

## Larvicidal Effects of *Tithonia diversifolia* (Hemsley) A. Gray Leaf Water Extract against *Culex sp.* Larvae

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### Abstract

**Background:** There are various vector mosquitoes that cause filariasis such as *Culex sp.* Controlling the number of vectors with larvicide can reduce the spread of this disease. The extracts of *Tithonia diversifolia* (Hemsley) A. Gray leaves are known to have larvicidal effects on *Aedes aegypti* larvae. The purpose of this study is to determine the larvicidal effects of *Tithonia diversifolia* (Hemsley) A. Gray leaf water extract against *Culex* larvae.

**Methods:** This was an experimental study conducted during the period of October to November 2012 at the Laboratory of Biochemistry, Faculty of Medicine, Universitas Padjadjaran. First trials were conducted to find the most effective larvicidal concentration of *Tithonia diversifolia* (Hemsley) A. Gray leaf water extract in killing 25 *Culex* larvae. A concentration of 100 ml was decided as the testing concentration which would be compared to a positive control containing Abate® and a negative control containing distilled water. The number of dead larvae was calculated every hour for 48 hours.

**Results:** Effective larvicidal concentration of *Tithonia diversifolia* (Hemsley) A. Gray leaf water extract was 60% ( $p=0.000$  compared to negative control), which was lower than Abate®. Result also showed LC50 and LC90 of 24% and 42%, respectively.

**Conclusions:** *Tithonia diversifolia* (Hemsley) A. Gray leaf water extract has a larvicidal effect on *Culex sp.* larvae, although it is not as effective as Abate®, Hence, this leaf may be used as an alternative larvicide as these plants are easily available in the community and inexpensive.

**Key words:** *Culex sp.*, filariasis, larvicide, *Tithonia diversifolia* (Hemsley) A. Gray

### Introduction

Filariasis is a disease caused by the filarial worm borne by vectors such as mosquitoes. There are 337 districts/cities in Indonesia that become endemic sites for filariasis.<sup>1</sup> One of the vectors is the genus of *Culex*.<sup>2</sup> One way to control the mosquito-borne diseases such as filariasis is by controlling the mosquitoes population itself.<sup>3</sup> Controlling the number of vector can be done by manipulating the mosquitoes breeding places or by using chemical materials.<sup>4</sup>

Abate® has been used for about 30 years, since it is provisioned as the eradication program of *Aedes aegypti*. With the use of Abate® in long run, it is possible that the resistance of larvae has emerged. Some studies mentioned that the resistance of mosquito *Culex sp.*, for some classes of insecticides, including organophosphates group occurs

more rapidly compared to *Aedes aegypti*.<sup>5</sup>

Developing pesticides from natural materials still continues to rise as there are limited side effects arised compared to those of chemical pesticides. Toxic effects that occur from the chemical larvicides are their effect to non-target organisms such as to humans, and there is also likelihood of mosquito resistance to the pesticide.<sup>6</sup>

The *Tithoniadiversifolia* (Hemsley) A. Gray leaves or better known as the flower leaf moon, is regarded by the public as the weeds which grow wildly. *Tithoniadiversifolia* (Hemsley) A. Gray leaves extract is well known to contain flavonoids, tannins, that possess larvicidal effect against larvae of the mosquito *Aedes aegypti*.<sup>7</sup>

The aim of this study is to investigate whether *Tithoniadiversifolia* (Hemsley) A. Gray leaves are potential to be a green larvicide that

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**Table 1 Effective Concentration of Water Extract of *Tithoniadiversifolia (Hemsley) A. Gray* leaves**

Concentration	Number of Larvae	Number of Dead Larvae	Time of Dead Larvae (Hours)
15%	25	8	-
30%	25	8	-
45%	25	19	-
60%	25	25	30
75%	25	25	33
90%	25	25	34
100%	25	25	39

can be used in the wider community or not. To determine the potential active compounds present in the *Tithoniadiversifolia (Hemsley) A. Gray* leaves, the values of LC<sub>50</sub> and LC<sub>90</sub> will also be determined.<sup>8</sup>

### Method

This was an experimental study conducted during the period of October to November 2012 at Laboratory of Biochemistry Faculty of Medicine Universitas Padjadjaran. First trials were conducted to find the most effective concentration of water extract of *Tithoniadiversifolia (Hemsley) A. Gray* leaves.

The 100 ml of decided concentration was then tested for larvicidal effect of 25 *Culex* larvae compared to a positive control containing Abate® and negative control containing distilled water. The number of dead larvae was calculated in 48 hours once every hour. Effective concentration was decided at most effective time of all larvae were found dead.

*Culex sp.*, larvae was obtained from the School of Life Sciences and Technology of Bandung Institute of Technology (SITH-ITB) with inclusion criteria larvae *Culex sp.* that reached instar III or IV.

The data were processed using SPSS 15.0 for Windows. The larvicidal effect was analyzed

**Table 2 Larvicidal Effect of Water Extract of *Tithoniadiversifolia (Hemsley) A. Gray* leaves**

Group		Number of Larvae	Number of Dead Larvae	Time of Dead Larvae (Hours)
60% concentration	1	25	25	34
	2	25	25	34
	3	25	25	32
	4	25	25	34
	5	25	25	33
Distilled water	1	25	1	-
	2	25	0	-
	3	25	0	-
	4	25	1	-
	5	25	0	-
Abate®	1	25	25	2
	2	25	25	2
	3	25	25	1
	4	25	25	2
	5	25	25	1

**Table 3 Statistical Analysis**

	Parameter	Significance Value (2-tailed) Alpha=5%
<i>Tithoniadiversifolia (Hemsley) A. Gray</i> leaves 60% Concentration-distilled water	number of dead larvae	0,000
<i>Tithoniadiversifolia (Hemsley) A.Gray</i> leaves 60% Water Extract-Abate	number of dead larvae	0,000

using Mann-Whitney test. P value <0.05 was considered as significant. LC<sub>50</sub> and LC<sub>90</sub> values were analyzed using probit test.

## Results

First, there were 7 different concentration of water extract of *Tithoniadiversifolia (Hemsley) A. Gray* leaves; 15, 30, 45, 60, 75, 90, and 100% Evaluated for the most effective concentration. Each concentration was tested into a well consists of 25 *Culex sp.*, larvae and the larvicidal effect was calculated in 48 hours once every hour. The 60% concentration was found to be the concentration that can kill all larvae *Culex sp.*, in most effective time.

The 100 ml of 60% concentration was then tested for larvicidal effect compared to a positive control containing Abate® and negative control containing distilled water. Although less effective than Abate®, there was a potential larvicidal effect of water extract of *Tithoniadiversifolia (Hemsley) A. Gray* leaves compared to negative control.

Statistically, the 60% water extract of *Tithoniadiversifolia (Hemsley) A. Gray* leaves concentration was effective in killing the *Culex* larvae (p=0.000 compared to negative control), although we found a lower effect compared to Abate®.

Research model probit  $Y = -2.505 + 5.425 X$ , where Y is the probit, and X is log concentration of the water extract was used to determine LC<sub>50</sub> and LC<sub>90</sub>. From the equation, the LC<sub>50</sub> of water extract of *Tithoniadiversifolia (Hemsley) A. Gray* leaves was 24%, while the LC<sub>90</sub> was 42%.

## Discussion

The purpose of this study is to determine the larvicidal effects of boiled water of *Tithoniadiversifolia (Hemsley) A. Gray* leaves against the mosquito larvae *Culex sp.* The study also included LC<sub>50</sub> and LC<sub>90</sub> of the water extract

as vegetable larvacide against larvae *Culex sp.* The results showed that the water extract has a significant effect to kill larvae *Culex sp.*, although it is not as effective as Abate®.

Based on these results, the active substances contained in the water extract of *Tithoniadiversifolia (Hemsley) A. Gray* leaves are flavonoids and tannins.<sup>9</sup> Flavonoids have larvicidal effects due to their mechanism of action to inhibit the respiratory system and disrupt the electron transport process in the larval body thus decreasing ATP production and reducing the use of oxygen by mitochondria. Besides, the tannins work by inactivating the enzymes and proteins in the larvae's body.<sup>10</sup> Although the leaves contain flavonoids and tannins with larvicidal effect, the contents of both substances in the water extract of *Tithoniadiversifolia (Hemsley) A. Gray* leaves are allegedly not too high, therefore, it takes a long time to kill all the larvae.

Wide variations in the number of dead larvae in each group may be due to the type instar of larvae which affects the larvae's sensitivity to the decoction. The study itself was carried out in the rainy season that may influence the destruction the larvae. The study on the active substances contained in the water extract of *Tithoniadiversifolia (Hemsley) A. Gray* leaves should be an important issue.

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