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Research Article

Lethal Concentration (LC50) of *Myristicin* (*Myristica fragrans* Houtt) on Larva *Aedes aegypti* Instar III

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Abstract: Myristicin is an active substance that can be found in nutmeg plants. The purpose of this study was to determine the LC50 myristicin against larval instar III of *Aedes aegypti*. The samples used were myristicin isolates with varying concentrations of 5 ppm, 10 ppm, 15 ppm, 20 ppm and Abate as a positive control and DMSO as as negative control against *Aedes aegypti* instar III larvae. The parameters observed were LC50 *Aedes aegypti* larvae for 24 hours. The results obtained were based on calculations using the probit formula it is known that LC50 isolate myristicin which could kill larvae at 12 hours is 10 ppm, while the positive control was 10 ppm. These results are better when compared with results in other studies with LC50 of 111,002 ppm and positive control at 24 hours. Based on the LC50 isolate of Myristicin (*Myristica fragrants* Houtt) against 50% *Aedes aegypti* Instar III larvae at the 12th hour is 10 ppm.

Keywords: *myristicin; Aedes aegypti larvae; larvacide*

Introduction

Based on data from the Directorate General of Disease Prevention and Control, the Ministry of Health of the Republic of Indonesia stated that the distribution of dengue suspects from the first week of 2018 to the first week of 2019 was highest in East Java with a total number of 700 DBD suspects, followed by Central Java 512 people, and West Java 401 people [1]. Eradicating *Aedes aegypti* mosquitoes is very difficult because they can adapt to the environment which makes them very resilient, even after disturbances due to natural phenomena or human intervention. On the other hand, the use of synthetic insecticides is very effective in killing mosquito larvae. However, its use can continuously cause negative impacts such as insects becoming resistant, environmental pollution, resurgence, and tolerance of pesticides [2]. With the negative impacts caused by synthetic insecticides, it is necessary to try to obtain alternative larvicides that are feasible to develop. One of them is myristicin which is derived from nutmeg seeds because the insecticidal compound from plants easily decomposes in the environment, does not leave residue in air, water, and soil, and has a higher level of security.

Many studies showed that Essential oils obtained from nutmeg mace using the steam distillation method were tested for their activity as larvacides. The results obtained from the test of essential oils on the third instar *Aedes aegypti larvae* showed LC50-6 hours value of 224.399 ppm, LC50-12 hours of 150.724 ppm, LC50-24 hours of 111.002 ppm [3]. In addition, a similar result was made by [4] about the bioactivity of insecticides from the origin of environmentally friendly plant chemicals against *Culex sp* and *Aedes aegypti* mosquitoes derived from nutmeg plants. Myristicin is the highest bioactive compound larvicide that produces LC50 against *Aedes aegypti* mosquitoes at a concentration of 22.9 ppm for 24 hours and 16.8 ppm for 48 hours. Whereas in *Culex sp* mosquitoes produce LC50 at a concentration of 22.9 ppm for 24 hours and 15.2 ppm for 48 hours [5].

According to Wicaksono's research [6] it was found that 96% ethanol extract of nutmeg (*Myristica fragrans* Houtt) effect as larvicide indicated by LC50 price was 0.0284% w / v, which means that at a concentration of 0.0284% w/v, it could give 50% of deaths test animals. Myristicin (5-allyl-1-methoxy-2,3 (methylenedioxy benzene) is a plant-flavoring constituent and has been known to produce significant psychopharmacological responses, is also active as an insecticidal activity. Metabolism of myristicin resembles safrole. In addition to natural sources, myristicin can be a significant source of psychopharmacological responses and is also active as

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an insecticidal activity [7]. The purpose of this study was to determine the LC50 myristicin against *Aedes aegypti* instar III larvae.

Materials and Methods

Materials and Methods

Materials used in this research were *Aedes aegypti* larvae instar III and myristicin from the nutmeg seed. The process was initially with the isolation of myristicin from the nutmeg seed referred to by Al Jumaily et al, 2012 [8]. The nutmeg seed was extracted using three eluents, polar, nonpolar, and semipolar. The extract was fractionated to get a specific active compound. The final step is myristicin in the fowder. Myristicin was identified by the GCMS method. It was divided into four groups of concentration: 5 ppm, 10 ppm, 15 ppm, and 20 ppm with DMSO as solvent. After that, we prepared *Aedes aegypti* larvae instar III for myristicin assay. Confirmation test for *Aedes aegypti* mosquito larvae: the larvae were taken with a pipette, put into a watch glass, and then the water was discarded, the larvae were killed with hot water in a watch glass, and then one larva with a pipette, store it in a glass object and straighten it. We used 20 larvas for each group and there were three replications.

Total groups of treatment were six groups; myristicin 5 ppm, 10 ppm, 15 ppm, and 20 ppm, also positive control (Abate powder 10 ppm), and negative control (DMSO). All of the larvas were put in the cup with 50 mL solution of each treatment group. Observation was performed for 24 hours, then every 6 hours observed and counted the larval death.

Test the effectiveness of myristicin against third-instar *Aedes aegypti* larvae. Measurement of LC50 value was done by counting the number of half from all populations of larvae instar III. Based on testing 20 larvae, Probit Analyst performed using the IBM SPSS 22.0 program to determine the killing power of Myristicin *(Myristica fragrans* Houtt) with concentrations of 5, 10, 15, and 20 ppm against the larvae of *Aedes aegypti* instar III.

Results and Discussion

Based on the results of this research, it can be seen that myristicin obtained from nutmeg han potential to kill third instar *Aedes aegypti* larvae. The number of larval deaths will increase according to the increasing concentration and duration of larval exposure to the substance being tested. The longer the exposure and the observation time, the greater the larval mortality. This happens because the chemical compound myristicin plays a very active role as a natural larvicide.Results from this reseach was showed at figure 1.

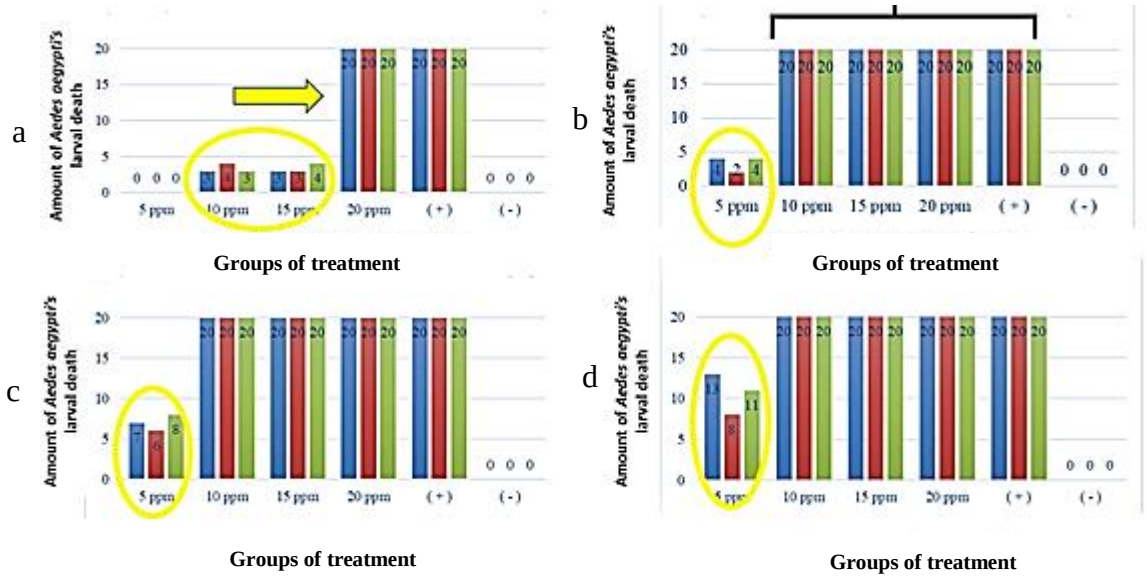

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Figure. 1 Amount of *Aedes aegypti's* larval death in variation concentration of myristicin and observation time. Groups of treatment: Myristicin concentration (5 ppm, 10 ppm, 15 ppm, and 20 ppm), (+) positive control (abate 10 ppm), (-) negative control (DMSO); blue, red and green bars are the repetition of treatments.
a: Amount of *Aedes aegypti's* larval death at 6 hours, b: Amount of *Aedes aegypti's* larval death at 12 hours, c: Amount of *Aedes aegypti's* larval death at 18 hours, d: Amount of *Aedes aegypti's* larval death at 24 hours

Aedes aegypti Instar III larvae that died have characteristics: inactive larvae, not moving when stimulated (the container is shaken/touched by a stick), and the body of the larvae turn pale. Many dead larvae are at the bottom of the solution, but some are floating on the surface of the test solution. Whereas larvae that almost die are those that are unable to rise to the surface or show no typical diving reaction when the water is disturbed [9].

The positive control (abate 10 ppm) showed the overall mortality of larvae at the 6th hour, while the negative control of the solvent did not show the death of larvae so it can be seen from these observations that the DMSO solvent did not have an effect to the treatments. Meanwhile, myristicin 20 ppm can cause 100% larval death similar to the positive control (abate 10 ppm), this fact showed that myristicin 20 ppm has the same potential as abate 10 ppm in killed *Aedes aegypti* larvae instar III at the same time. Then, myristicin 10 ppm and 15 ppm showed the potential to kill 100% of *Aedes aegypti* larvae instar III after 12 hours of observation. This matter showed that getting lower the concentration of myristicin increasingly needs time longer to kill *Aedes aegypti* larvae instar III.

The yellow cyrcle showed that at lower concentrations not give yet effect to kill half of the population larvae at the beginning time, but it can rise at the end of time observation. This data was analyzed using probit analysis. Based on calculations using the probit formula it is known that the LC50 that can kill larvae at 12 hours is 11,655 ppm and at 24 hours is 15,230 ppm. Mechanisms of myristicin to kill *Aedes aegypti* larvae instar III are still not known, but according to Ghosh et al, 2012 , we can predict that myristicin can influence the respiratory system of larvae of these mosquitoes. Myristicin was predicted can enter through the body wall of the larva or mouth when the larvae take food from their place of life. These bioactive compounds enter the larvae 's body at certain levels and can act as contact poisons, stomach poisons, and respiratory poisons so that they damage the body system of *Aedes aegypti* larvae [10, 11].

Conclusions

The concentration of LC50 of myristicin which can kill *Aedes aegypti* Instar III larvae at 12 hours is 10 ppm.

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