THE POTENTIAL TEST OF ETHANOL CELERY LEAF (*Apiumgraveolens*) EXTRACT AS ANINSECTICIDEON HOUSEFLY (*Musca domestica*)USING SPRAYING METHOD

FINAL ASSIGNMENT

To Fulfill the Requirements for the Degree of Bachelor of Medicine

By:
TAN SHU ZHEN
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MEDICAL PROGRAMME
FACULTY OF MEDICINE
UNIVERSITY OF BRAWIJAYA
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2012

APPROVAL PAGE

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CERTIFICATION PAGE

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December 2012,

TAN SHU ZHEN

ABSTRACT

Shu Zhen. Tan 2012. **The Potential Test of Ethanol Celery leaf (*Apium graveolens)* Extract as an Insecticide on housefly (*Musca domestica)* Using Spraying Method*.*** Final Assignment, Faculty of Medicine, Brawijaya University. Supervisors:
(1) Dr.Aswin D.Baskoro,MS, Sp.ParK (2) Dr. Endang Asmaningsih, M.S

Musca domestica can act as a vector for many diseases. One of the methods to fight against this vector is insecticide. The usage of plant as bio-insecticide has better safety level because its molecules are easy to break down and become less dangerous compound. *Apium graveolens* contains *flavonoid* and *saponin* that are predicted to have the potential as insecticide. The design of this experiment was *true experimental-posttest only control group design.* The samples were 10 flies for each treatment. There were three study groups with different concentration of *Apium graveolens* extract, 1 group as positive control (malathion 0.28%) and another group as negative control (water). This study was repeated 4 times at 5 times interval (1st, 2nd, 4th, 6th and 24th hour). Concentration used in this experiment are 20%, 25% dan 30%. The result of this experiment revealed that higher percentage of *Apium graveolens* extract had greater potential as an insecticide. There was significant difference between the 20%, 25% and 30% concentration with the concentration of 26.27% at 24th hour as the lowest concentration that was able to kill 100% *Musca domestica*. From the *Pearson correlation* test, it was found that there was significant relation between *Apium graveolens* extract and the death of *Musca domestica*. (p=0,000), which meant there was significant correlation upon higher concentration and higher potential of insecticide. Based on this result, the conclusion is *Apium graveolens* extract has the potential as an insecticide against *Musca domestica.*

Keywords: *Apium graveolens, Musca domestica*, Insecticide

ABSTRAK

Shu Zhen. Tan 2012 **Uji Potensi Ekstrak Ethanol Daun Seledri (*Apium graveolens*) Sebagai Insektisida Terhadap Lalat *Musca domestica* Dengan Method Semprot.** Tugas Akhir Fakultas Kedokteran Universitas Brawijaya. Pembimbing: (1)Dr.Aswin D.Baskoro, MS,Sp.ParK (2)Dr. Endang Asmaningsih, M.S

Lalat *Musca domestica* merupakan vektor dari berbagai penyakit. Salah satu pengendalian vektor dilakukan dengan insektisida. Penggunaan tumbuhan sebagai insektisida umumnya menunjukkan tingkat keamanan yang tinggi karena molekulnya mudah dipecah menjadi senyawa tidak berbahaya.*Apium graveolens* mengandungi senyawa aktif iaitu flavonoid dan saponin yang diduga mempunyai potensi sebagai insektisida. Tujuan penelitian ini adalah untuk membuktikan potensi ekstrak daun Seledri (*Apium graveolens*) sebagai insektisida terhadap lalat *Musca domestica*. Penelitian ini menggunakan *true experimental-post-test only control group design.* Lalat yang digunakan sebagai sampel sebanyak 10 ekor untuk setiap perlakuan. Dilakukan 3 perlakuan dengan konsentrasi ekstrak daun seledri yang berbeda, 1 perlakuan kontrol positif (malathion 0,28%) dan 1 perlakuan kontrol negatif (aquades). Perlakuan diulang empat kali dan dilakukan pengamatan pada 5 interval waktu yaitu 1, 2, 4, 6, dan 24 jam. Konsentrasi yang digunakan adalah 20%, 25% dan 30%. Hasil penelitian menunjukkan bahawa semakin besar konsentrasi ekstrak daun seledri semakin besar pula potensinya sebagai insektisida. Kesimpulan dari penelitian ini adalah daun Seledri (*Apium graveolens*) mempunyai potensi sebagai insektisida terhadap lalat *Musca domestica.*

Kata kunci : *Apium graveolens, Musca domestica*, Insektisida

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CHAPTER 1

INTRODUCTION

1.1 Background

The most important housefly family is the genera *Musca* where they live in close association with humans. *Musca domestica*, the housefly, is the species found most commonly throughout the world and is the main focus of this section. The breeding sites of flies are animal and human excreta and a wide variety of other organic matter, particularly domestic rubbish. They are capable of traveling up to 8 km in 24 hours to find food and reproductive sites and easily move from heavily contaminated to human populated areas. This fly species closely associated with humans can become an important disease vector of the microorganisms that cause diseases. Epidemics of these diseases can be common where high human and fly population densities are associated with unsanitary conditions. (WHOPES,2006)

Until now, the housefly eradication in Indonesia has not been a priority. One of the most easy and effective way is by using insecticides. The use of chemical insecticide is effective and provides optimal results, but it will cause many negative impacts to both the living organisms and the environment. According to WHO more than 20,000 people die per year due to pesticide poisoning, besides it also cause fatal effects, such as cancer and infertility. (WHOPES, 2006)

The natural insecticide products derived from plants have been used successfully since ancient times to control a variety of insect pests that directly or otherwise endanger human survival. Interest in their use has been growing due to their safety and desirable properties. The demand for new precautionary strategies and improved health education is overwhelming hence the supreme need for safe, efficient and cost-effective alternative approaches. (Darman,2005)

One of the alternative natural plants is the celery leaf (*Apiumgraveolens*).In Indonesia, celery leaf (*Apiumgraveolens*) is widely known as DaunSeledri (Indonesia). Celery leaf can be found in North Sumatra and West Java easily and it is affordable. Itcan grow well in low-and high plains. Normally, celery leaf used in Indonesia is for complement the vegetables (example for soup) due to its strong aromatic smell.Besides that, it is a very good source of dietary fibre, potassium, folate, vitamin A, vitamin C, molybdenum, and manganese.

Celery leaf contains active ingredients such as flavonoid, saponin, alkaloid, tlavonoida, polifenol, tannin 1%, coline, lipase and others. The content of flavonoid and saponinon celery leaf can be utilised as insecticide. Flavonoid plays a role as respiratory inhibitor of insects which will damage the spiracle resulting in respiratory problem in insects. Saponin disturbs the digestive system of the insect by decreasing surface tension of digestive tract which results in digestive tract corrosion. By using the extraction method, the active substances can be obtained in high concentration which can produce highly effective result.(Dalimartha, 2008)

1.2 ResearchProblem

Does the extract ofethanol celery leaf (*Apiumgraveolens*) have the insecticidal potentialon housefly (*Musca domestica*) using spraying method?

1.3 Researchobjective

1.3.1 General objective

This research aims to investigate the potential test of ethanol celery leaf extract (*Apiumgraveolens*) as an insecticide on housefly (*Musca domestica*) using spraying method.

1.3.2 Specific objective

To test the relationship between ethanol celery leaf extract (*Apiumgraveolens*) and the potential of it as an insecticide on housefly (*Musca domestica*) based on different concentration and time of exposure.

1.4 Significance of the Research

1.4.1 Academic significance

To give the information on the potential of ethanol celery leaf's extract (*Apiumgraveolens*) as an insecticide on housefly (*Musca domestica*) using spraying method based on different concentration and time of exposure.

1.4.2 Application significance

1. As a source of informationfor the society to know the potential of ethanol celery leaf's extract (*Apiumgraveolens*) as an insecticide on housefly (*Musca domestica*).
2. This study also seeks to develop a new potential source of plant-based insecticide that would be available environmentally safe.

CHAPTER 2

REVIEW OF RELATED LITERATURE

2.1 Fly

Fly isthe insect of the order of Diptera or two-winged insect that is often the vector of organisms causing disease (Dorland, 2009). There are four types of common species which can be found, (Darman,2005)

1. Housefly (Musca sp.)
2. Green bottle fly (Luciliasericata sp.)
3. Blue blowfly (Calliphora erythrocephala sp.)
4. Fruitfly (Drosophila sp.)

2.2 *Musca domestica* (Housefly)

2.2.1 Taxonomy

Kingdom	: Animalia
Phylum	: Arthropoda
Class	: Insecta
Order	: Diptera
Suborder	: Cyclorrhapha
Family	: Muscidae
Genus	: Musca
Species	: *Musca domestica*

Subspecies :*M.domesticacalleva Walker,* 1849

2.2.2 Morphology

The housefly has a well-differentiated head, thorax and abdomen and two broad wings. Its length ranges between 5 - 8 mm long with a spread wingspan of 13-15 mm. The female is usually larger than the male.The head of the adult fly is oval-shaped and has reddish-eyes. Between the eyes there is a pair of short and thick antennae. The abdomen is grey or yellowish with dark midline and irregular dark markings on the sides.The female can be distinguished from the male by the relatively wide space between the eyes (in males, the eyes almost touch). House flies have sponging mouthparts (proboscis) and can only ingest liquids. However, they can eat solid food (e.g., sugar, flour, pollen) by first liquefying it with their saliva.They are most abundant in late summer and early autumn and have a life cycle of 7-45 days. (Baskoro dkk,2007).

Figure2.1: Adult *Musca domestica sp.* (MacKean, 2004)

2.2.3 Life cycle

Housefly has a complete metamorphosis with distinct egg, larva or maggot, pupa and adult stages. Forty-eight hours after emergence as an adult, the female

commences eggs' laying. It is capable of producing 4-5 batches of 100-150 eggs during its adult life of 1-3 months.

The pearly-white cylindrical eggs, 1mm in length, are laid in moist decaying matter such as household refuse, compost or dung. The eggs hatch in 8-48 hours, giving the smooth, white, legless maggot larvae and after three months reach maturity at a length of 10-12mm.

The larvae leave the breeding site for the cooler surrounding areas, e.g. soil. Here they develop as yellow, brown or black pupae 6mm long. Depending upon conditions, adults emerge three days to four weeks later.

The full cycle is generally completed between one to four weeks, depending upon temperature. It is clear that there is considerable potential for the development of huge populations. As many as 12 generations of flies may breed in one season, and in heated environments even this rate of reproduction may be exceeded. (Keiding,2001)

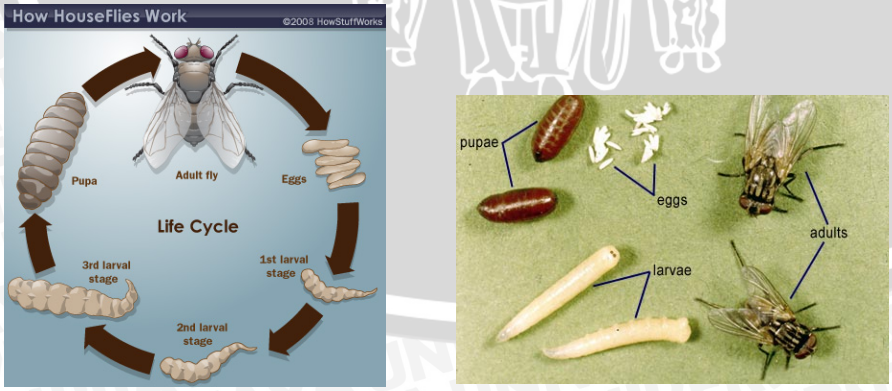


Figure 2.2: Life cycle of the house fly, *Musca domestica sp*. (Jerry F.Butlerand MattAubuchon,2008)

2.2.4 Breeding place

Musca domestica is world-wide in distribution and lives in close association with human dwellings. They are found wherever suitable breeding conditions exist, usually rotting, fermenting, or at least moist organic matters, preferably of a high protein content. They are particularly common around moist faeces and decaying organic matter.

Breeding continues throughout the year in warm parts of the country. In colder climates the larvae or pupae over winters and adults enter a resting state (diapause) in sheltered situations (Keiding, 2001).

2.2.5 Habits

The adult flies feed on a wide range of organic matter including faeces and many types of liquids, but can eat solid foods, such as sugar. To digest solid foods, house flies liquefy food by spitting out saliva on solid foods to predigest it, and then suck it back in. They also regurgitate partly digested matter and pass it again to the abdomen. During feeding, they also defecate on the food. Because of these habits, house flies can pose serious health threats by transmitting disease organisms.(MacKean, 2004)

2.2.6 Medical importance

Because they have sponging mouthparts, house flies cannot bite; however,the control of *Musca domestica* is vital to human health and comfort in many areas of the world.Flies can spread diseases because they feed freely on human food and filthy matter alike. The fly picks up disease-causing organisms while crawling and feeding. Those that stick to the outside surfaces of the fly may survive for only a few hours,but those that are ingested with the food may survive in the fly's crop or gut for several days. Transmission takes place when the fly makes contact with people or their food.

The most important damage related with this insect is the annoyance and the indirect damage produced by the potential transmission of pathogens (viruses, bacteria, fungi). (WHO,2009)

2.2.7 Control of *Musca domestica*

The control of *Musca domestica* can be done by many methods. Generally, these methods are divided into two types, which are natural control and artificial control. Flies have rapid, prolific breeding habits and high mobility.

Satisfactory hygiene is necessary to reduce or eliminate fly's breeding sites. Solid concrete floors with drains should be constructed; dung should be cleaned out and floors should be flushed daily. Domestic refuse must be stored in well-sealed bins, for early removal to disposal sites.High-risk material should be sealed in bags and burnt wherever possible. Farm manure should be kept as dry as possible, especially in poultry houses,where leaking water feeders can provide ideal, moist breeding conditions.

Prevention of contact between flies and disease-causing germs is important. The sources of germs include human and animal excrement, garbage, sewage, infected eyes, and open sores and wounds. Measures to eliminate fly breeding also reduce contact between flies and germs.

The most important are:

- the installation and use of proper latrines and toilets where flies cannotmake contact with faeces;
- the prevention of contact between flies and sick people, their excreta, open sores, and infected eyes;

In order to obtain the best results, insecticidal control measures should be integrated with good hygiene by protection of food, eating utensils and people from contact with flies. Flies can be killed directly by insecticides or physical means such as traps, sticky tapes, fly swats and electrocuting grids.

Chemical method is a method of attacking the habitats of insects with the usage of insecticides. This way is cheaper because it covers more space and can be done easily. This method can be divided into natural insecticide and non-natural insecticide (Dinata,2006)

(i) Baygon or Hit isthe example of non-natural insecticide. These insecticides are made from non-natural substances.

(ii) Examples of natural insecticide are extracts from plants.

There are few ways for insecticide to enter the insects body (Darman,2005):

a. Stomach poison

Stomach poison in an Insecticide which enters the digestion system of insects through their food and kill them. From here, the insecticide will enter the digestion organ of the insects and travel to target area where the active substance works. As an example, insecticide enters the respiration organs through nervous system of the insects and poisons them.

b. Skin contact poison

Skin contact insecticidesare insecticides which enters the insect body through pores on their skin, natural holes (trachea) or directly through their mouth. Particular insect will die due to direct contact with the insecticide. The mechanism of the skin contact poison is almost same as stomach poison.

c. Respiratory poison

Respiratory poison is an insecticide which enters the body through trachea in micro particle form in the air. Insects will die when they inhale these micro particles in a big amount. Most of the respiratory poison will be in a form of gas, smoke, or vapor from liquid poison.

2.3 Celery Leaf (*Apiumgraveolens*)

2.3.1 Taxonomy

Kingdom : *Plantae* – Plants

Subkingdom :*Tracheobionta* – Vascular plants

Superdivision:*Spermatophyta* – Seed plants

Division : Dicotyledonae–Plants having embryos with two cotyledons

Class : Umbelliflorae – Aromatic plants with hollow stems.

Subclass : Umbelliferae

Order : Apiaceae – Carrot/ parsley family

Family : *Apium*

Genus : *Apiumgraveolens*

Species : Celery

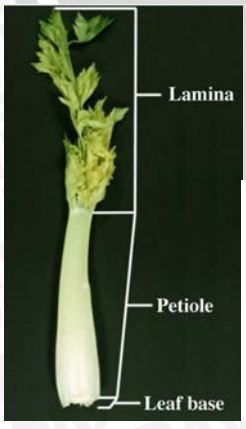


Figure 2.3: Celery leaf (*Apiumgraveolens*) (California Celery Research, 2004)

2.3.2 History

The celery that we know today was derived from wild celery. While thought to have its origins in the Mediterranean regions of northern Africa and southern Europe, it was also native to areas extending east to the Himalayas.

Celery has a long and prestigious history of use, first as a medicine and then later as a food. It is claimed medicinal purposes were probably attributable to its volatile oils, contained in all portions. The initial mention of the medicinal properties of celery leaves dates back to the 9th century B.C. The Ancient Greeks used the leaves as decoration while the ancient Romans used it as a seasoning, a tradition that has carried through the centuries. It was not until the Middle Age that celery's use expanded beyond medicine and seasoning into consideration as a food. (California Celery Research, 2004)

In Indonesia, celery leaf (*Apiumgraveolens*) is known as *DaunSeledri* (Indonesia);*DaunSledri* (Jawa), *DaunSaledri* (Sunda). Itcan grow well in low-and

high plains. In category celery plants as vegetables, celery plantations in Indonesia mostly in Berastagi, North Sumatra and West Java spread inPacet, Pangalengan and Cipanas. Normally, celery used in Indonesia is for complement the vegetables (example for soup). (Cherepanov S.K, 1995)

2.3.3 Morphology

Celery grows to a height of 12 to 16 inches and is composed of leaf-topped stalks arranged in a conical shape that are joined at a common base. The flowers are creamy-white, 2–3 mm diameter, produced in dense compound umbels. The seeds are broad ovoid to globose, 1.5–2 mm long and wide.It is a biennial vegetable plant that belongs to the Umbelliferae familywhose other members includes carrots, fennel and parsley. The leaves, roots and seeds can also be used as a food and natural medicine remedy.

2.3.4 Contents

The celery leaf contains flavonoids, saponin, alkaloid, polyphenol, tianin 1%, choline, lipase, nocotonic acid. (Dalimartha, 2008)

Besides that, celery is an excellent source of vitamin K. It is a very good source of dietary fiber, potassium, folate, vitamin A, vitamin C, molybdenum, and

manganese. Celery is also a good source of calcium, vitamin B1, vitamin B2, vitamin B5, vitamin B6, and magnesium. It also contains approximately 35

Celery, (Apiumgraveolens), Fresh,
Nutrient value per 100 g

milligrams of sodium per stalk. (Ensminger AH,1985)

Principle	Nutrient Value	Percentage of Recommended daily allowance (RDA)
Energy	16 Kcal	<1%
Carbohydrates	3 g	5.5%
Protein	3.46 g	6%
Total Fat	1.12 g	4.5%
Cholesterol	0 mg	0%
Dietary Fiber	2.10 g	5.5%
Vitamins		
Folates	36 µg	9%
Niacin	0.320 mg	2%
Pantothenic acid	0.246 mg	5%
Pyridoxine	0.074 mg	6%
Riboflavin	0.57 mg	4%
Thiamin	0.021 mg	2%
Vitamin A	449 IU	15%
Vitamin C	3.1 mg	5%
Vitamin K	29.3 µg	24%
Electrolytes		
Sodium	80 mg	5%
Potassium	260 mg	5.5%
Minerals		
Calcium	40 mg	4%
Copper	0.35 mg	4%
Iron	0.20 mg	2.5%
Magnesium	11 mg	3%
Manganese	0.103 mg	4.5%
Phosphorus	24 mg	3%
Zinc	0.13 mg	1%
Phyto-nutrients		
Carotene-ß	270 µg	--
Crypto-xanthin-ß	0 µg	--
Lutein-zeaxanthin	283 µg	--

Table 2.1 In- depth analysis of nutrient of *Apiumgraveolens (California Celery Research)*

2.3.5 Usage and benefits

Exotic celery herb is known for its strong aromatic flavour.

One of the very low calorie herbal plants, celery leaves contain only 16 calories per 100 gram weight and lots of non-soluble fibre which when combined with other weight loss regimens may help to reduce body weight and blood cholesterol levels.

Celery is a functional food. Its leaves are rich source of flavonoid antioxidants such as lutein and beta-carotene, which have anti-oxidant, cancer-protective, and immune-boosting functions.It is also good source of vitamin-A. Vitamin-A and beta-carotene are natural flavonoid antioxidants. Vitamin A is also required for maintaining healthy mucus membranes and skin, and benefit for eye and vision.

Its leaves and seeds contain many essential volatile oils such as limonene while its characteristic fragrance is due to chemical compounds known as phthalides in them.Essential oil obtained from extraction of celery plant has been used in soothing remedies for nervousness, osteoarthritis, and gouty-arthritis conditions.

In addition, its seeds and root has diuretic (removes excess water from body through urine), stimulant and tonic properties. (Dalimartha, 2008)

2.3.6 Active substances in *Apium graveolens*

2.3.6.1 Flavonoid

Flavonoid is a form of poison found in *Apium graveolens* leaf. It is a form of glucoside from glucose and flavon. Flavonoid is a biggest group of phenol. Flavonoid covers most of the pigments found in almost all the plants.

Flavonoid has a very strong smell. The yellow pigment in it dissolves in water and organic solvent and its breakable under high temperature.

Flavonoid enters the body of insect through respiratory system and weakens the nervous system and cause damage to the spiracle. This will lead to death of the insect. (Friedly, 2000)

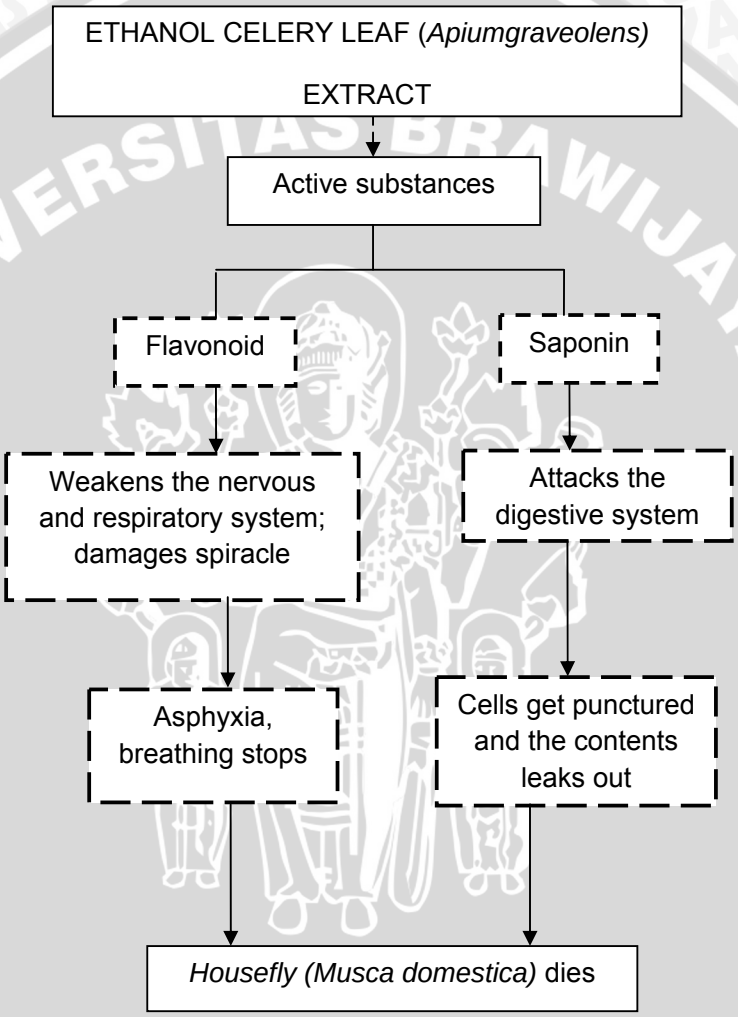
2.3.6.2 Saponin

Saponin damages structure and permeability of cell membrane of insect. This will lead to puncture of the cells which eventually cause death of houseflies. Saponin will also cause asphyxia and stop the breathing of the insect.(Friedly, 2000)

CHAPTER 3

CONCEPTUAL FRAMEWORK AND HYPOTHESIS

3.1 Framework



Where:

Not experimented

Experimented

Explanation of framework:

Celery leaf (*Apium graveolens*) contains flavonoid and saponin. Flavonoid causes the paralysis of the nervous system. This will cause the insect, housefly (*Musca domestica*) to suffocate and eventually die. Saponin can also attack the digestion system and damages cell membrane of *Musca domestica*.

3.2 Hypothesis

Ethanol celery leaf (*Apiumgraveolens*) extract has an insecticide potential onhousefly (*Musca domestica*) using spraying method.

CHAPTER 4

STUDY METHOD

4.1 Study design

This is a true laboratory experimental study with a design true experimental-post-test only control group. The purpose of the study is to know the insecticide potential of ethanol celeryleaf (*Apium graveolens)* extract on housefly (*Musca domestica*).

4.2 Population and Sample

The study population used in this experiment:

- All living houseflies (*Musca domestica*)

- Freely moving houseflies

This sample was taken from a habitat of houseflies (*Musca domestica*) near parasitological lab of University of Brawijaya (Unibraw). Sample was divided into 1 negative group (without extract),1 positive group and 3 study groups. Each study group represents one dose (concentration) of extracts with the same amount of samples. Each group consists of 10housefly (*Musca domestica*). Sample size estimation was done based on the following formula:

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

P = number of trial

n = number of repetition of each sample

(p) = 5,

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

$$5n-5 \geq 16$$

$$5n \geq 26$$

$$n \geq 4.2$$

Hence from the calculation, to be more accurate the test was done 4 times with different concentrations but the same extract.

4.3 Place and time of study

Experiment was carried out at Parasitological Laboratory of the medical faculty in Universitas Brawijaya. Extract processing was done in Faculty of Polytechnique, University of Brawijaya.

4.4 Variable identification

4.4.1 Dependent variable

Dependent variable in this research was the amount of dead housefly (*Musca domestica*).

4.4.2 Independent variable

Independent variable in this research was the dose or concentration of celeryleaf (*Apium graveolens)*extract with dosage of 20 %, 25 % and 30 %.

4.5 Operational definiton

- Concentration of celeryleaf (*Apium graveolens)*extract: Exploration technic was used. Ethanol 96% was used as a solvent. The extract was prepared by using “Technique of Simple Extraction” in faculty of Polytechnique, University of Brawijaya.
- Houseflies *(Musca domestica)*for the experiment were obtained from parasitological lab of UniversitasBrawijaya. 50 houseflies wereplaced in 5 glass containers (each container contained 10 houseflies)
- Dead *housefly (Musca domestica)*: The insect didn’t move when touched by using pin set.
- The insecticide potential of celeryleaf (*Apium graveolens)*extract was observed from the number of dead *housefly (Musca domestica)* from the extract’s concentration of 20%, 25% and 30%.

4.6 Instrumental Studies (Substances and tools)

Substances and tool needed are syringes, 5 glass cages (containers) with cover, pin set, celeryleaf (*Apium graveolens)* extract, adult housefly *(Musca domestica),* Malathion 0.28% and aquades(water).

4.7 Study work plan

4.7.1 Extraction process

Extraction was done by using the "Technique of Simple Extraction" as in the organic chemistry book, "An introduction to Modern Experimental Organic Chemistry" (H.William, 2003). The solvent used is ethanol 80%. The process of extraction was as followed:

1. 100g celeryleaf (*Apium graveolens)* werewashed and rinsed with clean running water.
2. Dried the leaves under hot sun and cut into small pieces and heated in oven under 60-80^0 C.
3. The leaves were blended into powder and weighed.
4. The blended powder was soaked with 250ml of ethanol in a 500ml bottle for 1 week until the active substance in the*Apiumgraveolens.* was dissolved in ethanol.
5. After finishing the extraction process, the active substance was separated from the ethanol using the extraction separator.

4.7.2 Evaporation process

1. The evaporator was set to a permanent pillar, so that it was in the slanting position at 30° to 40° from the experimental table.
2. The soaked extract-ethanol solution was transferred to the extraction separation container.
3. This container was connected to the base of evaporator while spiral cooler was connected above the evaporator.
4. Water pump was placed in the container which contains aquadest (water). It was connected to electrical source causing aquadest to flow and filled the spiral cooler (wait until water was well distributed).
5. The extract was evaporated until half of the separated extract was covered with aquadest in the water bath.
6. The rotary vacuum pump and water bath were connected to the source of electricity. The temperature of water bath was increased to 87°C (boiling point of ethanol).
7. The process occurred until evaporated solution accumulated in the evaporation separation container for approximately 6 hours.
8. The process of evaporation was followed by heating in oven at 50°C for 1-2 hours.
9. At the end of evaporation process, a very concentrated extract of *Apiumgraveolens*was obtained. This extract used in the experiment was kept in bottle.

4.7.3Study procedure

- Aquades (Water) was added to dilute 100% concentration of stok solution.
- Stoksolution of celeryleaf (*Apium graveolens)* was prepared in three different concentrations, 20 %, 25 % and 30 %.

Dosageof the stok solution was prepared by using the formula below:

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

Where:

M1 : Concentration of stok solution (100%)

M2 : Concentration of needed solution (20%,25%,30%)

V1 : Volume of stok solution

V2 : Volume of experimental solution(4ml) (Lukito, 1998).

4.7.4 Working method

1. Experimentswere done by using 5 glass containers of 25cm x 25cm x 25cm in size.

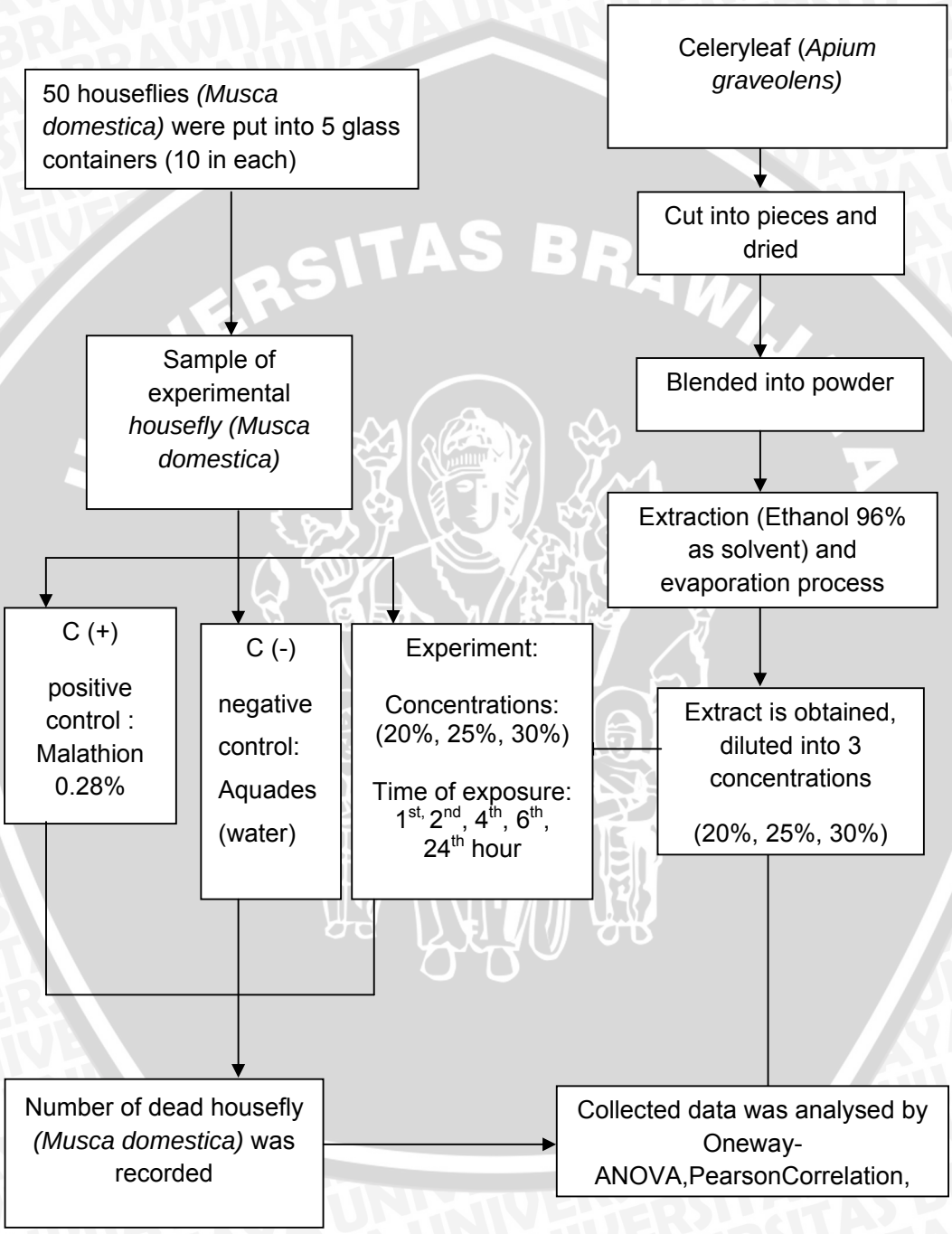
2. The celery leaf's extractwas prepared in three differentdosages, 20%, 25% and 30% and was filled in the sprayer bottle.

3. Each solution was sprayed to every container until the solution in each bottle was finished. The specific explanation is as below:

1. Container 1: 4.0 ml Malathion 0.28% solution (positive control)
2. Container 2: 4.0 ml of celery leaf's extractsolution 20%
3. Container 3: 4.0ml of celery leaf's extractsolution 25%
4. Container 4: 4.0ml of celery leaf's extractsolution 30%
5. Container 5: 4.0 ml aquades/water (negative control)
4. The number of flies that fell in each experiment was counted on 1st hour, 2ndhour, 4thhour, 6th hour and 24th hour.
5. This research were done with 4 times repetition for each experiment

Data of the dead adult housefly *(Musca domestica)*for each extract concentration and time interval were analysed. This was to find out the insecticide potential of the extract with different concentrations.

4.7.5 Framework of experiment



4.8 Data collection

The collected data was classified into table form according to the amount of dead housefly *(Musca domestica)*, repetition and concentration. Statistic test was done from the table.

4.9 Data analysis

Analysis was done according to the amount of dead housefly *(Musca domestica)* for each concentration of celeryleaf (*Apium graveolens)*by using Abbot formula. Analysis was done by using One-way Anova method and then followed withPost Hoc Test.

CHAPTER 5

RESEARCH RESULT

5.1 Research Result Data

In this research, three concentrations were used in this experiment; 20%, 25% and 30% together with Malathion 0.28% as the positive control and aquades (water) solution as the negative control. The experiments were repeated four times. Each experiment was being observed for every 1st hour, 2nd hour, 4th hour, 6th hour, and 24th hour. The results of the number of *Musca domestica* diedfrom the first to the fourth repetition can be seen from Table 5.1 until Table 5.4.

Table 5.1 : Number of housefly died in the 1st repetition

Concentration / Hours	Extract 20%	Extract 25%	Extract 30%	Positive Control	Negative Control
1	0	2	5	10	0
2	2	4	7	10	0
4	3	6	8	10	0
6	5	8	10	10	0
24	8	9	10	10	0

Table 5.2: Number of housefly died in the 2nd repetition

Concentration / Hours	Extract 20%	Extract 25%	Extract 30%	Positive Control	Negative Control
1	1	2	4	10	0
2	2	4	7	10	0
4	4	5	9	10	0
6	5	8	10	10	0
24	8	9	10	10	0

Table 5.3: Number of housefly died in the 3rd repetition

Concentration / Hours	Extract 20%	Extract 25%	Extract 30%	Positive Control	Negative Control
1	0	1	5	10	0
2	3	4	7	10	0
4	4	6	9	10	0
6	6	8	10	10	0
24	7	9	10	10	0

Table 5.4: Number of housefly died in the 4th repetition

Concentration / Hours	Extract 20%	Extract 25%	Extract 30%	Positive Control	Negative Control
1	1	2	4	10	0
2	2	4	6	10	0
4	3	5	8	10	0
6	5	8	9	10	0
24	7	10	10	10	0

Positive control : 4ml of malathion 0.28%

Negative control : 4ml of aquades(water) solution

Based on tables above, different concentration gives different number of dead *Musca domestica* in each repetition.

5.2 The insecticide potential of ethanol *Apium graveolens* extract on *Musca domestica* sp based on the concentration and time of exposure

The data of the total number of dead *Musca domestica*in each experiment was used to calculate the potential of the celery leaf as an insecticide by applying the *Abbott* formula as below:

$$A1 = \frac{A - B}{100 - B} \times 100\%$$

Where:

A1 : Percentage of dead housefly *(Musca domestica)* after correction

A : Percentage of deadhousefly *(Musca domestica)* with different concentration celeryleaf (*Apium graveolens)* Extract.

B : Percentage of deadhousefly *(Musca domestica)* with negative control

(Lukito, 1998)

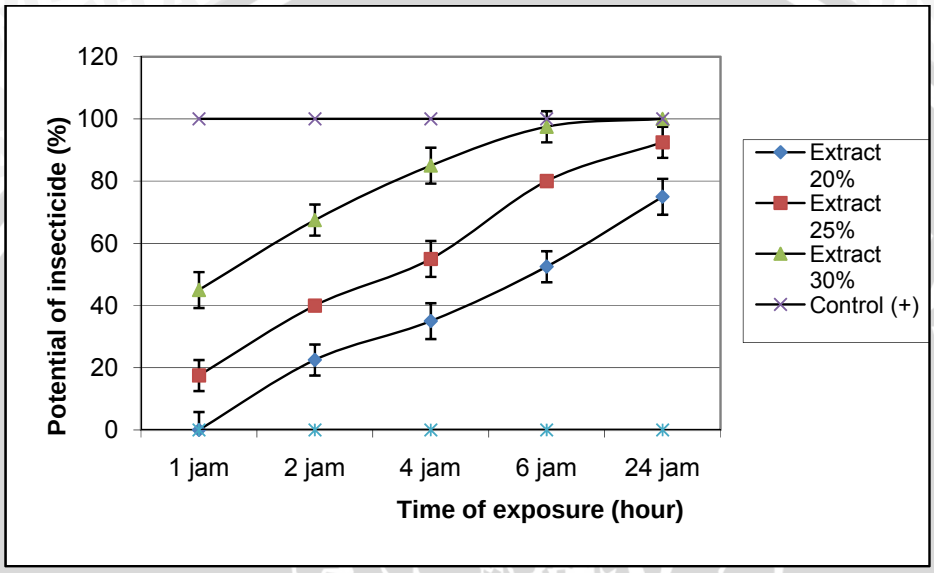
After the data was converted into potential percentage using *Abbott* formula, it was then put into a table and a graph as below:

Table 5.5 **Insecticide potential of *Apium graveolens* extract on *Musca domestica* sp based on the concentration and time of exposure**

Hours	Repetition	Extract 20%	Extract 25%	Extract 30%	Control (+)	Control (-)
1	1	0%	20%	50%	100%	0%
	2	10%	20%	40%	100%	0%
	3	0%	10%	50%	100%	0%
	4	10%	20%	40%	100%	0%
Mean		5%	18%	45%	100%	0%
Standard deviation		0.06	0.05	0.06	0.00	0.00
2	1	20%	40%	70%	100%	0%
	2	20%	40%	70%	100%	0%
	3	30%	40%	70%	100%	0%
	4	20%	40%	60%	100%	0%
Mean		23%	40%	68%	100%	0%
Standard deviation		0.05	0.00	0.05	0.00	0.00
4	1	30%	60%	80%	100%	0%
	2	40%	50%	90%	100%	0%
	3	40%	60%	90%	100%	0%
	4	30%	50%	80%	100%	0%
Mean		35%	55%	85%	100%	0%
Standard deviation		0.06	0.06	0.06	0.00	0.00
6	1	50%	80%	100%	100%	0%
	2	50%	80%	100%	100%	0%
	3	60%	80%	100%	100%	0%
	4	50%	80%	90%	100%	0%
Mean		53%	80%	98%	100%	0%
Standard deviation		0.05	0.00	0.05	0.00	0.00
24	1	80%	90%	100%	100%	0%
	2	80%	90%	100%	100%	0%
	3	70%	90%	100%	100%	0%
	4	70%	100%	100%	100%	0%
Mean		75%	93%	100%	100%	0%
Standard deviation		0.06	0.05	0.00	0.00	0.00

From table 5.5, a graph was plotted to see the potential differences between concentrations and the time of exposure.

Diagram 5.1 Graph of the mean insecticide potential of different concentration versus time of exposure



From the graph above, it can be observed that thereare differences in the number of dead *Musca domestica*with different concentration ofcelery leaf (Apium graveolens)extract and duration of time. It can be seen that the higher extract's concentration,the higher the insecticide potential as the time of exposure increases.

5.3 Data analysis

The result of this research was statistically analysed using SPSS 16.0 version for Windows. Before analyzing the data using One-way ANOVA statistic, there are some criterias that should be fulfilled such as the data distributions which must be normal, the data points must be independent from each other and the variances of the samples were not different.

First step in this process is to test the normality distribution of datausing Normality test such as Kolmogorov-Smirnov. After the normality is known, the Homogeneity of Variance Test is used to see if the data has different variance.

5.3.1 Normality Test

Based on the Kolmogorov-Smirnov Test, it can be seen that the data distribution from the research showed a significant value of p=0.083 (p>0.05). Hence, the data had a normal distribution.

Table 5.6 Data of Normality Test

One-Sample Kolmogorov-Smirnov Test

		potential
N		100
Normal Parameters[a,,b]	Mean	54.8000
	Std. Deviation	39.45282
Most Extreme Differences	Absolute	.159
	Positive	.138
	Negative	-.159
Kolmogorov-Smirnov Z		1.585
Asymp. Sig. (2-tailed)		**.083***

a. Test distribution is Normal.

b. Calculated from data.

5.3.2 Homogeneity of Variance Test

The significant value from the Test of Homogeneity of Variance was p=0.059(p>0.05), so it can be concluded that the variance of the population was homogenous.

Table 5.7 Data of Homogeneity of Variance Test

Levene's Test of Equality of Error Variances[a]

Dependent Variable: potential

F	df1	df2	Sig.
1.310	25	74	.059*

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + time + study method group + time *study method group

5.3.3 One – Way ANOVA Analysis

Sincethe data had a normal distribution and was homogenous, thus, it was eligibled for further One-Way ANOVA test.

Based on the test, it can be concluded that, the extract's concentration was shown that it had a significant value of 0.000 (p<0.005) which meant different concentration gave different effect on number of dead flies.

Table 5.8 Data of One-way ANOVA test

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	38004.173	2	19002.087	74.411	.000[a*]
	Residual	14555.827	57	255.365		
	Total	52560.000	59			

a. Predictors: (Constant), Concentration, Time

b. Dependent Variable: Potential

5.3.4 Pos Hoc Tukey Test

After analysing the data using One Way Anova method, Post Hoc Tukey test which is a multiple comparisons test was done to know exactly which group shows differences. It can be said that there is significant differences between the groups if the p value < 0.05.

Based on table 5.8 (Appendix 3: Homogeneous Subsets), it can be seen that the group of positive control, negative control and the group of 20%, 25% and 30%, each is on different subset group. This meant that there's significant difference.

5.3.5 Pearson Correlation Test

Pearson Correlation test was used to know the correlation and how strong is the relationship between the two variables. If $p < 0.05$, it means that there's a significant correlation between the two variables.

The Pearson correlation coefficient, *r*, shows the strength of correlation which can range from +1.00 to -1.00. If $r < 0.500$, it means that the correlation is weak. If $0.500 < r < 0.599$, the correlation is moderate. If $0.600 < r < 0.799$, the correlation is strong and if $r > 0.799$, the correlation is very strong.

Based on the Table 5.9, the result shows that:

1. p value =0.000 ($p<0.05$) means there is a significant correlation between the concentration of the extract and the number of housefly dies.

2. Pearson correlation coefficient, *r* = 0.566;Variable of concentration has a moderate correlation with the variable of potential. In other words, as the concentrationincreases, the total number of dead*Musca domestica*increases.

Explanation	**r**	**p**	**Conclusion**
Insecticide potential of ethanol *Apiumgraveolens*extract with concentration activity	0.566	0.000	There's a significant moderate correlation (++)

Table 5.9 The result data ofPearson Correlation Test

Correlations

		Time	Concentration	Potential
Time	Pearson Correlation	1	.000	.635
	Sig. (2-tailed)		1.000	.000
	N	60	60	60
Concentration	Pearson Correlation	.000	1	.566**
	Sig. (2-tailed)	1.000		.000
	N	60	60	60
Potential	Pearson Correlation	.635	.566**	1
	Sig. (2-tailed)	.000	.000	
	N	60	60	60

**. Correlation is significant at the 0.01 level (2-tailed).

To know about this relationship mathematically in detail, the regression test was conducted.

5.3.6Regression Test

Regression test is the next step up after correlation. It is used to know the influenceof external factor that affects the death of *Musca domestica*.This was tested in percentage (%).

Table 5.10 Regression model summary showing R square value

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.850[a]	.723*	.713	15.98016

a. Predictors: (Constant), Concentration, Time

According to table 5.10, the R square was equals to 0.723,which meant that 72.3% of dead houseflies were influenced by the concentration of extract. Whilst, there was 27.7% number of dead houseflies were influenced by external factor.

From the linear regression test (see appendix), an equation can be derived as the follows:

$$Y= -60.909+ 2.217\ x_1+4.100\ x_2$$

Where:

Y = insecticide potential of celery leaf (*Apium.g*)

x_1 = time of exposure(hour)

x_2 = extract's concentration(%)

CHAPTER 6

DISCUSSION

This research was conducted in order to test the potentialof the ethanol celery leaf (*Apium.g*) extract as an insecticide towards *Musca domestica.*The celery leaf was used in this study because it can be found easily in Java area (Malang) and it is affordable.

The research is carried out by using ethanol celery leaf (*Apium.g*) extract with the concentration of 20%, 25%,30%, a positive control(Malathion0.28%) and a negative control (aquades). The experimentwas observed for every 1sthour, 2nd hour, 4th hour, 6th hour, and 24th hour. This test was done with repetition as much as 4 times for each treatment.

Based on the analysis of One-Way ANOVA test, it can be concluded that there were significant differences between the varied concentrations and the total number of dead *Musca domestica* in each experiment, which the result's value was $p=0.000$ ($p<0.05$).

The analysis was proceeded with Post Hoc Tukey test. As the significances of the means were obtained through the ANOVA test, specific information of these data was further tested in this test. Based on this analysis, the significances in each interval in comparison with the concentrations; 20%, 25% and 30%, were shown differently.

On the 1st hour, the 20% extract's concentration had 5% potency, 25% concentration had 17.5% potency and 30% concentration had 45% potency.

During the 1st hour, it can be seen that there were significant differences between each subset groups.

On the 2nd hour, the 20% extract's concentration had 22.5% potency, 25% concentration had 40.0% potency and 30% concentration had 67.5% potency. During the 2nd hour, it can be seen that there were significant differences between each subset groups.

On the 4th hour, the 20% extract's concentration had 35% potency, 25% concentration had 55% potency and 30% concentration had 85% potency. During the 4th hour, it can be seen that there were significant differences between each subset groups.

On the 6th hour, the 20% extract's concentration had 52.5% potency, 25% concentration had 80% potency and 30% concentration had 97.5% potency. During the 6th hour, it can be seen that there were significant differences between each subset groups.

On the 24th hour, the 20% extract's concentration had 75% potency, 25% concentration had 92.5% potencywhile the 30% concentration had 100% potency (which is same as the potency as control group). During the 6th hour, it can be seen that there were significant differences between each subset groups except in concentration 30%.

Correlation test was performed to see the relation of extract's concentration and the number of dead housefly died. From the result of the test, the relation between these two variables is significant ($p<0.05$) and the strength

of correlation is moderate. The number of dead*Musca domestica*was increased simultaneously with the increased in extract's concentration.

Although the celery leaf extract has the potential as an insecticide and could kill the mosquito up to 100 % in 24 hours but the extract still could not compete with Malathion as an insecticide that could be used by the community.

Malathion (0.28%) is a yellow to brown liquid that is insoluble in waterand it is a man-made organophosphate insecticidewhichis toxic through skin contact, ingestion, and inhalation exposure.Malathion binds to the enzymeacetyl-cholinesterase (AChE) at nerve endings of the bodies of insects and other organisms. Without AChE functions, ACh accumulates at the nerve junction and results in overstimulation of the nervous systemsuch as convulsion, paralysis and results in death.

The celery leaf (*Apium graveolens*)contains substance such as, flavonoids, saponin, alkaloid, polyphenol, tianin 1%, choline, lipase, nocotonic acid. The active substance of the celery leaf which accounts for its toxicity flavonoid, saponin and alkaloid. Flavonoid affects the respiratory system and causes paralysis of the nervous system. This will cause the insect, *Musca domestica* to suffocate and eventually dies. Saponin can also attack the digestion system and damages cell membrane of *Musca domestica*.

CHAPTER 7

CONCLUSIONS AND SUGGESTIONS

7.1 Conclusions

From this research, it can be concluded that:

1. Ethanol celery leaf (*Apiumgraveolens*) extract has thepotential as an insecticide on housefly (*Musca domestica)* using spraying method.
2. The higher concentration of ethanol celery leaf (*Apiumgraveolens*) extract, the higher the potential as an insecticide on housefly *(Musca domestica).*
3. The longer the time of exposure, the higher the potential of celery leaf (*Apiumgraveolens*) as an insecticide on housefly *(Musca domestica).*

7.2 Suggestions

1. Further conduct research can be projected to determine the extract's toxicity towards human and the environment.
2. It is suggested to conduct research on long time effect of the insecticide storage and the reduction of the killing effect.
3. Further investigation can be conducted in revising the potential concentration of the *Apiumgraveolens*extract using different strain of *Musca domestica.*

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APPENDICES:

Appendix 1: One way Anova

Oneway

ANOVA

1st hour

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	26980.000	4	6745.000	367.909	.000
Within Groups	275.000	15	18.333		
Total	27255.000	19			

ANOVA

2nd hour

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	24330.000	4	6082.500	608.250	.000
Within Groups	150.000	15	10.000		
Total	24480.000	19			

ANOVA

4th hour

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	25400.000	4	6350.000	317.500	.000
Within Groups	300.000	15	20.000		
Total	25700.000	19			

ANOVA

6th hour

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	27530.000	4	6882.500	688.250	.000
Within Groups	150.000	15	10.000		
Total	27680.000	19			

ANOVA

24th hour

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	28680.000	4	7170.000	614.571	.000
Within Groups	175.000	15	11.667		
Total	28855.000	19			

Appendix 2: Post Hoc Tukey Test

Post Hoc Tests

Multiple Comparisons

1st hour
Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Extract 20%	Extract 25%	-12.50000*	3.02765	.007	-21.8492	-3.1508
	Extract 30%	-40.00000*	3.02765	.000	-49.3492	-30.6508
	Control (+)	-95.00000*	3.02765	.000	-104.3492	-85.6508
	Control (-)	5.00000	3.02765	.490	-4.3492	14.3492
Extract 25%	Ekstrak 20%	12.50000*	3.02765	.007	3.1508	21.8492
	Extract 30%	-27.50000*	3.02765	.000	-36.8492	-18.1508
	Control (+)	-82.50000*	3.02765	.000	-91.8492	-73.1508
	Control (-)	17.50000*	3.02765	.000	8.1508	26.8492
Extract 30%	Extract 20%	40.00000*	3.02765	.000	30.6508	49.3492
	Extract 25%	27.50000*	3.02765	.000	18.1508	36.8492
	Control (+)	-55.00000*	3.02765	.000	-64.3492	-45.6508
	Control (-)	45.00000*	3.02765	.000	35.6508	54.3492
Control (+)	Extract 20%	95.00000*	3.02765	.000	85.6508	104.3492
	Extract 25%	82.50000*	3.02765	.000	73.1508	91.8492
	Extract 30%	55.00000*	3.02765	.000	45.6508	64.3492
	Control (-)	100.00000*	3.02765	.000	90.6508	109.3492
Control (-)	Extract 20%	-5.00000	3.02765	.490	-14.3492	4.3492
	Extract 25%	-17.50000*	3.02765	.000	-26.8492	-8.1508
	Extract 30%	-45.00000*	3.02765	.000	-54.3492	-35.6508
	Control (+)	-100.00000*	3.02765	.000	-109.3492	-90.6508

*. The mean difference is significant at the 0.05 level.

a. Uses Harmonic Mean Sample Size = 4.000.

Post Hoc Tests

Multiple Comparisons

2nd hour
Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Extract 20%	Extract 25%	-17.50000*	2.23607	.000	-24.4048	-10.5952
	Extract 30%	-45.00000*	2.23607	.000	-51.9048	-38.0952
	Control (+)	-77.50000*	2.23607	.000	-84.4048	-70.5952
	Control (-)	22.50000*	2.23607	.000	15.5952	29.4048
Extract 25%	Extract 20%	17.50000*	2.23607	.000	10.5952	24.4048
	Extract 30%	-27.50000*	2.23607	.000	-34.4048	-20.5952
	Control (+)	-60.00000*	2.23607	.000	-66.9048	-53.0952
	Control (-)	40.00000*	2.23607	.000	33.0952	46.9048
Extract 30%	Extract 20%	45.00000*	2.23607	.000	38.0952	51.9048
	Extract 25%	27.50000*	2.23607	.000	20.5952	34.4048
	Control (+)	-32.50000*	2.23607	.000	-39.4048	-25.5952
	Control (-)	67.50000*	2.23607	.000	60.5952	74.4048
Control (+)	Extract 20%	77.50000*	2.23607	.000	70.5952	84.4048
	Extract 25%	60.00000*	2.23607	.000	53.0952	66.9048
	Extract 30%	32.50000*	2.23607	.000	25.5952	39.4048
	Control (-)	100.00000*	2.23607	.000	93.0952	106.9048
Control (-)	Extract 20%	-22.50000*	2.23607	.000	-29.4048	-15.5952
	Extract 25%	-40.00000*	2.23607	.000	-46.9048	-33.0952
	Extract 30%	-67.50000*	2.23607	.000	-74.4048	-60.5952
	Control (+)	-100.00000*	2.23607	.000	-106.9048	-93.0952

*. The mean difference is significant at the 0.05 level.

Post Hoc Tests

Multiple Comparisons

4th hour
Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Extract 20%	Extract 25%	-20.00000*	3.16228	.000	-29.7649	-10.2351
	Extract 30%	-50.00000*	3.16228	.000	-59.7649	-40.2351
	Control (+)	-65.00000*	3.16228	.000	-74.7649	-55.2351
	Control (-)	35.00000*	3.16228	.000	25.2351	44.7649
Extract 25%	Extract 20%	20.00000*	3.16228	.000	10.2351	29.7649
	Extract 30%	-30.00000*	3.16228	.000	-39.7649	-20.2351
	Control (+)	-45.00000*	3.16228	.000	-54.7649	-35.2351
	Control (-)	55.00000*	3.16228	.000	45.2351	64.7649
Extract 30%	Extract 20%	50.00000*	3.16228	.000	40.2351	59.7649
	Extract 25%	30.00000*	3.16228	.000	20.2351	39.7649
	Control (+)	-15.00000*	3.16228	.002	-24.7649	-5.2351
	Control (-)	85.00000*	3.16228	.000	75.2351	94.7649
Control (+)	Extract 20%	65.00000*	3.16228	.000	55.2351	74.7649
	Extract 25%	45.00000*	3.16228	.000	35.2351	54.7649
	Extract 30%	15.00000*	3.16228	.002	5.2351	24.7649
	Control (-)	100.00000*	3.16228	.000	90.2351	109.7649
Control (-)	Extract 20%	-35.00000*	3.16228	.000	-44.7649	-25.2351
	Extract 25%	-55.00000*	3.16228	.000	-64.7649	-45.2351
	Extract 30%	-85.00000*	3.16228	.000	-94.7649	-75.2351
	Control (+)	-100.00000*	3.16228	.000	-109.7649	-90.2351

*. The mean difference is significant at the 0.05 level.

Post Hoc Tests

Multiple Comparisons

6th hour
Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Extract 20%	Extract 25%	-27.50000*	2.23607	.000	-34.4048	-20.5952
	Extract 30%	-45.00000*	2.23607	.000	-51.9048	-38.0952
	Control (+)	-47.50000*	2.23607	.000	-54.4048	-40.5952
	Control (-)	52.50000*	2.23607	.000	45.5952	59.4048
Extract 25%	Extract 20%	27.50000*	2.23607	.000	20.5952	34.4048
	Extract 30%	-17.50000*	2.23607	.000	-24.4048	-10.5952
	Control (+)	-20.00000*	2.23607	.000	-26.9048	-13.0952
	Control (-)	80.00000*	2.23607	.000	73.0952	86.9048
Extract 30%	Extract 20%	45.00000*	2.23607	.000	38.0952	51.9048
	Extract 25%	17.50000*	2.23607	.000	10.5952	24.4048
	Control (+)	-2.50000	2.23607	.795	-9.4048	4.4048
	Control (-)	97.50000*	2.23607	.000	90.5952	104.4048
Control (+)	Extract 20%	47.50000*	2.23607	.000	40.5952	54.4048
	Extract 25%	20.00000*	2.23607	.000	13.0952	26.9048
	Extract 30%	2.50000	2.23607	.795	-4.4048	9.4048
	Control (-)	100.00000*	2.23607	.000	93.0952	106.9048
Control (-)	Extract 20%	-52.50000*	2.23607	.000	-59.4048	-45.5952
	Extract 25%	-80.00000*	2.23607	.000	-86.9048	-73.0952
	Extract 30%	-97.50000*	2.23607	.000	-104.4048	-90.5952
	Control (+)	-100.00000*	2.23607	.000	-106.9048	-93.0952

*. The mean difference is significant at the 0.05 level.

Post Hoc Tests

Multiple Comparisons

24th hour
Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Extract 20%	Extract 25%	-17.50000*	2.41523	.000	-24.9580	-10.0420
	Extract 30%	-25.00000*	2.41523	.000	-32.4580	-17.5420
	Control (+)	-25.00000*	2.41523	.000	-32.4580	-17.5420
	Control (-)	75.00000*	2.41523	.000	67.5420	82.4580
Extract 25%	Extract 20%	17.50000*	2.41523	.000	10.0420	24.9580
	Extract 30%	-7.50000*	2.41523	.048	-14.9580	-.0420
	Control (+)	-7.50000*	2.41523	.048	-14.9580	-.0420
	Control (-)	92.50000*	2.41523	.000	85.0420	99.9580
Extract 30%	Extract 20%	25.00000*	2.41523	.000	17.5420	32.4580
	Extract 25%	7.50000*	2.41523	.048	.0420	14.9580
	Control (+)	.00000	2.41523	1.000	-7.4580	7.4580
	Control (-)	100.00000*	2.41523	.000	92.5420	107.4580
Control (+)	Extract 20%	25.00000*	2.41523	.000	17.5420	32.4580
	Extract 25%	7.50000*	2.41523	.048	.0420	14.9580
	Extract 30%	.00000	2.41523	1.000	-7.4580	7.4580
	Control (-)	100.00000*	2.41523	.000	92.5420	107.4580
Control (-)	Extract 20%	-75.00000*	2.41523	.000	-82.4580	-67.5420
	Extract 25%	-92.50000*	2.41523	.000	-99.9580	-85.0420
	Extract 30%	-100.00000*	2.41523	.000	-107.4580	-92.5420
	Control (+)	-100.00000*	2.41523	.000	-107.4580	-92.5420

*. The mean difference is significant at the 0.05 level.

Appendix 3: Homogeneous Subsets

Homogeneous Subsets

1st hour

TukeyHSD[a]

Group	N	Subset for alpha = 0.05			
		1	2	3	4
Control (-)	4	.0000			
Extract 20%	4	5.0000			
Extract 25%	4		17.5000		
Extract 30%	4			45.0000	
Control (+)	4				100.0000
Sig.		.490	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

2nd hour

TukeyHSD[a]

Group	N	Subset for alpha = 0.05				
		1	2	3	4	5
Control (-)	4	.0000				
Extract 20%	4		22.5000			
Extract 25%	4			40.0000		
Extract 30%	4				67.5000	
Control (+)	4					100.0000
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

4th hour

TukeyHSD[a]

Group	N	Subset for alpha = 0.05				
		1	2	3	4	5
Control (-)	4	.0000				
Extract 20%	4		35.0000			
Extract 25%	4			55.0000		
Extract 30%	4				85.0000	
Control (+)	4					100.0000
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

6th hour

TukeyHSD[a]

Group	N	Subset for alpha = 0.05			
		1	2	3	4
Control (-)	4	.0000			
Extract 20%	4		52.5000		
Extract 25%	4			80.0000	
Extract 30%	4				97.5000
Control (+)	4				100.0000
Sig.		1.000	1.000	1.000	.795

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

24th hour

TukeyHSD[a]

Group	N	Subset for alpha = 0.05			
		1	2	3	4
Control (-)	4	.0000			
Extract 20%	4		75.0000		
Extract 25%	4			92.5000	
Extract 30%	4				100.0000
Control (+)	4				100.0000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Appendix 4: Regression Test

Variables Entered/Removed

Model	Variables Entered	Variables Removed	Method
1	Concentration , Time[a]	.	Enter

a. All requested variables entered.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.850[a]	**.723***	.713	15.98016

a. Predictors: (Constant), Concentration , Time

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	38004.173	2	19002.087	74.411	**.000[a]**
	Residual	14555.827	57	255.365		
	Total	52560.000	59			

a. Predictors: (Constant), Concentration , Time

b. Dependent Variable: Potential

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	**-60.909***	12.927		-4.712	.000
	Time	**2.217***	.243	.635	9.110	.000
	Concentration	**4.100***	.505	.566	8.113	.000

a. Dependent Variable: Potential

Correlations

Correlations

		Time	Concentration	Potential
Time	Pearson Correlation	1	.000	.635**
	Sig. (2-tailed)		1.000	.000
	N	60	60	60
Concentration	Pearson Correlation	.000	1	.566**
	Sig. (2-tailed)	1.000		.000*
	N	60	60	60
Potential	Pearson Correlation	.635**	.566**	1
	Sig. (2-tailed)	.000	.000	
	N	60	60	60

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 5: Pictures

Substances and tools used

5 glass containers/ cages

The solution of celery leaf extract was sprayed to every container.

STATEMENT OF ORIGINALITY

Name : TAN SHU ZHEN

NIM : 0910714015

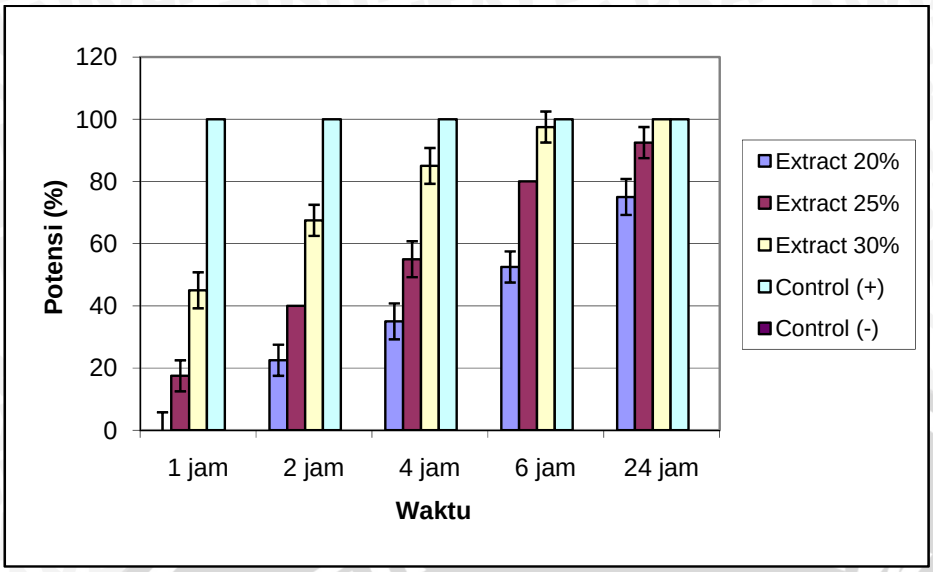
Study Program : Medical program, Faculty of Medicine, University of Brawijaya

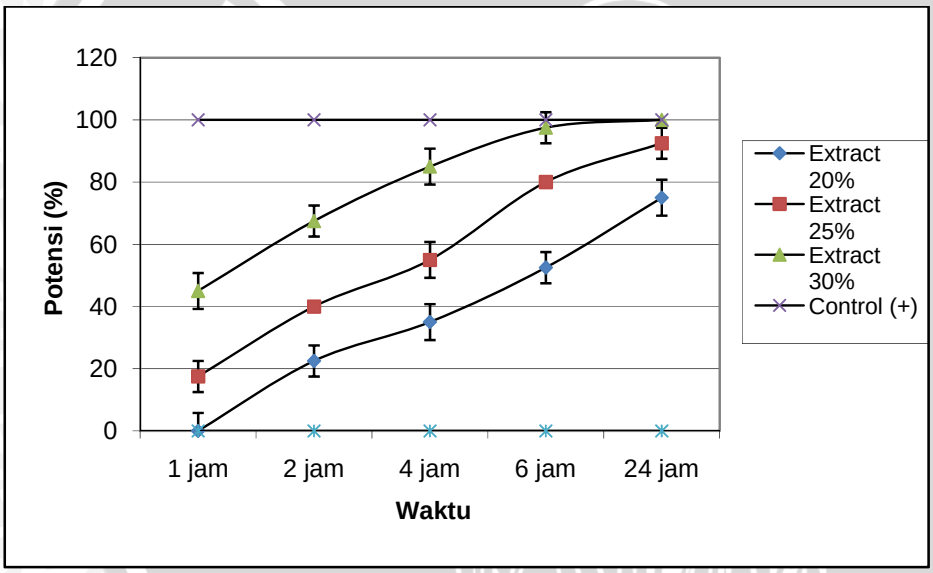
I hereby verify that this thesis is done by me. It is my original work and not based on any form of plagiarism. In the future, if my thesis is proven as the work of others, I am willing to be punished as stated by the rules.

Malang, 1ST December 2012

Sincerely,

(TAN SHU ZHEN)


120
100
80
60
40
20
0
Potensi (%)
1 jam
2 jam
4 jam
6 jam
24 jam
Waktu
Extract 20%
Extract 25%
Extract 30%
Control (+)
Control (-)


120
100
80
60
40
20
0
Potensi (%)
1 jam
2 jam
4 jam
6 jam
24 jam
Waktu
Extract 20%
Extract 25%
Extract 30%
Control (+)

Hours	Repetition	Extract 20%	Extract 25%	Extract 30%	Control (+)	Control (-)
1	1	0	2	5	10	0
	2	1	2	4	10	0
	3	0	1	5	10	0
	4	1	2	4	10	0
Mean		0.50	1.75	4.50	10.00	0.00
sd		0.58	0.50	0.58	0.00	0.00
2	1	2	4	7	10	0
	2	2	4	7	10	0
	3	3	4	7	10	0
	4	2	4	6	10	0
Mean		2.25	4.00	6.75	10.00	0.00
sd		0.50	0.00	0.50	0.00	0.00
4	1	3	6	8	10	0
	2	4	5	9	10	0
	3	4	6	9	10	0
	4	3	5	8	10	0
Mean		3.50	5.50	8.50	10.00	0.00
sd		0.58	0.58	0.58	0.00	0.00
6	1	5	8	10	10	0
	2	5	8	10	10	0
	3	6	8	10	10	0
	4	5	8	9	10	0
Mean		5.25	8.00	9.75	10.00	0.00
sd		0.50	0.00	0.50	0.00	0.00
24	1	8	9	10	10	0
	2	8	9	10	10	0
	3	7	9	10	10	0
	4	7	10	10	10	0
Mean		7.50	9.25	10.00	10.00	0.00
sd		0.58	0.50	0.00	0.00	0.00

Hours	Repetition	Extract 20%	Extract 25%	Extract 30%	Control (+)	Control (-)
1	1	0%	20%	50%	100%	0%
	2	10%	20%	40%	100%	0%
	3	0%	10%	50%	100%	0%
	4	10%	20%	40%	100%	0%
Mean		5%	18%	45%	100%	0%
sd		0.06	0.05	0.06	0.00	0.00
2	1	20%	40%	70%	100%	0%
	2	20%	40%	70%	100%	0%
	3	30%	40%	70%	100%	0%
	4	20%	40%	60%	100%	0%
Mean		23%	40%	68%	100%	0%
sd		0.05	0.00	0.05	0.00	0.00
4	1	30%	60%	80%	100%	0%
	2	40%	50%	90%	100%	0%
	3	40%	60%	90%	100%	0%
	4	30%	50%	80%	100%	0%
Mean		35%	55%	85%	100%	0%
sd		0.06	0.06	0.06	0.00	0.00
6	1	50%	80%	100%	100%	0%
	2	50%	80%	100%	100%	0%
	3	60%	80%	100%	100%	0%
	4	50%	80%	90%	100%	0%
Mean		53%	80%	98%	100%	0%
sd		0.05	0.00	0.05	0.00	0.00
24	1	80%	90%	100%	100%	0%
	2	80%	90%	100%	100%	0%
	3	70%	90%	100%	100%	0%
	4	70%	100%	100%	100%	0%
Mean		75%	93%	100%	100%	0%
sd		0.06	0.05	0.00	0.00	0.00