CHAPTER 4

RESEARCH METHOD

4.1 Study Design

This study was a laboratory experimental study with a design of true experimental-post test only control group. The purpose of this study was to know the potential insecticidal effect of red pepper, *Capsicum annuum* towards fire ants, *Solenopsis sp.*

4.2 Location and Time of Study

This study was conducted at the Laboratory of Parasitology, University of Brawijaya within the month of March 2013 until it completed.

4.3 Study Population

The study population being used for this study was fire ants, *Solenopsis sp*. *(Solenopsis invicta)* that fulfilled the inclusion and the exclusion criteria. The inclusion criteria for this study were as follows:

- Ants from genus *Solenopsis* that were alive.
- Ants must be able to move freely or active.

The exclusion criteria for this study were the ants that died during the course of experiment.

The sample used was *Solenopsis sp.* worker ants that did not die during the sample selection and fulfilled the inclusion criteria. As much as15 ants were palced inside each set of sample group.

There are total 5 sample groups which were divided into 1 negative control group which was exposed to water without the exposure of red pepper

extract, 1 positive control group which was exposed to malathion (0.28%) and 3 study groups. Each of the 3 study groups represented 10%, 15% and 20% dose of red pepper extract concentrations respectively and they were to be tested on to the same amount of ant sample each. Each of 5 sample group would consist of 15 ants. The experiment was repeated 4 times.

4.4 Estimation of Number of Repetition

The number of repetition for each sample was calculated using the following equation:

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

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

$$5n - 5 \geq 16$$

$$5n \geq 21$$

$$n \geq 4$$

Explanation: p= number of treatment

n= number of repetition needed (Loekito.1998)

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

4.5 Variable Identification

4.5.1 Dependent Variable

Dependent Variable in this study was the number of fire ants that died during the study.

4.5.2 Independent Variable

Independent variable in this study was the concentration of red pepper extract in percentage which was given to each study group and the duration of the insecticidal effect in each sample.

4.6 Operational Definitions

i. Red pepper, *Capsicum annuum* to be used in this study were bought from the market.

ii. Fire ants, Solenopsis sp. to be used in this experiment were searched and collected by the staff of the Laboratory of Parasitology.

iii. Water was used as a solvent for red pepper extract in the preparation of red pepper spray.

iv. The negative control to be used to make contact with the ant was water.

v. An insecticide is any pesticide used to kill, deter, or control insects (IUPAC 2006) and malathion, an insecticide was used in the experiment.

4.7 Instruments and Materials

The instruments used in this research were divided into two groups. The first group consisted of instruments and materials used for the extraction of red pepper, *Capsicum annuum.* The second group of instruments and materials were those used in testing of insecticidal effect of red pepper, *Capsicum annuum* towards fire ants, *Solenopsis sp.*

Instruments and materials for the extraction of red pepper:

- Electronic Balance
- conical funnel
- filter paper, cloth
- Beaker
- Measuring cylinder
- spatula
- stove
- oven
- Ground red pepper
- water

Instruments and materials used in testing of insecticidal effect of red pepper towards fire ants:

- 5 wide mouthed clear containers made of glass or plastic
- Holes were made on the lid of the container to allow ventilation
- mask and gloves for personal safety
- About 400 fire ants for 15 in each study group
- Red pepper extract mixed with water in the concentrations of 10%, 15% and 20%
- Spray bottles

4.8 Research Procedure

4.8.1 Red Pepper Extraction

The following was the process to make extraction from red pepper, *Capsicum annuum* by infusion method.

Red peppers were ground and the water was boiled to 90 degree Celsius first.

Ground red pepper →steeped in boiled water

→ Leave about (15 minutes)

→ Filter using paper, cloth

→Get the extract

→Heat in oven in order to remove remaining water to get 100% concentration (Methods of preparing herbal remedies, 2013)

4.8.2 Working Method

1) 15 fire ants were placed in 5 clear containers of the same size for different concentrations of red pepper extract solution and control as well.
2) Each bottle was closed by a cover with holes and labelled as I, II, III and IV and V.
3) Red pepper extract was mixed with water to prepare solution with desired concentration.
4) To obtain the desired concentration of red pepper solution, the following equation was used:

$$M_1 V_1 = M_2 V_2$$

Explanation: M_1 = 100 % concentration of red pepper extract solution

M_2 = desired concentration of red pepper extract solution

V_1 = volume of red pepper extract solution that should be diluted

V_2 = the desired volume of red pepper extract solution

5) The red pepper extract solution with concentrations of 10%, 15% and 20% were sprayed in bottle I, II and III and bottle IV and V were used with water as negative control and malathion as positive control for the study.
6) The number of dead fire ants in each bottle was calculated every 1 hour for 6 hours and then in 24 hour.
7) The potency of insecticide means the data of dead fire ants during the study. To evaluate the number of fire ants which were killed by insecticide (red pepper extract), the Abbot formula must be used:

$$A_1 = [(A - B) / (100 - B)] \times 100\%$$

Explanation:

A_1 = number of dead fire ants after correction

A = number of dead fire ants in different concentration of red pepper extract respectively

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

4.9 Experimental Framework

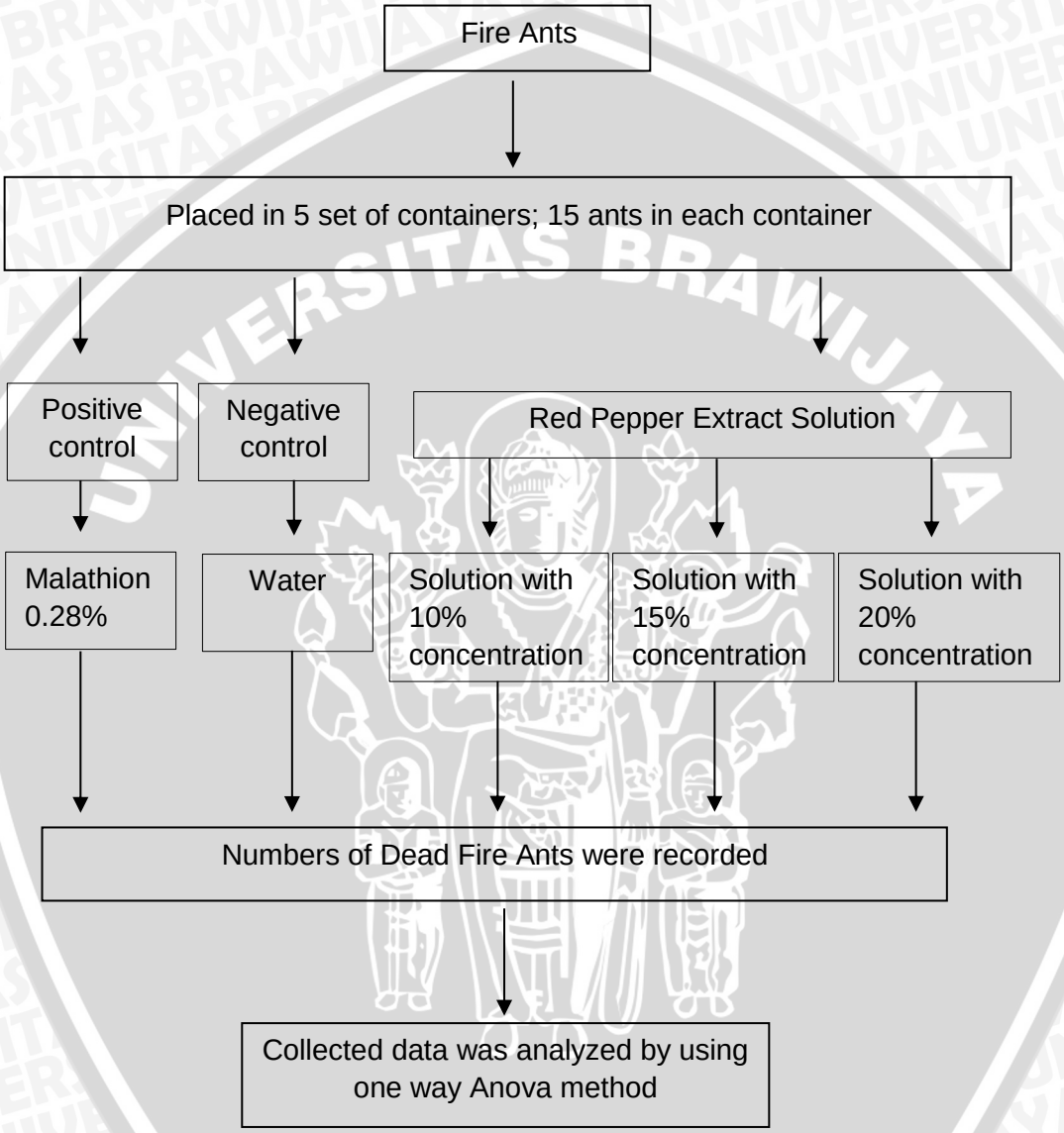

Figure 4.1 Experimental Framework

4.10 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 red pepper, *Capsicum annuum* extract solution.

4.11 Data Analysis

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