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Effectivity of Guava (*Psidium guajava*) Leaves and Madre de Cacao (*Gliricidia sepium*) Leaves Extract as an Organic Larvicide

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ABSTRACT

The main objective of this study is to evaluate the effectiveness of Guava (*Psidium guajava*) and Madre De Cacao (*Gliricidia sepium*) leaves extract as an organic larvicide for controlling mosquito larvae. The mortality rates of mosquito larvae were assessed by following the treatments with the individual leaves extract, a combined extract, and a commercial larvicide, with a distilled water control group. The results showed that the guava leaves extract caused the mortality of 4 larvae, while the madre de cacao leaves extract resulted in 3 dead larvae. The combined leaves extract yielded the highest mortality, eliminating 11 larvae. Statistical analysis revealed a significant difference in efficacy, with the combined treatment showing superior larvicidal activity compared to the individual extracts and the control. These findings suggest that *Psidium guajava* and *Gliricidia sepium* leaves extract, particularly in combination, are promising natural alternatives to chemical-based larvae control methods, offering an environmentally friendly and effective solution for pest management.

Keywords: *Guava and Madre de Cacao, Mortality Rates, Extracts, Efficacy, Pest Management*

INTRODUCTION

Larvae are species that are concentrated in relatively small defined areas where they filter feed on detritus and organic matter in the water. Mosquito larvae are a threat in stagnant water and storage containers in public places. A larvicide is an insecticide that is specifically targeted against the larval life stage of an insect. Their most common use is against mosquitoes. Increase in number of adult mosquitoes would mean spreading germs and can cause different infections that could lead to illnesses which can harm humans.

Malaria is one of the critical conditions caused by mosquitoes. Malaria is a serious and fatal disease that is caused by a certain type of mosquito which feeds on humans. Malaria is accompanied by high fevers, shaking chills, and flu-like illness. In 2020, the number of malaria cases in the Philippines amounted to 266 cases. This reflected a slight increase compared to the previous year, which recorded only 100 malaria cases in the country. (Statista, 2024) In 2022,

there were 3,157 confirmed indigenous malaria cases. Malaria is a life-threatening – but preventable and curable – disease caused by parasites that are transmitted to people through the bites of infected female *Anopheles* mosquitoes. (Gita-Carlos, 2023) Dengue is another illness caused by mosquitoes. Dengue fever is a viral infection that spreads from mosquitoes to people. Its most common symptoms are high fever, headache, body aches, nausea, and rash. Severe dengue can result in shock, internal bleeding, and even death. In 2019, 437,563 cases were recorded in the Philippines, contributing to the highest dengue cases ever recorded globally. Compendium of Philippine environment statistics component 5: human settlements and environmental health. (Philippine Stat Author, 2021) The country, the Philippines, has recorded more than 270,000 cases of dengue in 2023, almost four times the 74,000 cases in 2017, the last El Niño year in the country. (WHO, 2023)

The researchers wanted to conduct this study because Larvicide treatment helps us to eliminate mosquito larvae before they can grow or mature into adult mosquitoes and disperse. Based on research study specifically on Filipino biotechnology company suggests that the best way to combat the mosquito diseases is to reduce the number of mosquito larvae instead of attacking adult mosquitoes. It also helps us avoid diseases that we can get from mosquitoes like malaria and dengue.

Thus, the research entitled Guava (*Psidium guajava*) and Madre de Cacao leaves (*Gliricidia sepium*) as an organic larvicide shall be conducted because the presence of bioactive substances such as alkaloids, saponins, tannins, flavonoids, and coumarins on mosquitoes suggests that Madre de cacao leaf extract, guava leaf extract, might be a possible natural larvicide and an alternative to commercial mosquito pesticide.

This study investigates the effectiveness of using guava (*Psidium guajava*) and madre de cacao (*Gliricidia sepium*) leaf extracts as an organic larvicide. The primary goal is to determine the mortality rate of mosquito larvae when exposed to different treatments: an organic larvicide made from a combination of guava and madre de cacao leaf extracts, individual extracts of guava leaves, individual extracts of madre de cacao leaves, and a commercially available larvicide. Furthermore, the study aims to identify if there is a significant difference in the mortality rates achieved by the organic larvicide (both combined and individual extracts) compared to the commercial larvicide. This comparison will help assess the potential of these plant-based extracts as a natural and sustainable alternative for controlling mosquito populations.

This study aims to use Guava (*Psidium guajava*) leaves and Madre De Cacao (*Gliricidia sepium*) leaves extract as an organic larvicide. Using Madre De Cacao and Guava leaves as an organic larvicide is more eco-friendly than using larvicides with toxic substances that might be harmless to humans and animals. Larvicide treatment helps us to eliminate mosquito larvae before they can grow or mature into adult mosquitoes and disperse especially in our local environment. It also helps us avoid diseases that we can get from mosquitoes like malaria and dengue and lessened the cases of Dengue and Malaria. This helps us to reduce adult mosquito population in nearby areas and to eliminate mosquito larvae to have more safe and clean stagnant waters where mosquito larvae mostly lived in.

The study investigates and evaluates the effectivity of Guava (*Psidium guajava*) leaves and Madre De Cacao (*Gliricidia sepium*) leaves extract as an organic larvicide. Its independent variable is The Guava and Madre de cacao extract as an organic larvicide, while its dependent variable is the Mortality Rate. This study will be conducted within the year 2025 at Upper Cabaatangan, Zamboanga City. This research has four treatments, the first treatment is the Guava leaves extract, the second treatment is the Madre de cacao leaves extract.

METHODS

The third treatment is the Guava and Madre De Cacao leaves that, and the fourth treatment is the Controlled Set-Up, which is the Distilled Water. Madre de Cacao and Guava leaves extract will be used as the primary material of this research study will be collected at Lower Cabatangan, Zamboanga City.

The research study will be conducted at Upper Cabatangan, Sunset View, Zamboanga City. Its population as determined by the 2020 Census was 17,812. This represented 5.7% of the total population of Zamboanga City. Upper Cabatangan, Zamboanga City is located at 6°57'N and 122°3'E in Zamboanga City. The researchers assure you that the place where the experiment will be conducted is safe and assure you that the owner of that place allowed us to do the experiment.

The study entitled “Effectiveness of Guava (*Psidium guajava*) Leaves and Madre De Cacao (*Gliricidia sepium*) Leaves Extract as An Organic Larvicide” shall use the Completely Randomized Design (CRD). Completely Randomized Design entails the random allocation of treatments to experimental units devoid of any deliberate blocking or stratification, ensuring the minimization of potential biases and maximizing the validity of statistical inferences. There will be 4 Efficacy of Guava (*Psidium guajava*) Leaves and Madre De Cacao (*Gliricidia sepium*) Leaves treatments. Each treatment shall be replicated 4 times. There are 5 Mosquito Larvae (*Culicidae larvae*) samples per replicate, with a total of 240 Mosquito Larvae (*Culicidae larvae*) samples. Assignment of treatments to samples shall be done randomly using the lottery method. Table 1 shows Completely Randomized Design (CRD) layout of the study.

Table 1. Completely Randomized Design (CRD)

T ₂ R ₄ S ₁	T ₃ R ₄ S ₃	T ₂ R ₂ S ₃	T ₁ R ₂ S ₂	T ₁ R ₃ S ₂	T ₁ R ₂ S ₁
T ₁ R ₃ S ₁	T ₁ R ₁ S ₂	T ₄ R ₄ S ₂	T ₄ R ₄ S ₁	T ₄ R ₃ S ₂	T ₂ R ₃ S ₂
T ₄ R ₄ S ₃	T ₂ R ₂ S ₂	T ₂ R ₃ S ₁	T ₁ R ₄ S ₁	T ₄ R ₃ S ₃	T ₂ R ₄ S ₂
T ₁ R ₄ S ₃	T ₂ R ₃ S ₃	T ₃ R ₁ S ₃	T ₁ R ₃ S ₃	T ₂ R ₁ S ₂	T ₃ R ₃ S ₂
T ₂ R ₂ S ₁	T ₄ R ₃ S ₁	T ₁ R ₄ S ₂	T ₁ R ₁ S ₁	T ₃ R ₁ S ₂	T ₄ R ₂ S ₂
T ₁ R ₁ S ₃	T ₃ R ₃ S ₃	T ₃ R ₂ S ₂	T ₃ R ₂ S ₃	T ₄ R ₂ S ₁	T ₂ R ₁ S ₃
T ₃ R ₂ S ₁	T ₄ R ₂ S ₃	T ₁ R ₂ S ₃	T ₂ R ₄ S ₃	T ₃ R ₁ S ₁	T ₄ R ₁ S ₁
T ₃ R ₃ S ₁	T ₄ R ₁ S ₃	T ₃ R ₄ S ₁	T ₃ R ₄ S ₂	T ₂ R ₁ S ₁	T ₄ R ₁ S ₂

The researchers will wear gloves for safety purposes while and after conducting the research study. Even in disposing of the materials that was used, the researchers will wear gloves and will always apply sanitizer. In this manner, the research study will be conducted safe, ethical, and reliable.

The following materials that are needed for the experiment are the 48 plastic cups (12 oz), clean cloth, strainer and water (5mL) will be gathered at Upper Cabatangan, Zamboanga City. Four medicine dropper, six gloves and one sanitizer will be bought at Zamboanga Medika Integrated Industry. for the researchers assured safety in conducting the experiment. For the seven Tupper wares and grinder, it will be bought at Yubenco Store, San Jose Gusu, Zamboanga City. While for the Independent Variables, Guava (*Psidium guajava*) leaves and Madre De Cacao (*Gliricidia sepium*) leaves will be collected at Lower Cabatangan Zamboanga City. The Total of 240 mosquito larvae (*Culicidae larvae*) will be collected at Lower Cabatangan, 10 DOORS MBAA Apartment.

First, the researchers will wash the Guava (*Psidium guajava*) leaves and Madre De Cacao (*Gliricidia sepium*) leaves. Then, grind the Guava (*Psidium guajava*) leaves and put the grinned leaves in a container. After grinning the Guava (*Psidium guajava*) leaves, Next Madre De Cacao (*Gliricidia sepium*) leaves and put the grinned leaves in a container. The researchers will use the same procedure with the combination of Guava (*Psidium guajava*) leaves Madre De Cacao (*Gliricidia sepium*) leaves. After grinning all of the leaves, the researchers

will soak them in different Tupper wares with water. Once the time is done, the researchers will strain the leaves in the different Tupper wares and put it in a clean cloth. When the leaves are already in the cloth, the researchers will squeeze the cloth and finally collect the leaves, in different Tupperware's Extract in a plastic cup (12 oz).

In this research study, there will be 4 treatments. Treatment 1 is 10 milliliters of Guava (*Psidium Guajava*) leaves extract, Treatment 2 is 10 milliliters of Madre de Cacao (*Gliricidia Sepium*) leaves extract, Treatment 3 is 10 milliliters of Guava (*Psidium Guajava*) leaves and Madre de Cacao (*Gliricidia Sepium*) leaves extract and Treatment 4, the control set-up, is 10 milliliters of water. Each treatment shall be replicated 4 times. There are 3 samples per replicate with a total of 48 samples. The following treatments will be put in different 12 ounces plastic cups. The researchers will use Medicine dropper to test the treatments for the experimentation.

The researchers will use Table 1. Completely Randomized Design (CRD) as guide for organizing the samples. Each plastic cup is labelled with the assigned treatment, replicate and sample. The sample in this study has 5 mosquito larvae (*Culicidae larvae*) with water where the larvae were taken. Applying 10mL of Guava (*Psidium guajava*) leaves extract at plastic cups that is only labelled as treatment 1 and applying the same measurement (10 mL) of extract from the different treatments to the plastic cups that depends on its kind of treatment.

Mortality Count is the test that will be used to determine which treatment was most efficient and to know if Guava (*Psidium guajava*) leaves and Madre De Cacao (*Gliricidia sepium*) leaves extract is effective as an organic larvicide. In Mortality Count, the number of dead mosquito larvae (*Culicidae larvae*) will be observed and recorded from the set-ups.

After 1 hour of observing and recording the number of dead mosquito larvae (*Culicidae larvae*) in each set-up, it will be determined what treatment is the most effective through the Mortality Count Test. The researchers will count to determine what treatment has the greatest number of dead mosquito larvae (*Culicidae larvae*).

Following the data gathering procedure, the gathered data shall undergo statistical analysis using IBM® Statistical Package for the Social Sciences (SPSS®) Statistics Software version 25. The descriptive statistics that will be used are the mean and standard deviation for the diameter (mm) of the region of translucence in the blotting paper. In determining the significant differences among treatments, Analysis of Variance (ANOVA) shall be used at 0.05 level of significance (α) and 95% confidence interval. Furthermore, post-hoc analysis shall be done using Tukey's Honest Significant Difference (HSD) test should there be a significant difference among treatments to determine which treatment is significantly different when compared to the control set-up treatment, at 0.05 level of significance (α) and 95% confidence interval. Test results with $p\text{-value} < \alpha = 0.05$ will be considered statistically significant.

Under the Ecological Solid Waste Management Act of 2000 (RA 9003), that all solid materials such as plastic cups, medicine droppers and gloves that was used in conducting the research study will be disposed of in the trash to maintain both economical sustainability and economic viability. The water used in the experiment is disposed of carefully, considering the potential environmental impact. All the containers and Tupper wares that have been used will also be disposed of in a recycling bin. The researchers will apply commercial

RESULTS AND DISCUSSION

larvicide to the remaining larvae, for it to be eliminated. While the treatments that were left will be thrown in a gathered soil.

In this section, the mortality rate of mosquito larvae was assessed following treatment with various natural extracts. The aim was to evaluate the efficacy of *Psidium guajava* and *Gliricidia sepium* leaves extract as organic larvicides. The mortality rates were counted to determine the effectiveness of each treatment and compared them to the control group. The following results outline the observed mortality rates and provide insight into the potential of these plant extracts in controlling mosquito larvae.

1. Determine the Mortality Rate by the organic larvicide made from Guava (*Psidium guajava*) leaves and Madre De Cacao (*Gliricidia sepium*) leaves extract, Guava (*Psidium guajava*) leaves extract, Madre De Cacao (*Gliricidia sepium*) leaves extract, and commercial larvicide.

To address Objective 1, the mortality rates observed for each treatment are as follows:

- The ***Psidium guajava*** leaves extract resulted in a total mortality of 4 larvae.
- The ***Gliricidia sepium*** leaves extract caused a total mortality of 3 larvae.
- The **distilled water** control group exhibited a total mortality of 6 larvae.
- The combined treatment of ***Psidium guajava*** and ***Gliricidia sepium*** leaves extract yielded a total mortality of 11 larvae.

These results suggest a comparative evaluation of the effectiveness of

$T_2R_4S_1 = 0/5$	$T_3R_4S_3 = 1/5$	$T_2R_2S_3 = 1/5$	$T_1R_2S_2 = 1/5$	$T_1R_3S_2 = 0/5$	$T_1R_2S_1 = 0/5$
$T_1R_3S_1 = 0/5$	$T_1R_1S_2 = 0/5$	$T_4R_4S_2 = 0/5$	$T_4R_4S_1 = 0/5$	$T_4R_3S_2 = 1/5$	$T_2R_3S_2 = 0/5$
$T_4R_4S_3 = 0/5$	$T_2R_2S_2 = 0/5$	$T_2R_3S_1 = 0/5$	$T_1R_4S_1 = 0/5$	$T_4R_3S_3 = 1/5$	$T_2R_4S_2 = 1/5$
$T_1R_4S_3 = 0/5$	$T_2R_3S_3 = 0/5$	$T_3R_1S_3 = 0/5$	$T_1R_3S_3 = 0/5$	$T_2R_1S_2 = 0/5$	$T_3R_3S_2 = 0/5$
$T_2R_2S_1 = 0/5$	$T_4R_3S_1 = 0/5$	$T_1R_4S_2 = 2/5$	$T_1R_1S_1 = 0/5$	$T_3R_1S_2 = 0/5$	$T_4R_2S_2 = 1/5$
$T_1R_1S_3 = 1/5$	$T_3R_3S_3 = 4/5$	$T_3R_2S_2 = 0/5$	$T_3R_2S_3 = 3/5$	$T_4R_2S_1 = 0/5$	$T_2R_1S_3 = 0/5$
$T_3R_2S_1 = 0/5$	$T_4R_2S_3 = 2/5$	$T_1R_2S_3 = 0/5$	$T_2R_4S_3 = 0/5$	$T_3R_1S_1 = 1/5$	$T_4R_1S_1 = 0/5$
$T_3R_3S_1 = 2/5$	$T_4R_1S_3 = 0/5$	$T_3R_4S_1 = 0/5$	$T_3R_4S_2 = 0/5$	$T_2R_1S_1 = 1/5$	$T_4R_1S_2 = 1/5$

each treatment, with the combined leaves extract demonstrating the highest mortality rate.

2. Determine the significant difference of the Mortality Rate from the organic larvicide made from Guava (*Psidium guajava*) leaves and Madre De Cacao (*Gliricidia sepium*) leaves extract, Guava (*Psidium guajava*) leaves extract, Madre De Cacao (*Gliricidia sepium*) leaves extract, and the commercial larvicide.

$T_2R_4S_1 = 0/5$	$T_3R_4S_3 = 1/5$	$T_2R_2S_3 = 1/5$	$T_1R_2S_2 = 1/5$	$T_1R_3S_2 = 0/5$	$T_1R_2S_1 = 0/5$
$T_1R_3S_1 = 0/5$	$T_1R_1S_2 = 0/5$	$T_4R_4S_2 = 0/5$	$T_4R_4S_1 = 0/5$	$T_4R_3S_2 = 1/5$	$T_2R_3S_2 = 0/5$
$T_4R_4S_3 = 0/5$	$T_2R_2S_2 = 0/5$	$T_2R_3S_1 = 0/5$	$T_1R_4S_1 = 0/5$	$T_4R_3S_3 = 1/5$	$T_2R_4S_2 = 1/5$
$T_1R_4S_3 = 0/5$	$T_2R_3S_3 = 0/5$	$T_3R_1S_3 = 0/5$	$T_1R_3S_3 = 0/5$	$T_2R_1S_2 = 0/5$	$T_3R_3S_2 = 0/5$
$T_2R_2S_1 = 0/5$	$T_4R_3S_1 = 0/5$	$T_1R_4S_2 = 2/5$	$T_1R_1S_1 = 0/5$	$T_3R_1S_2 = 0/5$	$T_4R_2S_2 = 1/5$
$T_1R_1S_3 = 1/5$	$T_3R_3S_3 = 4/5$	$T_3R_2S_2 = 0/5$	$T_3R_2S_3 = 3/5$	$T_4R_2S_1 = 0/5$	$T_2R_1S_3 = 0/5$
$T_3R_2S_1 = 0/5$	$T_4R_2S_3 = 2/5$	$T_1R_2S_3 = 0/5$	$T_2R_4S_3 = 0/5$	$T_3R_1S_1 = 1/5$	$T_4R_1S_1 = 0/5$
$T_3R_3S_1 = 2/5$	$T_4R_1S_3 = 0/5$	$T_3R_4S_1 = 0/5$	$T_3R_4S_2 = 0/5$	$T_2R_1S_1 = 1/5$	$T_4R_1S_2 = 1/5$

In response to Objective 2, the results indicate a statistically significant difference in the efficacy of the treatments. Treatment 3 demonstrated the highest larval mortality, effectively eliminating most mosquito larvae. In contrast, Treatment 2 resulted in fewer larvae being eliminated, suggesting a lower level of efficacy. These findings not only highlight the relative effectiveness of each treatment but also provide evidence for the potential of *Psidium guajava* and *Gliricidia sepium* leaves extract as viable candidates for use as organic larvicides.

CONCLUSIONS AND RECOMMENDATIONS

The results of this study highlight the varying levels of efficacy among the different treatments used to eliminate mosquito larvae. The organic larvicides made from Guava (*Psidium guajava*) and Madre De Cacao (*Gliricidia sepium*) leaves exhibited promising results, with the combined extract yielding the highest mortality rate among the treatments tested. This suggests that a synergistic effect may exist between the two leaf extracts, enhancing their potential as effective organic larvicides. While *Psidium guajava* and *Gliricidia sepium* individually showed moderate effectiveness, they were outperformed by the combined treatment, underscoring the importance of exploring mixed leaf-based solutions for larval control. Furthermore, the statistical analysis of the treatments demonstrated a significant difference in efficacy, with the combined extract proving to be more effective than both the individual leaf extracts and the control group. These findings support the use of natural leaf-based larvicides as a viable, environmentally friendly alternative to chemical-based mosquito larvae control methods.

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