CHAPTER 4

METHODOLOGY

4.1 Research Design

This is a laboratory experimental study with a true experimental-post test only control group design. This study is aimed to know the potential insecticide effect of sulfur towards fire ants, *Solenopsis sp.*

4.2 Location and Time of Study

The study was carried out in the Parasitology Laboratory of University of Brawijaya. The study began in February 2013 until it completed in December 2013.

4.3 Sample and Estimation of Sample Size

4.3.1 Number of Sample Size

The sample used in this study was the fire ant (*Solenopsis sp.*) which fulfilled the inclusion criteria and the exclusion criteria.

- Inclusion criteria of this study were:
  - Live fire ants
  - Actively moving fire ants
- Exclusion criteria of this study were:
  - Dead fire ants during the study

There were a total of 5 sample groups which were divided into 1 positive control group which was exposed to malathion 2.8% without exposure to sulfur, 1 negative control group which was exposed to water without the exposure to sulfur and 3 study groups which were exposed to different concentrations of sulfur,

0.5%, 1.5% and 2.5% respectively. As much as 15 ants were placed in each sample group. Experiment was repeated for 4 times to each sample group.

4.3.2 Estimation of Number of Repetition

Repetition of Estimation was done according to the following formula:

$$p\,(n-1) \geq 16$$

$$p\,(n-1) \geq 16$$

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

$$5n-5 \geq 21$$

$$n \geq 4.2 \sim 4 \text{ (Loekito, 1998)}$$

where:

p= number of treatment

n= number of repetition needed (Loekito, 1998)

Therefore, in this study the number of repetition needed is 4.

4.4. Variable Identification

4.4.1 Dependent Variable

Dependent Variable in this study is the number of fire ants that die during the study.

4.4.2 Independent Variable

Independent Variable in this study is the concentration of sulfur which is given to each study group and the time of exposure.

4.5 Operational Definitions

- Sulfur, an element used in this study, was bought from the market.

- Aquades was used as a solvent for sulfur in the preparation of sulfur spray.

- Fire ants (*Solenopsis sp.)* were taken from the surrounding areas of the laboratory of Parasitology, Brawijaya University.

- Insecticide is any pesticide which functions to kill insects including ants in the environment.

- Positive control → a known insecticide (malathion 0.28%)

- Negative control → solvent for sulfur (Aquades)

The experiment was repeatedly done for 4 times for all the concentrations of sulfur.

4.6 Apparatus and Materials

4.6.1 Apparatus

- 10 wide mouthed clear bottles made of glass or plastic

- Covers that contain tiny holes to allow ventilation

- Spraying bottles

- Mask and gloves for personal safety

4.6.2 Materials

- About 300 fire ants for 15 in each study group

- Sulfur mixed with sterile water in the concentrations of 0.5%, 1.5% and 2.5%.

4.7 Study Procedure

1. 15 fire ants were placed in 5 clear bottles of the same size for different concentrations of sulfur solution and positive and negative controls as well.

2. Each bottle was closed with a cover with tiny holes to allow fire ants breathe and labeled as 0.5%, 1.5%, 2.5%, malathion 0.28%.

3. Sulfur powder was mixed with water to prepare solution with desired concentration.

4. To obtain the desired concentration of sulfur, the following equation is used:

$$M_1 V_1 = M_2 V_2$$

where:

M_1 = 100% concentration of sulfur solution

M_2 = desired concentration of sulfur solution

V_1 = volume of solution that should be mixed with solvent

V_2 = desired volume of sulfur solution

5. The sulfur solution with concentrations of 0.5%, 1.5% and 2.5% were sprayed into bottle I, II and III and bottle IV was sprayed with malathion 2.8% as positive control while bottle V was sprayed with sterile water as negative control for the study.

6. The number of dead fire ants in each bottle was calculated every 1 hour for first 6 hours and then in 24th hour.

7. Calculation of potency of insecticide: The potency of insecticide means the data of dead fire ants during the study. To evaluate the number of fire ants which are killed by insecticide (sulfur), the Abbot formula was used:

$$A_1 = \frac{A - B}{100 - B} \times 100\%$$

where:

A_1 = number of dead fire ants after correction

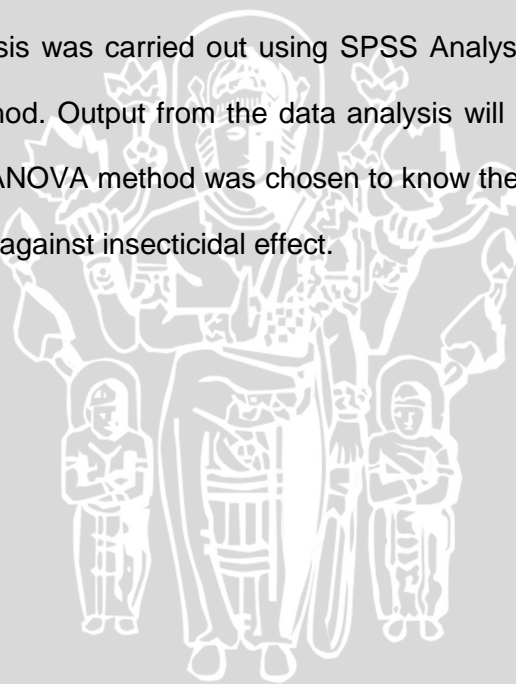A = number of dead fire ants in different concentration of sulfur

respectively

B= number of dead fire ants in the negative control group

4.8 Data Collection

The data collected was classified into table forms according to the amount of dead fire ants, *Solenopsis sp.* at the repetition tests and different concentrations of sulfur solution.

4.9 Data Analysis

The data analysis was carried out using SPSS Analysis 17.0 version by One-way ANOVA method. Output from the data analysis will be included in the attachment. One-way ANOVA method was chosen to know the difference among each treatment groups against insecticidal effect.

4.10 Experimental Framework

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┌─────────────────┐
│     Sulfur      │
└────────┬────────┘
         │
         ▼
┌─────────────────┐              ┌──────────────────────┐
│ Mixed with Aquades │           │   15 Soenopsis sp.   │
│ into 3 concentrations │        │  were placed in each │
│  (0.5%, 1.5%, 2.5%) │          │   of  5 containers   │
└────────┬────────┘              └──────────┬───────────┘
         │                                  │
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Experiment:
Sulfur Spray
(0.5%, 1.5%, 2.5%)

Positive
Control
(Malathion 0.28%)

Negative
Control
(Aquades)

Collected data were
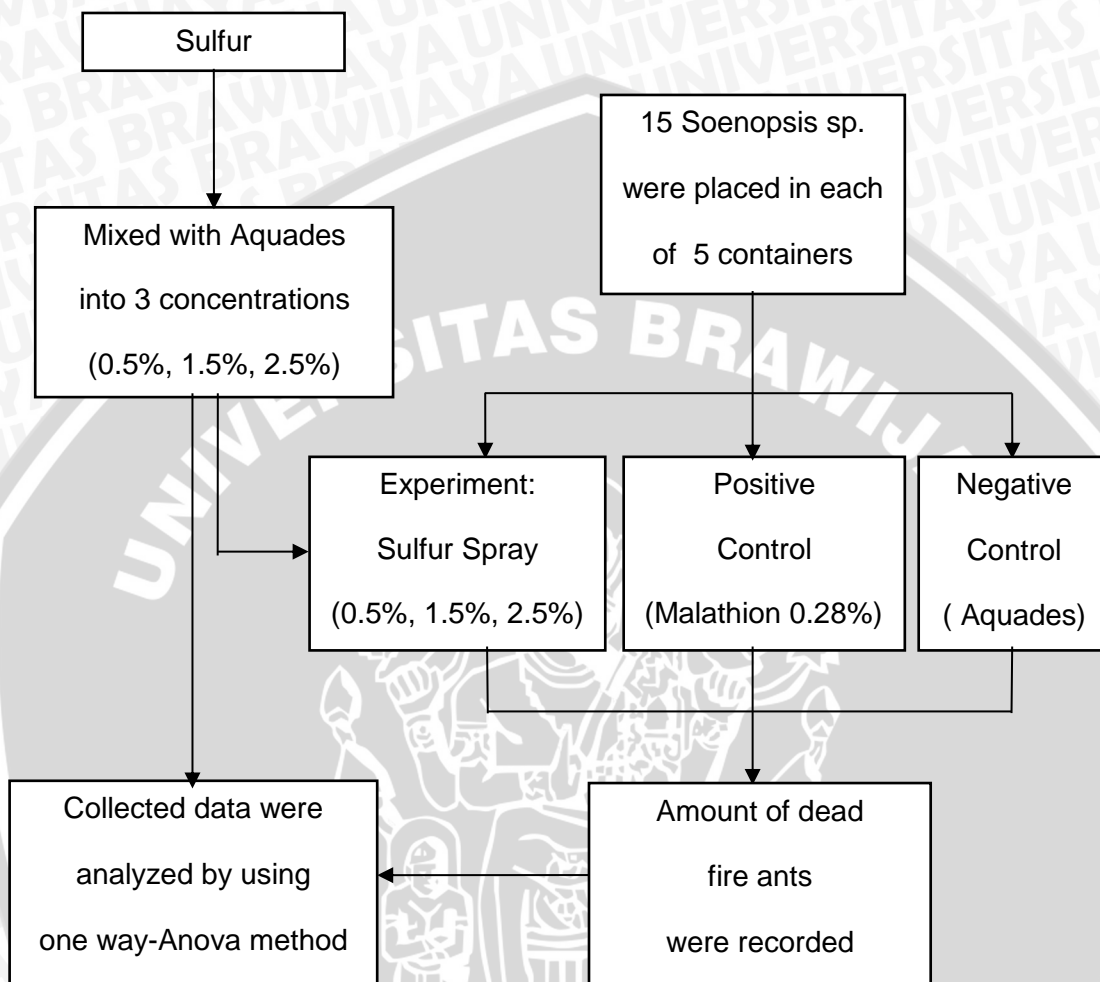analyzed by using
one way-Anova method

Amount of dead
fire ants
were recorded

Figure 4.1: Experimental Framework