NATURAL MITICIDES FOR CONTROLLING OF *TROPILAELAPS* SP. IN *APIS MELLIFERA* L.

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MASTER OF SCIENCE
IN
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Kritsana Narapong

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ABSTRACT

Tropilaelaps mites cause damage to *Apis mellifera* L. and lead to great economic loss in beekeeping. Many control methods, such as chemical agents, resulted in the mite resistance and accumulation of acaricidal residues in bee products. Botanical essential oils have been shown to provide an efficient alternative method to replace synthetic acaricides for the control of the mites. In this study, the efficacies of three botanical essential oils from *Curcuma longa* L., *Zingiber officinale* Roscoe and *Alpinia galanga* L. with the concentrations of 25%, 50%, and 75% were investigated to control the *Tropilaelaps* mites both under laboratory and field (apiary) conditions. Under laboratory conditions, the results demonstrated that both essential oils of *Z. officinale*, and *A. galanga* showed the same potential in controlling the mites and had higher efficacy in the increase of mite mortality than *C. longa* essential oil did at every tested concentration. The mite mortality occurred within 1-6 hours after being exposed to *Z. officinale* and *A. galanga* essential oils at every concentration. In contrast, all concentrations of *C. longa* essential oil showed significantly slower action (24-36 hours) ($P<0.05$) in causing mortality rate after the exposure compared to the previous two botanical essential oils. When observing the effect of these botanical essential oils

on the honeybees in the laboratory, the result showed that the 50% and 75 of *Z. officinale* and *A. galanga* oils had a negative effect on the bees and resulted in death within 2 days whereas 25% concentration of both botanical essential oils showed significantly lower mortality of honeybees ($P<0.05$) and the honeybees could survive for 14 to 36 days. *C. longa* essential oil at every tested concentration had a significant difference in honeybee mortality compared to the control (water) treatment ($P<0.05$).

According to the lower efficacy of *C. longa* essential oil in controlling the mites, only *Z. officinale* and *A. galanga* essential oils were tested for efficacy in the control of the mites in the apiary. The result showed that both botanical essential oils at 25%, 50%, and 75% concentrations had a significant acaricidal effect on the mites when compared to the control treatment ($P<0.05$). However, all concentrations of *A. galanga* essential oil caused significant differences in mortality rate of the mites when compared among the months ($P>0.05$). Moreover, *A. galanga* essential oil was found to increase mortality of honeybees. *Z. officinale* essential oils at all concentrations gave no significant efficacy in controlling the mites ($P>0.05$) and caused no negative effect on the honeybee population. Our results suggested that *Z. officinale* essential oil has a higher potential than *A. galanga* essential oil for controlling *Tropilaelaps* mites in apiculture.

Keywords: *Apis mellifera* L., Honeybees, *Tropilaelaps* mites, *Curcuma longa* L., *Zingiber officinale* Roscoe., *Alpinia galanga* L., Essential Oil

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CHAPTER 1

INTRODUCTION

1.1 Background and Significance of the Research Problem

Bees are social insects which are divided into caste system (queen, drone and worker) according to their functions and roles in a colony. They are important to global agricultural economy, food industry and other cosmetic industry (Chantawannakul, 2017; Wakhal et al., 1999). Bee products (honey, brood propolis, bee pollen, bee wax, bee venom and royal jelly) are accepted as healthy foods and often used in medicine (Martinello & Mutinelli, 2021; Mizrahi & Lensky, 2013). They play an important role in biodiversity, being pollinators of various vegetable crops, flowers and fruits. Currently the most popular species of honey bee which the beekeepers tend to rear is European honeybee, *Apis mellifera* L. which originated in Europe (Alvarez-Suarez, 2017).

The European honeybees were first introduced to Thailand in 1940's and reared in the Chulalongkorn University (Vongsamanode et al., 1986). Later in 1977's the European honeybee keeping became famous for commercial purposes. Nowadays, European honeybee keeping in Thailand has become a good business giving a lot of profit to the beekeepers because of a lot of honey yield which can be harvested with this species (Chantawannakul, 2018). However, the serious problem occurs to beekeepers in rearing the European honey bee is mite infestation. Nowadays, beekeepers have been concerned about the mite infestation because it is difficult to get rid of.

In Asia, the European honeybee industry is highly exposed to ectoparasite mites. The mites most commonly found in this area are *Varroa* and *Tropilaelaps* mites. Both mites generally feed on the bee brood (hemolymph) which can lead to weight loss, wing deformities, death of the bees and migration or swarming (Chantawannakul, 2017).

When colony is infested, mated females of mites enter the capped brood cells containing a stage honeybee larva. Eggs are laid around 3-10 eggs within 24 h, then egg develops from protonymph, deutonymph into an adult mite within 6-9 days (de Guzman et al., 2017; Ritter & Akratanakul, 2006). Adult mites emerge from the brood cells when the adult honeybee emerges. At this point, *Varroa* mite required a phoretic stage (1-2 day) while *Tropilaelaps* mites can re-infest to new host immediately (Buawangpong et al., 2015; Woyke, 1987). Moreover, *Tropilaelaps* could build up population rapidly as compared to Varroa mites (Buawangpong et al., 2015; Chantawannakul, 2017; de Guzman et al., 2017; Woyke, 1987).

Both *Varroa* and *Tropilealaps* mites also occur in Thai beekeeping. Thai beekeepers have controlled both mite species by chemical or colony manipulation techniques. In a honeybee colony infested with *Varroa,* it is preferred not to use chemicals miticides to manipulate the brood rearing cycle and remove the mites from the colony within the capped brood cell to reduce the mite population (de Guzman et al., 2017). However, chemical control is the most popular method of mite control in beekeeping which must be used after honey harvest or before the blooming season to avoid contamination of honey or other bee products, including bee colonies and side effects on bee behavior (de Guzman et al., 2017). Beekeepers still tend to choose this method because it gives quick results and is easy to use to treat colonies with broods by fumigation or spraying. Examples of chemical products include hard acaricides such as Apistan (fluvalinate), Checckmite+ (coumaphos), Bayvarol (flumethrin), Hopguard® and Apivar ® (amitraz), and soft acaricides such as organic acids (formic acid, oxalic acid, and lactic acid) and essential oils (Rosenkranz et al., 2010). However, in Thailand, *Tropilaelaps* mites, especially the species of *T. mercedesae*, were the dominant brood parasites of European honeybees (Buawangpong et al., 2015; de Guzman et al., 2017). The study of controlling the *Tropilealaps* mites is still limited.

Using chemical has risk of contamination, accumulation of chemical residues in bee product and environment. Moreover, the development of resistance to the chemical product of the mites can occur. Another alternative method of mite control is to use botanical plant extracts such as essential oils due to the properties of natural compounds which hardly caused no negative effect on honeybee colonies. Moreover, there is low risk of contamination from the plant extracts in honeybee product because

they are volatile substances (Rosenkranz et al., 2010). Moreover, they can also kill the mites in the capped brood cells. Essential oils from plant extracts nowadays especially the ones with strong pungent or peppery aroma such as peppermint essential oil, cinnamon oil, neem oil, thyme oil and lemon grass oil, were reported to have efficacies against mites (de Guzman et al., 2017; Islam et al., 2017; Rosenkranz et al., 2010). Moreover, turmeric rhizome, ginger rhizomes and galangal rhizome have been reported to have ability to deter behaviors of some insect pests. (Maedeh et al., 2012; Pandey et al., 2021; Raina & Abraham, 2017)

Turmeric rhizome (*Curcuma longa* L.), chemical compounds are gingerols, shogaols, and essential oils and resin (oleoresin) which contain 1.0-3.3% of essential oils. Moreover, the major constituent is 30-70% composed of sesquiphellandrene hydrocarbon (aromatic) such as (-)-zingiberene, (+)-ar-curcumene, (-)-beta-sesquiphellandrene, beta-bisabolene, camphene, alpha-pinene, nerol, geranyl acetate, linalool, borneol (Govindarajan & Stahl, 1980). Turmeric has the efficacy against insect pest such as cabbage looper larvae that used turmeric powder from the rhizomes (de Souza et al., 2016).

Ginger rhizome (*Zingiber officinale* Roscoe) contains 1-3% essential oils based on growing and storing. Ginger essential oil compound contain zingiberene, bisabolene, camphene and oleoresin (aromatic). The major constituents of oleoresin are gingerol, shogaol, and zingerine (Zachariah, 2008). Ginger rhizomes can repel adult maize weevil (*Sitophilus zeamais*) (Ukeh et al., 2009).

Galangal rhizome (*Alpinia galanga* L.) contains about 2.5-4% essential oil. The main components of the oil are ethyl cinnamate (25%), ethyl-p-methoxycinnamate (30%) and pmethoxycinnamic acid and a monoterpene ketone compound such as 3-carene-5-one. Galangal rhizome was reported to have efficacy against two species of termites which are *Coptotermes gestroi* (Wasmann) and *Coptotermes curvignathus* (Holmgren) (Abdullah et al., 2015).

However, the appropriate concentration is important and also must be able to kill the mites without side effects to the honeybees as well as honeybee product contamination. Therefore, the goals of this study were first to screen the potential of selected native herbal plant essential oils having strong pungent or spicy odors in

getting rid of mite infestation in European honeybee colonies. Secondly, the optimized concentrations of each plant essential oil were also investigated.

1.2 Research Objectives

1.2.1 To investigate the potential of selected herbal plant extract essential oils in the control of *Tropilealaps* mite infestation

1.2.2 To investigate the optimal concentration of herbal plant extract essential oils contributing the highest efficacy in the control of *Tropilealaps* mite infestation without any negative effect on honeybee health

1.3 Scope of Research

The candidate herbal plants to be investigated for miticidal properties were collected from Chiang Rai Province. The criteria to select these plants was based on the common use and availability and from the literature review being reported for the potential of these plants as insecticides. The chosen plants were *Curcuma longa* L., *Zingiber officinale* Roscoe and *Alpinia galanga* L. The samples of chosen plants were then extracted by hydro-distillation. The essential oils were tested for miticidal properties in laboratory condition and in Apiary. The chemical compositions of each essential oils were identified using GC-MS.

1.4 Expected Outcomes

1.4.1 Potential essential oil for mite control

1.4.2 Effective dosage of essential oil for mite control

1.4.3 Alternative green method of mite control for beekeepers

CHAPTER 2

LITERATURE REVIEW

2.1 Honeybee (*Apis mellifera* L.)

Apis mellifera L. originated in European and African countries but later spread worldwide via human transportation and activities (Chantawannakul, 2017). *Apis mellifera* L. is a social insect that belongs to the Kingdom Animalia, Phylum Arthropoda, Class Insect, Order Hymenoptera and Family Apidae (Chantawannakul, 2017).

Honeybees are generally most important pollinators for maintaining plant biodiversity and agricultural crops in the world. Most countries such as European countries, North American countries including Asian countries introduced these honeybees to be used as pollinators and apiculture (Chantawannakul, 2017). Around the middle of the 20th century, they were introduced into Thailand (Wongsiri et al., 2000). Honey, pollen, royal jelly, propolis, wax, bee brood, and bee venom are some of the export products of beekeeping (Martinello & Mutinelli, 2021; Wongsiri et al., 2000; Wongsiri et al., 2012).

2.1.1 Biology of *A. mellifara* L.

Honeybees are social insects. They perform different tasks in their colonies base on their caste and age. There are three castes of honeybees, queen, drone and worker. In a colony, the queen usually lays eggs and produces chemical signals known as pheromone to control activities in colony. A drone's only purpose is to mate with a virgin queen from another colony (Chantawannakul, 2018; Gurung et al., 2012). Worker honeybees are the main labors performing different colony maintenance roles depending on their ages. This is called age-related polytheism (Chantawannakul, 2018; Gurung et al., 2012). The youngest workers take care of the brood, eggs, larvae, and pupae, while older workers make wax comb, ensure the food storage within the colony

and watch for the enemies not to enter the colony. The oldest workers are foragers; these are the honeybees which people find flying around most often (Chantawannakul, 2018; Gurung et al., 2012).

Colony activities are organized by complex communication between individuals, through both pheromones and the dance language. The pheromones released from various glands such as queen mandibular glands which release pheromones to assist a colony to detect where the queen is. The immature bees release brood pheromone in order to be taken care of. Nasanov pheromone released from worker bees is to guide the foraging bees to come back to the colony without getting lost. Dance language can imply the location and direction where the food is (Camiletti et al., 2014).

One of the most dominant honeybee behaviors is stinging. Stinging is a defensive behavior worker bees use to protect the colony. When a colony is entered by the enemies, guard bees release an alarm pheromone that evoke a defensive response. After stinging, the workers lose their stings and die shortly (Camiletti et al., 2014).

2.1.2 Value of Honeybee

Bees are considered as economic insects that provide great valuable products such as honey, beeswax, royal jelly, pollen, propolis and bee's venom. These products can be divided into two groups for examples, products obtained from raw material that bees collect from outside their hive such as honey, propolis and bee pollen. The other products created by bees on their own such as beeswax, royal jelly and bee venom (Chouhan et al., 2017; Mizrahi & Lensky, 2013).

2.1.3 Products of Honeybee

2.1.3.1 Honey

Honey comes from nectars of flowers and fruit trees. Moreover, another source of honey can be honey dew which bees collect from aphids. This honey which bees collect goes through chemical and physical process until it is converted into honey and then stored inside a bee comb. The use of honey can be applied in cosmetic industry such as soap, shampoo and cream (Wongsiri et al., 2000; Wongsiri et al., 2012). The most recognized use of honeybee is in food industry such as making dessert, bread, candies, ice-cream including mixing in beverage for example lemon juice (Wongsiri et

al., 2000; Wongsiri et al., 2012). Honey was found to contains high value of nutrition and can be used to replace sugar in many kinds of food. (Wongsiri et al., 2000; Wongsiri et al., 2012).

2.1.3.2 Bee Pollen

Bee pollen comes from the pollens of flowers from many plants that bees collect. Once these pollens are collected, they are then generated through bee mixing with anthers. Bee pollen is considered a high protein nutrient for the bees in the entire colony especially the broods (Di Pasquale et al., 2013).

Other components found in bee pollen are fat, carbohydrate, enzyme, minerals, and all vitamins (Campos et al., 1997; Wongsiri et al., 2012). Bee pollens are used as medicine to treat allergies and also increase immunity in patients with allergies (Wongsiri et al., 2012). Additionally, bee pollen can be used as an ingredient in many cosmetics such as facial cleansers, foundations, and care skin creams. Not only that it can be used as a hair treatment to make the hair remain sleek and glossy and also antidandruff by adding shampoo and pomade (Mizrahi & Lensky, 2013).

2.1.3.3 Beeswax

Beeswax is composed of several types of chemicals which are released from a beeswax gland located on the dorsal of the bee's abdomen. The application of beeswax is found commonly in cosmetic products such as facial cleansers, skin-care oil, and lipstick. Moreover, it is used in the production of candles, glue, gum, and crayons (Wongsiri et al., 2012). When beeswax is combined as a component of candles, it helps the candles to release little smoke and gives a pleasant fragrance (Wongsiri et al., 2012).

2.1.3.4 Royal Jelly

Royal jelly is the most valuable in bee products. It is secreted by the hypopharyngeal glands of young workers or nurse bees and is used to feed the young larvae (queen bee). Royal jelly is used in the cosmetics industry as well as the medical industry. It can be used as a natural treatment for viral infections and has a regenerate effect (Bogdanov, 2011; Wongsiri et al., 2000).

2.1.3.5 Propolis

Propolis is also considered one of important bee products. The way propolis is made comes from the resin from different trees collected by honeybees. Honeybees

bring this resin back to the bee hive in order to seal the hive to protect members of the colony from bacterial and fungal infections. Propolis can be used as medicine such as antibiotic, anti-inflammatory and immune system booster. The active ingredient of propolis is available for human used in the form of capsule, herbal drink and ointment or cream (Mizrahi & Lensky, 2013).

2.1.4 Honeybee Diseases

The decline of the honeybee population has recently become problem combination of factors including diseases caused by viruses, bacteria, fungi and protozoa, and ectoparasitic mites (Chantawannakul, 2017; de Guzman et al., 2017). The honeybee is susceptible to all kinds of diseases. Moreover, the ectoparasitic mites, *Varroa* mites and *Tropilaelaps* mites share life history traits and both infect honeybee colonies, *A. mellifera* which are also a serious problem to the European honeybees due to two mite species are biological vectors of several honeybee viruses such as Deformed Wing Virus (DWV), Sacbrood virus (SBV) and Black queen cell virus (CBPV) (Khongphinitbunjong et al., 2015; Sanpa & Chantawannakul, 2009).

2.1.4.1. Deformed Wing Disease

Deformed Wing Virus (DWV) is one of a few bee viruses that cause symptoms in infected honeybees. Typical disease symptoms of DWV infection include shrunken, crumpled wings, decreased body size, and discoloration in adult bees. In addition, from the adult stage, DWV infection is also detected in other stages of bee development including egg, larvae, and pupae. When pupae at the normally multiply slowly and rarely kills the pupae, instead mostly causing deformation and early death in newly emerged adult bees. Adult honeybees infected with DWV usually present normal symptoms but may cause a decrease life span (Chen & Siede, 2007).

2.1.4.2 Sacbrood Disease

Sacbrood disease caused by *Morator aetotulas* is the most common viral disease of honeybees (Ritter & Akratanakul, 2006). Sacbrood is affecting the brood of the honeybee and resulting in larval death. The anterior section of the infected larva, consisting of its head and thorax, is the first part of the larva body to change color, changing from pearly white to pale yellow and finally to dark brown and black (Bailey, 1975; Bailey et al., 1964; Grabensteiner et al., 2001). Sacbrood virus (SBV) affects

adult bees without causing clear signs of disease but the infected adult bees may have a decreased life span (Chen & Siede, 2007). Sacbrood disease occurs most frequently in spring when the colony is growing most rapidly and large numbers of susceptible larvae and young adults are available (Bailey et al., 1969; Khongphinitbunjong et al., 2015; Sanpa & Chantawannakul, 2009).

2.1.4.3 Black Queen Cell Disease

Black queen cell virus (BQCV) mainly affects developing queen larvae and pupae in the capped cell stage. A high prevalence of this virus infection is observed in queen-rearing colonies in spring and early summer (Laidlaw, 1979). The infected larvae show the symptom as a pale-yellow appearance and tough saclike skin. BQCV usually multiplies in the pupal stage of honeybees. Infected pupae show a dark appearance and die shortly after being infected. If BQCV infects the queen cell, the cell will become a dark color. Worker bees can also be attacked by BQCV but do not show the symptoms obviously (Chen & Siede, 2007).

2.2 Ectoparasite Mites

The regular infestation of *A. mellifera* colonies is caused by the two parasitic mite species which are *Varroa* and *Tropilaelaps* mites. In Asia countries, *Tropilaelaps* mites are considered to be more influential parasite of *A. mellifera* than *Varroa* mites (Buawangpong et al., 2015; Burgett et al., 1983; de Guzman et al., 2017).

Tropilaelaps mites, belong to the order Mesostigmata and family Laelapidae, are ectoparasite that feed on the hemolymph (larvae and pupae) of developing honey bee brood. The original hosts of *Tropilaelaps* spp. are the giant Asia honeybee species, *Apis dorsata*, *Apis laboriosa* and *Apis breviligula* (Anderson & Roberts, 2013; Chantawannakul, 2017). *Tropilaelaps* mites have been identified, characterized and classified into four species *Tropilaelaps clareae*, *Tropilaelaps koenigerum*, *Tropilaelaps mercedesae* and *Tropilaelaps thaii* (Anderson & Morgan, 2007; de Guzman et al., 2007; de Guzman et al., 2017). Each species is closely associated with a part of giant honeybees in Asia. But two species, *T. clareae* and *T. mercedesae*, are successfully shifted to *Apis mellifera* (Chantawannakul, 2017; Ritter & Commission,

2008). These mites are a major threat to managed European honeybees (Anderson & Morgan, 2007; Pettis et al., 2013).

In Thai beekeeping, *Varroa destructor* and *Tropilaelaps mercedesae* are two mite species typically found in *A. mellifera* colonies (Chantawannakul et al., 2016). Even though it is rarely (< 0.1%), these two mites can infest *A. mellifera* colonies at the same time. *T. mercedesae* was the more common *A. mellifera* brood parasite in Thailand (Buawangpong et al., 2015).

2.2.1 Morphology of *Tropilaelaps* Mites

Both sexes of the mites are elongated and their bodies are covered with numerous, short, spine-like setae. *Tropilaelaps* mites normally show reddish brown color. However, the males show less sclerotized. *Tropilaelaps* mites tend to be smaller, flatter, and more oval-shaped compared to *Varroa* mites (Warrit & Lekprayoon, 2011). For some species, an adult female of *T. mercedesea* can be differentiated from *T. clareae* by the characteristic of the plate. Female *T. clareae* show bluntly pointed plate whereas *T.* mer*cedesea* has the plate in the form of a bluntly to sharply pointed plate (Warrit & Lekprayoon, 2011).

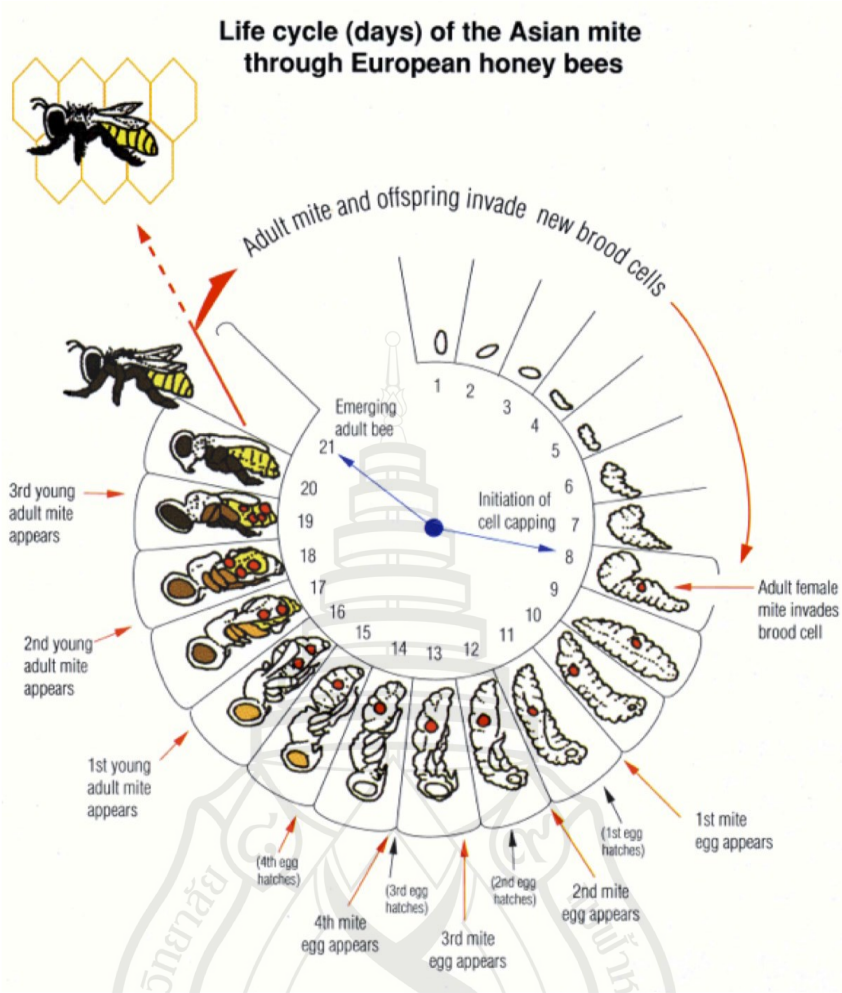
The males of *T. mercedesae* and *T. clareae* have similar characteristics that their moveable digit of the chelicerae, which acts as a spermatodactyl organ appears in a long corkscrew-like coiled structure (Anderson & Morgan, 2007; de Guzman et al., 2017; Warrit & Lekprayoon, 2011). The nymphal stages of the two mites are white in color.

Source Anderson and Roberts (2013)

Figure 2.1 Comparison of mounted specimens of a *T. mercedesae* (a) male and (b) female

2.2.2 Life Cycle of *Tropilaelaps* Mites

The life cycle of *Tropilaelaps* mites begins when a gravid female mite enters honeybee worker or drone brood cells containing a last stage honeybee larva before capping, feeding on larva hemolymph for 2 days after that lays the first egg (Anderson & Roberts 2007; de Guzman et al., 2017; Warrit & Lekprayoon, 2011). One to four eggs can be found in a capped brood cell. The development time of *Tropilaelaps* mites has three stages from egg, larva (protonymph and deutonymph) to adult mite within 6-9 days (de Guzman et al., 2017; Chantawannakul, 2017). *T. mercedesea* presents to lay eggs every 24 hours unlike Varroa mites lay eggs in 30 hours. Also, *Tropilaelaps* mites have a short life cycle, and also the population of this genus increase faster than *Varroa* mites (Anderson & Roberts, 2013).



Source Anderson and Roberts (2013)

Figure 2.2 Life cycle of *T. mercedesae* on *A. mellifera* L.

2.2.3 Effects of *Tropilaelaps* on Honeybee Health

2.2.3.1 Damage at the Individual Level

The individual honeybee is damaged in a variety way. When high infestation in *A. mellifera* brood by *Tropilaelaps* mite occurs, it often causes the abdomen of bees to survive mite attacks being reduced in body size as distorted abdomens, deformed or missing wings and legs, and the infected honeybees have shorter life-span than healthy bees (de Guzman et al., 2017; Anderson & Robert, 2007; Ritter & Akratanakul, 2006; Ritter & Commission, 2008).

2.2.3.2 Damage at the Colony Level

Untreated infestation rapidly increases to high levels in *A. mellifera* colonies and constantly leads to colony decline or absconding (Mahmood et al., 2011; de Guzman et al., 2017; Anderson & Roberts, 2007). When brood cells were infested with *Tropilaelaps* mites the infestation can be high more than 90% then this can lead to low honey yield (de Guzman et al., 2017)**.**

2.2.4 Detection of *Tropilaelaps* Mite's Infestation

According to the method of Ritter and Commission (2008), mite collections include ether or sugar powder. About 100-200 bees can be collected in a wide-mouthed jar with a lid. Once the bees are collected, they are knocked to the bottom of the jar with a sharp blow. After that, the lid is removed and sprayed with ether starter fluid for 2 seconds. Another way to detect the mite infestation is to use 70% alcohol or soapy water for covering the bees or adding around 25 g of powdered sugar all over the bee. In case of using the ether, the lid needs to be replaced and the jar needs to be rolled for about 10 seconds then the mites will stick to the walls of the jar. If using soap or alcohol, agitate and then strain out the bees with a mesh strainer. Mites will be found in the liquid. In the case of using powder sugar, the screening material will need to be put on top of the jar and the jar will be shaken then the mites will fall down on the white paper. In addition to brood infestation, *Tropilaelaps* infestation can be detected on the bottom broad by using sticky traps placed on the bottom (de Guzman et al., 2017; Ritter & Commission, 2008).

2.2.5 Management and Control of *Tropilaelaps* Mites

2.2.5.1 Chemical Control

Chemical control is the most popular method of mite control in beekeeping which has to be conducted after honey harvest or before the blooming season to avoid contamination of chemicals in honey, or in other bee products including bee colonies and side effects on bee's behavior (de Guzman et al., 2017). Among chemical products are hard acaricides like Apistan (fluvalinate), Checckmite+ (coumaphos), and Bayvarol (flumethrin) have been used to treat *A. mellifera* colonies against Tropilaelaps mites (de Guzman et al., 2017). The dosage is 2-3 strips of Apistan per colony. Strips of filter paper soaked in 15% potassium nitrate with 12.5% amitraz solution which is also used

as a hive fumigant to get rid of the mites (Anderson & Roberts, 2013). Moreover, organic acids such as formic acid, thymol, and a combination of thymol and oxalic acid showed high efficacy against *Tropilaelaps* mites (de Guzman et al., 2017; Mahmood et al., 2012; Mahmood et al., 2011). According to the study done by de Guzman et al. (2017) and Ritter and Akratanakul (2006), formic acid can be used successfully in controlling *Tropilaelaps* mites. However, the dosage per comb should not be more than 2 ml is applied on a sponge per a Langstroth hive (de Guzman et al., 2017; Ritter & Akratanakul, 2006).

2.2.5.2 Essential Oils

Essential oils are blends of approximately 20-80 different plant metabolites which are usually extracted from plants through steam distillation (Bakkali et al., 2008). They have been applied to replace synthetic chemical acaricides because the consumers are concerned about chemical residue in bee products. Essential oils are the common term for liquid, highly volatile plant compounds, characterized by an intensive, characteristic odor. They are present in almost all plant species, but only plants containing more than 0.1 % oil can be called essential oils (Imdorf et al., 1999; Regnault-Roger, 1997; Sararit & Auamcharoen, 2020). Essential oils are plant products found in the flowers, fruits, seeds, leaves, roots, or wood and they can be present in all parts of a plant or only in specific parts (e.g., flowers of roses) (Imdorf et al., 1999; Sangwan et al., 2001). They perform various functions in plants such as being attractants for insect pollinators. Moreover, they can be repellent or protectant against phytophagous insects. Many also exhibit fungicidal and bactericidal activities and protect plants from microorganisms (Abd El-Wahab & Ebada, 2006; Hikal et al., 2017).

The application of essential oils for example, essential oils of cade (*Juniperus oxycedrus*), clove (*Eugenia caryophyllata*), coriander (*Coriandrum sativum*), horseradish (*Armoracia rusticana*), and mustard (*Brassica juncea*), applied onto mesh-ended cylinders containing the poultry red mite *(Dermanyssus gallinae*) into sealed vials revealed the successfully in 100% mortality of the poultry red mite (*D. gallinae*) after insect were exposed for 24 hours (Kim et al., 2004). *In vitro* contact assays using the dog ear mite, *Otodectes cynotis* (Hering), found that the essential oil constituent of geraniol killed all dog ear mites within 1 hour at concentrations of ≥5% (v/v) (Traina et al., 2005). The potential of essential oils of thyme, horsemint (*Mentha*

longifolia), and *Dorystoechas hasata* against tick larvae (*Rhipicephalus microplus*) which the results showed 100% mortality in tick larvae 24 hours after 5 mins soaking in 0.1% solutions of each oil (Koc et al., 2012). *In vitro* investigation about the efficacy of essential oil of tea tree against sheep lice (*Bovicola ovis*) showed that exposure to 1% concentration of tea tree essential oil caused 100% mortality of sheep lice adults and eggs in contact with dipped wool and mortality was also high in adults exposed to tea tree essential oil vapors (James & Callander, 2012a, 2012b). The citronella (*Cymbopogon nardus*) essential oil repels mosquitoes and flies, and garlic (*Allium sativum*) essential oil can control many insect herbivores (Regnault-Roger et al., 2012).

In Thailand, the common plants that show the pungent aroma can be found in *Curcuma longa* L., *Alpinia galanga* L, and *Zingiber officinale* Roscoe.

Turmeric essential oil (*Curcuma longa* L.) was shown to have efficacy against insect pests such as cockroaches (Dictyoptera: Blattidae, Blattellidae, and Blaberidae) (Thavara et al., 2007). Moreover, this essential oil was used in the management of different species of dengue mosquitoes (Bushra & Tariq, 2014). Additionally, the turmeric essential oil was found to have efficacy against rice weevil (*Sitophilus zeamais*) (Ishii et al., 2010) and also against diamondback moth (*Plutella xylostella* Linnaeus) (Javier et al., 2016) and the cabbage worm (*Crocidolomia pavonana*) (Javier et al., 2018).

Effect of galangal essential oil (*Alpinia galanga* L.) against insect pests was found in some insects such as two species of termites, *Coptotermes gestroi* (Wasmann) and *Coptotermes curvignathus* (Holmgren) (Abdullah et al., 2015).

Ginger essential oil (*Zingiber officinale* Roscoe) was shown to have efficacy against insect pests such as the whitefly (*Bemisia argentifolii*) on tomato seedlings (Zhang et al., 2004), mosquito larvicidal, against the filarial vector *Culex quinquefasciatus* (Pushpanathan et al., 2008), and maize weevil (*Sitophilus zeamais*) (Ukeh et al., 2009). The essential oil was also found to repel domiciliary cockroach (*Periplaneta Americana*) (Ahmad et al., 1995), adults of the red flour beetle (*Tribolium castaneum*), larvae of the Mediterranean flour moth, (*Ephestia kuehniella*) (Maedeh et al., 2012) and also maize weevil (*S. zeamais*) (Ishii et al., 2010).

2.2.5.3 Management

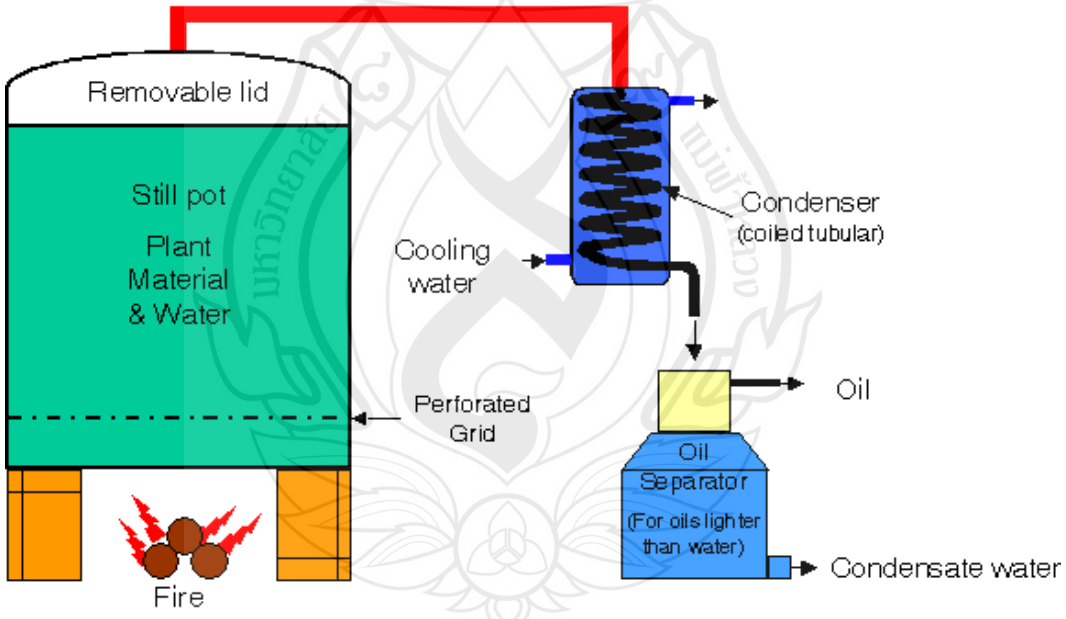
Beekeeping management techniques are specifically designed to reduce mite infestation in a colony. Biotechnical methods used commonly are drone brood removal and trapping, artificial swarm and open mesh floors (Zemene et al., 2015).

Open-screen floors in hives may interfere with mite population growth by decreasing the rate at which mites invade brood cells, leading to fewer mites, a lower percentage of mites in brood cells and more cells of capped brood compared with hives with wooden floors (Harbo & Harris, 2004). A high proportion of mites can be removed from bee colonies by creating an artificial swarm. This involves moving the parent colony approximately 4 meters from the original colony area. A second hive containing newly drawn combs and the queen is placed on the original site, causing foragers to return to this hive, creating an artificial swarm (Zemene et al., 2015).

Brood removal and trapping according to *Varroa* mite method for control of *Tropilaelap* mite is based on the understanding that mites are limited in brood cell when the cell are capped. The mites can be easily removed from the colony without the mites being able to escape back onto the adult bees. Removal of worker brood can also reduce mite levels, but it greatly affects colony productivity and is labor intensive (Anderson & Roberts, 2013).

2.3 Hydro Distillation

Hydro distillation is a tradition method for the extraction of essential oil from plants. The main advantages of this method are that less steam is used, shorter processing time and a higher oil yield (Rudin et al., 2015). In this method, plant materials are added in a still segment (pot) then water is added in sufficient amount and heated to the point of boiling (Hasbay & Galanakis, 2018). On the other hand, direct steam may also be injected into the plant sample. The vapor mixture of water and oil is condensed by indirect cooling with water. Condensed mixture flows from condenser to a separator, where essential oils are separated consequently from the water (Hasbay & Galanakis, 2018). Essential oils are lighter than water will float on the surface (Rudin et al., 2015).

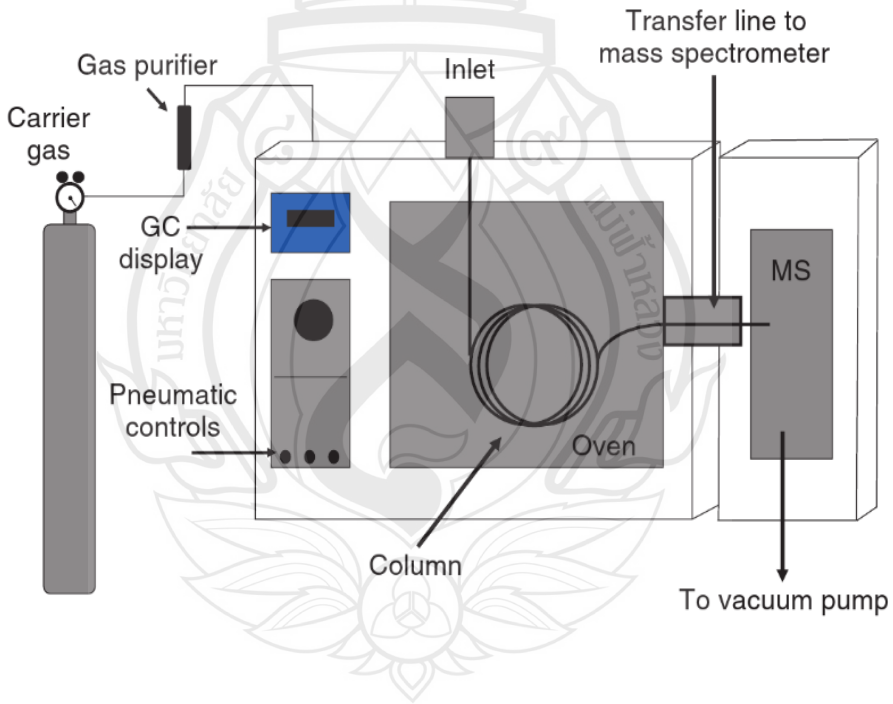


Source Douglas et al. (2005)

Figure 2.3 Schematic of diagram of the Hydro distillation method

2.4 Gas Chromatography-Mass Spectrometry (GC-MS)

Gas Chromatography–Mass Spectrometry (GC-MS) is a hyphenated analytical technique that combines the separation properties of gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances within a test sample (Figure 2.4). GC is used to separate the volatile and thermally stable substitutes in a sample whereas GC-MS fragments the analyte to be identified based on its mass (Chauhan et al., 2014).



Source Sparkman et al. (2011)

Figure 2.4 Schematic of a typical simple GC-MS

CHAPTER 3

MATERIALS AND METHODS

3.1 Herbal Plant Extraction

Fresh roots of curcumin, galangal, and ginger (15.0 kg per sample) were bought from Ban-Du market, Chiang Rai, Thailand. Herb plant extraction was conducted at the Center of Excellence in Medicinal Plants and Thai Traditional Medicine, Mae Fah Luang University, Thailand. The fresh plants were cut into small pieces. The hydro-distillation of the sample was performed for 6 to 8 hours. The only oils were collected and separated from other solutions (water) by using hexane and then evaporated solvent (hexane) by using an evaporator. The essential oil was stored in essential oil bottles in a refrigerator at 4°C (Rudin et al., 2015).

3.2 Experiment in Laboratory

3.2.1 Sample Collection

Adult *Tropilaelaps* mites from the highly infested colonies were collected from bee broods using a fine paintbrush and were placed into Petri dishes. Only adult female mites were selected for further investigation.

3.2.2 Effects of Essential Oils on Life Span of Honeybees

The experiment was designed by using a Completely Randomized Design (CRD) with 3 replications. The *C. longa, Z. officinale*, and *A. galanga* essential oils treatments in this experiment consisted of 25%, 50%, and 75% concentrations and distilled water (control). Thirty adult honeybees were collected and put into the plastic cages (12.5 × 6.5 cm). After that a Whatman filter paper No.1 (1 × 3 cm) was applied with each concentration of the essential oil. All the volumes of essential oils per volume

of plastic container were made to be 0.88 µl/cm^3. The volume of each plastic cage was 797.67 cm^3. Therefore, in each plastic cage, the amount of prepared concentration of essential oils applied to the filter paper to be placed in each plastic cage was approximately 702 µl. After the prepared filter paper of each concentration of essential oils was completely dried, then it was placed in each assigned cage. Then, the cages were placed in the dark conditions at room temperature. The number of individual dead bees was observed every 24 hours or until all bees died.

3.2.3 Effects of Essential Oils on the Life Span of *Tropilaelaps* Mites

The experiment was designed by using a Completely Randomized Design (CRD) with 3 replications. The *C. longa, Z. officinale*, and *A. galanga* essential oils treatments in this experiment consisted of 25%, 50%, and 75% concentrations and distilled water (control). Eleven *Tropilaelaps* adult female mites were collected and put into the middle of the glass tube (1.2 × 20 cm). After that a Whatman filter paper No.1 (1 × 3 cm) was applied with each concentration of the essential oil. All the volumes of essential oils per volume of glass tube were made to be 0.88 µl/cm^3. The control (the other side of the glass tube) of each treatment was distilled water. Then, glass tubes were placed at 32±1°C in an incubator. The number of individuals moving and dead mites was observed at 1, 2, 3, 6, 12, 24, and 36 hours, respectively.

3.2.4 Data Analysis

Statistical analyses were performed using SPSS (version 23.0). Differences in honeybee and *Tropilaelaps* mite survival among treatment groups were compared using a Keplan-Meier survival analysis; Log Rank multiple comparison tests were used to determine differences among treatments.

3.3 Experiment in Apiary

The experiment was conducted in Chan Chawa Tai Subdistrict, Mae Chan District, Chiang Rai, Thailand (geographic location: 20˚N, 99˚E) from 18th January 2021 to 3rd April 2021. The average temperature and humidity were 23.83˚C and 74.6%, respectively. All treatments were arranged in a Complete Randomized Design (CRD) with 3 replications. Twenty-one honeybee colonies in Langstroth standard honeybee colonies (41.5 × 50.5 × 24.5 cm) were naturally infested with *Tropilealaps* mites and each colony had 7 frames (workers and brood frames).

3.3.1 Investigation of Mite Infestation on Honeybee Colony from Each Treatment

The honeybee population was examined according to Delaplane et al. (2013). The adult honeybee population density was examined by taking photos with a digital camera because adult honeybees always walk in the bee hive. The mite infestations in capped brood cells were evaluated before and after treatment by randomly opening 100 capped brood cells per colony. Honeybee population and mite infestations data were recorded for 3 months.

3.3.2 Efficacies of Essential Oils on *Tropilaelaps* Mites in Honeybee Colony

The treatments consisted of three concentrations, 25%, 50%, and 75%, and the control (water) for both *Z. officinale* and *A. galanga.* All the volumes of essential oils per volume of Langstroth beehive were made to be to be 0.88 µl/cm^3 in the same manner as in laboratory experiment. The Langstroth beehive has the standard volume of 51,345.88 cm^3. Therefore, in each Langstroth beehive, the amount of prepared concentration of essential oils applied to each beehive was approximately 45.20 ml. Each concentration of each essential oil with the amount of 45.20 ml was applied to a piece of paper towel and placed into Zip lock bag (20 × 30 cm) with punctured holes. Each hole was 1 cm apart. Each prepared Zip lock bag was placed on top of the brood frame under the top cover of the assigned bee hive colony. The number of mite infested cells was observed by opening the capped brood cells every month for 3 months. The mortality of adult honeybees was observed every month for 3 months.

3.3.3 Measuring of Fallen Mites

The number of fallen mites were examined by using white sticky board (30 × 40 cm) were applied with mesh screen (to protect the bees contact the debris of mite) and placed on the deep bottom of bee hive in every colony. The number of falling mites were recorded at every week intervals for 3 months.

3.3.4 Data Analysis

Statistical analyses were performed using SPSS (version 23.0). Analysis of Variance (ANOVA) was used to analyze data. Comparisons between means were made by using the Duncan's Multiple Range Test (DMRT) at 0.05 probabilities

3.4 The Determination of Chemical Composition from *C. longa*, *Z. officinale* and *A. galanga* Essential Oils

The essential oils were obtained from curcumin, ginger, and galangal to identify the chemical composition that was investigated by gas chromatography-mass spectrometer with an Agilent 19091S-433 instrument equipped. The column was a capillary column with a dimension of 30 m × 0.25 mm i.d. × 0.25 μm film thickness, HP-5MS (5% phenyl-polymethylsiloxane). The GC-MS was operating under the oven temperature program started at 60°C (5 min) and ramped up to 60°C to 240°C (3°C/min). The carrier gas was Helium with 20 ml/min. The injection volume was 1 μL in split mode with a 100:1 split ratio. Quadrupole temperature was 150°C and transfer-line temperature 230°C. The identification of the volatile compounds was operated by comparing the mass spectra with the database using Wiley 7n.1 and NIST08 mass spectra libraries. To confirm the identification of essential oil compounds was conducted by comparison of Kováts retention indices relative to C_9-C_{18} *n*-alkanes and comparison of mass spectra of individual components with corresponding the references standard mass spectra in Wiley275 and NIST05 database according to Adam (2007). The data percentage is a report as the mean values. Identification of essential oil compounds were conducted by using Kováts retention index with the formula Eq.1.

$$RI = 100[C+[[RtX-RtC] / [Rt_{(C+1)}-RtC]]] \quad \text{Eq.1}$$

Where:

RI = Retention index

C = The number of the carbon atom of the *n*-alkanes peak that before sample peak

RtX = Retention time of the sample peak

RtC = Retention time of *n*-alkanes before sample peak

$Rt_{(C+1)}$ = Retention time of *n*-alkanes after sample peak

CHAPTER 4

RESULTS

4.1 Experiment in Laboratory

4.1.1 Effects of Essential Oils on Life Span of Honeybees

For the effect of treatments on honeybees, the highest survival rate was found in *A. galanga* essential oils at 25% concentration and in control treatments which the honeybees could survive for 33 to 36 days, and there was no significant difference between these two treatments (P=0.0440). The lowest survival rate was found in the treatments of 50% *A. galanga*, 75% *A. galanga*, 50% *Z. officinale*, and 75% *Z. officinale* essential oils with no significant differences in survival rate among treatments (P>0.05), and the honeybees appeared to die after 2 days. In addition, *Z. officinale* essential oil at 25% concentration, and *C. longa* essential oil at 75% concentration showed no significant difference (P=0.814) in the survival rate which honeybees could survive within 11 to 14 days. However, *Z. officinale* essential oil at 25% concentration and *C. longa* essential oil at 50% concentration had no significant difference (P=0.480) in survival rate of the honeybees and the survival of the honeybees lasted within 8 to 14 days. Also, *Z. officinale* and *C. longa* essential oils at 25% concentration showed no significant difference (P=0.153) in survival rate and the honeybees could survive no longer than 12 to 14 days (Figure 4.1).

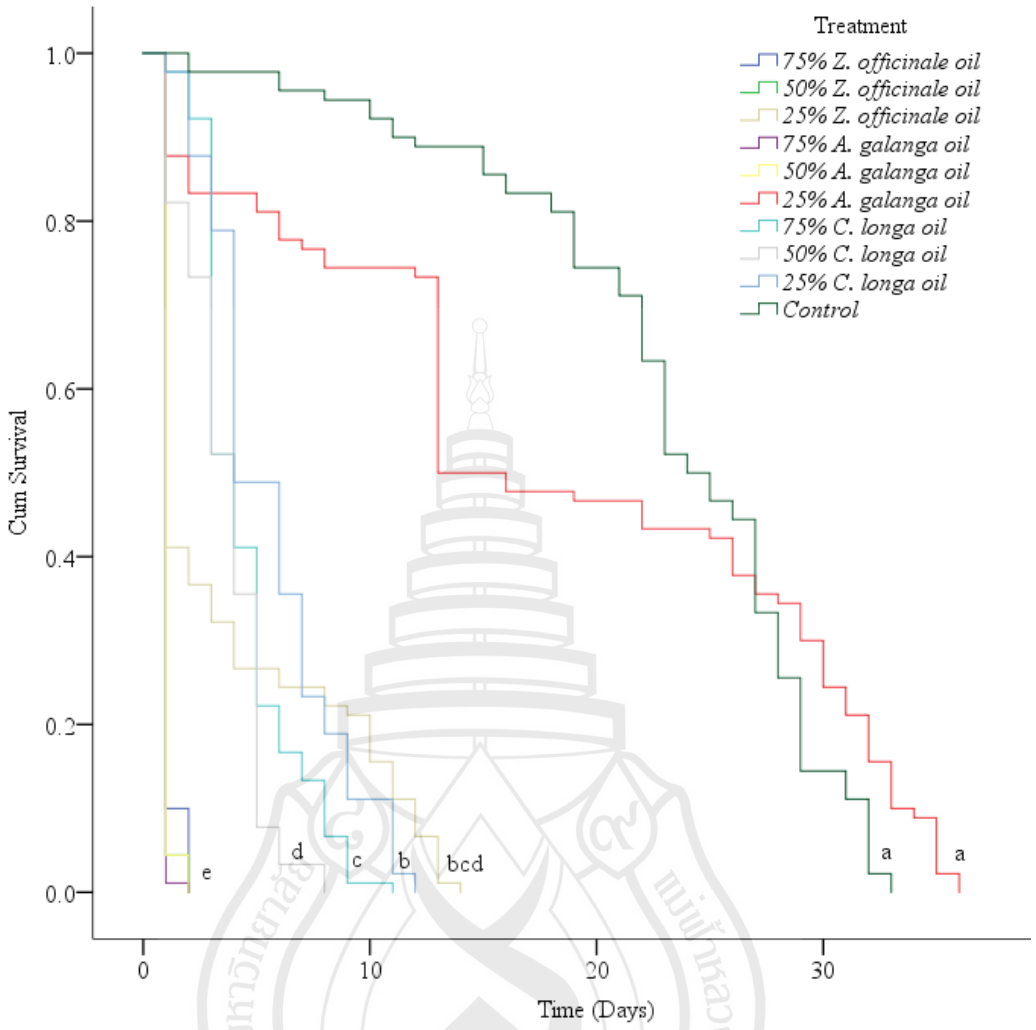


Figure 4.1 Number of day honeybees survived in plastic cage among treatments. Different letters are significantly different from each other (Log rank test, $P<0.05$)

4.1.2 Effects of Essential Oils on Survival of *Tropilaelaps* Mites

The highest survival rate was found in control (P<0.0001) which the mites could survive until the 48th hour. The second highest survival rate was found in *C. longa* essential oil at 25% concentration. The *C. longa* essential oil at 50% and 75% concentrations did not show significant differences in survival rate (P=0.495) and the mites could survive within 24 to 36 hours. The lowest survival rate was found in 75% *Z. officinale* and 50% *A. galanga* essential oils and both essential oils of the mentioned concentrations did not differ significantly (P=0.526) which caused the mites to die within 1 hour. However, *Z. officinale* essential oil at the concentration of 25% and 50% did not show significant differences in the survival rate of the mites (P=0.448) and the mortality of the mites occurred within 3 hours. Moreover, *Z. officinale* essential oil at 50%, and *A. galanga* at 75% showed no significant difference in reducing the rate of survival of the mites (P=0.526) and the mites could survive for 3 hours. Additionally, 25% of *Z. officinale*, and 75% of *A. galanga* essential oils showed no significance (P=0.182) in survival rate which the mites could survive no longer than 3 hours (Figure 4.2).

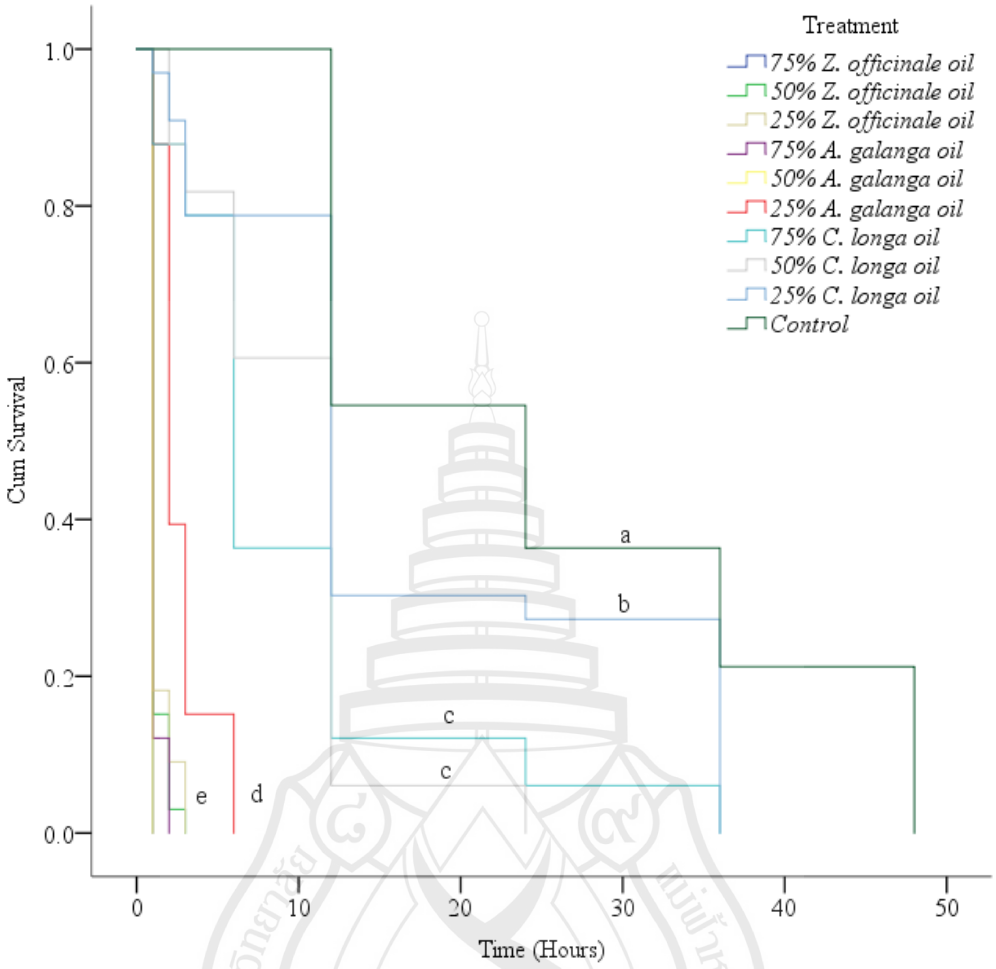


Figure 4.2 Number of hours *Tropilaelaps* mite survived in glass tube among treatments. Different letters are significantly different from each other (Log rank test, $P<0.05$)

4.2 Experiment in Apiary

4.2.1 Effects of *Z. officinale* Essential Oil on Honeybee Population

For the effect of treatment on the honeybee population, the result showed that there were no interactions between treatments and the months. Regardless of month, there were no significant differences in honeybee population among concentrations ($F_{3,32}$=1.130, P=0.352, Figure 4.3). There were no significant differences in the honeybee population in each month. ($F_{3,32}$=1.733, P=0.180, Figure 4.3).

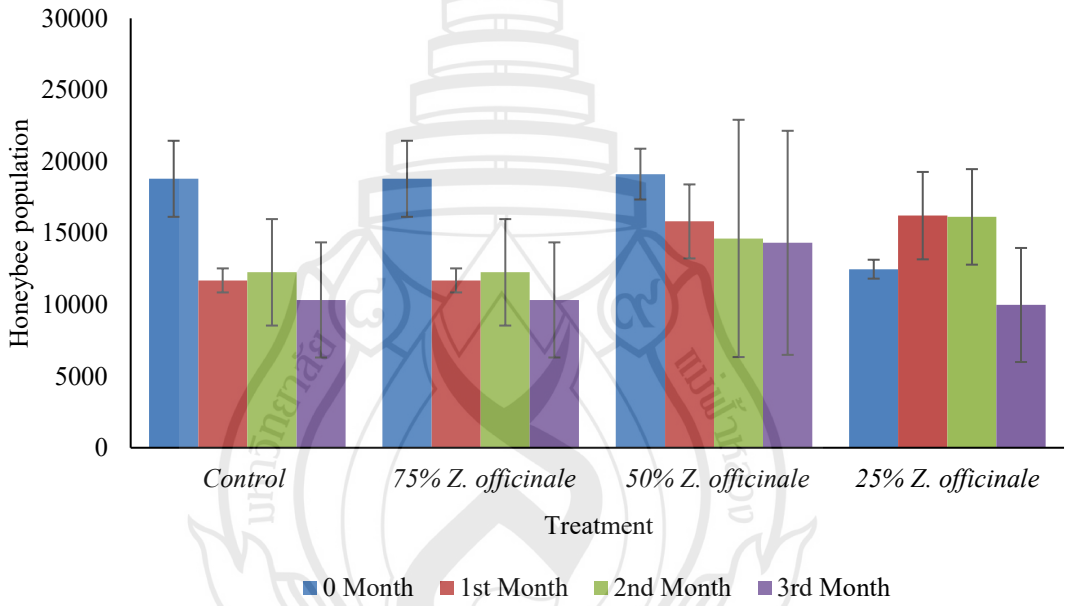


Figure 4.3 Mean ± SE of honeybee population in honeybee colonies after treatment with *Z. officinale* essential oil at 25%, 50% and 75% concentrations in 3 months. Different letters are significantly different from each other (P<0.05)

4.2.2 Effects of *Z. officinale* Essential Oil on Mite Infestation

For the effect of *Z. officinale* treatments on mite infestation, there were interactions among treatments and months ($F_{9,32}$=2.738, *P*=0.017). After the treatments, the number of mite infestations decreased gradually to the lowest infestation in the third month ($F_{15,47}$=2.632, *P*=0.011).

Compared to the control, the mite infestation reduction trend showed that *Z. officinale* essential oil of 75% concentration was the highest efficacy in reducing mite infestation. The second highest efficacy in reducing mite infestation was the concentration of 50%. The lowest efficacy in reducing mite infestation was the concentration of 25%. However, the effectiveness of the concentrations of 75%, 50%, and 25% in reducing mite infestation was not significantly different ($F_{3,32}$=0.845, *P*=0.480) (Figure 4.4). The trend of concentration of 75% showed continuously reducing mite infestation every month while the concentration of 50% was able to reduce mite infestation in the first month, but infestation increased again in the second month and decreased again in the third month (Figure 4.4). At a concentration of 25%, the percentage of mite infestation decreased in the first two months but infestation increased again in the third month (Figure 4.4).

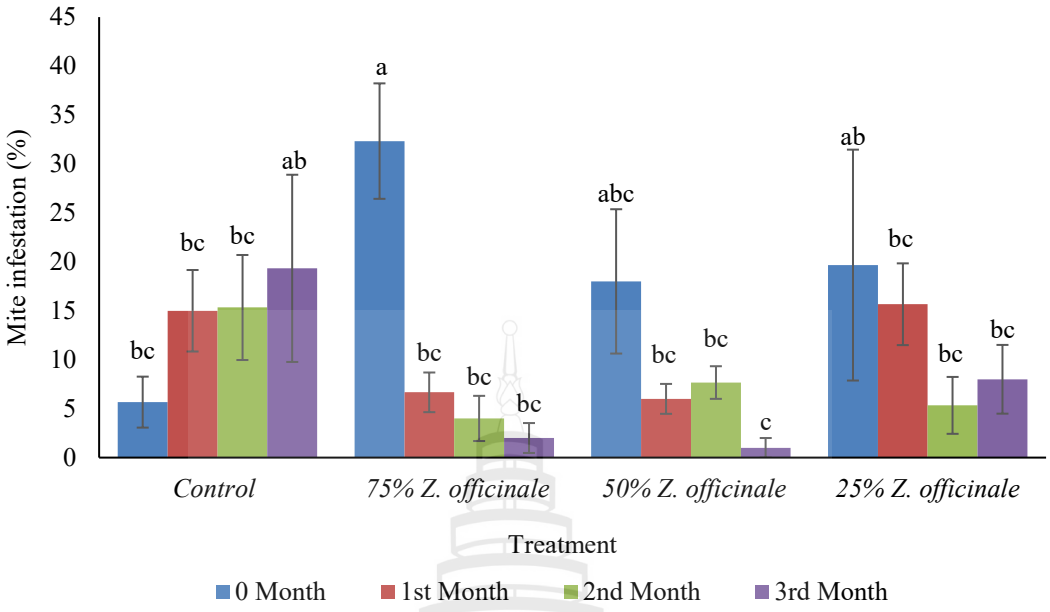


Figure 4.4 Mean ± SE of mite infestation in honeybee colonies after treatment with *Z. officinale* essential oil at 25%, 50% and 75% concentrations in 3 months. Different letters are significantly different from each other (P<0.05)

4.2.3 Quantitative Measurement of Fallen Mites after Being Exposed to *Z. officinale* Essential Oil

For the effect of *Z. officinale* essential oil treatments on the number of fallen mites, the result showed that there were interactions among treatments and months ($F_{9,32}$=0.016, P=0.000). After the treatments, *Z. officinale* essential oil at 75% concentration caused the maximum number of fallen mites on the sticky board in the first month ($F_{15,47}$=3.426, P=0.002) (Figure 4.5) and then the number of fallen mites decreased during the second and third months. At 50% and 25% concentrations, the number of fallen mites decreased in the first two months but increased again in the third month (Figure 4.5).

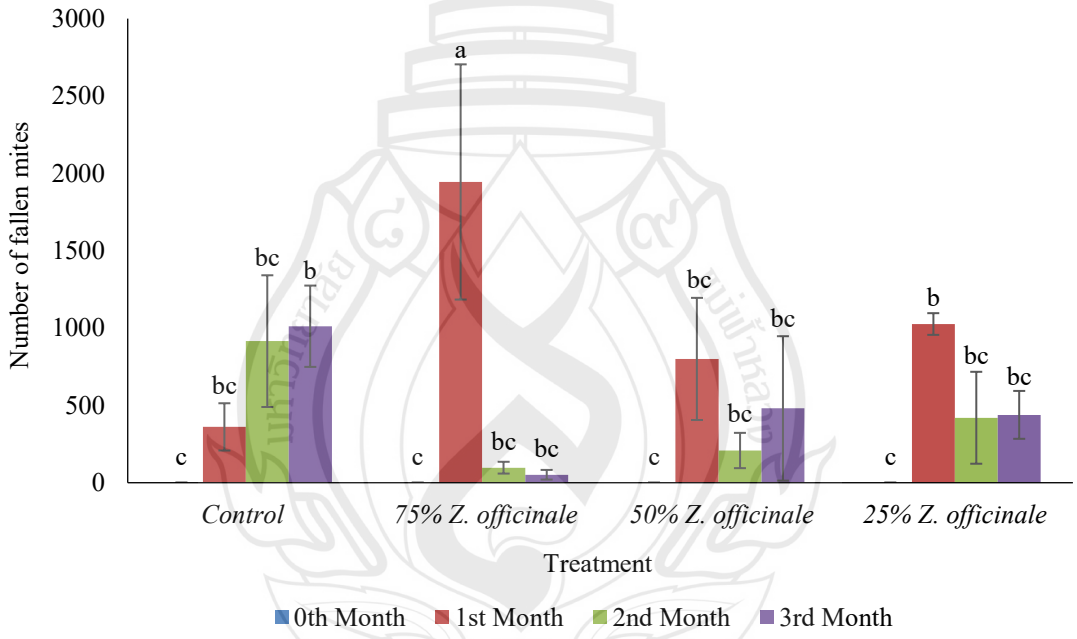


Figure 4.5 Mean ± SE number of fallen mites in honeybee colonies after treatment with *Z. officinale* essential oil at 25%, 50% and 75% concentrations in 3 months. Different letters are significantly different from each other (P<0.05)

4.2.4 Effect of *A. galanga* Essential Oil on Honeybee Population

As for the effect of treatment on the honeybee population, the result showed that there was no interaction between treatments and months ($F_{9,32}$=0.502, P=0.862). Regardless of treatments, there were significant differences between months ($F_{3,32}$=6.494, P=0.001). There were no significant differences in concentrations ($F_{3,32}$=0.768, P=0.5.20) (Figure 4.6).

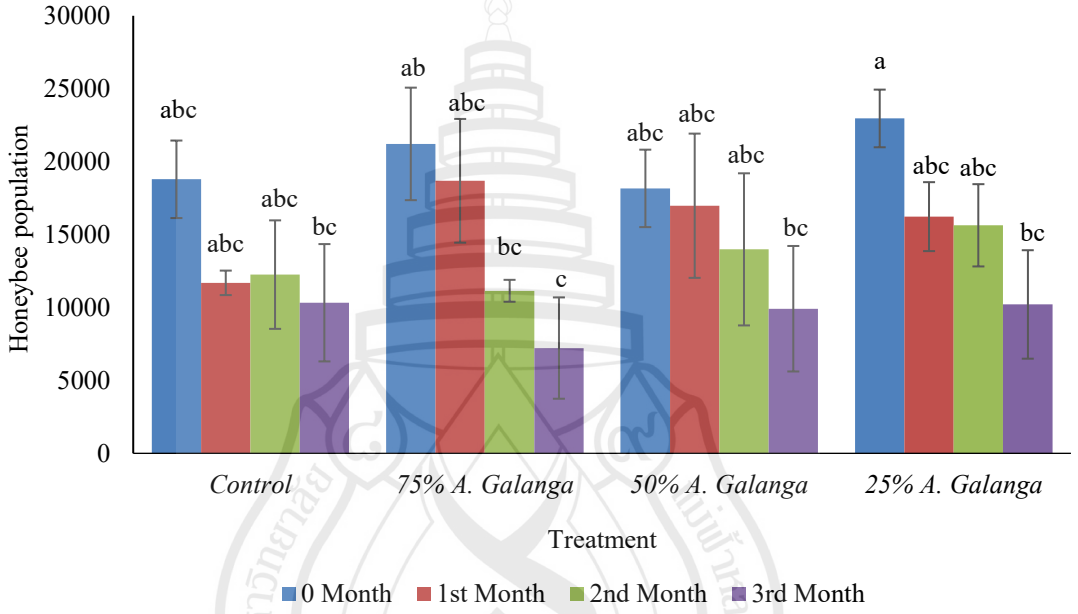


Figure 4.6 Mean ± SE of honeybee population in honeybee colonies after treatment with *A. galanga* essential oil at 25%, 50% and 75% concentrations in 3 months. Different letters are significantly different from each other (P<0.05)

4.2.5 Effect of *A. galanga* Essential Oil on Mite Infestation

There were interactions among treatments and months in the effect of treatment on mite infestation ($F_{9,32}$=2.428, P=0.031). The highest efficacy against mite infestation was observed at 75% concentration of *A. galanga* essential oil ($F_{15,47}$=2.509, P=0.014). However, mite infestation gradually decreased with every concentration (25%, 50%, and 75%) and was lowest in the third month, in contrast to mite infestation in the control group (Figure 4.7).

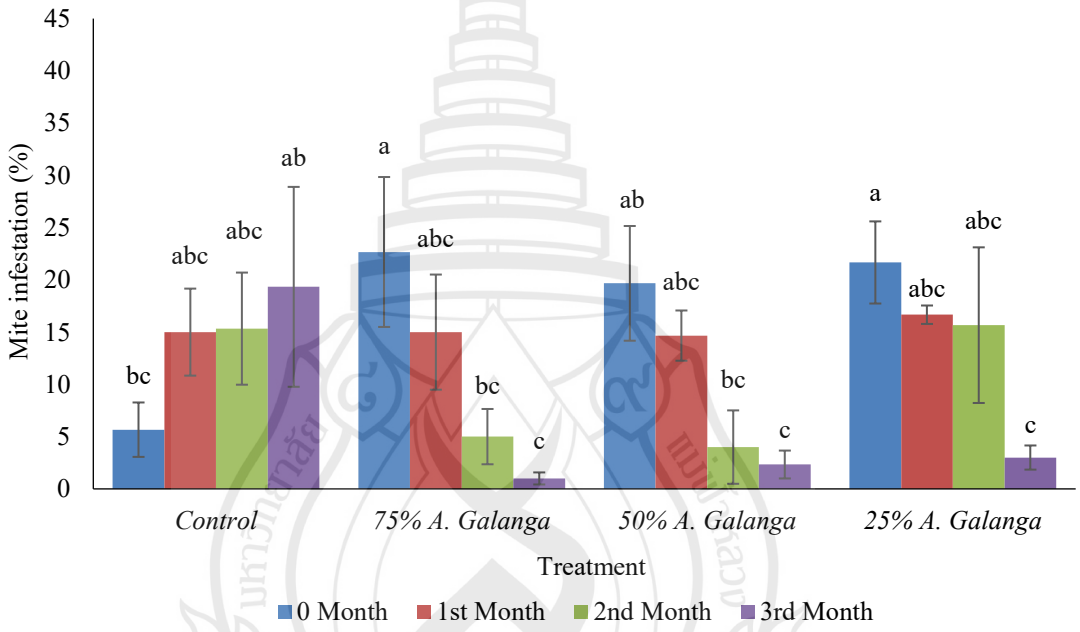


Figure 4.7 Mean ± SE number of mite infestation in honeybee colonies after treatment with *A. galanga* essential oil at 25%, 50% and 75% concentrations in 3 months. Different letters are significantly different from each other (P<0.05)

4.2.6 Quantitative Measurement of Fallen Mites after Being Exposed to *A. galanga* Essential Oil

For the effect of *A. galanga* essential oil treatments on the number of fallen mites, there were interactions among treatments and months ($F_{9,32}$=0.038, P=0.003). After the exposure, the maximum number of fallen mites drop on sticky board of all concentrations (25%, 50%, and 75%) occurred in the first month ($F_{15,47}$=2.769, P=0.008).

As shown in Figure 4.8, the number of mites decreased after treatment with essential oils of *A. galanga*. The concentration of 75% in the first month caused significant differences in reducing the number of fallen mites when compared with the second and third months. However, the number of fallen mites at the concentrations of 25% and 50% decreased gradually from the first until the third month and there was no significant difference in each month (Figure 4.8).

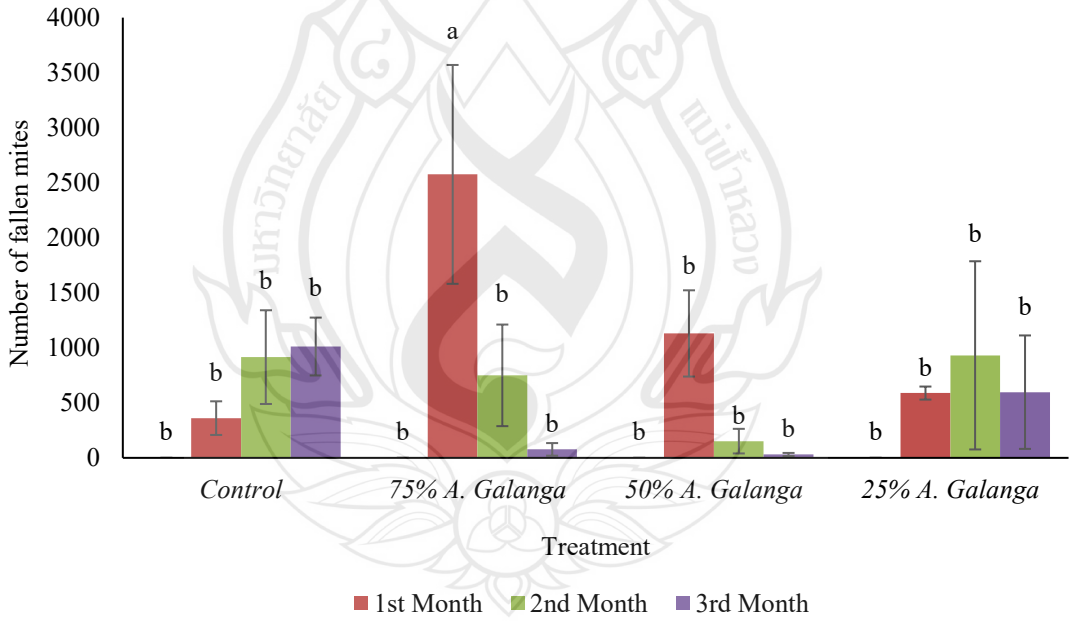


Figure 4.8 Mean ± SE number of fallen mites in honeybee colonies after treatment with *A. galanga* essential oil at 25%, 50% and 75% concentrations in 3 months Different letters are significantly different from each other (P<0.05)

4.2.7 Comparison of the Effectiveness of *Z. officinale* and *A. galanga* Essential Oils in Controlling the Mites

When comparing the effectiveness of *Z. officinale* and *A. galanga* essential oil treatments on mite infestation, there were interactions among treatments and months ($F_{18,56}$=1.970, *P*=0.028, Table 4.1). There were significant differences between months regardless of treatment (F18,56=12.606, *P*=0.000, Table 4.1). However, there were no significant differences in concentration (F18.56=0.779, *P*=0.590, Table 4.1).

Table 4.1 Comparison of the effectiveness of *Z. officinale* and *A. galanga* essential oils in controlling the mites (Mean ± SE)

	Mean of mite infestation (%Mean ± SE)			
Treatments	**0 Month**	**1st Month**	**2nd Month**	**3rd Month**
Control	5.67±2.60^{cdefg}	15.00±4.16^{bcdefg}	15.33±5.36^{bcdefg}	19.33±9.56^{abcde}
75% *Z. officinale*	32.33±10.21^{a}	6.67±3.51^{bcdefg}	4.00±4.00^{defg}	2.00±2.65^{fg}
50% *Z. officinale*	18.00±12.77^{abcdef}	6.00±2.65^{cdefg}	7.67±2.89^{bcdefg}	1.00±1.73^{g}
25% *Z. officinale*	19.67±20.43^{abcd}	15.67±7.23^{bcdefg}	5.37±5.03^{cdefg}	8.00±6.08^{bcdefg}
75% *A. galanga*	22.67±7.17^{ab}	15.00±5.51^{bcdefg}	5.00±2.65^{defg}	1.00±0.58^{g}
50% *A. galanga*	19.67±5.49^{abcd}	14.67±2.40^{bcdefg}	4.00±3.51^{defg}	2.33±1.33^{fg}
25% *A. galanga*	21.67±3.93^{abc}	16.67±0.88^{abcdefg}	15.67±7.45^{bcdefg}	3.00±1.15^{efg}

Note Means in column followed by the same letter were not significantly different (*P*>0.05) according to Duncan's Multiple Range Test (DMRT).

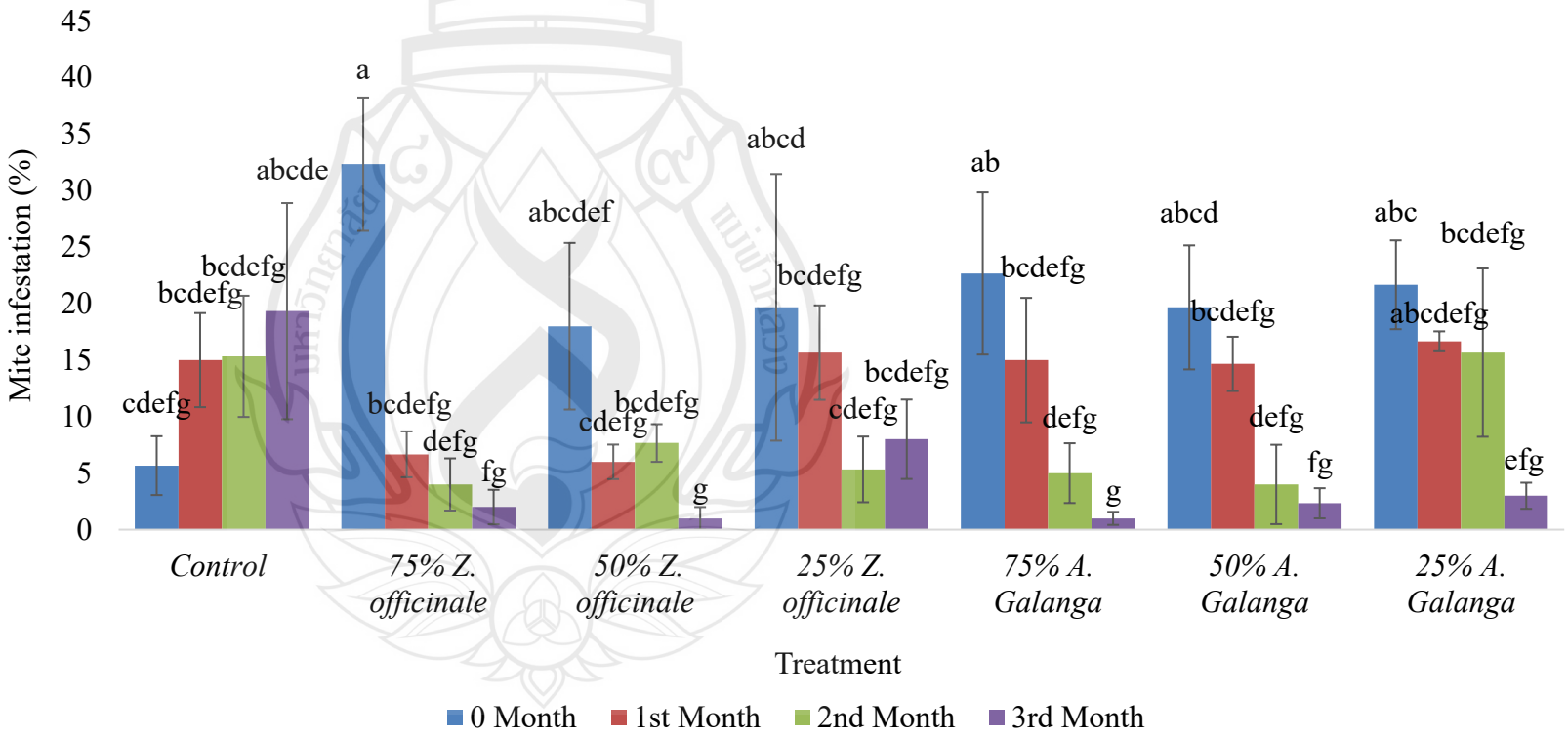


Figure 4.9 Means ± SE number of mite infestation between about *Z. officinale* and *A. galanga* essential oils at 25%, 50% and 75% concentrations in 3 months. The same letter indicates no significant difference (P>0.05)

4.3 The Determination of Chemical Composition from *C. longa*, *Z. officinale* and *A. galanga* Essential oils

4.3.1 Chemical Composition of *C. longa*

In the Gas chromatogram of essential oils obtained from *C. longa* in which the major constituents detected was β-Tumerone (49.94%), α-Tumerone (10.43%), and AR-Tumerone (18.32%) respectively (Figure 4.10, Table 4.2).

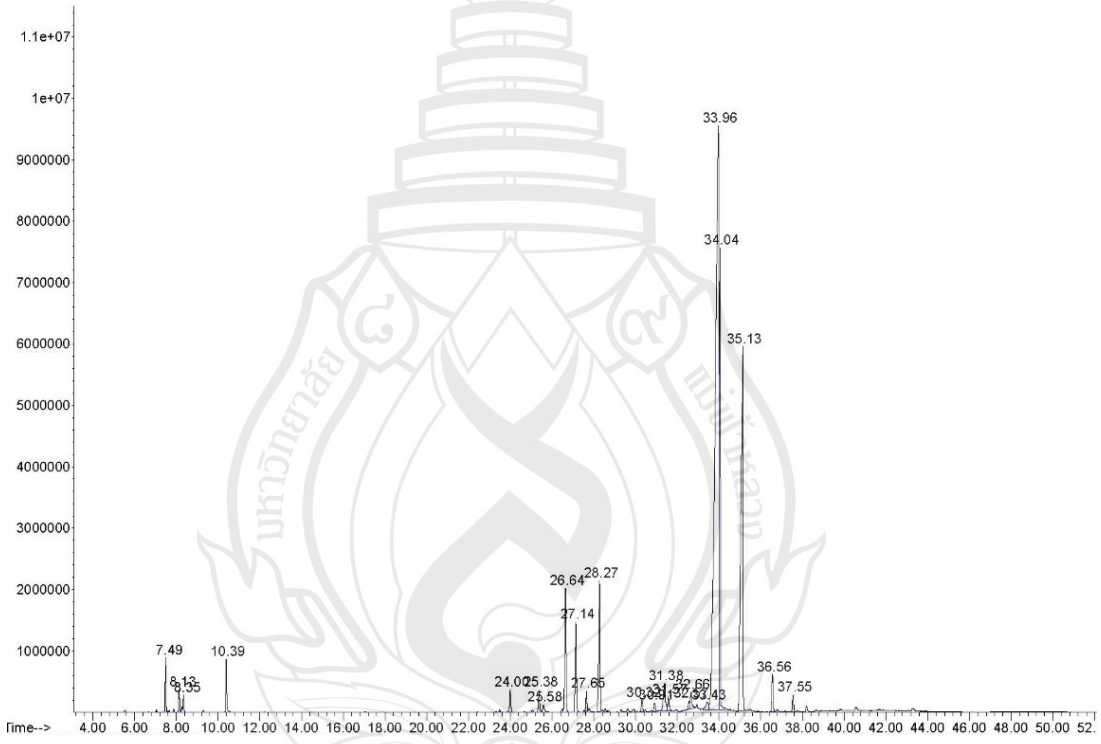


Figure 4.10 The GC-MS chromatogram of *C. longa*

Table 4.2 The chemical constituents and their relative peak area percentage of volatile compounds of *C. longa* by GC-MS

Peak	RT	Components	RI*	RI**	% Peak area
1	7.49	1-Phellandrene	999.60	1002	1.02
2	8.13	Benzene, 1-methyl-2-(1-methylethyl)-	1017.65	1042	0.46
3	8.35	1,8-Cineole	1023.81	1026	0.48
4	10.39	α-Terpinolene	1080.95	1086	1.28
5	24.00	trans-Caryophyllene	1409.34	1408	0.67
6	25.38	α-Humulene	1443.24	1452	0.64
7	25.58	trans-β-Farnesene	1448.16	1440	0.29
8	26.64	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	1474.20	1474.4	3.69
9	27.14	1,3-Cyclohexadiene, 5-(1,5-dimethy l-4-hexenyl)-2-methyl-	1486.49	1487.76	2.64
10	27.65	β-Bisabolene	1499.02	1505	0.54
11	28.27	β-Sesquiphellandrene	1514.95	1521	3.95
12	31.38	Benzene, 1-methyl-3-(1-methylethyl)-	1567.53	1562	1.37
13	33.43	γ-curcumene	1650.00	1481	0.52
14	33.96	β-Turmerone	1664.25	1699	49.94
15	34.04	AR-Turmerone	1666.40	1664	10.43
16	35.13	α-Turmerone	1695.70	1668	18.32
17	37.55	(+)-α-Atlantone	1764.02	1777	0.49

Note RI*; calculated retention indices

RI**; literature retention indices from Adam (2007)

4.3.2 Chemical composition of *Z. officinale*

In the Gas chromatogram of essential oils obtained from *Z. officinale* in which the major constituents detected were Zingiberene or 1,3-cyclohexadiene 5-(1,5-dimethyl-4-hexenyl)-2-methyl (12.96%), 2,6-Octadienal, 3,7-dimethyl- (12.68%), and Z-citral (9.73%) respectively (Figure 4.11, Table 4.3).

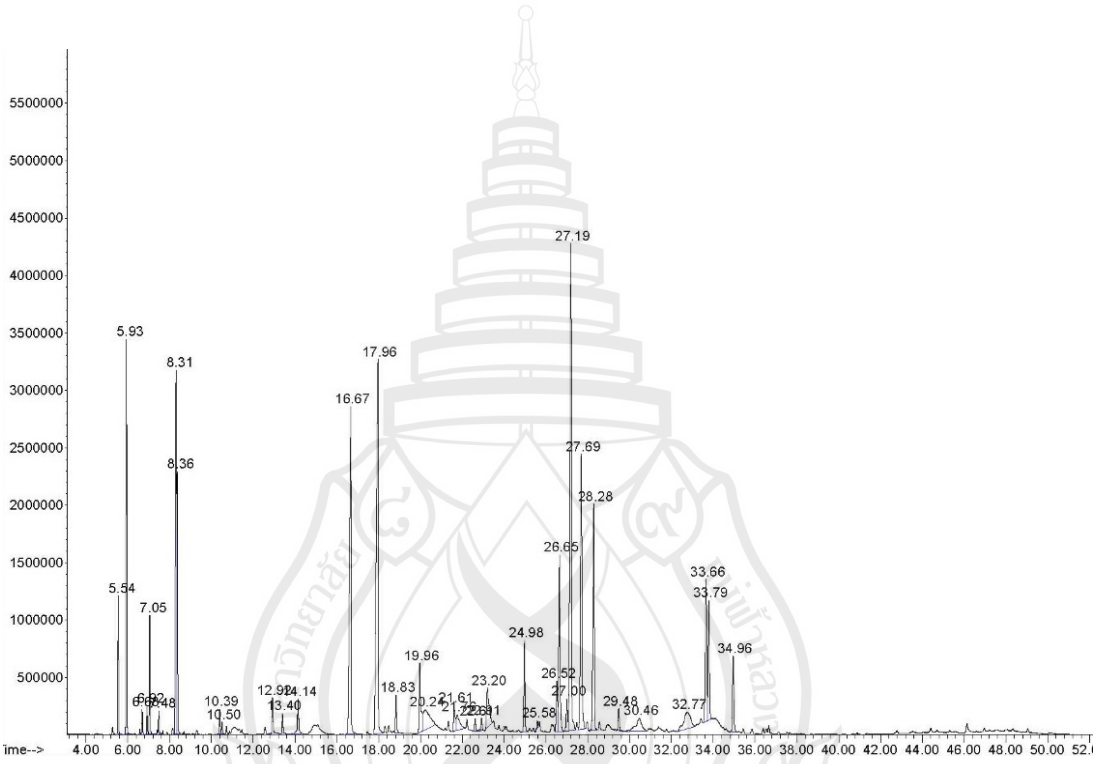


Figure 4.11 The GC-MS chromatogram of *Z. officinale*

Table 4.3 The chemical constituents and their relative peak area percentage of volatile compounds of *Z. officinale* by GC-MS

Peak	RT	Components	RI*	RI**	% Peak area
1	5.54	α-Pinene	922.22	932	1.67
2	5.93	Camphene	937.70	994	5.13
3	6.68	β-Pinene	967.46	974	0.34
5	7.05	β-Myrcene	982.14	988	1.63
6	7.49	1-Phellandrene	999.60	1002	0.35
7	8.31	Sabinene	1022.69	696	7.36
8	8.36	1,8-Cineole	1024.09	1026	3.35
9	10.39	α-Terpinolene	1080.95	1086	0.44
10	10.5	2-Nonanone	1084.03	1087	0.22
11	12.92	6-Octenal, 3,7-dimenthyl-	1145.23	1140	0.68
12	13.4	Cyclohexane, ethenyl-	1156.97	1143	0.36
13	14.14	α-Thujone	1175.06	1102	0.9
14	16.67	Z-Citral	1235.45	1114	9.73
15	17.96	2,6-Octadienal, 3,7-dimethyl-	1265.73	1235	12.68
16	18.83	2-Undecanone	1286.15	1293	0.75
17	22.91	β-Elemene	1383.10	1390	0.29
18	26.52	Germacrene D	1471.25	1484	1.19
19	27.00	1H-Cyclopropa[a]naphthalene	1483.05	1431	0.75
20	27.19	Zingiberene or 1,3-cyclohexadiene 5-(1 5-dimethyl-4-hexenyl)-2-methyl	1487.71	1493	12.96
21	27.69	α-Farnesene	1500.00	1505	7.27
22	28.28	β-Sesquiphellandrene	1515.21	1521	5.35
23	29.48	Germacrene B	1546.13	1559	0.5
24	30.46	Nerolidol	1571.39	1561	1.43
25	32.77	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1α,7β,8aα)]	1632.26	1630	2.04

Table 4.3 (continued)

Peak	RT	Components	RI*	RI**	% Peak area
26	33.66	β-Tumerone	1656.18	1699	3.58
27	33.79	AR-Tumerone	1659.68	1664	2.61
28	34.96	α-Tumerone	1691.13	1668	1.8

Note RI*; calculated retention indices

RI**; literature retention indices from Adam (2007)

4.3.3 Chemical composition of *A. galanga*

In the Gas chromatogram of essential oils obtained from *A. galanga* in which the major constituents detected were 1,8-Cineole (81.11%), trans-β-Farnesene (3.73%), and β-Bisabolene (2.26%) respectively (Figure 4.12, Table 4.4)

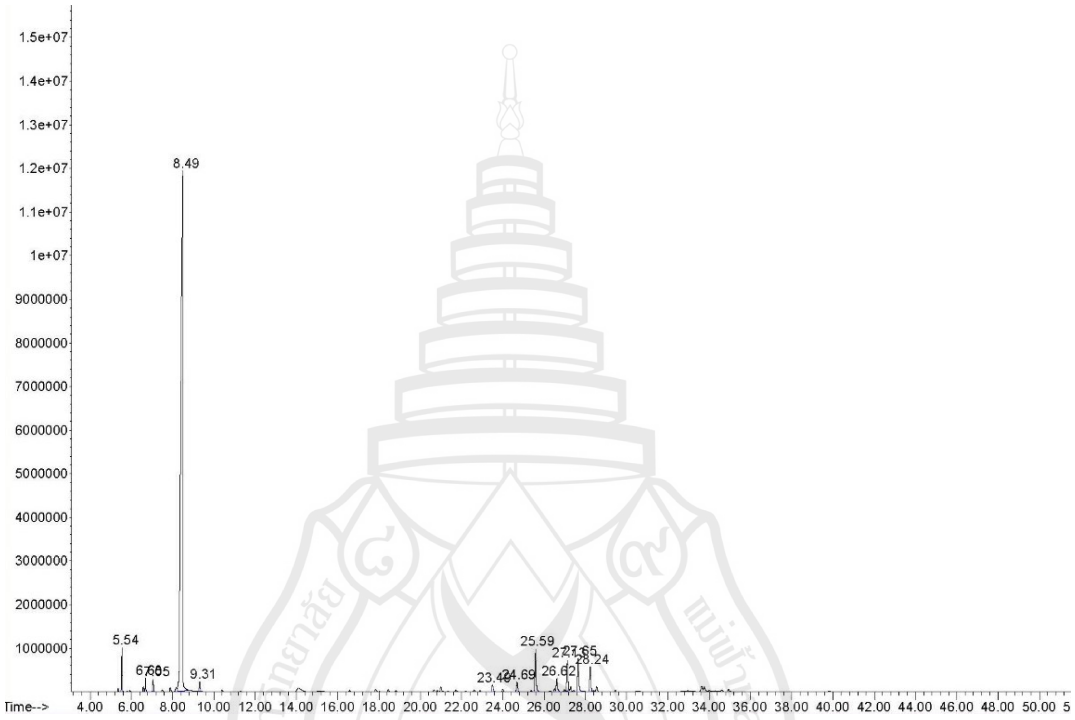


Figure 4.12 The GC-MS chromatogram of *A. galanga*

Table 4.4 The chemical constituents and their relative peak area percentage of volatile compound of *A. galanga* by GC-MS

Peak	RT	Component	RI*	RI**	% Peak area
1	5.54	α-PINENE	927.03	939	2.12
2	6.68	β-PINENE	969.58	979	0.74
3	7.05	β-Myrcene	983.39	988	0.74
4	8.49	1,8-Cineole	1027.73	1026	81.11
5	9.31	γ-Terpinene	1050.70	1014	0.62
6	23.49	Benzene	1396.90	1374	0.65
8	25.59	trans-β-Farnesene	1448.40	1456	3.73
10	27.13	Zingiberene	1486.24	1493	2.78
11	27.65	β-Bisabolene	1499.02	1505	3.14
12	28.24	β-Sesquiphellandrene	1514.18	1522	2.26

Note RI*; calculated retention indices

RI**; literature retention indices from Adam (2007)

CHAPTER 5

DISCUSSION

Some plant species are called aromatic plants because they contain volatile compounds that give them an odor and a characteristic taste (Regnault-Roger, 1997; Sararit & Auamcharoen, 2020). Some of the aromatic plants have shown promising for use in integrated pest management (IPM), namely some of the volatile compounds normally known as essential oils (Moharramipour & Negahban, 2014; Regnault-Roger, 1997). Essential oil compounds can disrupt basic behavioral functions, metabolic, biochemical, and physiological functions of some arthropods (Abd El-Wahab & Ebada, 2006; Hikal et al., 2017). In addition, the volatile compounds produce a strong odor that can block or penetrate the respiratory system of insects, resulting in their deaths (Abd El-Wahab & Ebada, 2006; Hikal et al., 2017; Pugazhvendan et al., 2012).

The chemical compositions of *C. longa* essential oil obtained from the rhizome contain a variety of monoterpenoids, sesquiterpenoids, and curcuminoids (Bushra & Tariq, 2014; Devkota & Rajbhandari, 2015). The main constituents were reported as β-turmerone, α-turmerone, ß-sesquiphellandrene, 1,4-dimethyl-2-isobutylbenzene, *ar*-turmerone, zingiberene, E-a-atlantone, and caryophyllene oxide (Devkota & Rajbhandari, 2015). These compounds have the potential of insecticidal, repellent and antifeedant effects against a variety of insect pests (Bushra & Tariq, 2014). The result of previous study done by Su et al. (1982) was similar to our result in the way that we also found β-turmerone, α- turmerone, *ar*-turmerone, and β-Sesquiphellandrene (Table 4.2). In addition, the *ar*-turmerone and turmerone also gave strong repellency to *T. castaneum* and *ar*-turmerone at 1000 ppm caused 100% mortality of *Nilaparvata lugens* female adults and *Plutella xylostella* (Lee et al., 2001). Tawatsin et al. (2006) reported that *C. longa* essential oil was an efficient mosquito repellent and oviposition deterrent.

The *Z. officinale* essential oil was found to exhibit repellent, insecticidal, anti-ovipositional, anti-egg hatching, and persistent insecticidal activities against *Callosobruchus chinensis* (Chaubey, 2013), *Sitophilus zeamais*, and *Tribolium castaneum* (Suthisut et al., 2011) under the laboratory condition. The study done by Loni and Panahi (2015) found that *Z. officinale* essential oil had a negative effect on egg hatching and adult emergence of *C. maculatus* (F.).

Plant essential oil of *A. galanga* has insecticidal and repellent properties (Abdullah et al., 2015). The extract was found to be both contact toxic and repellent to *Coptotermes gestroi and C. curvignathus* (termites) (Abdullah et al., 2015). Other studies have shown that *A. galanga* essential oil can also be used to control *Bactrocera dorsalis* (Sukhirun et al., 2009), and *Plutella xylostella* and *Callosobruchus chinensis* (Riyanto, 1998).

From our laboratory testing, the survival rate of *Tropilaelaps* mites showed that the concentrations of 75% and 50% of both *Z. officinale* and *A. galanga* essential oils resulted in mite death within 1 hour and revealed the fastest mortality compared to other treatments (Figure 4.2). The longest survival rate of *Tropilaelaps* mites was 36 to 48 hours after exposure at 25% *C. longa* essential oil and control (Figure 4.2). The highest survival rate of honeybees was a concentration of 25% *A. galanga* essential oil and control, where the honeybees survived 33 to 36 days after exposure (Figure 4.1). In contrast, the concentrations of 50% and 75% of the essential oils of *Z. officinale* and *A. galanga* resulted in the lowest survival rate of honeybees and the honeybees died after 2 days of exposure. From our results in laboratory testing, it was presented that, *C. longa* essential oil did not show as much efficacy in controlling the *Tropilaelaps* mites when compared to *Z. officinale* and *A. galanga* essential oils. According to that we chose only *Z. officinale* and *A. galanga* essential oils to be tested for efficacy in controlling *Tropilaelaps* mite in the field environment (Apiary).

Our field experiment revealed that *Z. officinale* essential oil was proved as the best to control *Tropilaelaps* mite infestation at 75% concentration (Figure 4.4). The second highest efficiency in controlling the mite infestation was the concentration of 50% while the concentration of 25% was the lowest in reducing the mite infestation in the honeybee colonies (Figure 4.4). All concentrations of Z. *officinale* essential oil did not adversely affect the honeybee population ($F_{3,32}$=1.130, P=0.352). However, the

result of the field experiment was not exactly the same as the result from the laboratory in the way that *Z. officinale* essential oil in tested concentrations (50%, and 75%) did have a negative effect causing mortality to honeybees. The reason could be from environmental factors such as the inconsistency of temperature and humidity during the month period when the field experiment was conducted. Moreover, the food (honey) that honeybees in apiary acquired came from different sources such as pollen and nectar from a variety of flowers containing more nutritional value. In contrast, the honeybees tested in the laboratory were fed only sugar water with a ratio of 1:1. Because of that honeybees in the apiary tended to be stronger and had more ability to tolerate the adverse effect of the *Z. officinale* essential oil than tested honeybees in laboratory. Additionally, the room space in which honeybees stayed could play important role in the survival of honeybees. The tested box in the laboratory was the size of 797.67 cm^3 and thirty individuals were released in each treatment box. The ventilation of each tested box was not good enough because each treatment box was kept in a closed dark condition. The apiary where the field experiment was conducted had better ventilation and more space for the honeybees in each colony.

The study conducted by Maedeh et al. (2012) reported the constituents of *Z. officinale* essential oil consisting of monoterpenes, (1,8- cineole, phellandrene, camphene, α-pinene, myrcene, citral, borneol) and sesquiterpenes (zingiberene, zingiberol, zingiberenol, β-bisabolene, β-sesquiphellandrene and ar-curcumene) (Maedeh et al., 2012). Their result was similar to our result in the way that we also found 1,8- cineole, camphene, α-pinene, β-sesquiphellandrene (Table 4.3). The insecticidal activity of Z. *officinale* essential oil is carried with insect species such as adult *Bactrocera dorsalis* (Sukhirun et al., 2011) and *Lasioderma serricorne* adults (Wu et al., 2014) did not respond to the essential oil at the same level. *Z. officinale* contains sesquiterpenes and the pungent odor which appear to be responsible for toxicity, and repellent effect on the insect pests (Maedeh et al., 2012). Moreover, the *Z. officinale* essential oil was found in many reports about insecticidal activity, antifeedant and growth inhibitory activities (Maedeh et al., 2012). Sahayaraj (1998) reported *Z. officinale* essential oil could exhibit antifeedant and growth inhibitory activities against the last instar larvae of *Spodoptera litura*. Although *Z. officinale* essential oil consists of many constituents, some of them may exhibit much higher activity than the whole

essential oil. For example, pure 1,8-cineole was reported as a toxic agent against *Tribolium confusum* adults and the last larval instar whereas other constituents did not seem to cause any effect (El-Aziz et al., 2009). When all chosen concentrations (25%, 50%, and 75%) of *Z. officinale* were tested in an apiary, these concentrations did not differ significantly compared to control (water) treatment. This means that *Z. officinale* essential oil at these concentrations did not seem to increase the mortality rate of honeybees (Figure 4.11).

The field experiment testing efficacy of *A. galanga* essential oil to control *Tropialelaps* mites demonstrated that the highest mortality of *Tropilaelaps* mites was found at a concentration of 75% (Figure 4.7). The second and third potential to get rid of the mites were concentrations of 50% and 25%, respectively. Moreover, all concentrations of *A. galanga* essential oil also showed a negative effect on the honeybee population because the number of honeybee population was dramatically reduced from the first until the third month of the experiment (Figure 4.6). Contrastingly, our result differed from what Lin et al. (2020) reported. The reason could be that their study used different volumes for testing of the impact of galangal essential oil on honeybees. Moreover, the species of galanga which they tested differ from the spices that we used in our experiment. Additionally, the volume of tested galangal (*A. officinarum* Hance) oil which they used was 4 µl and it did not show any negative effect on honeybees. For our study, the volume of 45.20 ml (per beehive) of essential oil applied to each treatment showed the toxicity to honeybees.

The main components of *A. galanga* essential oil were found in the rhizome, namely 1,8-cineole, fenchyl acetate, and α-pinene (Raina & Abraham, 2017). Their result was similar to our result in the way that we also found 1,8-cineole and α-pinene (Table 4.4). According to Tunón et al. (2006), α-pinene showed strong repellent activity against *Ixodes ricinus* (tick). In addition, the constituents 1,8-cineole and α-pinene were reported to be toxic and against *T. confusum* (Ojimelukwe & Adler, 1999; Tapondjou et al., 2005).

The comparison of the effectiveness of *Z. officinale* and *A. galanga* essential oils under the field condition showed that all concentrations of the two botanical essential oils demonstrated no significant difference in controlling the mite infestation (Table 4.1). However, when compared in each month, *Z. officinale* essential oil at the

concentration of 75%) showed that the mortality rate of the mites tended to decrease continuously whereas the concentration of 50% demonstrated the reduction of the mite infestation in the first month then the mite infestation started to increase again during the second month and declined again at the third month (Figure 4.9). The *Z. officinale* essential oil at 25% reduced the mite infestation during the first two months but it slightly increased again in the period of the third month (Figure 4.4). On the other hand, *A. galanga* essential oil at all concentrations (25%, 50%, and 75%) resulted in a continuous monthly reduction in mite infestation (Figure 4.7).

The number of fallen mites after exposure to *Z. officinale* essential oil at a concentration of 75% caused the highest number of fallen mites in the first month and gradually decreased until the third month (Figure 4.5). *Z. officinale* essential oil at concentrations of 50% and 25%, the number of fallen mites was highest in the first month and decreased in the second month but increased again in the third month (Figure 4.5). The number of fallen mites on the sticky board, when treated with the essential oil of *A. galanga* showed that the essential oil of *A. galanga*, at concentrations of 25% and 50% had no significant differences (Figure 4.8).

Toxic symptoms have noted in honeybees at high concentrations or dosages of essential oils (Lin et al., 2020; Rattan, 2010). Although almost all essential oils are toxic to honeybees (Gashout & Guzmán-Novoa, 2009; Lin et al., 2020; Ruffinengo et al., 2005), their toxicity is lower than synthetic acaricides (Kostyukovsky et al., 2002). The results from these studies implied that we can choose the appropriate dosages of botanical essential oils which have no effect on honeybees but still can control mite infestation.

CHAPTER 6

CONCLUSION

This study was conducted to investigate the efficacy of three plant essential oils *Curcuma longa, Zingiber officinale and Alpinia galanga* in controlling *Tropilaelaps* mites infesting honeybee colonies. Under laboratory conditions, *Z. officinale*, and *A. galanga* revealed higher potential to reduce the mite population than *C. longa* did. Because of that only *Z. officinale* and *A. galanga* were chosen to investigate for efficacy in controlling the mites in the apiary. The result showed that both *Z. officinale* and *A. galanga* at the concentrations of 25%, 50%, and 75% were able to reduce mite infestation in honeybee colonies significantly compared to the control treatment. However, *A. galanga* at every concentration showed a negative effect on honeybees. Therefore, the recommended essential oil to be used in the apiary to reduce mite infestation should be *Z. officinale* with the least concentration of no lower than 25%. As such, *Z. officinale* can be used as an alternative approach in the control of *Tropialealaps* mites in organic beekeeping.

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