CHAPTER 6

DISCUSSION

This experiment was done in order to prove the insecticidal potency of Clove extract on *Aedes aegypti* mosquitos. Clove was chosen for this experiment due to it being easily available in most tropical countries including Indonesia and its potential as well as perceived benefits for health as well as a number of other uses in our daily lives. Clove extract is obtained via technique of simple extraction using ethanol to produce a strongly aromatic and spicy aroma (Burdock, 2009). The spraying method is chosen to administer the insecticide because it is easier and similar to the potential real life application of insecticidal sprays sold to the general populace.

This study uses five containers measuring 25cm x 25cm x 25cm, each containing 25 *Aedes aegypti* mosquitoes with solutions used as follows: 1 container acts as the negative control in this case using aquades, 1 container for positive control using malathion 0.28%, and 3 other containers for Clove extract each with a concentration of 20%, 30% and 40%. The concentration is used based on preliminary research that has been done before hand. These concentrations are chosen by taking into account the effect and its potential as an insecticide before repeating the experiment four times from the formula contained in research methods. Observations for each treatment carried out every 10 minutes until the peak of 60 minutes or 1 hour.

The results obtained for the clove extract concentrations are all the *Aedes aegypti* mosquitoes died after the 24th hour. Therefore the purpose of this study was to prove that the ethanol extract of Clove (*Syzgiyum aromaticum)* has potential as an insecticide against *Aedes aegypti* mosquitoes.

However, as most of the mosquitoes seem to have died by the 1st hour, it can be surmised that the clove extract also exhibits a knockdown effect. It is an effect produced by certain insecticides that induces a state of partial intoxication and partial paralysis which precedes death (Wickham et. al., 1974).

From the WHO 2013 insecticide score table, one can determine the score of the knockdown effect and its interpretation based on the knockdown time:

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

Table 6.1 Interpretation of Knockdown Time (KT50)

Observations of the number of knocked-down mosquitoes are made during the hour-long exposure period. A mosquito is considered knocked down if it is unable to stand or fly in a coordinated way; it will usually fall to the bottom of the container. It is recommended that observations are made at regular intervals, usually after 10, 15, 20, 30, 40, 50 and 60 minutes into the exposure period. If, after 60 minutes, the observed knockdown rate is less than 80%, another count at 80 minutes should be made of the mosquitoes in the container. The holding

container may be tapped a few times before this final determination is made. In very susceptible populations, the recording of knock down time should be done more frequently, every 3 minutes (WHO, 2013).

KT50 (50% Knockdown Time) is the time it takes for 50% of all mosquitoes in the container to be knocked down. In the context of this experiment, it is found that 40% clove extract on average causes the knockdown effect in 50% of all mosquitoes in the first 10 minutes. Therefore based on Table 6.1, it can be surmised that clove extract has a strong knockdown effect.

Then the potential of the extract on the 24th hour were observed. At the 24th hour, the concentration of 40% extract has a potency of 100% which is equal to that of the positive control Malathion 0.28%. This proves that the ethanol extract of Clove 40% concentration has potential as an insecticide for reaching potential of 100% in 60 minutes if the knockdown effect is taken into consideration.

Administration of 20% Clove extract gave a significantly different effect on the number of dead *Aedes aegypti* mosquitoes between each observation time ($p = 0.000$). Likewise with Clove extract at a concentration of 30% ($p = 0.000$) and a concentration of 40% ($p = 0.000$). With the trend of increasingly longer observation time, it is found that more *Aedes aegypti* mosquitoes die. The concentration of Clove extract also causes significantly different effects on the number of dead *Aedes aegypti* mosquitoes at each observational time. In the 10^{th} minute there are significant differences in the number of *Aedes aegypti* mosquitoes that die between groups of different concentration of Clove extract with concentrations of 20%, 30% and 40% ($p = 0.001$). Similar results were obtained for the 20^{th} minute ($p = 0.000$) and 30th minute ($p = 0.000$). Different

results were found for the 40th minute (p = 0.005), 50th minute (p = 0.004), and the 60th minute (p = 0.002) with the trend of the number of *Aedes aegypti* mosquitoes that die increase more and more with increasing concentrations of clove extract.

From the correlation test results obtained, there is a significant relationship between the concentration of clove extract with insecticide potency at each observation time (p = 0.000, R = 0.342). Which means that the higher the concentration of clove extract, the more the number of *Aedes aegypti* mosquitoes that die. It also showed that there is a significant correlation between the length of time of observation with insecticide potential at each concentration (p = 0.000, R = 0.265), where the longer the time of observation, the more the number of *Aedes aegypti* mosquitoes that die.

Clove extract possesses several active ingredients and chief among them are: thymol, eugenol, pulegone, terpineol, and citronellal. These components are widely used in numerous fields such as traditional medicine, used as perfume, as antimicrobials and even insect repellent. When clove extract is administered in the form of spray, the aerosolized clove extract droplets mix with the air particles and are breathed into the mosquito via spiracles located on its abdomen. This is a far more effective insecticidal delivery system as it does not depend on contact or if the insect needs to consume the insecticide for it to take effect. The clove extract then travels from the mosquito's respiratory system and into its circulatory system where the extract is distributed throughout the body. The active ingredients in clove extract may act in synergy with each other or in the presence of piperonyl butoxide which is another organic compound to inhibit cytochrome P450-mediated oxidation and stop the detoxification process involving glutathione S-transferase in *Aedes aegypti* mosquitoes. Eugenol in particular inhibits the

electron transport process in the mitochondrial respiratory chain. Inhibition of mitochondrial electron transport causes failure of ATP production (Waliwitiya et. al., 2012).

Even though Clove oil is generally thought of as safe and even beneficial to humans, there have been isolated cases where an excessive ingestion of clove oil can lead to children having acute liver damage (Hartnoll, 1993). Aside from this there is little or no evidence that clove related products are inherently harmful towards humans in small doses. (Prashak, 2012)

As clove extract is safe in low doses and environmentally friendly, hopefully clove extract can one be widely used as a candidate for a natural pesticide that is safe for both humans and the environment. Naturally a lot more research and experimentation must be done to make certain of this fact before clove extract as a pesticide is widely accepted by the general populace.

Among the many limitations faced throughout this study are limited tools and means, so the mechanism of active substances from the ethanol extract of clove cannot be studied in more detail. In addition, there are other limitations which had unknown influence on this experiment including environmental conditions such as air temperature, humidity, and storage time of the ethanol extract of clove against its potential as an insecticide. Other influences include hormones and behavior by the mosquitoes that were used in this experiment.