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Research Article
Effects of *Carica papaya* Seed and Leaf Extracts on *Anopheles* sp. Larval Mortality

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Abstract

Background and Objective: Insecticide resistance and environmental damage are impacts of the continuous application of synthetic larvicides; therefore, alternative larvicides are necessarily for Malaria vector control. The study aimed to analyze the effect of *Carica papaya* seed and leaf extracts on *Anopheles* sp. larval mortality. **Materials and Methods:** The study method involved a post-test only control group experimental design. Third and fourth instar larvae of a field strain of *Anopheles* sp. were collected from a paddy field habitat. Fresh *C. papaya* seeds and leaves were obtained from a garden located in the Tanete subdistrict. The *C. papaya* seeds and leaves were extracted with 70% methanol using a Soxhlet apparatus. A bioassay test was carried out in three different concentrations of each extract and a control. Larval mortality was observed during 12 h in three replicates. Further, a field trial on each extract as a larvicide was conducted in *Anopheles* sp. habitat in Tanete, Bulukumba, South Sulawesi, Indonesia. **Results:** Phytochemical screening of the *C. papaya* seed extract revealed the presence of tannins and terpenoids. The *C. papaya* leaf extract revealed the presence of flavonoids, saponins and steroids. The LC_{50} value of the *C. papaya* seed extract reached a 3.9% concentration and The LT_{50} value was 5 min ($p<0.05$). The LC_{50} value of the *C. papaya* leaf extract reached a 2.8% concentration and The LT_{50} value was 60 min ($p>0.05$). The LC_{50} value of the mixed *C. papaya* seed and leaf extract reached a 2.6% concentration and The LT_{50} value was 5 min ($p>0.05$). The field trial of the *C. papaya* extract showed that the seed and mixed seed and leaf extracts both demonstrated a 100% larval density reduction, whereas the leaf extract only showed a 91% reduction (Mulla's formulae). **Conclusion:** The *C. papaya* seed extract had a significant effect on the *Anopheles sp.* larval mortality, whereas the other extracts (leaf and mixed seed and leaf extracts) had no significant effect. The *C. papaya* seed and mixed seed and leaf extracts were indicated as effective larvicid for Malaria vector control.

Key words: *Anopheles* sp., bioassay, *Carica papaya* extracts, larvicide, malaria cases, papaya leave extract

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Since 2008, Indonesia has consistently been in the top 5 ranking ASEAN countries with the highest number of malaria cases[1,2]. There were 207 million malaria cases and 627,000 deaths reported around the world in 2012. A total of 3.4 billion people were reported to be at risk of malaria, especially in Africa and Southeast Asia. In 2013, malaria cases rose to 216 million cases, with a mortality rate of approximately 655,000[1,2].

The malaria incidence was still above the 3.1 incidence rate and the 8.1 malaria prevalence rate in South Sulawesi, Indonesia in 2013[3,4]. From 2009-2011, The malaria prevalence in Bulukumba was higher in comparison to other districts in South Sulawesi[5]. A 14.34% annual malaria incidence (AMI) rate and a 4.29% annual parasite incidence (API) rate were reported in 2009. In 2010, the AMI reached 22.0% and the API reached 5.3%. In 2011, the AMI rate was 22.0% and the API was 0.29%[5]. In 2012, the AMI was 11.89% and the API was 0.09%, while the API in 2013 showed malaria-positive patients amounting to 135 people[5].

According to World Health Organization[6], larval control is one way to control the spread of *Anopheles* sp. in addition to using spraying and insecticide-treated nets. In general, larval control involves synthetic chemicals, although this method can effectively reduce the larval density but it can cause adverse effects to the environment, public health, non-target organisms, the chemicals are not readily biodegradable and the larvae have shown increasing resistance[6]. These reasons have encouraged research efforts to identify environmentally friendly larvicides that have low cost, are effective and are easy to apply as a substitute for synthetic chemical larvicides.

One plant that can be used as a larvicide is *C. papaya*. Alkaloids, flavonoids and saponins were extracted from *C. papaya* seeds and leaves and these compounds can be used as larvicides against *Anopheles* sp. This study aimed to determine the effects of the *C. papaya* seed, leaf, mixed seed and leaf extracts against *Anopheles* sp. larval mortalities in the Tanete Subdistrict, Bulukumba, South Sulawesi, Indonesia.

MATERIALS AND METHODS

The study method had a post-test only control group experimental design. Third and fourth instar larvae of a field strain of *Anopheles* sp. were collected from a paddy fields habitat in the Tanete Subdistrict, Indonesia. The *C. papaya* seeds and leaves were obtained from a garden located in the Tanete Subdistrict. The *C. papaya* seeds were cleaned of husks, washed and dried at room temperature and then placed in an oven until they reached a constant weight. The *C. papaya* leaves were washed, cut into pieces and dried at room temperature and then placed in the oven until they reached a constant weight. The *C. papaya* seeds and leaves were extracted with 70% methanol using a Soxhlet apparatus. The crude papaya leaf and seed extracts were evaporated to dryness in rotary vacuum evaporator (Sigma Scientific Glass Pvt. Ltd, India). Phytochemicals of the *C. papaya* seeds and leaves extracts were screened using Harborne[7,8]. Bioassay tests[9] were carried out at three different concentrations of each extract. Third and fourth instar larvae of *Anopheles* sp. were placed in a plastic cup, with each cup containing 10 larvae and 5-10 cm of water. The control group was given 1 mL of the 70% methanol solution. Three replicates were performed for each concentration. Larval mortalities were recorded for 12 h at the Laboratory of the Chemistry Department, Faculty of Mathematics and Natural Sciences, Hasanuddin University. Further, a field trial[8] was conducted for each extract in puddles in the paddy field habitat of *Anopheles* sp. in the Tanete Subdistrict. The *C. papaya* seed extract was applied a 3.9% concentration for the field trial. The *C. papaya* leaf extract was applied a 2.8% concentration. The mixed *C. papaya* seed and leaf extract was applied a 2.6% concentration. The amount of testing extract applied was 480 mL of the seed extract, 660 mL of the leaf extract and 364 mL of the mixed *C. papaya* seed and leaf extract. Based on puddle width, the puddles that were used were categorized as medium puddle (>2-10 m^2). The field trial was conducted during 1 week with observations on day 2, 4 and 7. The percentage reduction in the *Anopheles* sp. larval density was calculated using Mulla's formulae[10].

Data for treatment outcomes each extract and control test were subjected to two way analysis of variance using a simple linear regression test. The 50% lethal concentration (LC50) and the 50% lethal time (LT50) values were determined using probit analysis[11]. Data processing was performed using SPSS 21.0 software (IBM Corp, USA) program.

RESULTS

Phytochemical analysis of the *C. papaya* seed and leaf extracts: Phytochemical analysis of the *C. papaya* seed extract indicated that it contained tannins and terpenoids. The *C. papaya* leaf extract contained flavonoids, saponins and steroids. The *C. papaya* leaf extracts contained flavonoids, saponins and steroids.

Bioassay test of *C. papaya* extracts: The preliminary test observations became the basis of the main bioassay test. The results of the bioassay test of the *C. papaya* extracts against *Anopheles* sp. larvae in the laboratory are presented in Table 1 (Seed), Table 2 (Leaf) and Table 3 (mixed seed and leaf).

Table 1: Effects of *Carica papaya* seed extract on *Anopheles* sp. larval mortality

Time	Control (0%)						Concentration 3.9%						Concentration 7.8%						Concentration 15.6%					
(min)	I	II	III	Mean	SD	%age	I	II	III	Mean	SD	%age	I	II	III	Mean	SD	%age	I	II	III	Mean	SD	%age
5	0	0	0	0	0	0	6	6	5	5.67	0.58	56.67	8	9	8	8.33	0.58	83.33	9	7	8	8.00	1.00	80.0
10	0	0	0	0	0	0	8	9	8	8.33	0.58	83.33	8	10	9	9.00	1.00	90.00	9	8	8	8.33	0.58	83.3
15	0	0	0	0	0	0	9	9	9	9.00	0.00	90.00	9	10	10	9.67	0.58	96.70	9	9	10	9.33	0.58	93.3
20	0	0	0	0	0	0	10	10	10	10.00	0.00	100.00	10	10	10	10.00	0.00	100.00	10	10	10	10.00	0.00	100.0

Table 2: The influence of Papaya leaves extract (*Carica papaya Linn.*) on the mortality of *Anopheles sp.* larvae

Time	Control (0%)						Concentration 1.4%						Concentration 2.8%						Concentration 5.6%					
(min)	I	II	III	Mean	SD	%age	I	II	III	Mean	SD	%age	I	II	III	Mean	SD	%age	I	II	III	Mean	SD	%age
5	0	0	0	0	0	0	0	0	0	0.00	0.00	0.00	0	0	0	0.00	0.00	0.00	0	0	0	0.00	0.00	0.0
30	0	0	0	0	0	0	0	0	0	0.00	0.00	0.00	0	0	0	0.00	0.00	0.00	0	0	0	0.00	0.00	0.0
60	0	0	0	0	0	0	4	4	5	4.33	0.58	43.33	5	5	6	5.33	0.58	53.33	5	6	6	5.67	0.58	56.7
120	0	0	0	0	0	0	5	6	6	5.67	0.58	56.67	7	6	7	6.67	0.58	66.67	7	8	7	7.33	0.58	73.3
180	0	0	0	0	0	0	6	7	7	6.67	0.58	66.67	8	8	8	8.00	0.00	80.00	8	9	8	8.33	0.58	83.3
240	0	0	0	0	0	0	7	8	9	8.00	1.00	80.00	9	9	9	9.00	0.00	90.00	10	10	10	10.00	0.00	100.0

Table 3: Effects of the mixed *Carica papaya* seed and leaf extract on the *Anopheles* sp. larval mortality

Time	Control (0%)						Concentration of 2.6%						Concentration of 5.6%						Concentration of 10.4%					
(min)	I	II	III	Mean	SD	%age	I	II	III	Mean	SD	%age	I	II	III	Mean	SD	%age	I	II	III	Mean	SD	%age
5	0	0	0	0	0	0	6	5	4	5.00	1.00	50.0	6	5	5	5.33	0.58	53.3	7	5	5	5.67	1.15	56.7
10	0	0	0	0	0	0	6	5	7	6.00	1.00	60.0	7	6	5	6.00	1.00	60.0	8	6	7	7.00	1.00	70.0
15	0	0	0	0	0	0	7	8	7	7.33	0.58	73.3	8	8	6	7.33	1.15	73.3	8	7	8	7.67	0.58	76.7
20	0	0	0	0	0	0	7	8	8	7.67	0.58	76.7	8	8	8	8.00	0.00	80.0	8	8	8	8.00	0.00	80.0
25	0	0	0	0	0	0	9	8	9	8.67	0.58	86.7	9	9	8	8.67	0.58	86.7	10	9	9	9.33	0.58	93.3
30	0	0	0	0	0	0	10	10	10	10.00	0.00	100.0	10	10	10	10.00	0.00	100.0	10	10	10	10.00	0.00	100.0

SD: Standard deviation, %age: Percentage

Table 4: Results of linear regression test and probit value of *Carica papaya* seed, leaf and mixed seed and leaf extracts

Carica papaya extracts concentrations	R^2	Standardized coefficients	Linear equation	Probit value
Seed				
Concentration of 3.9%	0.762	-1.048	Y = 19.022-1 .048x	
Concentration of 7.8%	0.741	0.176	Y = 19.022+0.176x	0.088
Concentration of 15.6%	0.709	0.000	Y = 19.022+0.000x	
Leaf				
Concentration of 1.4%	-0.052	1.056	Y = 28.582-1 .056x	
Concentration of 2.8%	-0.027	-0.973	Y = 28.582-0.973x	4.049
Concentration of 5.6%	-0.100	-0.029	Y = 28.582-0.100x	
Mixed seed and leaf				
Concentration of 2.6%	0.121	0.161	Y = 22.622+0.161x	
Concentration of 5.2%	0.132	-0.078	Y = 22.622-0.078x	0.013
Concentration of 10.4%	0.207	-0.655	Y = 22.622-0.655x	

As shown in Table 1, the *C. papaya* seed extract at a 3.9% concentration was found to cause *Anopheles* sp. larval mortality as high as 56.67% after 5 min. Mortality as high as 83.33% was found for the 7.8% concentration. Mortality as high as 80.0% was found at a concentration of 15.6%. As shown in Table 2, mortality as high as 43.33% was found after 60 min for the *C. papaya* leaf extract at a 1.4% concentration. Mortality as high as 53.33% was found at a concentration of 2.8%. Mortality as high as 56.7% was found at a concentration of 5.6%. As shown in Table 3, mortality as high as 50.0% was found for the mixed *C. papaya* seed and leaf extract at a 2.6% concentration. At a 5.2% concentration, mortality as high as 53.3% was found. Mortality as high as 56.7% was found at a concentration of 10.4%.

As shown in Table 4, the probit value of the *C. papaya* seed extract was found to be 0.088. The probit value of the *C. papaya* leaf extract was found to be 4.049. Probit value of the mixed *C. papaya* seed and leaf extract was found to be 0.013. The LC_{50} value results was obtained from a linear equation (Table 4). The LC_{50} and the LT50 values were determined using probit analysis. The Probit mortality of the *Carica papaya* seed, leaf and mixed extracts are shown in Fig. 1-3 respectively. The LC_{50} value for the *C. papaya* seed extract was obtained at a 3.9% concentration, in which the *Anopheles* sp. mortality reached 56.67% within 5 min. The LC_{50} for the *C. papaya* leaf extract was obtained at a 2.8% concentration, in which the *Anopheles* sp. mortality reached 53.33% within 60 min. The LC_{50} for the mixed *C. papaya* seed and leaf extract was obtained at a 2.6% concentration, which the *Anopheles* sp. mortality reached 50% within 5 min.

Observation of the LT_{50} indicates the time necessary to kill 50% of the larvae. The LT_{50} value of the *C. papaya* seed extract was found to be 5 min for 3.9% concentration. The LT^{50} value was 3 min for the seed extract at a 7.8% concentration and 4 min for the 15.6% concentration. The LT_{50} value of the

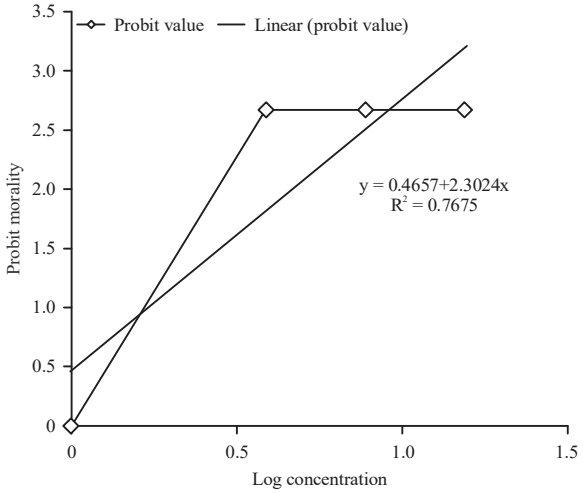

Fig. 1: Probit mortality of *Carica papaya* seed extract

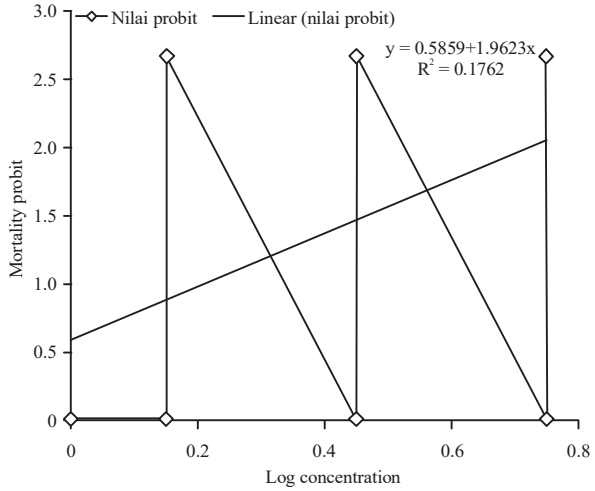

Fig. 2: Probit analysis of mortality papaya leaves extract (*Carica papaya* Linn)

C. papaya leaf extract was found to be 4 h for a 1.4% concentration. It was found to be 1 h for both the 2.8 and 5.6%

Table 5: Field trial of the *Carica papaya* seed, leaf and mixed seed and leaf extracts in Bulukumba District, South Sulawesi, Indonesia, 2014

Carica papaya extracts	Medium puddle	*Anopheles* sp. Larval density for test (no/dip)	Day 2		Day 4		Day 7	
			L. Density	Reduction (%)	L. Density	Reduction (%)	L. Density	Reduction (%)
Papaya seed	Control	2.1	2.1	61.4	2.00	78.91	0.70	100
	Test	11.4	4.4		2.29		0.00	
Papaya leaf	Control	2.1	2.1	88.0	2.00	92.0	0.70	91
	Test	9.4	1.1		0.71		0.28	
Mixed papaya seed and leaf	Control	2.1	2.1	28.0	2.00	60.0	0.70	100
	Test	6.7	4.8		2.57		0.00	

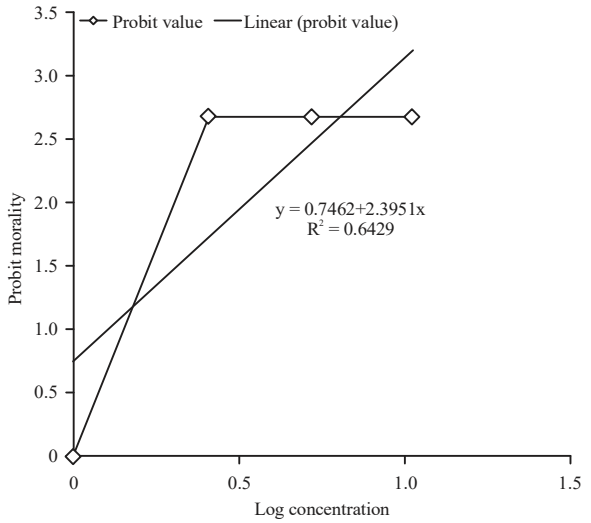

Fig. 3: Probit mortality of the mixed *Carica papaya* seed and leaf extract

concentrations. The LT_{50} value of the mixed *C. papaya* seed and leaf extract was found to be 5 min for the 2.6, 5.2 and 10.4% concentrations

***Carica papaya* extract test in the breeding habitat:** The LC_{50} values obtained from each extract were used as a test concentration in the original habitat of the *Anopheles* sp. larvae. As shown in Table 5, the *Anopheles* sp. larval density was reduced on day 2 after the extract application. The *C. papaya* seed extract at a 3.9% concentration caused a 61.4% reduction in the *Anopheles* sp. larvae. The *C. papaya* leaf extract at a 2.8% concentration caused an 88.0% reduction.

The mixed *C. papaya* seed and leaf extracts at a 2.6% concentration caused a 28.0% reduction. The *C. papaya* seed extract caused a 78.91% reduction in *Anopheles* sp. larvae on day 4. The *C. papaya* leaf extract caused a 92.0% reduction in the *Anopheles* sp. larvae. The mixed *C. papaya* seed and leaf extracts caused a 60% reduction in the *Anopheles* sp. larvae. In terms of the observations on day 7, the reduction in *Anopheles* sp. larvae reached 100% for both the *C. papaya* seed and mixed seed and leaf extracts. The *C. papaya* leaf extract caused only a 91.0% reduction in *Anopheles* sp. larvae.

DISCUSSION

Phytochemical analysis: In this study phytochemical analysis[12] showed differences when compared to previous studies. According to Sukadana *et al.*[13] and Warisno[14], *C. papaya* seeds contain phenol, triterpenoids, alkaloids and saponins. The *C. papaya* leaves contain active ingredients[15,16] that have the potential for use as a larvicide, namely papain enzyme, saponins, flavonoids and tannins. Based on research conducted by Kovendan *et al.*[17], *C. papaya* leaf extracts contain chemical components such as alkaloids, tannins, anthraquinone, steroids, saponins, phenolic and flavonoid. Oladimeji *et al.*[18] reported that *C. papaya* leaves contain phytochemicals alkaloids, saponins, tannins, cardiac glycosides and flavonoids. Awais[19], also reported that *C. papaya* leaves contain phytochemicals steroids, saponins, triterpenoids, lipids, coumarin and organic acids. These differences are caused by the phytochemicals targeted for identification, the different ages of the plant parts, differences in sampling locations and the different solvents used for maceration.

Bioassay test: Rawani *et al.*[20] found that the liquid extract of *C. papaya* seeds becomes effective as mosquito larvicides at 0.20, 0.15, 0.11 and 0.07% concentrations but the larvae used were those of *Cx. quinquefasciatus*. Ariesta[21] found that the liquid extract of *C. papaya* leaves caused 95% mortality of *Aedes aegypti* larvae at a concentration of 10%. Lafiani[22] reported that a *C. papaya* leaf extract effectively kills 50% of the larvae of *Anopheles aconitus* at 883.29 ppm concentration over 24 h of exposure and that the concentration of 1456.79 ppm kills 90.0% of the larvae. Additionally, Valiant *et al.*[23] also reported the larvicidal effects of papaya leaf extract against the larvae of *Culex* at a 2.0% concentration. Fathonah[24] reported that the ethanol extract of *C. papaya* seeds and leaves showed larvicidal activity on

Anopheles aconitus larvae at concentrations of 3.9 and 1.4%, respectively. These studies illustrate that liquid extracts of the *C. papaya* seeds and leaves can be used as mosquito larvicides, which became the basis for selecting *C. papaya* seeds and leaves for evaluation in this study. The Effect of the mixed *C. papaya* seeds and leaves against *Anopheles* sp. larvae is unknown. Therefore, in this study, not only were *C. papaya* seed and leaf extracts applied but also the mixed extract of the *C. papaya* seeds and leaves.

The effects of extracts on *Anopheles* sp. larval mortality have been determined based on various factors such as larval instar, food, exposure period, air quality, extract concentration and larval sensitivity. In this study, the results of the linear regression suggest that the *C. papaya* seed extract concentration of 3.9, 7.8 and 15.6% had significant effects on the *Anopheles sp*. larvae mortality ($p = 0.004$). The *C. papaya* leaves ($p = 0.698$) and the mixed extracts ($p = 0.116$) had not significant effect on the Anopheles sp. larval mortality at any of the concentrations.

The mortality of *Anopheles sp.* larvae found in this study is closely related to the phytochemical content of the *C. papaya* seed (tannins and terpenoids) and leaf (flavonoids, saponins and steroids) extracts, which were previously identified[12]. A similar result was found by Yoshiki *et al.*[25] whereas, the mixed extract of the *C. papaya* seeds and leaves contains important phytochemicals, namely tannin, terpenoids, flavonoids, saponins and steroids[12-19,25]. This combination can increase the potential effect of the extract on the mortality of *Anopheles* sp. Larvae. The observed mortality was so much faster than that of the extracts of the *C. papaya* seeds (5 min) and leaves (60 min). This study showed that the mixed *C. papaya* seed and leaf extract (2.6%) was more effective in terms of mortality than that of the seed extract (3.9%) observed after 5 min. When it was compared with the leaf extract, mortality of 2.8% was found after 60 min for the leaf extract, whereas the mixed extract (2.6%) only took 5 min.

In addition to the phytochemical content of the extracts, the mortality of *Anopheles* sp. larvae can be caused by the physical appearance of the extract. The larvicidal oil layer of the seed extract cover the water surface, making it difficult for the larvae to breathe. A similar result was also found by Astuti *et al.*[26].

Field trial: In this study, the *C. papaya* extract was applied to medium-sized puddles, showing positive results. A similar study was conducted by Widyastuti *et al.*[27] over 3 weeks. The reduction of larval population density was found more than 70%. Jayadipraja[28] conducted a similar field trial in an unproductive fishponds in coastal areas. The larval reduction after seven days was found 84%.

The *C. papaya* seed and mixed seed and leaf extracts only took 7 days to reduce the *Anopheles* sp. larvae by 100%. The *C. papaya* leaf extract was only able to reduce the *Anopheles* sp. larval density by 91%. The effect on mortality of the *C. papaya* seed extract was higher than that of the mixed extract of the *C. papaya* seeds and leaves. This is because the *C. papaya* seed extract has a rather oily physical appearance and the oil covers the water surface, making it difficult for the *Anopheles* sp. larvae to breathe.

The *C. papaya* seed extract and the mixed seed and leaf extract have potential as larvicides in malaria vector control, specifically the larval control of *Anopheles* sp. in puddle rice in the Tanete subdistrict, Bulukumba, Indonesia.

CONCLUSION

Based on the results obtained, it can be concluded that the seed extract of papaya (*Carica papaya Linn.*) had a significantly effect on the larval mortality of *Anopheles* sp. (at a concentration of 3.9% at 5 min observation). The papaya leaves extract (*Carica papaya* Linn.) had no effect on the larval mortality of *Anopheles* sp. (at a concentration of 2.8% at 60 min observation). The mixed extract of the seeds and leaves of papaya (*Carica papaya* Linn.) had no significant effect on the mortality of *Anopheles* sp. larvae (at a concentration of 2.6% at 5 min observation).

SIGNIFICANCE STATEMENT

This study discovers the possible synergistic effect of the mixed *C. papaya* seed and leaf extracts that can be beneficial for Malaria vector control. This study will help the researcher to uncover the critical area of biolarvicide that many researchers were not able to explore. Thus, a new theory on these plant extract combination and possibly other combinations, may be arrived at.

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