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(CASE REPORT)

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The toxicity of mixture of earring leaf and root extract (*Acalypha indica* L.) against *Aedes aegypti* mosquito larvae mortality

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Abstract

Tropical regions such as Indonesia are known to have optimal conditions of humidity and temperature that are very conductive for the survival of insects, including the *Aedes aeypti* mosquito which contains the Dengue virus. Until now, people still use chemical control using chemically active larvicides such as temephos. The continued use of temephos can cause the mosquito vector to become resistant to the next generation and be harmful to the environment. One way to control vectors can be done biologically by using natural insecticides, a mixture of leaf and root extracts of the earrings plant (*Acalypha indica* L.). The study used a mixture of leaf and root extracts of the earrings (*Acalypha indica* L.) plant using 7 treatments consisting of 5 serial concentrations of 250ppm, 500ppm, 750ppm, 800ppm and 1000ppm, 1 positive control using Aedes and 1 negative control using Abate. The concentration that can kill 50% of *Aedes aegypti* larvae or (LC_{50}) within 24 hours of exposure is 766,000ppm. The plants contain secondary metabolites such as alkaloids, flavonoids, triterpenoids and steroids which is toxic to the larvae of the *Aedes aegypti* mosquito.

Keywords: Aedes aeypti; *Acalypha indica* L.; Toxicity; Root Extract; Larvae Mortality

1. Introduction

Indonesia has humidity and warm temperatures which are very beneficial for the survival of insects, including mosquitoes. Mosquitoes are considered harmful to humans because they are vectors of infectious diseases. One of them is dengue hemorrhagic fever (DHF) which is transmitted by the *Aedes aegypti* mosquito which contains the Dengue virus (Oroh et al., 2020 Dengue Hemorrhagic Fever (DHF) is a very dangerous disease because it can cause the sufferer to die within a few days (Suryowati et al., 2018). The Dengue virus develops a lot in urban, suburban and rural areas with a low level of environmental hygiene so that currently DHF is still a national health problem and often has the status of an Extraordinary Event (EV) with a fairly high number of cases in a number of areas in Indonesia. (Anisak & Dewi, 2019). The high number of dengue cases increases during the rainy season, due to an increase in the *Aedes aegypti* mosquito population. In 2021 there were 37,127 cases recorded. The number of deaths due to DHF was recorded as many as 361 cases spread over 472 regencies/cities in 34 provinces, while deaths occurred in 210 regencies/cities, therefore it is necessary to control the *Aedes aegypti* mosquito vector. (RI Ministry of Health, 2021). To date, the control of the *Aedes aegypti* mosquito vector still often uses biological, chemical and mechanical methods. The community is still using chemical control using larvicides with chemical active ingredients such as fogging and Abate (temephos). (Suwandi & Halomoan, 2017). Temephos can kill the DHF mosquito vector in a short period of time and a wide range because it has the basic ingredients of synthetic organic chemicals, but temephos can cause the DHF mosquito vector to become resistant to the next offspring. Temephos contains chemicals that are difficult to decompose in nature and insecticide residues, so temephos is dangerous for the environment because it can cause water pollution and poisoning several types of animals. (Kurniawan et al., 2019). One way to control the DHF vector can be done biologically using bioinsecticides. Bioinsecticides come from plant parts that contain active compounds such as secondary metabolites which are toxic and can affect the behavior and nervous system of the DHF vector but have no impact on the

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environment or the people around them. (Fuad, 2021). Earring plant (*Acalypha indica* L.) is considered a weed because of its large number and it is often found on roadsides, unkempt grassy fields and agricultural land, because of its large number it can provide opportunities for this plant to increase its use value as an insecticide naturally by utilizing the leaves and roots which contain several secondary metabolites such as alkaloids, flavonoids, triterpenoids and steroids (Kirom & Ramadhania, 2017).

2. Material and Methods

2.1. Types of research

The study "Totoxicity of leaf and root extracts of the Anting-anting (*Acalypha indica* L.) plant on the mortality of *Aedes aegypti* mosquito larvae" is a laboratory and quantitative experimental study. This research was conducted using a completely randomized design (CRD).

2.2. Place and time of research

This research was carried out at the Toxicology Sub-Laboratory of the Biology Education Study Program, Faculty of Teacher Training and Education (FKIP), University of Jember and the Entomology Laboratory of the Surabaya Provincial Health Office. The time for research to be carried out is between October 2021 and completion.

2.3. Tools and materials

The tools used in this study were baking trays, blenders, glass jars, stirrers, filters, funnels, filter paper, rotary evaporators, ovens, plates, measuring cups, beaker glass, tissues, spoons, aluminum foil, analytical balance, refrigerators, bottles. glass, plastic cups, pipettes, microscopes, object glass, digital cameras and label paper. The materials used in this study were the leaves and roots of the earrings plant (*Acalypha indica* L.), 97% ethanol *Aedes aegypti* mosquito larvae in late III and early IV instars, eosin, distilled water as larval habitat when tested and negative control and abate as positive control . Used branded Abate 1 gr.

2.4. Research Sample

The *Aedes aegypti* mosquito larvae used in this study were ± 500 larvae. The Preliminary Test did not repeat, using 20 larvae for each test concentration in the preliminary test. Meanwhile, in the final test, three repetitions were carried out using 20 larvae at each test concentration. The samples used in this study were late third and early fourth instar *Aedes aegypti* mosquito larvae. The larvae of the *Aedes aegypti* mosquito used were healthy, had agile movements, the spines on the chest were clear, and the selected black breathing funnel was healthy and agile. Research Sample This study used test larvae of *Aedes aegypti* late third and early IV instars which had already formed organs that were relatively stable against environmental influences. The subject of this study was a mixture of leaf and root extracts of the earrings plant (*Acalypha indica* L.) as a natural insecticide. Positive control with abate and negative control using distilled water. The object of this research was the late third and early fourth instar *Aedes aegypti* mosquito larvae. Determination of the number of repetitions is obtained from the Federer formula, as follows:

$$(t-1)\ (r-1) \geq 20$$

Information:

t : *treatment* (number of treatments)

r : *replication* (number of repetitions)

20 : free amount for Full Random Design

3. Results

3.1. Research result

This study used five series of concentrations obtained based on the results of the preliminary test. The study used a mixture of leaf and root extracts of the earrings (*Acalypha indica* L.) plant using 7 treatments consisting of 5 serial concentrations of 250ppm, 500ppm, 750ppm, 800ppm and 1000ppm, 1 positive control using *Aedes* and 1 negative control using Abate, with 4 repetitions for each treatment. Each test cup contained 20 *Aedes aegypti* larvae and was observed within 24 hours of exposure. The final test was carried out to determine the concentration value of a mixture

of leaf and root extracts of the *Acalypha indica* L. plant which could kill 50% of the test larvae or **LC_{50}** within 24 hours of exposure. The Preparation of Earring Leaf and Root Extract (*Acalypha indica* L.) show in Figure 1 below.

Collection of leaves and roots of earrings (*Acalypha indica* L.)

Drying of leaves and roots of earrings (*Acalypha indica* L.)

Smoothing of leaves and roots of earrings (*Acalypha indica* L.)

Weighing the powder of the leaves and roots of the earrings (*Acalypha indica* L.)

Maceration of earring leaves (*Acalypha indica* L.)

Maceration of the root of the earring (*Acalypha indica* L.)

Process rotary evaporators

Results of the earring leaf rotary evaporator (*Acalypha indica* L.)

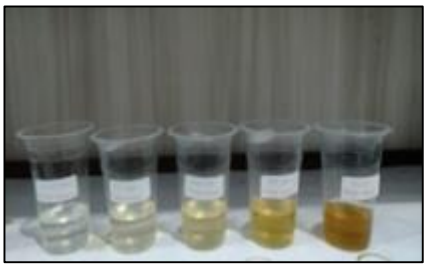
Results of earring root rotary evaporator (*Acalypha indica* L.)

Figure 1 The Preparation of Earring Leaf and Root Extract (*Acalypha indica* L.)

The Preliminary Test and Final Test shown on Figure 2 Below.

Extract weighing

Selection of third instar larvae



Test Preliminary Mixture of leaf and root extracts of earrings

Final Test A mixture of leaf and root extracts of earrings

Microscopic observation of *Aedes aegypti* larvae

Figure 2 The Preliminary Test and Final Test

The mixture of leaf and root extracts of the earrings (*Acalypha indica* L.) plant with a 1:1 ratio uses serial concentrations of 250ppm, 500ppm, 750ppm, 800ppm and 1000ppm obtained based on preliminary test results. 20 test larvae were used and observations were made within 24 hours of exposure

Table 1 Mortality of *Aedes aegypti* mosquito larvae in the final test after administration of a mixture of leaf extract and root extracts of the *Acalypha indica* L. plant with a ratio of 1:1 in 24 hours exposure time

Consentration (ppm)	***Aedes aegypti* Larvae Mortality**				**mortality rate ± SD**
	Repetition				
	1	2	3	4	
K(+)	0	0	0	0	0 ± 0
K(-)	20	20	20	20	20 ± 0
250	1	1	2	2	2 ± 0,5
500	6	5	4	4	5 ± 0,829
750	9	11	11	8	10 ± 1,299
800	12	12	12	9	12 ± 1,299
1000	15	17	18	17	17 ± 1,089

Based on the calculation results shown in Table. 1 showed that the higher the concentration of the mixture of leaf and root extracts of the *Aedes aegypti* mosquito larvae, the higher the mortality rate of *Aedes aegypti* mosquitoes. The lowest mortality of *Aedes aegypti* mosquito larvae was at a concentration of 250 ppm with an average number of deaths of 2 larvae, while the highest mortality of *Aedes aegypti* mosquito larvae was at a concentration of 1000 ppm with an average total number of deaths of 17 larvae.

Table 2 Probit Analysis LC_{50} of a mixture of leaf and root extracts of the Anang (*Acalypha indica* L.) plant

Lethal Concentration (LC_{50})	**LC_{50}**	**Lower limit**	**Upper limit**
	(ppm)	(ppm)	(ppm)
Extract of a mixture of leaves and roots of the earring plant (*Acalypha indica* L.)	756,709	686,520	812,486

Probit analysis in the final test was carried out to determine the Lethal Concentration 50 (LC_{50}) extract of a mixture of leaves and roots of the earrings plant (*Acalypha indica* L.) on the mortality of *Aedes aegypti* mosquito larvae within 24 hours of exposure. Probit analysis was performed using SPSS. Based on the results of the probit analysis in table 2, it can be seen that the concentration of a mixture of leaf and root extracts of the Anting-anting plant (*Acalypha indica* L.) required to kill 50% of the test larvae within 24 hours of exposure time is 765.709 ppm with a lower limit of 686.520 ppm and a lower limit of 686.520 ppm. over 812.486 ppm. The lower concentrations are the lowest concentrations of an extract that can kill 50% of the test larvae within 24 hours of exposure while the upper concentrations are the highest concentrations of an extract that can kill 50% of the test larvae within 24 hours.

4. Discussion

Toxicity is the ability of a chemical substance to cause damage to organisms. Toxicity in this study is the toxic effect caused by compounds contained in a mixture of leaf and root extracts of the earing plant (*Acalypha indica* L.), after application to the test larvae causing death and damage to the *Aedes aegypti* mosquito larvae. (Kurniawidjaja et al., 2021). The magnitude of the toxic effect produced by an extract will be directly proportional to the mortality of *Aedes aegypti* larvae so that the higher the concentration of the extract, the higher the mortality of the test larvae, conversely, the lower the concentration, the lower the mortality of the test larvae. In addition, the toxic effect depends on the compounds contained in the extract. The late third instar *Aedes aegypti* mosquito larvae show on Figure 3 below.

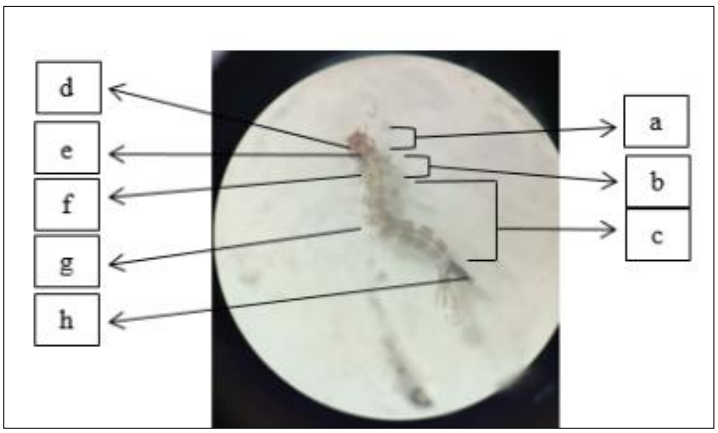


Figure 3 Late third instar *Aedes aegypti* mosquito larvae. (a) Chief (Chepal); (b) Chest (Thorax); (c) Stomach (Abdomen); (d) Antenna; (e) Eyes; (f) Thorn (Bristle); (g) Lateral Hair; (h) Breathing funnel (Siphon). (Microscope magnification: 40x)

The study of the toxicity test of a mixture of leaf and root extracts of the Anting-anting (*Acalypha indica* L.) plant on the mortality of *Aedes aegypti* mosquito larvae showed the death of the larvae, this was due to the toxic compounds contained in each extract. According to Kirom, 2017. the leaves and roots of the earrings plant (*Acalypha indica* L.) contain several compounds that are equally toxic to *Aedes aegypti* mosquito larvae, some of these compounds include alkaloids, saponins, tannins, steroids and flavonoids (Silalahi, 2019), All of these compounds can be obtained through the leaves and roots of the earrings plant by the extraction process.

The LC_{50} of a mixture of leaf and root extracts of the earrings (*Acalypha indica* L.) plant with a ratio of 1:1 on the mortality of *Aedes aegypti* mosquito larvae within 24 hours of exposure time can be determined by probit analysis using SPSS. Based on the results of the probit analysis that was carried out using SPSS, the concentration value that could kill 50% of the test larvae or LC_{50} obtained was 766,000 ppm with a lower limit of 100,000 ppm and an upper limit of 800,000 ppm. The data from the analysis shows that to kill 50% of the test larvae requires a mixed concentration of leaf and root extracts of the earrings (*Acalypha indica* L.) plant, namely 766,000 ppm, the lower limit indicates that if the extract concentration is less than 100,000 ppm, it is likely that the mortality of the test larvae is reaches 50%, while the upper limit indicates that if the concentration of the extract exceeds 800 ppm then it is likely that the mortality of the test larvae can exceed 50%. The study of a mixture of leaf and root extracts of the Anang-anting (*Acalypha indica* L.) plant on the mortality of *Aedes aegypti* mosquito larvae within 24 hours of exposure also made observations of third instar *Aedes aegypti* larvae before and after treatment to determine changes that occur in the body of the larvae after treatment was carried out. Based on the observations, the body condition of the larvae can be seen in Figure 4.

a b

Figure 4 *Aedes aegypti* mosquito larvae (a) Third instar *Aedes aegypti* mosquito larvae without treatment (b) Third instar *Aedes aegypti* mosquito larvae after treatment with a mixture of leaf and root extracts of the earrings plant (*Acalypha indica* L.) (Microscope magnification: 40x0.67)

Based on the observations in Figure 2, Figure (a) is the third instar *Aedes aegypti* mosquito larvae before being treated. The body morphology of third instar *Aedes aegypti* mosquito larvae that can be observed under normal conditions includes clearly visible spines on the chest, the respiratory funnel begins to blacken, the horns on the head begin to be clearly visible. Figure (b) is the third instar larvae of *Aedes aegypti* mosquitoes after being treated with a mixture of leaf and root extracts of the earrings plant (*Acalypha indica* L.). *Aedes aegypti* mosquito larvae showed the death of the larvae, this was caused by the toxic compounds contained in each extract. According to Kirom, 2017 the leaves and roots of the earrings plant (*Acalypha indica* L.) contain several compounds that are equally toxic to *Aedes aegypti* mosquito larvae, some of these compounds include alkaloids, saponins, tannins, steroids and flavonoids (Silalahi, 2019) , all of these compounds can be obtained through the leaves and roots of the earrings plant by the extraction process.

The body morphology of third instar *Aedes aegypti* mosquito larvae that can be observed after being given treatment includes the mid gut (middle digestive tract) starting to break down and crack this is caused by alkaloid compounds which are antifeedant which prevents the larvae from eating by making the larvae lose receptors taste in the mouth area which causes the larvae to fail to recognize their food so that the larvae do not get nutrition, this causes the death of the larvae (Yuliasih, 2017). Alkaloid compounds work by entering the digestive tract towards the front (stomodeum), middle (mesenteron) and rear (proctoderum) digestive tract so that they will pass through the peritropic membrane and then bind to special receptors found on the microvilli of epithelial cells of the middle digestive tract (mesenteron).) which is composed of lipids and proteins. The binding of compounds that are stomach poisons with special receptors found in the microvilli of the epithelial cells of the middle digestive tract (mesenteron) results in the three-dimensional structure of the protein being disrupted and opened, causing denatured protein, damaged biological activity, and damaged digestive cell walls. Disturbance that occurs in the mesenteron results in disruption of the body's metabolic processes which causes inhibition of ATP production and unable to repair cells that have been damaged, so that the larvae will experience death. The thickening of chitin in the larvae is caused by disruption of growth hormone which affects the thickening of chitin cells in the larvae so that the larvae cannot change their skin and develop which will eventually cause death in the lava, this is caused by steroid compounds (Yuliati, 2017).

The chiffon begins to break down caused by flavonoid compounds because these compounds enter the larvae's body through the respiratory funnel (siphon). The mechanism of action of flavonoid compounds as nerve poisons and respiratory poisons begins with the entry of flavonoid compounds through siphons to the larvae. Flavonoid compounds

can cause wilting of the nerves and cause damage to the chiffon. Flavonoids as nerve poisons will attack the nervous system in several vital organs of the larvae, such as the respiratory organs. Flavonoids act as amikolinesterses which cause the cholinesterase enzyme to phosphorylate and become inactive, so that the electron transport process stops and causes muscle spasms which cause disturbances in the respiratory system (Rahman et al., 2020), (Wahyani, et al., 2020). The cholinesterase enzyme is an enzyme that plays a role in keeping nerve cells, muscles, and glands in the body working in an organized manner. When the larvae experience muscle spasms in their respiratory system, the larvae will have difficulty moving to find oxygen. As respiratory inhibitors, flavonoids can reduce the rate of chemical reactions thereby disrupting the energy formation mechanism in the mitochondria by inhibiting the electron transport system, as a result the respiratory system will be damaged and the larvae will die because they cannot breathe to take in oxygen (Wahyani, et al., 2020).

Utilization of mixed extracts from the leaves and roots of the earrings plant as a vegetable insecticide can have advantages if it has properties where synergistic properties mean it can increase its effect and effectiveness on test larvae and will be detrimental if the mixture of the two extracts is antagonistic because the mixture of plant extracts which are antagonistic will reduce the effect and effectiveness of the insecticide mixture on the larvae. In this study, a mixture of leaf and root extracts of the earrings plant showed that the two extracts combined had an antagonistic effect. It can be seen that the LC_{50} results of the combination of extracts 1 and 2 are not greater and toxic than the single extract which is equal to 756.709 ppm while the single extract has an LC_{50} value of 246.219 ppm. synergistic effect (Taufika et al., 2020).

5. Conclusion

The LC_{50} of a mixture of leaf and root extracts of the earrings plant (*Acalypha indica* L.) on the mortality of *Aedes aegypti* mosquito larvae in 24 hours of exposure time was 756.709 ppm, the two extracts after being combined gave an antagonistic effect, due to the similarity in the content of the compounds in the extracts.

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