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Efficacy of Ethanol Extract of Papaya Leaves (*Carica papaya* var California L.) on Mortality of Instar III Larvae of *Aedes aegypti* Mosquito

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ABSTRACT

This study aims to evaluate the efficacy of Papaya var California leaves extract and identify its effective concentration in inducing mortality among third-instar larvae of *Aedes aegypti* mosquitoes. The study utilized adult *Ae. aegypti* mosquitoes as the experimental subjects. This study was an experimental study with a comparative Completely Randomized Design The test groups include negative control, 2.5 mg/mL, 5 mg/mL, 10 mg/mL and positive control. Each test used 20 third instar larvae of *Ae.aegypti* mosquitoes, with four repeats.. The test groups were 2.5 mg/mL, 5 mg/mL, 10 mg/mL, and the control groups were A negative control contained distilled water and Tween 20, and a positive control contained abate (temephos) Based on Kruskall-wallis test shows a significant difference. The results indicated that the optimal concentration was 10 mg/mL, with the highest effectiveness observed at the 30th time hours.

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INTRODUCTION

A disease that is still of particular concern today is Dengue hemorrhagic fever. In Indonesia, dengue hemorrhagic fever has emerged as an extraordinary event. The prevalence of dengue fever continues to rise, eliciting widespread concern within communities due to its rapid transmission and associated mortality risk. According to the East Java Provincial Health Office in 2017, the incidence of dengue fever continued to increase from 21,092 (in 2015) to 25,336 people.

One of the efforts made in tackling the threat of DHF is by controlling vector or the breeding sites. Generally, *Ae. aegypti* control is carried out using chemical larvicides. Larvicide application in the form of 1% temephos sand granules has been proven to be effective in controlling *Ae. aegypti* mosquito larvae for 8-12 weeks. The 1% temephos sand granules are known as abate, and the administration of abate itself is called abatement.

The application of chemical larvicides for controlling *Ae.aegypti* has resulted in the development of resistant populations, necessitating higher doses that pose toxic risks to humans, animals, and the environment. Temephos is a larvicide widely used as dengue vector control in Martinique that has shown resistance in Southeast Asia, South America, and the Caribbean (Ramayanti *et al*., 2016)

Papaya plant or in Latin name known as *Carica papaya* L. is a plantthat comes from Tropical America, but this fruit is very well known by Indonesians from various walks of life this because papaya in Indonesia is available throughout the year and papaya cultivation does not recognize the season (Aliyudin *et al*., 2017). Papaya production in East Java in 2021 reached 253,700 tons. The large amount of production is proportional to the natural waste produced, one of which is leaf.

This study aimed to evaluate the efficacy of ethanol extract from papaya var. caliifornia at

specific concentrations in inducing mortality among third-instar larvae of *Ae. aegypti* mosquitoes.

MATERIALS AND METHODS

After being collected and identified, the selected papaya var California leaves were washed under running water, aerated, then pulverized and sieved (Lazuardi, 2019).

The fine powder of papaya leaves (*Carica papaya L. var* California) was processed through maceration by weighing 1 kilogram of the powder and adding 2 liters of 96% technical ethanol as a solvent. In a sealed container, the mixture was allowed to stand for two days, stirring every four hours for five minutes. After that, followed by filtering. Filtrate were placed in a rotary evaporator until a thick extract was produced. An Evaporator ran at 3000 rpm and between 40°C and 50°C (Meles *et al*., 2012).

The extract was subsequently diluted with distilled water and emulsified with Tween 20 to prepare liquid extract formulations at various concentrations. The concentration of the ethanol extract from papaya var California leaves was calculated using the dilution formula, as follows:

$$V_1 \times M_1 = V_2 \times M_2$$

Description:
V1 = Volume of first solution (mL)
M1 = First concentration (mg/mL)
V2 = Volume of solution after dilution (mL)
M2 = Concentration after dilution (mg/mL)

Samples of *Ae. aegypti* mosquito larvae were obtained from the Institute of Tropical Disease (ITD) Laboratory, Airlangga University Surabaya. The sample criteria for *Ae.aegypti* mosquito larvae consisted of 20 third-instar larvae, characterized by a body length of 4-5 mm, the beginning of clear spines (spinae) in the thoracic region, and a blackish-brown respiratory funnel (siphon).

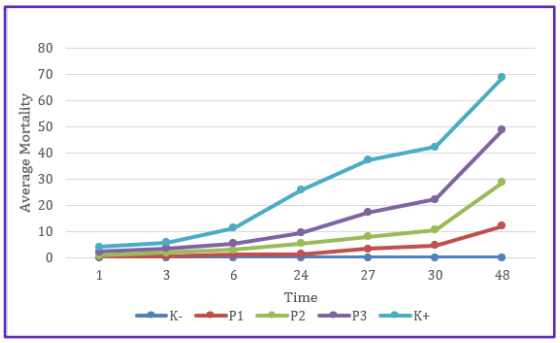
In the activity test of the ethanol extract from papaya var California leaves, third-instar *Ae.aegypti* larvae were introduced into media treated with various concentrations of the ethanol extract using a plastic pipette, with approximately 20 larvae per media with four repeats. The treatments included: Treatment 1 (P1) with a concentration of 2.5 mg/mL, Treatment 2 (P2) with 5 mg/mL, Treatment 3 (P3) with 10 mg/mL, a positive control (K+) containing abate (temephos), and a negative control (K-) containing distilled water and Tween 20.

Following an initial 24-hour observation period, if 50% of the test larvae have not died, the observation time extended to 48 hours (Nurhaifah and Sukesi, 2015). Lethal Time (LT_{50} and LT_{90}) and Lethal Concentration (LC_{50} and LC_{90}) were determined by exposing third instar *Ae. aegypti* larvae to various concentrations of the extract, and observing larval mortality at specific time intervals (Nurhaifah and Sukesi, 2015).

The data obtained from the experiment was carried out with the number of larvae that died in the experiment recorded and analyzed using the *Kruskall-wallis* test analysis method which was then followed by the *Mann whitney* test to see the average difference of each treatment using the SPSS statistical program IBM version 26.

RESULTS AND DISCUSSION

The five ethanol extract treatments demonstrated different mortality rates, which which were influenced *Carica papaya* extract concentration. These results suggest that as the concentration of the extract increases, larval mortality also increases.



Figures 1. Average mortalitiy of third instar larvae of *Ae.aegypti* larvae

This was similar with the findings of of Candido, Cavalcanti and Beserra (2013), who stated that higher concentrations and prolonged exposure times lead to increased larval mortality. The variation in larval mortality among *Aedes aegypti* is influenced by the concentration level, with higher extract concentrations resulting in a greater mortality (Karima and Ardiansyah, 2021).

According to World Health Organization (2016), larval mortality testing is usually done within 48 hours. This is because insecticides need time to work for the larvae to die. Testing over a longer period of time allows for more accurate measurement of mortality and LC_{50}.

According to research by Mahatriny *et al*. (2014), the papaya leaves contain compounds including fatty acids, alkaloids, flavonoids and terpenoids. Larvae exposed to alkaloids have effects as contact poisons and stomach poisons, which in histopathology show degeneration and proliferation in the digestive tract (Hastutiek and Sunarso, 2015)

The probit test was conducted on the three concentrations of papaya var California leaves extract extract at the 30th and 48th hour of observation, because the extract on the 30th hours at the concentration of 2.5 mg/mL, 5 mg/mL and 10 mg/mL showed the half mortality of the test larvae.

Tables 1. LC_{50} and LC_{95} values at 30 hours and 48 hours respectively.

Time	Probit	
	LC50 (mg/mL)	**LC95 (mg/mL)**
30 hours	10.16	27.12
48 hours	4.04	8.02

The probit LC_{50} test results at the 30_{th} hour were 10.16 mg/mL and 4.04 mg/mL at the 48th hour. Considering to these results a dose interval of 4.04 mg/mL to 10.16 mg/mL can be was to kill 50% of *Ae.aegypti* mosquito larvae. The probit test results for LC_{95} at hour 30 were 27.12 mg/mL and 8.02 mg/ml at hour 48. These results indicated that 8.02 mg/mL - 27.12 mg/mL was the interval that can be used to kill 95% of *Ae. aegypti* mosquito larvae.

Table 2. LT_{50} and LT_{95} values at various concentration

Concentration	Probit	
	LT_{50} (Hours)	**LT_{95} (Hours)**
2.5 mg/mL	43.11	83.20
5 mg/mL	36.01	65.28
10 mg/mL	29.44	49.29

The papaya leaves (*Carica papaya* L. var California) extract at a concentration of 10 mg/mL exhibited the fastest ability to achieve 50% and 95% mortality in the third-instar larvae of *Ae.aegypti* mosquitoes, followed by the 5 mg/mL concentration. In contrast, the extract at a concentration of 2.5 mg/mL required the longest duration to reach 50% and 95% mortality in the larvae.

The test results indicated that the time required for the papaya var California leaves extract to achieve 50% mortality of third-instar *Ae.aegypti* larvae ranged from 29.44 to 43.11 hours, while the time to reach 95% mortality 49.29 to 83.20 hours. The mortality of third instar *Aedes aegypti* larvae was attributed to the bioactive compounds present in papaya var California leaves. According to Ardahidayani (2020), GC-MS analysis revealed that California papaya leaves contain several phytochemical constituents, including alkaloids, flavonoids, and terpenoids.

CONCLUSION

In third-instar *Ae. aegypti* larvae, the ethanol extract of papaya leaves (*Carica papaya* L. var California) effectively induces mortality. To reach the LC_{50}, a concentration of 4.04 mg/mL must be used. At doses of 2.5 mg/mL, 5 mg/mL and 10 mg/mL, the papaya leaves ethanol extract takes 43.11 hours, 36.01 hours, and 29.44 hours, to achieve the LT_{50}. The highest effective period is 30 hours, and the most effective concentration is 10 mg/mL.

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AUTHORS' CONTRIBUTIONS

COSN performed the experiment, data collection, data analysis, and writing of the manuscript. PH conceptualized, developed, and supervised the research and proofreading of the manuscript. DKM was involved in developing the research methodology, ethical considerations, and proofreading the manuscript. EBAH, ML, and AS guided data analysis, data interpretation, and literature evaluation of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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