CINNAMON (*Cinnamomum Burmannii)* EXTRACT HAS WEAK KNOCKDOWN EFFECTS TOWARDS *Culex sp.*

Final Project

To fulfill the requirement for the degree of Bachelor in Medicine

By:

Nurul Faizah Binti Jusoh

0610714021

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APPROVAL PAGE

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CERTIFICATION PAGE

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Examined on: 22th Dec 2011

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Date: 12.30 pm

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All praise to Allah, The Almighty, for His gift so that I can finish the final project, Cinnamon (*Cinnamomum burmannii)* Extract has Weak Knockdown Effects towards *Culex sp.*

This topic was chosen because the use of chemical insecticides caused so many harms to human and other living creature. Eventhough, 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 cinnamon, one of the most common biosecticides 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.

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Malang, Jan 2012

Author

ABSTRACT

Jusoh, Nurul F. 2011. Cinnamon (*Cinnamomum burmannii)* Extract has Weak Knockdown Effects towards *Culex sp.* Final project, Faculty of Medicine, Brawijaya University. Supervisors: (1) Prof Dr. dr. Teguh W. Sardjono, DTM&H, MSc, SpParK, (2) dr. Danik Agustin P., M.Kes

Chemical insecticides which had been widely used to control mosquitoes as vector of many diseases might have negative effects towards human being. Therefore, natural insecticide is starting to be developed to get effective insecticide but safer towards environment. Cinnamon (*Cinnamomum burmannii*) possessed eugenol and cinnamaldehyde that had been known as active substances for insecticide. This true experimental study using post-test only control group design was conducted in order to measure the knockdown effects of cinnamon (*Cinnamomum burmannii*) extract towards *Culex sp.* mosquitoes. One hundred adult mosquitoes were divided into four cages consist of 25 mosquitoes each. Three cages sprayed with 12.5%, 25% and 50% cinnamon extract respectively and the fourth cage was sprayed with aceton as control. Those procedures repeated for five times. Result showed that crude extract with 50% concentration of cinnamon (*Cinnamomum burmannii*) could kill more than 50% population of the mosquitoes within 30 minutes after spraying while the lesser concentration could not reach 50% population until 60 minutes observation. This study showed that crude extract of cinnamon (*Cinnamomum burmannii*) has a weak knockdown effect towards adult *Culex sp.* with score 2 according to WHO Insecticide Score.

Keyword: *Culex* sp, Bioinsecticides, *Cinnamomum burmannii*, Weak knockdown effect

ABSTRAK

Jusoh, Nurul F. 2011. Ekstrak Kayu Manis (*Cinnamomum burmannii*) Mempunyai Efek Weak Knockdown Terhadap *Culex sp*, Tugas Akhir, Fakultas Kedokteran, Universitas Brawijaya. Pembimbing: (1) Prof Dr. dr. Teguh W. Sardjono, DTM&H, MSc, SpParK, (2) dr. Danik Agustin P.,M.Kes

Insektisida kimia yang saat ini telah banyak digunakan untuk mengendalikan nyamuk yang merupakan vektor dari pelbagai penyakit mungkin memiliki efek negatif terhadap manusia. Oleh karena itu, insektisida alami mulai dikembangkan untuk mendapatkan insektisida yang efektif tetapi aman terhadap lingkungan. Kayu manis (*Cinnamomum burmannii*) mengandungi *eugenol* dan *cinnamaldehyde* yang dikenal sebagai zat aktif yang memiliki effek insektisida. Studi true experimental yang menggunakan desain *post-test only control group* ini dilakukan untuk mengukur efek *knockdown* dari ekstrak kayu manis terhadap nyamuk *Culex sp.* Seratus nyamuk dewasa dibagi menjadi empat kandang yang terdiri dari 25 ekor nyamuk di masing-masing kandang. Tiga kandang masing- masing disemprot dengan 12.5%, 25% dan 50% ekstrak kayu manis dan kandang keempat disemprot dengan aseton sebagai control. Prosedur ini diulang sebanyak lima kali. Hasil penelitian menunjukkan bahwa ekstrak kasar dengan konsentrasi 50% dari kayu manis (*Cinnamomum burmannii*) bisa membunuh lebih dari 50% populasi nyamuk dalam waktu 30 menit setelah penyemprotan sementara konsentrasi yang lebih rendah tidak mencapai populasi 50% hingga 60 menit waktu pengamatan. Studi ini menunjukkan bahwa ekstrak kasar dari kayu manis (*Cinnamomum burmannii*) memiliki efek *knockdown* yang lemah terhadap nyamuk *Culex sp.* dewasa dengan skor 2 menurut WHO skor insektisida.

Kata kunci: *Culex* sp, bioinsektisida, *Cinnamomum burmannii*, *Weak Knockdown Effect*

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LIST OF ABBREVIATION

- WHO: World Health Organization
- KT: Knockdown Time
- sp. : Species
- Ach : acetylcholine
- GABA : Gamma Aminobutyric Acid
- PNS : Peripheral nervous system
- CNS : central nervous system
- cm : centimeter
- ml : milliliter
- mg : milligram

CHAPTER 1

INTRODUCTION

1.1 Background

Mosquitoes are insects in the family *Culicidae*. They have a pair of scaled wings, a pair of haltered, a slender body, and long legs. The males may have beaks, or probosces, but cannot pierce, and they feed upon fruit and plant juices. The females have piercing and sucking mouth parts and apparently they must feed at least once upon mammalian blood before their eggs can develop properly. During blood meals the females may either acquire or transmit various disease organisms (Britannica, 2010).

Diseases such as lymphatic filariasis, Japanese B encephalitis, West Nile virus, and St. Louis encephalitis are transmitted by *Culex* species of mosquitoes (Jefferson, St 2008). The most frequent disease that being spread by *Culex sp.* is filariasis. Populations at high risk for contracting or developing a lymphatic filariasis infection are primarily poor, and majority of the cases are concentrated in rural areas. Lymphatic filariasis is often associated with areas that have poor sanitation and housing quality (Stanford, 2006).

Besides filariasis, Japanese encephalitis (JE) also frequently spread by *Culex sp*. The incidence of JE in humans varies by country and is usually seasonal, coinciding with the rains. Encephalitis causes will be left with residual neurological deficits, including paralysis, ataxia, and speech difficulties. Approximately one third of patients with clinically manifestation of JE were died (Nathnac, 2010).

Considering of how dangerous it is for the spreading of those diseases, people should be more aware and know how to reduce the transmission of the disease. There are many methods used for mosquito control. Some target the larval stage, while others are used to kill or repel adults. The common ways in handling adult mosquitoes are by spraying insecticide such as malathion, parathion, propoxur, pyrethroid, and DDT. Although these insecticides were used to kill the mosquitoes, some of these chemical substances have negative effects on organisms especially human being. These synthetic insecticides have slow rate of biotransformation and degradation of its chemical substances. Thus these chemical substances will persist in the environment, bioaccumulation, and biomagnification through the food chains (EPA, 2007). Because of these, there should be other alternative substance that is farther save and more environmental friendly that can be used to replace the chemical substances.

To reduce the negative effects of chemical insecticide, people developed insecticide based on natural substances that can be obtained from the environment for example plants, fruits, and others. There are about 300,000 types of plants in the world, 30,000 types of which is estimated growing in Indonesia and only 1000 types of materials that have been used as herbal drugs and insecticides (Aminah, 2001). The use of natural substance in insecticide showed the positive effects on environments and safe for human being. So, it is hoped that the positive effects of the natural substance on this natural insecticide becoming a good insecticide where the negative effects of chemical insecticide removed and the effects in killing the mosquitoes maintained.

Cinnamon *(Cinnamomum burmannii)* has been found to be effective in killing mosquito, mosquito larvae and also as an insect repellent. The compounds

Eugenol in Cinnamon will disturb the peripheral nervous system and the central nerve of the mosquitoes and will increase the excitation phase from the neuron body cell and finally it will cause nerve cell paralyze (Ware & Whitacre, 2005).Cinnamon spice use globally by people in making dishes and herbal medicine showed promise as fragrant, and environmental friendly (Howard, 2008).

Research on Cinnamon *(Cinnamomum burmannii)* extract has been proven to have insecticidal properties, for example, research carried out by Seoul National University has shown that Cinnamon *(Cinnamomum burmannii)* oil is effective as insecticide against mites, nematodes and mosquito larvae (Park *et.al*, 2000). Another research was conducted by Fahmi from Brawijaya University has shown that Cinnamon *(Cinnamomum burmannii)* extract have ability in killing adult mosquitoes (Fahmi, 2008). One of the methods to measure the effectivity of insecticide is by its quick knockdown time. However, there is one of the questions that is to know the concentration of Cinnamon *(Cinnamomum burmannii)* extract that has the effect of Quick Knockdown Time on adult *Culex sp* mosquito.

1.5 Statement of research problem

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

What is the concentration of cinnamon (*Cinnamomum burmannii*) extract that has an effect of Quick Knockdown Time on adult *Culex sp* mosquito?

1.6 Objective of the research

1.6.1 General objective

To know what is the concentration of cinnamon (*Cinnamomum burmannii*) extract has an effect of Quick Knockdown

1.6.2 Specific objective

1. To determine the KT50 of Cinnamon *(Cinnamomum burmannii)* extract that can give the effectivity of knockdown time toward *Culex sp*.
2. To determine score of cinnamon as insecticide according to WHO insecticide score.
3. To determine whether cinnamon have fulfils all the criteria needed as a good insecticide.

1.7 Significance of the research

1. Give the information to publics about knockdown effect and knockdown time from the Cinnamon *(Cinnamomum burmannii)* extract toward *Culex sp*.
2. As altenative way on killing mosquitoes especially biological vector mosquitoes (*Culex sp*.)
3. Increasing the undestanding and information about the mosquitoes born disease on publics
4. Give the information about the wondeful things about herbal plant especially collerate with medical properties.

CHAPTER 2

LITERATURE REVIEW

2.4 Mosquitoes

Mosquitoes are a bloodsucking insect of the family Culicidae (Dorland, 2004). It is known as slender, delicate flying insects of evil reputation. The bloodsucking mosquitoes of the family Culicidae are the carriers of malaria, dengue, filariasis, encephalitis and other dangerous diseases that can cause death, brain damage or physical deformity (Yong, 2008). Most taxonomic keys to identify mosquitoes are based on morphological characters (Rueda, 2007).

There are 3 subfamilies in the *culicidae* family:

1. Toxorhynchitinae
2. Culicinae: *Culex, Aedes, Mansonia*
3. Anophelinae: *Anopheles*

2.5 *Culex* sp.

2.5.1 Taxonomy

The taxonomy of *Culex* sp. that will use in this research as below:

Kingdom:	Animalia
Phylum:	Arthropoda
Class:	Insecta
Order:	Diptera
Sub order:	Nematocera
Family:	Culicidae
Sub family:	Culicinae
Genus:	*Culex*
Species:	*Culex* sp.

2.5.2 Morphology and life cycle of the *Culex* sp.

Mosquitoes experienced holometabola that is the adult, egg, the larva, and the pupa. This was also valid for *Culex sp*., below this will be explained morphology from every stage in the development metamorfosa of *Culex sp*.

Figure 1 *Culex sp.* Mosquitoes (NSW,2008)

2.5.2.1 Adult *Culex sp*.

Culex body consists of thre parts which are head, thorax and abdomen that are clearly separated. The colour of the adult *Culex* is light brown. The head is round or spherical form. The head is composed of eyes, antenna, and mouth part. One pair of compound eyes are united (holoptic) in male and clearly separated (dichoptic) in female. It has long, 15 jointed antennaes which are plumose in the males and pilose in the females. The elongated mouth part, adapted in female for piercing and sucking blood. The mouth part of blood sucking female consists of the grooves lower labium, the upper labrum-epipharynx, the hypopharynx, the styletlike paired maindibles and the serrated maxilla. The maxillary palps of the female are slender and hairy, while those of the male are long and ornamented like the antennae with tufts of hair, giving a plumed appearance. *Culex* thorax has three segments in which each segment is completed with one pair of legs. The rigid thorax, covered by a dorsal scutum, bears three pairs of long, slender legs (Neva and Brown, 1994). In addition, the mesothorax has a pair of functional wings, and the metathorax, a pair of wings represented by knobbed structures called halteres. The dorsal part of the thorax is ovoid or rectangle shape, covered with hairs, the mesonatum is separated with trilobular scutellum by a transversal line.(Figure 1). (Rueda, 2007)

Figure 2 *Culex sp.* Eggs (Floore,2010)

2.5.2.2 Eggs

The eggs of *Culex sp.* have the shape of like bananas (banana shape) which was placed in a row like raft in the surface of water. Each raft consisted of 100 – 200 eggs (Floore, 2010).

Figure 3 *Culex sp.* Larvae (NSW,2008)

2.5.2.3 The Larva

The larva (plural - larvae) lives in the water and comes to the surface to breathe. Larvae shed (molt) their skins four times, growing larger after each molt. Most larvae have siphon tubes for breathing and hang upside down from the water surface. The larvae feed on microorganisms and organic matter in the water. During the fourth molt the larva changes into a pupa (Floore, 2010).

Figure 4 *Culex sp.* Pupa (NSW,2008)

2.5.2.4 The pupa

The pupa was one of the constructions that resembled the comma, was the stage non feeding (did not eat). The head and thorax joined called cephalothorax. His movement was typical likes jerky movement and when at rest it will approach the surface of water to breathe with breathing tube (breathing trumpet) that was met to the dorsal side of thorax, the form of trumpet on *Culex sp.* are long and slim. The last segment of the abdomen there are a pair paddles for swim (Brown & Neva, 1994).

2.5.3 Breeding place

Every species of *Culex* have different place for breeding such as *Culex pipiens* it more prefer to breed on polluted or foul water high in organic content. However, it can occur in fresh water but not common. While *Culex tarsalis* more prefer in all freshwater sources except treeholes. Can occur in polluted water but not common (Richard, 2003).

2.5.4 Life cycle

Mosquitoes experienced holometabola that is the egg, the larva, the pupa and adult. The life cycle takes 10 to 14 days. Mosquitoes lay their eggs, singly or in rafts, on or near standing water or along the margins of rivers that are subject to periodic flooding. Under suitable environmental conditions, the eggs hatch within a few hours into tiny larvae, which feed on microscopic organic suspended materials.

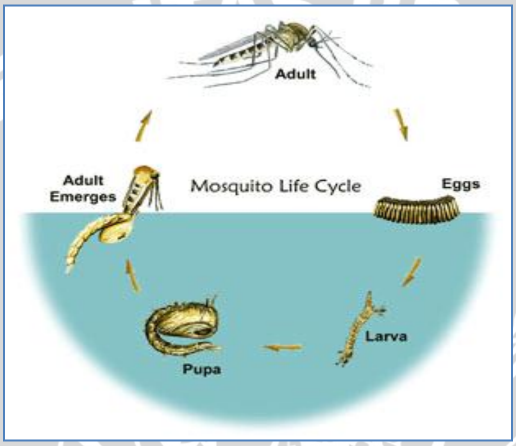

Figure 5 life cycle of mosquitoes (Floore, 2010)

The larvae undergo four different stages of development (instars) within an average of ten days, depending on the water temperature and availability of resources. The final stage is usually the longest, lasting several days, after which pupation occurs. The 'comma-shaped' pupa swims efficiently just under the water surface and breathes air but is no longer able to feed. After several days, the adult mosquito then emerges from the pupal case and rests above water for a short time until blood is pumped into the wings. Mating usually follows soon after the first flight and most females then immediately search for a blood meal. After ingestion of sufficient protein, the eggs develop in the female and she searches for a suitable egg-laying site. Female mosquitoes have a life span of anywhere from a couple of weeks to several months (Floore, 2010).

2.5.5 Medical importance

The diseases that are spread by *Culex* mosquito include West Nile virus, filariasis, Japanese encephalitis, St. Louis encephalitis, Western equine encephalomyelitis, and California encephalomyelitis (Brown & Neva, 1994). From all diseases stated above, Japanese encephalitis and filiariasis are the most occurant in Asia (Stuart, 2008).

2.5.5.1 Japanese encephalitis

Japanese encephalitis is the leading cause of viral encephalitis (brain infection) in Asia. The disease occurs chiefly in China, Korea, Southeast Asia and the Indian subcontinent. Japanese encephalitis is transmitted by *Culex* mosquitoes, which breed in ground pools, especially flooded rice fields, and bite primarily after dusk. The virus lives principally in domestic pigs and Ardeid birds. Transmission of Japanese encephalitis is therefore greatest in rural, agricultural areas where rice paddies and pig farming co-exist. The risk may be increased by heavy rainfall and irrigation. Most infections are asymptomatic. But encephalitis, when it occurs, is severe and frequently leads to death or permanent brain damage (CDC, 1998).

2.5.5.2 Filariasis

Lymphatic filariasis or elephantiasis is one of the most debilitating and disfiguring scourge among all diseases. It is a major public health problem in many South-East Asian countries. Nine out of the 11 countries in the Region are known to be endemic for filariasis. The infection is caused by helminthic worms inhabiting the lymphatic (Turkington, 2006).

All the three lymphatic filaria parasites such as *Wuchereria bancrofti*, *Brugia malayi* and *B. timori* are prevalent in the region. Bancroftian filariasis transmitted by the ubiquitous principal vector, *Culex quinquefasciatus*, is the most predominant infection in South Asia while Brugian infections transmitted by *Mansonia* and *Anopheles* vectors predominate in Indonesian Archipelago (Turkington, 2006).

Lymphatic filariasis is one the only six infectious diseases considered eradicable by WHO with the available tools. Though the disease is not fatal, it is responsible for considerable morbidity causing social stigma among men, women and children. It is usually acquired during early childhood. It mainly afflicts poor people in both urban and rural areas. (WHO, 2006)

2.5.6 Mosquitoes Control

Since nineteenth century, it was discovered that certain species of insects, other arthropods and freshwater snails were responsible for the transmission of some important diseases. Since effective vaccines or drugs were not always available for the prevention or treatment of these diseases, control of transmission often had to rely mainly on control of the vector. Vector is controlled by screening of houses, the use of mosquito nets, the drainage or filling of swamps and other water bodies used by insects for breeding, and the application of oil or Paris green to the breeding places (Rozendaal, 1997).

Mosquito control manages the population of mosquitoes to reduce their damage to human health, economic, and enjoyment. There are many methods used for mosquito control. Some target the larval stage, while others are used to kill or repel adults. Much of modern mosquito control is no longer dependent on pesticides but specialized organisms that eat mosquitoes, or infect them with a disease. Such methods can even be used in conservation areas, like the

"Forsyth refuge", where some major mosquito control is performed and monitored using "killifish" and juvenile eels. The success is documented with most advanced underwater microscopes like the ecoSCOPE. However, outbreaks of human mosquito-borne diseases may still result in fogging with products that are less toxic than those used in the past (Baietto, 2007).

2.5.7 Insecticide

An insecticide is any substance or mixture of substances intended to prevent, destroy, repel, or mitigate any insects, rodents, nematodes, fungi, weeds, or other forms of life declared to be pests. Based on the insect stage, it has ability as an imagocide that was aimed at mature insects, larvacide that was aimed at the insect larva and ovacide that was aimed to kill his egg. Based on the place of the entry of insecticide it could be classed onto contact poison which entered the insect body through skin, stomach poison that entered through the mouth and fumigans that entered the insect body through the insect respiratory tract (Soedarto, 1990).

Several factor like the insect species, stage of mosquitoes, mosquitoes habitat, and mosquitoes biological characteristics should be take care to choose the right insecticide that can be used to killed the mosquitoes. This is because different stage of mosquitoes, so different method of killing it (Sharkarama, 2008).

The good insecticide has several characteristics such as has big and fast power killed but safe for humankind and the animal when applies to them. The structure of chemistry chain was stable and was not easy to be burnt, easy to use and also easy to be mixed with solvent. Another characteristic should be care is cheap and easy to get and they are not coloured and have the stimulating smell (Gandahusada, 2003).

One of the conditions to be able to be said as good insecticide was if this insecticide has Quick Knockdown Effect which is having capacity to kill insects in the big number and in fast time (Astari, 2005). Knockdown Time (KT) was time that was needed by insecticide to drop mosquitoes. It was measured by counting the number of mosquitoes that fell for the interval of specific time until the entire mosquito died. Time that was needed to all the mosquitoes fall was mentioned as KT100, whereas time that was needed to half of the number of mosquitoes fell was mentioned as KT50 or the Knockdown Time Median. This Knockdown Time median could be afterwards used to know the effectiveness from an insecticide by using Insecticide Score (Astari, 2005).

Tabel 2.1. Insecticide Score based on KT50 (WHO.2006)

KT50(minutes)	Score	Knockdown effect	Interpretation
>50	0	-	-
31-49	1	+	Weak Knockdown
16-30	2	+	Weak Knockdown
11-15	3	+	Weak Knockdown
5-10	4	++	Strong Knockdown
<5	5	+++	Quick Knockdown

An insecticide were stated had knockdown effect if the score of the median knockdown valuable 1–5. Thought 3 meant the median knockdown was in extension 11- 15 minutes that were interpreted had knockdown effect scrutinised weak. Thought 4 meant the median knockdown was in extension 5 - 10 minutes that were interpreted had knockdown that was strong. And thought 5 meant the median knockdown was in extension < 5 minutes that were interpreted that this insecticide had “Quick Knockdown Effect” (WHO, 2003)

2.6 Cinnamon (*Cinnamomum burmannii)*

Cinnamon *(Cinnamomum burmannii)* is native to Southeast Asia and Indonesia. It is normally found in West Sumatra in the region of the city of Padang. It is an introduced species in other parts of the subtropical world, particularly in Hawaii, where it is naturalized and invasive, spreading slowly on several islands (Starr, 2003). *Cinnamomum burmannii*, along with other Cinnamomum species is cultivated for a variety of purposes. The aromatic bark is used for making spices such as cinnamon, perfumery, and medicine (Starr, 2003). In the medicine, the Cinnamon *(Cinnamomum burmannii)* is used as astringent in the treatment of diarrhea and flatulence (Harvey, 2010).

2.6.1 Taxonomy

The taxonomy of the *Cinnamomum burmannii* is:

Kingdom : Plantae

Phylum : Magnoliophyta

Class : Magnoliopsida

Order : Laurales

Family : Lasiocampoidea

Subfamily : Lauroideae

Genus : Cinnamomum

Species : *Cinnamomum burmannii* (Backer et. al, 2008).

Figure 6 Stick of *Cinnamomum Burmannii (Harvey, 2010)*

2.6.2 Morphology of Cinnamon *(Cinnamomum burmannii)*

Cinnamomum burmannii is a small evergreen tree 10–15 metres (32.8–49.2 feet) tall, belonging to the family Lauraceae, and is native to India, Sri Lanka, Bangladesh, and Nepal. The bark is widely used as a spice due to its distinct odour. The leaves are ovate-oblong in shape, 7–18 cm (2.75–7.1 inches) long. The flowers, which are arranged in panicles, have a greenish colour, and have a distinct odour. The fruit is a purple one-centimetre berry containing a single seed. Its flavour is due to an aromatic essential oil that makes up 0.5% to 1% of its composition. The pungent taste and scent come from cinnamic aldehyde or cinnamaldehyde and, by the absorption of oxygen as it ages; it darkens in colour and develops resinous compounds. Chemical components of the essential oil include ethyl cinnamate, eugenol, cinnamaldehyde, beta-caryophyllene, linalool, and methyl chavicol (Grieve, 2010).

2.6.3 The bioactive compounds of Cinnamon *(Cinnamomum burmannii)*

The use of herbal remedies, including the herb Cinnamon *(Cinnamomum burmannii)* are popular as an alternative to standard Western Allopathic Medicine for a variety of problems such as fungal infections, diabetes, menaopouse, diarrhea, and others (Duke, 2010).

There are four main compounds in Cinnamon *(Cinnamomum burmannii)* which are cinnamaldehyde, cinnamyl acetate, eugenol and anethole. Eugenol is one of the Cinnamon *(Cinnamomum burmannii)* main components that may potentially act as bioinsecticides and have quick knockdown effect on mosquitoes (Park, 2000). Cinnamon *(Cinnamomum burmannii)* consists of 5% to 10% eugenol. Eugenol is a chemical substance with $C_{10}H_{12}O_2$ dissolve in water H_2O and solvent. The pungently sweet spice and great aroma in

Cinnamon *(Cinnamomum burmannii)* with yellowish in coloured are the specific characteristic of eugenol (Peter, 2004).

Eugenol is very neurotoxic for mosquitoes. This substance will effect and disturb insect sensory nerve at the PNS and CNS. The toxicity of eugenol will disturb the nerve axon and increase the excitation phase from the neuron body cell and finally it will cause nerve cell of the insect paralyze (Ware & Whitacre, 2005). Besides that, eugenol acts as Octopamine receptor blocker, a kind of sympatomimetic receptor that control sympathetic activity. This activation will cause bronchoconstriction and lead to death of the insect (Enan, 2001).

Although eugenol is neurotoxic to mosquitoes by its fast acting contact insecticide, but it is effective on a wide variety of household pests such as cockroaches, ants, dust mites, flies, wasps, spiders, crickets, and fleas. It is also used on some ornamental plant pests such as armyworms, thrips, aphids and mites. Eugenol has little or no residual activity, so that why products based on eugenol are considered minimum risk pesticides with very low risk of damage to the environment and user (Russ, 2008).

Cinnamaldehyde, or 3-phenylprop-2-enal, is an oily yellow liquid at room temperature with a boiling point of 246 °C. This pale yellow viscous liquid occurs naturally in the bark of cinnamon trees and other species of the genus Cinnamomum. Cinnamaldehyde can be made synthetically but is more commonly obtained from the steam distillation of the oil of Cinnamon *(Cinnamomum burmannii)* which is a much more efficient process. Cinnamaldehyde is reported to be a good insecticide. It disturbs energy production possibly by interference with glucose uptake or utilization of glucose which is disrupting the movement of mosquitoes (Brown, 2006). Cinnamon *(Cinnamomum burmannii)* consists of 65% to 75% cinnamaldehyde. It is non-toxic but can irritate skin if in contact for too long. As with many components of essential oils cinnamaldehyde displays antiviral, antibacterial and antifungal properties. These properties support the medicinal and soothing properties of Cinnamon (Burnham, 2006).

CHAPTER 3

CONCEPTUAL FRAMEWORK

3.1 Conceptual framework diagram

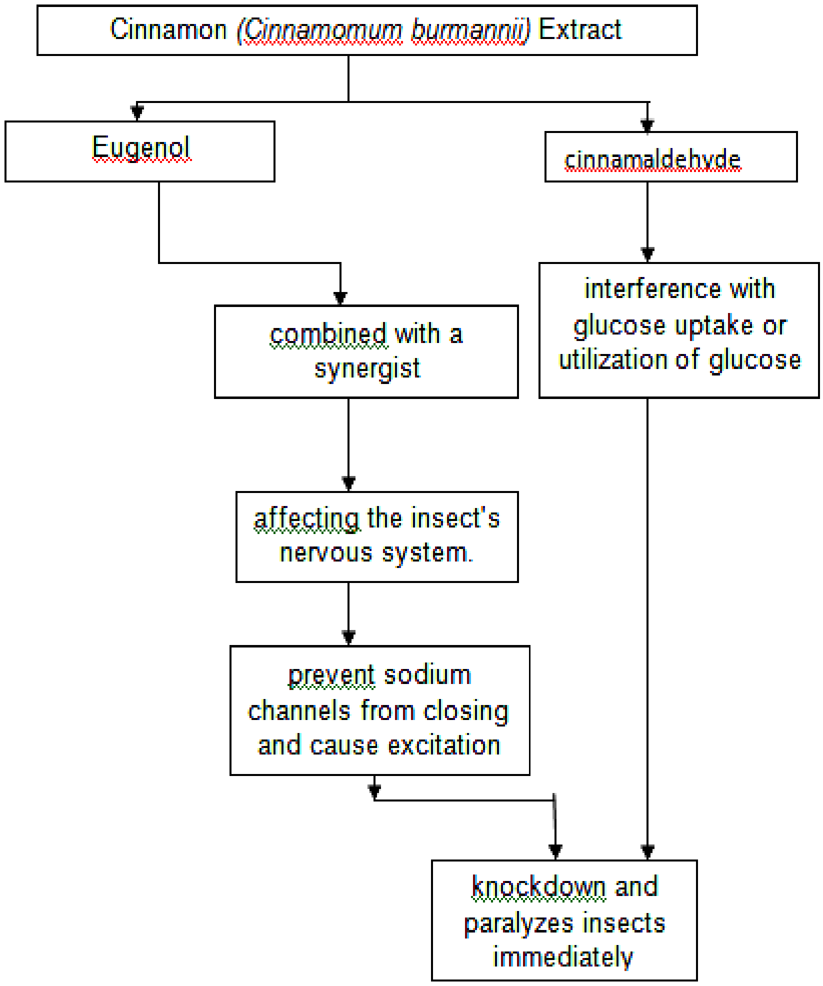

Figure 3.1: Conceptual framework diagram

Eugenol is one of the Cinnamon *(Cinnamomum burmannii)* main components that act as bioinsecticides and have quick knockdown effect on mosquitoes. Eugenol is very neurotoxic for mosquitoes. Eugenol affects and inhibits the nervous system of insects by causing multiple action potential in the nerve cells by delaying the closing of an ion channel. Nerve cell membranes have a specific electrical charge. Altering the amount of ions (charged atoms) passing through ion channels causes the membranes 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.

Eugenol acts as a contact poison, affecting the insect's nervous system. Eugenol, when combined with a synergist that works by restricting an enzyme insects use to detoxify it, then becomes one of the fastest acting insecticides known. Even before it kills, it knocks down and paralyzes insects almost immediately.

While cinnamaldehyde, it act on energy production by interference with glucose uptake or utilization of glucose that needed by insects for activities. Those may cause the knockdown and paralyzed the insects.

3.2 Research hypothesis

3.2.1 Cinnamon *(Cinnamomum burmannii)* extract has a Quick Knockdown Effect towards *Culex sp.*

CHAPTER 4

METHODOLOGY

4.1. Research Design

The research design that was being used is a true-experimental post test of control group design. The purpose is to measure the concentration of Cinnamon *(Cinnamomum burmannii)* that has quick knockdown effect towards *Culex sp.*

4.2. Population and Sample

The research population is adult mosquitoes of *Culex sp*. from Parasitology Laboratory of Brawijaya University. The mosquitoes must have inclusion and exclusion criteria. The inclusion criteria include:

1. Adult *Culex sp*. To identify the Culex, it can be seen from the way of its landing. *Culex* sp lands parallel with the horizontal line. The colour of the adult *Culex* is light brown with the proboscis, thorax, wings, and tarsi darker than the rest of the body. (Neva and Brown, 1994)The head is light brown. The scales of the thorax are narrow and curved. The abdomen has pale, narrow, rounded bands on the basal side of each tergite. *Culex* sp is nighttime-active, so they are easily to be catch during dawn or night.(Larrick and Conelly, 2009)
2. Active movement of mosquitoes.

Other than that, exclusion criteria are adult *Culex sp*. mosquitoes that are died during experimental and do not include in inclusion criteria.

The sample of this research is adult *Culex sp*. mosquitoes that have been selected and fulfill inclusion criteria. 25 adult *Culex sp*. mosquitoes were used for each experiment. (WHO, 2006)

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

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

E1: Spraying of Cinnamon *(Cinnamomum burmannii)*12.5% extract solution

E2: Spraying of Cinnamon *(Cinnamomum burmannii)*25% extract solution

E3: Spraying of Cinnamon *(Cinnamomum burmannii)*50% extract solution

Repeat of the experiment based on the formula below (Tjokonegoro, 2001):

$$\boxed{P\,(n-1) \geq 16}$$

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

Experiment was held in Parasitology Laboratory of Brawijaya University in August to September 2010.

4.4. Identification of Variable

4.4.1. Independent Variable

The independent variable in this research is a Cinnamon *(Cinnamomum burmannii)* extract concentration in percent (%).

4.4.2. Dependent Variable

The dependent variable in this research is knockdown time in minute.

4.5 Research Material and Equipment

4.5.1 Ingredient for Preparing Cinnamon *(Cinnamomum burmannii)* Extract:

- Cinnamon *(Cinnamomum burmannii)*
- Ethanol 96%
- Aquadest
- Aceton

4.5.2 Instrument for Preparing Cinnamon (*Cinnamomum burmannii)* Extract:

- Oven
- Blender
- Filter
- Filter paper

- Analytical weight apparatus
- Extraction glass
- Evaporation vacuum
- Ethanol container
- Evaporator container
- Rotary evaporator
- Water pump
- Water bath
- Plastic pipe
- Evaporator

4.5.3 Instrument for Preparing Cinnamon (*Cinnamomum burmannii)* Test:

- mosquitoes container
- mug
- mosquitoes net
- acetone

4.6. Operational Definition

1. Cinnamon *(Cinnamomum burmannii)* extract is an evaporation product from extraction of dried Cinnamon *(Cinnamomum burmannii)* using ethanol. This process produced oil which is water insoluble and was assumed to have 100 % concentration. (Astari, 2005)
2. Acetone solution 1% is used for negative control.
3. Adult mosquitoes of *Culex sp.* were obtained from their breeding place in Parasitology Laboratorium of Brawijaya University.
4. Knockdown Time is a data of mosquitoes falling per-interval time by using Abbot formula. KT50 (Median knockdown) is a time require to paralyze

50% of mosquitoes population at certain concentration. Mosquitoes were considered successfully knockdowned after being paralysed for 5 minutes. (Tze, 2007)

5. Mosquitos' container: the container is square shape with the box size is 25cm x 25cm x 25cm. At each side of the box is covered by glass and at one part of the box is covered by net for spraying process and to prevent the mosquitoes escape from the box (Brown, 1994)
6. Glucose solution 10% is used for mosquitoes feeding.
7. Cinnamon *(Cinnamomum burmannii)* was obtained from Dinoyo Market in Malang.

4.7 Research Procedure

4.7.1 Cinnamon (*Cinnamomum burmannii)* Extraction (Vogel, 1946)

Extraction process of Cinnamon *(Cinnamomum burmannii)* was done according to "Technique of Sample Extraction" where ethanol 96% was used as solvent. The process of extraction was as following:

1. Cinnamon *(Cinnamomum burmannii)* was dried under the sun for 1 hour and heats it in oven 60-89 degree for 12 hour until it contains ±5% water.
2. Blend the dried Cinnamon *(Cinnamomum burmannii)* in order to get 300 mg of a very fine size of Cinnamon *(Cinnamomum burmannii)* (like a powder).
3. The blended Cinnamon *(Cinnamomum burmannii)* is soak with ethanol in a bottle for a week until the active substance in the Cinnamon *(Cinnamomum burmannii)* is solute in ethanol.

4. Stop the extraction and put into the other bottle.

5. After finished the extraction process, this extract is evaporate to separate the active substance from the ethanol.

4.7.2 Evaporation of Cinnamon *(Cinnamomum burmannii)* Extract

1. Set evaporator to a permanent pillar, so that it will be in slantingly position at 30° to 40° from experimental table.
2. The ethanol soak is transfer to the extraction separation container
3. This container is connected to the base of evaporator where else spiral cooler is connected above the evaporator.
4. Water pump is placed in the container which contains aquadest. Water pump is connected to electrical source which causing aquadest to flow and fills the spiral cooler (wait until water are well distributed)
5. Put one set of evaporation, until half of the extraction separation covered with aquadest in a water bath.
6. Vacuum and water bath are connected to the source of electricity. The temperature of water bath is increase to 70°C (suitable with boiling point of ethanol).
7. Let the process to occur until evaporated solution accumulates 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 will be 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 Cinnamon *(Cinnamomum burmannii)* was obtained. This extract was used in the experiment.

4.7.3 Acclimatization

The Acclimatization of Culex sp is 3-5 days in Laboratorium condition. After 4 hour and before the experiment, these mosquitoes were fasting.

4.7.4 Stock Solution Preparation

1% is a dose where the 1ml that dissolve in 99ml solvent. 1% of acetone was used as the solvent of Cinnamon *(Cinnamomum burmannii)* extract. The stock solution of the Cinnamon *(Cinnamomum burmannii)* extract is produce to make it easier in processing test solution (Astari, 2005).

4.7.5 Processing Stock Solution

The stock Solution of Cinnamon *(Cinnamomum burmannii)* Extract was diluted with acetone 1% solution until getting the required dosage, by using the dilution formula as below:

$$\boxed{M1 \times V1 = M2 \times V2}$$

Explanation:

M1 =Concentration of Stock Solution (10000ppm)

M2 = Concentration of Required Solution

V1 = Volume of Stock That Will Be Diluted

V2 = Total Volume of Solution

The end volume of the solution that is needed in each experiment was 4ml. To get 4ml volume stock solution was being added with acetone 1% until it reached the required concentration.

4.7.6 Preparation of the Sample.

The adult *Culex sp*. that was used as the sample was being catched by using plastic bottles, in area of Parasitology Laboratorium Medical Faculty of Brawijaya University. All this adult *Culex sp.* was directly entered to mosquito container.

i. Measurement Time of Insecticides Knockdown Time

1. Trials were done by using 5 glass boxes with the size 25cm x 25cm x 25cm.
2. The solution of Cinnamon *(Cinnamomum burmannii)* extract with 12.5%, 25%, and 50% 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 be sprayed to every container until the solutions in each bottle were finished. The specific explanation are as below:
5. container 1 was sprayed by using 4ml acetone 1% solution (negative control)
6. container 2 was sprayed using 4ml of Cinnamon *(Cinnamomum burmannii)* extract solution 12.5%
7. container 3 was sprayed using 4ml of Cinnamon *(Cinnamomum burmannii)* extract solution 25%
8. container 4 is sprayed using 4ml of Cinnamon *(Cinnamomum burmannii)* extract solution 50%.
9. The number of mosquitoes that fall in each experiment was counted after the spraying on 0 minute, 5^{th} minute, 10^{th} minute, 15^{th} minute,

20^{th} minute, 25^{th} minute, 30^{th} minute, 35^{th} minute, 40^{th} minute, 45^{th} minute, 50^{th} minute, 55^{th} minute and 60^{th} minute.

10. This research was done with 5 times repetition for each experiment.

4.7.8 Observation

The observations were done on 0 minute, 5^{th} minute, 10^{th} minute, 15^{th} minute, 20^{th} minute, 25^{th} minute, 30^{th} minute, 35^{th} minute, 40^{th} minute, 45^{th} minute, 50^{th} minute, 55^{th} minute and 60^{th} minute. The conditions of each mosquito group were observed to find the changes of falling mosquitoes. The numbers of falling mosquito were counted and this data were inserted into the table.

Quick knockdown of insecticides was determined by the number of mosquito that fall in 60 minute with 5 minute interval. (WHO, 2006)

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 mosquito in certain dosage while KT100 is the needed for the paralysis of 100% of mosquito certain dosage.

To evaluate the differences between definite and indefinite data, Oneway Anova analysis was used. This can be 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 has four numerical variable (12.5%, 25%, 50%) added with one negative control (acetone,1%). The second group was the interval of subtype time (0-60 minute with 5 minute interval). The Oneway Anova statistic test is chosen due to the complexity of the data and to fulfill the standard of the statistic (miniTab V13.30).

The entire above test was ended by Post Hoc Tuckey Test (Duncan test - miniTab V13.30) to know the significant data at each experiment and sub experiment depends on the concentration (numeric) and time (interval).

Flow Chart of Work

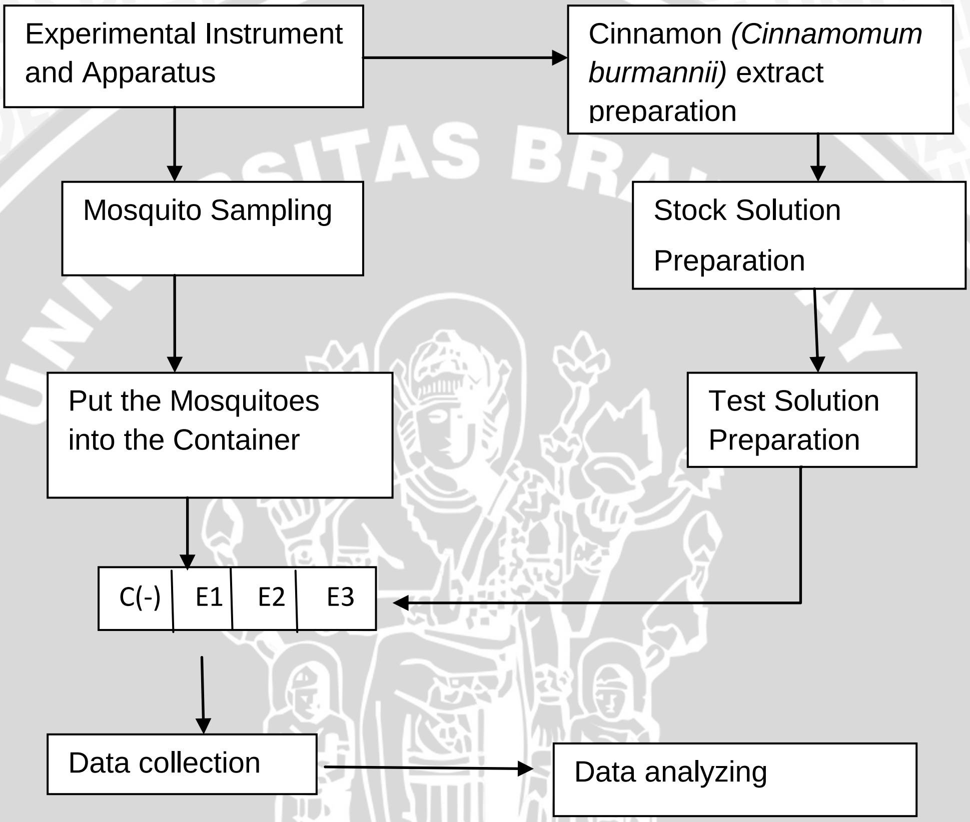

Figure 4.1: Flow chart of work

Explaination :

C(-): Spraying of acetone solution 1%

E1: Spraying of 12.5% Cinnamon *(Cinnamomum burmannii)* extract solution

E2: Spraying of 25% Cinnamon *(Cinnamomum burmannii)* extract solution

E3: Spraying of 50% Cinnamon *(Cinnamomum burmannii)* extract solution

CHAPTER 5

RESEARCH RESULT

5.1 Preliminary Study

For the knockdown effect of Cinnamon (*Cinnamomum burmannii*) towards adult *Culex* sp, preliminary study was done to find the optimum dose to knockdown the mosquitoes. For the preliminary study, three concentrations were used, which were 12.5%, 25% and 50%. The result of preliminary study is shown in Table 5.1 below:

Table 5.1 Number of *Culex* sp knockdowned (N:25)

Time (minute)	Concentration		
	12.5%	25%	50%
5	0	6	10
10	0	6	10
15	1	7	12
20	1	8	13
25	2	8	13
30	2	9	13
35	3	9	14
40	3	9	14
45	3	9	14
50	4	10	15
55	5	10	15
60	5	10	16

As the number of mosquitoes' knockdown were obtained, the lowest concentration that was used, 50%, caused more than 50% of mosquitoes' knockdown at twentieth minute. So from this result, the three concentrations were used for the true experiment since the KT_{50} was successfully obtained.

5.2 True Experiment

Four concentrations of extract in the form of solution were used, which were 12.5%, 25%, and 50%, together with acetone 1% as the negative control. The calculations of mosquitoes knockdown were done on 5th minutes, 10th minutes, 15th minutes, 20th minutes, 25th minutes, 30th minutes, 35th minutes, 40th minutes, 45th minutes, 50th minutes, 55th minutes, and 60th minutes. The repetitions were done for five times. Result of numbers of *Culex* knockdown from the First Repetition until Fifth repetition can be obtained from Table 1.

5.3 Knockdown effect of cinnamon (*cinnamomum burmannii* extract) towards adult *Culex sp*. Based On The Concentration and Time Interval

Based on the Appendix 1, the different concentration gives the different effect towards the number of mosquitoes knockdown in each of the repetition. However, no knockdown effect was seen for the repetition using the negative control. Result of the analysis for each concentration using 5 minutes interval is simplified as below:

Table 5. 2: Number Of *Culex* Knockdown in Mean **±** SD (N:25 *Culex* sp)

Time (minutes)	Number of *Culex* Knockdown from 25 Adult Culex sp in each group Mean ± SD				P value
	Control (-) (N:25)	12.5% (N:25)	25% (N:25)	50% (N:25)	
5	0±0.00	0.00 ± 0.00	6.00 ± 0.71 (0.00)**	10.80 ± 0.84 (0.00)**	0.00*
10	0±0.00	0.00 ± 0.00	6.20 ± 0.84 (0.00)**	11.00 ± 0.71 (0.00)**	0.00*
15	0±0.00	0.80 ± 0.84	6.80 ± 0.7184 (0.00)**	12.00 ± 1.00 (0.00)**	0.00*
20	0±0.00	1.80 ± 0.84	7.20 ± 0.84 (0.00)**	12.20 ± 0.84 (0.00)**	0.00*
25	0±0.00	2.20 ± 0.84	7.80 ± 0.84 (0.00)**	12.20 ± 0.84 (0.00)**	0.00*
30	0±0.00	2.20± 0.84	8.00 ± 0.71 (0.00)**	13.00 ± 0.00 (0.00)**	0.00*
35	0±0.00	2.80 ± 0.84	8.20 ± 0.84 (0.00)**	13.80 ± 0.84 (0.00)**	0.00*
40	0±0.00	3.00 ± 0.71	9.00 ± 0.00 (0.00)**	14.20 ± 0.84 (0.00)**	0.00*
45	0±0.00	3.20 ± 0.84	9.80 ± 0.84 (0.00)**	14.80 ± 0.84 (0.00)**	0.00*
50	0±0.00	4.00 ± 0.00	10.00 ± 0.71 (0.00)**	15.80 ± 0.84 (0.00)**	0.00*
55	0±0.00	4.80 ± 0.84	10.60 ± 1.14 (0.00)**	15.80 ± 0.84 (0.00)**	0.00*
60	0±0.00	5.00 ± 0.71	11.20 ± 0.84 (0.00)**	16.20 ± 1.30 (0.00)**	0.00*

*The values of significancy of each concentration were being compared between groups

**The values of significancy of each concentration were being compared with the lowest concentration (12. 5%). These shows significancy for all minutes and concentration.

The table above shows no knockdown was observed when the mosquitoes were sprayed with the negative control, acetone 1%. Besides that, starting with the 5th minute, using the 50% concentration, half were knockdown but on the 60th minute, not all 25 mosquitoes were knockdown.

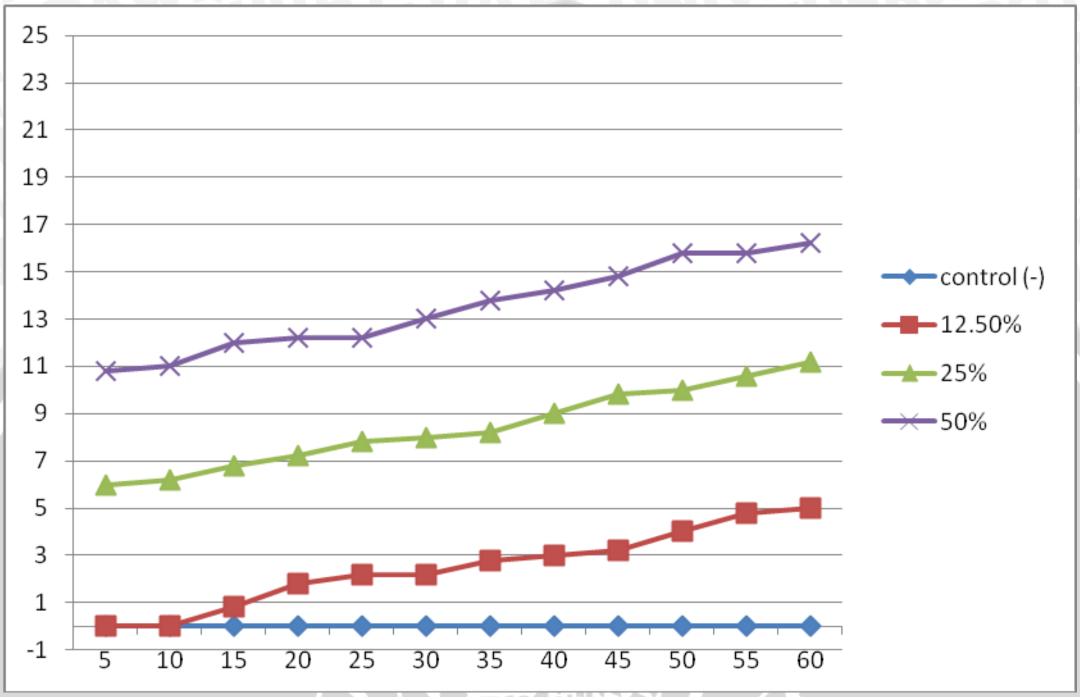

Figure 5.3 Number of *Culex*'s knockdown for each concentration during each time interval

The diagram above shows that there were 0 knockdown starting with 12.5% concentration on the 5th minute. The number of mosquitoes knockdown slowly increased for this concentration which reached 5 knockdown at the 60th minute. For the 25% concentration, there were 6 knockdown at the 5th minute and it gradually increase until it reached 12 knockdown at the 60th minute. As for the 50% concentration, it starts with 11 knockdown at the 5th minute and 16 knockdown at the 60th minute.

5.4 Data Analysis with Normality Test (Kolmogorov-Smirnoff)

After analyzing the data with the Normality Test (Kolmogorov-Smirnoff), the significant values were all found to be 0.20 (>0.05). Thus, the distribution of the data is normal.

5.5 Data Analysis with Homogeneity of Variances Test (Levene Statistics)

The data were then analyzed with the Homogeneity of Variances Test (Levene Statistic) and the significant values were found to be between 0.05 – 0.08 (>0.05). Thus the set of data was said to be homogenous.

5.6 Data analysis with ANOVATest

Result of this research was analyzed using the statistical analysis, SPSS 16.0 version For Windows. Data of the result were grouped and put into the table, and the significances were tested using One-way ANOVA (Analysis of Variance). From the Appendix 5, it is can be seen that there were significant result started from 5th, 10th, 15th, 20th, 25th, 30th, 35th, 40th, 45th, 50th, 55th, and 60th minute, which is 0.00. The significant results were obtained as there were influences from the different concentrations (12.5%, 25%, and 50%) that were used in the experiment.

5.7 Data analysis using Post Hoc TUKEY Test

After analyzing the data using the One-Way ANOVA method, double comparison towards different concentration and time was done using Post Hoc TUKEY method. The mean differences were all significant for all of the concentrations being tested during 5th, 10th, 15th, 20th, 25th, 30th, 35th, 40th, 45th, 50th, 55th, 60th minutes. In the homogenous subset analysis, for the 5th, 10th, 15th, 20th, 25th, 30th, 35th, 40th, 45th, 50th, 55th and 60th minute, all concentrations are in their respective subsets.

CHAPTER 6

DISCUSSION

Cinnamon (*Cinnamomum burmannii*) has found to be effective in killing mosquito, mosquito larvae and also as an insect repellent (Starr, 2003). The compounds eugenol in Cinnamon will disturb the peripheral nervous system and the central nerve of the mosquitoes and will increase the excitation phase from the neuron body cell and finally it will cause nerve cell paralyze (Ware & Whitacre, 2005). The objective of this research is to gain more specific information through the knockdown effect. One of the conditions that are needed for the good insecticide is to have quick knockdown effect that means the insecticide has the ability to cause mosquito knockdown in large amount and in rapid time.

The data was analyzed with Normality Test (Kolmogorov-Smirnov) and Homogeneity Test (Levene Test). The data showed that the result was normal distribution and homogeny variance. Therefore, One-Way ANOVA were analyzed and the result was significantly different among the three concentrations of Cinnamon (*Cinnamomum burmannii*) extract and time interval towards knockdown of *Culex sp*. mosquitoes.

Post Hoc Tukey HSD Test resulted that between the concentration and time, for every concentration of Cinnamon (*Cinnamomum burmannii*) extract showed that at 5th minutes, 10th minute, 15th minute, 20th minute, 25th minute, 30th minute, 35th minute, 40th minute, 45th minute, 50th minutes 55th minute, and 60th minute the significant value (p) was 0.00. When $p < 0.05$ its mean that the data was significantly different.

In the homogenous subset analysis, for 5th, 10th, 15th, 20th, 25th, 30th, 35th, 40th, 45th, 50th, 55th and 60th minute, all concentrations are in different subsets which means that the knockdown mosquitoes were significant for each different concentration during those minutes.

According to the result from table 5.2, the *Culex* mosquitoes knockdown to be caused by the active ingredients from Cinnamon (*Cinnamomum burmannii*) extract. Eugenol is very neurotoxic for mosquitoes. This substance will effect and disturb insect sensory nerve at the PNS and CNS. The toxicity of eugenol will disturb the nerve axon and increase the excitation phase from the neuron body cell and finally it will cause nerve cell of the insect paralyze (Ware & Whitacre, 2005). Besides that, eugenol acts as Octopamine receptor blocker, a kind of sympatomimetic receptor that control sympathetic activity. This activation will cause bronchoconstriction and lead to death of the insect (Enan, 2001). Although eugenol is neurotoxic to mosquitoes by its fast acting contact insecticide, but it is effective on a wide variety of household pests such as cockroaches, ants, dust mites, flies, wasps, spiders, crickets, and fleas. It is also used on some ornamental plant pests such as armyworms, thrips, aphids and mites. Eugenol has little or no residual activity, so that why products based on eugenol are considered minimum risk pesticides with very low risk of damage to the environment and user (Russ, 2008).

There are few factors that may influence the result of this research. For example, the time intervals of keeping the cinnamon extract. It is believed that the longer the extract being kept, the ability to cause mosquito's knockdown might decrease hence affecting the result of the research (Gardiner, 2008). This was done by doing experiment in short interval between each repetition.

Besides that, the environment in which the experiment being held might influence the result, for example, the exposure of mosquitoes to other chemical contents in the experiment room. There may be other particles from other experiment coincidently being conducted that day that still in the air at the time this research being done. This condition excluded by researcher by cleaning the wall of experiment room using acetone 1%. As for the spraying method, the researcher had tried to ensure that the mosquitoes were not being affected by the spraying technique. This was done by spraying the extract solution at the area of the container before entering the adult *Culex sp.* However, these entire factors have been tried to be minimized.

From WHO Insecticide Score 2006 (table 2.1), the effectiveness of cinnamon (*Cinnamomum burmannii*) concentration (12.5%, 25% and 50%), can be defined based on KT50. Quick knockdown effect will be achieved if KT50 was found in the lower concentration and less than 5 minutes which according to WHO insecticide score equals to 5. Cinnamon (*Cinnamomum burmannii*) extract with 50% concentration caused knockdown of 13 mosquitoes at 30^{th} minute. It can be said that the KT_{50} of cinnamon (*Cinnamomum burmannii*) extract towards adult *Culex sp* were reached at 30^{th} minutes of 50% cinnamon concentration which when interpreted using WHO insecticide score will equals to 2. Thus, this bioinsecticide has weak knockdown effect.

According to the study of Fahmi, 2008, cinnamon extract has been proven to have insecticidal effect toward adult *Aedes sp.* Result of the study showed that 15% cinnamon concentration killed 72% Aedes sp. in 24 hours while 25% cinnamon concentration killed 97% Aedes sp, in 24 hours.

Compared to researcher's study the 50% cinnamon extract concentration cause knockdown of 13 mosquitoes at 30th minute and in one hour, there are only 16 mosquitoes knockdown. There are different in result between the study done by Fahmi and this study because in his study he observe the insecticide effect of cinnamon in 24 hours while in this study the knockdown effect of cinnamon in one hour was observed. Based on this research, it can be said that cinnamon extract is more effective as insecticide instead of as knockdown.

When compared with chemical insecticide, cinnamon (*Cinnamomum burmannii*) extract fulfilled five out of ten criteria of good insecticide. The comparison between cinnamon (*Cinnamomum burmannii*) and chemical insecticide are simplified in table below.

Table 6.1 Comparison of Chemical Insecticide and Cinnamon (*Cinnamomum burmannii*)

Criteria of good insecticide	Chemical insecticide (malathion)	Cinnamon (*Cinnamomum burmannii*) extract	Conclusion
1) Ability to kill rapidly	+	-	Cinnamon has insecticidal effect but takes time to work
2) Safe	-	+	Cinnamon has no known adverse side effects
3) Cheap	+	-	Chemical insecticide more affordable than cinnamon extract.
4) Easy to get	+	+	Easily found in Indonesia & many parts of the world.
5) Chemically stable	+	-	Chemical insecticide more stable than cinnamon extract
6) Inflammable	-	+	Cinnamon has lower boiling point
7) Easy to use	+	+	Both can be use by spraying method
8) Easy to be diluted	+	-	Chemical insecticide easily diluted in water. But in cinnamon extract, it can dilute in aceton 1%.
9) Colourless	+	-	Cinnamon extract is brownish in color
10) Odour	-	+	Cinnamon has a fragrant smell

From the table above, there were five criteria that were not fulfilled were the ability to kill mosquito in rapid time and in large amount, affordable to get, stability of chemical substance, solubility and the colour of insecticide colourless. This is because cinnamon extract has insecticidal effect but it takes longer time to work, not chemically stable in storage of the substance in a long period because it is easily oxidize in the presence of oxygen. It also expensive to obtain, difficult to dilute and is brownish in colour. From the table above, it is shown that cinnamon (*Cinnamomum burmannii*) extract is still qualified as bioinsecticide even it is a weak bioinsecticide.

CHAPTER 7

CONCLUSION AND SUGGESTION

7.1 Conclusion

From this experiment, it can be concluded that:

1. From all the concentration being used in the experiment, concentration 50% is the lowest concentration that has the effect of KT50 at the 30th minute and has score of 2 from WHO Insecticide Score.
2. Cinnamon (*Cinnamomum burmannii*) fulfilled five out of ten criteria needed as a good insecticide and it is qualified as bioinsecticide.
3. Cinnamon (*Cinnamomum burmannii*) extract has weak knockdown effect as the insecticide towards adult *Culex sp.* because the active substances from crude extract of cinnamon are in small amount.

7.2 Suggestion

1. Further study should be done to reveal the exact active substance of cinnamon (*Cinnamomum burmannii*) that causes the knockdown effect of the adult *Culex sp.,* an experiment using purified of cinnamon can be held.

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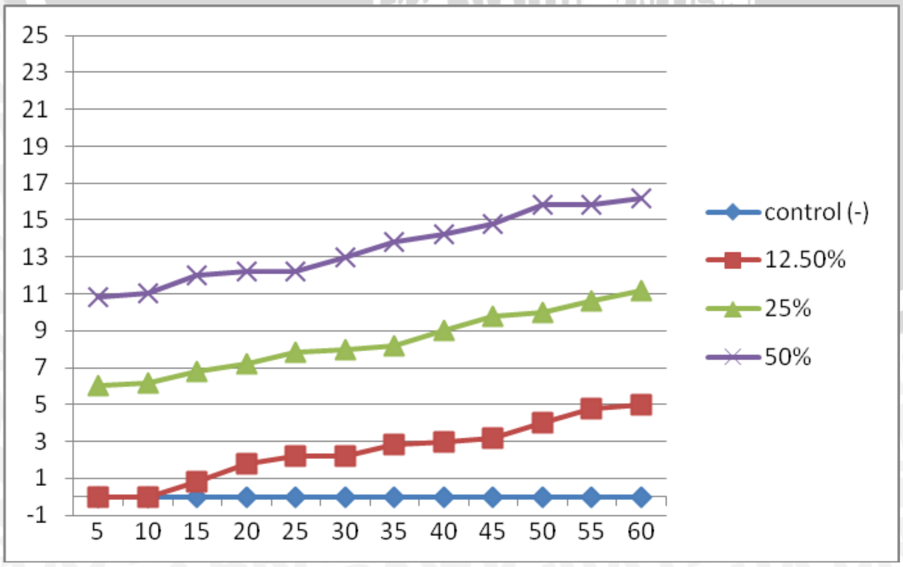
APPENDICES

Appendix 1: Result of Knockdown Time of Cinnamon Extract (*Cinnamomum burmannii*) as Bioinsecticides towards *Culex* sp Experiment

Concentration	12.5% concentration						25% concentration						50% concentration					
Time (minute) / repetition	1	2	3	4	5	M	1	2	3	4	5	M	1	2	3	4	5	M
5	0	0	0	0	0	0	5	6	6	6	7	6	10	10	11	11	12	10.8
10	0	0	0	0	0	0	5	6	7	6	7	6.2	10	11	11	11	12	11
15	1	0	2	0	1	0.8	6	7	7	6	8	6.8	11	11	13	12	13	12
20	2	1	3	1	2	1.8	7	8	7	6	8	7.2	12	11	13	12	13	12.2
25	3	1	3	2	2	2.2	7	8	8	7	9	7.8	12	11	13	12	13	12.2
30	3	1	3	2	2	2.2	8	8	8	7	9	8	13	13	13	13	13	13
35	4	2	3	2	3	2.8	8	9	8	7	9	8.2	13	15	14	13	14	13.8
40	4	3	3	2	3	3	9	9	9	9	9	9	14	15	15	13	14	14.2
45	4	3	4	2	3	3.2	9	10	10	9	11	9.8	14	15	15	14	16	14.8
50	4	4	4	4	4	4	9	10	10	10	11	10	15	16	15	16	17	15.8
55	5	5	4	6	4	4.8	9	10	11	11	12	10.6	15	16	15	16	17	15.8
60	5	5	4	6	5	5	10	11	12	11	12	11.2	15	16	15	17	18	16.2



Appendix 2 Normality Test

Tests of Normality[b,c,d,e,f]

	concentration	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
5 minutes	25%	.300	5	.161	.883	5	.325
	50%	.231	5	.200*	.881	5	.314
10 minutes	25%	.231	5	.200*	.881	5	.314
	50%	.300	5	.161	.883	5	.325
15 minutes	12.5%	.231	5	.200*	.881	5	.314
	25%	.231	5	.200*	.881	5	.314
	50%	.241	5	.200*	.821	5	.119
20 minutes	12.5%	.231	5	.200*	.881	5	.314
	25%	.231	5	.200*	.881	5	.314
	50%	.231	5	.200*	.881	5	.314
25 minutes	12.5%	.231	5	.200*	.881	5	.314
	25%	.231	5	.200*	.881	5	.314
	50%	.231	5	.200*	.881	5	.314
30 minutes	12.5%	.231	5	.200*	.881	5	.314
	25%	.300	5	.161	.883	5	.325
35 minutes	12.5%	.231	5	.200*	.881	5	.314
	25%	.231	5	.200*	.881	5	.314
	50%	.231	5	.200*	.881	5	.314
40 minutes	12.5%	.300	5	.161	.883	5	.325
	50%	.231	5	.200*	.881	5	.314
45 minutes	12.5%	.231	5	.200*	.881	5	.314
	25%	.231	5	.200*	.881	5	.314
	50%	.231	5	.200*	.881	5	.314
50 minutes	25%	.300	5	.161	.883	5	.325
	50%	.231	5	.200*	.881	5	.314
55 minutes	12.5%	.231	5	.200*	.881	5	.314
	25%	.237	5	.200*	.961	5	.814
	50%	.231	5	.200*	.881	5	.314
60 minutes	12.5%	.300	5	.161	.883	5	.325
	25%	.231	5	.200*	.881	5	.314
	50%	.221	5	.200*	.902	5	.421

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.

b. 5 minutes is constant when concentration = 12.5%. It has been omitted.

c. 10 minutes is constant when concentration = 12.5%. It has been omitted.

d. 30 minutes is constant when concentration = 50%. It has been omitted.

e. 40 minutes is constant when concentration = 25%. It has been omitted.

f. 50 minutes is constant when concentration = 12.5%. It has been omitted.

Appendix 3 Homogeneity of Variances

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
5 minutes	3.213	2	12	.076
10 minutes	3.213	2	12	.076
15 minutes	.222	2	12	.804
20 minutes	.000	2	12	1.000
25 minutes	.000	2	12	1.000
30 minutes	3.213	2	12	.076
35 minutes	.000	2	12	1.000
40 minutes	3.213	2	12	.076
45 minutes	.000	2	12	1.000
50 minutes	3.213	2	12	.076
55 minutes	.407	2	12	.675
60 minutes	1.876	2	12	.196

Appendix 4 Descriptive Test

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
5 minutes	12.5%	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	25%	5	6.0000	.70711	.31623	5.1220	6.8780	5.00	7.00
	50%	5	10.8000	.83666	.37417	9.7611	11.8389	10.00	12.00
	Total	15	5.6000	4.61055	1.19044	3.0468	8.1532	.00	12.00
10 minutes	12.5%	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	25%	5	6.2000	.83666	.37417	5.1611	7.2389	5.00	7.00
	50%	5	11.0000	.70711	.31623	10.1220	11.8780	10.00	12.00
	Total	15	5.7333	4.69752	1.21289	3.1319	8.3347	.00	12.00
15 minutes	12.5%	5	.8000	.83666	.37417	-.2389	1.8389	.00	2.00
	25%	5	6.8000	.83666	.37417	5.7611	7.8389	6.00	8.00
	50%	5	12.0000	1.00000	.44721	10.7583	13.2417	11.00	13.00
	Total	15	6.5333	4.80872	1.24161	3.8704	9.1963	.00	13.00
20 minutes	12.5%	5	1.8000	.83666	.37417	.7611	2.8389	1.00	3.00
	25%	5	7.2000	.83666	.37417	6.1611	8.2389	6.00	8.00
	50%	5	12.2000	.83666	.37417	11.1611	13.2389	11.00	13.00
	Total	15	7.0667	4.46361	1.15250	4.5948	9.5385	1.00	13.00
25 minutes	12.5%	5	2.2000	.83666	.37417	1.1611	3.2389	1.00	3.00
	25%	5	7.8000	.83666	.37417	6.7611	8.8389	7.00	9.00
	50%	5	12.2000	.83666	.37417	11.1611	13.2389	11.00	13.00
	Total	15	7.4000	4.30614	1.11184	5.0153	9.7847	1.00	13.00
30 minutes	12.5%	5	2.2000	.83666	.37417	1.1611	3.2389	1.00	3.00
	25%	5	8.0000	.70711	.31623	7.1220	8.8780	7.00	9.00
	50%	5	13.0000	.00000	.00000	13.0000	13.0000	13.00	13.00
	Total	15	7.7333	4.60538	1.18910	5.1830	10.2837	1.00	13.00
35 minutes	12.5%	5	2.8000	.83666	.37417	1.7611	3.8389	2.00	4.00
	25%	5	8.2000	.83666	.37417	7.1611	9.2389	7.00	9.00
	50%	5	13.8000	.83666	.37417	12.7611	14.8389	13.00	15.00
	Total	15	8.2667	4.71270	1.21681	5.6569	10.8765	2.00	15.00
40 minutes	12.5%	5	3.0000	.70711	.31623	2.1220	3.8780	2.00	4.00
	25%	5	9.0000	.00000	.00000	9.0000	9.0000	9.00	9.00
	50%	5	14.2000	.83666	.37417	13.1611	15.2389	13.00	15.00
	Total	15	8.7333	4.77294	1.23237	6.0902	11.3765	2.00	15.00
45 minutes	12.5%	5	3.2000	.83666	.37417	2.1611	4.2389	2.00	4.00
	25%	5	9.8000	.83666	.37417	8.7611	10.8389	9.00	11.00
	50%	5	14.8000	.83666	.37417	13.7611	15.8389	14.00	16.00
	Total	15	9.2667	4.97805	1.28533	6.5099	12.0234	2.00	16.00
50 minutes	12.5%	5	4.0000	.00000	.00000	4.0000	4.0000	4.00	4.00
	25%	5	10.0000	.70711	.31623	9.1220	10.8780	9.00	11.00
	50%	5	15.8000	.83666	.37417	14.7611	16.8389	15.00	17.00
	Total	15	9.9333	5.02091	1.29639	7.1528	12.7138	4.00	17.00
55 minutes	12.5%	5	4.8000	.83666	.37417	3.7611	5.8389	4.00	6.00
	25%	5	10.6000	1.14018	.50990	9.1843	12.0157	9.00	12.00
	50%	5	15.8000	.83666	.37417	14.7611	16.8389	15.00	17.00
	Total	15	10.4000	4.73286	1.22202	7.7790	13.0210	4.00	17.00
60 minutes	12.5%	5	5.0000	.70711	.31623	4.1220	5.8780	4.00	6.00
	25%	5	11.2000	.83666	.37417	10.1611	12.2389	10.00	12.00
	50%	5	16.2000	1.30384	.58310	14.5811	17.8189	15.00	18.00
	Total	15	10.8000	4.82849	1.24671	8.1261	13.4739	4.00	18.00

Appendix 5 ANOVA Test

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
5 minutes	Between Groups	292.800	2	146.400	366.000	.000
	Within Groups	4.800	12	.400		
	Total	297.600	14			
10 minutes	Between Groups	304.133	2	152.067	380.167	.000
	Within Groups	4.800	12	.400		
	Total	308.933	14			
15 minutes	Between Groups	314.133	2	157.067	196.333	.000
	Within Groups	9.600	12	.800		
	Total	323.733	14			
20 minutes	Between Groups	270.533	2	135.267	193.238	.000
	Within Groups	8.400	12	.700		
	Total	278.933	14			
25 minutes	Between Groups	251.200	2	125.600	179.429	.000
	Within Groups	8.400	12	.700		
	Total	259.600	14			
30 minutes	Between Groups	292.133	2	146.067	365.167	.000
	Within Groups	4.800	12	.400		
	Total	296.933	14			
35 minutes	Between Groups	302.533	2	151.267	216.095	.000
	Within Groups	8.400	12	.700		
	Total	310.933	14			
40 minutes	Between Groups	314.133	2	157.067	392.667	.000
	Within Groups	4.800	12	.400		
	Total	318.933	14			
45 minutes	Between Groups	338.533	2	169.267	241.810	.000
	Within Groups	8.400	12	.700		
	Total	346.933	14			
50 minutes	Between Groups	348.133	2	174.067	435.167	.000
	Within Groups	4.800	12	.400		
	Total	352.933	14			
55 minutes	Between Groups	302.800	2	151.400	168.222	.000
	Within Groups	10.800	12	.900		
	Total	313.600	14			
60 minutes	Between Groups	314.800	2	157.400	162.828	.000
	Within Groups	11.600	12	.967		
	Total	326.400	14			

Appendix 6 Post Hoc Test

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) concentration	(J) concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
5 minutes	12.5%	25%	-6.00000*	.40000	.000	-7.0671	-4.9329
		50%	-10.80000*	.40000	.000	-11.8671	-9.7329
	25%	12.5%	6.00000*	.40000	.000	4.9329	7.0671
		50%	-4.80000*	.40000	.000	-5.8671	-3.7329
	50%	12.5%	10.80000*	.40000	.000	9.7329	11.8671
		25%	4.80000*	.40000	.000	3.7329	5.8671
10 minutes	12.5%	25%	-6.20000*	.40000	.000	-7.2671	-5.1329
		50%	-11.00000*	.40000	.000	-12.0671	-9.9329
	25%	12.5%	6.20000*	.40000	.000	5.1329	7.2671
		50%	-4.80000*	.40000	.000	-5.8671	-3.7329
	50%	12.5%	11.00000*	.40000	.000	9.9329	12.0671
		25%	4.80000*	.40000	.000	3.7329	5.8671
15 minutes	12.5%	25%	-6.00000*	.56569	.000	-7.5092	-4.4908
		50%	-11.20000*	.56569	.000	-12.7092	-9.6908
	25%	12.5%	6.00000*	.56569	.000	4.4908	7.5092
		50%	-5.20000*	.56569	.000	-6.7092	-3.6908
	50%	12.5%	11.20000*	.56569	.000	9.6908	12.7092
		25%	5.20000*	.56569	.000	3.6908	6.7092
20 minutes	12.5%	25%	-5.40000*	.52915	.000	-6.8117	-3.9883
		50%	-10.40000*	.52915	.000	-11.8117	-8.9883
	25%	12.5%	5.40000*	.52915	.000	3.9883	6.8117
		50%	-5.00000*	.52915	.000	-6.4117	-3.5883
	50%	12.5%	10.40000*	.52915	.000	8.9883	11.8117
		25%	5.00000*	.52915	.000	3.5883	6.4117
25 minutes	12.5%	25%	-5.60000*	.52915	.000	-7.0117	-4.1883
		50%	-10.00000*	.52915	.000	-11.4117	-8.5883
	25%	12.5%	5.60000*	.52915	.000	4.1883	7.0117
		50%	-4.40000*	.52915	.000	-5.8117	-2.9883
	50%	12.5%	10.00000*	.52915	.000	8.5883	11.4117
		25%	4.40000*	.52915	.000	2.9883	5.8117
30 minutes	12.5%	25%	-5.80000*	.40000	.000	-6.8671	-4.7329
		50%	-10.80000*	.40000	.000	-11.8671	-9.7329
	25%	12.5%	5.80000*	.40000	.000	4.7329	6.8671
		50%	-5.00000*	.40000	.000	-6.0671	-3.9329
	50%	12.5%	10.80000*	.40000	.000	9.7329	11.8671
		25%	5.00000*	.40000	.000	3.9329	6.0671
35 minutes	12.5%	25%	-5.40000*	.52915	.000	-6.8117	-3.9883
		50%	-11.00000*	.52915	.000	-12.4117	-9.5883
	25%	12.5%	5.40000*	.52915	.000	3.9883	6.8117
		50%	-5.60000*	.52915	.000	-7.0117	-4.1883
	50%	12.5%	11.00000*	.52915	.000	9.5883	12.4117
		25%	5.60000*	.52915	.000	4.1883	7.0117
40 minutes	12.5%	25%	-6.00000*	.40000	.000	-7.0671	-4.9329
		50%	-11.20000*	.40000	.000	-12.2671	-10.1329
	25%	12.5%	6.00000*	.40000	.000	4.9329	7.0671
		50%	-5.20000*	.40000	.000	-6.2671	-4.1329
	50%	12.5%	11.20000*	.40000	.000	10.1329	12.2671
		25%	5.20000*	.40000	.000	4.1329	6.2671
45 minutes	12.5%	25%	-6.60000*	.52915	.000	-8.0117	-5.1883
		50%	-11.60000*	.52915	.000	-13.0117	-10.1883
	25%	12.5%	6.60000*	.52915	.000	5.1883	8.0117
		50%	-5.00000*	.52915	.000	-6.4117	-3.5883
	50%	12.5%	11.60000*	.52915	.000	10.1883	13.0117
		25%	5.00000*	.52915	.000	3.5883	6.4117
50 minutes	12.5%	25%	-6.00000*	.40000	.000	-7.0671	-4.9329
		50%	-11.80000*	.40000	.000	-12.8671	-10.7329
	25%	12.5%	6.00000*	.40000	.000	4.9329	7.0671
		50%	-5.80000*	.40000	.000	-6.8671	-4.7329
	50%	12.5%	11.80000*	.40000	.000	10.7329	12.8671
		25%	5.80000*	.40000	.000	4.7329	6.8671
55 minutes	12.5%	25%	-5.80000*	.60000	.000	-7.4007	-4.1993
		50%	-11.00000*	.60000	.000	-12.6007	-9.3993
	25%	12.5%	5.80000*	.60000	.000	4.1993	7.4007
		50%	-5.20000*	.60000	.000	-6.8007	-3.5993
	50%	12.5%	11.00000*	.60000	.000	9.3993	12.6007
		25%	5.20000*	.60000	.000	3.5993	6.8007
60 minutes	12.5%	25%	-6.20000*	.62183	.000	-7.8589	-4.5411
		50%	-11.20000*	.62183	.000	-12.8589	-9.5411
	25%	12.5%	6.20000*	.62183	.000	4.5411	7.8589
		50%	-5.00000*	.62183	.000	-6.6589	-3.3411
	50%	12.5%	11.20000*	.62183	.000	9.5411	12.8589
		25%	5.00000*	.62183	.000	3.3411	6.6589

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

Appendix7 Homogeneous Subsets

5 minutes

Tukey HSD

concentration	N	Subset for alpha = 0.05		
		1	2	3
12.5%	5	.0000		
25%	5		6.0000	
50%	5			10.8000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

10 minutes

Tukey HSD

concentration	N	Subset for alpha = 0.05		
		1	2	3
12.5%	5	.0000		
25%	5		6.2000	
50%	5			11.0000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

15 minutes

Tukey HSD

concentration	N	Subset for alpha = 0.05		
		1	2	3
12.5%	5	.8000		
25%	5		6.8000	
50%	5			12.0000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

20 minutes

Tukey HSD

concentration	N	Subset for alpha = 0.05		
		1	2	3
12.5%	5	1.8000		
25%	5		7.2000	
50%	5			12.2000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

25 minutes

Tukey HSD

concentration	N	Subset for alpha = 0.05		
		1	2	3
12.5%	5	2.2000		
25%	5		7.8000	
50%	5			12.2000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

30 minutes

Tukey HSD

concentration	N	Subset for alpha = 0.05		
		1	2	3
12.5%	5	2.2000		
25%	5		8.0000	
50%	5			13.0000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

35 minutes

Tukey HSD

concentration	N	Subset for alpha = 0.05		
		1	2	3
12.5%	5	2.8000		
25%	5		8.2000	
50%	5			13.8000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

40 minutes

Tukey HSD

concentration	N	Subset for alpha = 0.05		
		1	2	3
12.5%	5	3.0000		
25%	5		9.0000	
50%	5			14.2000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

45 minutes

Tukey HSD

concentration	N	Subset for alpha = 0.05		
		1	2	3
12.5%	5	3.2000		
25%	5		9.8000	
50%	5			14.8000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

50 minutes

Tukey HSD

concentration	N	Subset for alpha = 0.05		
		1	2	3
12.5%	5	4.0000		
25%	5		10.0000	
50%	5			15.8000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

55 minutes

Tukey HSD

concentration	N	Subset for alpha = 0.05		
		1	2	3
12.5%	5	4.8000		
25%	5		10.6000	
50%	5			15.8000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

60 minutes

Tukey HSD

concentration	N	Subset for alpha = 0.05		
		1	2	3
12.5%	5	5.0000		
25%	5		11.2000	
50%	5			16.2000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Appendix 8 Experiment Documentation

Figure 1 mosquito's container with the size of 25cm x 25cm x 25cm

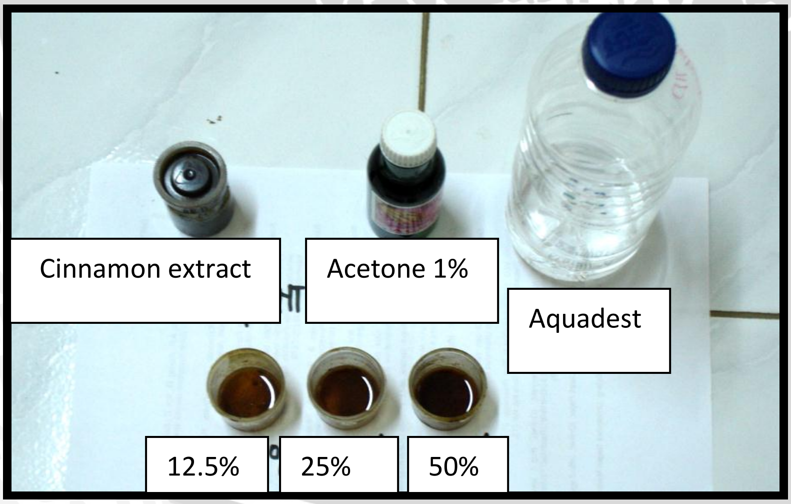


Figure 2 Solutions for the Experiment

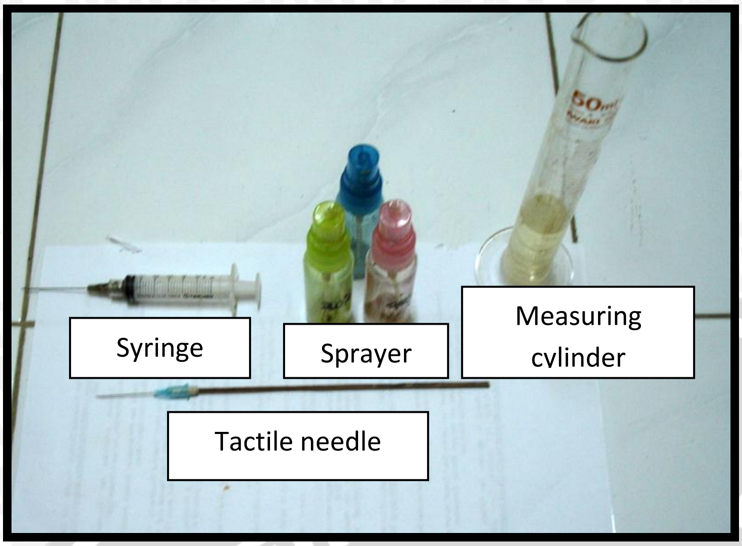


Figure 3 Equipments for the Experiment

Figure 4 Knockdown of Adult *Culex* sp.

VERIFICATION OF FINAL PROJECT

I, hereby

Name : Nurul Faizah binti Jusoh

NIM : 0610714021

Study Program: Medicine, Medical Faculty, University of Brawijaya

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

Malang, 13th Dec 2011

Sincerely,

(Nurul Faizah binti Jusoh)

0610714021