

## CHAPTER 4 STUDY METHOD

### 4.1 Study design

This is a true laboratory experimental study with a design true experimental-posttest only control group. The purpose of this study is to determine the insecticidal effect of Clove extract (*Syzygium aromaticum*) on *Aedes aegypti* mosquito.

### 4.2 Population and Sample

The study population of *Aedes aegypti* has the following requirements:

- Live mosquitoes (*Aedes aegypti*)
- free moving

The sample was taken via random hand catching from a habitat of mosquitoes obtained from Dinas Kesehatan Surabaya. The sample was divided into 1 positive control group (without exposure to extract but exposed to store bought insecticides as control), 1 negative control group (exposed to water/acetone) and 3 study groups. Each study group represented one dose (concentration) of extract with the same amount of samples. Each group consisted of 25 *Aedes aegypti* mosquitoes.

Sample size estimation was done based on the following formula:

$$P(n-1) \geq 16.$$

P = number of trial

n = number of repetition of each sample

$$(p) = 5,$$

$$5(n-1) \geq 16$$

$$5n-5 \geq 16$$

$$5n \geq 21$$

$$n \geq 4,2 \quad (\text{Lukito, 1998})$$

Hence from the calculation, the test was done 5 times with different concentrations but the same extract.

Amount of *Aedes aegypti* needed:

$$(25 \times 5 \text{ repetiton} \times 5 \text{ groups}) = 625$$

### 4.3 Place and time of study

Experiment was carried out at the Parasitology laboratory in University of Brawijaya.

### 4.4. Variable identification

#### 4.4.1 Dependent variable

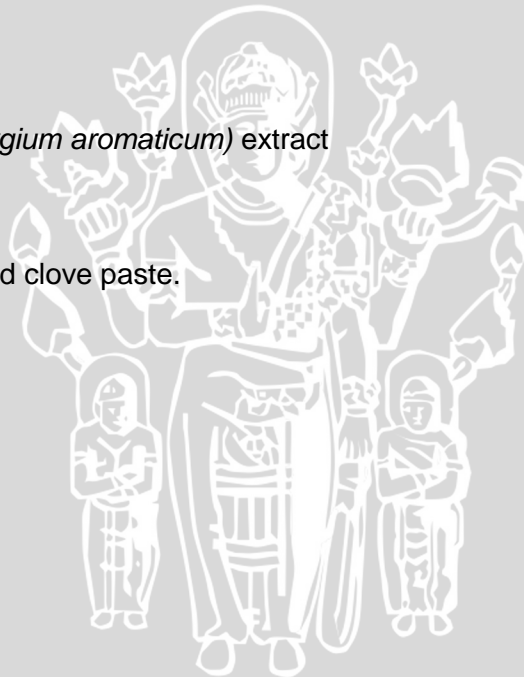
Dependent variable in this research is the number of *Aedes aegypti* that were found dead.

### 4.5 Materials and Equipment

#### 4.5.1 Research Equipment

❖ To make the Clove (*Syzygium aromaticum*) extract

1. Blender
2. Test tube to place blended clove paste.
3. Filter
4. Filter Paper
5. Extraction Glass
6. Analytic Scales
7. Static Klem
8. Oven
9. Balance

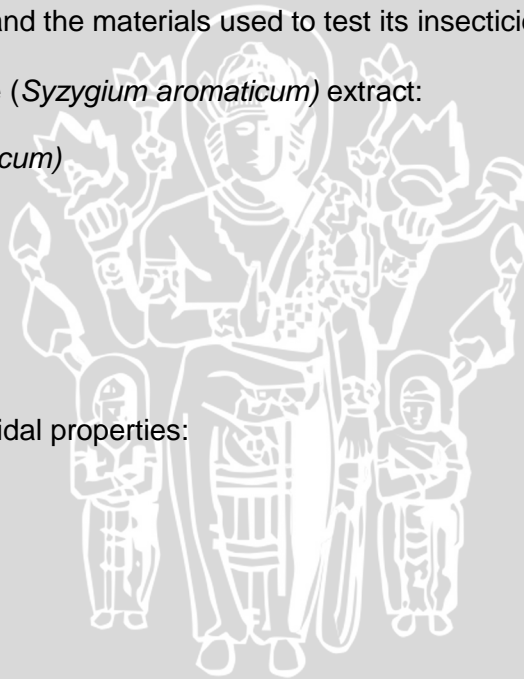


- ❖ To test the insecticides on *Aedes aegypti*.
  1. A simple container with measurements 25 cm x 25 cm x 25cm
  2. Spray
  3. Pinset
  4. Face Mask
  5. Glove

#### 4.5.2 Research Materials

The materials used in this research were divided into materials used to produce clove (*Syzygium aromaticum*) extract and the materials used to test its insecticidal properties.

- Materials used to make clove (*Syzygium aromaticum*) extract:
  1. Clove (*Syzygium aromaticum*)
  2. Aquades
  3. Ethanol 96%
  4. Acetone 1%
- Materials used to test insecticidal properties:
  1. Clove extract
  2. Aquades
  3. *Aedes aegypti*
  4. Malathion



## 4.6 Research Procedure

### 4.6.1 Extraction of Clove (*Syzygium aromaticum*)

#### 4.6.1.1 Extraction process

Extraction was done by using the "Technique of Simple Extraction" as demonstrated in the organic chemistry book, "An introduction to Modern Experimental Organic Chemistry". The solvent used is ethanol 96%. The process was started using 500g clove (*Syzygium aromaticum*) that were washed and rinsed with clean running water. The cloves were then dried under in the hot sun and cut into small pieces. It was then dried once again in the oven under the temperature of 60-80<sup>0</sup> C. Once it was completely dried, the leaves were blended into powder with the blender and weighed. This powder which weighed 100g was put into a 1.5 L bottle with 1 L of ethanol for 1 week. The substance in this bottle was vaporized to separate the extract and ethanol (Royston et. al., 1969).

#### 4.6.1.2 Evaporation process

First, the evaporator was fixed in such way so that the temperature of the experiment area is 30<sup>0</sup> C – 40<sup>0</sup> C. The ethanol solution of the extract was transferred to extraction separator. Extraction separator was connected to the lower part of evaporator while the spiral cooler was connected to the top part of evaporator. The spiral cooler was connected to vacuum by using a plastic pipe. A water pump was placed in a container which consisted of aquades. The water pump was then connected to an electrical source so that the aquades fills the spiral cooler. The vacuum and water bath were connected to an electrical source and the temperature of the water bath was increased until 70<sup>0</sup> C (according to the boiling point of ethanol). The circulation was left until the evaporated substance is collected in extraction separator for 2 to 3 hours. Continued with drying in oven under temperature of 50-60<sup>0</sup> C for 1 to 2 days. The product which looks like a solid oil with a special smell is the extract (Royston et. al., 1969).

#### 4.6.2 Method of obtaining different concentration of extract

Temperature of the thick extract which was kept in the freezer was equalized with room temperature by keeping it out for 15 minutes. The extract paste is mashed until it became homogenized. It was then mixed with acetone until 100% of pure concentration was obtained.

#### 4.6.3 Study procedure

Distilled water & 1% acetone were added to dilute 100% concentration of Clove extract.

- ❖ Stock solution of Clove extract is prepared in three different concentrations: 20%, 30% & 40%.
- ❖ Dosages of the stock solution are prepared by dilution method. Below is the method :
  - I. Mark sterile tubes (1-3)
  - II. Take the clove extract of 100 %.
  - III. Total volume used in this experiment is 4 ml. Thus for each experiment, the volume of 100% concentration of clove extract has to be calculated.
  - IV. This is done by dilution method based on the formula below.

$$M1 \times V1 = M2 \times V2$$

Explanation:

M1: 100% concentration of Clove extract.

M2: Percentage of concentration of clove extract needed.

V1: Volume of 100% concentration of clove extract needed to be used to obtain M2.

V2: Volume needed for the experiment to be carried out.

- V. After the needed volume for 20%, 30% & 40% concentration were calculated; the volume of distilled water needed to obtain 4ml was calculated. (WHO, 2006).

Concentration needed	Volume of 100% concentration Clove extract	Volume of distilled water and 1% acetone needed
20%	1.0 ml	3.0 ml
30%	1.2 ml	2.8 ml
40%	1.6 ml	2.4 ml

#### 4.6.4. Working method

- ❖ Trials were done by using 5 containers with the size of 25 cm x 25 cm x 25 cm
  - ❖ (A4 sized box) under room temperature.
  - ❖ The stock solutions of Clove extract were prepared in three different concentrations (20%, 30% and 40%) and were then placed into their respective spray containers.
  - ❖ The contents of the spray containers are emptied into each box.
  - ❖ Box 1 was sprayed with aquades (negative control).
  - ❖ Box 2 was sprayed with Malathion (positive control)
  - ❖ Box 3 was sprayed with 4.0 ml of 20 % clove extract.
  - ❖ Box 4 was sprayed with 4.0 ml of 30 % clove extract.
  - ❖ Box 5 was sprayed with 4.0 ml of 40 % clove extract.
  - ❖ The number of dead mosquitoes were noted every 10 minutes for the first hour then every 3 hours over a 24 hour time period
- This test is repeated 4 times for each 3 different concentrations.
  - Data of the killed *Aedes aegypti* for each extract concentration and time interval was analysed. This is to find out the insecticidal effect of the extract with different concentrations and the effect of time towards the strength of insecticidality. Percentage of insecticidality to *Aedes aegypti* is calculated by this formula :

$$A1 = (A - B / 100 - B) \times 100 \%$$

Explanation:

- A1 : Percentage of *Aedes aegypti* deaths after correction  
A : Percentage of dead *Aedes aegypti*  
B : Percentage of dead negative control *Aedes aegypti* (WHO, 2006).

#### 4.6.5 Experimental Framework

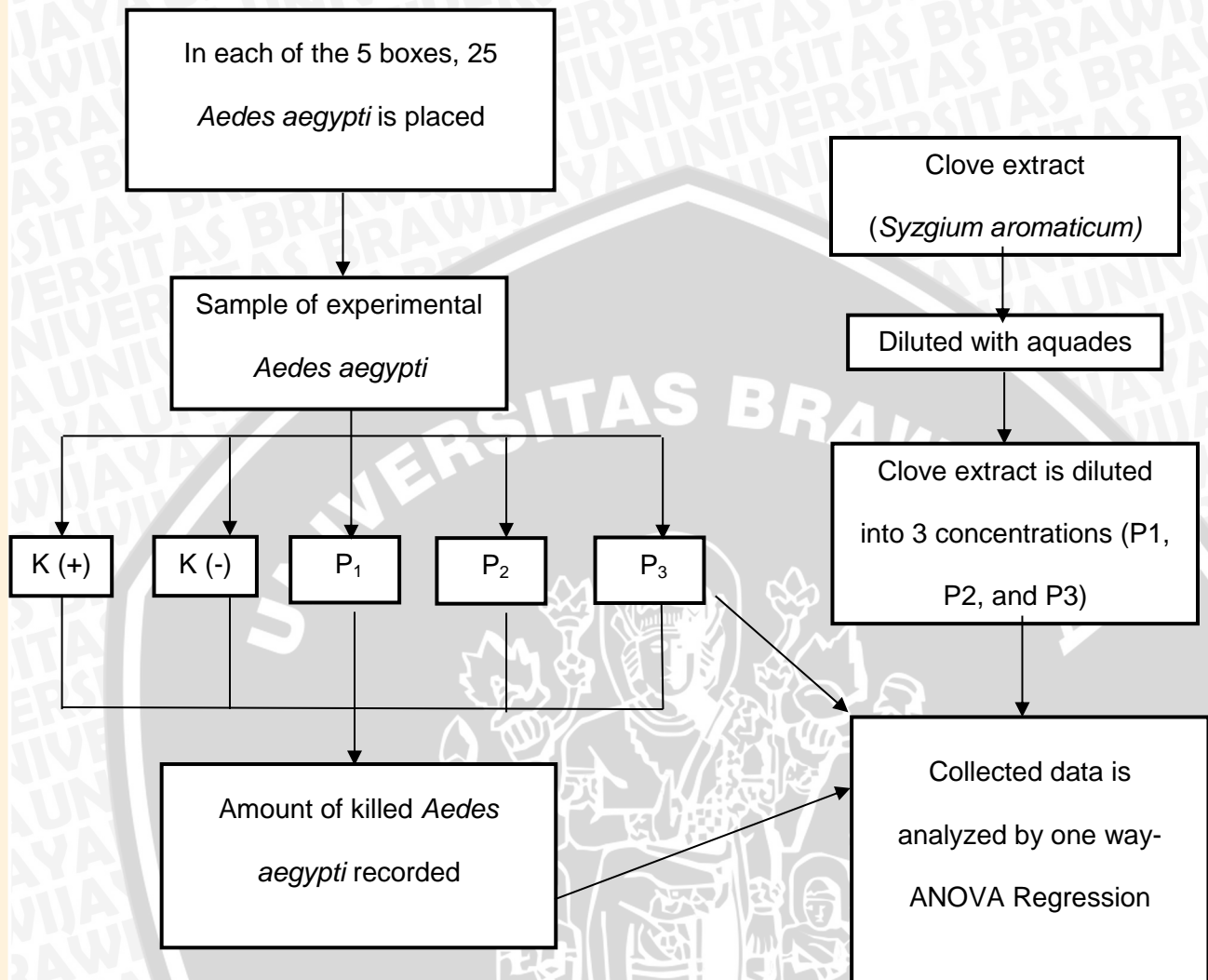


Figure 4.3: Experiment framework

Explanation:

K (+) : positive control: (Malathion 0.28%)

K (-) : negative control (Aquades)

P<sub>1</sub> : 20% concentration Clove extract

P<sub>2</sub> : 30% concentration Clove extract

P<sub>3</sub> : 40% concentration Clove extract

#### 4.7 Data Collection

The data that was collected including the information was classified into table form according to the concentration of clove extract, killed *Aedes aegypti*, and number of repetitions. Statistic tests were also performed.

#### 4.8 Data processing & Analysis

This analysis was done based on the one-way ANOVA method based on the ANOVA condition fulfillment, which are tested by these steps:

1. Evaluate the requirement for one- way ANOVA testing which is: There are one or more independent variables namely time and concentration.

The assumptions that were made include:

- a) The populations from which the samples were obtained must be normally or approximately normally distributed.
  - b) The samples must be independent.
  - c) The variances of the populations must be equal.
  - d) The groups must have the same sample size.
2. One-way ANOVA testing is done to know if there is different insecticidal potential for clove extract at different concentration or time.
  3. Post Hoc Test (Turkey Test) to know which concentration of Clove extract that has insecticidal potential significantly different and not significant.
  4. Correlation test to know the correlation between concentrations or time with the insecticidal potential of clove extract towards *Aedes aegypti*.
  5. Regression test, to know the effect of concentration or time towards the insecticidal potential of clove extract towards *Aedes aegypti* (WHO, 2006).