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**NEW BIOINSECTICIDES FROM COMBINATION EXTRACT OF LEAF AND FLOWER OF BREADFRUIT (ARTOCARPUS ALTILIS) ON MORTALITY OF AEDES AEGYPTI LARVAE**

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INFO ARTICLE	ABSTRACT
Accepted 5 Februari 2022	The main vector for the spread of dengue fever is the female mosquito <i>Aedes aegypti</i> . In Indonesia, chemical larvicides are commonly used to control <i>Aedes aegypti</i> larvae. Bioinsecticides made from plants are one option for environmentally friendly larvae control. Leaves and flower of breadfruit <i>Artocarpus altilis</i> contains toxic compound that can function as larvicides. This experimental study used combination of leaf and flower of breadfruit expect that the combination of the two extracts was synergistic and it was more effective in killing <i>Aedes aegypti</i> larvae. The third instar <i>Aedes aegypti</i> larvae was used and divided into control group and test group with concentrations 1000, 2750, 4500, and 6250 ppm and replicated in 3 times with observations every 6, 9, and 24 hours. To identify the significant difference, the mortality data was analyzed using ANOVA and Duncan's test. In probit analysis, LC50 value of breadfruit leaf extract was 1871 ppm with LT50 was 4.2 hours. LC50 value of breadfruit flower extract was 2531 ppm with LT50 was 10.8 hours. LC50 value of the combination of breadfruit leaf and flower extract was 903 ppm with LT50 was 3.7 hours. Based on these studies, it may be indicated that breadfruit leaves and flowers was more effective in killing <i>Aedes aegypti</i> larvae than single extracts.
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**Keywords:**

health center;  
accredited; ISO  
9001:2008 certified

**Introduction**

Dengue fever is an infectious disease caused by the dengue virus. Dengue fever is a virus spread by the *Aedes aegypti* mosquito, the world's fastest growing mosquito, which infects about 390 million people each year (Kementerian Kesehatan RI, 2018). Data obtained from the Ministry of Health of the Republic of Indonesia (2018), the incidence of dengue fever in Indonesia has continued to increase since 1968 - 2015. This is due to several factors such as climate change, temperature, humidity, and the direction of the air that is suitable for vectors of this disease. In addition, the increasing population density and the lack of public participation in vector prevention and eradication have caused

the spread of this disease to spread more quickly (Kementerian Kesehatan RI, 2018). The dengue fever morbidity rate in East Java in 2019 was 47 per 100,000 population, an increase compared to 2018 which was 24 per 100,000 population. The case fatality rate (CFR) for dengue fever in 2019 was 1%, which indicates that the mortality rate due to dengue fever in East Java is still above the target of <1%. Meanwhile, the larvae-free rate was 78.2%, which was lower than the target that had been set, which was 95%. Cases of dengue fever in Jember in 2019 were 988 cases, with a CFR of 0.7% (Dinkes Jawa Timur, 2020).

One method of reducing or suppressing vector populations is with control. The use of

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synthetic pesticides is not accompanied by consideration of the potential negative effects. The use of sublethal doses stimulates the adaptation of insects to insecticides. This feature will be passed on to future generations, resulting in a new population of insecticide-resistant insects (Sembel, 2009). Insecticides containing hazardous chemicals such as organochlorines have been banned from use in Indonesia because of their persistent and bioaccumulative nature, which is stated in the Regulation of the Minister of Agriculture No. 24/Permentan/SR.140/4/2011. Insecticides generated from plants or other natural materials are one option for dealing with these issues (Pertanian, 2011)

Breadfruit (*Artocarpus altilis*) is one of Indonesia's most common plants and has the potential to be a mosquito repellent. In people living in rural areas, breadfruit flowers are often used as mosquito repellents (Narulita, W. Anggoro, B.S. And Novitasari, 2019). Based on the research that has been done, the flowers and leaves of breadfruit contain several compounds such as saponins, flavonoids, polyphenols, which have a sequential mechanism effect, namely inhibiting the stimulation of eating insects, and respiratory inhibitors. Meanwhile, saponin have a damaging effect on insect cell membranes, causing them to activate enzymes and degrade cell proteins (Nikmah, 2016). Maharani (2014) found that the methanol extract of dried breadfruit leaves includes alkaloids, flavonoids, tannins, phenols, and saponins, according to the results of phytochemical screening (Maharani et al., 2014)

Research conducted previously by Edi (2011), showed that breadfruit flowers were able to immobilize 10 mosquitoes with an average time of 15.6 minutes for 5 repetitions (Hamsir & Fahmi, 2019). Lumowa (2011) found that breadfruit flower powder, which acts as an anti-inflammatory, was able to kill

the most mosquitoes with a content of 2 grams of breadfruit flowers, killing an average of 15.6 insects (78%) (Lumowa, 2011). Kurniawan (2020) concluded that extracts from breadfruit stems and leaves can be used as natural insecticides because they show a fairly high mortality rate of *Anopheles sp* mosquitoes. Although they are not as effective as synthetic insecticides, breadfruit stem and leaf extracts are safer for human health and environment (Kurniawan et al., 2020). The combination of several active compounds which is a several extracts is able to provide effects such as synergistic, antagonist, and addictive. The use of plant extracts as vegetable insecticides can be used alone or in combination (Shalan EA, Canyon D, Younes MW, Abdel-Wahab H, 2005). Previous research by Vastrad (2002) explained that combining of two plant extracts that have synergistic bioactive compounds is more effective as an agent for controlling insect pests and disease vectors than a single extract (Taufika et al., 2020). So if the active compounds from the leaves and flowers of breadfruit which are toxic are combined, it is expected to increase the mortality of *Aedes aegypti* larvae. Several previous studies only used *Anopheles sp* larvae as subjects, and only used a single extract. Therefore, this is attracted researchers to compare the capacity each of extracts from breadfruit leaves and flowers, and combination extracts of breadfruit leaves and flowers, as a bioinsecticide to kill *Aedes aegypti* larvae which are environmentally friendly and safe for humans.

## Method

This is a true experimental study with a post-test only control group design. This study used a posttest-only control group design, in which two groups were randomly assigned to each other. The subjects were *Aedes aegypti* instar III larvae taken from 860 larvae reared in the Entomology Laboratory,

with a total of 20 larvae in each test for four treatments and three replications. Leaves and flower of breadfruit were extracted at Phytochemical Laboratory UPT. Materia Medika, Batu City, East Java. Larval rearing and effectiveness tests starting from 15 November 2021 to 02 December 2021, was carried out at the Entomology Laboratory, Institute for Tropical Diseases, Airlangga University, Surabaya.

Breadfruit leaves and flowers were collected from gardens in Banyuwangi, East Java, and dried before being extracted with 96% ethanol using the maceration method. To achieve a thick extract, the extraction process takes around 14 days. A total of 860 *Aedes aegypti* larvae in the third instar were collected and fasted for 24 hours. The third instar larvae were chosen because they have a stronger ability to neutralize harmful substances than the first and second instar larvae. Whereas in instar IV it is closer to being a pupa, so there can be bias during the study.

The third instar of larvae that had been collected were then put into each plastic cup containing 20 ml of distilled water along with breadfruit leaf extract, breadfruit flower, and a combination of breadfruit leaf and flower extract with concentrations of 1000, 2750, 4500, and 6250. ppm. Every 6, 9, and 24 hours after treatment, the mortality of the larvae was observed and recorded. When a stick is pressed against the larvae's body, and

they stop moving and do not respond to stimuli, indicating that they have died. The percentage of larval mortality was determined using the Abbott formula:

$$\text{Mortality} = \frac{\text{Number of Dead Larvae}}{\text{Number of Test Larvae}} \times 100\%$$

One Way ANOVA test was used to analyze the observed data. Duncan's follow-up test was used to determine the significant difference in the effectiveness of each concentration of the test extract if the results of the study demonstrated significance (Gomez, 2015). To find out the values of LC<sub>50</sub> and LT<sub>50</sub> using Probit Analysis in the SPSS program. To find out the value of the Combination Index (CI) use the formula :

$$CI = \frac{LCx^{1(cm)}}{LCx^1} + \frac{LCx^{2(cm)}}{LCx^2} + \left( \frac{LCx^{1(cm)}}{LCx^1} \times \frac{LCx^{2(cm)}}{LCx^2} \right)$$

Description :

CI : Combination Index

LCx<sup>1 (cm)</sup> : Lethal Concentration x

Combination Extract on the First Single Extract

LCx<sup>2 (cm)</sup>: Lethal Concentration x

Combination Extract on the Second Single Extract

LCx<sup>1</sup> : Lethal Concentration x First Single Extract

LCx<sup>2</sup> : Lethal Concentration x Second Single Extract.

**Table 1**  
**Category of Combination Extract Interaction Properties According to Chou and Martin (2004)**

Combination Index Value Range	Characteristics of Combination Interaction
< 0,1	Very Strong Synergist
0,1 – 0,3	Strong Synergist
0,3 – 0,7	Synergistic
0,7 – 0,85	Moderate Synergy
0,85 – 0,9	Low Synergistic
0,9 – 1,1	Additive
1,1 – 1,2	Low Antagonist

1,2 – 1,45	Medium Antagonist
1,45 – 3,3	Antagonist
3,3 – 10	Very Antagonist
> 10	Very Strong Antagonist

Source: (Chou & Martin, 2004)

**Results and Discussion**

After the final test and observation, extract of leaf, flower, and combination leaf

and flower of breadfruit in the following concentration can kill *Aedes aegypti* larvae:

**Table 2**  
**Number of *Aedes aegypti* Larvae Mortality After Giving Breadfruit Leaf Extract**

Concentration	Replication	Number of Dead Larvae			Mortality Presentation in 24 hours (%)
		6 hours	9 hours	24 hours	
1000 ppm	I	6	6	7	38,3
	II	4	6	8	
	III	5	7	8	
2750 ppm	I	8	9	11	53,3
	II	8	9	10	
	III	9	7	11	
4500 ppm	I	10	11	12	65
	II	11	12	13	
	III	12	13	14	
6250 ppm	I	17	17	16	86,6
	II	16	18	19	
	III	17	17	17	

After processing the data through the Kolmogorov-Smirnov normality test, it was discovered that the mosquito larvae mortality data were normally distributed with a significance value of >0.05. The leaf extract of the breadfruit *Artocarpus altilis* was then analyzed using One Way ANOVA with a significance value of 0.05, indicating that it has an influence on the mortality of *Aedes aegypti* larvae.

Duncan's test was used to determine whether there was a significant difference in each extract concentration, with the finding that at a concentration of 6250 ppm, there was a significant difference with the highest value of 51.33. As a result, when compared to other concentrations, the concentration of 6250 ppm had the highest effect.

**Table 3**  
**Number of *Aedes aegypti* Larvae Mortality After Giving Breadfruit Flower Extract**

Concentration	Replication	Number of Dead Larvae			Mortality Presentation in 24 hours (%)
		6 hours	9 hours	24 hours	
1000 ppm	I	1	2	3	16,6
	II	2	2	3	
	III	3	3	4	



2750 ppm	I	7	7	12	50
	II	8	8	8	
	III	7	8	10	
4500 ppm	I	10	11	13	70
	II	10	11	14	
	III	11	14	15	
6250 ppm	I	15	17	17	90
	II	16	17	18	
	III	17	18	19	

After processing the data through the Kolmogorov-Smirnov normality test, it was discovered that the larvae mortality data were normally distributed with a significance value of  $>0.05$ . The flower extract of breadfruit was then analyzed using One Way ANOVA with a significance value of 0.05, indicating that it has an influence on the mortality of *Aedes aegypti* larvae. Duncan's test was

used to determine whether there was a significant difference in each extract concentration, with the finding that at a concentration of 6250 ppm, there was a significant difference with the highest value of 51.33. As a result, when compared to other concentrations, the concentration of 6250 ppm had the highest effect.

**Table 4**  
**Number of *Aedes aegypti* Larvae Mortality After Giving Combination Breadfruit Leaf and Flower Extract**

Consentration	Replication	Number of Dead Larvae			Mortality Presentation in 24 hours (%)
		6 hours	6 hours	6 hours	
1000 ppm	I	2	6	11	55
	II	4	7	12	
	III	3	7	10	
2750 ppm	I	10	12	16	83,3
	II	10	14	17	
	III	12	13	17	
4500 ppm	I	15	16	19	91,6
	II	16	18	18	
	III	16	17	18	
6250 ppm	I	18	18	20	98,3
	II	17	18	20	
	III	17	19	19	

After processing the data through the Kolmogorov-Smirnov normality test, it was discovered that the larvae mortality data were normally distributed with a significance value of  $>0.05$ . Combination of breadfruit leaf and flower extracts was then analyzed using One Way ANOVA with a significance value of 0.05, indicating that it has an influence on the mortality of *Aedes aegypti* larvae. Duncan's

test was used to determine whether there was a significant difference in each extract concentration, with the finding that at a concentration of 6250 ppm, there was a significant difference with the highest value of 55.33. As a result, when compared to other concentrations, the concentration of 6250 ppm had the highest effect.

#### LC<sub>50</sub> and LT<sub>50</sub> Values

A Probit analysis was performed using SPSS for Windows 16.0 software to determine the values of  $LC_{50}$  and  $LT_{50}$  in each extract. According to the results of the probit analysis of breadfruit leaf extract, the estimated  $LC_{50}$ , which can kill 50% of larvae in 24 hours, is 1871 ppm, with an interval between 1253 and 2437 ppm, and the  $LT_{50}$ , which can kill the larvae up to 50%, is estimated to be 4.2 hours with a 0 to 8-hours interval after treatment. From probit analysis of breadfruit flower extract, the estimated  $LC_{50}$ , which can kill 50% of larvae in 24 hours, is 2531 ppm, with an interval between 2120 and 2959 ppm, and the  $LT_{50}$ , which can kill the larvae up to 50%, is estimated to be 10.8 hours with a 6 to 19.8 hours interval after treatment. From probit analysis of combination breadfruit leaf and flower extract, the estimated  $LC_{50}$ , which can kill 50% of larvae in 24 hours, is 903 ppm, with an interval between 558 and 1204 ppm, and  $LT_{50}$ , which can kill the larvae up to 50%, is estimated to be 3.7 hours with a 2 to 5 hours interval after treatment.

At the  $LC_{50}$ , the result for the combined index value is 0.82. According to [Chou & Martin \(2004\)](#), this result shows that the combination of leaf and flower extracts of *Artocarpus altilis* has low synergistic properties in the category of interaction characteristics of the combination extracts. Meanwhile, the  $LC_{90}$  result was 0.82, indicating that the combination of breadfruit leaf and flower extracts has a low synergistic character, according to *Artocarpus altilis*.

#### **Effect of Breadfruit's Leaf Extract on Mortality of *Aedes aegypti* Larvae**

After giving the breadfruit leaf extract to the test larvae, the maximum concentration of 6250 ppm caused the most mortality, with 86.6% of the test larvae was died. As a result, breadfruit leaf extract can be utilized as an alternative bioinsecticide. The high amount of active compounds such as alkaloids, tannins, and flavonoids was assumed to be the cause

of the test larvae's mortality. The alkaloids contained in breadfruit leaves are able to slow down the growth hormone of the larvae, which causes the larvae to be unable to metamorphose, causing death due to a decrease in the formation of nitrate which is used to synthesize protein, and is able to restrain the distribution of sucrose into the small intestine ([War et al., 2012](#)). There are two methods for tannin compounds to enter the body of a larva: through penetrating the larva's body wall and through the digestive tract ([Tiwari, 2012](#)). Flavonoids work as inhibitors of the respiratory system in larvae. Flavonoids enter through the respiratory tract of the larvae, it causes nerve weakness and damages the respiratory tract which causes the larvae to be unable to breathe. Flavonoids also play a role by slowing the action of the acetylcholinesterase enzyme, the enzyme is useful in the breakdown of acetylcholine into acetyl Co-A and choline on nerve cell impulses. Due to the decrease in the work of the acetylcholinesterase enzyme, it causes a buildup of acetylcholine which can result in disturbances in the impulse conducting system from nerve cells to muscle cells, and causes muscles to spasm, paralysis and then ends in the death of the larvae. ([Rattan, 2010](#)). According to [Kurniawan \(2014\)](#) research, the breadfruit leaf can be utilized as a natural larvicide because it kills mosquito larvae. The high rate of larval mortality indicates this, although it cannot be compared with chemical larvicides. Bioinsecticides, such as those made from breadfruit leaves, are better for the environment, animals, and humans ([Kurniawan et al., 2020](#)). As a result, breadfruit leaf extract has the potential to be a natural larvicide by reducing the number of mosquito larval *Aedes aegypti* mortality. According to a study conducted by [Rosmawaty \(2013\)](#), the crude extract of the leaves of breadfruit contains various secondary metabolite chemicals, including alkaloids, steroids, terpenoids, and

flavonoids. These chemicals are thought to be effective at killing *Aedes aegypti* larvae (Rosmawaty, 2013). From this study which using breadfruit leaf extract, it was found that breadfruit flower extract can be used as an alternative bioinsecticides.

#### **Effect of Breadfruit's Flower Extract on Mortality of *Aedes aegypti* Larvae**

The data from the experiment indicated from the treatment of breadfruit flower extract showed that the highest concentration of 6250 ppm was capable of killing 90% of the test larvae. As a result, breadfruit flower extract can be used as a alternative bioinsecticides. High amounts of saponins and flavonoids were assumed to be the causes of the test larvae's mortality. Saponins can have a bitter effect on the larvae, causing them to lose their interest in food and eventually die. Saponin can harm the waxy coating that protects the outer body of the larvae, causing the larvae to lose a lot of fluid and eventually die (Prakoso, Gandung, 2016). In larvae, flavonoids act as respiratory system inhibitors. This substance enters the larvae's respiratory tract, causing neurological weak and damage the respiratory tract, stopping the larvae from breathing (Rattan, 2010). Jones (2012) identified fatty acids that are more effective than N,N-diethyl-m-toluamide (DEET) in an identification test of chemical compounds found in male breadfruit flowers, suggesting that male breadfruit flower could be one of the natural ingredients to repel mosquitoes (Jones AM, Klun JA, Cantrell CL, Ragone D, Chauhan KR, Brown PN, 2012). DEET is the active ingredient commonly used in many insect repellent products (Medicine, 2021). Breadfruit flower extract was shown to be a potential option for developing bioinsecticides to control mosquito larvae *Culex* in a previous study by (Gladys & Bukola, 2019).

#### **Effect of Combination Breadfruit's Leaves and Flower Extract on Mortality of *Aedes aegypti* Larvae**

The data from the experiment indicated from the treatment of breadfruit flower extract showed that the highest dose of 6250 ppm was capable of killing up to 98.3% of the test larvae. The combination index calculation shows that the combination of leaf extract and breadfruit flower in LC<sub>50</sub> and LC<sub>90</sub> is weakly synergistic. However, at the maximum dose, the combined toxicity of the two extracts was higher than the toxicity of each single extract. At the highest concentration, a single extract of breadfruit leaves was able to kill an average of 86.6%, a single extract of breadfruit flowers was able to kill an average of 90%, and the combination of breadfruit leaves and flowers was able to kill up to 98.3%. The synergistic aspect of the two extracts may be due to the fact that each extract has the same amount of active substances, such as flavonoids and saponins. The toxic effect of combination extracts from breadfruit leaves and flowers can be detected in the clinical symptoms that show in larvae, which include slowed movement, shriveled, weak bodies, and eventually death. Treatment with combination extracts of breadfruit leaves and flowers was expected to get a more toxic effect than treatment with single extracts in this research. Breadfruit leaves contain a variety of secondary metabolites, including alkaloids, steroids, terpenoids, flavonoids, and saponins, according to Rosmawaty (2013). Breadfruit flowers contain saponins, polyphenols, and flavonoids, according to Nikmah (2016). Several types of toxic compounds contained in single extracts of breadfruit leaves and flowers have similarities because they are still the same type of plant, so that if the two single extracts are mixed it is expected that the content of toxic compounds will be stronger. Although the combination index value showed minimal synergistic outcomes, the results of this study showed that the mortality of larvae was higher when given a combination of two

extracts when compared to the mortality of *Aedes aegypti* larvae in a single extract.

From the probit analysis,  $LT_{50}$  of combination extract leaves and flowers breadfruit obtained at 2 hours after giving the treatment, the value is much faster when compared to the  $LT_{50}$  for each single extract. The results of this study are have similarity with the statement of Vastrad (2002) in Taufika (2020), that the combination of two plant extracts containing bioactive compounds that have synergistic properties is more effective as a larvicide and controlling disease vectors when compared to a single extract. Because plant sources of bioinsecticides are not always available in large quantities in an area, the use of a synergistic combination of extracts from bioinsecticides can reduce the amount of raw material used compared to single extracts of bioinsecticides, allowing them to overcome if there is a shortage of raw materials at the farm level. The use of a combination of bioinsecticides at lower doses can also reduce unwanted effects on non-target organisms and the environment. Resistance can also be prevented by using a combination of natural pesticides with various modes of action (Abizar & Prijono, 2010). Bioinsecticides have various advantages over chemical insecticides, including a relatively simple manufacturing procedure and the ability to make them individually. Bioinsecticides also leave less residue in the environment, making them safer than chemical pesticides. Bioinsecticides have toxic chemicals that decompose quickly, preventing resistance at the target. The disadvantage of utilizing bioinsecticides is that they include various active ingredients or complex active chemicals that are sometimes difficult to detect and cannot be generalized due to the influence of diverse plant growth places, temperatures, plant ages, soil types, and harvest periods. It makes the active

compounds become very varied (Hamsir & Fahmi, 2019).

### Conclusions

Based on the study of Bioinsecticide from Combination Extract of Leaf and flower of Breadfruit (*Artocarpus altilis*) on Mortality of *Aedes aegypti* larvae, it concluded that using leaf extract, flower extract, and combination of breadfruit leaf and flower extract has an effect on *Aedes aegypti* larvae mortality. When compared to single extract, combination of breadfruit leaf and flower extracts showed the highest mortality in killing *Aedes aegypti* larvae after 24 hours of treatment with the same serial concentration of each extract. At the highest concentration of 6250 ppm, combination of breadfruit leaf and flower extracts was able to kill larvae up to 98.3%. The  $LC_{50}$  value was reached at concentration 903 ppm in a combination of breadfruit leaf extract and flower extract, and the  $LT_{50}$  value of breadfruit leaf extract was 3.7 hours after treatment.

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