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The Effectiveness of *Derris elliptica* (Wall.) Benth Root Extract Against Temephos-resistant *Aedes aegypti* Larvae

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The Effectiveness of *Derris elliptica* (Wall.) Benth Root Extract Against Temephos-resistant *Aedes aegypti* Larvae

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Abstract. *Derris elliptica* (Wall.) Benth is a wild plant or weed in agricultural fields that was traditionally used for poisoning fish and plant pests. Several studies showed the low lethal concentration of *Derris elliptica* root extract to control the laboratory strain *Aedes aegypti* larvae. This study aimed to evaluate the effectiveness of *Derris elliptica* root extract against the temephos-resistant *Aedes aegypti* larvae from the Dengue endemic areas. This experimental study used post-test only control group design with five different concentrations of methanolic *Derris elliptica* root extract, namely 0.09%, 0.13%, 0.17%, 0.21%, and 0.25%, respectively. Twenty-five of F1 temephos-resistant of wild-caught *Aedes aegypti* larvae were subjected to each group of the five different concentrations, and two control groups with aquadest. The temephos-resistant strain of *Aedes aegypti* larvae was determined by bioassay test with temephos -0.02 ppm compound. Mortality rate and lethal concentration (LC50 and LC90) of the larvae were calculated after 24-h contact. Average of mortality rate in each concentration after 24-h contact with *Derris elliptica* root extract were 32.8%, 49.6%, 72.0%, 95.2%, and 100% with LC50 and LC90 were 1,600 and 2,040 ppm, respectively. The methanolic *Derris elliptica* root extract have larvacidal potential to control the temephos-resistant *Aedes aegypti* larvae. Further research to obtain secondary metabolites is needed.

Keywords: *Derris elliptica, Aedes aegypti* larvae, temephos-resistant, Dengue vector

1. Introduction

Derris elliptica (Wall.) Benth or tuba is one of the wild plants found in farmland of South and Southeast Asia [1]. The root of the tuba plant was traditionally used as the fish poison and crop pest insecticide [2,3]. This plant contains many kinds of toxic chemical compounds [4], mainly flavonoid group [5] including rotenone, toxicarol, elliptone, sumatrol, tephrosin, and deguelin [6,7]. Recently, using tuba root extract has developed in various bioactive compounds exploration not only for the agricultural field but also public health intervention, especially in Dengue control programs.

Chemical compounds of tuba root extract have been tried to control the diseases vector mosquitoes including the Dengue vector, *Aedes aegypti* and *Aedes albopictus* [8,9] since this disease affected a half of world population, mainly in the tropic and subtropical regions [10]. Dengue prevention is done by combining the vector control program and vaccination [11], although the vaccine is still under researched [12], and the chemical methods use are preferred by people in dengue endemic areas rather than others [13]. Temephos is one of the chemical compounds that world widely used to control *Aedes aegypti* larvae since the last three decades [14-17]. This phenomenon caused the emergence of temephos-resistant strains of *Aedes aegypti* world widely.

The resistance of *Aedes aegypti* larvae to temephos was reported from several countries in the world, such as Brazil [18,19], Columbia [20], Argentina [21], India [22], French West Indies [23]. In Indonesia, temephos resistant populations of this Dengue vector were reported from Jakarta [24], West Java Province [25,26], Surabaya [27], Tanjung Emas Harbor of Semarang [28], and Kendari – Southeast Sulawesi [29]. The emergence of temephos-resistant strains of *Aedes aegypti* larvae inhibits

Dengue control program in the endemic areas. Trial in using herbal compounds is an alternative effort for finding a new solution to control the resistant-strains of *Aedes aegypti* larvae.

Several previous studies about using a bioactive compound of tuba root extract to control *Aedes aegypti* larvae were done in different origin and with different extraction solvents. The studies used laboratory strain mosquito and showed various results. Our study in Semarang using four different of ethanolic extract of tuba root ranged from 0.5 to 4 percent showed that mortality rate of *Aedes aegypti* larvae ranged from 86 – 100 percent, and the effective concentration was 2% above [8]. Another study from Manado, North Sulawesi also used ethanol solvent and laboratory strain of *Aedes aegypti* larvae and showed the lethal concentration of 50% (LC50) was 44.75 ppm [9]. In Malaysia, a similar study use four solvent variations of methyl chloride and methanol comparison showed that the 0.05 mg/ml of tuba root extract caused 83.33% of larvae mortality [30].

The use of different extraction solvent of tuba root resulted in different lethal concentration of laboratory strain of *Aedes aegypti* larvae mortality. Another study showed that methanol solvent results the highest extractable solid rather than hexane, ethanol, and acetone [31]. This study aimed to study the effectiveness of methanolic extract of tuba root to control the field strain *Aedes aegypti* larvae which resistant to temephos 0.02 mg/L.

2. Material and methods

2.1. Derris elliptica origin, determination, and extraction.

Root of tuba plant was obtained from Jepara district, Central Java Province, Indonesia. Determination test for this plant was conducted at the Chemical Laboratory of Mathematical and Natural Sciences Faculty of Universitas Padjadjaran, Bandung, Indonesia. Extraction process based on the previous method [32] by using methanol solvent was done at the Chemical Laboratory of Mathematical and Natural Sciences Faculty of Universitas Negeri Semarang, Semarang, Indonesia.

2.2. Aedes aegypti larvae collection and rearing.

Larvae of Aedes mosquitoes were collected from perimeter areas of Tanjung Emas Harbor, Semarang, Indonesia. Based on the previous study [28], the *Aedes aegypti* population was resistant to temephos 0.02 mg/l. Larvae were reared into adult stage and subjected to the morphological species identification based on the Walter Reed Biosystematics Unit procedure [33]. The eggs of *Aedes aegypti* mosquito resulted before from the identification test were reared in the cage and allowing the mating and resulting the first generation (F1). The 3-5 days of F1 larvae were subjected to temephos 0.02 mgl/l bioassay test for determining the recent resistance status [34] of the population to the temephos compound. The temephos bioassay test resulted from the 73% of mortality of *Aedes aegypti* larvae; indicating the resistance status to the compound.

2.3. Experimental design.

The posttest-only control group design experiment was conducted at the Epidemiology and Tropical Diseases Laboratory of Public Health Faculty of Universitas Muhammadiyah Semarang. Preliminary bioassay test using the tuba root extract was designed based on the previous study [9] based on concentration ranged from 10, 50, 100, 500, and 1,000 ppm, and resulting the concentration 1,000 ppm (0.1%) causing the 52% of mortality. Based on the results, experiments were performed with concentration ranged from 0.09 %, 0.13 %, 0.17 %, 0.21 %, dan 0.25 %. As many as the 25 of third instar of *Aedes aegypti* larvae (temephos-resistant strain from Tanjung Emas, Semarang) in each group were subjected to the five groups of experiment with five replications, and accompanied by the 25 larvae of control in the cup with tap water. Larvae were contacted for 24 hours to the solution of tuba root extract, and the knockdown larvae were recorded at the 30, 60, 120, 240, 480, and 1440 minutes. Mortality rate of *Aedes aegypti* larvae were recorded after 24 hours contacting with the tuba root extract solution.

2.4. Data analysis.

Descriptive and analytical data analysis was performed by the SPSS statistical software using the one-way analysis of variance and probit test. Results of the data analysis were presented in the tables and figures.

2.5. Ethical consideration.
Ethics approval was obtained from the Ethic Committee of Health Research of Public Health Faculty of Universitas Muhammadiyah Semarang with registration number 125/KEPK-FKM/UNIMUS/2018.

3. Results
Derris elliptica root extract with the tested-concentration range has the larvacidal effect to the temephos-resistant strain of *Aedes aegypti* larvae. Average of the knockdown *Aedes aegypti* larvae increased based on the concentration of the extract, and one hundred percent of knockdown rate was reached at the 24 hours exposure by the concentration of 0.25% (Figure 1).

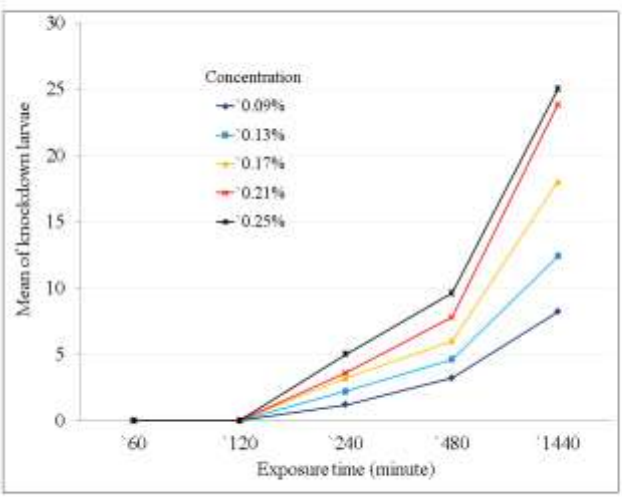

Figure 1. The knockdown of *Aedes aegypti* larvae based on exposure time and tuba root extract concentration.

Different concentration of tuba root extract caused significantly difference of the temephos-resistant of *Aedes aegypti* larvae mortality after 24-h exposure (Figure 2). The lowest and highest mortality rates (32.8% and 100%) of *Aedes aegypti* larvae were showed by the 0.09% and 0.25% of concentrations (Table 1). Probit analysis showed that the effective concentrations (LC50 and LC90) were 0.160 % (0.142 % - 0.170 %) and0.204% (0.194 % – 0.218 %).

Table1 Mortality rate (%) of the temephos-resistant *Aedes aegypti* larvae after 24 hours exposed to tuba root extract

Concentration (%)	Minimum (%)	Maximum (%)	Average (%)
0,09	20	44	32.80
0,13	40	60	49.60
0,17	64	84	72.00
0,21	88	100	95.20
0,25	100	100	100.00
0 (control)	0	0	0

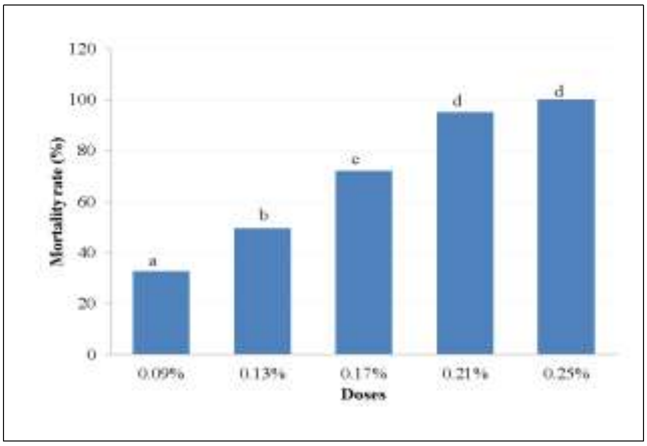

Figure 2.Mortality rate difference of *Aedes aegypti* larvae after 24 hours exposure of tuba root extract concentrations

4. Discussion

Larvacidal potency of tuba root extract for control the primary Dengue vector, *Aedes aegypti* mosquito has been studied and reported in the last decades with various results [7-9]. Two previous studies using laboratory strain of *Aedes aegypti* larvae with different solvent extraction, ethanol and methanol showed the equal result [7,8], but a study from North Sulawesi using ethanol solvent resulted in the lowest LC50, namely 44.75 ppm. The result arose a new hypothesis that the chemical compound containing of the tuba plant is not influenced by solvent, but different geographic habitat. It is similar to the previous report that the different geographic result different chemical compound quantity [35].

This study is the first report that this trial was conducted to increase the efficacy of larvacidal effect of tuba root extract against temephos-resistant *Aedes aegypti* larvae. Knockdown effect of each concentration level of tuba root extract was started at the 2nd hour of observation showing the slow toxicity mechanism. The main result showed that the effective dose of tuba root extract for control this strain is 36 folds higher than the laboratory strain. The lowest LC50 of the previous study was 44.75 ppm equal with 0.0045% [9]. It can be understood that the current study uses temephos-resistant *Aedes aegypti* larvae as the research subject so that higher larvicidal doses were needed. Although the resistant ratio of *Aedes aegypti* to organophosphate insecticide was not available yet, but a study in Semarang showed the resistance ratio of this species to pyrethroid compound was 119.75 folds [36]. A comprehensive study to test the larvacidal effect of tuba root extracts by comparing susceptible and temephos-resistant larvae at the same time and place is needed to strengthen these findings.

Toxicity of flavonoids and fatty acid compounds containing in the *Derris elliptica* root extract to mosquito larvae by a unique mechanism affecting both of acetylcholinesterase site and octopaminergic systems [37]. Another compound, saponin affect mosquito larvae by causing the change of food taste, digestive disorders, and cell damage resulting in disruption of growth and death [38,39].

In the past, the resistance of *Aedes aegypti* larvae to temephos has been reported world wide, included Indonesia [18-29]. This condition inhibits the Dengue vector control program based on chemical method, and necessary to be solved. This study provided the new promising results to obtain the new effective chemical compounds for control the Dengue vectors, especially the temephos-resistant strains in the Dengue endemic areas. Further investigation is needed to understand the kinds, role, and quantity of the secondary metabolites that have the high potency for control the resistant mosquito vectors as well as the larvacidal potency for controlling larvae of the other mosquito vectors. Several aspects are necessary to be understood such as larvacidal efficacy of tuba root extract based on degradation period, residual effect, persistence to temperature, and water replacement effect to the toxicity of the extract to *Aedes aegypti* and the other species larvae as well as the microbes in the household water container. The most important advantage of the use of *Derris elliptica* root extract is easily degradable with short residual effect.

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