The Efficacy of Euphorbia Milii (Crown-Of-Thorns) Stem Extracts as Potential Larvicide of the *Aedes Aegypti* Mosquito

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ABSTRACT

Diseases like dengue, malaria, and chikungunya Zika fever are common in the Philippines during the rainy season. These fatal illnesses are carried by Aedes aegypti or Yellow fever mosquito which is abundant in the country. Since, operational fogging/misting, insecticides, and even mosquito repellents do not provide enough prevention, the researchers formulated organic alternatives that may have the capability of killing mosquito larvae. Two formulations were made: (1) extracts from the crown-of-thorns plant (Euphorbia milii) through the pounding of stem using mortar and pestle then submerging it in a 120 mL distilled water and (2) by simply soaking the stem in 120 mL of water. These two formulations were separated into two concentrations; 10 mL and 20 mL. Both formulations were allowed to settle for 48 hours. Afterward, these treatments were applied to different containers with 10 mosquito larvae each. In addition to the treatments, a positive control (cooking oil) and a negative control (acetone) were also used against mosquito larvae. The mortality percentage was computed after 24 hours. The results show that the treatments caused a 0-50 percent mortality rate against mosquito larvae. Culex quinquefasciatus cover most of the fatalities. Meanwhile, the control groups yielded a 100 percent mortality rate. There is also a significant difference between the control groups and experimental groups (P =.0008) at 0.05 significance level. Therefore, the stem extracts of Euphorbia milii were found to be not significantly effective against mosquito larvae.

Key words: Dengue, Euphorbia milii, mosquito larvae, Aedes aegypti, Culex quinquefasciatus

INTRODUCTION

Rainy season in the Philippines does not only bring non-stop precipitation, flood, and low temperature, the proliferation of dengue and other similar diseases are also widespread. *Aedes Aegypti* or Yellow Fever mosquitos are the main vectors that transmit the viruses which can cause dengue, chikungunya, Zika fever, and yellow fever viruses. This species of mosquito has a great population and high tendencies of reproduction which lead to widespread illnesses. In fact, according to the Department of Health (DOH), over 216,000 cases have been reported in the year 2018 alone and a total of 106, 630 dengue cases have been recorded from January 2019 to June 2019. This figure is 85 percent higher than the dengue cases recorded in the same period from the previous year. It is evident that operational misting and fogging of DOH were not successful and obviously, it needs other ways on how to prevent the continuous growth of the *Aedes aegypti* population.

Organic substances show greater efficacy and lesser environmental threat, also a more convenient process since it is all-natural. Plant extracts can be used as larvicide and a common succulent here in the Philippines may have the potential in doing so. The crown-of-thorns plant (*Euphorbia milii*) is the chosen subject to test whether its stem and its extract might affect the growth and development of mosquito larvae. *Euphorbia milii* plants can be seen in multiple gardens as they bear beautiful and tiny pink flowers. The thorns on its stem signify the family they belong in, the Euphorbiaceae. Recent studies have discovered that aveloz's (*Euphorbia tirucalli*) stem extracts, another species of Euphorbiaceae, can be an agent of reducing mosquito population for its larvicidal potential. Therefore, *Euphorbia milii* may contain the same properties of *Euphorbia tirucalli* that can kill *Aedes aegypti* larvae.

This matter leads to the formulation of larvicide attacking mosquitoes in its larval stage which inhibits further growth. Generally, the genus Euphorbia is the largest genus of medicinal plants widely distributed in the tropical country. In fact, *Euphorbia milii* is an ornamental shrub native in the

Philippines which is believed to be containing multiple health benefits and disease prevention agents. Their population here in the country is very diverse and vast which will benefit the people for its convenience in health and preventive purposes.

Review of Literature

Dengue fever is globally considered as the most common arthropod-borne virus occurring in at least 128 countries and putting four billion people at risk. Dengue is a viral disease transmitted by female *Aedes aegypti* mosquitoes. The World Health Organization (WHO) annual report has stated that dengue cases have significantly increased from an average of less than a thousand cases in the 1950s to more than three million cases in 2015 globally. There are four various serotypes of dengue fever, hence, a person can be infected more than once. Dengue fever could also evolve into dengue hemorrhagic fever (DHF) or dengue shock syndrome which are more complex (de Los Reyes & Escaner, 2018). Vaccine is still not available for the four dengue virus serotypes which is why the disease is hard to control (Charisma, et al., 2015).

Moreover, dengue has been strongly related to the rainy season in the Philippines starting from June to October. Different species of mosquitoes breed mainly in stagnant water and transmit different diseases (WHO, 2014). Several studies from the Philippines have indicated that climate change has increased the trend of dengue. It is predicted that climatic changes may affect the vector organism and pathogen, therefore, the risk of acquiring dengue is higher. In addition, mosquitoes are starting to reach mature stage at a faster rate due to warmer temperatures (Charisma, et al., 2015).

The main focus of this study is to potentially produce a larvicide against *Aedes aegypti* larvae which is the dengue-carrying mosquito. Therefore, the researchers have to differentiate *Aedes aegypti* larvae from the other different mosquito species. *Aedes aegypti* larvae were observed under a florescent microscope and can be identified as having a single siphon and a single rowed comb in the tail region (Gutierrez, Antepuesto, Eugenio, & Santos, 2014; Soon, 2000). Moreover, *Aedes aegypti* larvae commonly feed through the water column while Anopheles larvae prefer feeding on the surface and thrives in shallow water. *Aedes aegypti* larvae and pupae also tend to cluster together. Temperature is the most important external factor that controls and affects the growth rates of the larvae. Larvae typically grow in water temperature that ranges from 26-28°C. Moreover, food availability also affects larval growth. The adult size is determined by the larval size which is why the amount of food is important (Clements, 1992). It is difficult to differentiate female and male *Aedes aegypti* larvae during 3rd instar due to similarities in size but females grow faster than males. By 4th instar, the female and male *Aedes aegypti* larvae can be distinguished based on size, males grow faster but females are larger as adults compared to males (Chambers, 2005; Clements, 1992).

Aedes aegypti population is difficult to control because they have become resistant to neurotoxic insecticides used. The intensive use of neurotoxic insecticides has resulted in the loss of effectiveness in several vector populations because of resistance acquisition (Farnesi, et al., 2012; Ghosh, Chowdhury, & Chandra, 2012). Resistance in mosquitoes is the result of increased activity of enzymes involved in insecticide metabolism and mutation in the target sites (Borase, et al., 2014). *Aedes aegypti* larvae are attracted to certain synthetic and natural compounds at a particular concentration. Hence, these compounds should be included in pest management programs. Water quality, formulation, and the vulnerability of the larvae are important factors to consider when formulating a larvicide (Gonzalez, González, Audino, & Masuh, 2015). The use of synthetic insecticides, however, has been limited due to lack of novel insecticides, expensiveness, harmful effects on environment and human health, and increasing insecticide resistance on a global scale (El, et al., 2012; Ghosh, et al., 2012). An insecticide does not just have to cause a high mortality rate against the targeted organism, it also has to be eco-friendly. Therefore, an alternative approach is to explore safer insecticides of botanical origin as a sustainable mosquito control method. There are more than 10,000 low-molecular mass compounds, known as secondary metabolites, of plants. For this reason, plants have become ideal as an alternative for chemical insecticides and improved defense against pests and diseases (Jeyasankar, 2017). Plant extracts that contain active toxic agents are non-toxic, biodegradable, cheap, and exhibit large target-specific activities against different species of mosquitoes. In addition, plants, as a new source of larvidical compounds, has been of great interest due to modern pharmacological testing (Ghosh, et al., 2012).

In a research study conducted by Gutierrez, et al. (2014), the researchers used three plant samples, namely: *Jatropha curcas*, *Citrus grandis*, and *Tinospora rumphii* as potential larvicides against *Aedes aegypti*. Results show high mortality compared to the control groups. The phytochemicals present in the plants such as alkaloids, flavonoids, tannins, saponins, and steroids are said to have caused toxic effects against the mosquito larvae.

Furthermore, studies show that plant latex and latex-synthesized silver nanoparticles are potential larvicides against mosquitoes. These could be an alternative larvicide that may also help decrease the harmful effects of toxic chemical insecticides (Borase, et al., 2014). Similarly, the stem bark and latex extracts of *Euphorbia tirucalli* contains methanolic, chloroform, and ether extracts that were tested for larvicidal activity against *Culex quinquefasciatus* larvae. Results show that the latex extracts contain stronger larvicidal compounds compared to the stem bark (Yadav, Srivastava, Chandra, Singh, 2002). In addition, *Euphorbia tirucalli* latex extract was also tested against Anopheles larvae. Results indicate that the latex of *Euphorbia tirucalli* can bring total mortality to Anopheles larvae. Lastly, Euphorbiaceae plants are known to contain phytochemicals like triterpenes, saponins, tannins, flavoloids, and alkaloids among others which have been found to be an efficient larvicide against species of mosquito (Mwine, Van Damme, & Jumba, 2010; Rahuman, Gopalakrishnan, Venkatesan, and Geetha, 2008).

Objectives of the study

The objective of this research is to promote the utilization of plant extracts as a larvicidal component against pests. This study specifically aims to formulate larvicide against *Aedes aegypti* larvae made from *Euphorbia milii* plant and to measure its efficacy as a potential larvicide.

Hypothesis

Several studies have stated that Euphorbiaceae plants are rich in phytochemicals that are found to cause a high mortality rate against different species of mosquito. Thus, *Euphorbia milii*'s plant extracts might also contain larvicidal properties that can be effective against *Aedes aegypti* larvae.

METHODOLOGY

The researchers used experimental research. The scientific research design must be strictly followed in experimental research design. It includes a hypothesis, a variable that can be manipulated by the researcher, and variables that can be measured, calculated, and compared. Experimental research is conducted in a controlled environment. The researcher collects data and results will either support or reject the hypothesis (Harland, 2015).

The materials needed for this research were collected in Lyceum of the Philippines–Laguna. The experiment was conducted in the NatSci Laboratory of Lyceum of the Philippines–Laguna.

Euphorbia milii was collected in Brgy. Palingon located in the city of Calamba, Laguna. The authentication of the *Euphorbia milii* was done by using three applications, specifically: PictureThis, PlantSnap, and PlantNet. The researchers took a picture of the plant material and uploaded it on the said three applications. The applications confirmed that it is indeed *Euphorbia milii*.

The researchers made two formulations: (1) pounded stem of *Euphorbia milii* using mortar and pestle, soaked in distilled water, and (2) the stem of *Euphorbia milii* soaked in distilled water. These two formulations were separated into two concentrations—10 mL and 20 mL. Both formulations were allowed to settle for 48 hours.

A control variable is an essential part of the methodology which is kept throughout the experiment though is not the primary concern in the experimental outcome. There was positive and negative control in addition to the plant extracts. Cooking oil is said to be an effective larvicide for mosquitos which the researchers considered as the positive control. On the other hand, Acetone $[(CH_3)_2CO]$ was used as a negative control that is not expected to produce results (Narayanaswamy, et al., 2014). Both positive and negative controls were applied directly to the study samples.

The larvae were collected in Lyceum of the Philippines–Laguna. The larvae that were gathered ranges from 1^{st} to 4^{th} instar larvae. Sixty larvae were used in this experiment and were transferred to the NatSci Laboratory and maintained at room temperature. The larvae were divided into six containers, ten larvae per container filled with 120 mL tap water.

The mosquito larva was identified using a compound microscope. A small amount of water with mosquito larvae was dropped in a slide to be able to view the specimen in the compound microscope.

The mosquito larvae for the two control groups were exposed to 20 mL cooking oil and 20 mL acetone. The experimental groups were exposed to the pounded stem of *Euphorbia milii* and the stem of *Euphorbia milii* soaked in water with 10 mL and 20 mL concentrations. Six droppers were used to apply the treatments in each experimental group. The effects of the plant extracts were monitored after 24 hours of treatment and the percentage mortality was computed.

The researchers utilized the following statistical techniques to ensure valid and reliable analysis and interpretation of data:

1. Percentage Mortality = $\frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$
2. Analysis of Variance (ANOVA)

ANOVA is a statistical test that analyzes variance. It is used to compare two or more means of groups which allows a researcher to draw various results and make predictions about two or more sets of data (Anova Formula, 2013).

RESULTS AND DISCUSSION

Table 1. Percentage mortality of mosquito larvae in control and experimental group after 24-hour observation

Experimental/Control Groups	Concentrations	Percentage Mortality
Pounded stem	10 mL	40%
	20 mL	0%
Soaked stem	10 mL	40%
	20 mL	50%
Cooking oil	20 mL	100%
Acetone	20 mL	100%

Table 1 shows the results of the larvicidal test at different concentrations against different mosquito larvae. It is evident that the prepared treatments of the stem of *Euphorbia millii* were not found significantly effective against the larvae, causing 0-40 percent mortality on all of the treatments except the 50 percent result on the soaked stem with 20 mL concentration. Meanwhile, the positive control group (cooking oil) and even the negative control group (acetone) caused 100 percent mortality on the mosquito larvae.

The researchers would like to emphasize that most of the dead larvae were identified as *Culex quinquefasciatus* larvae under a compound microscope (see Figure 1). The researchers also identified that some of the larvae that were still alive were *Aedes aegypti* larvae (see Figure 2).

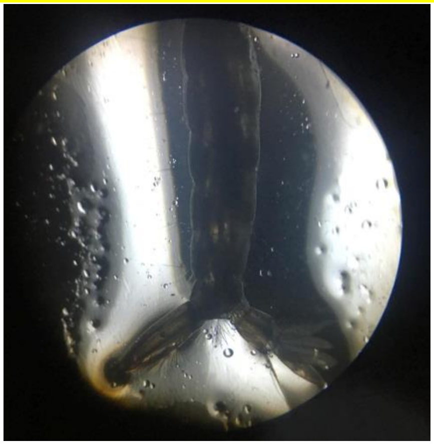

Figure 1. Dead larvae

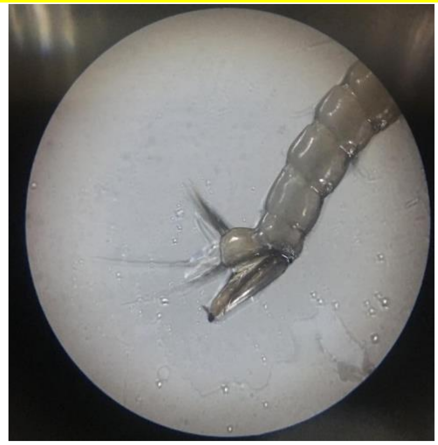

Figure 2. Live larvae

Moreover, 1st instar larvae are the ones which mostly died while those in the 4th instar larvae remained alive, like what was observed in the experimental group of the pounded stem with 20 mL concentration. Through the development stage of the larvae, it is only during the 3rd and 4th instar where mosquito larvae start to develop chitin in their epidermis; 1st to 2nd instar periods do not (Farnesi, et al., 2012). Chitin provides the tough structure of the outermost layer of the body of the *Aedes aegypti* larvae and as it reaches the 3rd instar period, the chitin component increases, protecting the thoracic organs and underlying parts.

Table 2. ANOVA results of mortality of mosquito larvae treated on the various concentrations from plant extract of Euphorbia milii after 24 hours

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P-value
Treatments	1	57.0	57.00	17.54	0.053
Error	2	6.5	3.25		
Total	3	63.5			

Table 2 indicates the one-way ANOVA that was conducted to compare the means of the two treatments: soaked stem and pounded stem of *Euphorbia milii*, on mosquito larvae. The F value is the ratio of the mean square treatments, 57, to the ratio of mean square error, 3.25, thus, the F value is 17.54 with a P-value or significance of 0.053. Therefore, at 0.05 level of significance, there is little significant difference between the two treatments that were made.

Table 3. ANOVA results of mortality of mosquito larvae treated on the various concentrations from plant extract of Euphorbia milii and the control group after 24 hours

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P-value
Control vs. Experimental	2	203	101.5	35.87	0.008*
Error	3	8.5	2.83		
Total	5	211.5			

*At 0.05 level of significance, *implies that there is a significant difference*

Table 3 reflects the one-way ANOVA that was conducted to compare the means of the killed mosquito larvae by the control groups and experimental groups. The F value is the ratio of the mean square control vs. experimental groups, 101.5, to the ratio of mean square error, 2.83, thus, the F value is 35.87 with a P-value or significance of 0.008. Therefore, at 0.05 level of significance, there is a significant difference between the experimental groups and control groups. The control groups included a positive control (cooking oil) and negative control (acetone) which both yielded 100 percent mortality rate on mosquito larvae. It is evident that the control groups were more effective than the treatments. Hence, the stem bark extracts from *Euphorbia milii* is not found to be significantly effective against mosquito larvae. As opposed to a study conducted by Borase, et al. (2014) wherein results show that the silver nanoparticles (AgNPs) derived from the latex of *Euphorbia milii* were highly effective against *Anopheles stephensi* larvae. The present study only used the stem bark of the plant material and did not utilize pure latex. A study was done by Oliveira-Filho & Paumgartten (2000), however, in which they tested *Euphobia milii's* latex against target snails and non-target aquatic organisms which showed that the latex was not toxic to mosquito larvae.

Furthermore, the present study is in contrast to the study conducted by Yadav, Srivastava, Chandra, and Singh (2002) wherein they tested stem barks and latex of *Euphorbia tirucalli* on the mosquito *Culex quinquefasciatus* larvae and yielded high mortality results. In addition, latex extracts contain more potent larvicidal components compared to stem bark extracts. This present study is also in contrast to the study conducted by Rahuman, Gopalakrishnan, Venkatesan, and Geetha (2008) wherein results showed that the petroleum ether extracts of *Euphorbia tirucalli* were highly effective against the larvae of *Aedes aegypti* and *Culex quinquefasciatus*.

The researchers were unable to separate the *Aedes aegypti* larvae, the targeted species of this study, from the other species of mosquito larvae which could have affected the results. In a study conducted by Santana-Martínez, Molina, & Dussán (2017), larval coexistence under food scarcity condition showed that *Aedes aegypti* had greater adult development than *Culex quinquefasciatus* larvae because they are deprived of the food needed to complete metamorphosis. This is due to *Aedes aegypti* larvae's scrape feeding habitats and fast larval development making them more successful in exploiting microhabitats and food is insufficient. In addition, *Culex quinquefasciatus* larvae were also more vulnerable to temperature fluctuations.

CONCLUSION AND RECOMMENDATION

The researchers were able to formulate a potential larvicide against mosquito larvae from the stem extracts of *Euphorbia milii*. Two formulations were produced: (1) soaked stem and (2) pounded stem both submerged in 120 mL of water. These two formulations were separated into two concentrations: 10 mL and 20 mL to test its efficacy against mosquito larvae. The targeted species of this study which is the *Aedes aegypti* larvae were not as affected as the *Culex quinquefasciatus* which holds the majority of the fatality. There is also a significant difference between the experimental and control groups. The control groups yielded 100 percent mortality while the treatments caused 0-50 percent mortality against mosquito larvae. This indicates that *Euphorbia milii* stem extracts were not significantly effective against mosquito larvae.

For further studies, the researchers recommend using a higher concentration of the formulas. Second, use the latex of the *Euphorbia milii* because it could be more effective since *Euphorbia tirucalli's* latex contains more potent larvicidal components and it could be the same with *Euphorbia milii*. Lastly, identify the species of mosquito larvae and separate them each.

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