The Influence of the Ethanol Extract of Bitter Vine (*Mikania micrantha Kunth*.) on the Mortality, the Hatchability of the Eggs and the Larval Growth of *Aedes aegypti Linn*.

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Abstract: The research on the impact of the ethanol extract of *M. micrantha* leaf on the mortality, egg hatchability and larval growth of *A. aegypti* had been conducted using a Complete Randomized Design (CDR) with five treatments and replications. The mortality tests on 3rd instar larva with concentration treatments of the ethanol extract of *M. micrantha* leaves at 0.2%,0.4%,0.6%,0.8%, and 0.1%generated a LC_{50} value of 0.58%.The ethanol extract of *M. micrantha* leaves at a sub-lethal concentration of 0,1%,0,2%,0,3%,0,4%,0,5%indicated a significant impact on the mortality, egg hatchability and larval growth ($p \leq 0,05$).A sublethal concentration at 0,4% of the plant was effective in suppressing the egg hatchability at a percentage of 41,6%, larval development into pupa at a percentage of 19.5% and pupae transformation into imago a percentage of 63,3%.

1 INTRODUCTION

The control of the vector-borne disease can be conducted using chemical compounds such as synthetic insecticides, but this may cause losses such as resistance, death of untargeted, or human poisoning. World Health Organization has advocated finding alternatives to control these issues through biological or environmental control methods, by using natural chemicals derived from the plants (Indonesian Department of Health, 2010). Floras in Indonesia have mass potentials to be utilized as an alternative for plant-based insecticides using the secondary metabolites they produce (Boesri et al., 2015). Organic insecticides are generally pesticides whose active ingredients come from plant parts that are toxic to insects and have secondary metabolites containing various bioactive compounds (Thamrin, M. et al., 2007).

Various plants, including weeds, have secondary metabolite compounds that can be used for self-defense against pests and diseases (Tampubolon, 2018). Bitter Vine (*Mikania micrantha*) is one of the potential weeds and has been proven as an effective plant-based insecticide because it contains secondary metabolites that can kill insects (Salam et al., 2014).

Based on the phytochemical analysis results, the leaf extract of *M. micrantha* contains active substances in the form of secondary metabolites such as alkaloids, saponins, flavonoids, steroids, tannins, and terpenoids (Polakitan et al., 2017). *M. micrantha* also has other specific active substances called mikanolide and dihydromichiolide. These substances belong to the *sesquiterpene* group commonly found in the plants of the *Asteraceae* family (Tripathi et al., 2012). Noshirma & Willa (2016), in their research, mentioned that these phenolic metabolites might cause stomach poisoning which can interfere the digestive system of *A. aegypti* larvae, so the larvae fail to develop and eventually die. “interfere with the digestive system”

Plant-based insecticides also work specifically by damaging the growth of eggs, larvae, and pupae; inhibiting skin turnover; disrupting insect communication; inhibiting the female reproduction; reducing appetite; blocking the insect ability to eat; and repelling the insects (Sudarmo, 2005). Only a few studies on the leaves of *M. micrantha* as an organic insecticide have been conducted, so a test is required to see the effect on the egg hatchability, mortality and development of *A. aegypti* larvae as one of the efforts in controlling the number of *A. aegypti mosquitoes* through monitoring in the larval

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phase. Thus, the object of this study was how the ethanol extract of *M. micrantha* leaves affect the egg hatchability, mortality, and development of *A. aegypti* larvae. The study was conducted to determine the effect of ethanol extract of *M. micrantha* leaves on larval mortality, egg hatchability, and development of *A. aegypti* larvae.

2 METHODS

2.1 Animal Subject Rearing

Rearing was carried out to keep and breed the animal subjects to provide the eggs and larvae of the *A. aegypti*. It was conducted at the Institute of Environmental Health and Infection Disease Control (BTKL-PPM) Class 1 Medan.

2.2 General Architecture

This research is an experiment using a completely randomized design (CRD) with five extract concentrations (with one control) and five iterations of 25 larvae and *A. aegypti* eggs.

2.3 Ethanol Extract of *M. micrantha* Leaves

Five kilograms of *M. micrantha* leaf samples were washed and dried for five days, then crushed using a blender to form a powder. The powder was weighed as much as 1 kg then macerated with ethanol for 144 hours and stored in Erlenmeyer. During the maceration process, the stirring was carried out every day until obtained macerate. The obtained macerate was then filtered and evaporated with a Vacuum Rotary Evaporator until all the ethanol evaporated into a thick extract. This extract would be stored in a silica gel desiccator (Hamidah et al., 2015).

2.4 Observation of Test Parameter

2.4.1 The Mortality Test on the Third Instar *A. aegypti* Larva

The toxicity test of the ethanol extract of *M. micrantha* leaves on the mortality of the third instar *A. aegypti* larvae was conducted using six concentration variants with five replications, namely K1 = 0.2%, K2 = 0.4%, K3 = 0.6%, K4 = 0,8%, K5 = 1%, and K0 = 0% (control). A total of 25 third instar *A. aegypti* larvae were put into separate exposure medium containing 100 ml of each extract. The temperature and humidity in the treatment room as and the exposure medium were set as the standard measurement. The observation was performed 24 hours after the exposure. The larval mortality rate can be calculated using the formula below.

$$Larval\ Mortality\ (\%) = \frac{Number\ of\ Dead\ Larvae}{Total\ Number\ of\ Larval\ Subjects}\ x\ 100 \quad (1)$$

Furthermore, the data analyzed for regression to obtain the LC50 value using Microsoft Excel 2013.

2.4.2 Egg Hatchability and Growth of *A. aegypti* Larvae at Sublethal Concentration

The sublethal test was conducted to determine the egg hatchability and larval development of *A. aegypti* until the imago phase. Twenty-five eggs of *A. aegypti* were put into a test cup containing 100 ml of ethanol extract of M.micrantha leaf with sublethal concentrations, based on the results of the mortality test, at P0 = 0% (control), P1 = 0.1%, P = 2, 0.2%, P3 = 0.3%, P4 = 0.4% P5 = 0.5% with five replications. The observation of the egg hatchability was monitored every 24 hours for 72 hours (3 days) (WHO, 2005).

$$Egg\ Hatchability\ (\%) = \frac{Number\ of\ hatched\ eggs}{Total\ of\ eggs}\ x\ 100 \quad (2)$$

The testing of the development of *A. aegypti* was observed from the hatched eggs into larval stages in 24 hours for ten days. It aims to determine the number of successful larvae transformed into pupae and pupae into the imago. This can be calculated using the formulas below.

$$Larve - Pupae(\%) = \frac{Number\ of\ Developed\ Pupae}{Total\ of\ Larvae}\ x\ 100 \quad (3)$$

$$Pupae - Imago\ (\%) = \frac{Number\ of\ Developed\ Imago}{Total\ of\ Pupae}\ x\ 100 \quad (4)$$

2.5 Statistical Analysis

The obtained data from each observation variables were recorded and arranged in tabular form. The

generated quantitative data (dependent variables) were tested for their significance on the impact of the treatment groups (independent variables) with the help of a statistical computer program, namely the SPSS (release 22). The test sequence began with a normality test, homogeneity test, one-way ANOVA test for data with repeated observations (more than two times).

If in the ANOVA test, there is a significant difference ($p < 0.05$) in the treatment group, then the test will be continued using the Post Hoc-Duncan analysis at a level of 5%. At the end of the study, it can be determined which concentration of ethanol extract of *M. micrantha* leaves has the most significant and practical effect on the egg hatchability and larval growth of *A. aegypti*.

3 RESULTS AND DISCUSSION

3.1 Larval Mortality

Based on the test results, the mortality rate of the third instar A aegypti larvae was obtained after being treated with ethanol extract of *M. micrantha* leaves. It can be seen in Figure 1.

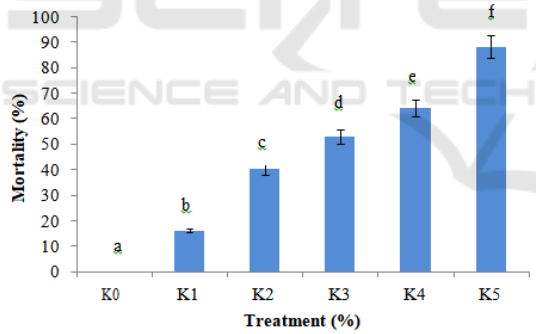

Figure 1: Influence of ethanol extract of M. micrantha leaves on the mortality rate of the third instar A. aegypti larvae (24-hour observation). Note: K0: 0% (control), K1: 0.2%, K2: 0.4%, K3: 0.6%, K4: 0.8% and K5: 1%. The number followed by the same letter in the figure was not significantly different in the Duncan test at a rate of 5%.

Figure 1 shows that the greater the concentration of the treatment given, the higher the percentage of larval mortality rate, compared to the K0 (control) treatment. In the K0 treatment, no larvae mortality was found. Larval mortality began to occur in the K1 treatment with the lowest percentage of mortality rate at 16%, while the highest mortality was in the K5 treatment at a percentage of 88%. Based on the results shown in Figure 1, the lethal concentration value of 50% (LC50) can be determined by using probit analysis.

The result of the probit analysis showed that the LC50 value is at 0.58% after 24 hours. The regression calculations illustrated the relationship between the extract concentration of *M. micrantha* leaf and larval mortality. This can be obtained using the equation of $y = 0.44 + 21.0x$ with a regression coefficient of $r2 = 0.98$, which is shown in Figure 2.

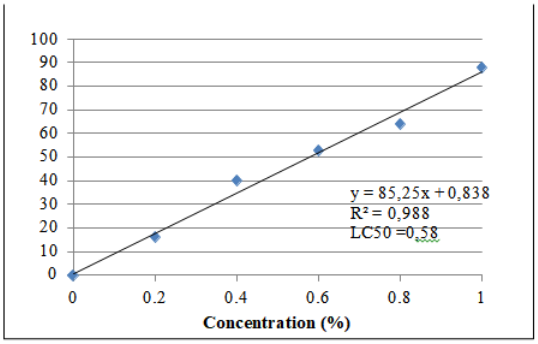

Figure 2: Graph of regression analysis on the effect of the ethanol extract concentration of M. micrantha leaves with the percentage of A. aegypti larval mortality.

Fitmaya (2006) stated that the higher the concentration of the insecticide given, the higher the content of the active substance so it can increase metabolic obstruction of the larvae subject which led to the increasing percentage of its death. Haisya, N. et al., (2013) mentioned that the leaves of *M. micrantha* contain several active compounds in the form of secondary metabolites such as alkaloids, flavonoids, tannins that are insecticides.

Each active substance has different work principles in impacting larval mortality. According to Cania (2013), alkaloids serve as a stomach poison. Alkaloids, in the form of salts, can degrade the cell membranes to damage the cells and also disrupt the larval nerve system by inhibiting the action of the acetylcholinesterase enzyme and causing larvae to undergo paralysis and die. Flavonoids act as a respiratory poison, causing larvae death. Tannins play a role in reducing the ability to digest the food by suppressing the activity of digestive enzymes (Haditomo, 2010).

The lower the LC50 value of a substance means that the substance has higher activity in killing experimental animals since it requires a lower concentration to kill the animals simultaneously (Chang, 2004).

3.2 Egg Hatchability and Development of *A. aegypti* Larvae in Sublethal Concentration

The tests on egg hatchability and larval development were performed using LC_{50} at sublethal concentrations (0.58%) of 0.5%, 0.4%, 0.3%, 0.2% and 0.1%.

Table 1: The influence of ethanol extract of M. micrantha leaf on the percentage of the egg hatchability, and the larvae-pupae and pupae-imago growth of A. aegypti in the 14-days observation.

Treatment	Number	Egg Hatchability (%)	Larvae-Pupae Development (%)	Pupae – imago Development (%)
P0	25	92^{a}	85.3^{a}	92.0^{a}
P1	25	80.8^{b}	68.4^{b}	87.0^{b}
P2	25	73.6^{b}	66.4^{b}	82.3^{b}
P3	25	65.6^{c}	44.3^{c}	78.3^{c}
P4	25	41.6^{d}	19.5^{d}	63.3^{d}

Note that P0= 0 % (control), P1= 0.1%, P2= 0.2%, P3= 0.3% , P4= 0.4%, and P5= 0.5%. The number followed by the same letter in the same column was not significantly different in the Duncan test at a rate of 5%.

3.2.1 The Egg Hatchability

Table 1. shows that the ethanol extract of *M. micrantha* leaf at treatments of P1, P2, P3, P4, and P5 can decrease the rate of the egg hatchability, and development of larvae-pupae and pupae-imago compared to P0 (control). The number of hatched eggs decreases with increasing concentration of the given treatment. The lowest number of hatched eggs was at P5 treatment at a percentage of 26.4%, while the highest hatchability rate at P1 treatment at 80.8%.

The difference in egg hatchability is thought to be caused by the secondary metabolite content of the extract which disrupts the metabolism in the egg, so the egg fails to hatch. Salam et al., (2014) stated that *M. micrantha* leaves contain active substances such as flavonoids and tannins. The decline in the percentage of the egg hatchability is due to the flavonoids that enter the egg through the diffusion process on the surface of the eggshell.

3.2.2 Development of Larvae - Pupae

Table 1 showed that the ethanol extract of *M. micrantha* leaf in all treatments was able to suppress the development of the larvae-pupa stage. The highest percentage of the successful growth of larvae-pupa was in P1 treatment at 68.4% while the lowest occurred in P5 treatment at 11.5%. This shows that during exposure, the ethanol extract of *M. micrantha* leaves affected the development of larvae into pupae. The higher concentration of ethanol extract of *M. micrantha* leaves led to the failure of larvae to become pupae, thus reducing the percentage of successful development

During the observation, the larval phase showed a tendency to require a longer time to develop into pupae, which was around 10-12 days, compared to the control treatment, which only took 8-10 days. The larvae also tend to experience changes in body size which is larger than the larvae in the control treatment group. This may be caused by the extracted content of *M. micrantha* leaf, in the form of Mikanolide, and belongs to the sesquiterpene group, which has a structural similarity to the juvenile hormone.

Bowers (1971) stated that the metamorphosis stage from larvae to pupae is sensitive and complex. The distribution of juvenile hormones in a long time can have effects such as the formation of large larvae. Elimam et al., (2009) also revealed that the levels of the juvenile hormone could directly determine the larval stage to become a pupa that will last a long time.

3.2.3 Development of Pupae - Imago

The living pupae were observed their development into the imago. The percentage of pupa-imago was obtained from the comparison of the number of imagoes formed with the total number of pupae. Table 1 shows that the highest percentage of successful pupa-imago development was in the P1 treatment at 87.0%, while the lowest was in the P5 treatment at 50.0%. All ethanol extracts of *M. micrantha* leaves tended not to affect the percentage of developmental failure of the pupae-imago stage compared to the control treatments.

This can be seen from the high percentage of pupae-imago. This may be related to the pupae that no longer needs food, which means that the pupae do not consume the extract solution anymore so that the pupa can avoid the toxic effects of the ethanol extract of *M. micrantha* leaves.

During the observation, it could be seen that the growth of pupae to imago needed a longer time,

which was around 5-6 days. (Yulidar & Wilya, 2015) stated that the normal time for pupae to develop into imago is 3-4 days.

The pupae stage is a fasting phase where the body is wrapped in a layer called the puparium. The increase in the time it takes for a pupa to become an imago may be due to the pupa trying to survive by extending its maturation period into an imago, so the pupa remains protected by a protective layer that wraps its body from exposure to toxic extracts. Typically, at the fourth instar larvae, there is a decrease in the secretion of juvenile hormone by corpora allata, but with the exposure to the ethanol extract of *M. micrantha* leaves, which was thought to have an effect like juvenile hormone, induced the pupae to prolong its development into an imago (Habibi, 2011).

Based on the results of the study on the effect of the ethanol extract of *M. micrantha* leaves on the egg hatchability and the development of *A. aegypti*, it can be determined that the most significant concentration which can reduce the rate of the egg hatchability and the larval development is the P4 treatment with a concentration of 0.4 %.

The same thing occurred in the research of Nursal & Hardiansyah (2018). The dichloromethane extract of the leaves of the bitter melon (Momordica charantia L.), basil (Ocimum basilicum L.), and lemongrass (Cymbopogon winterianus) can reduce the percentage of the egg hatchability and the larval development (larvae- pupae and pupae – adult) of Aedes aegypti. Likewise, with Nursal & Yeanny (2019), the ethanol extract of the leaves of bitter melon (Momordica charantia L.) and basil (Ocimum basilicum L.) can also reduce the hatchability of the eggs and the growth (larvae-pupae and pupae-adult) of Aedes aegypti mosquitoes.

4 CONCLUSIONS

Based on the test results, it can be concluded that the LC50 concentration of the ethanol extract of *M. micrantha* leaves on the mortality of third instar larvae was at 0.58%. The sublethal concentration of the ethanol extract of *M. micrantha* leaves has a significant influence on the eggs hatchability and larval development. The ethanol extract concentration of *M. micrantha* at 0.4% was effective in reducing the rate of the eggs hatchability and the larval development into pupae, and the pupae development into imago by 41.6%, 19.5%, and 63.3% respectively.

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