Larvicidal Effect of *Carica papaya* (L.) Leaf Extract Against *Aedes aegypti* (Linnaeus,1762) Larvae Under Laboratory Condition

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Abstract

Dengue fever is the one danger disease caused by dengue virus which is infected through species of mosquito *Aedes aegypti*. Chemical insecticides has been considered to the development of the resistance in the mosquito species as the impact in vector control. This study aims to determine the ability of papaya extract in controlling the *Aedes aegypti* mosquito. Extraction of *Carica papaya* was conducted at laboratory of Zoology Department, Dagon University at East Dagon Township, Yangon region. This study carried out from January, 2019 to September, 2019. The effects of larvae have been investigated in 4thinstar for 48 hours with four different concentration 100ppm, 200ppm, 300ppm and 400ppm. Larval mortality and lethal concentration of larvae was investigated. Larvae of 4th instar that require for experiment reared in laboratory. One gram of plant residue was dissolved in 5ml of ethanol mixed well then, it was dissolved in 95ml of the distilled water. It was to be 1% of stock solution. Larval mortality was calculated by using Abbott's formula. Lethal concentration (LC_{50}) was determined by using profit analysis by linear regression equation between log concentration (log base 10) and probit mortality using SPSS 23.0 software. The highest larval mortality (100%) of the ethanol leaf extract of *Carica papaya* against the 4th instars larvae were reached in four different concentrations (100,200,300,400 ppm) for 24 hours. The bioassay showed that LC_{50} of Papaya (*Carica papaya*) leaf has an effect to an *Aedes aegypti* LC_{50} of 500.997 ppm and 232.293 ppm for 3 hours and 6 hours respectively. The chi-square values were significant at 5% level.

Keywords: *Carica papaya*, *Aedes aegypti* (larva), Larvicides

INTRODUCTION

Mosquitoes are one type of insects belonging to the Order Diptera and Family Culicidae (Kardinan, 2003). Mosquitoes have two scaly wings, a slender body, and six legs. Mosquitoes has proboscis, both male and female have different function. In male mosquito proboscis has function to feed nectar, meanwhile female mosquito used proboscis to pierce and suck the blood to provide nutrient for egg (Natadisastra, 2005).Mosquitoes constitute a major public health problem as vector of serious human diseases (Hag *et al*., 1999). Comprising approximately 3,500 species of mosquitoes are grouped into 41 genera and found beyond the tropical and sub region of the world with they are classically associated. Several species belong the *Aedes*, *Anopheles* and *Culex* vectors for the pathogen of various diseases like *Anopheles*-malaria, *Aedes*-yellow fever, dengue, chikungunya and *Culex*-West Nile, Japanese encephalitis filariasis (White, 2004).

Mosquitoes have a holometabolous type of development; that is, having four distinct stages in their life cycle: egg, larva, pupa, and adult. Larvae and pupae of mosquitoes require an environment with standing or flowing water for proper development. The female adult lays either single eggs (e.g., *Aedes, Anopheles*) or in clusters (e.g., Culex, Culiseta), up to several hundred at a time, on the surface of the water, on the upper surface of floating vegetation, along the margins of quiet water pools, on the walls of artificial containers or in moist habitat subject to flooding. The larvae (called wrigglers) undergo shedding (or molting) of the skin four times before becoming pupa. Larvae of most species usually filter out and feed on organic matter and other microorganisms, in the water for about 1–3 weeks or longer depending on the water temperature. In some predatory species, the first instar is a filter feeder, and the predaceous feeding structures are not developed until the second instar. The pupae (called tumblers), or resting stage, appear after the fourth larval molt. Unlike larvae,

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pupae do not feed, and live for 1–3 days before becoming adults. Only adult female mosquitoes bite humans and animals. Male mosquitoes feed primarily on flower nectars, while the females require the blood meal to produce viable eggs (Harris *et al*., 1969).

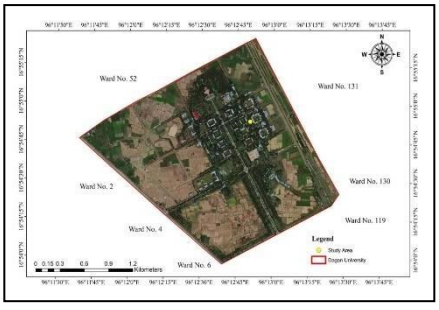
Carica papaya, belongs to the family of Caricaceae, and several species of Caricaceae have been used as remedy against a variety of diseases (Mello *et al.,* 2008).Many scientific investigations have been conducted to evaluate the biological activities of various parts of *C. papaya*, including fruits, shoots, leaves, rinds, seeds, roots or latex. The leaves of papaya have been shown to contain many active components that can increase the total antioxidant power in blood and reduce lipid peroxidation level, such as papain, chymopapain, cystatin, tocopherol, ascorbic acid, flavonoids, cyanogenicglucosides and glucosinolates (Seigler *et al*., 2002).Insecticidal activity from content of secondary metabolites in the leaves and seeds of papaya its nature is toxic such as saponin, flavonoid and triterpenoid. Saponin is a toxin that is polar, soluble in water, and when it enters the body in larvae can result in hemolysis in the blood vessels. Flavonoids works as a stomach poison that lowers appetite larvae because larvae fail to recognize food stimulus, so that over time the larvae will die of starvation. Triterpenes is acute toxic compounds. Triterpenoid causing reduced feeding and increased mortality. The use of leaf extracts and papaya seed extract as larvicides relatively safer for the environment because it is a natural substance and its nature is not toxic, but extract of papaya leaf into a body of water will affect the color and flavor (Wahyuni, 2015).

Larvicide activity measured by larval mortality. In this study, *Aedes aegypti* larvicidal activity was investigated using leaf of papaya (*Carica papaya*) and fourth instar larvae. Larvae of 4thinstar that require for experiment were reared in laboratory of Zoology department, Dagon University atEast Dagon Township, Yangon Region. The objectives of the present study:to obtain the larvae of equal developmental stage (fourth instar) for using the larvicidal activity test in laboratory,to analyze the effect of papaya leaf extracts as larvicide against the *Aedes aegypti* larvae (fourth instar) and to determine the lethal concentration (LC_{50}) and lethal time of larvicide (bioinsecticide) on mosquito larval instars .

MATERIALS AND METHODS

Study site

The present experiment was conducted at laboratory of Zoology Department, Dagon University, East Dagon Township, and situated at Yangon region, located between 16˚54' 35" N Latitude and 96˚12' 30" E Longitude (Plate 1)



Source : Geography department, 2019
16˚54'35" N / 96˚ 12' 30" E

Figure. 1 Map showing the study area of Dagon University at East Dagon Township

Study period

The study period lasted from December, 2018 to September, 2019.

Study design

Collection and identification of reared mosquito's species, rearing of mosquitoes in study sites, preparation of larvicide extract from *Carica papaya* leaf, testing with the different concentration and different times of 4th instar larvae of *Aedes aegypti* and analyzing the recorded data were conducted in study period.

Collection and Identification of mosquitos' species

Mosquito's larvae were collected from the environment of Shwe Pauk Kan Township at Yangon region. The collected larvae were brought to the laboratory of Zoology Department, Dagon University, East Dagon Township, and Yangon Region. Then, the larvae were reared until to reach the adult stages. The emerged adults were identified by Rattanarithikul and Panthusiri (1994) to certain the *Aedes aegypti* species. Pairs of *Aedes aegypti* were reared until when they laid eggs.

Maintenance of larvae

The eggs of *Aedes aegypti* were placed in 60 cm ×3cm glass Petri dish containing water for hatching.The emerged larvae were maintained in 500 ml of beaker with of tap water at 27 °C in the laboratory. The larvae were fed with a diet of dried beef liver. The feeding was continued till the larvae transformed into the pupae stage. The first instar larvae develop into pupae in about 9-11days through four stages.

Maintenance of pupae and adult

The pupae were transferred to 500 ml of beakers with of tap water with the help of a plastic tube. The pupae containing glass breaker were kept in 45cm × 45 cm × 45 cm size mosquito cage for adult emergences. The newly emerged adult mosquitoes were fed with 10% sugar solution soaked in cotton wool for the period of three days before they were provided an animal for blood feeding. The cotton was always kept moist with the solution and changed every day.

Blood feeding of adult *Aedes aegypti*

After three days of emergence, the adult female were fed on the blood of albino mice for egg laying. The blood feeding was repeated for 3 days, each feeding session lasting 3 hours.100 ml of beaker lined with filter paper containing water was placed at the corner of the cage for gravid female to deposit their eggs. This arranged made collection of eggs easier.

Preparation of leaves powder

Papaya leaves obtained fresh from the environment of Shwe Pauk Kan Township at Yangon region. Then the collected fresh leaves were transferred to the laboratory of Zoology Department of Dagon University located East Dagon Township, and Yangon Region. Then, the collected fresh papaya leaves were washed, cut into pieces and dried at room temperature for 15 days. In making leaf extract, after drying the plant material was powdered with the help of electrical blender. The fine particles were separated and stored in clean container and used for further analysis.

Preparation of ethanol extract

10 g of blender powder was weighed and was soaked in the 100 ml of the ethanol for ethanol solvent. They are kept at room temperature for 168 hours (1 week) to dissolve the

active components of plant leaf materials. Each mixture was stirred every hours using sterile glass rod, then it was filtered through the Whatman. No.1 filter paper the extracting procedure was done twice for complete extraction of the active compounds. Firstly, beaker was weight and the filtered extract solution was pull into the beaker. The beaker with filtrates was put into the aluminum pot containing little water. Then, this aluminum pot was heated slowly by electric stove at room temperature to evaporate the filtered extract solution. After about two hours, the condensed extract were got and stored in another breaker for preparation of stock concentration.

Preparation of stock solution

A stock emulsified water solution of the extract of 1000 ppm was prepared by using 1 gram of plant residue was dissolved in 5 ml of the ethanol mixed well then it was dissolved in 95 ml of the distilled water (Sakthovadivel *et al* ., 2012).It was said to be 1% of stock solution (Jayapal Subramanian *et al*., 2012).

Preparation of working solution

From the 1 % stock solution 4 different concentrations were prepared ranging from 100 ppm, 200 ppm, 300 ppm and 400 ppm respectively.

Larvicidal effect

The immature mosquitoes particularly fourth instar larvae were used for the present study and they fed with a diet of beef liver. The larvae were collected by using a plastic tube into the 500ml of beaker with tap water. The test concentrations were made with the ethanol extract of *Carica papaya* .From the each stock solution, different concentrations were prepared as 100 ppm, 200 ppm, 300 ppm and 400 pm. 20 numbers of 4th instar larvae per concentration were exposed to each dose, the number of dead larvae was recorded for 12hours, 24 hours, 36 hours and 48 hours for two days at room temperature. The larvae considered to dead when they showed no sign of movement *ie,* immobile and unable to reach the water surface. They were disposed with the probed using a needle and mortality data was analyzed by using Abbott's method (Abbott's, 1925). Each concentration had three replicate with appropriate control. The stock control was prepared by mixing 5 ml of ethanol.

$$\text{Percentage Mortality} = \frac{\text{Number of dead Larvae}}{\text{Number of larvae introduced}} \times 100$$

Determination of median lethal concentration

LC_{50} (Lethal concentration 50%) is the concentration of any toxic substance reducing by mortality the number of tested individuals to 50% in a prefixed time (Ravera, 1986). According to Rand and petrocelli (1985) the LC50 (median lethal concentration) is estimated to produce mortality in 50% of a test solution over a specific period of time. Preliminary toxicity tests were carried out to find the median lethal tolerance limit of *Aedes aegypti* larvae to *Carica papaya* for 24h.The value of LC_{50} was calculated by using probit analysis (Finney, 1971). Larval mortality was calculated by using Abbott's formula (Abbott's, 1925). Lethal concentration (LC_{50}) was determined by using profit analysis (Finney, 1971) by linear regression equation between log concentration (log base 10) and probit mortality using SPSS 23.0 software.

A. Mosquito cage

B. Cage with pupa, adults, mice and oviposotion substrates

B. Hatching mosquitoes in Petri dish

Plate 1. Larva collection and rearing of *Aedes aegypti*


A. Papaya dried leaves


B.Powder with electrical blender


C. Papaya leaves powders


D. Powders soaked in ethanol


E. Filtering the solution


F. Heating the solution

Plate 2. Procedure of papaya leaves extract

RESULTS

Developmental stages of *Aedes aegypti*

The developmental stages of *Aedes aegypti* were given in Plate.3.

(a) Egg

A freshly laid egg is single, small, light in color and shiny black within a few hours. It is oval shape and about (0.6 to 0.7 mm) long (Plate 3 .C).

(b) Larvae

Larvae are entirely aquatic holometabolic insects. Larvae emerge from eggs within 2-3 days in 25˚C-27˚C. All larvae go through four developmental stages called instars. The first instar larvae are barely noticeable to the human eye and the other larval instars were easily seen. Last instar was approximately (5.5-6.0 mm) long. The larvae have a well-formed head, thorax, nine segmented abdomens and lack legs. They breathe through an air tube at the rear of their bodies. *Aedes*larvae hang almost vertically to the water surface. The larval stage is completed in 6-12 days (25˚C-27˚C) (Plate.3 D,E,F,G).


A. Adult male


B. Adult female


C. Eggs


D. First instar larva


E. Second instar larva


F. Third instar larva


G. Fourth instar larva

Plate 3. Developmental stages of *Aedes* mosquitoes

Table 1. Biomorphometrics and duration of various larvae stages of *Aedes aegypti* (Linnaeus, 1762)

No	Life stage	Number of observed (n)	Length(mm)			Duration(days)		
			Mini	Maxi	Mean±SD	Mini	Maxi	Mean±SD
1	Egg	30	0.6	0.8	0.67±0.07	2	3	2.67±0.58
2	First instar	30	1.2	2.1	1.76±0.30	1	2	1.67±0.58
3	Second instar	30	2.5	3.5	2.88±0.31	1	3	2±1
4	Third instar	30	4	5.3	4.83±0.36	1	4	2.33±1.53
5	Fourth instar	30	5.5	7	5.98±0.50	2	5	3.67±1.53

Mortality (%) of *Aedes aegypti* fourth instar

The present experiment was made during 24 hours and larval death registration is done by during 3 hours, 6 hours and 24 hours. The potential effect of papaya leaf extract was tested at concentration of 100 ppm, 200 ppm, 300 ppm, and 400 ppm, respectively (Table 2)

In the concentration of 100 ppm of papaya leaf extract, 5% mortality of *Aedes aegypti* larvae was found in the first 3 hours; 20% mortality was found in 6 hours and 100% mortality was occurred in 24 hours. In the concentration of 200 ppm of papaya leaf extract, 30% mortality of *Aedes aegypti* larvae was noted in the first 3 hours; 50% mortality was found in 6 hours and 100% mortality was occurred in 24 hours. In the concentration of 300 ppm of papaya leaf extract, 30% mortality of *Aedes aegypti* larvae was noted in the first 3 hours; 55% mortality was found in 6 hours and 100% mortality was occurred in 24 hours. In the concentration of 400 ppm of papaya leaf extract, 40% mortality of *Aedes aegypti* larvae was noted in the first 3 hours; 70% mortality was found in 6 hours and 100% mortality was occurred in 24 hours.Control was not exposed to concentrations of papaya extract given treatment. In the control group was given 1ml of 70% ethanol solution. There are no dead *Aedes aegypti* larvae in the first 3 hours of control (ethanol solution); 5% of mortality was found in 6 hours and10% of mortality was occurred in 24 hours (Table 2).The peak mortality of *Aedes aegypti* larvae was founded in concentration of 400 ppm of papaya leaf extract, 40% of mortality was noted in the first 3 hours; 70% of mortality was found in 6 hours and 100% of mortality was found in 24 hours. Thus, mortality was increased as the concentration was increased in specific time.

Table 2. Larval death (mortality %) based on dose (ppm) and the experiment hours

Replication			I	II	III	Total number	Average	Mortality (%)
Times interval	Number of Larva (n)		20	20	20	60	20	
3 hours	Concentration (ppm)	Control	0	0	0	0	0	0
		100	0	1	1	2	1	5
		200	6	8	3	17	6	30
		300	6	9	3	18	6	30
		400	7	12	5	24	8	40
6 hours	Concentration (ppm)	Control	0	1	0	1	1	5
		100	3	6	4	13	4	20
		200	10	13	6	29	10	50
		300	10	15	7	32	11	55
		400	16	17	10	43	14	70
24 hours	Concentration (ppm)	Control	0	6	1	7	2	10
		100	20	20	20	60	20	100
		200	20	20	20	60	20	100
		300	20	20	20	60	20	100
		400	20	20	20	60	20	100

Number of death *Aedes aegypti* larvae after exposure papaya leaf extract

In the present experiment, replication is performed three times for each concentration. Larval death registration was done by 3 hours, 6 hours and 24 hours respectively. 20 individuals of *Aedes aegypti* larvae (4th instar) per concentration were used to test experiments.In the first replication, at the controls (ethanol solution) was not found dead *Aedes aegypti* larvae. Papaya leaf extract of 200 ppm and 300 ppm concentration for 6 hours, *A.aegypti* larvae dead reached 10 individuals (50%).In the second replication, at the controls (ethanol solution) *Aedes aegypti* larvae were dead in only one individual for 3 hours while six individuals for 24 hours. Papaya leaf extract of 200 ppm, 300 ppm and 400 ppm concentration for 6 hours, *A. aegypti* larvae dead reached in 13 (65%), 15(75%) and 17(85%) individuals respectively.In the third replication, at the controls (ethanol solution) was not found dead *Aedes aegypti* larvae while only one individual dead for 24 hours. Papaya leaf extract of 400 ppm concentration for 6 hours, *A. aegypti* larvae dead reached 10 individuals(50%).At the concentration of 100 ppm, 200 ppm, 300 ppm and 400 ppm of papaya leaf extract, 20 individuals were dead in 24 hours.

Number of death *Aedes aegypti* larvae after exposure papaya leaf extract for different concentration

In the present experiment, the number of *Aedes aegypti* larvae was average dead in one individual within 3 hours and four individuals within 6 hours in 100 ppm. In 200 ppm concentration, the number of *Aedes aegypti* larvae was average dead in six individual within 3 hours and 10 individuals within 6 hours while six individual within 3 hours and 11 individuals for 300 ppm concentration.In 400 ppm concentration, the number of *Aedes aegypti* larvae was average dead in eight individual within 3 hours and 14 individuals within 6 hours. All of the 20 larvae were dead in the highest concentration of 400 ppm.

Table 3 Number of death *Aedes aegypti* larvae after exposure papaya leaf extract for (100 ppm, 200 ppm, 300 ppm and 400 ppm concentration

Lethal dose	Exposed time	First replication	Second replication	Third replication	Average
(ppm)	(Hour)	No. of dead larvae	No. of dead larvae	No. of dead larvae	No. of dead larvae
100	3 h	0	1	1	1
	6 h	3	6	4	4
	24 h	20	20	20	20
200	3 h	6	8	3	6
	6 h	10	13	6	10
	24 h	20	20	20	20
300	3 h	6	9	3	6
	6 h	10	15	7	11
	24 h	20	20	20	20
400	3 h	7	12	5	8
	6 h	16	17	10	14
	24 h	20	20	20	20

Papaya leaf extracts Potential Test for Larvicides against *Aedes* larvae mortality

Testing the potential of papaya leaf extract at a concentration was 100 ppm, 200 ppm, 300 ppm, and 400 ppm respectively. Value of Lethal Concentration (LC_{50}) of papaya extract on 3 hours test period is at 500.997 ppm dose and value Lethal Concentration (LC_{90}) at 2211.299 ppm ; the regression equation was Y=-5.97+2.23x with a value of R=0.886. While the 6 hours test period, the value of Lethal Concentration (LC_{50}) at 232.293 ppm, and the

value of lethal concentration (LC_{90}) at 924.467 ppm, the regression equation was Y=-5.11+2.16x, and the value of R=0.964.R value indicates a very strong correlation between the *Aedes aegypti* mortality of larvae with papaya extract concentration.The value of Lethal Time (LT_{50}) on the clock to 3.016 h, meaning that the time required killing 50% of larvae of *Aedes aegypti*; is over 3.016 hours. Value Lethal Time (LT_{90}) on the clock to 6.958 h, meaning that the time required to kill 90% of larvae of *Aedes* is over 6.95 h. Base on the probit regression, regression equation Y= -1.64+3.44x, and the value of R= 1. Rated R shows a very strong correlation between mortality of larvae of *Aedes* sp. and papaya extract exposure time.

Table 4. Lethal Concentration and Lethal Time of Papaya leaf Extracts against larvae of *Aedes aegypti*

No.	Period of Bioassay Test (hour)	Lethal Concentration (ppm)		Regression Equation	R value	Lethal time Description
		LC_{50}	LC_{90}			
1	3	500.997	2211.299	Y=5.97+2.23x	0.886	(LT_{50})
2	6	232.293	924.467	Y=5.11+2.16x	0.964	(LT_{90})

DISCUSSION

In the present study, egg, larva, and pupal and adult stage and an entire aquatic cycle of *Aedes aegypti* was completed 8-20 days.Vythilingam *et al*., 1992 similarly recorded that the life cycle takes about 9 to 10 days to complete.Their finding was slightly different with the present study. It may be due to the environmental factors, such as, temperature, moisture and food.In the present study, the female mosquito of *Aedes aegypti* lay the eggs approximately 3 days subsequent to feeding on blood. Females produce 100-140 eggs per batch and about 5 batches of eggs for the duration of her lifetime.Vythilingam *et al*., 1992, recorded that *A. aegypti* is able to lay about 102 eggs per female.Their finding was slightly different with the present study. It may be the size of the blood meal which determines the quantity of eggs produced (Nelson, 1986).In the present study, the duration of the eggs developed from two to three days (25-27˚ C).The eggs are white but very rapidly turn to shiny black. Embryonic development is usually completed within 48 hours if the environment is humid and warns, but it may take up to 5 days at lower temperatures (Nelson, 1986).

In the present study, the larvae pass through four instar, spending time of the developing period was taken about 5-14 days.After a day or two feeding and growth, moulting occurs and the second instar emerges. The first three instars develop quickly, while the fourth instar takes longer and increases more in size and weight.Under optimal conditions, the time from hatching to pupation can be as short as 5 days but more commonly lasts 7-14 days (Nelson, 1986). This observation was similar to the present study. *A.aegypti* adults can stay alive for months in the laboratory but they usually live only a few weeks in nature. Many adults die at the time of emergence or soon after. (Nelson, 1986). The present study was conducted to test the potential of the extracts from the leaves of papaya(Carica papaya) as larvicides against Aedes aegypti larvae mortality. Testing was conducted larvicides against of *A. aegypti* larvae instar IV. Experiments were made during 24 hours and larval death registration is done by during 3 hours, 6 hours and 24 hours. The potential of papaya leaf extract was tested at concentration of 100 ppm, 200 ppm, 300 ppm, and 400 ppm, respectively.The papaya leaf extract effectively used as larvicides for Lethal Concentration values ≤1% (10,000 ppm) (Sesanti *et al*., 2014).The present study, mortality of *Aedes aegypti* larvae was noted to be 5%, 30%, 30% and 40% at the concentration of 100 ppm, 200 ppm, 300 ppm and 400 ppm respectively in the first 3 hour. Mortality of *Aedes aegypti* larvae was

found to be 20%, 50%, 55% and 70% at the concentration of 100 ppm, 200 ppm, 300 ppm and 400 ppm respectively in 6 hour. The present study showed that, the concentration of 100 ppm, the extract killed 20% of larvae in 6 hours while at the concentration of 400 ppm; the extract killed 70% of larvae in 6 hours.

The present study found the Lethal Concentration (LC_{50}) of papaya leaf extract against *Aedes aegypti* was 500.997ppm and (LC_{90}) was 2211.299 ppm in 3 hours. Lethal Concentration (LC_{50}) of papaya leaf was 232.293 ppm, and (LC_{90}) value was 924.467 ppm in 6 hours. Ravichandran *et al*., (2014) examined that the larvicidal activity of five different organic solvent extract of Papaya against *Culex quinquefasciatus*. The result was reported that the LC_{50} and LC_{90} value of ethanol extract of *C. papaya* was found to be 790.53 and 7207.31 ppm in 24 hrs. Sesanti *et al*., (2014) studied that the potential of papaya leaf and seeds extracts as larvicides against the *Anopheles* sp. The experiment described that the Lethal Concentration (LC_{50}) papaya leaf extract was 1497.658 and (LC_{90}) was 13089.781 ppm in 12- hour. The Lethal Concentration (LC_{50}) papaya leaf extract was 422.311 ppm, and (LC_{90}) was 1399.577 ppm in 24 hours.

Different Lethal concentration values between the present study and previous studies may be caused by different types of organic solvents, different solution, mosquito's species, different concentration and exposure time.LC_{50} *Aedes aegypti* larvae were tested to determine the value of the effective concentrations of granules of extract for papaya seed and leaf at 0, 30, 60, 120 and 150 ppm respectively under simulated conditions. The toxicity of *A. aegypti* LC_{50} :107 ppm and LC_{90} :150ppm. The lower value of LC_{50} will be the more toxic the substance (Wahyuni, 2015). Insecticidal effects of plants extracts vary not only according to plant species, mosquito species, geographical varities and parts used, but also due to extraction methodology adopted and the polarity of the solvents used during extraction (Wahyuni, 2015).Based on WHO guidelines (2005), it can be said that papaya leaf extract effectively used as larvicides for Lethal Time < 48 hours (Sesanti *et al*., 2014).

In the present study, (LT_{50}) of papaya extract and (LT_{90}) were 3.016 hours and 6.958 hour.Value Lethal Time (LT_{50}) papaya extract LT_{90} value of 13.579 hours and 23.478 hours, meaning 13.579 within hours of papaya leaf extract is able to kill 50% of larvae, and 23.478 within hours, papaya extract is able to kill 90% of larvae of *Anopheles* sp (Sesanti *et al*., 2014).Value Lethal Time is much different between this studies with previous studies. This may be caused by the use of different raw materials, such as the level of maturity leaves, papaya varieties, method of extraction, which is a different type of solution, also the concentration of the solution used. The papaya (*C. papaya*) seed and leaf extract showed excellent using granule of extract from papaya. The papaya (*C. papaya*), is one of the most commonly studied plants for the control of mosquitoes; it contain several biologically active principles, and papain being the predominant insecticide (Wahyuni, 2015).According to the study, it may suggest that using of the papaya extract can affect in the control of target species (*Aedes aegypti*) and the use of leaf extracts and papaya seed extract as larvicides relatively safer for the environment because it is a natural substance and its nature is not toxic to aquatic animals.

CONCLUSION

Based on the analysis results, it is seen that, the smaller the concentration of papaya extract then require a longer contact time with the larvae can cause death to the larvae of *A. aegypti*, the higher the concentration of papaya extract requires a shorter the contact time in killing the larvae of *Aedesaegypti*. Mortality was increased as the concentration increased. In conclusion, the result showed the effective (ethanol) *Carica papaya* leaf extract have the potential to be used as an eco-friendly approaches for the control of *Aedes aegypti*.

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