

## ***Durio zibethinus* Murr Extracts as Potential Larvacide Against *Anopheles aconitus* Donitz and *Anopheles maculatus* Theobald (Diptera: Culicidae)**

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**Abstract:** Malaria is endemic in most parts of Indonesia. *Anopheles aconitus* and *Anopheles maculatus* are examples of dominant malaria vectors in Indonesia. Plant secondary metabolites can be used as malaria vector control that is safe for the ecosystem, one of which is *Durio zibethinus* leaves which are thought to have active compounds so that they can be used as botanical larvicides. This study aimed to determine the potency of *D. zibethinus* leaf extract against *An. aconitus* and *An. maculatus* and to determine the concentration, type of extract and content of secondary metabolites of *D. zibethinus* leaves. Determination of LC<sub>50</sub> and LC<sub>90</sub> using probit analysis. Maceration and remaceration were used to produce the extract and phytochemical screening to determine the active compound of *D. zibethinus*. The WHO insecticide bioassay testing procedure was used for the larvicide test. Ethanol extract of *D. zibethinus* leaf as a larvicide for *An. aconitus* second and third instar and larvae of *An. maculatus* second and third instar had LC<sub>50</sub> respectively: 480; 520; 510; and 540 ppm and LC<sub>90</sub> respectively: 750; 760; 760; and 810 ppm. *D. zibethinus* leaf aqueous extract as larvicides against larvae of *An. aconitus* second and third instar and larvae of *An. maculatus* second and third instar had LC<sub>50</sub> values respectively 14,500; 16,400; 22,100; and 23,300 ppm and LC<sub>90</sub> respectively, 26,100; 27,200; 30,600; and 33,700 ppm. Ethanol extract of *D. zibethinus* leaves was more effective as a larvicide. Alkaloid, saponins and tannins were found in the leaves of *D. zibethinus* through phytochemical screening.

**Keywords:** *An. aconitus*, *An. maculatus*, botanical larvicide, *Durio zibethinus*.

### Introduction

Insects have various roles in the ecosystem which humans classify into beneficial or harmful insects. Insects that are considered harmful include insects that act as disease vectors. *Anopheles* is a genus of members of the Order Diptera which is a vector for malaria (Gillot, 2005; Munif, 2019; Sinka, 2020). *An. aconitus* and *An. maculatus* is 2 of 10 malaria vectors in Indonesia. The use of chemical insecticides to control malaria vectors has had a negative impact, for example the case of *Anopheles* experiencing resistance (Soerono et al., 1965; Namountougou et al, 2019; Chukwuekezie, 2020).

Plants have a variety of secondary

metabolite compounds so that they can be used as alternative insecticides that are safer. Santi, (2011) found that durian skin extract (*Durio zibethinus* Murr) was effective as a controller of *Aedes spp.* imago (adult) phase. However, durian bears fruit according to the season so it cannot be obtained all the time. Thus, one of the organs that is always available from the durian plant, the leaves, needs to be investigated to be used as an alternative. The leaves, stems and bark of *D. zibethinus* contain secondary metabolites including alkaloids, flavonoids, saponins, and tannins (Brown, 1997; Nurliani, 2007). These various active compounds are expected to be used as botanical larvicides. Phytochemical screening was carried out to determine the secondary

metabolite content of *D. zibethinus* leaves.

Ethanol is often used as a solvent in the extraction process because it is safer than other organic solvents. Generally, people use water as a solvent. Our study was conducted to determine which type of *D. zibethinus* leaf extract was more effective as a larvicide. This study tested the effectiveness of *D. zibethinus* leaf extract against *An. aconitus* and *An. maculatus* after 24 hours of exposure as indicated by the values of LC<sub>50</sub> and LC<sub>90</sub>.

## Material and Methods

### Materials of research

The material that have been used are larvae of *An. aconitus* and *An. maculatus* which were the second and third instars obtained from the B2P2VRP Salatiga laboratory culture and the leaves of *D. zibethinus* Menoreh *Kuning* variety from Kulonprogo Regency, Yogyakarta. The materials used for the extraction were ethanol and water solvents (aquades). The materials used for the phytochemical test were Mg powder, concentrated HCl, HCl 2N, bismuth subnitrate, KI, FeCl<sub>3</sub> and aquades.

### Procedure

#### 1. Preparation of *D. zibethinus* leaf extract

The leaves of *D. zibethinus* were washed and dried and then powdered. Powder weighing 200 g was put into a glass jar and then 2 L of ethanol was added and macerated for 48 hours and then filtered. The extract solution was then evaporated. For water solvents, remaceration was carried out 2 times. Leaf powder as much as 500 g was macerated using 4 L of water for 48 hours. The extract solution was then evaporated. Stock solution was made according to the standard procedure of WHO (2005) with some modifications. The volume of the stock solution of 10,000 ppm ethanol extract was 240 mL, which was in the form of 2.4 g of extract added to 240 mL of ethanol solvent. The stock solution of aqueous extract was 40,000 ppm as much as 4,000 mL.

#### 2. Preliminary Test

There were 6 concentrations of ethanol extract solution, 62.5; 125; 250; 500; 1,000; and 2,000 ppm (v/v) and 6 concentrations of aqueous extract of *D. zibethinus* leaf extract, 10,000;

15,000; 20,000; 25,000; 30,000; and 35,000 ppm. Seven plastic cups were prepared (six for treatment and one for control). Each treatment glass was filled with different concentrations of *D. zibethinus* leaf extract. The control medium for the ethanol extract was 99 mL of water plus 1 mL of ethanol, while the control medium for the aqueous extract was 100 mL of water. Ten larvae were added to each test glass. Furthermore, the number of larvae mortality was observed after 24 hours.

#### 3. Advanced Test

From the 6 pre-test concentrations, 4 concentrations were selected for the toxicity test of *D. zibethinus* leaf extract. The principle used in the follow-up test was the same as the preliminary test. However, further tests used 3 replications.

#### 4. Phytochemical Screening

Phytochemical tests were carried out based on the method of Imaniar *et al.* (2013) with some modifications. To determine the presence of flavonoids in *D. zibethinus* leaves, 1 mL of sample extract was put into a test tube, then 1 gram of Mg powder and concentrated HCl solution were added. The color change of the solution to red indicates the extract contains flavonoid compounds. To determine the presence of alkaloids, 1 mL of sample extract was put into a test tube, then Dragendorff's reagent was added to it. How to make Dragendorff's reagent: 0.6 g of bismuth subnitrate was put into 2 mL of concentrated HCl and 10 mL of distilled water and 6 g of KI was put into 10 mL of distilled water. The two solutions were mixed with 7 mL of concentrated HCl and 15 mL of distilled water. The presence of a brick red precipitate indicates that the extract contains compounds belonging to the alkaloid group. To determine the presence of tannins (polyphenols), 1 mL of sample extract was put into a test tube, then 2-3 drops of FeCl<sub>3</sub> solution was added. The color change to black indicates that the extract contains tannins. To determine the presence of saponins, 2 mL of sample extract was put into a test tube, then added with distilled water and shaken vigorously for 10 minutes. If foam is formed which is stable for 30 minutes and does not disappear on the addition of 1 drop of 2 N HCl, it indicates the presence of a compound of the saponin group.

## Data Analysis

Data were analyzed by Analysis of variance (ANOVA), Duncan Multiple Range Test (DMRT) and Probit analysis (Finney, 1971). ANOVA was carried out to determine the difference in the killing power of *D. zibethinus* leaf extracts against larvae of *An. aconitus* and *An. maculatus*. DMRT was carried out to determine the difference significant effect of extract between concentration and control. Probit analysis was carried out by comparing LC<sub>50</sub> and LC<sub>90</sub> of each leaf extract of *D. zibethinus* on mortality of mosquito larvae.

## Results and discussion

### *Durio zibethinus* leaf extract and phytochemical screening

The percentage of yield aqueous extract was higher than of ethanolic extract, indicating that the secondary metabolite compounds of *D. zibethinus* leaves were more polar or the particles of compounds that could be attracted by the water solvent were larger than the compound particles which were attracted by the ethanol solvent. Isolation of compounds from green tissue using ethanol was associated with the withdrawal of chlorophyll, causing the extract to have a dark green color. The brown water extract of *D. zibethinus* leaves was thought to be contributed by the extraction of natural polar dye compounds (pale yellow-dark brown) (Harborne, 1987).

Table 1. Extraction results of *D. zibethinus* leaves

Solvent	Extraction Method	Weight of leaf powder	Yield result	Percentage	Total
Etanol	Maceration	200 g	12,8 g	6,4 %	12,8 g
Water	Re-Maceration I	500 g	84,5 g	16,9 %	169,1 g
	Re-Maceration II	500 g	80,9 g	16,18 %	

Table 2. Content of secondary metabolites of *D. zibethinus* leaves

Compound	Ethanol extract	Water extract
Flavonoid	–	–
Alkaloid	+	–
Tannin (polyphenols)	+	+
Saponin	–	+

Description: (+) contains compounds; (–) contains no compounds.

### Preliminary Test Toxicity of *D. zibethinus* leaf extract

### extract

Table 3 shows the number of mortality among larvae *An. aconitus* and *An. maculatus* did not differ much, probably because it was not repeated so that there was no mean value that could better indicate the difference in the number of mortality between the two types of larvae. From the results of the preliminary test of the ethanolic extract of the leaves of *D. zibethinus*, a concentration of 250-1,000 ppm was used as a benchmark to make 4 concentrations in the further test.

Table 3. Observations of the preliminary toxicity test of the ethanol extract of *D. zibethinus* leaves after 24 hours of exposure.

Species	Number of larval deaths (%) at various concentration (ppm)						
	0	62,5	125	250	500	1.000	2.000
Second Instar of <i>An. aconitus</i>	0	0	10	20	40	100	100
Third Instar of <i>An. aconitus</i>	0	0	0	10	30	100	100

Second Instar of <i>An. maculatus</i>	0	0	10	10	30	100	100
Third Instar of <i>An. maculatus</i>	0	0	0	10	30	100	100

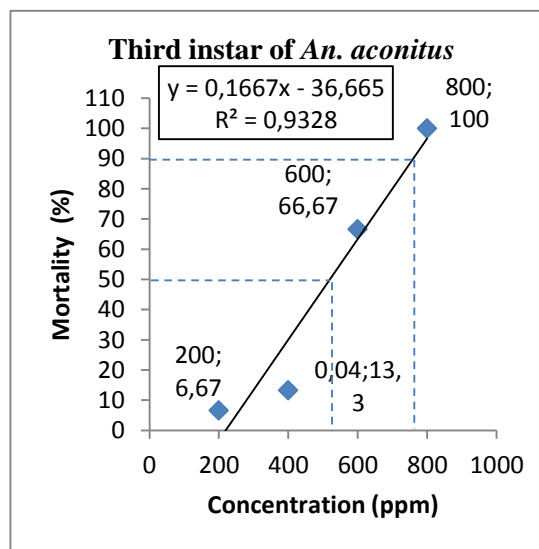
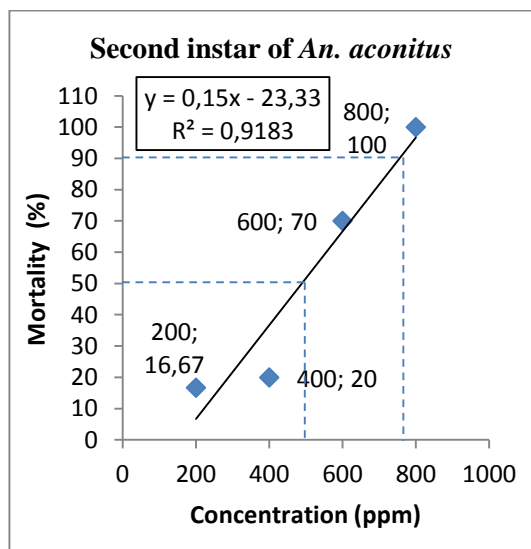
Table 4. The results of the preliminary test of the toxicity of the aqueous extract of *D. zibethinus* leaves after 24 hours of exposure.

Species	Number of larval deaths (%) at various concentration (ppm)						
	0	10.000	15.000	20.000	25.000	30.000	35.000
Second Instar of <i>An. aconitus</i>	0	20	60	90	100	100	100
Third Instar of <i>An. aconitus</i>	0	10	40	90	100	100	100
Second Instar of <i>An. maculatus</i>	0	10	40	40	50	60	100
Third Instar of <i>An. maculatus</i>	0	10	10	20	60	80	100

A striking difference in the number of larval mortality was seen in each concentration, where the larvae of *An. aconitus* were more susceptible to the aqueous extract of *D. zibethinus* leaves than the larvae of *An. maculatus*. Different species have different abilities in responding to poisons or toxic substances. Species differences are related to genetic factors, namely the ability of cells to respond to toxins. Cells are organized in tissues and have specific instructions that must be obeyed. These

specific instructions differ between the cells that make up the network in a species because there are differences in the flow of information, especially in cells between two different species (Hayes, 2011). The concentration of 10,000-35,000 ppm was used as a benchmark to make 4 concentrations in the follow-up test.

#### Advanced Test (Efficacy of *D. zibethinus* Leaf Extract)



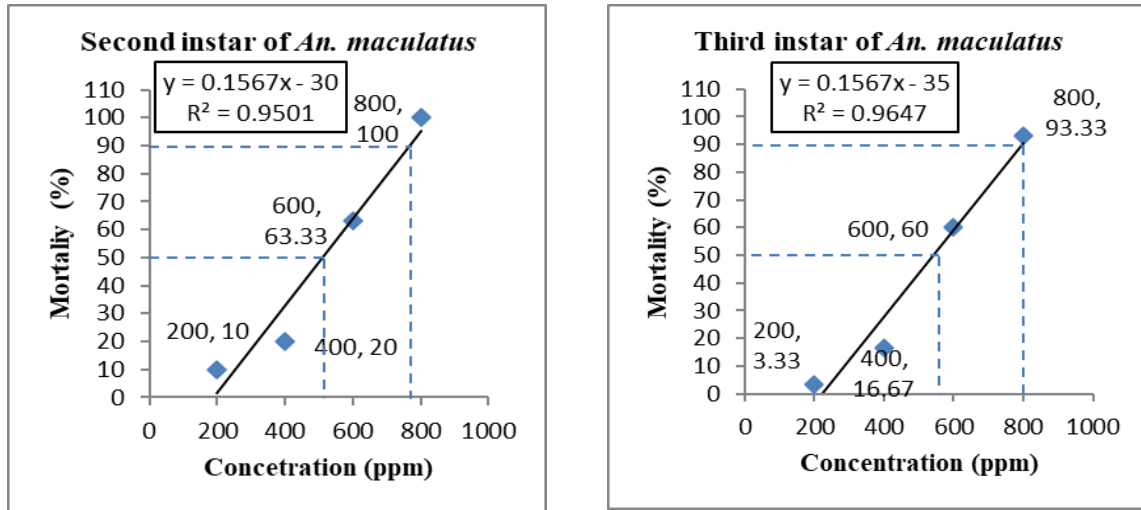


Figure 1. Linear regression equation curve for the concentration of *D. zibethinus* leaf ethanol extract with Probit % larval mortality

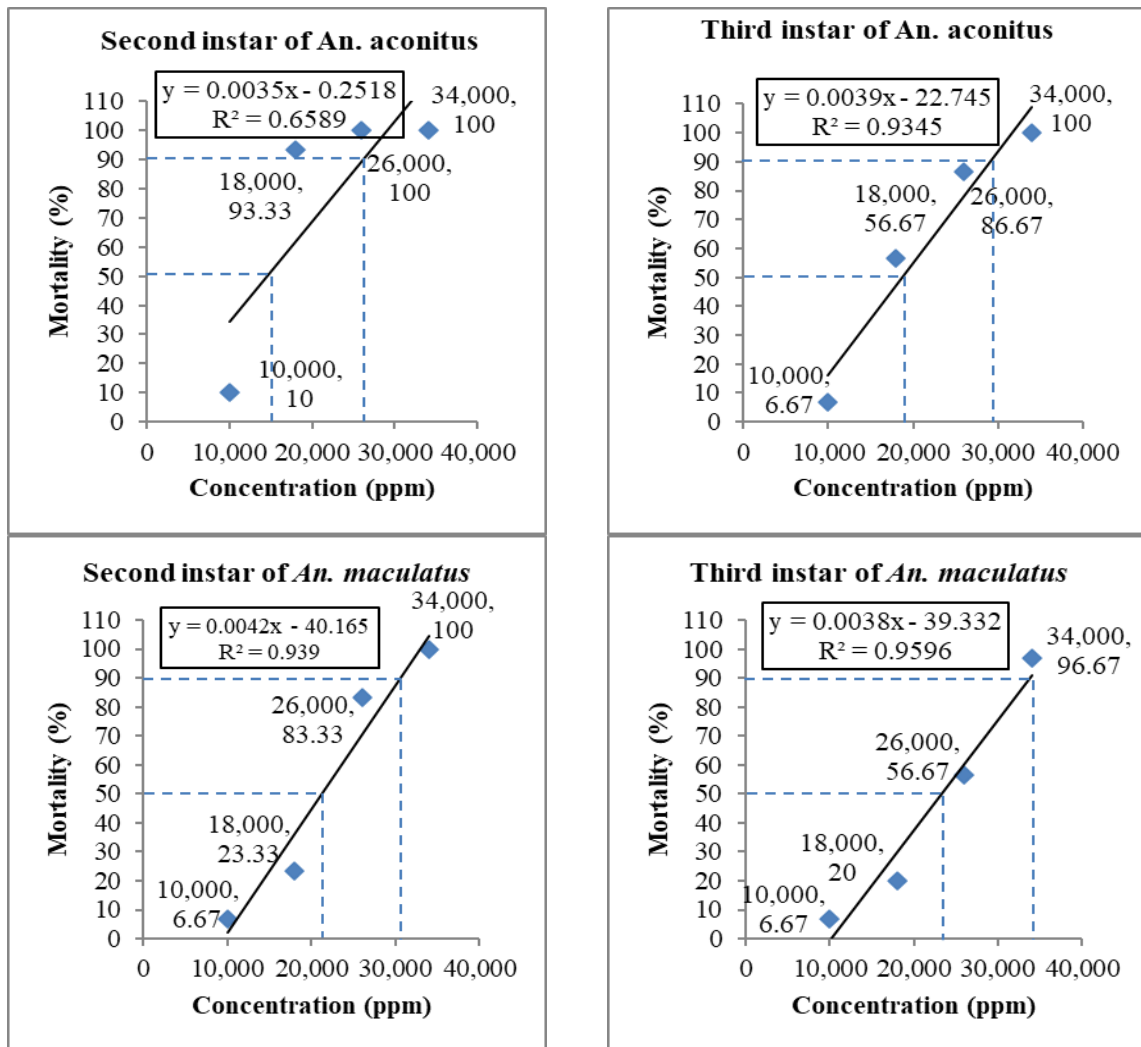


Figure 2. Linear regression equation curve of *D. zibethinus* leaf aqueous extract concentration with Probit % larval mortality

Table 5. Statistical analysis of larval mortality of *An. aconitus* and *An. maculatus* second and third instars after 24 hours of exposure to *D. zibethinus* leaf ethanol extract

Species		Mean larval mortality (%) at various concentrations of Ethanol (ppm) ± standard deviation				
		0	200	400	600	800
<i>An. aconitus</i>	Second instar	0,00±0,00 <sup>a</sup>	16,67±5,77 <sup>b</sup>	20,00±0,00 <sup>b</sup>	70,00±10,00 <sup>c</sup>	100,00±0,00 <sup>d</sup>
	Third Instar	0,00±0,00 <sup>a</sup>	6,67±5,77 <sup>ab</sup>	13,33±5,77 <sup>b</sup>	66,67±5,77 <sup>c</sup>	100,00±0,00 <sup>d</sup>
<i>An. maculatus</i>	Second instar	0,00±0,00 <sup>a</sup>	10,00±0,00 <sup>b</sup>	20,00±0,00 <sup>c</sup>	63,33±5,77 <sup>d</sup>	100,00±0,00 <sup>e</sup>
	Third Instar	0,00±0,00 <sup>a</sup>	3,33±5,77 <sup>a</sup>	16,67±5,77 <sup>b</sup>	60,00±0,00 <sup>c</sup>	93,33±5,77 <sup>d</sup>

Table 6. Statistical analysis of larval mortality of *An. aconitus* and *An. maculatus* second and third instars after 24 hours of exposure to aqueous extract of *D. zibethinus* leaves

Species		Average larval mortality (%) at various concentrations (ppm) ± standard deviation				
		0	10.000	18.000	26.000	34.000
<i>An. aconitus</i>	Second instar	0,00±0,00 <sup>a</sup>	10,00±10,00 <sup>b</sup>	93,33±5,77 <sup>c</sup>	100,00±0,00 <sup>c</sup>	100,00±0,00 <sup>c</sup>
	Third Instar	0,00±0,00 <sup>a</sup>	6,67±5,77 <sup>a</sup>	56,67±5,77 <sup>b</sup>	86,67±5,77 <sup>c</sup>	100,00±0,00 <sup>d</sup>
<i>An. maculatus</i>	Second instar	0,00±0,00 <sup>a</sup>	6,67±5,77 <sup>a</sup>	23,33±5,77 <sup>b</sup>	83,33±5,77 <sup>c</sup>	100,00±0,00 <sup>d</sup>
	Third Instar	0,00±0,00 <sup>a</sup>	6,67±5,77 <sup>a</sup>	20,00±10,00 <sup>b</sup>	56,67±5,77 <sup>c</sup>	96,67±5,77 <sup>d</sup>

Table 7. LC<sub>50</sub> and LC<sub>90</sub>

Extract	Species	LC <sub>50</sub> (ppm)	LC <sub>90</sub> (ppm)
Ethanol	<i>An. aconitus</i>	Second instar	480
		Third Instar	510
	<i>An. maculatus</i>	Second instar	510
		Third Instar	540
Water	<i>An. aconitus</i>	Second instar	14.530
		Third Instar	18.770
	<i>An. maculatus</i>	Second instar	21.210
		Third Instar	23.300

The increase in the number of larval deaths occurred along with the increase in extract concentration. The second instar of *An. maculatus* was more susceptible to larvicides of ethanol extract and aqueous extract of *D. zibethinus* leaves than the third instar, as well as the larvae of *An. aconitus*. Younger individuals are often more susceptible to food poisoning because younger individuals eat more than older individuals in the proportion of food to body weight. If the food of both individuals is given a toxic substance, the young individual receives a higher dose of the poison than the older

individual (Hayes & Laws, 2010; Kheloul, 2020).

The ethanol extract contains alkaloids and tannins, while the aqueous extract of *D. zibethinus* leaves contains tannins and saponins. Alkaloids are a class of compounds that have several modes of action as insecticides, one of which according to Copping, (2004) is that it has neurotoxic activity by slowing the Na<sup>+</sup> channel to close and disrupting membrane depolarization. This causes paralysis (larvae are not actively moving despite being disturbed) before death. Tannins are also widely known to have a toxic effect on insects, as stated by

Barbehenn & Constabel, (2011) in Mrdaković *et al.*, (2013), in insects with a high gut pH, tannins are prone to act as prooxidants. They produce reactive oxygen species, which can damage nutrients and/or midgut tissue and accordingly influence insect performance. If the larvae nutrients and/or midgut tissue is damaged, their development will be disrupted and then die. Saponins have toxicity that can harm insects, such as anti-feeding, disrupting the molting process, regulating growth, mortality, and others (Chaieb, 2010; Cui 2019). The insecticidal activity of saponins that interfere with the molting process is based on their interaction with cholesterol which causes disruption of the synthesis of ecdysteroids, one of the important hormones in the molting process. Saponins are also protease inhibitors. The inhibited protease enzymes can cause digestion or protein formation in the larvae to be disrupted so that the larvae are malnourished and then die. Another characteristic of saponins is that they are toxic to cells (cytotoxic).

In general, the results showed that ethanol extract was more toxic to both types of *Anopheles* larvae. Similar results are also the same as the results of several researchers who compared the effectiveness of ethanol and water extracts from various plants to kill mosquito larvae, such as those stated by Ivoke *et al.*, (2010); Nagappan, (2012); and Ubulom *et al.*, (2012). The toxicity of a material is influenced by the content of the compound it has (Hayes & Laws, 2010; Lushchak, 2018). Alkaloids and tannins as active compounds dissolved by ethanol may be more toxic or in greater quantity when compared to saponins and tannins produced by water solvents. For this reason, it is necessary to carry out further research to determine the toxicity of each of these compounds to *An. aconitus* and *An. maculatus*.

## Conclusion

*D. zibethinus* leaf extract contains alkaloids, saponins and tannins which have the potential as larvicides to kill second and third instar of *An. aconitus* and *An. maculatus*. Compared to aqueous extract, the ethanolic extract of *D. zibethinus* leaves was more effective as a botanical larvicide. Ethanol extract of *D. zibethinus* leaf as a larvicide for *An. aconitus* second and third instar and larvae of *An. maculatus* second and third instar had LC<sub>50</sub> respectively: 480; 520; 510; and 540 ppm and

LC<sub>90</sub> respectively: 750; 760; 760; and 810 ppm. *D. zibethinus* leaf aqueous extract as larvicides for larvae of *An. aconitus* second and third instars and larvae of *An. maculatus* second and third instar had LC<sub>50</sub> values respectively 14,500; 16,400; 22,100; and 23,300 ppm and LC<sub>90</sub> respectively, 26,100; 27,200; 30,600; and 33,700 ppm.

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