization of the mosquito larvae. The mosquito larvae in the current study have shown different characterization and behavior in different types of treatment exposure.

Objective 2. Observe the effects of the expression crude extracts of *Carica papaya Linnaeus* **(papaya),** *Azadirachta indica A. Jussieu* **(neem) and mixed extracts to mosquito larvae.**

Expression Extract

Plate 8. *Carica papaya Linnaeus* (papaya), *Azadirachta indica* (neem) crushed leaves and mixed extract placed in separate reagent bottles.

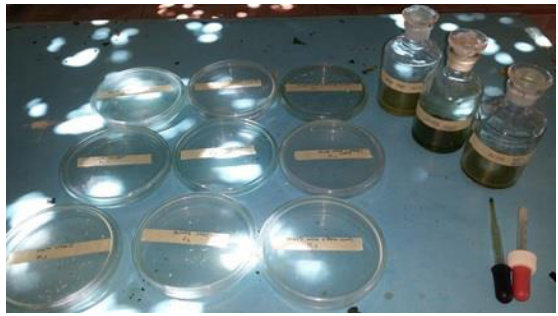

Plate 9. Five (5) mosquito larvae are placed in each petri dish.

Plate 10. Adding 0.5 mL of *Carica papaya Linnaeus* (papaya) crude extract to each 3 replicates, adding 0.5 mL of *Azadirachta indica A. Jussieu* (neem) crude extract to each 3 replicates and adding 0.5 mL of mixed extract to each 3 replicates.

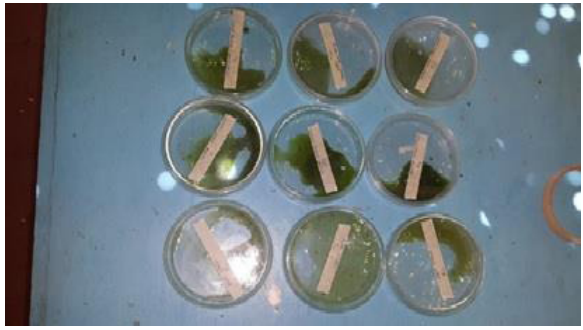

Plate 11. The reactions of the mosquito larvae were observed.

Table 2. Number of Dead Mosquito Larvae After Treatment

Plant Extract	Number of Dead Mosquito Larvae		
	R1 (5 min)	R2 (20 min)	R3 (35 min)
Carica papaya Linnaeus (papaya) crude extract	5	5	5
Azadirachta indica A. Jussieu (neem) crude extract	0	0	0
Mixed Extract	3	3	1

Table 2 shows the number of dead mosquito larvae after treatment. There were 15 out of 15 mosquito larvae expired after being exposed to *Carica papaya Linnaeus* (papaya) crude extract in the three replicates. All the mosquito larvae expired when exposed to the papaya crude extract during the 5, 20, and 35 minutes of exposure. There were no mosquito larvae found dead in the *Azadirachta indica A. Jussieu* (neem) crude extract sample replicates. However, 3 out of 5 mosquito larvae expired after being exposed to mixed extracts of *Carica papaya Linnaeus* (papaya) and *Azadirachta indica A. Jussieu* (neem) for 5 and 15 minutes. On the other hand in Replicate 3, after 35 minutes, only one mosquito larvae was found dead.

Plate 14. Mosquito Larvae exposed to *Azadirachta indica A. Jussieu* (neem) crude extract

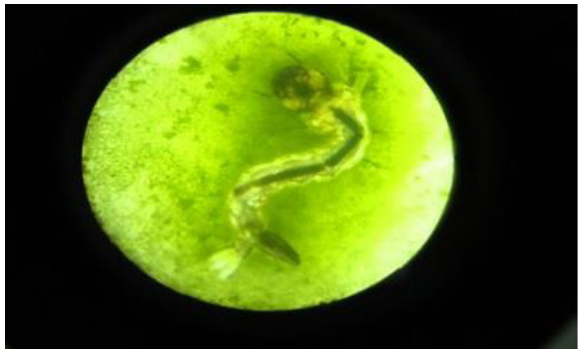

Plate 15. Mosquito Larvae exposed to *Carica papaya Linnaeus* (papaya) crude extract

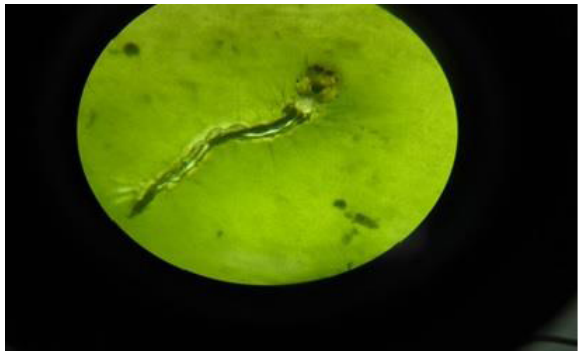

Plate 16. Mosquito Larvae exposed to Mixed extracts.

Objective 3. Know the genera of mosquito larvae.

Having the knowledge of the type of mosquito helps the community prepare better against diseases. The Aedes can transmit yellow fever, chikungunya, filarias and even dengue fever. Anopheles is commonly vectors to malaria disease. Culex is another genus of the mosquito which could transmit Japanese encephalitis.

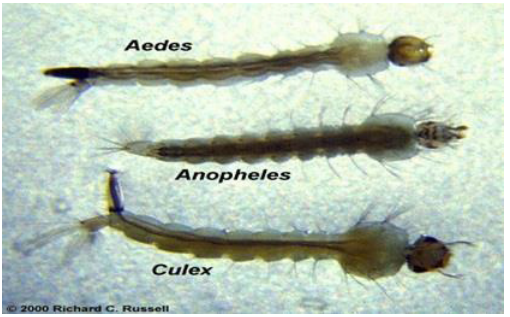

Plate 17. Types of mosquito larvae according to their genera.

In the study, the following genera of mosquito were identified:

Plate 18. T0 Control Sample Mosquito larvae: The mosquito larvae had large head and slim thorax and abdomen. This larvae is indicative of an Aedes.

Plate 19. T1 Department of Science and Technology (DOST) Ovitrap pellets exposed Mosquito larvae: The color is darker and have a larger thorax and abdomen comparing to the control group larvae. This larvae is indicative of an Anopheles.

Plate 20. T1 Department of Science and Technology (DOST) Ovitrap pellets exposed Mosquito pupa: T1 contained many pupa stage mosquito larvae. The pupa had very large heads and no visible thorax. The abdomen were shorter. The movement of the pupa were fast and very mobile.

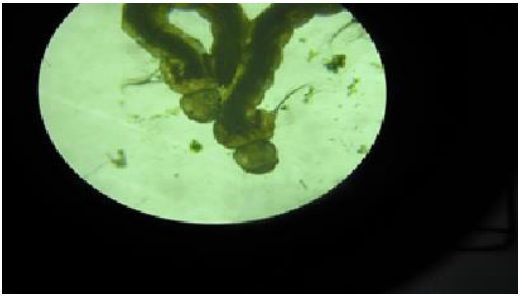

Plate 21. Mosquito larvae in sample replicates with crushed *Carica papaya Linnaeus* (papaya) leaves: The color of the larvae is greenish and more transparent. The abdomen has prominent abdominal segments. This larvae is indicative of an Aedes.

Plate 22. Mosquito larvae in sample replicates with crushed *Carica papaya Linnaeus* (papaya) leaves: The abdomen of the larvae is darker and had a large thorax. There are two long hairs along the thorax of the larvae. This larvae is indicative of a Culex.

Plate 23. T4 Mosquito larvae in sample replicates with crushed *Azadirachta indica A. Jussieu* (neem) leaves: The abdomen is longer and slimmer compared to the T2 & T3 larvae. There are no visible abdominal hairs of the larvae. This larvae is indicative of an Aedes.

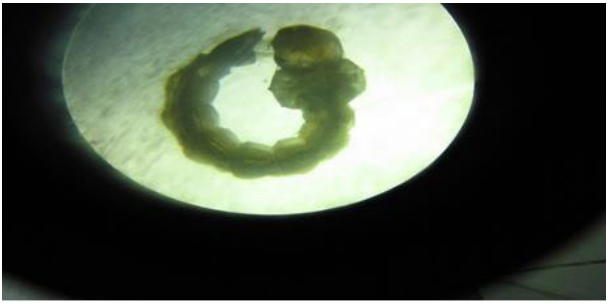

Plate 24. T5 Mosquito larvae in sample replicates with crushed *Azadirachta indica A. Jussieu* (neem) leaves: The thorax is very large and the abdominal segments are very prominent. The color is light green. This larvae is indicative of an Anopheles.

The genera of the mosquito larvae found in the experimental set-ups were mostly Aedes, then followed by Anopheles and Culex. The Aedes mosquitoes are best known to be carriers of dengue fever.

CONCLUSIONS

The mosquito larvaes in the treatment replicates thrived due to the presence of plant debris containing phytoplanktons and algae, which are part of their diet. The crushed *Carica papaya Linnaeus* (papaya) and *Azadirachta indica A. Jussieu* (neem) leaves served as food source for the developing mosquito larvaes. However, it is evident in the study that more mosquito larvae expired when treated with pure papaya crude extract. The specific phytoplanktons and algae contained in the crushed *Carica papaya Linnaeus* (papaya) and *Azadirachta indica A. Jussieu* (neem) leaves are yet to be determined because it is not part of the scope of the study.

There exists certain phytochemicals in *Carica papaya Linnaeus* (papaya) crude extract that may become potential larvicide. The exact chemical composition of the phytochemicals is yet to be determined and is not part of the scope of the study.

Aedes were the most dominant genera found in the experimental set-ups. The physical attributes of the mosquito larvae were observed through the dissecting microscope of Liceo de Cagayan University Biology Laboratory.

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