

CHAPTER 4

RESEARCH METHOD

4.1 RESEARCH DESIGN

This study is a laboratory experimental study with true experimental-post test only control group design. The purpose of this study is to know the potential repellent effect of cucumber extract towards fire ants, *Solenopsis* sp.

4.2 Location and Duration of Study

This study was conducted in the Parasitology Laboratory of University of Brawijaya. This study started during the month of June 2013 until it completed.

4.3 Sample and Estimation of Sample Size

4.3.1 Number of Samples

The sample being used in this study was the fire ant, *Solenopsis* sp, which fulfils the inclusion criteria and exclusion criteria.

- The inclusion criteria for this study are as follow:

- ✓ Ants of *Solenopsis* sp which are alive.
- ✓ Ants which can move freely.

- The exclusion criteria of this study are as below:

- ✓ The ants which died during the course of experiment.

The study population being used was worker ants form *Solenopsis* sp which did not die during the sample selection and which met the inclusion criteria and 15 worker ants were put in each set of sample group.

4.3.2 Procedure

The procedure of this research is:

There were 5 sample groups, in which sugar was put and they were divided into ;

1. Sample I : The negative control group without any exposure to repellent.
2. Sample II : Exposure to the repellent, extract of cucumber 65%
3. Sample III : Exposure to the repellent, extract of cucumber 70%
4. Sample IV : Exposure to the repellent, extract of cucumber 75%
5. Sample V: The positive control group which exposed to naphthalene (Chemical repellent) without the exposure of cucumber extract.

4.3.3 Repetition

The estimated number of repetition for each sample is calculated by using the following equation :

$$[(n \times p - 1) - (p - 1)] \geq 15 \quad (\text{Loekito, 1998})$$

Where, n = number of repetition for each sample

p = number of trial

In this study, p = 5 since the total number of the different samples here is 5.

Therefore, the number of repetition needed for each sample (n) is :

$$[(n \times p - 1) - (p - 1)] \geq 15$$

$$[(5n - 1) - 4] \geq 15$$

$$5n - 1 - 5 \geq 15$$

$$5n \geq 20$$

$$n \geq 4$$

Conclusion: According to the calculation above, the number of repetition required for this study is at least 4 times.

4.4 Variable Identification

4.4.1 Dependent Variable

In this study, the dependent variable was the number of ant which did not cross through the pipe which was exposed to different cucumber etanol extract concentration.

4.4.2 Independent Variable

In this study, the independent variables were the cucumber ethanol extract of concentrations of 65%, 70% and 75%, naphthalene powder which is used as positive control, and the duration of the repellent effect in each sample.

4.5 Operational Definition

- The extract of cucumber is the product result of the maceration and evaporation method. The cucumbers are bought from the traditional market.
- Naphthalene power is get from grinding the the naphthalene ball. Naphthalene is used as the positive control.
- Ants form *Solenopsis* sp. The ants which are found near the Faculty of Medicine, Brawijaya University is used in this experiment.
- Repellent is an agent or a method that wards off, as insect-repellent to repel insects. (Biology online dictionary, 2010)

- Positive control uses the experimental treatment that is already known to produce that effect. Naphthalene is used in this experiment.
- Negative control is a group that has not been treated with the cucumber extract or any other repellent.
- The number of ants which is found inside the bottle where they were placed from the start without ever crossing the pipe which has been treated with the repellent is defined as the repelled *Solenopsis* sp.
- The formula of counting the repelled ants is as follow :

$$\text{Repellency (\%)} = 100 - (T \times 100) / N$$

(Thavara, et al, 2007)

Where,

T = the number of ants found inside the bottle which contains the sugar food source

N = the total number of ants used

4.6 Tools and Materials

The materials and tools which were used in this research are ;

1. Extract of cucumber
2. Naphthalene powder
3. Sugar food source
4. Blender
5. Sieve
6. Filter Paper

7. Aquades
8. Laboratory beaker
9. 400 *Solenopsis* sp fire ants
10. 10 clean, clear, same size and same shaped plastic bottles.
11. 5 clean and clear pipes of same diameter and same length
12. Mosquito net
13. Latex gloves
14. Rubber bands
15. 1ml and 5ml syringes
16. Timer
17. Refrigerator

4.7 Research Procedure

4.7.1 Method of Cucumber extract

- Cucumber extract was obtained by macerating the crushed fruit in hydro-alcoholic mixture, then filtering and concentrating it on rotary evaporator. (Akhtar, et al,2011)
- The cucumber which were in proper condition was cut and dried under direct sunlight. When the cucumber became dry, it was blended in a blender into fine powder. The cucumber powder was collected in a tightly sealed bottle.
- After that, the cucumber powder was immersed in ethanol 96% for approximately seven days.
- The product was evaporated to separate ethanol from cucumber from ethanol.

Evaporation

- The evaporator was installed to a permanent pole at the angle of 30° - 40° from the surface of the table.
- The product from ethanol immersion was transferred into an extraction separator.
- The extraction separator was connected to the lower part of evaporator, and to the vacuum via a plastic tube.
- The water pump was connected to a basin which contained aquadest. And then it was connected to electric source where aquadest flowed to fill the spiral cooler.
- The evaporation was placed in a position so that, part of the extraction separating flask was under the water (aquadest) in the waterbath.
- Vacuum and waterbath were connected to the electric source and the temperature of waterbath was increased 0-10 degree (according to the boiling point of ethanol).
- The circulation was continued so that the remaining product of evaporation inside the separated extraction flask was there for 2-3 more hours.
- It was then continued by heating in an oven with the temperature of 50° - 60° for 1-2 days.
- The end product of cucumber extract was produced as a paste. This end product is the extract of cucumber 100% being used in this research.

4.7.2 Method to Obtain Different concentrations of Cucumber Extract

The different concentrations of cucumber were obtained by using the dilution equation:

$$C_1 \times V_1 = C_2 \times V_2 \quad (\text{Stephenson, 2010})$$

Where:

C_1 = Initial concentration or molarity.

V_1 = Initial volume.

C_2 = Final concentration or molarity.

V_2 = Final volume

4.7.3 Acclimatization

Solenopsis sp workers were starved for five hours before the experiment.

Ants are cold blooded so they slow down when they get cold. Ants were placed in the refrigerator for about 5 minutes at the temperature of 4°C to 5°C to slow them down before being placed into the bottle to make it easier and safer to get the ants into the bottle. (Life Studies, 2006)

4.8 Working Plan

1. This research was conducted by using 10 clean, clear, same size and same shaped bottles which were placed in room temperature of $27 \pm 2^\circ\text{C}$.
2. Each of the bottle was cut in the half and only the upper end was used to make five sets of apparatus, each of which had 2 bottles.

3. The 15 starved and cooled *Solenopsis* sp worker ants were placed in the first bottle through the cut opened end, which was later be covered with mosquito net for stopping the ants form escaping and for the ventilation of the ants. The mosquito net is then secured by rubber band.
4. The sugar food source was placed in the second bottle which was also sealed with mosquito net and rubber band.
5. Step 3 and 4 were repeated for other sets of apparatus.
6. After this, 5 clean and clear pipes of same diameter and same length were treated with 0%, 65%, 70%, 75% of cucumber extract and naphthalene powder. 4 ml of each solution was used. The pipe was shaken after the solution was applied into the pipe so that it was evenly distribute on the pipe wall. The pipe was shaken while both ends of the pipe were covered tightly by gloved hands to prevent from spilling the solution.
7. One end of the pipe was inserted into the mouth of the bottle which contained the ants and the other end into the bottle of the sugar food source.
8. Each pipe was assembled in all five apparatus.
9. The number of ants found in the sugar food source bottle was noted at every one hour until 6 hours. This experiment was repeated for 4 times for every five sample groups.

4.9 Experimental Framework

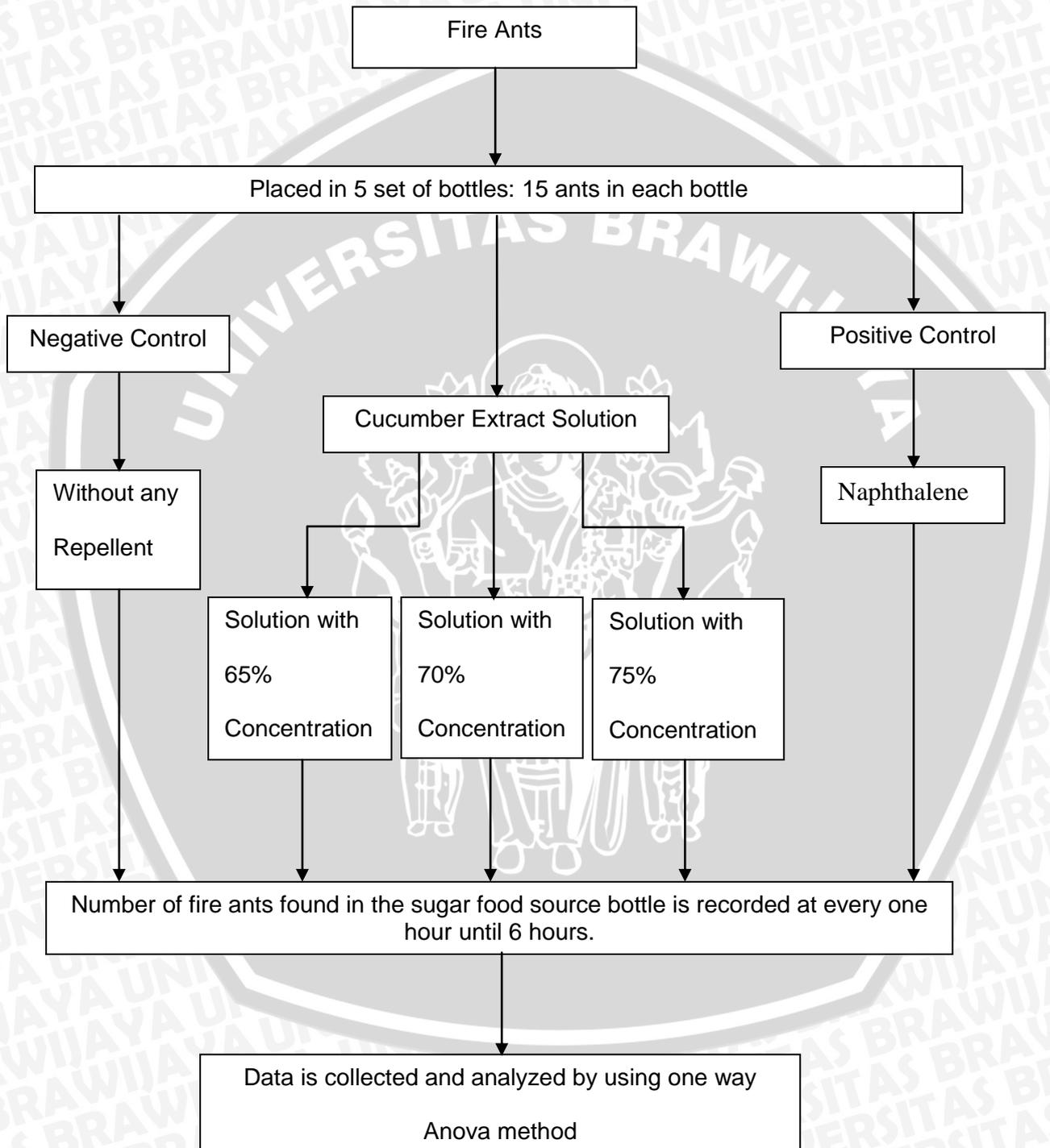


Figure 4.1 Experimental Framework

4.10 Data Collection

The data collected was classified according to the amount of ants repelled at the specific time, concentration of cucumber extract and repetition tests. The data was classified in the form of tables.

The formula of counting the repelled ants is as follow :

$$\text{Repellency (\%)} = 100 - (T \times 100) / N$$

(Thavara, et al., 2007)

Where,

T = the number of ants found inside the bottle which contains the sugar food source

N = the total number of ants used

4.10.1 Data Analysis

The data analysis was done by using *One Way Anova (One Way Analysis Of Variance)* method .

