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Larvicidal Activity of Papaya (Carica papaya) and Madre de Cacao (Gliricidia sepium) Leaf Extracts Against Aedes aegypti

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Abstract— Resistance to chemical insecticides and the harm these insecticides pose to the target, and non-target organisms, including humans and the environment, have been considered obstacle in vector control. This motivated researchers to focus their attention towards natural products of plant origin with insecticidal properties for control of insect pests and vectors. This study aimed to determine the independent larvicidal activity of two plant species, Carica papaya, and Gliricidia sepium, and the combined effect of these plant extracts against the dengue vector, Aedes aegyti lavae.The researchers prepared three extracts: Carica papaya, Glaricidia sepium, and the combination of the two. These extracts were set up in four concentrations/doses namely; 25mg/mL, 50mg/mL. 75mg/mL and 100mg/mL. Mosquito larvae bioassay determined the percentage of mortality. Results of the experiment revealed that the Carica papaya and Glaricidia sepium contain saponins, alkaloids, and tannins. Likewise, the two plant extracts and their combined extract exhibited variable efficacy. Carica papaya and Glaricidia sepium extracts were efficient at 100mg/mL concentration while the combined extract of the two was effective at 75mg/mL and very effective at 100mg/mL. Furthermore, a significant difference exists between and among the different treatments. Finally, the combined plant extracts exhibited the best mortality effect killing 50% of the larvae at 35.48mg/mL concentration in 24 hours and 5.13mg/mL concentration in 48 hours.

Keyword: larvicidal activity, Carica papaya, Glaricidia sepium, Leaf extract, Aedes aegypti

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INTRODUCTION

Dengue fever/dengue hemorrhagic fever is a dreadful disease or infection, which is common in the sub-tropical and tropical countries. Any of the four closely related but genetically distinct arbovirus (DENV-1, DENV-2. DENV-3, and DENV-4) caused the disease and transmitted between people by mosquitoes belonging to the species Aedes aegypti and Aedes albopictus.

Arboviral infections are responsible for millions of diseases every year, most of these are due to dengue with its severe and often fatal syndromes of dengue hemorrhagic fever and dengue shock syndromes (DHF/DSS) which is a problem in more than 100 countries (WHO, 1996).

The World Health Organization estimates 50-100M infections occur annually including, 500,000 dengue hemorrhagic fever (DHF) cases and 22,000 deaths, mostly among children. The WHO reports that the year 2015 was characterized by large dengue outbreaks worldwide, with the Philippines listing more than 169,000 cases, representing a 59.5% increase in case numbers compared to the previous year. This information makes dengue fever/dengue hemorrhagic fever the most significant viral vector-borne disease affecting humans at this present time. However, despite high figures in mortality, the infection/disease is preventable and curable if treated promptly and adequately.

Although the Philippines was one of the first countries where the newly developed dengue vaccine (Dengvaxia) was made available, its use has been put on halt by the current Department of Health Secretary Pauline Ubial. Secretary Ubial earlier announced she was subjecting Sanofi's anti-dengue vaccine, Dengvaxia, to further medical review to make sure it is safe for use among children. The Department of Health (DOH) noted many side effects when Sanofi introduced the vaccine in the Philippines. Thus, Senator Dick Gordon in one of his past privilege speeches in the House of Senate expressed with a strong conviction that the immediate procurement of the vaccines had turned the Philippines into the "number one guinea pig in Asia" and exposed Filipino schoolchildren to danger.

Thus, as of now, the control of the transmission of the dengue virus depends solely on the management of the vector mosquitoes (Gosh, et al. 2012). The most common method used by the public to control mosquitoes is through the use of pesticides/insecticides. Pesticides are synthetic chemical solutions that can destroy the growth and development of the different stages in the life cycle or kill the adult mosquitoes. However, pesticides are dangerous to health. They are known to be carcinogenic. Pesticides also leave toxic residues in food products and are not biodegradable (Remia et al. 2010). In short, pesticides both affect the target and non-target organisms, including humans and the environment (Pavel, 2007).

Carica papaya Linn. is a tall (6-20 feet), and slender plant with a single trunk. Papaya, as it is commonly known, has its origin in tropical America and has propagated to different tropical countries. Although some literatures consider it as a tree, the Hortus Third (dictionary of plants) identifies it as a giant herb because it does not produce a woody tissue. An umbrella-like canopy of lobed leaves topped the trunk. Common folks prepared papaya leaves into a tea as a treatment for malaria, but the mechanism is not understood well, and the treatment method is not proven scientifically.

Gliricidia sepium (Madre de cacao), belonging to the family Fabaceae, grows throughout the Philippines and other tropical countries. It is a medium-sized tree that grows 10-12 meters high. It has composite leaves that can be 30 cm long. Flowers are light pink to lilac colors with a white tinge, and are located

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at the end of the branches. It has a pod-bearing fruit of about 10-15 cm long, which is green when unripe and becomes yellow-brown when it reaches maturity. Each pod produces 4 to 10 brown seeds. In the Philippines, Glaricidia sepium is used mainly for live fencing, fodders, firewood, green manure, intercropping, rat poison, and insect-repellant. Crushed leaves are pasted on the body of livestocks to ward off animal flies, and leaf extract is used to remove external parasites.

This present study was conducted to determine the biological activities of Carica papaya, and Gliricidia sepium against the dengue vector, Aedes aegypti. The result would be useful in promoting the conduct of more studies aiming at the development of new agents for mosquito control based on bioactive compounds from indigenous plant sources.

Materials and Methods

Experimental Design and Treatment

This study utilized the experimental method of research. It consisted of six treatments (Negative control which is distilled water, 25mg/mL, 50mg/mL, 75mg/mL, 100mg/mL and positive control which is Malathion solution) with three replications that were arranged in a parallel group design. Thus, 54 petridishes were used and a total of 10 larvae for each treatment per replication or a total of 540 larvae for the entire experiment.

Collection of Plant and Storage of the Extract

The Barang-ay Demo Farm in Labnig, San Juan, Ilocos Sur provided the leaves of Gliricidia sepium and Carica papaya. The resident botanist of the Bureau of Plant Industry, Department of Agriculture, Manila, authenticated the leaves.

The mature leaves of the plants were washed clean with tap water separately, allowed to dry in the shade at room temperature for at least five days and pulverized manually using the hands.

Extraction of the pulverized leaves

The College of Pharmacy, Centro Escolar University, Manila was the venue of the leaf extraction. Three hundred fifty (350) grams of powdered samples of each leaf was placed in the soxhlet chamber along with cotton on both upper and lower parts of the powder. The extractant used was analytical grade, 95% Ethanol. After extraction, the extract was concentrated by evaporating the 95% Ethanol using a Rotavap for 30 minutes until the extract became pasty in form. The resulting pasty extract was dehydrated through evaporation in the oven maintained at 500C. Carica papaya weighed 23.5g while Gliricidia sepium weighed 27.8g. They were stored in air tight desiccators and used in the experiment.

Phytochemical screening of plant extracts

Identification of the active components of the plant extracts was limited to the qualitative screening tests. The Natural Sciences Research Unit of the Saint Louis University in Baguio City performed the phytochemical screening test. Evaluation of the major phytochemicals like alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenolic compounds, flavonoids, and proteins was done following the standard procedure as described by Harborne (1998).

Culture of experimental organisms

The Department of Entemology, College of Agriculture, University of the Philippines, Los Banos, Laguna provided the eggs of the Aedes spp. To culture the mosquito eggs, two wide-mouthed wash basins were filled with unchlorinated water. Mosquito net sealed the opening of the containers. The water was allowed to "age" for three days. After the aging process, the filter papers containing the mosquito eggs were immersed in wide-mouthed containers. The containers were resealed to prevent mosquitoes from entering. The preparation was allowed to stand undisturbed until the eggs were transformed to larvae. Dog biscuit and yeast power in 3:1 ratio were sprinkled on the water to provide nourishment to the growing larvae. The environmental condition was maintained at 25 – 300C and 70 - 90% humidity. The IV Instar larvae were used in the experiment. They were identified by their age and morphological characteristics.

Confirmation of the Aedes aegypti Larvae

A compound microscope confirmed the identity of the larvae of the Aedes aegypti mosquitoes. One drop of water with a larva was gently placed at the middle of a clean glass slide and examined under the microscope for its morphological characteristics.

There are three main parts of the larval body: head, thorax, and abdomen. Morphologically, the larva has no legs, and the thorax is wider than the head and abdomen. Moreover, it has a single hair, a three-branch hair tufts on each side of the air tube. When the hair tuft has 2 or more branches, all branches arise from the same socket. Other species have 2 or more hairs, branches and hair tufts on each side of the air tube or siphon.

Bioassay

The experiment utilized six petridishes labelled with the different treatments (T1, T2, T3, T4, T5, and T6). Fifteen mL of the prepared solutions were transferred to the respective petridishes: 15mL distilled water to T1, 15mL of 25mg/mL extract to T2, 15mL of 50mg/mL extract to T3, 15mL of the 75mg/mL extract to T4, 15mL of the 100mg/mL extract to T5, and 15mL of the Malathion solution to T6. The researchers arranged the petridishes in a sequential series. The researchers introduced ten larvae in each petridish of both plant extracts and the combination of the two. The larvae were observed for 24-48 hours at room temperature (28-320C) for any changes in their behavior. Dead larvae were removed from the treatment solutions immediately after death to prevent de-

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composition that may alter the composition of the solutions.

The researchers monitored the effects of the plant extracts by counting the number of dead larvae after 24 hours of treatment, and the percentage mortality was computed.

Statistical Tools in the treatment of Data

Arithmetic mean was used to compute the average number of dead mosquito larvae. Analysis of variance (ANOVA) determined if significant difference exists on the mortality of mosquito larvae between the control and the experimental groups. Pairwise comparisons determined where the significant effects/differences are, and Probit analysis determine the lethal concentration of the plant extracts where 50% (LC50) of the Aedes aegypti mosquito larvae died after 24 and 48 hours of treatment.

Furthermore, to describe the level of effectiveness of the extract preparations, the following qualitative description was likewise utilized:

Table 1
The level of effectiveness of the leaf extracts based on percentage mortality

Descriptions	The average number of mosquito larvae died within 24 hours
Not effective	0.0-2.0
Less effective	2.1-4.0
Moderately effective	4.1-6.0
Effective	6.1-8.0
Very Effective	8.1-10.0

RESULTS AND DISCUSSION

Phytochemical Screening

The result of the phytochemical screening of the plants is presented in Table 2.

Table 2
Qualitative Phytochemical Analysis Result of the Two Plant Specimens

Constituents Detected	*Carica papaya*	*Gliricidia sepium*
Alkaloids	(+)	(+)
Tannins	(+)	(+)
Saponins	(-)	(+)
Carbohydrates	(+)	(+)
Glycosides	(-)	(-)
Phytosterols	(+)	(+)
Phenolic compounds	(+)	(+)
Flavonoids	(-)	(+)
Proteins	(+)	(-)

**Tests were done at the Natural Sciences Research Unit, Saint Louis University.

Table 2 shows the phytochemicals present in the ethanolic extact of Carica papaya and Gliricidia sepium. Common to both extracts were alkaloids, tannins, carbohydrates, phytosterols, and phenolic compounds. Proteins were specific to C. papaya while saponin and flavonoids to G. sepium. Both extracts lacked glycosides.

According to Howard (2007), phytochemicals serve as a massive reservoir of compounds that have biological actions. Alkaloids, saponins, and tannins are known to possess pesticidal properties (Azmathullah, 2011). Likewise, the three phytochemicals mentioned, plus flavonoids are not only insecticidal but toxic to other animals as well (Nweze, 2004; Akinyemi, 2005).

The study conducted by Bagavan et al. (2008) revealed that saponins isolated from Achyranthes aspera have a high larvicidal effect against Aedes aegypti and Culex quinquefasciatus. Moreover, Kotkar et al. (2002) in their study on Annona squamosa, revealed that flavonoids isolated from the aqueous extract of the plant are effective in killing 80% of the tested Culex chinensis.

Larvicidal Efficacy of the Plant Extracts

Efficacy after 24 hours of exposure

Table 3
Mean and percentage mortality of Aedes aegypti mosquito larvae in the control and experimental groups after 24 hours of exposure

Extract	Preparation	Ave no. of mosquito larvae used	Ave. no. of mosquito larvae died	% Mortality	Level of Effectiveness
Carica papaya	T1 - 0mg/mL	10	0	0	Not Effective
	T2 - 25mg/mL	10	0	0	Not Effective
	T3 - 50mg/mL	10	2.55	25.56	Less Effective
	T4 - 75mg/mL	10	4.22	42.22	Moderately Effective
	T5 - 100mg/mL	10	7.22	72.22	Effective
	T6 – Malathion	10	10	100	Very Effective
Gliricidia sepium	T1 - 0mg/mL	10	0	0	Not Effective
	T2 - 25mg/mL	10	0.77	7.78	Not Effective
	T3 - 50mg/mL	10	3.0	30	Less Effective
	T4 - 75mg/mL	10	4.88	48.89	Moderately Effective
	T5 - 100mg/mL	10	7.55	75.56	Effective
	T6 – Malathion	10	10	100	Very Effective
Combined *Carica papaya-Gliricidia sepium* extract	T1 - 0mg/mL	10	0	0	Not Effective
	T2 - 25mg/mL	10	3.66	36.67	Less Effective
	T3 - 50mg/mL	10	6.0	60	Moderately effective
	T4 - 75mg/mL	10	7.88	78.89	Effective
	T5 - 100mg/mL	10	9.66	96.67	Very Effective
	T6 – Malathion	10	10	100	Very Effective

The larvicidal efficacy of the ethanolic extracts

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of Carica papaya and Glaricidia sepium against the larvae of the dengue vector, Aedes aegypti was determined through larval bioassay. The observed the mean and percent mortality of the Aedes aegypti larvae in 25mg/mL, 50mg/mL, 75mg/mL and 100mg/mL after 24 hours and 48 hours of treatment.

Table 3 presents the mean and percentage mortality as well as the level of the level of effectiveness of the extracts in killing the Aedes aegypti larvae. The researchers observed the variations in the mean and percentage mortality among the different concentrations of the plant extracts. The 25mg/mL (25%) concentration exhibited the least percentage mortality while the 100mg/mL (100%) concentration manifested the highest percentage of mortality. This result shows that the three extract preparations exhibited a concentration-dependent activity against mosquito larvae. This finding further implies that as the concentration of the extract increases, the percentage mortality of the larvae also increases. This data agree well with the study of Gutierrez et al. (2014).

The Carica papaya extract was effective only at 100mg/mL (100%) with percentage mortality equal to 72% of the mosquito larvae. The Glaricidia sepium, on the other hand, was effective also at the same concentration but the percentage mortality was higher than the papaya extract with % mortality equal to 75.56% of the mosquito larvae. When combined, the extract became effective even at 75mg/mL or 75% concentration with percentage mortality equal to 78.89% of the mosquito larvae. The 100mg/mL or 100% concentration of the combined extract recorded a very effective activity with a mortality rate of 96.67% of the mosquito larvae.

The percentage mortality effect of each concentration of the different extracts after 24 hours is illustrated better in the bar graph below.

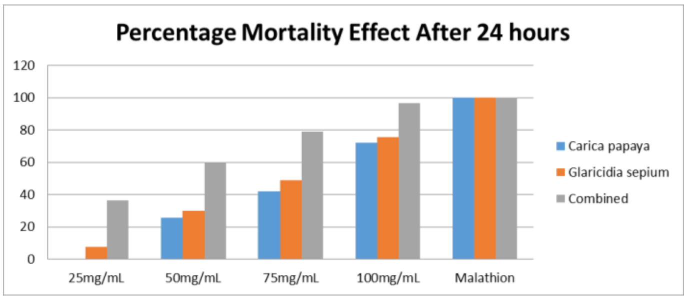

Figure 1. % Mortality of Aedes aegypti larvae after treatment with the different extract concentrations after 24 hours.

Efficacy after 48 hours of Exposure

Table 4
Mean and percentage mortality of Aedes aegypti mosquito larvae in the control and experimental groups after 48 hours of exposure

Extract	Preparation	Ave no. of mosquito larvae used	Ave. no. of mosquito larvae died	% Mortality
Carica papaya	T1 - 0mg/mL	10	0	0
	T2 - 25mg/mL	10	6.66	66.6
	T3 - 50mg/mL	10	7.66	76.6
	T4 - 75mg/mL	10	9.33	93.3
	T5 - 100mg/mL	10	9.77	97.7
	T6 – Malathion	10	10	100
Gliricidia sepium	T1 - 0mg/mL	10	0	0
	T2 - 25mg/mL	10	7.33	73.3
	T3 - 50mg/mL	10	8.55	85.5
	T4 - 75mg/mL	10	9.66	96.6
	T5 - 100mg/mL	10	10	100
	T6 – Malathion	10	10	100
Combined Carica papaya-Gliricidia sepium extract	T1 - 0mg/mL	10	0	0
	T2 - 25mg/mL	10	8.55	85.5
	T3 - 50mg/mL	10	9.44	94.4
	T4 - 75mg/mL	10	10	100
	T5 - 100mg/mL	10	10	100
	T6 – Malathion	10	10	100

Table 4 shows the average and percentage mortality of Aedes aegypti mosquito larvae treated with the various concentrations of the plant extract and the control groups after 48 hours. It revealed that the least percentage mortality was observed in the 25mg/mL Carica papaya extract (66.6%) while the highest percentage mortality was noted in the 100mg/mL of the combined plant extracts. The 75mg/mL of the combined extract and the 100mg/mL concentrations of the three experimental extracts were comparable to the effect of the Malathion (positive control). This finding means that the 100mg/mL Carica papaya extract, the 100mg/mL Glaricidia sepium extract, 75mg/mL combined extract and the 100mg/mL combined extract were as effective as the Malathion after 48 hours of treatment.

The presence of the phytochemicals, Alkaloids, Tannins, Saponins, and Flavonoids, which are known to have pesticidal properties have caused the high larvicidal activity of the three extracts. All four (4) phytochemicals were isolated from the Glaricidia sepium, and this may have caused its very potent larvicidal activity. The absence of saponin may have likewise caused the Carica papaya to have a lesser larvicidal effect than Glaricidia sepium. The synergistic effects of the two extracts may have caused their very efficient larvicidal activity against the mosquito larvae. As N'dung-u (2014) rightly stated that plants with larvicidal activity could act in combination or independently.

The percentage mortality effect of each concentration of the different extracts after 48 hours is illustrated better in the bar graph below.

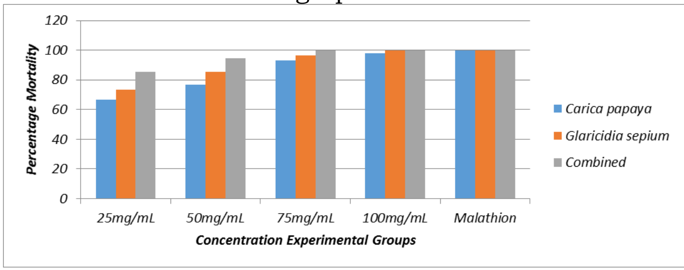

Figure 2. % Mortality of Aedes aegypti larvae after treatment with the different extract concentrations after 48 hours

Table 5
Tests of Within-Subjects Effects

Source	F-computed	P-value	Partial eta squared (n_p^2)	Implication of the n_p^2
Time	2229.044	0.000	0.984	0.14 & > = great effects
Time*extract	70.911	0.000	0.798	0.06-0.13 = medium effects
Time*treatments	319.501	0.000	0.978	0.01-0.05 = little effects
Time*extract*treatments	8.533	0.000	0.703	

** Tests done at 0.05 level of significance.

The researchers performed test within subject effect (Table 5) to show which of the independent variables (time, extract, treatment concentrations) caused the death of the larvae.

Table 5 reveals that time has significant effect on percentage mortality. This finding means that if the other variables were ignored, the percentage mortality significantly differs between 24 hours and 48 hours of measurement periods. Likewise, there is significant interaction between time and extract, meaning that the type of extract had a significant effect on the percentage mortality overtime. Similarly, there is a significant interaction effect between time and treatments. This means that the level of treatment had a significant effect on percentage mortality overtime. Lastly, there is a significant interaction effect between time, extract, and treatment.

The researchers used the Cohen's guideline to describe the magnitude of effect or the measure of effect of the variable in the interaction. The result shows that all variables in the interactions produced great effects.

Table 6
Pairwise comparison on the mortality of Aedes aegypti mosquito larvae treated on the various concentrations of the plant extracts and the control group after 24 hours of exposure

Variables compared	P-values		
	Carica papaya	*Gliricidia sepium*	Combined *C. papaya-G. sepium* extract
Malathion vs. 25mg/mL	0.000*	0.000*	0.000*
Malathion vs. 50mg/mL	0.000*	0.000*	0.000*
Malathion vs. 75mg/mL	0.000*	0.000*	0.000*
Malathion vs. 100mg/mL	0.000*	0.000*	1.000
25mg/mL vs. 50mg/mL	0.000*	0.000*	0.000*
25mg/mL vs. 75mg/mL	0.000*	0.000*	0.000*
25mg/mL vs. 100mg/mL	0.000*	0.000*	0.000*
50mg/mL vs. 75mg/mL	0.000*	0.000*	0.000*
50mg/mL vs. 100mg/mL	0.000*	0.000*	0.000*
75mg/ml vs. 100mg/mL	0.000*	0.000*	0.000*

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

Table 6 presents the Pairwise comparison on the mortality of Aedes aegypti mosquito larvae treated on the various concentrations of the plant extract and the control group after 24 hours of exposure. It shows that significant differences exist between and among the different treatments in each of the three extracts. This finding means that as the concentration increases, the killing effect of the extract also increases. Surprisingly, the malathion solution and the 100% combined C. papaya-G. sepium extract has statistically similar effects, implying that the latter is as effective as the former in terms of their larvicidal activity.

Table 7
Pairwise comparison on the mortality of Aedes aegypti mosquito larvae treated on the various concentrations of the plant extracts and the control group after 48 hours of exposure

Variables compared	P-values		
	Carica papaya	*Gliricidia sepium*	Combined *C. papaya-G. sepium* extract
Malathion vs. 25mg/mL	0.000*	0.000*	0.000*
Malathion vs. 50mg/mL	0.000*	0.000*	0.062
Malathion vs. 75mg/mL	0.012*	1.000	1.000
Malathion vs. 100mg/mL	1.000	1.000	1.000
25mg/mL vs. 50mg/mL	0.000*	0.000*	0.000*

25mg/mL vs. 75mg/mL	0.000*	0.000*	0.000*
25mg/mL vs. 100mg/mL	0.000*	0.000*	0.000*
50mg/mL vs. 75mg/mL	0.000*	0.000*	0.062
50mg/mL vs. 100mg/mL	0.000*	0.000*	0.062
75mg/ml vs. 100mg/mL	1.000	1.000	1.000

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

The table above shows the Pairwise comparison on the mortality of Aedes aegypti mosquito larvae treated on the various concentrations of the plant extract and the control group after 48 hours of exposure. It reveals that significant differences exist between and among the different treatments in each of the three extracts.

For the Carica papaya extract, the 75mg/dL and 100mg/dL are statistically of similar effect while the 100mg/dL is as effective as the malathion solution, but when 75mg/dL is compared to the malathion solution, the latter is still more effective than the former. For the Glaricidia sepium, all three solutions (75mg/dL, 100mg/dL, and Malathion) are statistically of similar effects. However when the two plant extracts are combined, even the 50mg/dL in addition to the 75 and 100mg/dL concentrations are statistically similar in effect and are as effective as the Malathion solution.

Table 8
Lethal concentration (LC50) values of the plant extracts on Aedes aegypti mosquito larvae after 24 and 48 hours treatment

Plant extracts	LC_{50} after 24 hours (mg/mL)	LC_{50} after 48 hours (mg/mL)
Carica papaya leaf extract	75.86	19.95
Glaricidia sepium leaf extract	67.61	14.79
Combined *Carica papaya-Glaricidia sepium* leaf extract	35.48	5.13

Table 8 summarizes the Lethal concentration (LC50) values of the three plant extracts on Aedes aegypti mosquito larvae after 24 and 48 hours. The combined Carica papaya and Glaricidia sepium extract exhibited the lowest LC50 values (35.48mg/mL and 5.13mg/mL) after 24 and 48 hours, respectively. This finding implies that this extract is the most effective in terms of pesticidal activity compared to the two extracts taken individually. On the contrary, the Carica papaya leaf extract reveals the least larvicidal activity with an LC50 of 75.86mg/mL for the 24 hours treatment period and 19.95mg/mL for the 48 hours treatment period. The table further reveals that the lethal concentration significantly decreased in time. This finding means that increasing the treatment period time increases the mosquito larvae mortality.

CONCLUSION

Mosquito control practices are mainly geared towards larvae and seldom against adult mosquitoes, because larvae are restricted in one place and are still very susceptible to larvicides. On the contrary, adults are transient, highly resistant, thus requiring more concentrated pesticide, which in turn could cause environmental pollution.

Results of this present study on the larvicidal property of two plant extracts and their combined extract revealed variable efficacy. Their difference has something to do with the plant extract used, the concentration of the extract, and the time of exposure. Likewise the presence of secondary metabolites like saponins, alkaloids, and tannins, all of which have known insecticidal properties add more to the killing effect of the extracts. The mechanism of action of these bioactive agents is by disrupting the cuticle membrane of the larvae causing an irreparable damage and eventually causing death.

The three extract samples exhibited larvicidal activity against Aedes aegypti mosquito larvae. This fact is manifested by a high percentage mortality rate compared to the control group. Of the three, the combined extract manifested the highest percentage mortality in both the 24 and 48 hours exposure time. Likewise, it is the most lethal, killing 50% of the larvae at 35.48mg/mL concentration for 24 hours and 5.13mg/mL concentration for 48 hours.

RECOMMENDATIONS

The researchers recommend the following:
1. Carica papaya leaf extract and Glaricidia sepium leaf extract may be used as larvicidal agents at 100% concentration, while the combined extracts may be used at 75% concentration.
2. A preservative may be added to the extracts to lengthen their shelf life.
3. The plant extracts may be combined at different concentrations to determine the most efficacious combination.

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