Conference Paper

# Toxicity of *Citrus mitis*, *Citrus aurantifolia*, and *Citrus maxima* leaf extract toward mortality of *Aedes aegypti* larvae (Diptera: Culicidae)

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## Abstract

Dengue Fever is a significant health issue in Indonesia for it is always been found and unresolved since 1968. Dengue Fever is transmitted through mosquito (*Aedes aegypti*) bite. Resistance of mosquito larvae towards temephos as consequence of chemical larvicide consumption has been reported in several countries. One of safe and environmentally friendly efforts to control mosquito is by using herbal larvicide which produced from plants. This study examines methanol extract of *Citrus mitis*, *Citrus aurantifolia*, and *Citrus maxima* leaf toward mosquito larvae *Ae. aegypti* instar III for 24 hours. Data of larvae mortality is analyzed using probit analysis by SPSS software. The result shows that *Citrus mitis* has the highest toxicity with the lowest lethal concentrations (LC) that are $LC_{50}$ = 1.547 ppm and $LC_{90}$ = 3.328 ppm. It followed by *Citrus aurantifolia* and *Citrus maxima* respectively.

**Keywords:** larvicide; *Aedes aegypti*; leaf extract; *Citrus mitis*; *Citrus aurantifolia*; *Citrus maxima*.

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## 1. Introduction

Dengue Fever is caused by dengue virus which classified in Arthropod-Borne Virus and Flaviviridae family. Dengue Fever is transmitted through mosquito bite, *Aedes aegypti*.[1, 2] Dengue Fever is always been found in every year since 1968.[3] The latest report from Minister of Health on Profil Kesehatan Indonesia 2015 (Health Profile of Indonesia 2015) confirmed that the number of Dengue Fever patients in 2015 was 129,650 with Incidence Rate 50.75 by 100.000 population; higher than previous year (2014) which was 39.80 by 100,000 population.[2]

The Minister of Health determined that Dengue Fever control in Indonesia is prioritized on vector control by eradicating mosquito breeding and the use of chemical

insecticides due to the unavailability of vaccines and anti-dengue drugs. Chemical vector control using larvicides and insecticides is still the main choice since 1980. The most widely used larvicide to control *Ae. aegypti* larvae is temephos.[3] Temephos is also recommended by WHO for killing *Ae. Aegypti* larvae in home water containers.[4] Fuadzy and Hendri (2015) mentioned that continuous and repeated exposure of insecticide during 2-20 years may lead to the emergence of resistant insects.[5] Several researchers have stated that resistant *Ae. aegypti* larvae are found in their country, as Mulyatno et al. in Indonesia – Surabaya, Bellinato et al. in Brazil, Singh et al. and Tikar et al. in India, Llinas et al. in Argentina, and Komalamisra et al. in Thailand.[6–11] Therefore, the discovery of a safer and more environmentally friendly way to control mosquito vectors is needed. One of them is by using herbal larviside which is produced from plants.

*Citrus* or well-known as citrus fruit, is one of magnoliopsida which is belong to Rutacea group and has high economical value because of its vitamin C. Citrus fruit is consumed as fruit and juice; while the leaves can be added to dishes as flavoring and fragrances. Citrus has much secondary metabolites that have the potential to be antibacterial, anti-inflammatory, anti-cancer, antioxidants, and maintain heart health so that it become medicinal plant.[12] The capability of citrus in killing mosquitoes larvae has been analyzed by Akram et al. He examined seed extract of 10 citrus variety, such as *Citrus aurantium*, *C. grandis*, *C. pseudolimon*, *C. paradisi*, *C. reticulata*, *C. limon*, *C. sinensis* var Musambi, *C. mitis*, *C. sinensis* var red blood, and *C. jambhiri* toward fatality of *Ae. albopictus* instar IV larvae. The result showed that all citrus mentioned are toxic toward mosquitoes larvae, *Citrus jambhiri* has the highest toxicity which causes mortality 95,6% of mosquitoes larvae within 24 hours, and the lowest concentration $LC_{50}$ among other *Citrus* (119.993 ppm).[13] Meanwhile, this present study use the leaves of *Citrus mitis*, *Citrus aurantifolia*, and *Citrus maxima* from Indonesia, specifically East Java, which were extracted with methanol solution and examined to mosquitoes larvae of *Ae. aegypti* instar III.

## 2. Material and Methods

This study is conducted in 5 stages, (1) collecting and drying *Citrus* leaves (*Citrus mitis*, *Citrus aurantifolia*, and *Citrus maxima*), (2) dissolving leaf powder in methanol solution for 2 weeks, (3) making extract of *Citrus* leaf using rotary evaporator, (4) colonization of tested larvae, and (5) bioassay. *Ae. aegypti* larvae are obtained from Entomology

Table 1: Score of Lethal Concentration ($LC_{95}$) of *Citrus* extract methanol after 24 hours.

| *Citrus* extract | $LC_{50}$ (ppm) 24 hours | $LC_{95}$ (ppm) 24 hours |
|---|---|---|
| *Citrus mitis* | 1.547 | 3.328 |
| *Citrus aurantifolia* | 2.197 | 3.660 |
| *Citrus maxima* | 2.938 | 6.369 |

Laboratory, Institute of Tropical Disease, Airlangga University, Surabaya. The instar used for examination is instar III.

Bioassay is executed by making the mother liquor (ppm): dissolving methanol extract of *Citrus* leaf (mg) with tween 20 solution and 1000 ml of distilled water. From the mother liquor, dilution is made of so that extract solution with various concentrations (ppm) are obtained. Each extract solution of various concentrations is measured so that the volume as much as 100 ml and placed in plastic glass. Furthermore, 20 larvae of *Ae. aegypti* instar III put in each glasses. Exposure is done for 24 hours. After 24 hours, the dead larvae is counted and recorded. The data is analyzed using SPSS software version of 16.0 with probit analysis.

## 3. Results

24 hours of observation toward *Ae. aegypti* larvae which exposed in methanol extract solution of *Citrus mitis*, *Citrus aurantifolia*, and *Citrus maxima* clearly showed the dead *Ae. aegypti* larvae. The evidence is that the larvae are not moving and sinking at the bottom of glass. The living *Ae. aegypti* are moving to surface and slowly down to the bottom repeatedly.

The result of probit analysis of the dead larvae after 24 hours exposure of extract methanol *Citrus mitis*, *Citrus aurantifolia*, *Citrus maxima* showed that extract *Citrus mitis* has the highest toxicity and the lowest Lethal Concentration among those three kind of *Citrus*. The score of Lethal Concentrations after 24 hours for each extract is shown on Table 1. The comparison of Lethal Concentrations is clearly shown in Pictuce 1.

Picture 1 explained that methanol extract of *Citrus mitis* leaf can kill 50% and 95% *Ae. aegypti* larvae instar III at the lowest concentration, for each 1.547 ppm and 3.328 ppm. Whereas methanol extract of *Citrus maxima* leaf is the lowest toxicity while it has high Lethal Concentrations compared to *Citrus mitis* and *Citrus aurantifolia*.

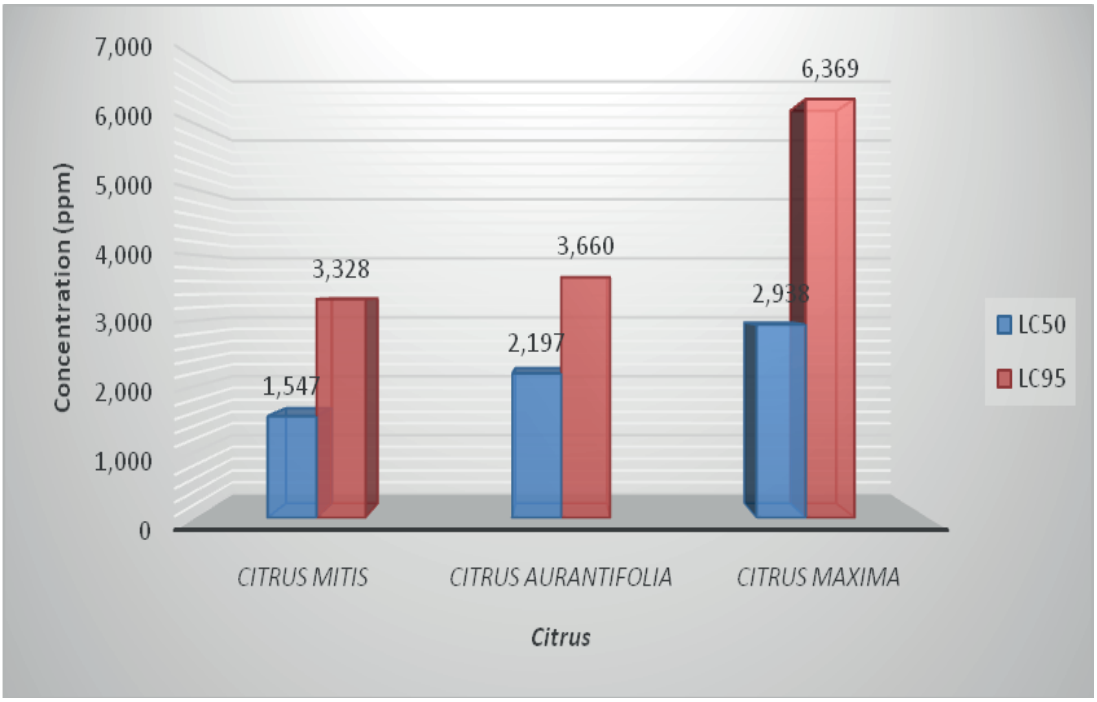


**Figure** 1: The activity of biolarvicide of Citrus leaf methanol extract toward *Ae. aegypti* larvae based on LC50 and LC90.

## 4. Discussion and Conclusion

This study used three extracts of *Citrus*, they are *Citrus mitis*, *Citrus aurantifolia*, *Citrus maxima,* which examined to *Ae. aegypti* larvae instar III. Plant can be used as an alternative of larvicide, which is good, decomposable, environmentally friendly, free-residue. *Citrus* contains of chemical compound of essential oil, flavonoid, saponin, steroid, and terpenoid, which compounds work as poison on mosquitoes larvae either contact poison or stomach poison.[14]

The potency of *Citrus* as biological control of mosquitoes is not in doubt. Murugan et al. (2012) discovered ability of *Citrus sinensis* becomes larvicide toward *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* instar III which gained $LC_{90}$ for each 659.31 ppm, 342.45 ppm, and 806.57 ppm.[15] Mya et al. (2015) reported that extract of *Citrus hystrix* is toxic and able to kill *Ae. aegypti* larvae.[14]

Gutierrez et al. (2014) proved on his study that methanol extract of *Citrus grandis* is more effective than *Jatropha curcas* extract. From screening result, phytochemical is found in 5 types of substances of secondary metabolite on methanol extract of *Citrus grandis* compared to *Jatropha curcas* which has only two secondary metabolites. Therefore, it might be found more poison from *Citrus grandis* which causes mortality of *Ae. aegypti* larvae instar III and IV.[14]

As it is shown in Table 1, those three extract of *Citrus* are toxic to *Ae. aegypti*. If it is examined to another species of mosquitoes, it must have the same result that causes mortality of mosquitoes. *Aedes aegypti* is included to the same class and order as *Anopheles stephensi*, *Aedes albopictus*, and *Culex quinquefasciatus*, are class of insect and order of diptera. Mallick et al. (2016) found that content of alkaloid, terpenoid, steroids, and flavonoids in *Citrus maxima* extract that can cause mortality of *Culex quinquefasciatus* larvae.[17]

Three of different mosquitoes species have been examined by Murugan et al. (2012) have been through mortality after it was exposed to ethanol extract of *Citrus sinensis*.[14] This study declared that methanol extract of *Citrus mitis* has the highest toxicity as bio-larvicide toward *Ae. aegypti* larvae, as it's $LC_{50}$ and $LC_{95}$ are the lowest.

The examination used *Ae. aegypti* larvae instar III because it has a fine endurance toward mechanics trouble and long lasted larvae stadium. Gutierrez et al. (2014) said that controlling mosquitos is prioritized on larvae stadium.[16]

This study showed that *Citrus mitis* has the highest toxicity with the lowest lethal concentrations (LC) among three kind of *Citrus* examined. That are $LC_{50}$= 1.547 ppm and $LC_{90}$ = 3.328 ppm. It followed by *Citrus aurantifolia* and *Citrus maxima* respectively.

For future study, it is hoped that potency of methanol extract of *Citrus mitis* can be tested in field or in laboratory toward adult mosquitoes.

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