# CHAPTER 4

# RESEARCH METHOD

## 4.1 Study Design

This research is a laboratory experimental study with true experimental-post test only control group.The reseach is purposed to know the potential repellent effect of turmeric extract (*Curcuma longa*) towards Fire ants (*Solenopsis sp*.)

## 4.2 Location and Time of Study

This study was conducted at Parasitology Laboratory of Medical Faculty, Brawijaya University, Malang starting from the month of March 2013 until it is finished.

## 4.3 Sample Selection and Sample Size Estimation

### 4.3.1 Study Population

The study population used in this study was Fire ants (*Solenopsis sp*.) that fufill the following inclusion and exclusion criteria.

The inclusion criteria for this study were:

- Fire ants (*Solenopsis sp)* that were alive.
- Fire ants that could move freely or active fire ants.

The exclusion criteria for this study was

- the fire ants that were died during the course of experiment.

The samples used in this study were *Solenopsis sp* worker ants that did not die during the sample selection and that fulfil the inclusion criteria.In this study, there were total 5 sample groups containing 15 ants in each.

### 4.3.2 Study method

The study method and action used in this study were

Sample I : Sugar filled bottle connected with a tube treated with aquades (no Turmeric extract repellant ) as a negative control

Sample II : Sugar filled bottle connected with a tube treated with the repellent Turmeric extract 30%

Sample III : Sugar filled bottle connected with a tube treated with the repellent Turmeric extract 40%

Sample IV : Sugar filled bottle connected with a tube treated with the repellent Turmeric extract 50%

Sample V : Sugar filled bottle connected with a tube treated with Napthalene powder as a positive control

### 4.3.3 Repetition

The number of repetition for each sample can be calculated by using the following formula:

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

p = number of trial

n = number of repitition for each sample ( Loekito, 1998 )

In this study, a total of 5 different sample groups (p) were used and hence the number of repetition for each sample (n) was:

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

$5n-1-4 \geq 15$

$5n - 5 \geq 15$

$5n \geq 20$

$$n \geq 4$$

Thus, based on this calculation, minimum repititions for each sample group in the research was 4 times.

### 4.4 Variable Identification

#### 4.4.1 Dependent Variable

The dependent variable in the study is the number of fire ants that are repelled.

#### 4.4.2 Independent Variable

Independent variables in the study are solutions with different turmeric extract concentration, naphthalene powder and the duration of repellency effect in hour.

### 4.5 Operational Definition

- Turmeric( *Curcuma longa*) extract used in this study was the result of evaporation and extraction of turmeric which raw material was old and dried turmeric rhizomes bought from the market. The solution resulted from extraction is considered to contain 100% concentration.
- Fire ants (*Solenopsis sp.)* are recognized by their three body features which are a pedicel with two nodes, copper brown head and body with a darker abdomen and antennae with 10 segments and two segmented clubs.
- Sugar acts as an attractant.
- Two equal transparent bottles that were closed with nets on the surface and attached by a paired tube between them were used for each set of sample.

- Repellent is defined as a substance which can be applied to human skin, clothing or other surfaces to prevent insects from landing or climbing on that surface.
- Repelled Fire ants (*Solenopsis sp*) is defined as the fire ants found inside the bottle that they were initially placed without even crossing the treated tube to reach the sugar placed inside the bottle on the other side of the tube.
- The repelled ants can be counted by using the formula :

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

whereas

T=represents the numbers of ants found in the bottle filled with attractant

N= total number of ants that are used.

- Repellency effect is defined as the effect of different concentrations of turmeric used that managed to repel the fire ants.
- The repellent effect of Turmeric extract is calculated using the number of repelled ants' formula for each concentration of 30%, 40%,50%, aquades and naphthalene powder for the time period of 1$^{st}$ hour, 2$^{nd}$ hour, 3$^{rd}$ hour, 4$^{th}$ hour, 5$^{th}$ hour and 6$^{th}$ hour. For each concentration of turmeric, the treatment must be repeated 4 times to conclude the result.

## 4.6 Equipment and Research Materials

### 4.6.1 Research Tools

The tools used in this study were divided into two groups: the tools used in production of turmeric extract and tools used for testing repellent effect of turmeric towards fire ants (*Solenopsis sp*.)

Tools for production of turmeric extract

i. Knife to slice turmeric rhizomes
ii. Blender : to grind sliced turmeric rhizomes into powder
iii. Tube to soak turmeric powder
iv. Filter paper
v. Static clamp
vi. Evaporation kit
    -Rotary evaporator
    -Vacuum pump
    -Cooling tubes and cold water circulation pump
    -Plastic tube
    -Water bath and vacuum
    -Water pump
    -Erlenmeyer flasks
vii. Aquadest basin
viii. Extract collecting bottle
ix. Refrigerator
x. Oven
xi. Scale

The tools used to test the effect of repellent are:

i. 10 clean and clear plastic bottles that are cut in halves
ii. 5 clean and transparent plastic tubes (length 15cm,inner diameter 2.8 cm and outer diameter 3 cm)
iii. Rubber bands
iv. Pieces of thin cloth as net
v. Timer
vi. Glove
vii. Sugar as attractant

**4.5.2 Materials used in research**

The materials used in this study were divided into two groups: the materials used in production of turmeric extract and materials used for testing repellent effect of turmeric towards fire ants (*Solenopsis sp*.)

Materials used for production of turmeric extract

i. Turmeric
ii. Ethathnol 96% as extract solvent
iii. Aquades

The materials used to test the effect of repellent are:

i. Fire ants (*Solenopsis sp*.)
ii. Turmeric extract in 3 different concentration
iii. Napthalene
iv. Sugar

## 4.7 Preparation

### 4.7.1 Turmeric ( *Curcuma longa*) extraction

At first, turmeric rhizomes weighed 400g was washed and thinly sliced and dried under the sun. Then the dried turmeric was later heated in oven with a temperature of 60C - 80C so that it became dried perfectly. Dried turmeric is ground using a blender to obtain powder. Then the turmeric was put into the bottle to be soaked in its solvent, which was 96% ethanol, for about 1 week. The result obtained from the above procedure will then be evaporated in order to separate turmeric extract with ethanol solvent.

### 4.7.2 Evaporation Process

Evaporation process was needed to separate the turmeric extract obtained above from its solvent ethanol. The procedures are as follows.

- Evaporator mounted on the permanent pole with a slope of 30- 40 degree on the experiment table.
- Turmeric mixed with ethanol which was resulted from the extraction procedures was moved into the extraction separator flask.
- The flask was connected to the bottom of the evaporator.
- Furthermore, the cooling spiral was connected to the top of the evaporator and also connected to a vacuum with a plastic hose, together with spiral cooling water pump with plastic tubing.
- Water pump was placed in a bath containing aquades, and connected to a power source so that aquades would flow to meet the cooling spiral.
- A set of evaporator was laid in that way so that a part of extraction separator flask was submerged in aquades in the waterbath.

- Then, the temperature was elevated to about 70.C which is the boiling point of ethanol and the circulation process was started.
- The process was continued about 2 to 3 hours to obtain crude extract of turmeric.
- The crude extract was heated again in oven at 50-60 .c for 1- 2 days to remove the remaining ethanol in it.
- Finally, the pure extract of turmeric which could be used in the study was obtained.
- The extract was then stored in the refrigerator to prevent deterioration ( Sastrohamidjojo, 2004)

### 4.7.3 Preparation of Stock Solution

A Stock Solution is a concentrated solution that will be diluted to some lower concentrated for actual use. Stock solutions are used to save preparation time, conserve materials, reduce storage space, and improve the accuracy with which working lower concentration solutions are prepared.In making turmeric stock solution, the solvent that is commonly used is distilled water ( Aquades ).

Preparation of test solution

Turmeric extract stock solution was diluted with distilled water to obtain the desired concentration using the formula shown below:

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

Where,

$M_1$ = concentration of the first turmeric solution

$V_1$ = volume of the first turmeric solution

$M_2$ = the desired concentration of the turmeric solution

$V_2$ = the desired volume of the second solution( final volume)

**4.8 Research procedure**

1. The experiment was performed by using bottles placed in room temperature.
2. Five sets of experimental apparatus were used and each set was prepared by using 2 clean and clear plastic bottles. Each of the plastic bottles was cut into half and only the upper half ot the bottle was used.
3. Fire ants ( workers) were starved for 3 hours. They were then placed inside a refrigerator at temperature 4.C to 5.C for 5 minutes before the experiment was started to make the ants easier to handle.
4. In the first bottle, 15 fire ants are placed through the cut open part and by using a rubber band,the opening is covered by a thin cloth to prevent the ants from escaping.
5. In the second one, sugar was placed and the opening was covered by a a thin cloth or net and secured by rubber band.
6. Procedure 3 and 4 were repeated for the rest sets of the apparatus.
7. Five clean and clear plastic tubes were then prepared. Each plastic tube was treated with different turmeric concentration of, 30%, 40% and 50% aquades and naphthalene powder.
8. Each tube was then inserted into the mouth of plastic bottles with an ants filled bottle at one end and sugar filled bottle at the other end.
9. Each set of apparatus was labeled as Sample I, II, III, IV and V.
10. The timer was needed to note the duration.

11. The numbers of ants found in the sugar- filled bottle was noted at 1st hour, 2nd hour, 3rd hour, 4th hour, 5th hour and 6th hour.
12. The experiment was then repeated for 4 times for each sample group.

## 4.9 Research Framework

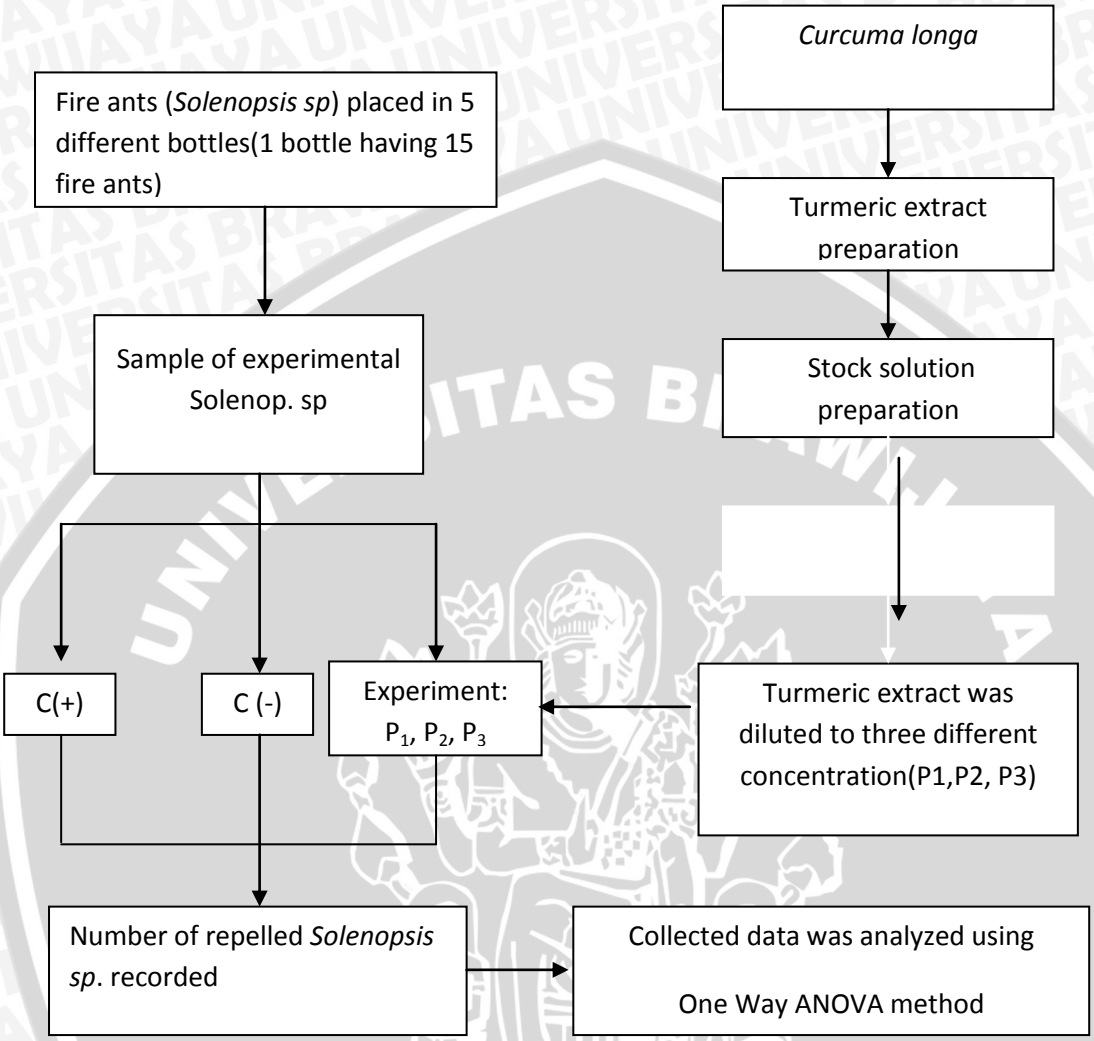


**Figure 4.1 Experimental framework**

Interpretation

C (+) = positive control (naphthalene powder)

C (-) =negative control (aquades)

P1 =30% turmeric concentration

P2 =40% turmeric concentration

P3 =50% turmeric concentration

### 4.10 Data Collection

The data collection was collected into table forms according to the number of repelled fire ants at the specified times and different concentrations of turmeric extract.

### 4.11 Data Analysis

The Data Analysis was carried out with the IBM SPSS Statistic Analysis software by using One- way Anova method. This method was chosen to know the difference among each sample group against concentrations of turmeric extract which can be used as fire ant repellent and the duration of the repellency. As the $p < 0.05$, the result was analyzed using Post Hoc Tukey Test, Pearson Correlation Test and Linear Regression Test to describe the strength of correction between the time and turmeric repellency as well as the dose and repellency towards fire ants ( *Solenopsis sp*) .