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Efficacy of Canarium ovatum (Pili Tree) Pulp Fixed Oil Extract as a Natural-Based Larvicide Against Aedes aegypti

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Abstract: *Aedes aegypti*, the primary vector of the endemic Dengue Fever (DENV) has always been a vector of concern in the Philippines due to its infectivity and known resistance against major commercial insecticide compositions. This challenge poses the need for an effective natural-based alternative, specifically the *Canarium ovatum* (Pili) exocarp, an unexplored biowaste that exhibits strong larvicidal potentials for its phytochemical and bioactive compositions. This research sought to determine the efficacy of *C. ovatum* pulp oil extract as a natural larvicide against *A. aegypti*. Five (5) different *C. ovatum* pulp oil concentrations in triplicates were tested against the positive control (Temephos) in the mortality assay through a modified application of the World Health Organization Guidelines. The results showed that the *C. ovatum* pulp oil exhibited positive dose- and time-dependent larvicidal potency against *A. aegypti*. Furthermore, the results indicate a similarity between the larval mortality induced by the *C. ovatum* pulp oil at 800µg/mL and 400 µg/mL in comparison to temephos, a commercial grade larvicide. The Lethal Concentration 50 (LC_{50}) and Lethal Concentration 90 (LC_{90}) were found at 287.869 ppm and 639.473 ppm, respectively. The results of the study concluded that the *C. ovatum* pulp oil is as significantly effective against *A. aegypti* larvae and an effective natural alternative to the commercially used larvicide. As such, the findings of this study address the known chemical-insecticide resistance among *A. aegypti* larvae and the mass bio waste product generated by the Pili industry.

Keywords: *Canarium ovatum*, Pili pulp oil, *Aedes aegypti*, larvicide, lethal concentration

1. INTRODUCTION

Dengue fever (DENV) is one of the most prominent vector-induced diseases across the globe and a constant health concern in the Philippines. Since its first incidence in the country in 1954, it has posed extensive endemicity leading to severe and fatal infections especially on children and adolescents aged 1 to 19 years (Agrupis et al., 2019; Bravo et al., 2014). The *Aedes aegypti* mosquito is one of more than 950 species of the genus *Aedes* that is specifically dominant in locations with tropical, subtropical, and temperate climates like the Philippines (Centers for Disease Control Prevention [CDC], 2020; Rogers, 2019). It is the primary vector of DENV. While the DOH exhausts its efforts to prevent the disease through programs and strategies including the eradication of mosquito breeding sites, *A. aegypti* was found to exhibit insecticide resistance against major insecticide components including dichlorodiphenyltrichloroethane (68%), permethrin (58%), and deltamethrin (27%) (Zulfa et al., 2022). This challenge poses the need to seek alternative larvicide components, which can be found in plants, which impose little to no risks on the target's surrounding organisms and the environment (Ojha & Pattabhiramaiah, 2013).

The *Canarium ovatum*, commonly called Pili is an indigent fruit of the Philippines that is identified as a *Canarium* species of the *Burseraceae* family (Coronel, 1996). It is commonly found in the Bicol region where 6,224.26 metric tonnes of *C. ovatum* fruits per annum are harvested (Department of Agriculture, 2021). The Pili nut is mostly consumed and used in the culinary industry. Whereas, the pulp is the outer covering that surrounds the shelled nut. It makes up 60% to 70% of the whole fruit and yet is only discarded as a biowaste in the Pili industry due to the lack of scientific

studies which hinder its full introduction to the commercial market (Millena & Sagum, 2018).

The phytochemical analysis of the *C. ovatum* pulp shows that it has high concentrations of phytosterols including camposterol and stigmasterol (Pham & Dumandan, 2020). Stigmasterol, specifically, is known to induce larval mortality by inhibition of acetylcholinesterase that disrupts the synaptic transmission of a mosquito, a major mechanism by most insecticides (Gade et al. 2016). Furthermore, the Pili pulp oil was also found to have Triterpenoids, specifically betulinaldehyde, which was proven to induce larvicidal toxicity against *A. aegypti* larvae (Dumandan et al., 2022). This favorable lipid composition of the *C. ovatum* exocarp strengthens the need to discover the potential larvicidal efficacy of *C. ovatum* on *A. aegypti*.

Other phytochemical compositions of the *C. ovatum* pulp also include Phenolics, Flavonoids, Tannins, Alkaloids, Cardiac glycosides, Terpenoids, and Saponins as previously provided by Recuenco et al. (2020) which were proven larvicidal potent contents in a similar study by Hayatie et al. (2015) on Carica papaya nut and husk.

As *C. ovatum* is indigenous and abundant in the Philippines, the mere presence of its biowastes in the form of unused pulp makes it an accessible and affordable larvicidal source. Data from 2017 shows that in the Bicol region alone, the primary producer of *C. ovatum* in the country, there are 142,405 hectares of Pili plantations that generate 6,224.26 metric tonnes of *C. ovatum* per annum which equates to 89% of the whole production in the Philippines (Department of Agriculture, 2021). However, the *C. ovatum* exocarp is a current waste product in the Bicol Region and the industry. As Millena and Sagum (2018) states, "The limited information available on the *Pili* nut hampers the full utilization of its potential to compete in the global market". This shows the inevitable need for the development and exploration of pili postharvest processing systems to lessen the *C. ovatum* wastes and achieve profitable industries for pili farmers in the country (Embuscado, 2010).

Given the significant lack of studies on the larvicidal capacity of *C. ovatum* exocarp, the present chemical insecticide resistance among *A. aegypti* mosquitoes, and the abundance of *C. ovatum* exocarp waste produce in the Philippines, this research aims to determine the larvicidal effectivity of *C. ovatum* pulp fixed oil against *A. aegypti*, as a cost-effective and efficient substitute for chemical-based commercial larvicides.

2. METHODOLOGY

2.1. *Aedes aegypti* Larvae Rearing

The *A. aegypti* larvae egg samples were sourced from the University of the Philippines Los Banos Institute of Weed Science, Entomology, and Plant Pathology (IWEP), Los Banos, Laguna, Philippines. The larvae were transported to and reared for 7 days, based on the recommendations by the Institute of Weed Science, Entomology, and Plant Pathology (IWEP), in the De La Salle Medical and Health Sciences Institute Angelo King Medical Research Center (DLSMHSI), Dasmarinas City, Cavite, Philippines.

As required by the WHO Larvicidal Bioassay (2005), the larvae were reared until their 3rd instar stage. Furthermore, the samples were grown adapting the rearing protocols applied by Dinh et al. (2020). As such, the larvae were stored in laboratory grade plastic boxes filled with 1-inch-deep distilled water inside the laboratory with a temperature range of 25-28 degrees Celsius with feed of ground fish flakes daily until the desired instar stage. The larvae samples were also exposed to a 9L:15D photoperiod, in modification of the adapted larvae rearing protocol.

2.2. *Canarium ovatum* nut pulp Oil Extraction

Ripe fruits of Pili (*Canarium ovatum* Engl.) were procured from a local seller located in La Medalla, Baao, Camarines Sur, Philippines. The samples were sent to the Bureau of Plant Industry (BPI) where it was screened and authenticated as a *C. ovatum* species.

Upon authentication, the fresh *C. ovatum* pulp samples were sent to the Pharmaceutical Section – Chemicals and Energy Division of the Department of Science and Technology (DOST), Taguig City, Metro Manila, Philippines for all necessary preparations, including the drying and grinding of the plant samples. for which it was dried for 7 days and underwent grinding with a Wiley Mill. The ground samples were processed at the Inorganic Chemistry Division (ICS) of DOST-ITDI using Solvent Extraction of Fixed Oil with analytical grade n-hexane as the solvent.

Through this oil extraction method, the researchers

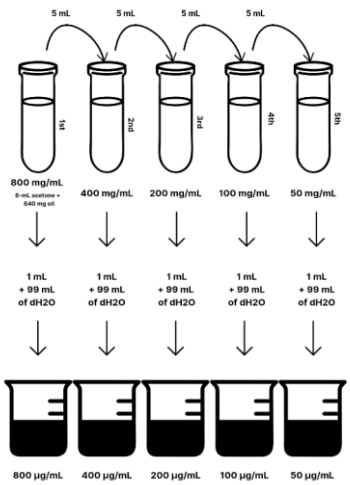
obtained 310.3 g of oil, with a total percent yield of 22.47% from the original 1,380.7 g of dried and ground plant samples.

2.3. Preparation of Stock Solution and Test Concentrations

This research applied the WHO Guidelines for Preparation of Stock Solutions and Test Concentrations (2005) with modifications adapted from Thomas et al. (2017). Prior to the formulation of the stock solution, 5 test cups were prepared with 5 mL of acetone in preparation for two-fold serial dilution. The *C. ovatum* fixed oil stock solution was formulated at an initial concentration of 1600 mg of oil to 10 mL of acetone. The mixture was shaken in a sealed test tube for proper content dispersion and the two-fold dilution followed. This procedure yielded five (5) *C. ovatum* fixed oil suspension at 800, 400, 200, 100, and 50 µg/mL.

Figure 2.1

Preparation of C. ovatum pulp oil Concentrations



2.4. Mortality Assay

In accordance with the WHO Guidelines for Laboratory and Field Testing of Mosquito Larvicides (2005) with minor modifications, the *A. aegypti* larvae were filtered for biological activity and separated into 20 larvae per 250 ml glass beaker filled with 99 mL of distilled water + 1ml of test concentration. The beakers were netted to prevent the escape of surviving mosquitoes. All the samples were kept in a controlled laboratory environment with the temperature ranging from 25°C-28°C and a 9L: 15D photoperiod, in modification of the provided 12L:12D protocol of the WHO (2005) due to the schedule limitations of the laboratory used.

There were 8 concentrations with 3 replicates each. First of the set ups are the *C. ovatum* solutions at 1 mL of 800, 400, 200, 100 and 50 µg/mL concentrations in 99 mL of distilled water (Figure 2.2). The control setup includes temephos (+) and distilled water (-). An additional solution with 99 mL of distilled water and 1 mL of acetone was also done to see if it contributes to the larvicidal potency of the solutions.

The larval mortality percentage per concentration was measured after the first 24h, 48h, and 72h of exposure. The number of dead, moribund, and active samples were counted, and the collected date were subjected to the mortality percentage formula:

$$Mortality\ (\%) = \frac{No.of\ dead\ larvae}{No.of\ larvae\ introduced}\ x\ 100\%$$

2.5. LC_{50} and LC_{90} Assay

The data collected for % mortality were used in the Linear Regression Probit Analysis to answer the LC_{50} and LC_{90} of the experimental concentrations on the *A. aegypti* larvae, these values are the minimum amount of concentration required to induce a 50% and 90% mortality on the test population were determined (Cruz et al., 2019; Fatimah et al., 2019).

3. RESULTS AND DISCUSSION

3.1 Percent Mortality of the Different *C. ovatum* Pulp Oil Concentrations

Considering the larvicidal efficacy of the *C. ovatum* pulp oil against *A. aegypti* larvae, the obtained results reveal that its efficacy is dependent on the concentration of the larvicide and the time of exposure to the treatment.

Table 3.1

Descriptive Statistics of Mortality Rate (%) of Different Concentrations of Canarium ovatum Pulp Oil against 3rd Instar Aedes aegypti Larvae

			95% Confidence Interval for Mean	
Concentration	M	SD	Lower bound	Upper bound
800 µg/mL	93.33	7.638	74.36	112.31
400 µg/mL	71.67	15.275	33.72	109.61
200 µg/mL	51.67	10.408	25.81	77.52
100 µg/mL	18.33	16.073	-21.59	58.26
50 µg/mL	11.67	5.774	-2.68	26.01

Among all the test concentrations of the *C. ovatum* pulp oil, 800 µg/mL is considered the most effective as it demonstrated a mean mortality rate of 93.33%. Following these are the lower concentrations which still induced larval deaths at lower extents. The data obtained proves the larvicidal efficacy of the *C. ovatum* pulp oil as a natural based larvicide against *A. aegypti* larvae. This is as verified by the attained Pearson r Correlation value of 0.894 (see Table 3.2). Therefore, the data presented shows enough scientific evidence that the larvicidal potency of the *C. ovatum* pulp oil on *A. aegypti* larvae is dependent on the amount of concentration in terms of mortality rate. This result corresponds to the results of a study by Ojha and Pattabhiramaiah (2013) which yielded the same trend of results as our study for its results verifies that higher concentrations of *J. curcas* seed oil, which contains the same phytochemical contents of Flavonoids, Tannins, and Saponins as the *C. ovatum* pulp oil, induces higher larval percentage mortality against *A. aegypti*.

Table 3.2

Correlation between the Mortality Rate (%) of Different Concentrations of Canarium ovatum Pulp Oil against 3rd Instar Aedes aegypti Larvae

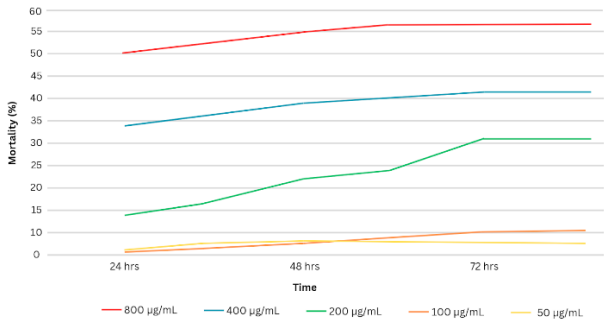
Test of Correlation	r	p
Pearson Correlation	0.894*	<.000

Note. *Significant at .05 level

3.2 Duration of Effect

Figure 3.1.

Cumulative Effect of Different Concentrations of Canarium ovatum Pulp Oil against 3rd Instar Aedes aegypti Larvae for 72 Hours



In addition to the strength of the treatment concentration, the duration of effect or the time of treatment exposure also is a significant contributing factor to the percent mortality induced by *C. ovatum* (See Figure 3.1, Table 3.3). As Table 3.3 presents, the 800 µg/mL concentration was found to have induced higher larval mortalities after longer exposures as its mortality rate reached only 83.33% in the first 24 hours of the treatment. This then increased through the 48 hours into 91.67% and reached its peak mortality rate by the 72-hour period at 93.33%. The same phenomenon is as observed in the other concentrations. The 400 µg/mL concentration mortality rates increased from 56.67% in the first 24 hours to 12.67% by the second 24-hour period, and finally 71.67% by the 72-hour period. Lower concentrations observed the same pattern of results. Similarly, as in the

concentration-based effectivity, the 50 µg/mL concentration induced the lowest mortality rate despite an increase of exposure to the treatment, obtaining only a peak mortality rate of 11.67% within the 72-hour period.

These results are enough to reject the first null hypothesis of the study, as the varying *C. ovatum* pulp oil concentrations generated significant differences in efficacy with respect to their induced mortality and time of exposure. Therefore, the effectiveness of *C. ovatum* pulp oil at higher concentrations lead to higher mortality rates of *A. aegypti* larvae over time. The found relationship is similar to the findings of Hayatie et al. (2015) on *C. papaya* pulp which contains similar photochemicals as C. ovatum including Tannins, Alkaloids, and Flavonoids.

Table 3.4

Cumulative Effect of Different Concentrations of Canarium ovatum Pulp Oil against 3rd Instar aedes aegypti Larvae for 72 Hours

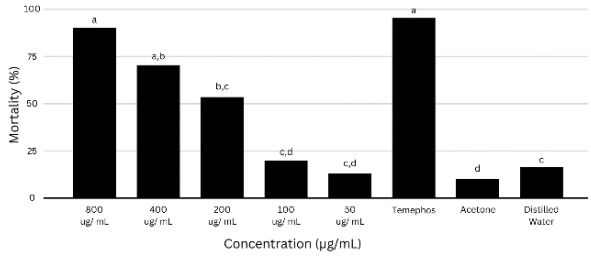
	24 Hours		48 Hours		72 Hours	
Concentrations	*M*	*%*	*M*	*%*	*M*	*%*
800 mg/mL	16.67	83.33	18.33	91.67	18.67	93.33
400 mg/mL	11.33	56.67	12.67	63.33	14.33	71.67
200 mg/mL	4.67	23.33	7.67	38.33	10.33	51.67
100 mg/mL	0.67	3.33	2.00	10.00	3.67	18.33
50 mg/mL	1.33	6.67	2.33	11.67	2.33	11.67

3.3. Comparison between the larvicidal efficacy of different C. ovatum pulp oil concentrations, distilled water, and Temephos

The results of the One-Way ANOVA showed that there is enough evidence to prove that there is a significant difference among the larvicidal effects of the varying test concentrations. This is proven by the <.000 p-value which is less than 0.05 p-value level of significance. The data subjected under the Tukey's Test on the 400 µg/mL revealed that the concentration has a highly significant difference in larvicidal efficacy as compared to the lower 50 µg/mL and 100 µg/mL concentrations. In the case of lower 200 µg/mL from 50 µg/mL to 100 µg/mL, there are no significant results. Acetone and distilled water have significant differences among *C. ovatum* pulp concentration. This supports the idea that there is a significant number of mortalities induced by the test concentration apart from the natural mortalities which occurred in the natural environment, as presented by the negative control distilled water, and the concentration of the diluting solution Acetone to distilled water.

Figure 3.2

Comparison between the Larvicidal Effect of the Canarium ovatum Pulp and Commercial Larvicide Based on Mortality Rate



Lastly, the data subjected to One-Way ANOVA showed that there is no significant difference observed among the mortality rates induced by the *C. ovatum* pulp at 800 µg/mL, 400 µg/mL concentration, and the positive control. The mean mortality rates recorded on the 800 µg/mL and the positive control all averaged above 90% at 93.33% and 96.67% respectively.

Thus, the *C. ovatum* test concentration showed similar larvicidal efficacy as the commercial grade larvicide.

3.4. LC_{50} and LC_{90}

Through the Linear Regression Analysis (See Table 3.1), the LC_{50} and LC_{90} values. The LC_{50} was found at 287.869 ppm. Whereas, the LC_{90} value is increased to 639.473 ppm. Therefore, the yielded results reflect that there is a significant increase in the larvicidal toxicity of the *C. ovatum* pulp oil based on mortality percentage as the concentration increases (see Table 3.5).

Table 3.5

Larvicidal Activity of Aedes aegypti using Canarium ovatum Pulp Oil Extract

Time	Treatment	LC_{50} (ppm) with 95% confidence interval	LC_{90} (ppm) with 95% confidence interval	Regression Equation
72 Hours	*Canarium ovatum* pulp oil extract	287.869 (208.278-1147.29)	639.473 (512.148-898.153)	Y = -1.049 + 0.004 X

Therefore, the proven larvicidal efficacy of the *C. ovatum* pulp oil at its most effective concentration does not make it a viable substitute to the commercial grade larvicide. However, due to the significant efficacy of the *C. ovatum* pulp oil in relation to the larval mortality induced by the commercial brand, it may be used as an effective natural-based alternative to the currently used commercial grade chemical-based larvicides.

The results generated by this study proves the *C. ovatum* pulp oil's suspected larvicidal efficacy grounded on its phytochemical compositions of Phenolics, Flavonoids, Tannins, Alkaloids, Cardiac glycosides, Terpenoids, and Saponins as previously provided by Recuenco et al. (2020). This is further supported by the larvicidal studies of Waris et al. (2020), Hayatie et al. (2015), and Ojha and Pattabhiramaiah (2013) which initially proved the larvicidal efficacy various plants including Ricinus communis (castor) leaf, Carica papaya seed and peel, and Jathropa curcas seed, respectively. These are effective natural bases with similar phytochemical components as the *C. ovatum* pulp oil.

The currently identified bioactive compound composition of the *C. ovatum* pulp oil is also a precursor of its larvicidal efficacy. As mentioned by Pham and Dumandan (2020), it contains stigmasterol, campesterol, and triterpenoids (Kagaoan et al., 2022) that were all found to have significant larvicidal inhibition and toxicity as supported by the studies of Gade et al. (2016) and de Silva et al. (2015). As such, this research corresponds and adheres to the results of the aforementioned larvicidal studies and has achieved the fullness of its research objectives.

Given the current lack of literature on the potentials of the C. ovatum and the current existence of insecticide resistance among the A. aegypti population, this study therefore fills the gap of knowledge on the larvicidal efficacy of the *C. ovatum* pulp. This study therefore concludes that *C. ovatum* pulp oil is an effective natural-based larvicide against *A. aegypti* larvae.

4. CONCLUSIONS

The research aimed to determine the efficacy of *Canarium ovatum* pulp fixed oil extract as a natural larvicide against *Aedes aegypti*. The results showed that the oil extract exhibited a positive larvicidal effect on *A. aegypti* larvae, dependent on the amount of treatment concentration and time of exposure.

The yielded LC50 value was found to be at 287.869 ppm, while LC90 is at 639.473 ppm. The larvicidal efficacy of the different concentrations was also found to increase along the length of exposure to the treatment. Among all test concentrations, 800 µg/mL was considered the most effective, inducing a mortality rate of 93.33%. Whereas, 50 µg/mL was considered the least at 11.67% mortality.

With *A. aegypti*'s possible insecticide resistance against major insecticide components (Zulfa et al., 2022), the data from this research supported the idea that the *Canarium ovatum* pulp oil is a natural-based larvicide against *A. aegypti* larvae, exhibiting a positive correlation with the mortality rate. In conclusion, this study suggests that the fixed oil extracted from the pulp of *C. ovatum* (Pili tree) has the potential to be a better naturally-derived larvicide alternative for commercial-grade larvicide against *A. aegypti*.

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