

# Differences in the Quality of Preserved Preservations of Aedes sp Larva in the Clearing Process Using Toluene and Clove Oil

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## Differences in the Quality of Preserved Preparations of *Aedes sp* Larva in the Clearing Process Using Toluene and Clove Oil

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### ABSTRACT

The purpose of making entomological preserved preparations was to identify insect morphology, which went through the stages of fixation, dehydration, clearing and mounting. Clearing is a step that aims to make the insect structure visible, clear and transparent, the materials used for the clearing process are toluene and clove oil which have carbon compounds and minimal toxicity. The purpose of this study was to determine the quality of preparations preserved by *Aedes sp* larvae in the clearing process using toluene and clove oil. This type of research was analytic with a cross-sectional approach with two treatments, namely toluene and clove oil, with 16 samples for each treatment. Preserved preparations of *Aedes sp* larvae were observed microscopically to assess the quality of preserved preparations which included the clarity of preparations, color quality, and the integrity of the parasite's limbs. The results showed that in the clearing treatment with toluene, 5 preparations were of good quality, 4 preparations of good quality, and 7 preparations of poor quality. Clearing treatment with clove oil obtained 12 preparations of good quality and 4 preparations of moderate quality. The Mann-Whitney statistical test showed a p-value of 0.032. It can be concluded that there is difference in the quality of preparations preserved by *Aedes sp* larvae in the clearing process using toluene and clove oil.

**Keywords:** quality of preparations; *Aedes sp*; toluene; clove oil

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### INTRODUCTION

Preserved preparations are samples of pathological and anatomical specimens that are placed or smeared on the surface of glass objects or slides with or without staining, which aims to identify, recognize, and observe the morphology of the parasite <sup>(1)</sup>. One of the parasites that still disturbs the environment and humans is *Aedes sp* larvae, because *Aedes sp* larvae can breed into adult mosquitoes which are one of the vectors of DHF (Dengue Hemorrhagic Fever) disease in humans which is transmitted through its bite on the human body <sup>(2)</sup>.

The process of making permanent preserved preparations begins with a fixation process using KOH solution for exoskeleton thinning, a dehydration process using a graded alcohol solution for the withdrawal of water molecules, a clearing process for clarification and a mounting process for tissue adhesion <sup>(1)</sup>.

The clearing process aims to clear the tissue preparations so that the insect structure looks clearer, clearer and transparent when observed using a microscope <sup>(3)</sup>.

The clearing process usually uses a solution of benzol, toluene, xylol, acetone and clove oil <sup>(1)</sup>. Clearing generally uses xylol solution, because xylol solution is a solution with a high refractive index and quickly attracts alcohol, but xylol solution has the disadvantage that it is flammable, if soaking is too long the tissue becomes dry and brittle, the preparations do not last long and xylol solution is a chemical that is toxic so that it can give side effects to the body when exposed continuously <sup>(4)</sup>. Then the xylol solution can be replaced with other materials that have the same solvent characteristics <sup>(1)</sup>. Toluene solution is a colorless aromatic hydrocarbon compound.

Toluene and xylol belong to the benzene group which has almost the same chemical formula <sup>(6)</sup>. In addition to toluene solution as a substitute solution, clove oil can be used as a clearing solution to clarify preparations.

Clove oil is an essential oil derived from the clove plant (*Syzygium aromaticum*) which is part of a hydrocarbon compound that has nonpolar properties so that it can attract alcohol in preparations.<sup>(1)</sup> Clove oil is obtained from clove bud oil, clove stalks oil, and clove leaf oil.<sup>(7)</sup> Clove oil has the advantages of faster purification, relatively cheaper prices, and does not contain toxins. The disadvantage of clove oil is that it can only clean the tissue transferred from 96% alcohol concentration <sup>(1)</sup>.

Based on this background, the researchers wanted to find out whether there was a difference in the quality of preparations for preserved *Aedes sp* larvae in the cleaning process using toluene and clove oil.

## METHODS

The type of research used was analytical research with a cross-sectional approach <sup>(9)</sup> it examines the difference between the clearing process using toluene and clove oil on samples of *Aedes sp*. The research was carried out at the Parasitology Laboratory of the Department of Health Analyst, Universitas Muhammadiyah Semarang in May 2022. The research population was *Aedes sp* larvae in the Gemah Sari area of Semarang. The sample in this study was *Aedes sp* larvae obtained from ovitraps placed in the Gemah Sari area, Tembalang sub-district, Semarang City by means of purposive sampling.

The research variables were the type of clearing material as the independent variable and the quality of the preserved preparations as the dependent variable. Variable measurement was done by: *Aedes sp* larvae as many as 32 samples were made to make quality preparations of preserved *Aedes sp* larvae with 2 treatments to see the color, clarity, and integrity of *Aedes sp* larvae preparations. The tools in this research were: microscope, tweezers, tissue, cotton, object glass, deck glass (cover glass), wide-mouthed bottle, beaker glass, measuring cup, dropper. The materials used for this study were: samples of *Aedes sp* larvae, graded alcohol ranging from concentrations of 30%, 50%, 70% and 96%, aquadest, 10% KOH solution, emtellan, clove oil and toluene.

How to make preserved preparations were: soak *Aedes sp* larvae in 10% KOH solution for 24 hours, second soak *Aedes sp* larvae in 30% alcohol for 5 minutes, third soak *Aedes sp* larvae in 50% alcohol for 5 minutes, fourth soak *Aedes sp* larvae into 70% alcohol for 5 minutes, the fifth *Aedes sp* larvae soaked in 96% alcohol for 5 minutes, the sixth *Aedes sp* larvae preparations were added to the toluene clearing agent for 5 minutes with 3 repetitions while the clove oil clearing agent was soaked for 15 minutes with 1 repetition, and finally mounting or closing the preparation using emtellan.

After obtaining data on the quality of preparations from clearing toluene and clove oil, the Mann-Whitney statistical test was performed to determine the difference.

## RESULTS

Table 1. The results of the percentage of the quality of the preserved preparations of *Aedes sp* larvae based on different clearing treatments

Clearing treatment	Quality of preserved preparations of <i>Aedes sp</i> larvae					
	Well	%	Pretty good	%	Bad	%
Toluene	7	43.75	4	25	5	3.25
Clove Oil	12	75	4	25	0	0

Based on table 1, it was found that the quality of preparations for preserved *Aedes sp* larvae with the clearing process using toluene showed that 7 preparations were good with a percentage of 43.75%, 4 preparations were quite good with a percentage of 25% and 5 preparations were bad with a percentage of 31.25%. While the clearing process using clove oil obtained 12 good preparations with a percentage of 75%, 4 preparations were quite good with a percentage of 25% and no results were obtained with poor quality preparations in the clove oil treatment. The results of the quality of preparations preserved by *Aedes sp* larvae with clearing treatment using clove oil have a higher score than the clearing treatment using toluene.

Then this study will determine whether the clearing treatment using clearing agent toluene and clove oil affects the quality of preparations preserved *Aedes sp* larvae can be used with the Mann-Whitney test, the following is the Mann-Whitney test from this study.

Table 2. Mann-Whitney test results on the quality of preparations preserved by *Aedes sp* Larvae

Clearing treatment	p
Toulen	0.032
Clove oil	

Based on table 2, the p-value of Mann-Whitney U test was 0.032, which means that there was a difference in clearing results between being given the toluene and clove oil treatment.

### DISCUSSION

Based on microscopic observation data on the preserved preparations of *Aedes sp* larvae based on the clearing process using toluene and clove oil, different results were obtained, in the table of the cleaning process using toluene obtained 7 (43.75%) preparations with good quality, 4 (25%) preparations with fairly good quality, and 5 (31.25%) preparations with poor quality. The clearing process using clove oil obtained 12 (75%) preparations with good quality, 4 (25%) preparations with moderately good quality, and no preparations with poor quality were found.

The preparations were said to be in good category including good color, clarity and morphological integrity of *Aedes sp* larvae, and quite good category because the incomplete clearing was still not transparent and the morphological color still looked black which made the morphology difficult to observe and the limbs of the *Aedes sp* larvae were still present. The loss can be caused by the concentration of KOH solution that is too high and the length of immersion time or other factors such as when sampling *Aedes sp* larvae<sup>(8)</sup>. Preserved preparations containing air bubbles are caused by the mounting process using less than perfect entellan which can result in poor quality preparations<sup>(1)</sup>.

Based on the results of the Mann-Whitney test showed that there is a difference between the treatment of toluene and clove oil.

### CONCLUSION

Based on the research that has been done, the following conclusions are obtained:

- 1) Larvae of *Aedes sp* in the clearing process using a clearing agent toluene produced 7 preserved preparations in the good category, 4 preserved preparations in the moderately good category, and 5 preparations in the poor category.
- 2) Larvae of *Aedes sp* in the clearing process using clove oil as a clearing agent produced 12 preserved preparations in good category, 4 preserved preparations in good enough category, and no preparations in poor category were obtained.
- 3) Based on the Mann-Whitney test, it was concluded that there is a difference in results between being given clearing treatment using toluene and clove oil clearing agent.

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