CHAPTER 2

LITERATURE REVIEW

2.1 Types and Spread of Mosquitoes

There are over 3500 species of mosquitoes, out of which few are considerd to be harmless or even useful to humans while the rest are vectors to any number of vector borne diseases. Among the best known species is the *Aedes Aegypti* mosquito*. Aedes aegypti*, is a mosquito that can spread the dengue fever, chikungunya, and yellow fever viruses, and other diseases. The mosquito can be recognized by white markings on its legs and markings in the form of a lyre on the thorax (Zettel & Kaufman, 2009).

There are approximately 98 species of anopheline mosquitoes and 248 culicine mosquitoes reported in Indonesia. As studies continue to be done on mosquitoes, these numbers can potentially increase. Most of the common species including anopheles and aedes can be found spread throughout the archipelago but certain species are only found localized on particular islands. Lombok Island in particular is home to more than 30 native species of mosquitoes (Ichiro et. al., 1994)

Aedes aegypti was previously found sporadically in Europe in the first half of the 20th century as far north as Brest and Odessa, but it disappeared from the Mediterranean region due to reasons unknown. It has since re-colonised Madeira and parts of Southern Russia and Georgia (Krasnodar Krai and Abkhazia and has been recently reported in the Netherlands, making it one of the most widespread mosquito species globally. The success of this invasive species has largely been due to globalisation. Historically, *Aedes aegypti* has moved from continent to continent via ships. It is suggested that *Aedes aegypti* evolved its domestic behaviour in West Africa and its widespread distribution and colonisation in the tropics led to the highly efficient inter-human transmission of viruses such as dengue. This domestic behaviour can provide protection against environmental conditions and numerous habitats suitable as oviposition sites, but can also result in increased sensitivity to control measures used to eliminate them (Powell & Tabachnick, 2013).

2.2 Aedes Aegypti

2.2.1 Taxonomy

Classification

Kingdom: Animalia

Subkingdom: Bilateria

Super Division: Ecdysozoa

Division: Arthropoda

Class: Insecta

Sub Class: Pterygota

Order: Diptera

Family: Culicidae

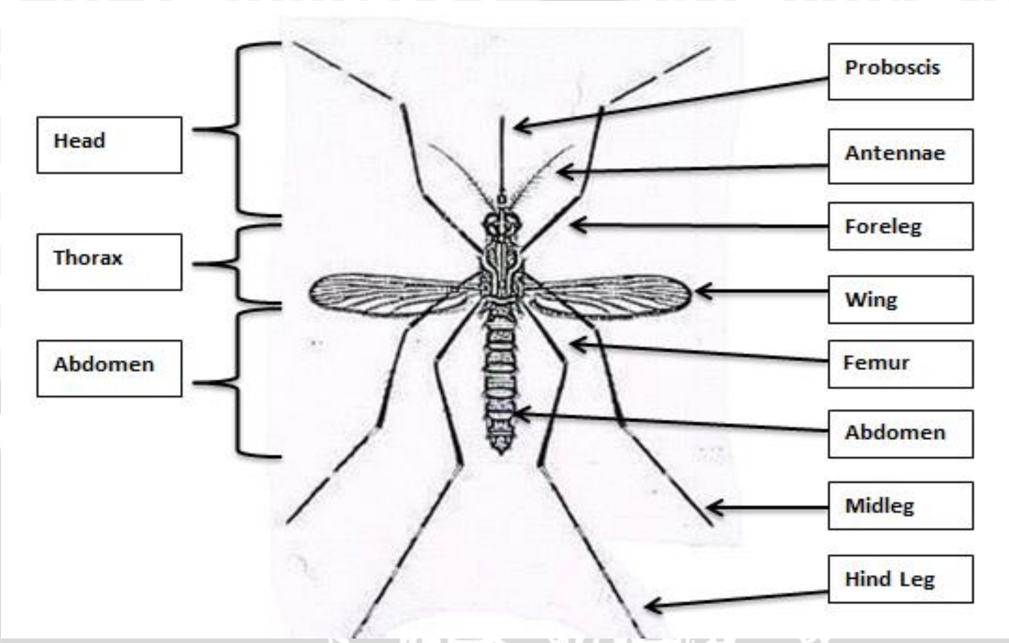
Genus: Aedes

Spesies: *Aedes aegypti*

(Gubler, 2014)

2.2.2 Morphology

The generally 3–6 mm long adult mosquitoes possess long slender legs. The head is globular, possessing 2 large compound eyes (no ocelli) and long filamentous antennae which contain sensory organs to recognize host and oviposition sites and the Johnston's organ in the basic segment by which males recognize wing beats of the females. The prominent mouthpart is equal in length to the head/thorax region and formed by the labium ensheathing the stylets which have developed from the labrum (building the food channel), the 2 mandibles and laciniae and the unpaired hypopharynx, the latter containing the salivary channel. The length, shape, and hairiness of the 5-segmented maxillary palps differ according to species and sex, being reduced in males which do not feed blood, but only sugars, e.g., honeydew or nectar. In addition, males and females can usually be separated according to the antennae, which are brush-like in males, the weaker developed mouth parts of males and the external genitalia of males, jointed claspers. In both sexes only the veins of the wings are covered with scales (Leopoldo, 2004).



Picture 2.1: Morphology of male *Aedes aegypti* mosquito (Leopoldo, 2004)

2.2.3 Life Cycle Of *Aedes Aegypti.*

Normally embryonic development is completed within a few hours after egg laying, and the first instar (larvae) hatch. Fully developed larvae of Aedes Aegypti remain in the eggshell until eggs are flooded, and can thereby be stored for a long period of time (depending on temperature and humidity up to 4.5 years). Larvae are aquatic, mainly occurring in fresh water, but some species also develop in salt water. The size of the habitat can be very small, e.g., tree holes. The total duration of the 4 larval instars varies greatly, even within one species, especially depending on temperature and food supply. In the tropics it can be completed within one week, in temperate regions many months, and even longer if a larval diapause exists. Some species are even frost-tolerant while others live at

50°C. The larvae feed on debris or plankton (filter feeders) or predate other larvae. The development of the also aquatic pupa is also temperature-dependent, lasting between 1 day or up to 3 weeks. If the pupae are disturbed, they actively swim downwards with their paddles at the end of the abdomen. The longevity of the adults strongly varies according to the climatic region, on average 1–2 weeks in the tropics and 4–5 weeks in temperate regions, but up to several months for females of hibernating or aestivating species. Thereby, the whole developmental cycle (egg to egg) can last about 7 days or up to several months in diapausing species (Zettel & Kaufman, 2009).

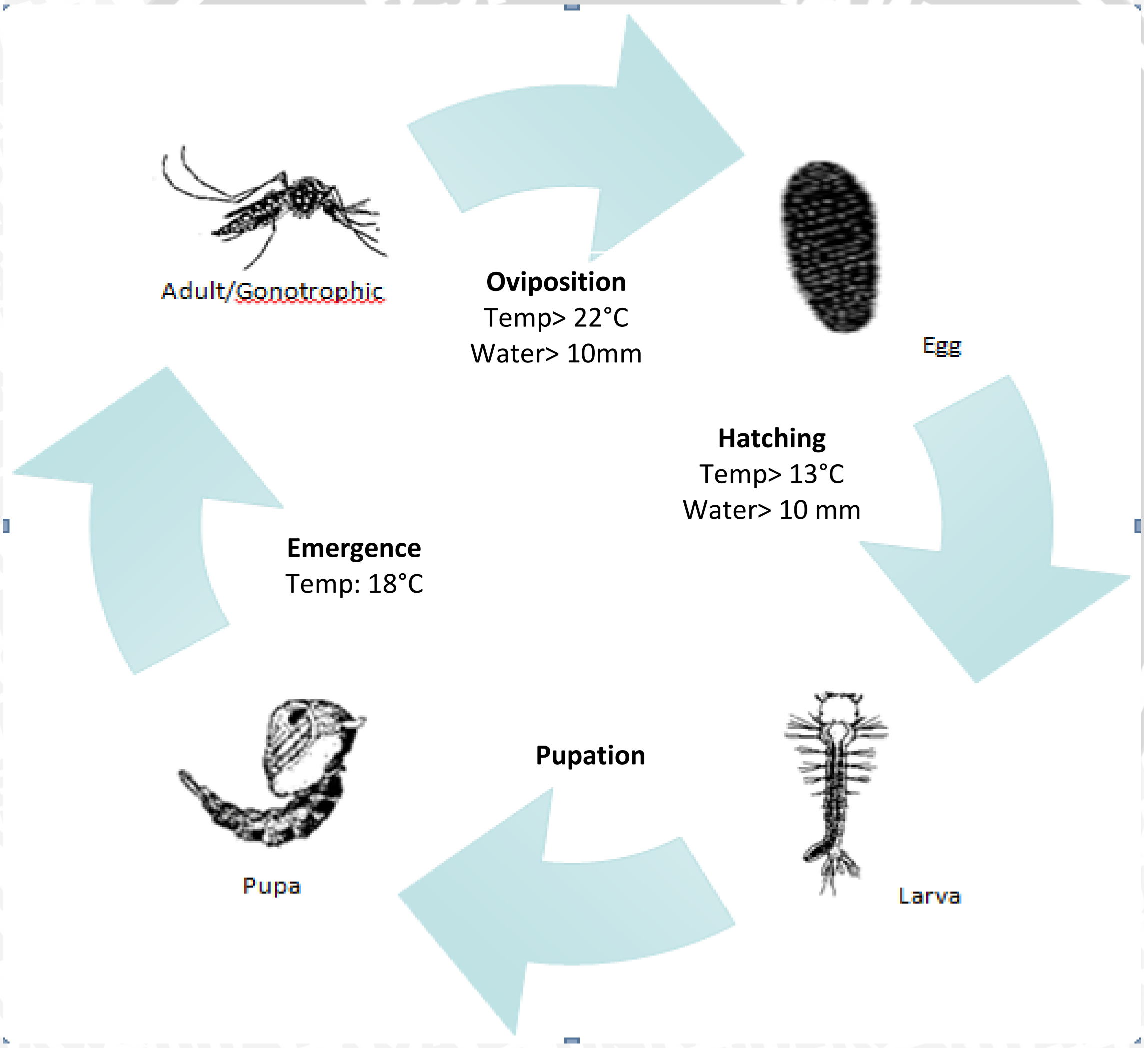


Figure 2.2 Life Cycle of Aedes *aegypti* (Hopp, 2001)
Observable change from an Egg hatching into a Larva, then turning into a Pupa before morphing into an Adult *Aedes aegypti* mosquito which then lays more eggs.

2.2.4 Habitat

The Aedes mosquito lays her eggs on the sides of containers with water and eggs hatch into larvae after a rain or flooding. A larva changes into a pupa in about a week and into a mosquito in two days. Aedes main aquatic habitats range from tree cavities to toilets and learn about the mosquito's life cycle. People also furnish shelter as Aedes aegypti preferentially rests in darker cool areas, such as closets leading to their ability to bite indoors (Levengood & Norrgren, 2012).

2.2.5 Nature

The Aedes mosquito has a variety of ways of finding it's prey, including chemical, visual, and heat sensors. The male mosquitoes feed on nectar and plant juices while the mouthparts of the females are adapted for piercing the skin of animal hosts and sucking their blood as ectoparasites. The female needs to obtain nutrients from a blood meal before she can produce eggs, whereas in many other species, it can produce more eggs after a blood meal. The feeding preferences of *Aedes aegypti* mosquitoes include those with type O blood, heavy breathers, those with a lot of skin bacteria, people with a lot of body heat, and the pregnant. Both plant materials and blood are useful sources of energy in the form of sugars, and blood also supplies more concentrated nutrients, such as lipids, but the most important function of blood meals is to obtain proteins as materials for egg production. Indoors, *Aedes aegypti* frequents darkened areas of a room, resting on available surfaces and biting whenever the host is available. Although chiefly diurnal in its biting activity, the species also is reported to be quite active at night is most active during daylight. It feeds for approximately two hours after sunrise and several hours before sunset as it is sensitive towards heat. The mosquito rests indoors, in closets and other dark places. When outdoors, they rest where it is cool and shaded (Robinson & Robinson, 2005).

2.2.6 Medical Importance

Aedes aegypti are pests. Bites cause minor localized itching and irritation to the skin, and can make an outdoor adventure very unpleasant. Most bites are not medically significant, but can be annoying. While many mosquitoes bite at night, dawn or dusk, Aedes. aegypti readily bite during the day and indoors as well as outdoors.It is also the primary vector of the dengue and yellow fever virus Yellow fever epidemics still occur frequently in the tropics, and can occur in temperate regions during summer months, although it is not the major threat it once was. Today, there is a very successful vaccine for yellow fever, which has contributed to the decline in cases in the United States. While the United States rarely experiences yellow fever cases, the most recent concern to the United States and Florida is the transmission of dengue virus. Dengue is also known as "break-bone fever" for the excruciating pain victims feel. Dengue is a dangerous disease due to four different serotypes: DEN-1, DEN-2, DEN-3, and DEN-4. Although a person can obtain immunity to one serotype, they are still susceptible to the others. The most deadly is dengue hemorrhagic fever (DHF), which is often fatal. Like yellow fever, dengue fever is caused by a flavivirus (attacks the liver), and can only be transmitted by female mosquitoes. A clear indication of dengue fever would be saddleback fever, progressive thrombocytopenia, increasing hematocrit and low albumin. The acute febrile illness (temperatures ≤40°C), like that of dengue fever, lasts approximately 2-7 days. However, in persons with dengue hemorrhagic fever, the fever reappears, giving a biphasic or saddleback fever curve (Zettel & Kaufman, 2009).

2.3 Insect Control

Control campaigns can be directed against larvae or adults. In attempts to control larvae, the best results have been obtained in Europe by draining marshes. This is without success if the vector species also breed in other habitats, if the high costs cannot be covered, or if environmental arguments exist. Other physical methods have to be used according to the respective vector species, e.g., intermittent irrigations, deforestation, or planting vegetation. In many tropical countries, education reduced the number of breeding places created by discarding old tyres, cans, or jars. Chemical control includes the spraying of mineral oils on the water surface, or applying the copper acetoarsenite dust, or many insecticides (carbamates, organophosphates, pyrethroids). Insect growth regulators arrest larval development or interfere with the formation of the cuticle. Control of adult mosquitoes includes methods such as the use of mosquito nets which can be impregnated with insecticides, the use of insect repellents, and anti-mosquito coils. Insecticides are often used against adult mosquitoes, e.g., in community treatments being applied by helicopters. Residual insecticides can also be sprayed in the house, but thereby populations of mosquitoes survive which leave the house directly after feeding and do not rest inside the house on the insecticide-impregnated walls (Ignacimuthu & Davi, 2009).

2.3.1 Non-Chemical Method

Homeowners should practice thorough sanitation in and around the house. The best way to reduce mosquitoes is to eliminate the places where the mosquito lays her eggs, like artificial containers that hold water in and around the home. Outdoors, clean water containers like pet and animal watering containers, flower planter dishes or cover water storage barrels. Look for standing water indoors such as in vases with fresh flowers and clean at least once a week (RISKESDAS, 2009).

2.3.2 Mechanical Method

Aside from non-chemical methods, mechanical methods are also applied. This includes wearing long sleeves and pants for additional protection when available. Also, ensure that all windows and door screens are secure and without holes. If available, use air-conditioning (RISKESDAS, 2009).

2.3.3 Biological Method

Biological control involves the introduction of organisms that prey upon dengue mosquitoes in order to reduce the size of the vector propagation. Examples include invertebrate copepods (small crustaceans) introduced into water storage tanks in Vietnam, and larvivorous (larvae-eating) fish. A larvivorous fish that is very effective and widely used in the USA is Gambusia, The biocide BTI (*Bacillus thuringiensis israelensis*) is a biologically derived insecticide that has been widely adopted in recent years. BTI is derived from *Bacillus thuringiensis* bacteria that produce spores which contain an endotoxin which induces the lysis of midgut cells of mosquito larvae. Biological control methods do not cause any chemical contamination of the environment and are often species specific so they do not adversely affect the rest of the ecosystem. However, the costs of rearing these organisms can be high and their use is often specific to a limited number of suitable locations (Ignacimuthu & Davi, 2009).

2.3.4 Chemical Method

In the 1940s the use of the insecticide DDT was the most common means of vector control, however by the 1960s most Aedes aegypti populations had developed resistance to this chemical. Since then other chemicals have been developed and two chemical methods have emerged as the most popular. Firstly, methods targeted at mosquito larvae (larvicidal methods) involve the application of low toxicity insecticides such as 1% temephos sand granules or methoprene briquettes to water storing containers necessary for domestic use. This method can be used to directly target known mosquito breeding sites (Jamison et. al., 2006).

2.3.5 Insecticides

Insecticides are substances that contain chemical compounds that are used to kill insects. Good insecticide has the following properties::

a) has a strong and fast killing ability and harmless to vertebrates including humans and livestock

b) cheap and available in large quatities

c) has a stable chemical structure and does not catch fire easily

d) easy to use and able to dissolve in a number of different solvents

e) colourless and odorless.

f) safe for the environment and crops

Some of the terms associated with insecticide are: (1) ovicides are insecticides to kill the egg stage; (2) larvicides is to kill the larvae or nymphs satdium; (3) adulticides are to kill the adult stage; (4) acaricides (miticides) is an insecticide to kill the mites and (5) pediculocides (lousecicide) is an insecticide to kill lice (Baskoro et al., 2005).

2.3.5.1 Factors to Consider in Choosing Insecticides

When selecting the insecticides, the first thing to remember is the type of pests that are to be contended with, the insect stage, the environment, and its way of life (Ignacimuthu & Davi, 2009).

2.3.5.2 Insecticide Administration

2.3.5.2.1 Based on Physical State of The Insecticide

a) Solid : (1) *dust*, measuring in 35-200 mikron; (2) *granules*, the size of a grain of sugar and unable to penetrate the mesh screen and (3) *pellets,* measuring at $1cm^3$ (Baskoro et al., 2005).

b) Liquid : (1) *aerosol* and *fog*, measuring at 0,1 dan 50 mikron; (2) *mis)*, measuring at 0 - 100 mikron dan (3) *spray*, measuring at 100 - 500 mikron (Baskoro et al., 2005).

c) Gas : (1) *fumes and smokes*, measuring at 0,001 – 0,1 mikron; (2) *vapours*, measuring less than 0,001 mikron (Baskoro et al., 2005).

2.3.5.2.2 Based on Method of Entry of Inscticide Into Insects

a) *Contact poisons*:

   a. Insecticides enter through the exoskeletons of insects into the body through the tarsus (toes) at rest on the surface containing residues. Generally used to combat insects that have a puncture-type suction mouth such as mosquitoes (Dreistadt, 2001).

b) *Stomach poisons*:

   a. Insecticides enter the insect body through the mouth, so it should be eaten. Insects usually eradicated by the use of these insecticides to have a mouth to bite, sticky suction, suction slices and shape suck (Dreistadt, 2001).

c) *Fumigants*:

   a. Insecticides enter through the respiratory system (spiracles) and also through the rest of the insect body surface. These type of insecticides can be used to combat all kinds of insects without having to pay attention to the shape of it's mouth (Dreistadt, 2001).

2.3.5.2.3 Based on Chemical Composition:

a) Inorganic insecticides consist of: (1) sulfur and mercury based compounds (SO_2, $CuSO_4$, $HgCl_2$) and (2) arsenicum based compounds (*Paris Green* = Cu ($C_2H_3O_{2.}$ 3-$Cu(As_3O_2)_2$, lead arsenate = $PbHAsO_4$, *Ca* arsenate = $Ca_3A_1F_3$,NaF) (Dreistadt, 2001).

b) Naturally occuring organic insecticides consist of: (1) plant based insecticides dan (2) soil based insecticides (kerosene, diesel, lubricants) (Dreistadt, 2001).

c) Synthetic organic insecticides consist of (1) chlorine based organic compounds (DDT, dieldrin, klorden, BHC, linden); (2) phosphorus based organic compounds (malation, paration, dizinon, phenotrition, temefos, DDVP, dipterex); (3) nitrogen based organic compounds (dinitrophenol); (4) sulphur based and (5) thiocyanate based organic compounds (letena, Tanit) (Dreistadt, 2001).

2.4 Cloves (*Syzgium aromaticum*)

2.4.1 Introduction

Cloves (*Syzygium aromaticum*, syn. *Eugenia aromaticum* or *Eugenia caryophyllata*) are the aromatic dried flower buds of a tree in the family Myrtaceae. Cloves are native to Indonesia and used as a spice in cuisines all over the world. The English name derives from Latin *clavus* 'nail' (also the origin of French *clou* 'nail') as the buds vaguely resemble small irregular nails in shape. Cloves are now harvested primarily in Indonesia, Madagascar, Zanzibar, Pakistan, and Sri Lanka; they are also grown in India under the name Lavang. In Vietnam, it is called đinh hương. In Indonesia it is called cengkeh or cengkih (Duke, 2002).

The clove tree is an evergreen which grows to a height ranging from 8–12 m, having large square leaves and sanguine flowers in numerous groups of terminal clusters. The flower buds are at first of a pale color and gradually become green, after which they develop into a bright red, when they are ready for collecting. Cloves are harvested when 1.5–2 cm long, and consist of a long calyx, terminating in four spreading sepals, and four unopened petals which form a small ball in the center (Duke, 2002).

2.4.2 Taxonomy of *Syzygium aromaticum*

<u>Synonym:</u>

Eugenia aromatica (L.)

Eugenia caryophyllata.

Eugenia caryophyllus.

Caryophyllus aromaticus L.

Picture 2.3: Clove Plant (Anonymous, 2014)

Common Name:

Indonesian:	Cengkeh, cengkih
English:	Clove
Vietnamese:	Hanh con
Thai:	Kaan phluu
Chinese:	Ding xiang
Japanese:	Kuroobu, shouji

Classification

Kingdom: Plantae

Subkingdom: Tracheobionta

Super Divisi: Spermatophyta

Divisi: Magnoliophyta

Kelas: Magnoliopsida

Sub Kelas: Rosidae

Ordo: Myrtales

Famili: Myrtaceae

Genus: Syzygium

Spesies: *Syzygium aromaticum*

(Prashak, 2012)

2.4.3 Origin

A native of Indonesia and the Malacca Islands, it is an evergreen tree that grows to about 10 meters (30 feet) tall and has bright green leaves and nail-shaped rose-peach flower buds which turn, upon drying, a deep red brown. These are then beaten from the tree and dried. The Latin word 'Clavus' means nail shaped, referring to the bud (Prashak, 2012).

It was often used by the Greeks, Roman and the Chinese, to ease toothache and as a breath sweetener, especially when talking to the Emperor. It has antiseptic properties and was used in the prevention of contagious diseases, such as the Plague. It was an important commodity in the spice trade and is still used in perfumes, mulled wines and liqueurs, love potions, dental products and, stuck in an orange as pomade, an insect repellant (Prashak, 2012).

2.4.4 Usage of Clove (*Syzgium aromaticum*)

Clove essential oil properties include being antiseptic, anti-viral, anti-biotic, a stimulant, an expectorant and an aphrodisiac. It is used in aromatherapy to treat digestive problems, exhaustion, infection, burns, cuts, arthritis, asthma, bronchitis, rheumatism and colds. Clove oil has also been used as an insect repellent, especially for mosquitoes. Clove stem oil is widely used in the perfumery industry; it is also substituted in the food industry for clove bud oil, due to its lower cost. Clove leaf oil is used in the chemical industry, primarily for the extraction of the chemical component eugenol. In addition to being used in the fragrance and food industries in cosmetics, drinks, dental products, perfumes and food, clove oil is also used to produce glue, varnish and to print ink (Lim, 2014).

Also used for treating bronchitis, colds, indigestion, toothache, infected wounds, mouth sores and muscle and nerve tensions. It also doubles up as an insect repellant and room disinfectant. Rinsing using a blend of cherry, nutmeg, cloves and cinnamon proves to be an effective mouthwash. If you are afflicted with chronic bronchitis or feel tired, rub a few drops into the chest 3 times a day. Clove oil is both a stimulant and a pain reliever. Carrying a tiny bag of cloves, fennel, or anise seeds to chew after odorous meals helps fight bad mouth odor (Lim, 2014).

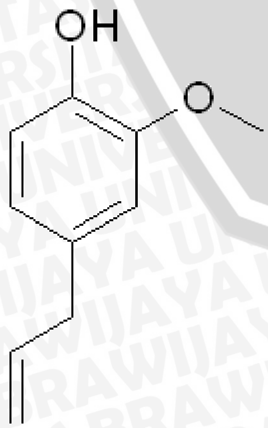
2.4.5 Chemical Content of Clove oil Extract (*Syzgium aromaticum*)

There are three types of clove oil:

- Bud oil is derived from the flower-buds of *Syzgium aromaticum*. It consists of 60-90% eugenol, eugenyl acetate, caryophyllene and other minor constituents.
- Leaf oil is derived from the leaves of *Syzgium aromaticum*. It consists of 82-88% eugenol with little or no eugenyl acetate, and minor constituents.
- Stem oil is derived from the twigs of *Syzgium aromaticum*. It consists of 90-95% eugenol, with other minor constituents (Alma et. al., 2007).

2.4.5.1 Eugenol

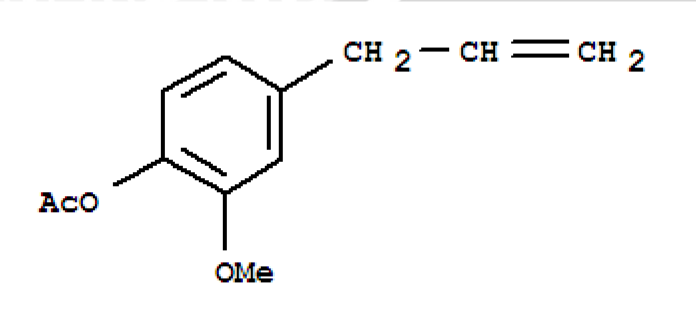
Clove flower buds contains a compound called eugenol in the form of a pale yellow, oily liquid Eugenol is a compound which belongs to the phenylpropene group, which are generally obtained in the form of phenol or phenol esterase. Due to this, eugenol exists primarily in the form of volatile liquids. If pure, simple phenol has a clear color, but it is usually oxidized and turns dark when in contact with air. Water solubility increases if the numbers of hydroxyl groups' increase, but it's solubility in organic solvents are generally polar. Solubility in water is <1 mg / ml and the boiling point is 254 ° C. Eugenol is known to have pronounced antiseptic and anesthetic properties (Agusta, 2000).



Picture 2.4 Eugenol (American Chemical Society, 2005)

2.4.5.2 Eugenyl Acetate

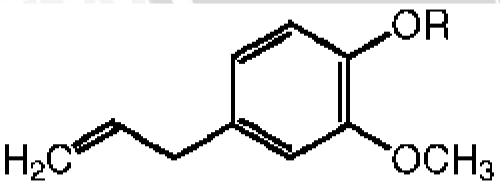
Eugenyl acetate (also known as acetyl eugenol), exists as a nearly colorless or pale yellow liquid. It smells spicy and sweet and it is soluble in alcohol and oils, but not soluble in water. It is widely used as berry, mint and vanilla flavoring. In the medical world, Eugenyl acetate is also known to have anti-inflammatory, ant prostaglandin, and antispasmodic effects. Also used in perfumery, and as trypsin-enhancers (Krupa, 2001).



Picture 2.5 Eugenyl acetate (American Chemical Society, 2005)

2.4.5.3 β-caryophyllene

β-caryophyllene oils form colorless or pale yellow liquids at room temperature. It is soluble alcohol and oils, slightly soluble in propylene glycol, but insoluble in water. Also widely used in flavoring spicy foods, providing citrus taste and as a flavoring. In the medical world it is used as a local anesthetic and gastric cytoprotective (Tambe et al, 1996).



Picture 2.6 β-caryophyllene (American Chemical Society, 2005)

2.4.5 Insect Repellent

An insect repellent is a substance applied to skin, clothing, or other surfaces which discourages insects (and arthropods in general) from landing or climbing on that surface. Insect repellents help prevent and control the outbreak of insect-borne diseases such as malaria, Lyme disease, Dengue fever, bubonic plague, and West Nile fever. Pest animals commonly serving as vectors for disease include fleas, flies, and mosquito; and cockroaches. Common insect repellents include:

a) DEET (*N*,*N*-diethyl-*m*-toluamide)
b) Essential oil of the lemon eucalyptus (*Corymbia citriodora*) and its active compound p-menthane-3,8-diol (PMD)
c) Icaridin, also known as picaridin, Bayrepel, and KBR 3023 Nepetalactone, also known as "catnip oil"
d) Permethrin
e) Neem oil
f) Clove oil

Synthetic repellents tend to be more effective and/or longer lasting than 'natural' repellents. The downside of essential oil repellents are they can be short-lived in their effectiveness, since essential oils can evaporate completely. Nevertheless, natural repellent are safe for the user and also environmental friendly compared to synthetic repellents (Debboun et. al., 2006).