CHAPTER 4

RESEARCH METHOD

4.1 Study Design

This study was a laboratory experiment that used true experimental – posttest only control group. The purpose was to determine the potential insecticidal activity of ethanol extract of garlic (*Allium sativum* Linn.) against flies (*Chrysomya sp*) by using spraying method.

4.2 Location and Time of Study

This study was processed at the Parasitology Laboratory, Faculty of Medicine, University of Brawijaya and started from the month of January 2015 until it had completed.

4.3 Study Population

For this study, the study population being used was flies (*Chrysomya sp*.) that fulfilled the inclusion and the exclusion criteria.

The inclusion criteria for this study were as follows:

- Adult flies, *Chrysomya sp* that still can move and fly

There were 5 sample groups in total which were divided into 1 negative control group which was exposed to water without garlic extract, 1 positive control group which was exposed to malathion (0.28%) and 3 study groups. Each of the 3 study groups represented 10%, 20% and 30% dose of garlic extract concentrations respectively and they were to be tested on to the same number of *Chrysomya* flies each. Each of 5 sample groups would consist of 10 flies. The experiment was repeated 4 times.

4.4 Estimation of Number of Repitation

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

- P (n - 1) ≥ 16
- 5 (n - 1) ≥ 16
- 5n – 5 ≥ 16
- 5n ≥ 21
- n ≥ 4,2

Explanation:

- p= number of treatment
- n= number of repetition needed (Loekito.1998)

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

10 flies x 5 container (3 concentrated, 1 control positive and 1 control negative) x 4 experiment = need 200 flies

4.5 Variable Identification

4.5.1 Dependent Variable

Dependent Variable in this study was the potential insecticidal activity of ethanol extract of garlic against flies by using spraying method.

4.5.2 Independent Variable

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

4.6 Operational Definitions

- Garlic, an element used in this study, the species *Allium sativum*, was bought from Traditional Central Market, Malang, East Java.

- Flies (*Chrysomya sp*.) used were searched and collected by laboratory of Parasitology, Brawijaya University.
- Aquades was used as a solvent for garlic in the preparation of garlic spray.
- The negative control used to make contact with the flies was water.
- An insecticide is any pesticide used to kill, deter, or control insects (IUPAC 2006) and malathion, an insecticide was used in the experiment for positive control.
- Potential insecticidal effect of garlic extract is that the ability to kill 100 % of flies within 2 hours. Potential effect can be evaluated with the Abbot formula.

4.7 Instruments and Materials

The instruments used in this research were divided into three different stages. The first stage consisted of instruments and materials which were used for the extraction of garlic, *Allium sativum.* The second stage included materials for collecting and storing sample. The third stage was preparing instruments and materials which were used in testing insecticidal effect of garlic (*Allium sativum)* against flies, *Chrysomya sp.*

- **Instruments and materials for the extraction of garlic**
  - Mortar
  - Analytic balance
  - Static clamp
  - beaker
  - test tube to soaked dried garlic which is grinded
  - water

- ethanol 96%
- acetone
- 100 gram of garlic
- filter paper , cloth
- bottle
- Measuring cylinder
- A set of vacuum evaporation equipment

- **Instruments and materials for identification and collecting flies**
  - Plastic bag
  - Magnifying glass
- **Instruments and materials used in testing of insecticidal effect of garlic against flies:**
  - 5 wide mouthed clear containers made of glass or plastic
  - Holes are made on the lid of the container to allow ventilation
  - mask and gloves for personal safety
  - Garlic extract wasl mixed with water in the concentrations of 10%, 20%, 30%
  - Spray bottles
  - Flies, *Chrysomya sp.*

4.8 Research Procedure

4.8.1 Preparation of study

4.8.1.1 Garlic extraction

Garlic extraction process was done by using the maceration process, by diluting it with 96% ethanol. The process was as followed: Garlic was sliced, cut and dried for approximately +/- 2 days, if not possible, dried using oven at a

temperature 30-40 degree Celsius. The dry garlic was grinded in a grinder until it was in a form of powder. Maceration process was used. And then 100 grams of garlic extract powder was poured into a bottle and soak with ethanol.

Ethanol was poured into the bottle until the powder, which was wrapped in filter paper, was entirely be submerged in the ethanol and left until turned into a dark brown colour (+/- 2 days) in room temperature and protected from sunlight. The soaking result in ethanol was placed in another bottle.

Extraction was stopped when the remaining of stored garlic extract product was crystal clear in storage (about one week). All the remains of soaking were placed in a bottle. The result was further evaporate which aims to separate the extract obtained by solvent ethanol.

4.8.1.2 Evaporation process

Vaporization tool were installed on a pole that was hung with a 30-40 degree slope to the table experiment. There was a water heating element, a container to store the vaporizing material, a rotary evaporator and a cooling tube. The cooling tube was connected with the cold water storage using a plastic pipe. The cooling tube was also connected to the vacuum pump and the storing container for evaporation. Storage container was filled with the remaining of the extraction process and then being strung together, rotary evaporator, cold water circulation pump and vacuum pump switched on.

Aquades heater was also switched on when vaporization container started to boil and the ethanol started to vaporize. After vaporization, the remaining went through condensation process to the ethanol storage container,

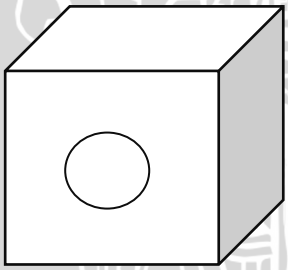
so that it separated from the vaporization result, the other steamed products was stuck by the vacuum pump.

The result of the extraction was rested vaporized until the volume are decreased and becomes thick, then process can be stopped. Vaporized remains was placed in the steam container, then placed in the oven with the temperature of 50-60 degree Celsius for 1-2 minutes. This step was to further vaporize the dilutant.

The extraction result which was going to be used in experiments was stored in -20 degree Celsius freezer to slow the damage.

4.8.2 Working Method

1) 10 flies was placed in 5 clear containers of the same size for different concentrations of garlic ethanol extract solution and controlled as well.



2) Each container was closed by a cover with holes and labeled as I, II, III, IV and V.
3) Garlic was mixed with water to prepare extract solution with desired concentration.
4) To obtain the desired concentration of garlic extract solution, the following equation was used:

$$M_1 V_1 = M_2 V_2$$

Explanation: M_1 = 100 % concentration of garlic extract solution
M_2 = desired concentration of garlic extract solution
V_1 = volume of garlic extract solution that should be diluted
V_2 = the desired volume of garlic extract solution

5) The garlic extract solution with concentrations of 10%, 20% and 30% was sprayed in container I, II and III and container IV and V was used with water as negative control and malathion as positive control for the study.
6) The number of dead flies in each container was calculated every 1 hour for 6 hours and then in 24 hour.
7) The insecticide potency was determined by the number of dead flies during the study. To evaluate the number of flies which were killed by insecticide (garlic extract), the Abbot formula must be used:

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

Explanation:

A_1 = percentage of dead flies after correction/ potential insecticidal effect

A = percentage of dead flies in different concentration of garlic extract respectively

B= percentage of dead flies in the negative control group

4.9 Experimental Framework

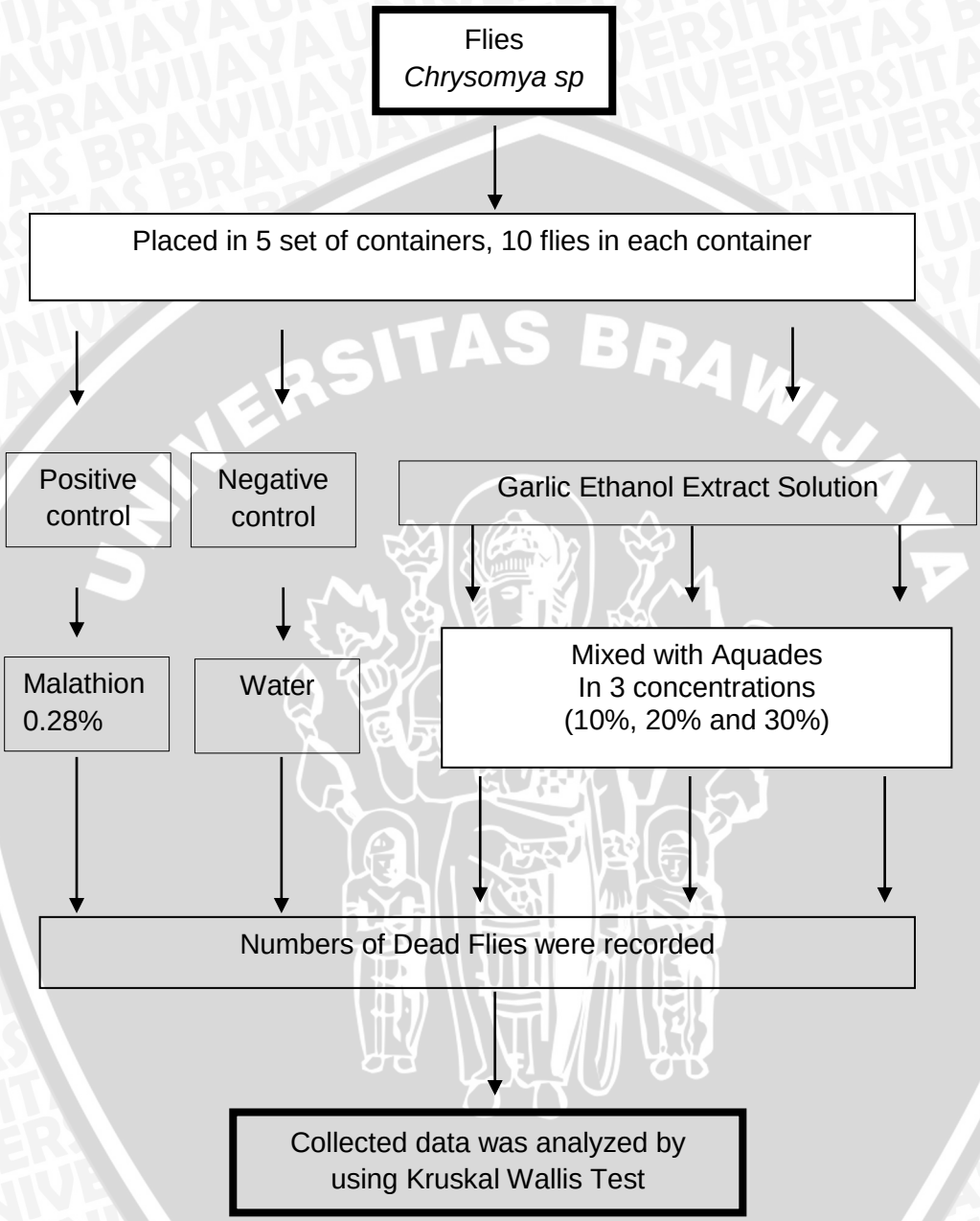


Figure 4.1 Experimental framework

4.10 Data Collection

The data collected were classified into table forms according to the amount of dead flies, *Chrysomya sp.* at repeated tests and different concentrations of garlic, *Allium sativum* extract solutions.

4.11 Data Analysis methods

The set of data obtained were analyzed by using various tests.

The normality test compares the scores in the sample to a normally distributed set of scores with the same mean and standard deviation; the null hypothesis is that “sample distribution is normal.” If the test is significant, the distribution is non-normal. The Shapiro-Wilk test is based on the correlation between the data and the corresponding normal scores. For hypothesis test, if data distribution is normal, parametric test is used. If data distribution is not normal, nonparametric test is used.

The independent t-test assumes the variances of the two groups which was measured to be equal. The assumption of homogeneity of variance was tested using Levene's Test of Equality of Variances, which is produced in SPSS when running the independent t-test. This test for homogeneity of variance provided an F statistic and a significance value (p-value). If the significance level was greater than 0.05, group variances can be treated as equal. However, if $p < 0.05$, the data were violated the assumption of homogeneity of variance.

To use a parametric test, 3 parameters of the data must be true or are assumed. First, the data need to be normally distributed, which means the data also need to have equal variance and have the same standard deviation. Finally, the data need to be continuous.

In Analysis of Variance (ANOVA), there are three assumptions:

- Observations are independent.
- The sample data have a normal distribution.
- Scores in different groups have homogeneous variances.

Statistical hypothesis in this study are:

H0: there is no difference in the percentage of deaths flies *Chrysomya sp* between treatment groups that are given garlic extract compared with the control group who did not receive the garlic extract.

H1: There is a significant difference between the percentage of deaths flies Chrysomyia sp. between treatment groups that are given garlic extract compared to the control group who did not receive the garlic solution extract.

If the differences are found significant, analysis was contineued with Tukey's post hoc test to test which groups who have a significant difference. Then proceed with the Pearson correlation analysis, where the analysis to look at the strength of the relationship between two variables, namely the concentration of garlic solution extract with the number of deaths. In addition, linear regression also performed.

If the data did not meet the criteria for a parametric test (normally distributed, equal variance, and continuous), it must be analyzed with a nonparametric test. The Kruskal-Wallis test is a rank-based nonparametric test that can be used to determine if there are statistically significant differences between two or more groups of an independent variable on a continuous or ordinal dependent variable. It is considered the nonparametric alternative to the one-way ANOVA, and an extension of the Mann-Whitney U test to allow the comparison of more than two independent groups.

The Mann-Whitney U test is used to compare differences between two independent groups when the dependent variable is either ordinal or continuous, but not normally distributed. The Mann-Whitney U test is often considered the nonparametric alternative to the independent t-test.

Like the Pearson product correlation coefficient, Spearman's Rho Correlation test is a nonparametric statistical test performed to determine the correlation between dependent variable (insecticidal potentials) and independent variables (time and concentrations).

The experiment result was analyzed using statistical analysis program, IBM SPSS (Statistical Products and Service Solutions) Statistics, version 22.0 for Windows.