

SUMMARY

Biolarvicidal Activities of Leaf Extracts of *Citrus* spp. and *Pandanus amaryllifolius* against *Aedes aegypti* Larvae Based On Biosistematic Numeric

Mosquitoes are the most important single group of insects well-known for their public health importance. *Aedes aegypti* is the major urban vectors of dengue, lymphatic filariasis, chikungunya, and japanese encephalitis. At present, no effective vaccine is available for dengue. Therefore, the only way of reducing the incidence of this disease is mosquito control. Essential oils, saponin, alkaloid, flavonoid, triterpenoid, and tannin from leaves, flowers, seed, rind, and roots of plants were found to have mosquito biolarvicidal activity. In this study, mosquito biolarvicidal activity was investigated using leaf extract of *Citrus* spp. (*Citrus hystrix*, *Citrus aurantifolia*, *Citrus amblycarpa*, *Citrus maxima*) and *Pandanus amaryllifolius*. *Citrus* has high diversity species so this study aims to find out the prime species and alternative species, which have the higher toxicity and effective among others. Biosistematic numeric is the answer to solve this problem.

The aims of this study are firstly to know biolarvicidal toxicity value from leaf extracts of *Citrus* spp. and *Pandanus amaryllifolius* against the 3rd instar larvae of *Aedes aegypti*, secondly to find out which is the most effective and alternative biolarvicidal from LC₉₀ results, thirdly to analyze their relationship, and finally to identify the similarity between the prime species and alternative species and their position in their relationship shown in biosistematic numeric.

This study was conducted using nested experiment design. The biolarvicidal activities, phytochemical screening, and relationship in biosistematic numeric of five plant, *Citrus hystrix*, *Citrus aurantifolia*, *Citrus amblycarpa*, *Citrus maxima*, and *Pandanus amaryllifolius* have been evaluated in this study. Firstly, methanol leaf extract of *Citrus* spp. and *Pandanus amaryllifolius* was characterized by phytochemistry content. Secondly, methanol leaf extracts of *Citrus* spp. and *Pandanus amaryllifolius* were tested at 500, 1375, 2250, 3125, and 4000 ppm concentrations, one control group (aquades), and five replicates against the 3rd instar larvae of *Aedes aegypti*. Sample size was 20 larvae for each treatment. Larvae mortality was observed after 24 hours and data were analyzed by probit. The effects from difference of five plant extract on mortality of *Aedes aegypti* were analyzed by Analyze of Variance (ANOVA).

The stages of biosistematic numeric are (1) characterize morphology and anatomy of leaf of five specimens, (2) analyze the data for grouping (group analysis). Data of morphology and anatomy *Citrus* spp. and *Pandanus amaryllifolius* were analyzed by SPSS programme (hierarchial cluster) and later by a dendrogram. After making a hierarchical grouping classification (Classify Hierarchial Cluster), it was followed by principal component analysis (PCA/ Principal Component Analyze). PCA analysis of morphology and anatomy characters was used to answer morphological character which makes a major contribution and separation of species.

The similarity between the prime species and alternative species of *Citrus* spp. and *Pandanus amaryllifolius* extract and their position in their relationship in biosystematic numeric would be explained by using lethal concentration 90% (LC₉₀) result, ANOVA result, and grouping result.

Phytochemistry screening in this study shows that leaves of *Citrus* spp. and *Pandanus amaryllifolius* contain chemical compounds: flavonoid, terpenoid, essential oil, and saponin. Theoretically chemical compounds have larvicidal property.

Larvicidal activity test was carried out using WHO protocol. The preliminary test was conducted in this study in order to get concentration value, which can kill 5% and 90% the 3rd instar larvae of *Aedes aegypti*. Concentrations of preliminary test in Irfan (2011) research, are 700, 1300, 1500, 1800, dan 2500 ppm. This study hope that this research have no false value or consistent with Irfan (2011) value. This study shows that the results are consistent with Irfan (2011) research result. The methanol leaf extract of *Citrus aurantifolia* at concentrations of 777,151 ppm and 3.154,690 ppm killed 5% and 90% the 3rd instar larvae of *Aedes aegypti*. Concentrations of real test five plants used for larvae of *Aedes aegypti* were 0, 500, 1375, 2250, 3125, and 4000 ppm. Larvae mortality from each five extracts was observed after 24 hours and was analyzed by probit to get lethal concentration 90% (LC₉₀).

The results of this biolarvicidal test show that lethal concentration 90% (LC₉₀) of methanol leaf extract of *Citrus hystrix* is 2.828,249 ppm; LC₉₀ of *Citrus aurantifolia* is 3.337,232 ppm; LC₉₀ of *Citrus amblycarpa* is 3.772,981 ppm; LC₉₀ of *Citrus maxima* is 5.611,454 ppm; and LC₉₀ of *Pandanus amaryllifolius* is 5.108,970 ppm. *Citrus hystrix* leaf extract has the highest toxicity and most effective among others with LC₉₀=2.828,249 ppm, followed by *Citrus aurantifolia* (LC₉₀= 3.337,232 ppm) and *Citrus amblycarpa* (LC₉₀= 3.772,981 ppm). ANOVA analysis shows that there is no significant difference at the 0.05 level in *Citrus hystrix*, *Citrus aurantifolia*, and *Citrus amblycarpa*. *Citrus hystrix* and *Citrus maxima* have significant difference at the 0.05 level. *Citrus hystrix* and *Pandanus amaryllifolius* have significant difference at the 0.05 level too.

The result of biosystematic numeric shows that *Citrus hystrix* clustered with *Citrus aurantifolia* at 61,8% similarity value, followed by *Citrus amblycarpa* at 48,3% similarity value and *Citrus maxima* at 44,3% similarity value, and *Pandanus amaryllifolius* made a single cluster at 15% similarity value.

According to LC₉₀, ANOVA result, and biosystematic numeric, there is similarity between the prime species (*Citrus hystrix*) and alternative species (*Citrus aurantifolia*) with their position in their relationship shown in biosystematic numeric.