Eco. Env. & Cons. 26 (4) : 2020; pp. (1713-1715)
Copyright@ EM International
ISSN 0971–765X

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

Hebert Adrianto[1]and Hamidah[2]*

[1]Department of Parasitology, Faculty of Medicine, Ciputra University, Surabaya, East Java, Indonesia
[2]Department of Biology, Faculty of Science and Technology, Airlangga University, Surabaya, East Java, Indonesia

(Received 12 January, 2019; Accepted 24 June, 2020)

ABSTRACT

This study examined methanol extract of *Citrus mitis*, *C. aurantifolia*, and *C. maxima* leaf toward mosquito larvae *Ae. Aegypti* instar III for 24 hours. Mortality data of larvae was analyzed using probit analysis by SPSS software. The result showed that *C. mitis* had the highest toxicity with the lethal concentration (LC), LC_{50} = 1.547 ppm and LC_{90} = 3.328 ppm. The lowest toxicity was shown by C. *maxima*.

Key words: *Citrus* sp, *Ae. Aegypti, Lethal concentration.*

Introduction

Dengue Fever is caused by dengue virus which classified as *Arthropod-Borne Virus* and *Flaviviridae* family. Dengue Fever is transmitted through mosquito bite of dipteran, *Aedes aegypti* (Achee *et al.,* 2012; Alkan *et al.,* 2015). Dengue Fever always been found every year since 1968 until present (MOH, 2012). The latest report from Minister of Health on Profile of Indonesia Health (Profil Kesehatan Indonesia) 2015 confirmed that Dengue Fever patients were 129,650 with Incidence Rate = 50.75 per 100,000 population were higher than previous year, 2014, with 39.80 per 100,000 population (MOH, 2012).

Minister of Health has determined that Dengue Fever control in Indonesia prioritizes on vector controlling, by eradicating mosquito breeding and chemical insecticide due to anti-dengue medication and vaccine are not available. Controlling vector chemically by using larvacide and insecticide always been chosen since 1980. The widest larvacide used to control *Ae. aegypti* larvae is temephos. Temephos is also recommended by WHO to kill *Ae. aegypti* in water container in houses. Insecticide exposure continuously and repeatedly during the last 2-20 years produce a resistant insect (Ishak and Ponno, 2018). Several researchers have stated that a resistant *Ae. aegypti* larvae are found in their country, as reported by Bellinato *et al.* (2016) in Brazil and Singh *et al.* (2014) in India. Regarding to this condition, it is emerged about how to control safe and environmentally friendly mosquito's larvae by using herbal larvacide which is produced from the plants.

Citrus or commonly known as citrus fruit is one of magnoliopsida plant which belongs to Rutacea group. This group has economic value because contains vitamin C. People usually consume *Citrus* as fruit and juice; the leaf can be added in dishes to enrich the flavor. Many secondary metabolites in

citrus fruit potentially to be used as anti-bacterial, anti-inflammatory, anti-cancer, antioxidant, antiaging, preservative and good for heart, therefore citrus becomes a medicinal plant (Nazliniwaty *et al.*, 2016; Nurwahyuni and Sinaga, 2018; Tambunan *et al.*, 2018). The capability of citrus fruit in killing mosquitoes larvae has been analyzed by Akram *et al.*, (2010), Pusparini (2017), and Ansori *et al.*, (2018), they 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* were toxic toward mosquitos' larvae. *C. jambhiri* has the highest toxicity which caused mortality 95.6% of mosquitoes' larvae within 24 hours with the lowest LC_{50}(119,993 ppm). The present study was conducted to examine the toxicity of methanol extracted *C. mitis*, *C. aurantifolia*, and *C. maxima* from East Java Indonesia to mosquito's larvae of *Ae. aegypti* instar III.

Methodology

This study was conducted in 5 stages, (1) collecting and drying leaf citrus (*Citrus mitis*, *Citrus aurantifolia*, and *Citrus maxima*), (2) dissolving leaf powder in methanol solution for 2 weeks, (3) made extract of citrus leaf using rotary evaporator, (4) colonization of tested larvae, and (5) bioassay. *Ae. aegypti* larvae were obtained from Entomology Laboratory, Institute Tropical Disease Universitas Airlangga Surabaya. The instar used to be examined was instar III because it had a fine endurance toward mechanics trouble, the larvae stage lasted longer and the controlling mosquitoes as vector prioritizes on larvae stadium.

Bioassay was executed by making mother solution (ppm), dissolved methanol extract of citrus leaf (mg) with tween 20 solution and 1000 mL of distilled water. Extract solutions of various concentrations (ppm) were made by diluting mother solution with distilled water. Each extract solution of various concentrationswas measured the volume as many as 100 mL and placed in plastic glass. The next step 20 instar III larvae of *Ae. aegypti* was put in each glass. Exposure was done for 24 hours. After 24 hours, the dead larvae was counted and recorded. The data was analyzed using SPSS software version of 16.0 with Probit analysis.

Results and Discussion

Observation during 24 hours to *Ae. aegypti* larvae which exposed in methanol extract solution of *C. mitis*, *C. aurantifolia*, and *C. maxima* clearly showed the dead *Ae. Aegypti* larvae. During the observation, the larvae did not move, however they sink at the bottom of the glass. The living *Ae. aegypti* moves to the surface and slowdowns to the bottom repeatedly.

The result of probit analysis showed that the extract *C. mitis* had the highest toxicity, followed by *C. aurantifolia* and *C. maxima*. The values of Lethal Concentration for 24 hours of each extract are shown in Figure 1.

The Figure 1 explained that methanol extracted *C. mitis* leaf could kill 50% and 95% *Ae. aegypti* larvae instar III at the lowest concentrations: 1.547 ppm (LC_{50}) and 3.328 ppm (LC_{95}). Whereas methanol extracted *C. maxima* leaf had the lowest toxicity with higher Lethal Concentration compared to *C. mitis* and *C. aurantifolia*.

This study showed that *C. mitis*, *C. aurantifolia*, and *C. maxima* potentially can be used as an alternative of larvacide of *Ae. aegypti*, because good, decomposable, environmentally friendly, and free-residue. *Citrus* contains chemical compounds such as essential oil, flavonoid, saponin, steroid, and terpenoid, which effectively work as poison to mosquitos' larvae through either contact poison or stomach poison (Mya *et al.*, 2015).

Mya *et al.* (2015) reported that extract of *C.hystrix* is toxic and can kill *Ae. aegypti* larvae effectively.

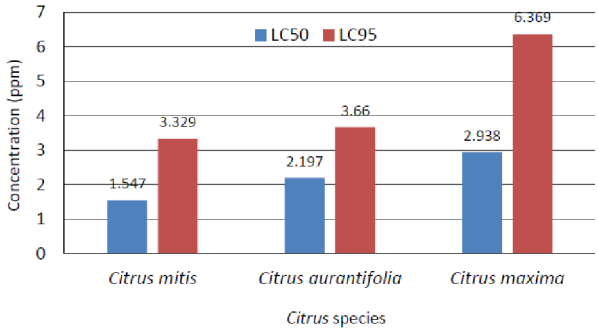

Fig. 1. The activity of biolarvacide of methanol extracted citrus leaf toward *Ae. aegypti* larvae based on LC_{50} and LC_{95}

Gutierrez *et al.* (2014) proved that methanol extracted *Citrus grandis* was more effective than *Jatropha curcas* extract. Screening results showed that

methanol extracted *C. grandis* had five types of secondary metabolite substances, while *J.curcas* had only two secondary metabolites. Due to its secondary metabolite properties, *C. grandis* was toxic to *Ae. aegypti* larvae instar III and IV (Table 1). The similar result could be occurred when *Citrus* extract apply as larvacite to another species of mosquitoes such as *Anopheles stephensi*, *Aedes albopictus*, and *Culex quinquefasciatus* which have the same class and order with *Ae. aegypti*. Mallick *et al*. (2016) found that the alkaloid secondary metabolite, terpenoid, steroids, flavonoid in *C. maxima* extract can cause mortality of *Culex quinquefasciatus* larvae. Gutierrez *et al.* (2014) demonstrated that the ethanol extract of *Citrus sinensis* caused significant mortality to three species of mosquitoes. Our study declares that methanol extract of *Citrus mitis* had the highest toxicity as bio-larvacide toward *Ae. aegypti* larvae. 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.

Conclusion

From three species of *Citrus*, *C. mitis* has the highest toxicity with the lowest lethal concentration (LC): LC_{50} = 1.547 ppm and LC_{95} = 3.328 ppm, followed *C.aurantifolia*, and *C. maxima*.

References

Achee, N., Masuoka, P., Smith, P., Martin, N., Chareonviryiphap, T., Polsomboon, S., Hendarto, J. and Grieco, J. 2012. Identifying the effective concentration for spatial repellency of the dengue vector *Aedes aegypti*. *Parasites & Vectors*.5 : 300. doi:10.1186/1756-3305-5-300.

Alkan, C., Zapata, S., Bichaud, L., Moureau, Lemey, P., Firth, A.E., Gritsun, T.S., Gould, E.A., Lamballerie, Z., Depaquit, J. and Charrel, R.N. 2015. Ecuador paraisoescondido virus, a new flavivirus isolated from new world sand flies in Ecuador, is the first representative of a novel clade in the genus flavivirus. *Journal of Virology*. 89 (23) : 11773-11785.

Ansori, A.N.M, Kusala, M.K.J., Irawan, H., Putri, N Fadholly, A., Proboningrat, A., Rukmana, S., Karni, I., Kaltaria, A. and Anisa, Hebert Adrianto, H. 2018. *Citrus reticulata* extract as biocides to control *Aedes aegypti*, the vector of dengue. *Bioscience Research*. 15(3) : 1661-1665.

Bellinato, D.F., Medeiros, P.F.V., Araujo, S.C., Martins, A.J., Lima, J.B.P. and Valle, D. 2016. Resistance status to the insecticides temephos, deltamethrin, and diflubenzuron in Brazilian *Aedes aegypti* populations. *BioMed Research International*. 1-12.

Cahyo, K., Mulyatno, Yamanaka, A., Ngadino, Konishi, E. 2012. Resistance of *Aedes aegypti* (L.) Larvae to Temephos in Surabaya, Indonesia. *Southeast Asian J Trop Med Public Health.* 43 (1): 29-33.

Gutierrez, P.M., Antepuesto, A.N., Eugenio, B.A.L. and Santos, M.F.L. 2014. Larvicidal activity of selected plant extracts against the dengue vector *Aedes aegypti* mosquito. *International Research Journal of Biological Sciences.* 3 (4) : 23-32.

Ishak, H. and Ponno, S. 2018. Resistance Status in *Aedes aegypti* Strain from North Toraja, Indonesia to Malathion and Temephos Insecticides. *Indian Journal of Public Health Research and Development.* 9(12) : 1345-1348. doi: 10.5958/0976-5506.2018.02039.9

Mallick, S., Mukherjee, D., Ray, A.S. and Chandra, G. 2016. Larvicidal efficacy of fruit peel extracts of *Citrus maxima* against *Culex quinquefasciatus*. *Journal of Mosquito Research*. 6 (20) : 1-8

Ministry of Health (MOH) Indonesia. (2012). Ministry of health regulation number 374/MENKES/PER/III/2010 (in Indonesia) about vector control. Jakarta: MOH.

Mya, M.M., Aye, Y.Y., Oo, A.W. and Saxena, R.K. 2015. Effect of *Citrus hystrix* DC leaves ethanol extract on larvae of *Aedes aegypti. Journal of Biological Engineering Research and Review*. 2(2) : 1-6.

Nazliniwaty, Karsono, Nilsya Febrika Zebua, N. F., Nerdy. 2016. Antioxidant activity and antiaging gel formulation grapefruit peel (*Citrus maxima* Merr.) ethanolic extract. *Der Pharmacia Lettre*, 8 (20) : 84-94

Nurwahyuni, I. and Sinaga, R. 2018. *In vitro* Propagation of Threatened Brastagi Citrus Variety Brastepu (*Citrus nobilis* Brastepu) CVPD Free Throughshoot Tips Subculture. *Pak. J. Bot*. 50(2): 667-678.

Pusparini, E.W. 2017. The Effect of Adding Varoius Doses Mat Powder Lime Peel (*Citrus aurantifolia*) Mosquito Aedes Sp. Mortality. *Jurnal Kesehatan Lingkungan*. 9(1) : 75–81.

Singh, R.K., Mittal, P.K., Kumar, G. and Dhiman, R.C. 2014. Insecticide susceptibility status of *Aedes aegypti* and *Anopheles stephensi*larvae against temephos in Delhi, India. *International Journal of Mosquito Research*. 1(3): 69-73.

Tambunan, R. Z., Rusmarilin, H. and Kaban, J. 2018. Antioxidant activity of tomato juice rich in lycopene antioxidant as degenerative chemopreventive agents using *Citrus aurantifolia* juice as a preservative. *IOP Conf. Series: Earth and Environmental Science*. 205 (2018) 012035. doi:10.1088/1755-1315/205/1/012035.