THE KNOCKDOWN EFFECT OF *Chrysanthemum morifolium ramat* FLOWER ETHANOLIC EXTRACT TOWARDS *Musca domestica.*

FINAL PROJECT
To fulfill the requirement for the degree of Bachelor in Medicine

By:

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Department of General Medicine
Faculty of Medicine
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Malang
2011

APPROVAL PAGE

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CERTIFICATION PAGE

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ACKNOWLEDGEMENT

All praise to Allah, The Almighty, for His gift so that I can finish the final project, The Knockdown Effect Of *Chrysanthemum morifolium ramat* flower ethanolic extract towards *Musca domestica.*

This topic was chosen because the use of chemical insecticides caused so many harms to human and other living creature. Even though, many natural insecticides were developed, most of them were not being proven to be the effective insecticides. So, it was the effort to prove that chrysanthemum, one of the most common natural insecticides being used, fulfill the criteria as an effective bioinsecticide.

This final project is one of the academic requirements for a medical student of Brawijaya University, Malang to qualify as the Medical Degree holder.

Here, I would like to express my highest appreciation to:

1. Dr.dr. Karyono Mintaroem, SpPA.as The Dean of Medical Faculty Brawijaya University, Malang for his support for the use of facilities in Medical Faculty, Brawijaya University.
2. Ibu Husnul Khotimah, S.si, MKes, as the first examiner of this final project for her inputs in completing this final project
3. dr Ashwin Djoko Baskoro, MS Sp.Park, as the first supervisor for his help, guidance and kindness until I can finished this final project successfully. Doctor, you are a great teacher and I had learned so many things from you. Thank you so much.
4. dr Drh Sri Muwarni, MS, as the second supervisor, for all the new knowledge especially from the statistical analysis part. Thank you for the time, support and encouragement,doctor.
5. My parents, Mohamad A.Majid and Kamaliyah Samuri, and not forgetting the rest of my family members. Your prayers are the most precious support that I would ever have.
6. To all ParasitologyTeaching Staff and Parasitology Laboratory Staff for their help and kindness.
7. My dearest friends especially my seniors (`Athirah Az-Zahra Abu Bakar and Abdul Hadi Che Zainol Ariff) for their contributions when time were not at my side. May we

become great doctors in the future and will be able to serve people in seeking His blessings. I would like to convey my gratitude to my lab partner, Zuhratun Nazihah Khairul Kamal, for her morale support during the ups and downs. Thank you, pal! Not to forget, my beloved classmates, thanks a million.

8. To my best friends in other part of Indonesia and Malaysia, who always give me support and motivate me so I would not fall down, we are always best friends even we are separated by the distance.
9. To all of the people who were so helpful in making this final project as a success.

I hope that this final project will be beneficial to the community in order to develop a better life. I would like to apologize if there is any unlisted name in this acknowledgement. This final project is not faultless yet might contain weaknesses. Any critics and suggestions are welcomed as this will improve this final project. Thank you.

Malang, December 2011

Author

ABBREVIATION

DDT = Dichlorodiphenyltrichloroethane

H, h = Hour

H_0 = Hypothesis null

Kg = Kilogram

KT = Knockdown Time

KT50 = Knockdown Time of half (50%) of total population

KT100 = Knockdown Time of all (100%) of total population

WHO = World Health Organization

MD = *Musca domestica*

Min = Minute

R = Correlation coefficient

R^2 = Coefficient determination

ABSTRACT

Wahida, Zulia Era . 2011 . **The Knockdown Effect Of *Chrysanthemum Morifolium Ramat* Flower Ethanolic Extract *Musca domestica*.** Final project, Faculty of Medicine, BrawijayaUniversity.Supervisors: (1) dr Ashwin Djoko Baskoro MS, Sp. Park, (2) Dr Drh Sri Murwani, MP.

Musca domestica is a vector of many diseases. To control it, natural insecticide which could kill these flies in high number, rapidly and safe towards human and other living species, was developed. These criteria were fulfilled by the *Chrysanthemum morifolium ramat* flower extract. The objective of this true-experimental post test control group design experiment is to know on which concentration of this *chrysanthemum sp.* extract has the effect of knocking down the *Musca domestica*. Forty *Musca domestica* were used and divided into four groups and was sprayed with 1% acetone (negative control) and *Chrysanthemum sp.* extract (50%, 60%,70%), and was repeated for five times. The spraying method was used as small molecule of droplets produced will be easily absorbed into the *Musca domestica* respiration system. The number of *Musca domestica* knockdown was observed in 30^{th} minute, 1^{st} , 2^{nd} , 3^{rd} , 4^{th}, 5^{th}, 6^{th}, 20^{th}, 21^{st} , 22^{nd} , 23^{th} and 24^{th} hour. The data collected was then analysed using Two Way Anova test where significant p value ($p=0.00$; $p<0.05$) was recorded for both variables. Next, the analysis was continued with the Post Hoc Tukey test and ended with the Correlation and Regression test. Significant values were recorded for all three tests. In this study, the knockdown number of *Musca domestica* was increase with the increase of extract concentration used and time of observation thus proving that *Chrysanthemum morifolium ramat* extract has the effect of knocking the *Musca domestica* down with the lowest concentration of 50%.

Keyword: *Musca domestica,* Natural insecticide, *Chrysanthemum morifolium ramat* flower , Knockdown time.

ABSTRAK

Wahida, Zulia Era, 2011, **Efek Knockdown Ekstrak Ethanol Bunga *Krisan Morifolium Ramat* Terhadap *Musca domestica,*** Tugas Akhir, Fakultas Kedokteran, Universitas Brawijaya. Pembimbing: (1) dr Ashwin Djoko Baskoro MS, Sp. Park, (2) Dr Drh Sri Murwani, MP.

Musca domestica merupakan vektor kepada berbagai penyakit. Untuk mengkontrolnya, insektisida alami yang dapat membunuh lalat dengan cepat dan banyak dan aman untuk manusia, telah dicipta. Hal-hal ini telah dipenuhi oleh krisan. Tujuan dari penelitian *"true-experimental post test control group"* ini adalah untuk melihat efek knockdown ekstrak K*risan morifolium ramat* terhadap lalat *Musca domestica.* Sebanyak empat puluh ekor lalat *Musca domestica* digunakan dan ini dibagi menjadi empat kelompok yang kemudian disemprot dengan aceton 1% (control negatif) dan ekstrak krisan (50%,60%,70%) dan diulang 5 kali. Metode semprot digunakan di dalam penelitian ini karena molekul-molekul *droplet* yang kecil dibentuk mudah diabsorbsi oleh sistem pernafasan lalat. Jumlah *knockdown Musca domestica* diobservasi dan dicatat pada menit ke-30, jam 1,2,3,4,5,6,20,21,22,23,24. Data diolah menerusi analisa *Two Way Anova.* Hasil dari analisa ini menunjukkan nilai p yang signifikan ($p=0.00$, $p<0.05$). Analisa diteruskan dengan uji *Post Hoc Tukey* dan diakhiri dengan uji korelasi dan regresi . Nilai p yang signifikan didapatkan bagi ketiga uji analisa ini. Didapatkan jumlah *knockdown Musca domestica* meningkat dengan meningkatnya konsentrasi dari ekstrak yang digunakan dan masa observasi penelitian. Dengan ini, telah terbukti bahwa *Krisan morifolium ramat* mempunyai efek *knockdown* terhadap lalat *Musca domestica* dengan konsentrasi terendah sebanyak 50%.

Kata kunci: *Musca domestica,* Insektisida alami, Bunga *Chrysanthemum morifolium ramat,* Masa *Knockdown.*

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CHAPTER 1

INTRODUCTION

1.1 Background

Flies can be found in almost all parts of this world. Because it is widely spread, flies-borne diseases have become one of the major health problems worldwide. *Musca domestica* , a cyclorrhapha suborder is a subspecies of flies that become a vector to many serious diseases. *Musca domestica,* also known as the housefly, is found to be one of the most distributed insects which accounts approximately 90% in human habitation (MacKean, 2004).

The diseases that are spread by the housefly include bacterial, viruses and parasitic diseases. For bacterial diseases, *Musca domestica* plays an important role in transmitting typhoid, cholera and pyogenic cocci while for viruses, the most common disease which are being transmitted are enteroviruses, poliomyelitis and viral hepatitis (hepatitis A and E). The cyst of protozoa (for example entamoeba histolytica and giardia lamblia) and the eggs of helminths (for example Ascaris lumbricoides and trichuris trichuria) are also being mechanically transmitted by the housefly which will significantly results in parasitic diseases (Campbell *et al*,2005).

Based on these problems brought by *Musca domestica*, many insecticides are developed to help human killing housefly. The most widely used are chemical insecticides. Even though the insecticides are useful, they bring negative effects to human. It is well known that the use of persistent organochlorines like DDT and of the acute toxic organo-phosphorous compounds have had hazardous effects on environment and human beings (Bloomquist, 2004). Based on these issues, natural plant-based insecticides are developed. More than 2,000 plant species have been known to have chemical factors and metabolites of value in pest control programs and among these plants, products of some 344 species have been reported to have a variety of activities against flies (Chansang *et al*, 2005). Pyrethrin (from

pyrethrum), nicotine, sabadilla, rotenone and neem are the examples of natural active compounds found in plants that are proven to be effective as the natural insecticides. Related to this issue, daisy or *Chrysanthemum morifolium ramat* contains pyrethrin. *Chrysanthemum morifolium ramat* has been used both as home-flower decorator due to its esthetic value and also as natural insecticides in many cultures for centuries. It is a perennial plant which is believed to come from the central of Crotia but today, it is cultivated all over the world (Chansang *et al*, 2005).

Research on pyrethrin from the *chrysanthemum morifolium ramat* has been proven to be effective in the control of insects such as bed-bugs by stimulating movement and thus resulting in better contact with residual insecticides (Davidson, 2000). Apart from that, pyrethrin spray has also been proven to have immediate effect in killing cockroaches via the same mechanism as in killing bed-bugs (McGovran & Piquett, 2001).In addition to that, pyrethrin is also effective in controlling mosquito-borne diseases. In the past, the usage of spray with pyrethrum extracts containing pyrethrins has achieved very satisfactory control of malaria in certain areas where the vector species was predominantly endophilic (Pal, 2003). Despite the effectiveness of pyrethrum extracts (pyrethrin) as insecticides to those listed species of insects, few questions are still remained. One of the questions is, whether *chrysanthemum morifolium ramat* extract has the same killing effect towards *Musca domestica* fly within a specific time.

1.2 Research problem

Based on the information above, the problem derived from this research is:

Does the *Chrysanthemum morifolium ramat* extract has the effect on knocking-down the *Musca domestica* fly?

1.3 Research objectives

1.3.1 General objective

To know what is the concentration of *Chrysanthemum morifolium ramat* extract that has an effect on knocking down of *Musca domestica*.

1.3.2 Specific objective

i. To determine the concentration that causes the KT50 of *Chrysanthemum morifolium ramat* extract towards *Musca domestica.*

ii. To determine the concentration that causes the KT100 of *Chrysanthemum morifolium ramat* extract towards *Musca domestica.*

iii. To determine whether *Chrysanthemum morifolium ramat* has all the conditions needed as an effective insecticide towards *Musca domestica* fly.

1.4 Significances of the research

i. To improve the knowledge of knockdown time in medical entomology field

ii. To improve the understanding about knock down effect of *Chrysanthemum morifolium ramat* as natural insecticides towards *Musca domestica.*

iii. To give more information of the usage of *Chrysanthemum morifolium ramat* as affordable, effective and safe natural insecticides of *Musca Domestica* among the public.

CHAPTER 2
REVIEW OF RELATED LITERATURE

2.1 Fly

Fly is a dipterous or two-winged insect that is often the vector of organisms causing disease (Dorland, 2009). It is one of the major insect orders both in terms of ecological and human (medical and economic) importance. Most toxonomic keys to identify flies are based on morphological characters (Reuda, 2007).

2.2 *Musca domestica*

2.2.1 Taxonomy

Kingdom : Animalia
Phylum : Arthropoda
Class : Insecta
Order : Diptera
Suborder : Cyclorrhapha
Family : Muscidae
Genus : Musca
Species : *Musca domestica* (Reuda, 2007)

2.2.2 Life Cycle and Morphology

The house fly has a complete metamorphosis with distinct egg, larva or maggot, pupal and adult stages. The house fly overwinters in either the larval or pupal stage under manure piles or in other protected locations. Warm summer conditions are generally optimum for the development of the house fly, and it can complete its life cycle in as little as seven to ten days. However, under suboptimal conditions the life cycle may require up to two months. As many as 10 to 12 generations may occur annually in temperate regions, while more than 20 generations may occur in subtropical and tropical regions (MacKean, 2004).

2.2.2.1 Adult

Musca domestica is 6 to 7 mm long, with the female usually larger than the male. The female and can be distinguished from the male by the relatively wide space between the eyes (in males, the eyes almost touch). The head of the adult fly has reddish-eyes and sponging mouthparts. The thorax bears four narrow black stripes and there is a sharp upward bend in the fourth longitudinal wing vein. The abdomen is gray or yellowish with dark midline and irregular dark markings on the sides. The underside of the male is yellowish. The house fly is often confused with the stable fly, *Stomoxys calcitrans* (Linnaeus), and the false stable fly, *Muscina stabulans* (Germar). All three are in the same family. Adults usually live 15 to 25 days, but may live up to two months. Without food, they survive only about two to three days. Longevity is enhanced by availability of suitable food, especially sugar. Access to animal manure does not lengthen adult life and they live longer at cooler temperatures. They require food before they will copulate, and copulation is completed in as few as two minutes or as long as 15 minutes. Oviposition commences four to 20 days after copulation. Female flies need access to suitable food (protein) to allow them to produce eggs, and manure alone is not adequate. The potential reproductive capacity of flies is tremendous, but fortunately can never be realized. The flies are inactive at night, with ceilings, beams and overhead wires within buildings, trees, and shrubs, various kinds of outdoor wires, and grasses reported as overnight resting sites. In poultry ranches, the nighttime, outdoor aggregations of flies are found mainly in the branches, and shrubs, whereas almost all of the indoor populations generally aggregated in the ceiling area of poultry houses (MacKean, 2004) (Figure 2.1)

Figure 2.1: Adult *Musca domestica sp.* (MacKean, 2004)

2.2.2.2 Egg

The white egg, about 1.2 mm in length, is laid singly but eggs are piled in small groups. Each female fly can lay up to 500 eggs in several batches of 75 to 150 eggs over a three to four day period. The number of eggs produced is a function of female size which, itself, is principally a result of larval nutrition. Maximum egg production occurs at intermediate temperatures, 25 to 30°C (MacKean, 2004). Often, several flies will deposit their eggs in close proximity, leading to large masses of larvae and pupae. Eggs must remain moist or they will not hatch (MacKean, 2004) (Figure 2.2).

2.2.2.3 Larva

Early instar larvae are 3 to 9 mm long, typical creamy whitish in color, cylindrical but tapering toward the head. The head contains one pair of dark hooks. The posterior spiracles are slightly raised and the spiracular openings are sinuous slits which are completely surrounded by an oval black border. The legless maggot emerges from the egg in warm weather within eight to 20 hours, and immediately feeds on and develop in the material in which the egg was laid (WHO,2006).

The larva goes through three instars and a full-grown maggot, 7 to 12 mm long, has a greasy, cream-colored appearance. High-moisture manure favors the survival of the house fly larva. The optimal temperature for larval development is 35 to 38°C, though larval survival is greatest at 17 to 32°C. Larvae complete their development in four to 13 days at optimal temperatures, but require 14 to 30 days at temperatures of 12 to 17°C (WHO, 2006).

Nutrient-rich substrates such as animal manure provide an excellent developmental substrate. Very little manure is needed for larval development, and sand or soil containing small amounts of degraded manure allows for successful belowground development. When the maggot is full-grown, it can crawl up to 50 feet to a dried, cool place near breeding material and transform to the pupal stage (MacKean, 2004) (Figure 2.2).

2.2.2.4 Pupa

The pupal stage, about 8 mm long, is passed in a pupal case formed from the last larval skin which varies in color from yellow, red, brown, to black as the pupa ages. The shape of the pupa is quite different from the larva, being bluntly rounded at both ends. Pupae complete their development in two to six days at 32 to 37°C, but require 17 to 27

days at about 14°C). The emerging fly escapes from the pupal case through the use of an alternately swelling and shrinking sac, called the ptilinum, on the front of its head which it uses like a pneumatic hammer to break throug the case (MacKean, 2004) (Figure 2.2).

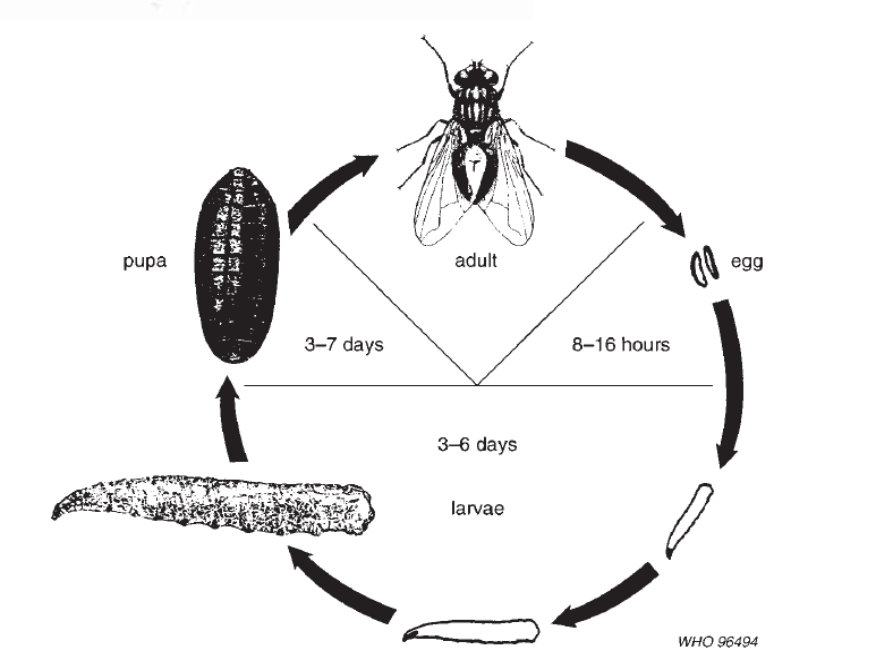


Figure 2.2 : The staging of *Musca domestica* fly (WHO, 2006)

2.2.3 Breeding Place

Musca domestica is world-wide in distribution and lives in close association with human dwellings. 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. According to a study conducted in Texas, USA, breeding site suitability (in descending order), was horse manure, human excrement, cow manure, fermenting vegetable, and kitchen waste. However, another study found that structures containing swine, horse, sheep, cattle, and poultry varied in fly abundance, with swine facilities containing the most and poultry the least. Fruit and vegetable cull piles, partially incinerated garbage, and incompletely composted manure also are highly favored sites for breeding (Keiding J, 2001).

2.2.4 Habits

Musca domestica feeds on feces, open sores, sputum, and moist decaying organic matter such as spoiled food, eggs and flesh (MacKean,2004). *Musca domestica* can take in

only liquid foods. They spit 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 (MacKean, 2004).

2.2.5 Medical Importance

Although this fly species does not bite, the control of *Musca domestica* is vital to human health and comfort in many areas of the world. 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, protozoa, and nematodes) associated with this fly. Pathogenic organisms are picked up by flies from garbage, sewage and other sources of filth, and then transferred on their mouthparts, through their vomitus, feces and contaminated external body parts to human and animal food. Of particular concern is the movement of flies from animal or human feces to food that will be eaten uncooked by humans. Also, when consumed by flies, some pathogens can be harbored in the mouthparts or alimentary canal for several days, and then be transmitted when flies defecate or regurgitate. In situations where plumbing is lacking, such as open latrines, serious health problems can develop, especially if there are outdoor food markets, hospitals, or slaughter houses nearby. Among the pathogens commonly transmitted by house flies are *Salmonella, Shigella, Campylobacter, Escherichia, Enterococcus, Chlamydia*, and many other species that cause illness. These flies are most commonly linked to outbreaks of diarrhea and shigellosis, but also are implicated in transmission of food poisoning and diarrhea, typhoid fever, tuberculosis and anthrax (Campbell *et al,* 2005).

2.2.5.1 Diarrhea

Diarrhea is the condition of having three or more loose or liquid bowel movements per day. It is a common cause of death in developing countries and the second most common cause of infant deaths worldwide. The loss of fluids through diarrhea can cause dehydration and electrolyte imbalances. The disease is spread throughout mostly rural areas of Asia by transmitting bacteria from the decayed materials which is adhered to its hairs on legs and body, to the foods. Apart from that, the bacteria may remain in the pseudotracheae or oesophagus and readily to be flushed out on to food with the next salivary flow (MacKean, 2004).

2.2.5.2 Typhoid Fever

Typhoid fever is a common worldwide illness, transmitted by the ingestion of food or water contaminated with the feces of an infected person, which contain the bacterium *Salmonella enterica enterica*, serovar Typhi (Keiding J, 2001). This disease is spread through the transmission of the bacterial-causing—typhoid fever of an infected person to the human's foods and drinks by the housefly. The bacteria then perforate through the intestinal wall and are phagocytosed by macrophages (MacKean, 2004). With this, symptoms such as slowly progressive high fever, profuse sweating, gastroentritis and bloody dysentry can occur.

2.2.5.3 Tuberculosis

Tuberculosis is a common and often deadly infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis* in humans. Tuberculosis usually attacks the lungs but can also affect other parts of the body. It is spread through the air when people who have the disease cough, sneeze, or spit. As in typhoid fever, this bacili bacteria may be transmitted from an infected person's fluids such as sputum and also sneeze which is carried on the hairs of *Musca domestica*. Systemic symptoms include fever, chills, night sweats, appetite loss, weight loss, pallor, and often a tendency to fatigue very easily, can occur due to the outbreak of this disease (WHO, 2006). These symptoms include chest pain, coughing up blood, and a productive, prolonged cough for more than three weeks.

2.2.5.4 Anthrax

Anthrax is an acute disease caused by the bacteria *Bacillus anthracis*. Most forms of the disease are lethal, and it affects both humans and other animals. Experiments in which flies have been allowed to walk over culture media in sterile dishes have resulted in the growth of over 100 bacterial and fungal colonies from bacteria and fungal spores which the fly deposited (MacKean, 2004). Many of these bacteria are harmless to humans but others may cause serious disease. In this context, the infection of herbivores (and occasionally humans) via the inhalational route normally proceeds as follows: once the spores are inhaled, they are transported through the air passages into the tiny air particles sacs (alveoli) in the lungs. The spores are then picked up by scavenger cells (macrophages) in the lungs and are transported through small vessels (lymphatics) to the lymph nodes in

the central chest cavity (mediastinum). Damage caused by the anthrax spores and bacilli to the central chest cavity can cause chest pain and difficulty breathing. Once in the lymph nodes, the spores germinate into active bacilli which multiply and eventually burst the macrophages, releasing many more bacilli into the bloodstream to be transferred to the entire body. Once in the blood stream these bacilli release three proteins named lethal factor, edema factor and protective antigen. All three are non-toxic by themselves, but the combination is incredibly lethal to humans (Guillemin, 2001).

2.2.6 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.

2.2.6.1 Natural Control

Natural control of flies usually occurs as the result of environmental factor, such as weather, topography, existence of predator or diseases that attack the flies. Weather influence toward fliesis more prominent during rainy season compared to dry season. For the high altitude area, the flies population are lesser compared to low altitude area. Vector, parasite, fungi, bacteria, virus and other factors also can influence the flies population (MacKean, 2004)

2.2.6.1.1 Reduction Or Elimination Of Fly Breeding Sites

2.2.6.1.1.1 Animal sheds, stables, pens and feed lots

Solid concrete floors with drains should be constructed; dung should be cleaned out and floors should be flushed daily (WHO, 2006)

2.2.6.1.1.2 Poultry Houses

Where birds are kept in cages and dung accumulates below them, fans should be used to dry it; leaking water pipes should be repaired, dung should be removed and the floors should be flushed at frequent intervals (WHO, 2006).

2.2.6.1.1.3 Garbage And Other Organic Refuse

This breeding medium can be eliminated by proper collection, storage, transportation and disposal. In the absence of a system for collection and transportation, garbage can be burnt or disposed of in a specially dug pit. At least once a week the garbage in the pit has to be

covered with a fresh layer of soil to stop breeding by flies. Flies are likely to breed in garbage containers even if they are tightly closed. In warm climates the larvae may leave the containers for pupation after only 3–4 days. In such places, garbage has to be collected at least twice a week. In temperate climates once a week is sufficient. When emptying a container it is important to remove any residue left in the bottom (WHO, 2006).

2.2.6.1.2 Reduction Of Sources That Attract Flies From Other Areas

2.2.6.1.2.1 Reduction of sources that attract flies from other areas

Flies are attracted by the odor emanating from breeding sites. In addition they are attracted by products such as fish-meal and bone-meal, molasses and malt from breweries, milk, and sweet-smelling fruit, especially mangoes. Attraction to waste can be prevented by cleanliness, the removal of waste, and its storage under cover. Industries using attractive products can install special exhausts for odors. One of the methods is by using the sanitary landfill (WHO, 2006) (Figure 2.3)

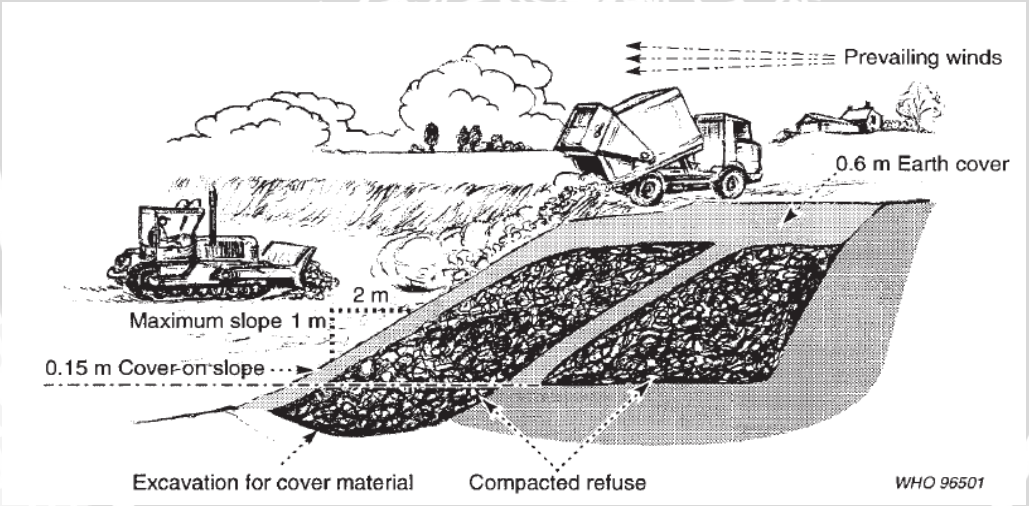


Figure 2.3 Sanitary landfill (WHO,2006)

2.2.6.1.2.2 Human Excreta

Breeding in open pit latrines can be prevented by the installation of slabs with a water seal and a fly screen over the vent pipe. If a water seal is not feasible, a tightly fitting lid may be placed over the drop hole. Installing a ventilated pit latrine can also reduce fly breeding. Defecation in the field, other than in latrines and toilets, may provide breeding places for filth flies (*Musca sorbens*). This is a common problem where large groups of people, e.g.

refugees, stay together in temporary camps. Installation of proper latrines should be given priority. In the absence of proper facilities, people could be asked to defecate in a special field at least 500m downwind of the nearest habitation or food store and at least 30 m from a water supply. This reduces the numbers of flies in the camp and makes it easier to remove exposed faeces. Covering the faeces with a thin layer of soil may increase breeding since the faeces are then likely to dry out more slowly (WHO, 2006).

2.2.6.1.2.3 Dung Heaps

Dung should be stacked to reduce the surface area and the zone in which the temperature is suitable for fly breeding. It should be covered with plastic sheets or other fly-proof material. This prevents egg-laying and kills larvae and pupae as the heat produced in the composting process can no longer escape. It is preferable to stack the dung on a concrete base, surrounded by gutters to prevent the migration of larvae to pupate in soil around the heap. In hot climates, dung may be spread on the ground and dried before the flies have time to develop (WHO,2006) (Figure 2.4)

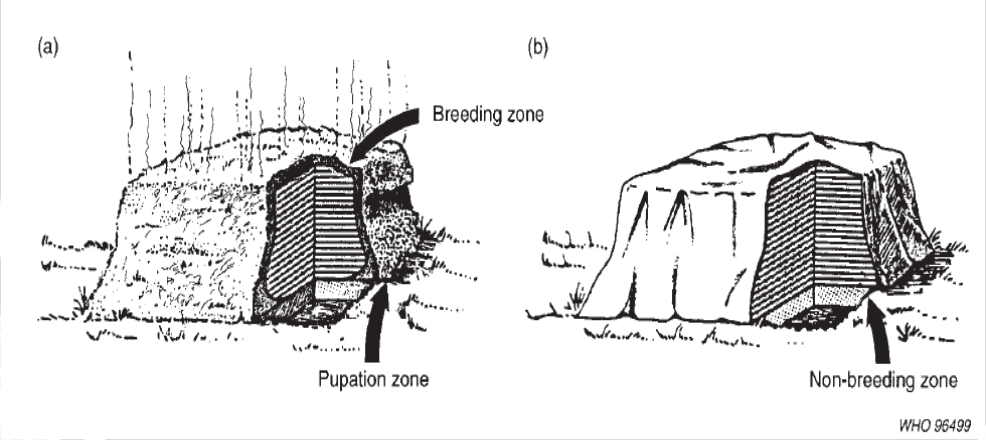


Figure 2.4: Fly breeding in dung heaps can be prevented by placing a light cover, e.g. a plastic sheet, over the dung; the sheet reduces heat loss and the surface layers become too hot for breeding (WHO, 2006).

2.2.6.1.3 Prevention Of Contact Between Flies And Disease-Causing Germs

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 cannot make contact with faeces;
— the prevention of contact between flies and sick people, their excreta, soiled baby nappies, open sores, and infected eyes;
— the prevention of access of flies to slaughter offal and dead animals (WHO, 2006)

2.2.6.2 Artificial Control

Flies can be killed directly by insecticides or physical means such as traps, sticky tapes, fly swats and electrocuting grids (WHO,2006)

2.2.6.2.1 Protection of Food, Eating Utensils And People From Contact With Flies.

2.2.6.2.1.1 Traps, sticky tapes and fly-proof container.

Food and utensils can be placed in fly-proof containers, cupboards, wrapping materials, etc. Nets and screens can be used on windows and other openings. Doors can be made self-closing. Doorways can be provided with anti-fly curtains, consisting of strings of beads or plastic strips which touch each other and prevent flies from passing through. Nets can be placed over babies to protect them from flies, mosquitoes and other insects, and can also be used to cover food or utensils. Electric fans create an air barrier across entrances or corridors that have to be kept open. The screening of buildings is the most important method but it may cause inconvenience because of reduced ventilation and light. Mesh with openings of 2–3mm is sufficient unless it is desired to exclude mosquitoes also, in which case the openings should be 1.5 mm or less. Plastic-coated material is preferable to metal because the latter may corrode. Flies that enter screened rooms can be killed with traps and also sticky tapes (WHO,2006) (Figure 2.5)

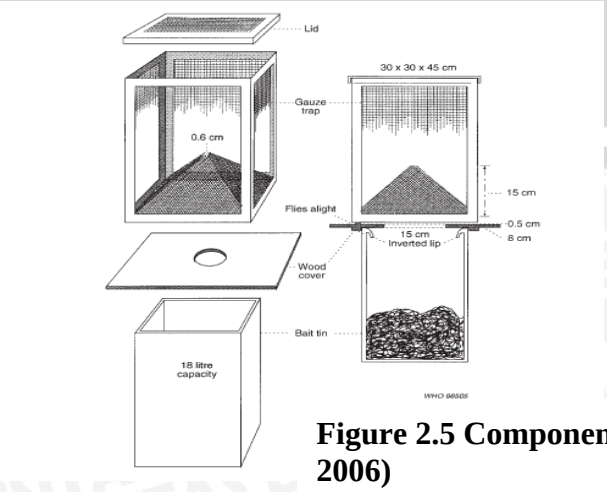


Figure 2.5 Components of fly trap (WHO, 2006)

2.2.6.2.1.2 Biochemical control by using chemical aerosol spray.

Nowadays, the usage of biochemical insecticides as one of the method in controlling flies have been widely incorporated in our society. The list of the biochemical insecticides is as stated in Table 2.6.

Table 2.6 Insecticides used in toxic baits for fly control (WHO, 2006)

Insecticide	Dry scatter	Liquid sprinkle	Liquid dispenser	Viscous paint-on
Organophosphorus compounds				
dichlorvos[a]	+[b]	+ +[b]	+ +	
dimethoate[a]		+	+ +	
trichlorfon[a]	+ +	+ +	+ +	+ +
azamethiphos	+			+ +
diazinon	+ +	+		+
fenchlorvos	+	+		+
malathion	+	+		+
naled	+	+		+
propetamphos				+ +
Carbamates				
bendiocarb	+ +	+		
dimetilan[a]		+	+ +	+
methomyl[c]				+ +
propoxur	+ +	+		
formaldehyde[a]			+	

[a] Aqueous suspension.
[b] + or + + indicates insecticides that are most suitable or have been most widely used for the particular type of application.
[c] Can also be used in the form of granules stuck on strips or boards.

Control with insecticides should be undertaken only for a short period when absolutely necessary because flies develop resistance very rapidly. The application of effective insecticides can temporarily lead to very quick control, which is essential during outbreaks of cholera, dysentery or trachoma (WHO, 2006).

Despite their many advantages, conventional insecticides are not ideal pest control agents. Indeed, one of their greatest strengths, broad-spectrum activity, is also one of their greatest weaknesses. While it is certainly an advantage to control multiple pest species with a single chemical treatment, the non-specificity of most conventional insecticides poses a serious threat to non-target organisms in the environment. High mortality among natural enemies can have an enduring impact on the ecological balance of any community. In the absence of biocontrol agents, more insecticide applications may be

the only recourse available to stop pest resurgence. Once we step onto this "insecticide treadmill", it can be very difficult to get off (Meyer, 2003).

The non-target effects of insecticides are not limited to natural enemies. Most of the organochlorine compounds (e.g., DDT) have been banned from use in the United States because of their indirect effects on reproduction in birds of prey (e.g., eagles, ospreys, condors, etc.). These environmentally stable compounds are highly soluble in lipids, making it possible for them to accumulate in the body fat of non-target organisms. Predators, particularly those at the top of a food chain, amass pesticide concentrations many times greater than anywhere else in the environment. This process, known as bioaccumulation (or biomagnification), was responsible for high levels of DDT and related compounds in birds of prey during the 1960's and 1970's. These pesticides did not injure the birds themselves, but caused thinning of their egg shells and high rates of breakage during incubation. Most organochlorine insecticides were banned during the 1970's and 1980's and many of the threatened bird species are now recovering from the brink of extinction (Meyer, 2003).

In some cases, sub-lethal concentrations of an insecticide can stimulate rather than suppress the growth of a pest population. This phenomenon, known as hormoligosis, has been observed in a number of pest species, including twospotted spider mites (Tetranychus urticae), western corn rootworms (Diabrotica virgifera), and brown planthoppers (Nilaparvata lugens). Low doses of pesticide seem to improve the nutritional quality of host plants, thereby increasing a pest's reproductive potential or decreasing its time of development (Meyer, 2003).

Based on these disadvantages, natural insecticides are preferable.

2.2.6.2.1.3 Biochemical Control By Using Natural insecticides.

Natural insecticides normally do not affect wildlife and water supplies and although it is good idea to still use precautionary measures while utilizing natural insecticides and to use them carefully in their application, compared to synthetic insecticides natural insecticides in general are milder and tend to oxidize and dissipate faster within the soil and off of the plant. Organic gardeners would never use chemically derived products if the use of insecticides is necessary they would concentrate on friendly insecticides homemade from safer substances that were less toxic to them (Natural insecticides, 2001).

2.2.6.2.1.3.1 Pyrethrum As Natural Insecticide.

Pyrethrum extract is the natural extract from the pyrethrum flowers. The extract contains about 25% of the active pyrethrins that make it effective. Pyrethrum and PBO are added to increase insect movement by stimulating their nervous systems. This brings the insects into contact with the silica dust more readily. Pyrethrum, discovered around 1800 in the Transcaucasian region of Asia, is the ground-up flowers of the daisy. Pyrethrins (most always plural) are the insecticidal components of the flowers (Natural Insecticides, 2001).

Pyrethrin is quickly broken down in the bodies of mammals. It also breaks down when exposed to sunlight or water. Pyrethrin compounds have been used primarily to control human lice, mosquitoes, cockroaches, beetles and flies. Some "pyrethrin dusts," used to control insects in horticultural crops, are only 0.3% to 0.5% pyrethrins, and are used at rates of up to 50 lb/A. Pyrethrins are also rapidly decomposed by mild acids and alkalis. Stored pyrethrin powders lose about 20% of their potency in one year (Natural Insecticides, 2001).

Pyrethrum marc is sold to farmers at about US$5 per 50 kg bag therefore it is quite cost effective. Pyrethrum is a relatively low toxicity natural insecticide and since it breaks down quickly, generally has a low environment impact as well. For these reasons it enjoys a reputation of being "safe" but it is deadly to insects and other "cold-blooded" life. Pyrethrum daisies have been used as an insecticide for over 2000 years. Found as a wild flower in the Balkans and in the mountains of Persia, Persians used the powder to protect stored grain (Natural Insecticides, 2001).

2.3 *Chrysanthemum Morifolium Ramat*

2.3.1 History

Chrysanthemums, often called mums or chrysanths, are of the genus (*Chrysanthemum sp.*) constituting approximately 30 species of perennial flowering plants in the family Asteraceae which is native to Asia and northeastern Europe. The name Chrysanthemum is derived from the Greek, *chrysos* (gold) and *anthos* (flower). Chrysanthemums were first cultivated in China as a flowering herb as far back as the 15th century BC. An ancient Chinese city (Xiaolan Town of Zhongshan City) was named Ju-Xian, meaning "chrysanthemum city". The plant is particularly significant during the Double Ninth Festival. It is believed that the flower may have been brought to Japan in the 8th century

CE, and the Emperor adopted the flower as his official seal. There is a "Festival of Happiness" in Japan that celebrates the flower. The flower was brought to Europe in the 17th century. Linnaeus named it from the Greekword χρυσός *chrysous*, "golden" (the colour of the original flowers), and □νθεμον -*anthemon*, meaning flower (Ombrello, 2001).

2.3.2 Taxonomy

Kingdom	:	Plantae
Division	:	Spermatophyta
Subdivisio n	:	Angiospermae
Class	:	Dicotyledonae
Order	:	Asterales
Family	:	Asteraceae
Genus	:	Chrysanthemum
Species	:	*Chrysanthemum morifolium ramat* (China Daily, 2003)

Figure 2.7 Chrysanthemum sp. flower (China Daily, 2003)

2.3.3 Common names:

Daisy, mums and chrysanths (English), krus anthemon (greek), krisantemum and bunga kekwa (Bahasa Malaysia), bunga krisan (Bahasa Indonesia) and Ju hua (Chinese) (China Daily, 2003).

2.3.4 Morphology of the plant

The morphological structure of the *chrysanthemum morifolium ramat* plant consists of root, stem, flower and leaves. Mum or chrysanthemum plants can grow to be 2-3 feet high, depending on the cultivar and growing conditions. Chrysanthemum is a perennial root-persistent herbage flower. The stalk is erect or semi-trailing with a height of 30 to 150 centimeters (Ombrello, 2001). The stalk is multiramose with fluffs. The leaf is monophyllous and alternate, ovoid or long circular, with indented leaf edge. Varying from different species, the lamina is lobed or partite. Chrysanthemums also come in many flower forms; that is, chrysanthemums are often grouped by the shape and arrangement of their petals. The flowers of a chrysanthemum are typically clustered over the top of the plant where they may be so abundant that they almost obscure the leaves beneath (China daily, 2003). Flowers of different plants may possess a wide range of visual characteristics. The petals may be daisy-like, narrow and lacy as in the spider form, flat and spoon-shaped, feathery, or even quill-shaped. The flower grows at the top of stalk and constitutes a capitulum. Female ligule flowers stand at the periphery of the capitulum, with diverse colors, sizes and shapes of pedals. At the center of the capitulum are bisexual tubulose flowers, glomerate and dish-shaped (Ombrello, 2001). Chrysanthemums come in a wide variety of colors, including white, off-white, yellow, gold, bronze, red, burgundy, pink, lavender and purple.

Figure 2.8: The leaf, flower and stem of chrysanthemum plant, C.Morifolium Ramat (China Daily, 2003)

In this experiment, *Chrysanthemum morifolium ramat is choosen.* This is based on the abundance of this flower species in this province (Malang), itself.

2.3.5 The Usage Of *Chrysanthemum Morifolium Ramat*

Chrysanthemum is widely used for many purposes. Chrysanthemums valued in many parts of the world for its pungent aroma and its esthetical value. Besides that, many investigations of chrysanthemum health benefits have considered its medicinal rather than other purposes. Chrysanthemum flowers known as Ju Hua, have been used in Traditional Chinese Medicine for centuries. They are found in many ancient formulas. But simple chrysanthemum flower tea is also a very common beverage in China, as the Chinese take into account the health benefits of the food and drink they consume. Ju Hua is classified as a cool, acrid herb which is good for relieving heat of the upper body-i.e. head and chest, being especially helpful for red, itchy eyes. It enters the Lung Meridian. It can help in the early stages of feverish type upper body flu. It may also help relieve certain types of headaches, blurred vision and dizziness, but the effect on those symptoms will vary dependent on the underlying cause.
Studies have shown Ju Hua may have a beneficial effect on high blood pressure (Smith, 2008).

2.3.6 Biochemistry

Chrysanthemum (pyrethrums) contains at least 13% of protein, 56% of carbohydrates, 23% of fibre, &% of minerals, 1% of oils and 0.1% of pyrethrins (Thijissen R, 2000). Potentially active chemical constituent of chrysanthemum is pyrethrins. The pyrethrin content in the chrysanthemum is proven to have insecticide effect (Singh and Singh, 2008)

2.3.6.1 Pyrethrin

Pyrethrins are insecticides that are derived from the extract of chrysanthemum flowers (pyrethrum). The plant extract, called pyrethrum contains pyrethrin I and pyrethrin II collectively, called pyrethrins. Pyrethrins are widely used for control of various insect pests (Klassen et al, 2000). Pyrethrin I and pyrethrin II are structurally related esters with a cyclopropane core, (+)-*trans*-chrysanthemic acid in the case of pyrethrin I. They differ by the oxidation state of one carbon. They are viscous liquids that oxidize to become inactivated. They are non-persistent, being biodegradable, and break down on exposure to light or oxygen. The chemical structure of pyrethrins is the basis for a variety of synthetic insecticides called pyrethroids such as bifenthrin, permethrin, and cypermethrin (Table 2.9).

Table 2.9: Relative proportions of six esthers in 50% extract of pyrethrum (*Chrysanthemum sp*) (The Royal Society Of Chemistry, 2001)

cinerin I	3.7%		
jasmolin I	2.0%	pyrethrins I 24.7%	
pyrethrin I	19.0%		
			total pyrethrins 50%
cinerin II	5.8%		
jasmolin II	2.0%	pyrethrins II 25.3%	
pyrethrin II	17.5%		

As pyrethrin is an oil-based substance, it (pyrethrin) can also be dissolved in alcohol-based solvent. In this experiment, ethanol was used. Ethanol as an alcohol-based solvent was preffered due to its high volality property. Volatile solvents have a higher penetration capacity which can be increased with pressure and temperature (Awang et al, 2008). Therefore, after the consideration was taken, ethanol was choosen as the solvent in the extraction process.

Pyrethrins are used in many varieties of insecticide, fogging products and in some pet products and have been used as an insecticide for over 100 years. They affect the flow of sodium out of the nerve cells in insects, resulting in repeated and extended firings of the nerves, causing the insects to die (The Royal Society Of Chemistry, 2001). Piperonyl butoxide, a synergist, is often used in combination with Pyrethrin, making the mixture more effective by not allowing the insect's system to detoxify the Pyrethrin. Although it is used as an insecticide, it also may be used as an insect repellent. Observations in food establishments demonstrate that flies are not immediately killed but are found more often on windowsills or near doorways (Gromley, 2010). This suggests that, due to the low dosage applied, insects are driven to leave the area before dying. Pyrethrin and the synergists are biodegradable and rapidly disintegrate in sunlight and air, thus assuring that there will be no excessive build-up of insecticides dispensed in the area being treated (NPIC, 1998).

Pyrethrins are one of the least poisonous insecticides to mammals. Pyrethrins are low in toxicity to mammals because they are quickly broken down into inactive forms andpass from the body in the urine and feces. Pyrethrum (the plant extract) may be absorbed by the digestive tract and the lungs. However, it is poorly absorbed

through the skin. Based on animal studies, any amount of pyrethrins absorbed by humans would be expected to be rapidly excreted (NPIC, 1998). Therefore, it is unlikely that pyrethrins would accumulate in humans. Pyrethrins are a collective of six molecules components of pyrethrin I, pyrethrin II, jasmolin I, jasmolin II, cinerin I and cinerin II, which chemically structured can be described as in Figure 2.9.

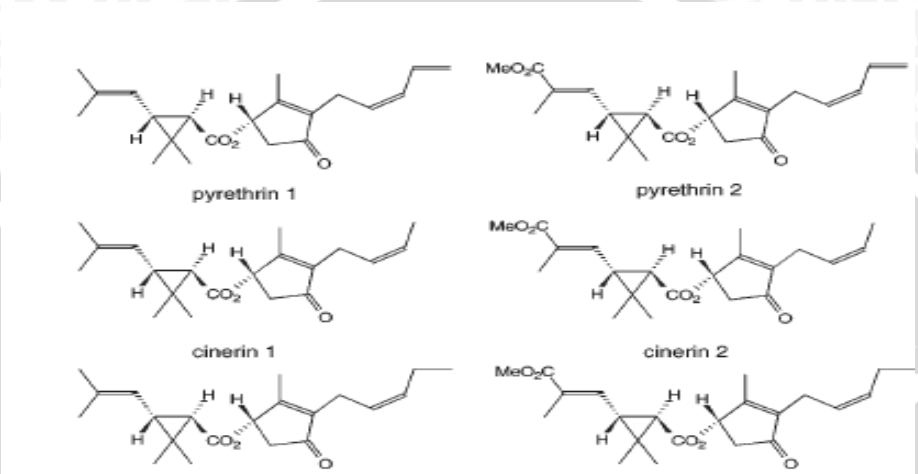


Figure 2.9 Structural formula of pyrethrin (NPIC, 1998)

2.4 Knock Down

2.4.1 Knock down effect

Knockdown effect can be defined as the ability of the insecticide to cause a rapid loss of normal posture and movement. One insecticide is consider as good insecticide if it has quick knock down effect that can effect a large number of insect in a short period (Astari, 2005).

2.4.2 Knock down time

Knockdown time (KT) is the time needed to cause the fall *Musca domestica*. It is measured by counting the number of fallen *Musca domestica* during certain period of time until all the *Musca domestica* die. KT100 is the time is needed to cause fall of all the *Musca domestica,* while KT is the time needed to cause half amount of the *Musca domestica* fly to fall (Astari, 2005).

2.4.3 The criteria for the effective insecticide

The criteria for the effective insecticide include:

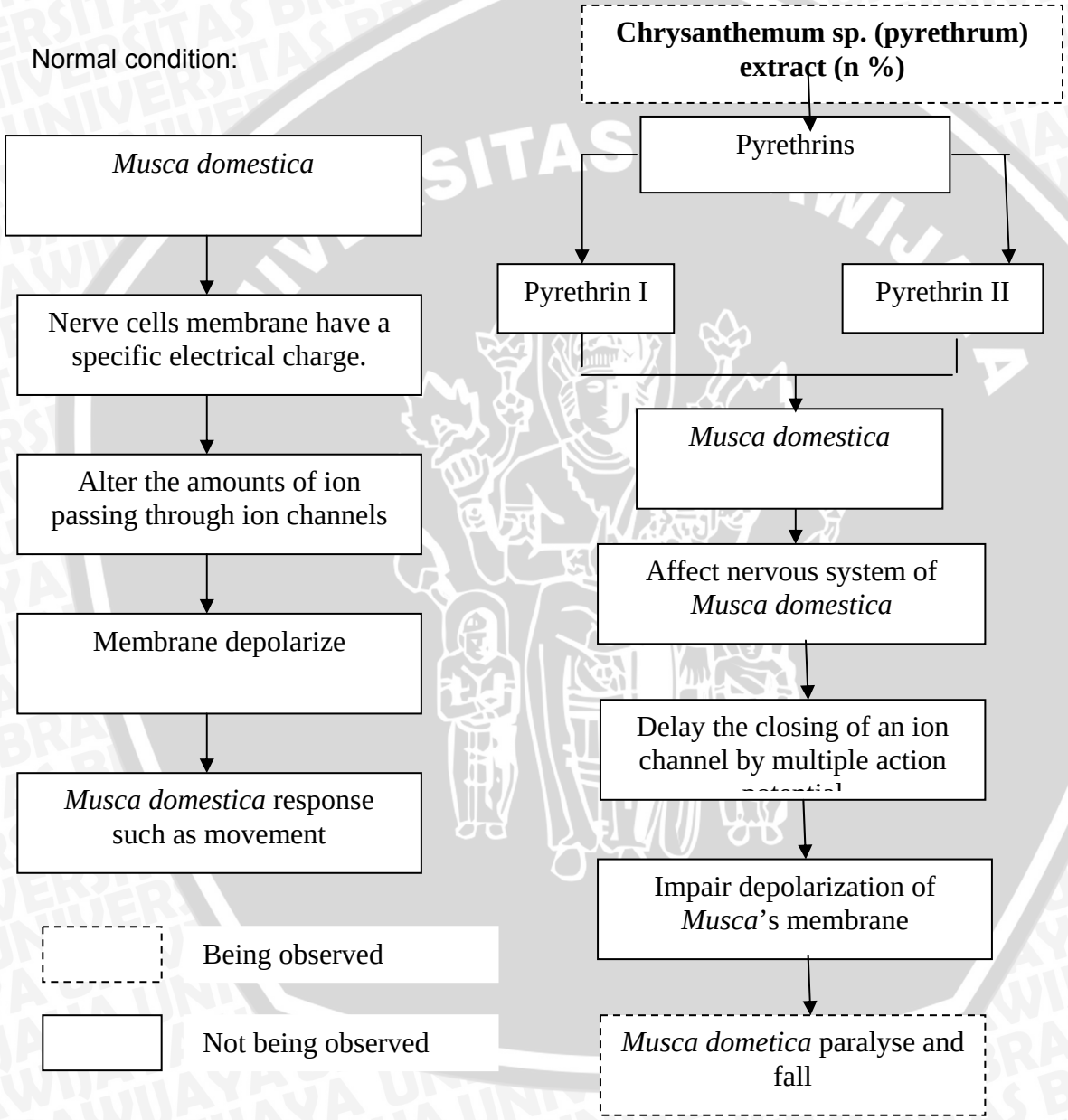
i. Have the ability to kill flies rapidly in high amount, but safe towards human and other animal
ii. Chemically stable and not easily inflammable
iii. Easy to use and being diluted
iv. Cheap and easy to be obtained
v. Colourless and not having a very strong odour

Another criteria that being proposed to an effective insecticide is having the the Quick Knockdown Effect (Astari, 2005).

CHAPTER 3

CONCEPTUAL FRAMEWORK AND HYPOTHESIS

3.1 Conceptual framework



Explaination of conceptual framework:

Generally, in normal condition, *Musca domestica* has a mechanism in order to respond to its surrounding. This mechanism is based on the *Musca domestica* specific electrical charges of its nerve cells membrane. The number of ion passing through the ion channel will be altered thus causing the membrane to depolarized. The depolarization of the membrane will initiate the response of *Musca domestica*.

Chrysanthemum morifolium ramat (pyrethrum) is proven to contain pyrethrin that can be classified into pyrethrin I and pyrethrin II. Both pyrethrins (pyrethrin I and pyrethrin II) work by affecting *Musca domestica* nervous system by delaying the closing of an ion channel (sodium channel) via multiple action potentials. With this, the depolarization of the *Musca domestica* membrane is impaired. The effect of active constituents in chrysanthemum sp. (pyrethrum) will cause the housefly (*Musca domestica*) to paralyzed thus resulting in knock down effect towards *Musca domestica*.

3.2 Research hypothesis

3.2.1 Alternative Hypothesis

3.2.1.1 *Chrysanthemum sp.* extract has an effective knock down effect towards *Musca domestica* fly.

3.2.1.2 The higher the concentration of *Chrysanthemum sp.* extract, the faster the Knockdown Time.

3.2.2 Hypothesis null

There will be no significant relationship between chrysanthemum sp. extract used and Musca domestica knockdown.

CHAPTER 4
METHODOLOGY

4.1. Research Design

The research design that was being used is a true-experimental post test control group design. The purpose is to measure the knockdown time of *chrysanthemum* extract as bioinsecticide towards *Musca domestica.*

4.2. Population And Sample

The research population was the adult flies of *Musca domestica*. The flies must have inclusion and exclusion criteria. The inclusion criteria included:.

1. Adult *Musca domestica* (male and female).
2. Active movement of the *Musca domestica* .

Other than that, the exclusion criteria was adult *Musca domestica* that were died during experimental and did not include in inclusion criteria.

The sample of this research was adult *Musca domestica* that have been selected and fulfilled inclusion criteria. Ten (10) adult *Musca domestica* were used for each experiment (Baskoro et al, 2005).

The experiment sample was divided into four experiments, as stated below:

C(-) : Spraying of acetone solution 1% (Astari, 2005)

E1 : Spraying of *Crysanthemum sp.* 50% extract solution

E2 : Spraying of *Crysanthemum sp.* 60% extract solution

E3 : Spraying of *Crysanthemum sp. 70*% extract solution

The experiment was repeated based on the formula below (Tjokonegoro, 2001):

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

Explanation: P → total experiment

n → total repetition

Calculation of total repetitions:

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

$$4(n-1) \geq 16$$

$$4n-4 \geq 16$$

$$4n \geq 20$$

$$n \geq 5$$

$$n = 5$$

Based on the formula above, the minimal repetition of this experiment is **5 times**.

4.3 Location Of Experiment And Time

The experiment was held in parasitology Laboratory of Brawijaya University in May 2011.

4.4 Identification Of Variable

4.4.1 Independent Variable

The independent variable in this research was the *Chrysanthemum morifolium ramat* extract concentration in percent (%) and time of observation.

4.4.2 Dependent Variable

The dependent variable in this research was the knockdown number of *Musca domestica*.

4.5 Research Material And Equipment

4.5.1 Material For The *Chrysanthemum morifolium ramat* Extract Preparation:

The ingredients needed in preparing the *Chrysanthemum sp.* extract were *Chyrsanthemum sp*. flower, ethanol 96%, aquadest and acetone.

4.5.2 Instrument For The *Chrysanthemum morifolium ramat* Extract Preparation:

The instruments needed in preparing the *Chrysanthemum morifolium ramat* extract were the oven, blender, filter, filter paper, analytical weight apparatus, extraction glass, evaporation vacuum, ethanol container, evaporator container, rotary evaporator, water pump, water bath, plastic pipe and evaporator.

4.5.3 Instrument For The *Crysanthemum morifolium ramat* Test Preparation:

The instruments needed in preparing the *Chrysanthemum sp.* test were the flies container, mug, flies net and acetone.

Operational Definiton

1. *Chrysanthemum morifolium ramat* extract was the evaporation product from the extraction of dried *Chrysanthemum morifolium ramat* using ethanol. This process produced oil which was water insoluble and was assumed to have 100 % concentration. (Astari, 2005)

2. The *chrysanthemum morifolium ramat.* flower used was the pinkish, full bloomed flower of *Chrysanthemum morifolium ramat;*.
3. The *chrysanthemum morifolium ramat* was obtained at Pasar Bunga, Malang.
4. Negative control was defined as a group of control which had no effect on the subject of the experiment (the result will not be affected by this group).
5. Acetone solution 1% was used for negative control.
6. An adult Musca domestica was 8–12 mm long, with gray thorax, four longitudinal dark lines on the back and the whole body was covered with hair-like projections.
7. Adult *Musca domestica* were manually obtained from the house compound.
8. Knockdown Time was a data of *Musca domestica* falling time per-interval time by using Abbot formula. KT50 (Median knockdown) was a time required to paralyzed 50% of *Musca domestica* population at a certain concentration while KT100 was a time required to paralyze all *Musca domestica* used.

9. Flies' container: the container is squared shape with size of 25cm x 25cm x 25cm. Each side of the box was covered by glass and one side of the box was covered by net for spraying process and to prevent the flies escape from the box (Brown, 1994)

10. *Chrysanthemum morifolium ramat* was obtained from Pasar Bunga in Malang.

4.7 Research procedure

4.7.1 *Chrysanthemum morifolium ramat* Extraction

The extraction process of *Chrysanthemum morifolium ramat* was done according to "Technique of Sample Extraction" where ethanol 96% was used as solvent. The process of extraction was as followed:

1. *Chrysanthemum morifolium ramat flower* was dried under the sun for 1 hour and heated in oven at 60-89 degree for 12 hour until it contained ±5% water.
2. The dried *Chrysanthemum morifolium ramat flower* was blended in order to get 300 mg of a very fine size of powdery *Chrysanthemum sp.*
3. The blended *Chrysanthemum morifolium ramat* was soaked with ethanol in a bottle for a week until the active substance in the *Chrysanthemum morifolium ramat* was dissolved in ethanol.
4. The extraction was stopped and put into a bottle.
5. After finishing the extraction process, this active substance was separated from the ethanol.

4.7.2 Evaporation of Chrysanthemum sp. Extract

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 ethanol was transfer 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 pump was connected to electrical source causing aquadest to flow and fills 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 vacuum and water bath were connected to the source of electricity. The temperature of water bath was increased to 70°C (boiling point of ethanol).
7. The process occurred until evaporated solution accumulated at the evaporation separation container for approximately 2-3 hours.
8. After doing step 1, 2, 3, 4, 5, 6 and 7, the process of evaporation was followed by heating in oven at 50°C to 60°C for one to two days.
9. At the end of evaporation process, a very concentrated and aromatic extract of *Chrysanthemum morifolium ramat* was obtained. This extract was used in the experiment.

4.7.3 Acclimization

The acclimization of *Musca domestica* was 3-5 days in laboratorium condition.

4.7.4 Stock Solution Preparation

One percent of acetone is a dose where the 1ml acetone that dissolved in 99ml aquadest (as the solvent). This solution was then used as the solvent of *Chrysanthemum sp.* extract. The stock solution of the *Chrysanthemum sp.* extract was produce to make it easier in processing the solution. (Astari, 2005).

4.7.5 Processing Stock Solution

The stock solution of *Chrysanthemum sp.* extract was diluted with acetone 1% solution until getting the required dosage, by using the dilution formula as below:

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

Explanation:

M1 =Concentration of Stock Solution (10000ppm)

M2 = Concesntration of Required Solution

V1 = Volume of Stock That Will Be Diluted

V2 = Total Volume of Solution

The end volume of the solution needed in each experiment was 3.5ml. To get the acquire concentration, 3.5ml volume stock solution was being added with1% acetone until it reached the required concentration.

4.7.6 Preparation of The Sample

The adult *Musca domestica* used as the sample were being catched by using plastic bottles, in area of Jalan Terusan Cikampek No.16. All this adult *Musca domestica* was directly put into the flies container.

4.7.7 Measurement Time of Insecticide Knockdown Time

1. Trials were done by using 5 glass boxes of 25cm x 25cm x 25cm in size.
2. The solution of *Chrysanthemum sp.* extract with 50%, 60% and 70% will be prepared in the sprayer.
3. The negative control solution (acetone 1%) was prepared (smith 2004).
4. Each solution was filled in sprayer bottle and sprayed to every container until the solutions in each bottle were finished. The specific explanation are as below:
   - container 1 was sprayed by using 3.5ml acetone 1% solution (negative control)
   - container 2 was sprayed using 3.5ml of *Crysanthemum morifolium ramat* extract solution 50%
   - container 3 was sprayed using 3.5ml of *Chrysanthemum morifolium ramat* extract solution 60%
   - container 4 was sprayed using 3.5ml of *Chrysanthemum morifolium ramat* extract solution 70%

5. The number of flies that fall in each experiment was counted after the spraying on 0 minute, 60^{th} minute (1 hour), 120^{th} minute (2 hour), 240^{th} minute (4 hour), 360^{th} minute (6 hour), 480^{th} minute (8 hour), 600^{th} minute (10 hour), 840^{th} minute (14 hour), 960^{th} minute

(16 hour), 1020th minute (18 hour), 1200th minute (20 hour), 1320th minute (22 hour) , and 1440th minute (24 hour).

6. This research were done with 5 times repetition for each experiment

4.7.8 Observation

The observations were done on 0 minute,30th minute, 60th minute (1 hour), 120th minute (2 hour), 240th minute (4 hour), 360th minute (6 hour), 480th minute (8 hour), 600th minute (10 hour), 840th minute (14 hour), 960th minute (16 hour), 1020th minute (18 hour), 1200th minute (20 hour), 1320th minute (22 hour) , and 1440th minute (24 hour). The conditions of each *Musca domestica* group were observed to find the changes in the number of falling *Musca domestica*. The number of falling *Musca domestica* were counted and the data were inserted into the table.

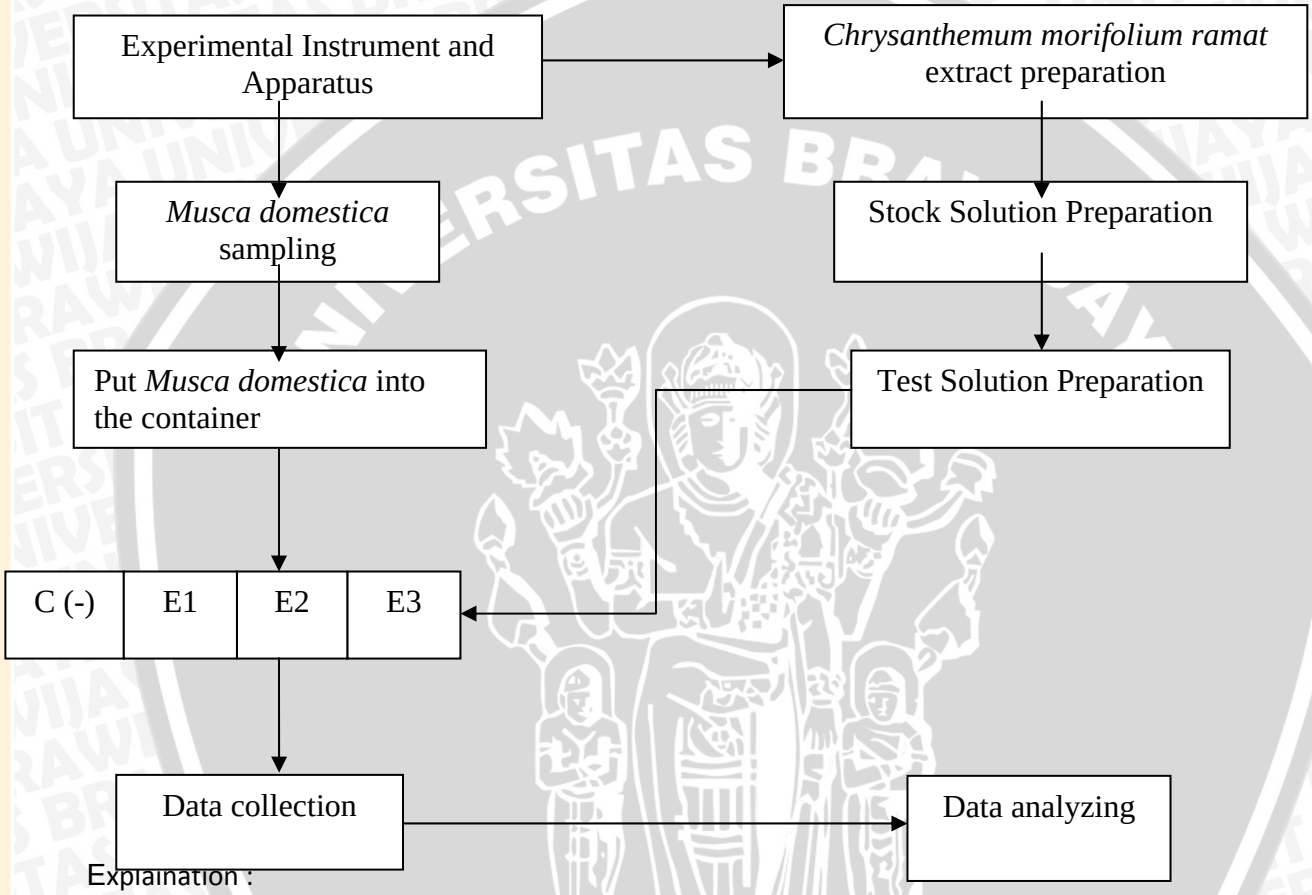
4.8 Data Analysis

To count the time of KT50 and KT100, analysis of linear regression miniTab 13.0 was used. KT50 is the time needed for the paralysis of 50% of *Musca domestica* at a certain dosage while KT100 is the time needed for the paralysis of 100% of *Musca domestica* at a certain dosage.

To evaluate the differences between definite and indefinite data, Two way Anova analysis was used. This was done as this research was using numerical variable which were two group of un-paired variable. The first group which was the concentration of the extract had three numerical variable (50%, 605 and 70%) added with one negative control (acetone,1%). The second group was the interval of subtype time (0-24th hour with 1 hour interval). The Two way Anova statistic test is chosen due to the complexity of the data and to fulfill the standard of the statistic.

The entire above test was proceeded by the Post Hoc Tuckey Test which was done to know the significancy of the data at each experiment, while sub experiment on the other hand, depended on two variables which were the concentration (numeric) and time (interval). The test ended with the correlation and regression test to know much specific relationship of the two variables (time-knockdown of *Musca domestica,* and concentration-knockdown of *Musca domestica).*

Workflow diagram



Explaination :

C(-) : Spraying of1% acetone solution.

E1 : Spraying of 50% *Chrysanthemum morifolium ramat* extract solution

E2 : Spraying of 60% *Chrysanthemum morifolium ramat* extract solution

E3 : Spraying of 70% *Chrysanthemum morifolium ramat* extract solution

CHAPTER 5

RESEARCH RESULT

5.1 Preliminary Study

For the knockdown effect of *Chrysanthemum* sp. towards adult *Musca domestica*, a eliminary study was done to find the optimum dose. For the preliminary study, three concentrations were used, which were 50%, 60% and 70%. The result of preliminary study is shown in Table 5.1 below:

Table 5.1: The result of the exploration. The concentration that was lowered to 50% knocked 50% of *Musca domestica* down .KT50 was obtained.

Minutes	Concentration		
	50%	60%	70%
0	0	0	0
30	3	3	3
1H	3	4	4
2H	3	4	4
3H	3	4	4
4H	4	4	5
5H	4	5	5
6H	5	5	7
20H	6	8	10
21H	7	8	10
22H	7	9	10

23H	8	10	10
24H	9	10	10

5.2 True experiment

Three concentrations were used in this experiment; 50%,60% and 70% together with acetone 1% as the negative control. The experiments were repeated five times which each experiment was being observed for every 30th minutes, 1st hour, 2nd hour, 3rd hour, 4th hour, 5th hour, 6th hour, 20th hour, 21st hour, 22nd hour, 23rd hour and 24th hour. The results of the number of *Musca domestica* knockdown from the first to the fifth repetition can be seen on Appendix 1.

5.3 Knockdown effect of Chrysanthemum sp. Extract towards *Musca domestica* sp Based On The Concentration and Time Interval

Based on Appendix 1, different concentration gives different effect towards the number of *Musca domestica* knockdown in each repetition. No knockdown effect was seen for the repetition using the negative control. The result of the analysis for each concentration is simplified in Table 5.2 in Appendix 1.

Based on the table, no knockdown effect was observed when the *Musca domestica* were sprayed with the negative control, acetone 1%. Starting in the 30th minute, 3 *Musca domestica* were knocked down by using 50% concentration. Meanwhile, by using 70% concentration, all ten *Musca domestica* were knocked down at 23rd hour.

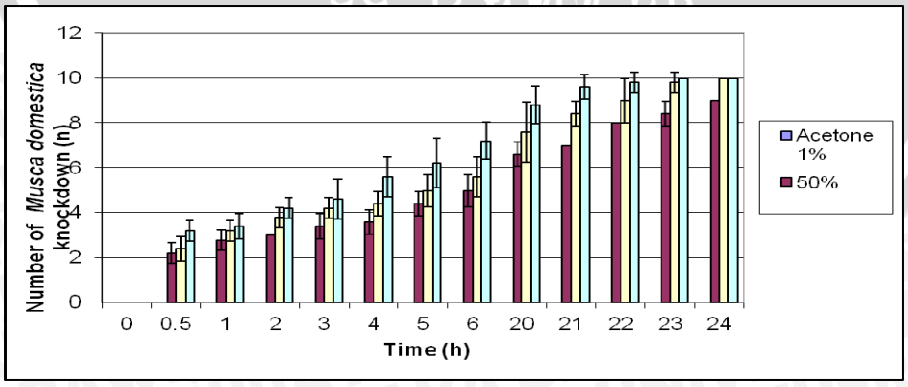


Figure 5.1: Number of Musca domestica knockdown for each concentration during each time interval with the standard deviation graph (the error bars).

The figure above shows the result of the number of *Musca domestica* been knocked down with the progression of time. During 0 minute, no *Musca domestica* was found to be knocked down. Later, as the time passed by, the figure shows significant increase in the number of *Musca domestica* knockdown for all three concentrations. During the 1st to the 6th hour, there is no significant increase of the knockdown number within the three concentrations used. Based on the figure, it is found that KT50 of *Musca domestica* was achieved during the 5th hour in 50% concentration, 4th hour in 60% concentration, and 3rd hour in 70% concentration. However, KT100 of *Musca domestica* is seen to be achieved only in 60% and 70% concentration at 24th and 23rd hour respectively. On the other hand, no *Musca domestica* was knocked down from the beginning to the end of the experiment when 1% acetone was used.

5.4 Data analysis

The result of this research was statistically analyzed using SPSS 16.0 version For Windows. As this study involving two numeric variables (the concentration and time against the number of *Musca domestica* knockdown), the statistical analysis of Two Way Anova was used. The analysis was then proceeded with the Post Hoc Tukey test whereby the differences of these significances were observed. The process continued with the Correlation and Regression test. This was ruled out in term of observing the relationship of these variables in more details.

5.4.1 Data Assumptions Test

Before the anova test can furtherly be conducted, few assumptions need to be postulated and taken into consideration. The postulated assumptions were; (1) The samples of data obtained must be normally distributed, (2) The samples must be independent, (3) the variances of the populations must be equal, and (4) the groups must have the same sample size (James,2011).

To verify that all these assumptions were fulfilled, few initial tests were conducted.

a) Normal distribution of data

Before the data was analysed statistically, the first assumption as postulated above need to be verified. Therefore, for this purpose, the Kolmogrov-Smimov Goodness of Fit Test was used for each variable respectively. A data was said to be normally distributed when; (1) the distribution consists of a bell-shaped density curve, and 2) the density curve is symmetrical, centered about its mean, with its spread determined by its standard deviation (Mackowiak,1992).

Based on the result as stated in Appendix 3, it was found that the variable used; the concentrations used against the number of *Musca domestica* knockdown, showed a significant result with the value of significancy (p) is 1.546 (p>0.05). As the p value is >0.05,the H_0 was accepted. This can be concluded that the data which was obtained had a normal distribution. In coherent with that, the first assumption of pre-anova testing fulfilled. The test was next proceeded with the analysis of variance test, the Two Way Anova test.

b) Homogenous test

To know whether there was any heterogeneity of the data subset, the Levene test homogeneity of variances was ruled out. The significant value,p which is 0.064 (p>0.05), thus this can be concluded that variance of the data obtained was homogenous. With this, the third and fourth assumptions were successfully verified. The next procedure of analysis by using the Two Way Anova test can be conducted.

5.4.2 Data analysis with Two Way Anova test

In this study, two independent groups of numeric variables were used. Along with this, there were two factors that needed to be distinguished from the number of *Musca domestica* knockdown. The first factor was the variety of extract concentrations; 50%, 60% and 70% concentration, that will be tested in the Parasitology laboratory. This was followed by the time of observation as the second factor. The experiment was observed from the 30^{th} minute to the last 24^{th} hour of the experiment, with an hour of interval time.

The process was proceeded with the data collection and analysis by using the statistical analysis of Two Way Anova test. This method of analysis was used in order for ones to know if there was any variant of influence towards the number of *Musca domestica* knockdown when different extract concentrations were used.

The table below shows the result of the anova analysis.

Tests of Between-Subjects Effects

Dependent Variable: Potential

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	7454.615[a]	16	465.913	237.639	.000
TIME	1212.354	12	101.029	51.530	.000
CONCENTR	1613.765	3	537.922	274.367	.000
Error	478.385	244	1.961		
Total	7933.000	260			

a. R Squared = .940 (Adjusted R Squared = .936)

Table 5.2 : The result of Two Way Anova

Based on the analysis in Table 5.2 above, the significancy of time which was 0.00 ($p<0.05$, H_0 was rejected) shown that there was a significant difference in the length of time used in observation.

On the other hand, for the second factor observed in this study which was the concentrations used, the value of significancy of this factor which was recorded as 0.00 ($p<0.05$, H_0 was rejected), shown that there was a significant difference in the various concentrations of the extract used.

As both factors (the time of observation and the concentrations used) showed the same significant value of 0.00, it can be postulated that there was a significant knockdown potential by the chrysanthemum extract with the consideration of the observation time. Hence, it can be concluded that the difference between the two variables were significant. As the result of previous test showed a great significancy, the process was continued with the Post Hoc Tukey test.

5.5 Data analysis using Post Hoc Tukey Test

After analyzing the data using One Way Anova method, double comparison between different concentrations with time was done using Post Hoc Tukey method. This analysis was meant in finding patterns and/or relationships between subgroups of sampled populations that would otherwise remain undetected and undiscovered were a scientific community to rely strictly upon *a priori* statistical methods (Jaccard,1984), which in this study

referring to the influence of various extract concentrations towards the number of *Musca domestica* knockdown at each observation intervals.

The mean differences were significant for almost all concentrations that were used during the observation time. However, during the 1st,2nd, 3rd, and 4th hour of observation time, the mean differences comparing the those hours with during 30th minutes, the values were recorded to be insignificant. The same thing happened when the 5th hour was compared to the 2nd, 3rd, 4th and 6th hour, where the values of mean differences were also being insignificant. In addition, the insignificant values can also be seen when the 20th hour of observation was compared with the 21st, 22nd, and 23rd hour of observation time.

On the other hand, the p value showed great significancy (as $p<0.05$) for all readings when the concentrations were compared with each other.

5.6 Data analysis with Correlation and Regression test

Correlation is a measure of the relation between two or more variables. The measurement scales used should be at least interval scales, but other correlation coefficients are available to handle other types of data. Correlation coefficients can range from -1.00 to +1.00. The value of -1.00 represents a perfect negative correlation while a value of +1.00 represents a perfect positive correlation. A value of 0.00 represents a lack of correlation. To know about this relationship mathematically in detail, the regression test was conducted.

Correlations

		Time	Concentration	Potential
Time	Pearson Correlation	1.000	.000	.900**
	Sig. (2-tailed)	.	1.000	.000
	N	195	195	195
Concentration	Pearson Correlation	.000	1.000	.201**
	Sig. (2-tailed)	1.000	.	.005
	N	195	195	195
Potential	Pearson Correlation	.900**	.201**	1.000
	Sig. (2-tailed)	.000	.005	.
	N	195	195	195

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

Table 5.3: The result of Pearson Correlation test

From Table 5.3,it can be seen that during each interval time of observation, the significant value, p=0.00 ($p<0.05$, H_0 was rejected) meant that the relationship of observation time and with the knockdown of *Musca domestica* was significant. The correlation coefficient, R = 0.9, showed that the correlation between the time observed and the number of *Musca domestica* knockdown can be said as strong enough and the vector went positive way, thus resulting in increase of the number of *Musca domestica* number with the increase of observation time interval.

The same thing happened when different concentrations of extract were compared, where the p value showed 0.00 ($p<0.05$, H_0 was rejected) which meant that the relationship between the concentrations used and the knockdown of *Musca domestica* was significant. On the other hand, the R value showed 0.201 where this correlation showed a weak association between the concentrations used in this study with the *Musca domestica* knockdown number, with the vector went positive. Hence, this can be concluded that the increase of concentration used will increase the knockdown potential of the chrysanthemum extract towards the *Musca domestica* knockdown number, even though the association was not strong enough.

To know how influential was the variation of the chrysanthemum extract concentrations and the observation time, the regression test was used. This was tested in percentage (%).

Based on the result of the Pearson Correlation Test in Table 5.3, it was shown that both factor; the chrysanthemum extract concentrations and the time of observation for the *Musca domestica* knockdown potential showed a significant result ($p<0.05$, H_0 was rejected). The result of the Regression Test (using the Regression equation) for each concentration and time of observation was shown as followed:

The Regression Equation	**R squared (R^2)**
$Y=0.123 + 0.276X_1 + 0.279X_2$	85%

Explaination:

Y = Knockdown potential of chrysanthemum extract/number of *Musca domestica* knockdown

X_1= Time of observation (in which time interval)

X_2= The chrysanthemum extract concentration

The regression model used was $Y=0.123 + 0.276X_1 + 0.279X_2$, with Y was the number of *Musca domestica* knockdown, X_1 as the observation time interval and X_2 was the concentration of the chrysanthemum extract. This can be defined as without the consideration of the length of observation time and the extract concentration (without manipulation), the knockdown potential of the chrysanthemum extract remain constant, which was 0.123 (as the constant value was positive). On the other hand, the knockdown potential increased 0.276% with every 1 hour increment of the observation time, whereby for the extract concentration, as much as 0.279% increment was noted with the increasing of every 1% concentration of extract used.

Based on the regression test result, the of R^2 coefficient value stated on how big was the association of the chrysanthemum extract with the length of observation time towards the knockdown potential (in percentage and the balance percentage, $1-R^2$), was influenced by other variable. Therefore, it can be said that the association of the extract concentrations used with the length of observation time was as big as 85% where as the balance of 15% was influenced by other factors (apart from the other two mentioned variables above), such as the self resistant mechanism by the housefly itself etc. This thus leading us to a conclusion where the higher the extract concentration that was used and the longer the observation time, the higher the influence of *Musca domestica* knockdown. With this, the significant values for both variables (factors) will be affected too.

Based on the result above, it can be predicted that the chrysanthemum extract potential or the observation time will be used in this study.

CHAPTER 6

DISCUSSION

Chrysanthemum is believed to have an insecticide effect towards *Musca domestica*. In 1917, the usage of chrysanthemum in repelling or killing the house pests such as the houseflies and mosquitoes, was widely incorporated in the community by the US Navy, even though at that time, the active ingredient(s) of the chrysanthemum used was still unknown (The Royal Society Of Chemistry, 2001).

This was then strongly proven with a few researches done by both students and professional researchers since decades. A study in 2008 by a medical student of Brawijaya University, had stated on the potential effect of chrysanthemum extract as an insecticide towards *Musca domestica.* In her research, the potential concentration mentioned was 30-40% (Kusumaningtyas, 2008). Meanwhile, for this study, higher concentrations were used. This was because of the latest finding by the New York State Agricultural Experiment Station at the Cornell University, in 2008, stating that there was a mechanism of mutation undergoing by these *Musca domestica* that prohibit them from being repelled or killed by the pyrethrin (the active substance extracted from a chrysanthemum species) with such low or moderate concentration (Soderlund, 2008). Thus, higher concentration than that of 30-40% is needed for the purpose of killing or knocked these flies down.

As what have been stated in Chapter 2, the main component of the chrysanthemum extract, the pyrethrins, were believed to play major contribution in enabling the chrysanthemum to be used effectively as a natural insecticide (Bennet, 2001). Scientifically, the nerve cell membranes have a specific electrical charge. Altering the amount of ions (charged atoms) passing through ion channels causes the membrane to depolarize which, in turn, causes a neurotransmitter to be released. Neurotransmitters help nerve cells communicate. Electrical messages sent between nerve cells allow them to generate a response, like a movement in an animal or insect (NPIC, 1998). Therefore, any alterations of

this process will consequently affecting the nervous system of the insect. In this study, the pyrethrins that composed of six molecules constituents; Cinerin 1, Cinerin 2, Jasmolin 1, jasmolin 2, Pyrethrin 1 and Pyrethrin 2, were proven to have the ability in altering the membrane depolarization of the Musca domestica by initiating multiple action potentials. This directly will resulting in the paralysis of the Musca domestica (NPIC, 1998).

As the potential of the chrysanthemum as an insecticide had been clearly proved and elaborated, the main highlight of this study was to gain more specific information of the *Musca domestica* through the Knockdown Time caused by the chrysanthemum extract towards these flies.

To know the effectiveness of this natural insecticide, the research was done using spraying method. This was chosen due to the feasibility and efficacy of the flies to be quickly knocked down and killed by mists or aerosols of insecticide solutions (WHO, 1991). In addition, the principle was to fill a space with a mist of small droplets that were picked up by the insects when they fly. As the sprayed droplet diameter is small (100-500n) and relatively smaller in gaseous form (0.001-0.01n), the absorbing process becomes easier (Baskoro et al, 2005). To add in, this method was similar with the one commonly used in the community. Due to this reason, the spraying method was preferable. For this study, the hand-operated sprayers were used.

The number of *Musca domestica* used for each experiment was 10 per cage. This was based on the recommendation by one of the journals been published in the World Health Organization, WHO website stating that the lowest number of *Musca domestica* which can be used in a study was 10 and certain other studies were using 25-30 flies per cage (IPCC, 2008). No lower number of that (10), was reported to be used in any studies. In addition, due to its minimal availability at that current time, instead of 25 houseflies (as what have been recommended), 10 houseflies were used. Therefore, this made a total of 200 houseflies which were used in this experiment (including the 5 repetitions). The number of repetitions for this experiment was determined by using a formula suggested by Tjokonegoro in 2001, as stated in Chapter 4 before.

After the experiment was done and the data was collected, the process continued with the data analysis. The statistical analysis used were the TwoWay Annova Test and Post Hoc Tuket Test.

As the study involved two or more sample means, theTwo Way Anova Test enabled the analysis of these data of all classes to be compared with each other stimultaneously rather than individually. Few assumptions need to be postulated and tested before the anova was conducted. The assumptions were; (1) the data was normally distributed, (2)the data variance was not high, and (3) the data was homogenous (Jamie Shutler, 2002). To ensure that these criteria were fulfilled, the NPar test and the Homogenous test were carried out. In this experiment, both criteria were fulfilled.As for the NPar test, the data was recorded to be normally distributed as p=1.546 (p>0.05), where as for the homogenous test, the p value recorded was 0.064 (p>0.05). This thus evidently showing that the data had fulfilled the second criteria for pre-anova testing which was the data subset should be homogenous.

In this experiment, the numbers of *Musca domestica* knocked down were analysed to evaluate the significances of each concentration in each time intervals (30^{th} minute, 1^{st} , 2^{nd} , 3^{rd} , 4^{th}, 5^{th}, 6^{th}, 20^{th}, 21^{st} , 22^{nd} , 23^{th} and 24^{th} hour). The data shows significant result as the P value are all 0.00 (<0.05).

The analysis was then being proceeded with Post Hoc TUKEY test. As the significances of the means were obtained through the ANNOVA test, specific information of these data was furtherly tested in this test. Based on this analysis, the significances in each interval in comparison with the concentrations; 50%, 60% and 70%, were shown differently. In this study, it was found that, for most readings that were tabulated, the p values recorded were insignificant when the first variable (the observation time interval) was compared. This might happened because of the active substance of the three concentrations of chrysanthemum sp. extract solution gives almost the same effect when being exposed to the *Musca domestica* for those hours. On the other hands, when the concentrations were compared to each other, the p value for all readings showed significant values.

There were a few limitation factors related to this study. For example, the sample of this research might include adult *Musca domestica* that were already exposed to other insecticides. The samples were supposedly grown from the egg (Chevillon *et al*, 1999). Other factors might also influence the immunity of *Musca domestica* before they were being exposed to the chrysanthemum sp. solution. The factors include air temperature, humidity and also the length of time the chrysanthemum sp. extract being kept. The longer the extract is being kept, the lower its ability to cause *Musca domestica* knockdown. Hence this will

affect the result of the research (Gardiner, 2008). These entire factors have been tried to be minimized. However, the limitation of time and tools are still being the main barriers. For the spraying method, the researcher had tried to ensure that the *Musca domestica* were not being affected by the spraying technique, but the exposure of the chrysanthemum sp. active substance. This was done by spraying the extract solution at the area of the container that was not encountered by the *Musca domestica*. Besides, the concentration that can cause KT50 (knockdown of 5 *Musca domestica*) might be lower than 50% as the concentration 50% had already knocked down 9 *Musca domestica*.

A few researches were conducted by professionals to find a suitable insecticide in knocking down the houseflies rapidly in a short period of time. One of them was by using the neem extract. In this research, the neem extract was introduced to the *Musca domestica* by using the same spraying method (as in this study) and the knockdown number of these *Musca domestica* was observed. For this research, the experiment was observed in 24 hours interval time (Scribd, 2011). Based on this research, the concentration of the neem extract that has the same quality of knockdown effect as in the chrysanthemum's was said to be 20-25%. Even though the chrysanthemum extract concentration used for this study was higher compared to the one used in the neem extract, the potential of this extract in knocking the *Musca domestica* down in rapid and high amount was still there.

There were few limitations of this study. The limitations were the accuracy of the spraying equipment used and the variety of strain and sex (males and females) of *Musca domestica* used. However, these limitations had not implicated the result of this experiment.

CHAPTER 7

CONCLUSION AND SUGGESTION

7.1 Conclusion

From this research, it can be concluded that:

1. Chrysanthemum sp. extract has the knockdown effect towards *Musca domestica.*

2. Concentration 50% is the lowest concentration that has the effect of KT50 on *Musca domestica.*

3. Concentration 60% and 70% are the concentration that has effect of KT100

7.2 Suggestion

1. Further study should be done to reveal the method of extracting the main active substance of chrysanthemum sp. that causes the knockdown effect of *Musca domestica.*

2. Further study can be conducted in revising the potential concentration of the *chrysanthemum sp.* extract using different strain of *Musca domestica* and the resistant mechanism of each *Musca domestica* used towards the active substance (pyrethrin) is carefully studied.

3. Specific research can be done to know the most effective spraying method in conjunction with time and distance of spraying together with the size of particle and storage mechanism of the extract as these can influence the result of the knockdown.

4. Further study can be done to suggest the mechanism of the active substance of chrysanthemum sp. extract as a natural insecticide.

5. Other study can also be held to compare which extracts of natural insecticides that have the quickest knockdown effect as this can be commercialized to be the most effective insecticide.

6. Further study focusing on the toxicology aspect of the active component of the extract (pyrethrin) be conducted in both in vivo and in vitro test.

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APPENDIX 1

Table 5.2 The result of *Musca domestica* knockdown with five (5) repetitions

	Ulangan	Acetone	Concentration		
		1%	50%	60%	70%
0	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
Mean		0	0	0	0
sd		0	0	0	0
30M	1	0	2	2	3
	2	0	2	2	3
	3	0	3	3	3
	4	0	2	2	3
	5	0	2	3	4
Mean		0	2.20	2.40	3.20
sd		0	0.45	0.55	0.45
1H	1	0	3	3	3
	2	0	3	3	3
	3	0	3	4	4
	4	0	2	3	3
	5	0	3	3	4
Mean		0	2.80	3.20	3.40
sd		0	0.45	0.45	0.55
2H	1	0	3	4	4
	2	0	3	3	4
	3	0	3	4	5
	4	0	3	4	4
	5	0	3	4	4

Mean		0	3.00	3.80	4.20
sd		0	0.00	0.45	0.45
3H	**1**	0	3	4	4
	2	0	4	4	4
	3	0	3	4	5
	4	0	3	4	4
	5	0	4	5	6
Mean		0	3.40	4.20	4.60
sd		0	0.55	0.45	0.89
4H	**1**	0	3	4	5
	2	0	4	4	5
	3	0	4	5	6
	4	0	3	4	5
	5	0	4	5	7
Mean		0	3.60	4.40	5.60
sd		0	0.55	0.55	0.89
5H	**1**	0	4	4	5
	2	0	5	5	5
	3	0	5	5	7
	4	0	4	5	7
	5	0	4	6	7
Mean		0	4.40	5.00	6.20
sd		0	0.55	0.71	1.10
6H	**1**	0	4	5	8
	2	0	5	5	6
	3	0	6	7	7
	4	0	5	5	7
	5	0	5	6	8
Mean		0	5.00	5.60	7.20
sd		0	0.71	0.89	0.84
20H	**1**	0	7	9	10

	2	0	6	6	8
	3	0	7	7	8
	4	0	6	7	9
	5	0	7	9	9
Mean		0	6.60	7.60	8.80
sd		0	0.55	1.34	0.84
21H	**1**	0	7	9	10
	2	0	7	8	9
	3	0	7	8	9
	4	0	7	8	10
	5	0	7	9	10
Mean		0	7.00	8.40	9.60
sd		0	0.00	0.55	0.55
22H	**1**	0	8	10	10
	2	0	8	8	9
	3	0	8	9	10
	4	0	8	8	10
	5	0	8	10	10
Mean		0	8.00	9.00	9.80
sd		0	0.00	1.00	0.45
23H	**1**	0	8	10	10
	2	0	9	10	10
	3	0	8	10	10
	4	0	9	9	10
	5	0	8	10	10
Mean		0	8.40	9.80	10.00
sd		0	0.55	0.45	0.00
24H	**1**	0	9	10	10
	2	0	9	10	10
	3	0	9	10	10
	4	0	9	10	10

	5	0	9	10	10
Mean		0	9.00	10.00	10.00
sd		0	0.00	0.00	0.00

Uji Normalitas

NPar Tests

One-Sample Kolmogorov-Smirnov Test

		Potential
N		260
Normal Parameters[a,b]	Mean	4.22
	Std. Deviation	3.57
Most Extreme Differences	Absolute	.189
	Positive	.189
	Negative	-.119
Kolmogorov-Smirnov Z		1.546
Asymp. Sig. (2-tailed)		.078

a. Test distribution is Normal.

b. Calculated from data.

Uji Homogenitas

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

Dependent Variable: Potential

F	df1	df2	Sig.
1.373	51	208	.064

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

a. Design: TIME+CONCENTR

Two-Way Anova

Tests of Between-Subjects Effects

Dependent Variable: Potential

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	7454.615[a]	16	465.913	237.639	.000
TIME	1212.354	12	101.029	51.530	.000
CONCENTR	1613.765	3	537.922	274.367	.000
Error	478.385	244	1.961		
Total	7933.000	260			

a. R Squared = .940 (Adjusted R Squared = .936)

Post Hoc Tests

Time

Multiple Comparisons

Dependent Variable: Potential
Tukey HSD

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	30 minute	-1.95*	.44	.001	-3.42	-.48
	1 hour	-2.35*	.44	.000	-3.82	-.88
	2 hour	-2.75*	.44	.000	-4.22	-1.28
	3 hour	-3.05*	.44	.000	-4.52	-1.58
	4 hour	-3.40*	.44	.000	-4.87	-1.93
	5 hour	-3.90*	.44	.000	-5.37	-2.43
	6 hour	-4.45*	.44	.000	-5.92	-2.98
	20 hour	-5.75*	.44	.000	-7.22	-4.28
	21 hour	-6.25*	.44	.000	-7.72	-4.78
	22 hour	-6.70*	.44	.000	-8.17	-5.23
	23 hour	-7.05*	.44	.000	-8.52	-5.58
	24 hour	-7.25*	.44	.000	-8.72	-5.78
30 minute	0	1.95*	.44	.001	.48	3.42
	1 hour	-.40	.44	1.000	-1.87	1.07
	2 hour	-.80	.44	.847	-2.27	.67
	3 hour	-1.10	.44	.385	-2.57	.37
	4 hour	-1.45	.44	.056	-2.92	1.68E-02
	5 hour	-1.95*	.44	.001	-3.42	-.48
	6 hour	-2.50*	.44	.000	-3.97	-1.03
	20 hour	-3.80*	.44	.000	-5.27	-2.33
	21 hour	-4.30*	.44	.000	-5.77	-2.83
	22 hour	-4.75*	.44	.000	-6.22	-3.28
	23 hour	-5.10*	.44	.000	-6.57	-3.63
	24 hour	-5.30*	.44	.000	-6.77	-3.83
1 hour	0	2.35*	.44	.000	.88	3.82
	30 minute	.40	.44	1.000	-1.07	1.87
	2 hour	-.40	.44	1.000	-1.87	1.07
	3 hour	-.70	.44	.936	-2.17	.77
	4 hour	-1.05	.44	.464	-2.52	.42
	5 hour	-1.55*	.44	.027	-3.02	-8.32E-02
	6 hour	-2.10*	.44	.000	-3.57	-.63
	20 hour	-3.40*	.44	.000	-4.87	-1.93
	21 hour	-3.90*	.44	.000	-5.37	-2.43
	22 hour	-4.35*	.44	.000	-5.82	-2.88
	23 hour	-4.70*	.44	.000	-6.17	-3.23
	24 hour	-4.90*	.44	.000	-6.37	-3.43

Based on observed means.

Multiple Comparisons

Dependent Variable: Potential
Tukey HSD

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
2 hour	0	2.75*	.44	.000	1.28	4.22
	30 minute	.80	.44	.847	-.67	2.27
	1 hour	.40	.44	1.000	-1.07	1.87
	3 hour	-.30	.44	1.000	-1.77	1.17
	4 hour	-.65	.44	.963	-2.12	.82
	5 hour	-1.15	.44	.312	-2.62	.32
	6 hour	-1.70*	.44	.008	-3.17	-.23
	20 hour	-3.00*	.44	.000	-4.47	-1.53
	21 hour	-3.50*	.44	.000	-4.97	-2.03
	22 hour	-3.95*	.44	.000	-5.42	-2.48
	23 hour	-4.30*	.44	.000	-5.77	-2.83
	24 hour	-4.50*	.44	.000	-5.97	-3.03
3 hour	0	3.05*	.44	.000	1.58	4.52
	30 minute	1.10	.44	.385	-.37	2.57
	1 hour	.70	.44	.936	-.77	2.17
	2 hour	.30	.44	1.000	-1.17	1.77
	4 hour	-.35	.44	1.000	-1.82	1.12
	5 hour	-.85	.44	.784	-2.32	.62
	6 hour	-1.40	.44	.079	-2.87	6.68E-02
	20 hour	-2.70*	.44	.000	-4.17	-1.23
	21 hour	-3.20*	.44	.000	-4.67	-1.73
	22 hour	-3.65*	.44	.000	-5.12	-2.18
	23 hour	-4.00*	.44	.000	-5.47	-2.53
	24 hour	-4.20*	.44	.000	-5.67	-2.73
4 hour	0	3.40*	.44	.000	1.93	4.87
	30 minute	1.45	.44	.056	-1.68E-02	2.92
	1 hour	1.05	.44	.464	-.42	2.52
	2 hour	.65	.44	.963	-.82	2.12
	3 hour	.35	.44	1.000	-1.12	1.82
	5 hour	-.50	.44	.996	-1.97	.97
	6 hour	-1.05	.44	.464	-2.52	.42
	20 hour	-2.35*	.44	.000	-3.82	-.88
	21 hour	-2.85*	.44	.000	-4.32	-1.38
	22 hour	-3.30*	.44	.000	-4.77	-1.83
	23 hour	-3.65*	.44	.000	-5.12	-2.18
	24 hour	-3.85*	.44	.000	-5.32	-2.38
5 hour	0	3.90*	.44	.000	2.43	5.37
	30 minute	1.95*	.44	.001	.48	3.42
	1 hour	1.55*	.44	.027	8.32E-02	3.02
	2 hour	1.15	.44	.312	-.32	2.62
	3 hour	.85	.44	.784	-.62	2.32
	4 hour	.50	.44	.996	-.97	1.97
	6 hour	-.55	.44	.991	-2.02	.92
	20 hour	-1.85*	.44	.002	-3.32	-.38
	21 hour	-2.35*	.44	.000	-3.82	-.88
	22 hour	-2.80*	.44	.000	-4.27	-1.33
	23 hour	-3.15*	.44	.000	-4.62	-1.68
	24 hour	-3.35*	.44	.000	-4.82	-1.88

Based on observed means.

Multiple Comparisons

Dependent Variable: Potential
Tukey HSD

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
6 hour	0	4.45*	.44	.000	2.98	5.92
	30 minute	2.50*	.44	.000	1.03	3.97
	1 hour	2.10*	.44	.000	.63	3.57
	2 hour	1.70*	.44	.008	.23	3.17
	3 hour	1.40	.44	.079	-6.68E-02	2.87
	4 hour	1.05	.44	.464	-.42	2.52
	5 hour	.55	.44	.991	-.92	2.02
	20 hour	-1.30	.44	.145	-2.77	.17
	21 hour	-1.80*	.44	.003	-3.27	-.33
	22 hour	-2.25*	.44	.000	-3.72	-.78
	23 hour	-2.60*	.44	.000	-4.07	-1.13
	24 hour	-2.80*	.44	.000	-4.27	-1.33
20 hour	0	5.75*	.44	.000	4.28	7.22
	30 minute	3.80*	.44	.000	2.33	5.27
	1 hour	3.40*	.44	.000	1.93	4.87
	2 hour	3.00*	.44	.000	1.53	4.47
	3 hour	2.70*	.44	.000	1.23	4.17
	4 hour	2.35*	.44	.000	.88	3.82
	5 hour	1.85*	.44	.002	.38	3.32
	6 hour	1.30	.44	.145	-.17	2.77
	21 hour	-.50	.44	.996	-1.97	.97
	22 hour	-.95	.44	.631	-2.42	.52
	23 hour	-1.30	.44	.145	-2.77	.17
	24 hour	-1.50*	.44	.039	-2.97	-3.32E-02
21 hour	0	6.25*	.44	.000	4.78	7.72
	30 minute	4.30*	.44	.000	2.83	5.77
	1 hour	3.90*	.44	.000	2.43	5.37
	2 hour	3.50*	.44	.000	2.03	4.97
	3 hour	3.20*	.44	.000	1.73	4.67
	4 hour	2.85*	.44	.000	1.38	4.32
	5 hour	2.35*	.44	.000	.88	3.82
	6 hour	1.80*	.44	.003	.33	3.27
	20 hour	.50	.44	.996	-.97	1.97
	22 hour	-.45	.44	.999	-1.92	1.02
	23 hour	-.80	.44	.847	-2.27	.67
	24 hour	-1.00	.44	.548	-2.47	.47
22 hour	0	6.70*	.44	.000	5.23	8.17
	30 minute	4.75*	.44	.000	3.28	6.22
	1 hour	4.35*	.44	.000	2.88	5.82
	2 hour	3.95*	.44	.000	2.48	5.42
	3 hour	3.65*	.44	.000	2.18	5.12
	4 hour	3.30*	.44	.000	1.83	4.77
	5 hour	2.80*	.44	.000	1.33	4.27
	6 hour	2.25*	.44	.000	.78	3.72
	20 hour	.95	.44	.631	-.52	2.42
	21 hour	.45	.44	.999	-1.02	1.92
	23 hour	-.35	.44	1.000	-1.82	1.12
	24 hour	-.55	.44	.991	-2.02	.92

Based on observed means.

Multiple Comparisons

Dependent Variable: Potential
Tukey HSD

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
23 hour	0	7.05*	.44	.000	5.58	8.52
	30 minute	5.10*	.44	.000	3.63	6.57
	1 hour	4.70*	.44	.000	3.23	6.17
	2 hour	4.30*	.44	.000	2.83	5.77
	3 hour	4.00*	.44	.000	2.53	5.47
	4 hour	3.65*	.44	.000	2.18	5.12
	5 hour	3.15*	.44	.000	1.68	4.62
	6 hour	2.60*	.44	.000	1.13	4.07
	20 hour	1.30	.44	.145	-.17	2.77
	21 hour	.80	.44	.847	-.67	2.27
	22 hour	.35	.44	1.000	-1.12	1.82
	24 hour	-.20	.44	1.000	-1.67	1.27
24 hour	0	7.25*	.44	.000	5.78	8.72
	30 minute	5.30*	.44	.000	3.83	6.77
	1 hour	4.90*	.44	.000	3.43	6.37
	2 hour	4.50*	.44	.000	3.03	5.97
	3 hour	4.20*	.44	.000	2.73	5.67
	4 hour	3.85*	.44	.000	2.38	5.32
	5 hour	3.35*	.44	.000	1.88	4.82
	6 hour	2.80*	.44	.000	1.33	4.27
	20 hour	1.50*	.44	.039	3.32E-02	2.97
	21 hour	1.00	.44	.548	-.47	2.47
	22 hour	.55	.44	.991	-.92	2.02
	23 hour	.20	.44	1.000	-1.27	1.67

Based on observed means.

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

Potential

Tukey HSD[a,b]

Time	N	Subset 1	2	3	4	5	6	7
0	20	.00						
30 minute	20		1.95					
1 hour	20		2.35					
2 hour	20		2.75	2.75				
3 hour	20		3.05	3.05	3.05			
4 hour	20		3.40	3.40	3.40			
5 hour	20			3.90	3.90			
6 hour	20				4.45	4.45		
20 hour	20					5.75	5.75	
21 hour	20						6.25	6.25
22 hour	20						6.70	6.70
23 hour	20						7.05	7.05
24 hour	20							7.25
Sig.		1.000	.056	.312	.079	.145	.145	.548

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 1.961.

a. Uses Harmonic Mean Sample Size = 20.000.

b. Alpha = .05.

Concentration

Multiple Comparisons

Dependent Variable: Potential

Tukey HSD

(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Acetone 1%	50%	-4.88*	.25	.000	-5.51	-4.25
	60%	-5.65*	.25	.000	-6.28	-5.02
	70%	-6.35*	.25	.000	-6.98	-5.72
50%	Acetone 1%	4.88*	.25	.000	4.25	5.51
	60%	-.77*	.25	.009	-1.40	-.14
	70%	-1.48*	.25	.000	-2.11	-.85
60%	Acetone 1%	5.65*	.25	.000	5.02	6.28
	50%	.77*	.25	.009	.14	1.40
	70%	-.71*	.25	.021	-1.34	-7.67E-02
70%	Acetone 1%	6.35*	.25	.000	5.72	6.98
	50%	1.48*	.25	.000	.85	2.11
	60%	.71*	.25	.021	7.67E-02	1.34

Based on observed means.

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

Potential

Tukey HSD[a,b]

Concentration	N	Subset 1	2	3	4
Acetone 1%	65	.00			
50%	65		4.88		
60%	65			5.65	
70%	65				6.35
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 1.961.

a. Uses Harmonic Mean Sample Size = 65.000.

b. Alpha = .05.

Correlations

Correlations

		Time	Concentration	Potential
Time	Pearson Correlation	1.000	.000	.900**
	Sig. (2-tailed)	.	1.000	.000
	N	195	195	195
Concentration	Pearson Correlation	.000	1.000	.201**
	Sig. (2-tailed)	1.000	.	.005
	N	195	195	195
Potential	Pearson Correlation	.900**	.201**	1.000
	Sig. (2-tailed)	.000	.005	.
	N	195	195	195

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

Regression

Variables Entered/Removed[b]

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

a. All requested variables entered.

b. Dependent Variable: Potential

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.922[a]	.850	.849	1.17

a. Predictors: (Constant), Concentration, Time

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1497.638	2	748.819	545.055	.000[a]
	Residual	263.777	192	1.374		
	Total	1761.415	194			

a. Predictors: (Constant), Concentration, Time

b. Dependent Variable: Potential

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-1.659	.629		-2.638	.009
	Time	.283	.009	.900	32.226	.000
	Concentration	.074	.010	.201	7.183	.000

a. Dependent Variable: Potential

Experiment documentation

Figure 1: Chrysanthemum (*Chrysanthemum sp.)* extract solution and the equipments.

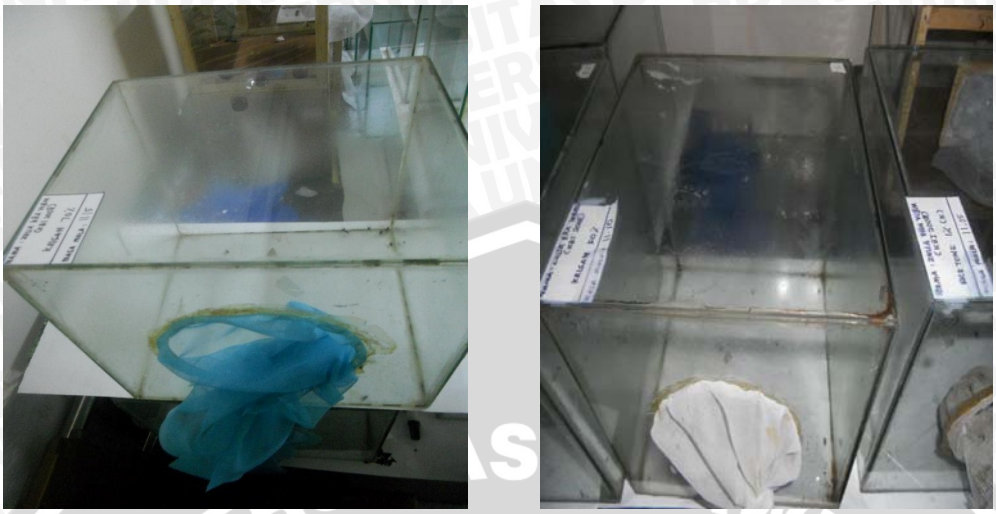

Figure 2: Fly container with the size of 25cm x 25cm x 25cm

Figure 3: The knockdown of *Musca domestica*

VERIFICATION OF THESIS

I, hereby

Name : Zulia Era Wahida Binti Mohamad

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would like to 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, 26th October 2011

Sincerely,

(Zulia Era Wahida Binti Mohamad)

0810714042