# Screening and Applying of Some Plant Essential Oils for Controlling Pulse Beetle, *Callosobruchus maculatus* (F.) on Mung Bean, *Vigna radiata* L. (Wilczek) Seeds

**Thein Naing Soe**

**A Thesis Submitted in Fulfillment of the Requirement for the Degree of Master of Science in Entomology**

**Prince of Songkla University**

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This is to certify that work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

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Candidate

I hereby certify that this work has not been accepted in substance for any degree, and is not being concurrently submitted in candidature for any degree.

…….Signature

(Mr. Thein Naing Soe)

Candidate

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**Academic Year** 2018

## ABSTRACT

*Callosobruchus maculatus* is one of the most serious pests brought into storage containers with harvested mung bean. It cause total loss of the stored crop in few months. In this study, five plant essential oils were compared for their residual contact and fumigant activities against *C. maculatus*. After getting the most effective essential oil, synergistic effect with sesame oil was investigated. Moreover, this study was created awareness of the value of plant products as the application method for pulse beetle in small holder farmers' storage facilities.

The insecticidal activities of plant essential oils extracted from lengkuas (*Alpinia galanga*) rhizome, citronella (*Cymbopogon nardus*) leaf, clove (*Syzygium aromaticum*.) flower bud, cinnamon (*Cinnamomum verum)* bark and kaffir lime (*Citrus hystrix*) peel were investigated against the *C. maculatus* adults under laboratory conditions. Insecticidal activities of plant essential oils varied with different essential oil, exposure period and concentration. In residual contact bioassay, clove oil exhibited the strongest toxicity against *C. maculatus* adults with $LC_{50}$ values of 16.05, 12.99 and 7.67 µl/ml at 24, 48 and 72 h, respectively. Moreover, clove oil was the most effective in the fumigation method followed by lengkuas, cinnamon, citronella. Its $LC_{50}$ values at 24, 48 and 72 h were 297.80, 221.69 and 136.20 µl/l air, respectively.

Synergistic toxicity of sesame oil and clove essential oils were investigated against *C. maculatus* adults, through residual contact and fumigation tests. The percent mortality of insect were 44.00±2.45 and 46.00±5.10 after exposure to clove oil alone at 24 h by the residual contact and fumigation methods, respectively. These values increased to 48.00±5.83 and 62.00±3.74 after exposure to the plant oil mixture of clove oil and sesame oil ratio (8:2), whereas there was no mortality after treating with sesame oil alone in both application methods. It suggests that sesame oil showed

the synergistic effect after mixing with clove oil at the ratio of 8:2 in both application methods. Synergistic toxicity was greater by fumigation than by residual contact application.

The most effective ratio of clove oil and sesame oil (8:2) was tested with three application methods of sack coating, seed dressing and fumigation against the *C. maculatus*. The results showed that inhibition percentage of plant oil mixture depended on concentration and day of exposure after sack coating method. At the 1st month, the movement inhibition of plant oil mixtures was less effective than 63.52 ± 1.47% to inhibit *C. maculatus* after 7 days of treatment. Plant oil mixtures 3.0% and 5.0% completely inhibited the F1 adult emergence and did not affect the weight loss of mung bean seeds. At the 2nd month, all concentrations of plant oil mixture showed the inhibition percentage less than 50% after 5 days. F1 adult emergence was reduced from 476.75±8.11 to 33.25±4.44 at the concentration of plant oil mixture ranging from 1.0% to 5.0%. The lowest weight loss of 0.27±0.09% was recorded at the concentration of 5.0%. In the seed dressing, the 3.5% concentration of plant oil mixture and chlorpyrifos completely suppressed the adult progeny of *C. maculatus*, no seed damage and lowest WPI values 0.00±0.00 were observed through the six months storage period. By fumigation with a burner, *C. maculatus* eggs were tolerant to plant oil mixture with the highest $LC_{50}$ values of 7.81% and the mortality percentage of 58.00±2.58%. On the other hand, *C. maculatus* adults were susceptible to plant oil mixture with the lowest $LC_{50}$ value of 3.64% after 72 h in plastic cup. This research provided a scientific basis in applying botanical insecticides against *C. maculatus*. Further studies should be done for the bioactivity of the plant oil mixture of clove oil and sesame oil (8:2) against other stored-product insect pests. Moreover, there is a need to assess the cost-effectiveness and feasibility of using the plant oil mixture on large scale seed storage.

**ACKNOWLEDGEMENT**

First and foremost, I would like to express my profound gratitude and respect to my thesis advisor, Assoc. Prof. Dr. Aran Ngampongsai, for his excellent professional guidance, close supervision, invaluable and constructive advice and patience in the preparation of manuscript that helped me to complete my work. My deep gratitude also goes to my co-advisor Asst. Prof. Dr. Wisut Sittichaya for his priceless comments, invaluable suggestion and encouragement. I really appreciate Asst. Prof. Dr. Narit Thaochan, Chairperson of the thesis examination committee and Asst. Prof. Dr. Patcharaporn Vanichpakorn, member of the committee, for their kindness, guidance, comments and suggestions generously offered in improving this work.

I would like to thank to Thailand's Education Hub for ASEAN Countries (TEH-AC) Grant for financial support and also Center of Excellent in Agricultural Biotechnology and Natural Resources, Faculty of Natural Resources, Prince of Songkla University for research fund. I would like to acknowledge the Department of Agriculture, Ministry of Agriculture, Livestock and Irrigation, Myanmar for giving permission to pursue master's degree program in the Thailand.

Moreover, my sincere thanks to Mrs Siriporn Prompat and Miss Parittiya Saejew who gave the great care for me such as dormitory, visa extension and valuable information. I am also equally grateful to Miss Pattamaporn Insuwonno and Siriporn Srichareon for their kindly help related to valuable information and documents preparation throughout my study.

I really thanks to my senior, Dr. Kanok-on Wuttiwong for her valuable advice, kindly support and cordial friendship. Many special thanks also go to all of my beloved entomology students-mates for kindly help, special friendship and encouragement.

I would like to extend my enormous thanks to my father U Maung Lay, my mother Daw Khin Thein, my wife Dr. Pan Ei Phyu and my daughter, May Mon Soe. I cannot possess this achievement without their endless support, endurance, sacrifice and mentally encouragement while I was in Prince of Songkla University.

Thein Naing Soe

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## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| ANOVA | = | Analysis of variance |
| cm | = | Centimeter |
| $LC_{50}$ | = | Lethal concentration required to kill 50% of the population |
| $LC_{90}$ | = | Lethal concentration required to kill 90% of the population |
| ml | = | Milliliter |
| RH | = | Relative humidity |
| S.E. | = | Standard Error |
| WPI | = | Weevil Perforation Index |
| µl | = | Microliter |
| $^{o}C$ | = | Degree Celsius |

# CHAPTER 1
# INTRODUCTION

## 1.1 Statement of the problem

Stored product pests are a great challenge in our economy because they infect and contaminate stored agricultural products and animal feed. Stored products are frequently damaged by insect pests and this may account to 5-10% in temperate zones and 20-30% in the tropics (Nakakita, 1998). Mung bean, *Vigna radiata* L. (Wilczek) is seriously infested by pulse beetles *Callosobruchus maculatus* (F.) and *C. chinensis* (L.) (Coleoptera: Chrysomelidae: Bruchinae) all over the world (Dimetry and Abbass, 2014; Ahmed, 2010 and Hafez *et al.,* 2013). *C. maculatus* is one of the most serious pests brought into storage containers with harvested mung bean that can cause total loss of the stored crop in a few months. The estimated post-harvest losses caused by bruchids to the pulses ranged from 30-40% within 6 months and when left unattended losses could be up to 100% (Dongre *et al.,* 1996; Mahendran and Mohan, 2002). In Thailand, two species of pulse beetles, *C. maculatus* and *C. chinensis,* seriously damage mung bean seeds during storage (Visarathanoonth and Promsatit, 1989). It is also a major storage insect pest of many crops in Myanmar (Htay *et al.,* 2002).

Mung bean is an important legume crop in South and Southeast Asia because it contains a high content of easily digestible protein, iron and folate (Bains *et al.,* 2003; Tang *et al.,* 2014; Weinberger, 2005 ). A hundred gram of mung bean gives 30 calories and consist of approximately 3 g proteins, 6 g carbohydrates, and 2 g dietary fibers. It supports about 45% of iron and 15% of calcium, respectively (Asif *et al*., 2013). It is suitable for the diabetes diet and consumers who want to lose weight because of its own glycemic index (GI) quite low (Mani *et al.,* 1993). It is also consumed as sprouts, which are an important source of vitamins and minerals (Somta *et al.,* 2007). It is grown widely in the South and Southeast Asia countries, mainly India, China, Pakistan, Myanmar, Thailand and Vietnam (Tomooka *et al.,* 2002).

Chemical controls such as fumigation with methyl bromide or phosphine are primary management systems of insect pests in many storage conditions. However, under the Clean Air Act and Montreal Protocol, the use of methyl bromide

has been prohibited in developed countries since 2005 and restricted in developing countries in 2010 because it potentially damages to the ozone layer. Moreover, some stored-product insects are found to develop resistance to phosphine in many countries, thus the further use of phosphine could be alarm by further development of resistant pests (Bell and Wilson, 1995; Collins, 2006). Therefore, use of methyl bromide and phosphine are expected to be more restricted in the future. Furthermore, the residual toxicity of insecticides increased in applying for stored product pests and traditional chemical insecticides were necessary for controlling stored product pest as the new approaches. (Yilsrim *et al*., 2001).

There is an urgent need to drive the search for less harmful effect and alternate to synthetic insecticide with no residual toxicity and harmful effects on non-target organisms. Plants are sources of natural compounds or secondary metabolites that can be utilized in the development of environmental safety methods for stored product pest control. An alternative to synthetic insecticides is the use of natural compounds such as essential oils resulted from secondary metabolism in plants. These compounds are volatiles with high insecticidal activity and quickly biodegradable. Most of the active constituents of plant essential oils are specific to particular insect groups (Huang *et al*., 1997) and are not harmful to mammals (Isman, 2006), many of them are not dangerous to humans. Hence, they should be considered in pest management strategies. In this regard, many plant products including essential oils and their constituents have been evaluated for their insecticidal properties against different stored grain pests (Kim *et al.*, 2003). The deleterious effects of essential oils on storage insect pests are manifested in several ways, feeding inhibition (Isman, 2006), oviposition deterrence (Tunc *et al.,* 2000) and fumigant action (Lee *et al.,* 2003). With these properties of essential oils, they could be alternatives to replace the synthetic chemical insecticides for controlling the stored product insect pests (Shaaya *et al.*, 1997).

Citronella oil and cinnamon oil were recorded as the strongest repellent and had the most toxic to *C. maculatus*. Moreover, these oils significantly reduced the oviposition rate and adult emergence of *C. maculatus* on mung bean seeds (Ratnasekera and Rajapakse, 2009). Lengkuas oil at 0.5% concentration caused 100% mortality of

*C. chinensis* adults and total reduction of oviposition on mung bean seeds (Ahmed and Ahmad, 1992). Additionally, clove oil also has biological properties such as antimicrobial activity in food and is a traditional flavoring ingredient (El-Maati *et al.*, 2016; Lee and Shibamoto, 2001). Kaffir lime peel oil also has a strong flavour and antibacterial activity against *Staphylococcus aureus* (Lertsatitthanakorn *et al*., 2014). Sesame oil has been reported as a good antioxidant and showed synergistic effect on the pesticide against *Spodoptera littoralis* (Biosd.) (Abd El-Hafez and Abd El-Aziz, 2010).

As there is much information on the antibacterial, medicinal and insecticidal activities of these plant essential oils, their bioactivities need to be investigated as alternative to synthetic insecticide for controlling stored product pests. Moreover, if found synergistic activity of sesame oil and the best ratio of plant oil mixture, it will be effective for controlling the stored product pest with low application cost. It can be created awareness of the value of plant products as the application method for controlling stored product pest, especially pulse beetle, *C. maculatus*, in mung bean storage for farmers in developing countries.

## 1.2 Literature Review

### 1.2.1 Importance of insect pests in mung bean storage

Mung bean is widely grown in south- and southeast Asia as a major crop. In developing countries, it is an important source of protein for human because there is very low consumption of animal protein. Since it is mainly grown in the tropical region, insect pests play an important role in crop production. The store product pests of bruchid species belonging to genus *Callosobruchus* are primary pests of mung bean, especially on storage condition. Gujar and Yadav (1978) reported that *C. maculatus* reduced the weight loss of 55.6% to 73.0% and *C. chinensis* reduced weight loss of 30.2% to 55.7% in one generation. Banto and Sanchez (1972) also reported that newly harvested mung bean seeds were totally destructed when infected mung bean seeds (9.9% seed damaged) were stored for three months. Infested seeds were not suitable for human consumption. Therefore, stored product pest control is very important to reduce avoidable losses.

### 1.2.2 Biology and ecology of *Callosobruchus maculatus*

*C. maculatus* adults do not feed on stored products and life duration is not more than 12 days under optimum conditions. Adult females can lay eggs up to 115 eggs, even though the oviposition rate can be decreased on the firstly infected seeds and they lay more egg on the large seeds than small seeds (Cope and Fox, 2003; Parr *et al.,* 1996). They also possess the ability to categorize their own oviposition markers, moreover, they tend to avert laying eggs on the seeds which have markers deposited already by any other females (Wijeratne and Smith, 1998). The optimal temperature for egg laying of *C. maculatus* is 30-35°C. Although the eggs are glued on the surface of the host seed, the smooth seed is more appropriate for oviposition than rough seed (Parr *et al*., 1996).

*C. maculatus* can be reared easily under laboratory condition and also as a model organism to observe several ecological studies. The development of pulse beetle depends on temperature, humidity, population source and host substrate (Messina and Slade 2002; Xu, 1999). The oviposition behavior of pulse beetle has been studied with some details inclusive of male size, multiple mating and interspecific interference on female lifetime fecundity (Parr *et al.,* 1996; Wilson *et al.,* 1999).

The optimum condition for the rapid development of *C. maculatus* is 32.5˚C and 90% RH and the shortest mean development period is 23 days. Female pulse beetle laid over 60% of the total number of eggs within the first three days. The eggs are oval shaped, shiny, clear and firmly glued to the surface of host bean seeds. The larva hatch from the egg take place within 5-6 days and then enter pupation within the seed 26 days after oviposition ( Barde *et al.,* 2014; Howe and Currie 1964). The larva burrows the seed coat and feeds on the endosperm of the seed. Meanwhile, they released detritus into the shell as the insect hatches, thereafter, the color of egg changed into white. As a single seed could be multiplied conspecific eggs, larval competition may be obvious (Horng, 1997). A life cycle of *C. maculatus* is shown in Figure 1.

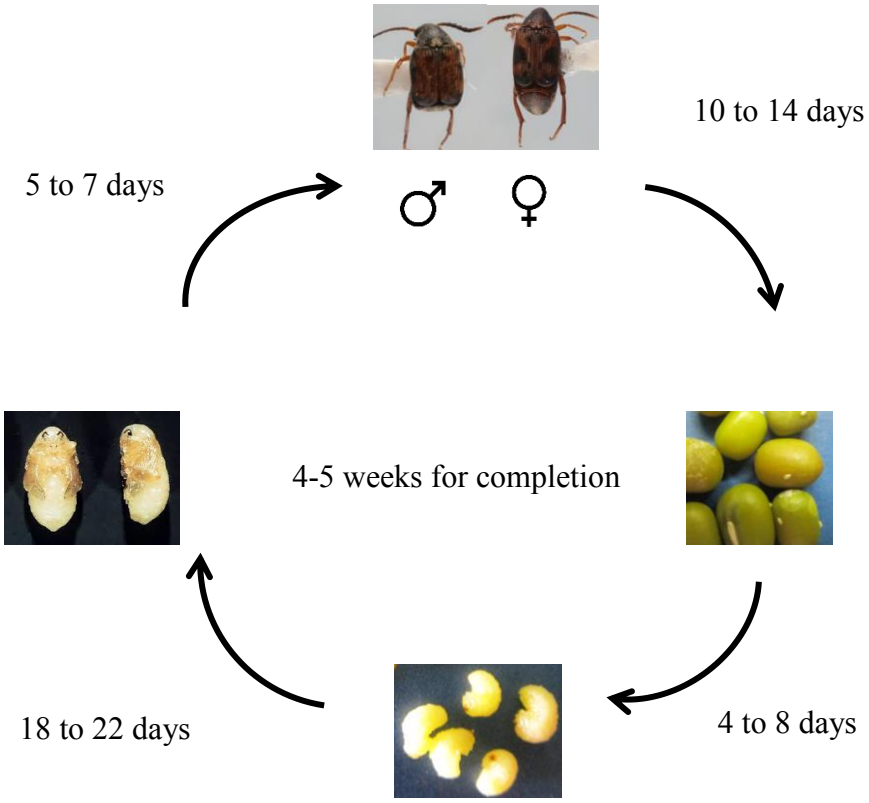


**Figure 1** Life cycle of *Callosobruchus maculatus*
Modified from: Beck and Blumber (2014)

### 1.2.3 Damage of *Callosobruchus maculatus* on mung bean

*C. maculatus* is the most common species that causing damage to mung bean by decreasing in an individual seed weight loss 56-73%, deterioration of nutritional quality, and reduction of seed viability (Booker, 1967). The larva penetrates into the host seed and feeds on the endosperm. The adult chews through the seed coat and emerges from the host bean (Beck and Blumer, 2014). The life cycle of C. maculatus is completed in the kernel and it is very difficult to detect the infestation without dissecting the seed. Insect infestation begins in the seeds before harvest time and continues to the storage time where considerable losses may occur.

Infestation rates are strongly influenced by the management of storage systems and the variety of seed (Ojimelukwe and Okoronkwo, 1999). During the storage condition, high levels of infestation will be caused at the high levels of moisture in seeds. Otherwise, storage temperature also influences the infestations level in local storage (Singh, 1999).

### 1.2.4 Chemical control and management for *Callosobruchus maculatus*

Over the years, the destructive activities and menace of *C. maculatus* and other storage pests have been effectively suppressed with synthetic organochlorine and organophosphate compounds like carbon disulphide, phosphine, malathion, carbaryl, pirimiphos methyl and permethrin (Adedire *et al.,* 2011). Organophosphates group insecticides are recommended for controlling this stored product pest (Ofuya and Lagunju, 1998).

Olubayo and Port (1997) found that infestation of *C. maculatus, C. chinensis, C. rhodesianus* and *A. obtectus* significantly reduced by planting intercropping maize with cowpeas, and not harvesting time late in Kenya. Patnaik *et al*. (1986) also reported that the sowing date of the seed influenced the levels of infestation in the field.

Moreover, hygiene is an important role in limiting the infestation of pests under storage condition. Generally, hygiene means the cleaning of infested residues from last harvest time. Mohamed (1996) revealed that solarization (sun drying and heating) is one of the useful methods to control infestations without affecting seed germination. Normally, sun-drying the small lot of beans regularly in a thin layer for periods of up to 4 hours can give enough protection. Seeds in transparent bags were dried under sunlight can also provide excellent control of insect infestations. (Ghaffar and Chauhan, 1999).

### 1.2.5 Biological control for *Callosobruchus maculatus*

Normally, biological control has not been widely used for controlling Callosobruchus species, even though C. maculatus natural population are often subjected to the high amount of parasitism, especially in West Africa (Ouedraogo *et*

*al.*, 1996). This may be due to the effect of climate (Ouedraogo *et al*., 1996), the complex biology of plant-host-parasitoid interactions (Monge and Cortesero, 1996) and host density (Sanon *et al*., 1998; Tuda, 1996) on the behavior of parasitoid. All of these factors influence the changes in storage practices to encourage parasitoids than the classical biological control implementation. Nonetheless, mass rearing methods for parasitoids (Islam, 1998) and strategy for controlling pests by inoculation of parasitoids (Sanon *et al*., 1998) have been developed.

### 1.2.6 Plant-based insecticides control for *Callosobruchus maculatus*

As a consequence of the harmful effects of chemical pesticides, there is a steadily increased to a more environmental oriented, sustainable agriculture with low of no usage of synthetic insecticides and other agricultural chemicals to preserve and protect the environment as well as living organisms. Thus, management of stored product pests by using substances of natural origin become the subject of many studies (Isman, 2006). Plant-based insecticides (PBIs) could be a promising source of insecticide against stored product pests because they are generally less toxic to man, readily biodegradable, suitable for use by small scale farmers and capable of protecting crops from attack by a wide range of insect pests (Rosenthal, 1986). Expect those natural plant products in current use such as pyrethrins, nicotine, sabadilla, rotenone, neem oil and ryania, several products of plant origin have been identified to exhibit repellent, toxic, antifeedant, growth and development inhibition potential against the arthropod pests. (Coats, 1994).

Plant oils are also important natural plant product of insecticides, which are generally considered broad-spectrum and safe for the environment because the array of compounds are quickly biodegradable (Bakkali *et al*., 2008; Cox, 2004). Moreover, the interest in essential oils has regained momentum during the last decade and is primarily due to the fumigant and contact insecticidal activities (Isman, 2006). Volatiles emanating from the various plant parts are reported to be toxic to stored grain pests (Ketoh *et al*., 2000).

Tarigan *et al*. (2016) reported the toxicity and physiological effect of cardamom, cinnamon and nutmeg oils against egg, larva, and adult of *Tribolium*

*castaneum* and *C. maculatus*. The cinnamon oil exhibited the highest efficacy against egg, larva, and adult of *C. maculatus* with an $LC_{50}$ of 0.01%, 0.132%, and 0.186%, respectively as compared with *T. castaneum*, which were recorded 1.051%, 0.109%, and 1.239%, respectively. Furthermore, all essential oils reduced the total carbohydrate, protein, and fat contents, and cinnamon oil demonstrated to be the most effective among the three essential oils. On the same note, cinnamon oil had a greater impact of inhibiting esterase and glutathione s-transferase activity compared to nutmeg and cardamom oils.

Al Yousef (2015) studied the effectiveness of the clove oil as a natural product against the adults of the cowpea seed weevil, *C. maculatus* under controlled laboratory conditions. Results indicated that the mortality percentage of the adult beetles increased with the increase of the oil concentration and the period after treatment. The percentage of mortality was 63.33% two days after treatment at the highest concentration (5 mg/l), increased to reach 96.667% four days after treatment at the same concentration. At the lowest concentration (0.12 mg/l), the percentage of mortality was 73.333% at the four days after treatment and the $LC_{50}$ value was 2.188 mg/l and the $LC_{90}$ was 75.445 mg/l after two days from treatment.

Dutra *et al.* (2016) investigated the bioactivity of essential oils extracted from fruit peels of *Citrus latifolia*, *Citrus reticulata*, *Citrus sinensis* and *Citrus paradisi* on *C. maculatus* adults. In the contact toxicity tests using treated cowpeas the $LC_{50}$ values ranged from 943.90 to 1037.70 ppm, with the lowest value for *C. latifolia* and the highest for *C. sinensis*. The number of eggs and newly emerged adults was inversely proportional to essential oil concentration increase. In the fumigant toxicity test, $LC_{50}$ values ranged from 10.20 to 12.98 ml/l air, with *C. latifolia* showing the best results. In the repellency test, the essential oils were classified as neutral at all concentrations. The percent of oviposition decreased from 29.74% to 71.66%, while reduction in emergence varied from 15.43% to 85.31%.

Huixim *et al*. (1998) investigated the effectiveness of 25 plant essential oils against *C. maculatus.* These plant essential oils were mixed with 0.1% of mung bean to control *C. maculatus*. The effectiveness of the essential oil of *Murmya paniculata, Cinnamomum cassra, Ocunum baeilicum, Chenopodium ambrosioides,*

*Pelarqomum graveolens, Carum carm, Cinnamomumi burmanna, Foeniculurn vulgare, Zanthoxlum bunqeanum* and *Ageratum conyzordes* was relatively ideal. The rate of population reduction, the rate of protection on insect penetration and the save rate of weight loss were all over 90%.

**Thesis objectives**

This study was conducted to accomplish the following objectives:

1. To compare the residual contact toxicity and fumigant toxicity of the five plant essential oils against *C. maculatus*
2. To study the synergistic effect of the selected plant essential oil with sesame oil
3. To evaluate the effectiveness of selected application methods with the selected plant essential oil which has the highest efficacy for protecting *C. maculatus* on mung bean

**Outcomes of the research**

1. Know the insecticidal toxicity of plant essential oil that can be used to control for storage of mung bean seed.
2. Know the synergistic effect of sesame oil with selected plant essential oil and can reduce the application cost.
3. Have the best and appropriate method for controlling stored product pest in mung bean storage by using the plant oil mixture and it could be contributed to alternative synthetic insecticide for small farmer storage facilities.

# CHAPTER 2
# RESEARCH METHODOLOGY

## 2.1 Experimental location

The experiment was carried out at toxicology laboratory of the Department of Pest Management, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

## 2.2 Insect culture

Pulse beetles, *C. maculatus* adults were collected from naturally infested mung bean seeds at the local market, Ratthakan Road, Hat Yai District, Songkhla Province, Thailand. To establish the stock culture of *C. maculatus,* mung bean seeds (*Vigna radiata* L) were used as a host. Seeds were kept in an oven for 4 hours at 55ºC for sterilization (Mookherjee *et al*., 1968). One hundred fifty grams of the sterilized seeds were put in 500 ml plastic containers and 25 pairs of *C. maculatus* adults were released in the containers. The containers were sealed by perforated plastic lids internally lined with muslin cloth to get ventilation and to prevent the escape of beetles. These parent beetles were allowed to lay egg for 7 days under the laboratory conditions (29 ± 3°C and 75 ± 5% RH) and then they were removed. These containers were stored in the laboratory until adult emergence (Figure 2). One to three days old *C. maculatus* adults were used for all experiments.

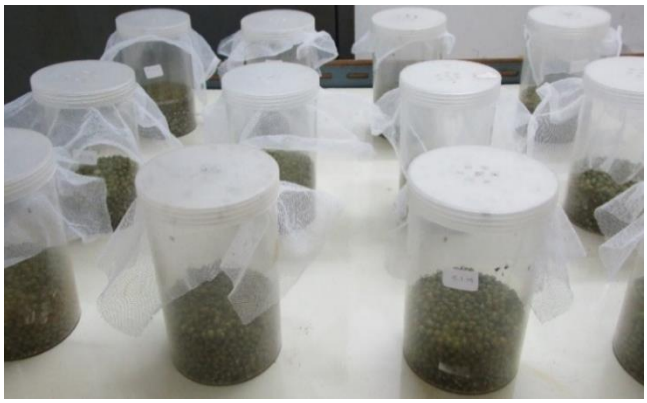

**Figure 2** Rearing the *Callosobruchus maculatus* in plastic container with mung bean seeds.

**2.3 Preparation of essential oils**

Essential oils were extracted from different parts of plants including unopened flower bud of clove (*Syzygium aromaticum*), bark of cinnamon (*Cinnamomum verum*), rhizome of lengkuas (*Alpinia galanga*), leaves of citronella (*Cymbopogon nardus*) and peel of kaffir lime (*Citrus hystrix*), respectively (Figure 3). The details of all five plant species used in experiments are given in Table 1. Plant parts were purchased from Trai-buri herbal shop in Hat Yai District, Songkhla Province, Thailand. They were cut into small pieces before extraction by steam distillation through a Clevenger type apparatus (Figure 4). The distillation process was conducted for 5-6 hours and water was eliminated by using anhydrous sodium sulphate. All samples were kept in refrigerator at 4ºC in airtight containers.

**Table 1** Plant samples evaluated for insecticidal activity against the pulse beetle, *Callosobruchus maculatus*

| **Scientific name** | **Common name** | **Family** | **Parts used** |
|---|---|---|---|
| Syzygium aromaticum | Clove | Myrtaceae | flower bud |
| *Alpinia galanga* | Lengkuas | Zingiberaceae | rhizome |
| *Cinnamomum verum* | Cinnamon | Lauraceae | bark |
| *Cymbopogon nardus* | Citronella | Poaceae | leaf |
| *Citrus hystrix* | Kaffir Lime | Rutaceae | peel |

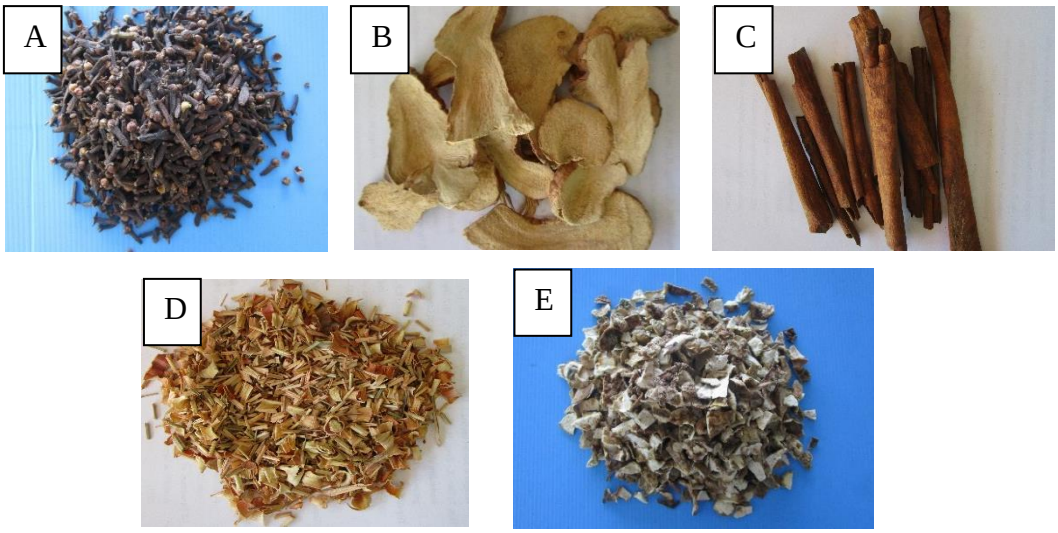


**Figure 3** Plant parts used in this study; clove flower bud (A), lengkuas rhizome (B), cinnamon bark (C), citronella (leaf) (D), kaffir lime peel (E)

**Figure 4** Clevenger type apparatus for extracting essential oil by steam distillation.

## 2.4 Bioassay test

### 2.4.1 Residual contact toxicity test

Residual contact toxicity of five plant essential oils against adults of *C. maculatus* was investigated by the bioassay method according to Kim *et al*. (2003) and Usha Rani and Rajasekharareddy (2010). Filter papers were treated with different concentrations of oil solution dissolved in acetone at 8, 16, 24, 32 and 40 µl/ml (equivalent to 0.13, 0.25, 0.38, 0.5 and 0.63 µl/cm$^2$ filter paper, respectively). A filter paper (9 cm diameter, surface area of 63.6 cm$^2$) was impregnated in 1 ml of the oil solutions mentioned above by micropipette (Figure 5A) and placed it in a glass petri dish (9 cm diameter) (Figure 5B). The control treatment was prepared using only acetone. Acetone was air-dried to evaporate for 10-15 minutes before releasing five pairs of adult male and female *C. maculatus* into each dish and covered with a lid (Figure 5C). The inside of the lids were coated with Vaseline® (pure petroleum jelly) to prevent the insects staying on the lid. All treatments were replicated five times and petri dishes were kept at room temperature. After treatment for 24, 48 and 72 h, insect mortality was recorded.

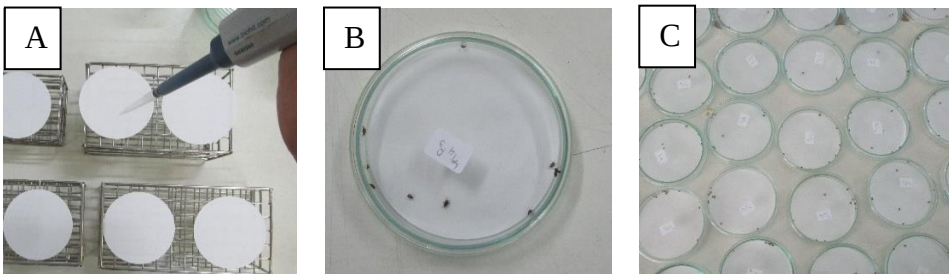


**Figure 5** Residual contact bioassay: 1 ml of plant oil solution were impregnated on filter paper (9 cm diameter) (A), five pairs of insects were placed on the treated paper (B), recorded the mortality of insects (C)

### 2.4.2 Fumigant toxicity test

Fumigant test method used in this study was described by Suthisut *et al.* (2011). Each filter paper cut in to 2 cm diameter pieces and then impregnated with the different oil concentrations (2, 4, 6, 8 and 12 µl equivalents to 100, 200, 300, 400 and

600 µl/l air) (Figure 6A). The impregnated filter papers were exposed to air drying for 2 min to evaporate the solvent, after that, these were attached under surface of the screw cap of each glass vials (20 ml). The caps were screwed tightly on each glass vials containing of *C. maculatus* adults (Figure 6B). Five pairs of adults were placed in vial without food before screw cap. Acetone treatment was used as a control. The inner side of the lids were coated with Vaseline® (pure petroleum jelly) to prevent direct contact of the impregnated filter paper with the insects. All tested glass vials were placed in the box and kept under laboratory conditions (Figure 6 C, D). All treatments were conducted with five replications. Mortality was checked for 24, 48 and 72 h after fumigation. The individual insects that have no movement of the antennal or legs were considered as a dead insect.

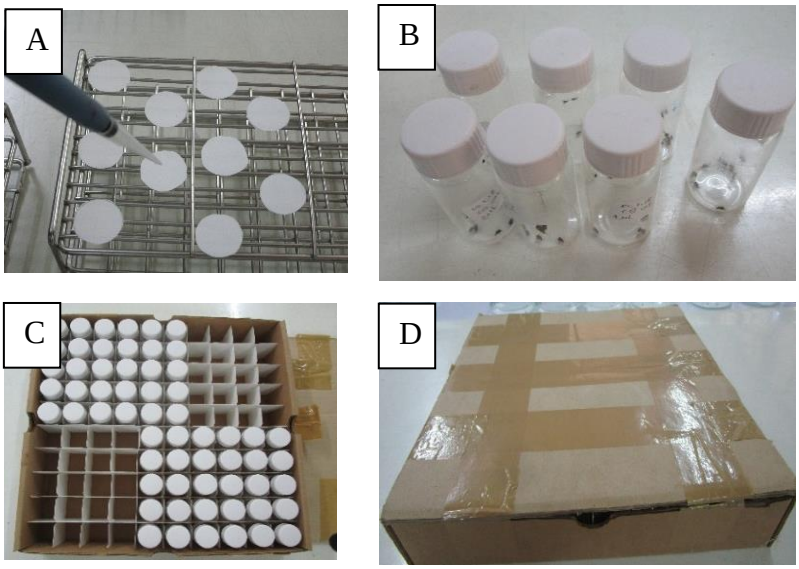


**Figure 6** Fumigation bioassay: impregnating plant oil solution on filter paper (2 cm diameter) (A), five pairs of insects were placed into the vial and closed the cap that was attached with treated filter paper (B), tested glass vials were placed inside the box (C) and before keeping the box under laboratory conditions (D)

## 2.5 Synergistic toxicity test of two plant oils

### 2.5.1 Residual contact toxicity test

Synergism in residual contact toxicity test was conducted according to method of residual contact bioassay (Kim *et al.*, 2003; Usha Rani and Rajasekharareddy, 2010) with some modifications. The different mixture ratios of clove oil and sesame oil (10:0, 8:2, 6:4, 5:5, 4:6, 2:8, 0:10) were used for this experiment. Based on the $LC_{50}$ values of the clove oil from the preliminary test, the 16 µl the oil mixtures in AR grade acetone (equivalent to 0.25 µl/cm$^2$) was observed in this treatment. Acetone was used as a solvent and negative control. The solution 1 ml was impregnated on Whatman filter paper no.1 (9 cm diameter, surface area of 63.6 cm$^2$) by micropipette and placed in a glass petri dish (9 cm diameter) (Figure 7). For control treatment, the filter paper was impregnated with 1 ml of acetone. The filter papers were air-dried for 10-15 minutes to evaporate the solvent. After that, five pairs of *C. maculatus* adult were placed into each petri dish and covered with a lid. Petroleum gel (Vaseline) (Hindustan Unilever Ltd, India) was coated at the inner side of the lid to prevent insect staying on lid. This treatment did not effect on tested insects. Five replications for treatment and control were done and insect mortality rates were recorded after 24, 48 and 72 h of treatment.

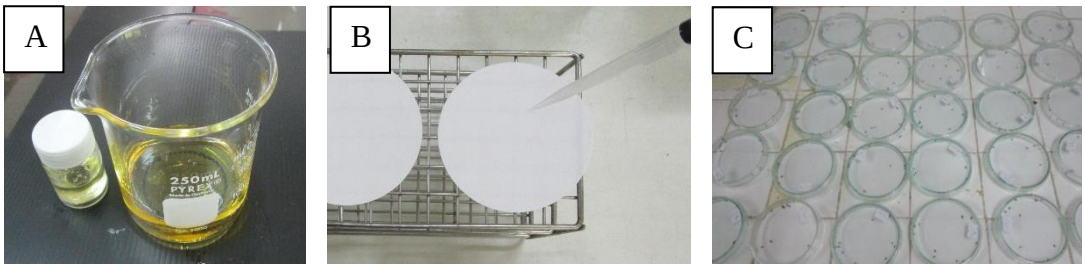


**Figure 7** Synergism in residual contact toxicity test: mixing the sesame oil and clove oil (A), impregnating the plant oil mixture solution 1 ml on the treated filter paper (9 cm diameter) (B), five pairs of insect were placed on the filter paper and mortality were recorded (C)

### 2.5.2 Fumigant toxicity test

Synergism in fumigant toxicity test method used in this study was well described by Suthisut *et al.* (2011). The plant oil mixture of clove oil and sesame oil (10:0, 8:2, 6:4, 5:5, 4:6, 2:8, 0:10) ratios were used for this experiment. Each filter-paper (Whatman no.1) cut into 2 cm diameter pieces and then impregnated with the oil mixture at the concentration of 6 µl/20 ml which was equivalent to 300 µl/l in air obtained by based on $LC_{50}$ values of clove oil after preliminary test (Figure 8A). Each filter-paper was air-dried for 2 min to evaporate the solvent (Figure 8B) and then the treated filter papers were attached to the under-surface of the screw cap of a glass vial (20 ml) (Figure 8C). Five pairs of *C. maculatus* adult were placed in vial without food before screw cap. The caps were screwed tightly on the vial containing of *C. maculatus.* Acetone was used as a control. The inner side of the glass vial was coated with petroleum gel (Vaseline®) (Hindustan Unilever Ltd, India) to prevent direct contact of the impregnated filter paper with the insects. The vials were placed inside the box (Figure 8D) and was kept under laboratory conditions (Figure 8E). Both treatment and control were replicated five times. Mortality was checked for 24, 48 and 72 h after exposure. When no leg or antennal movements were observed, insects were considered as dead.

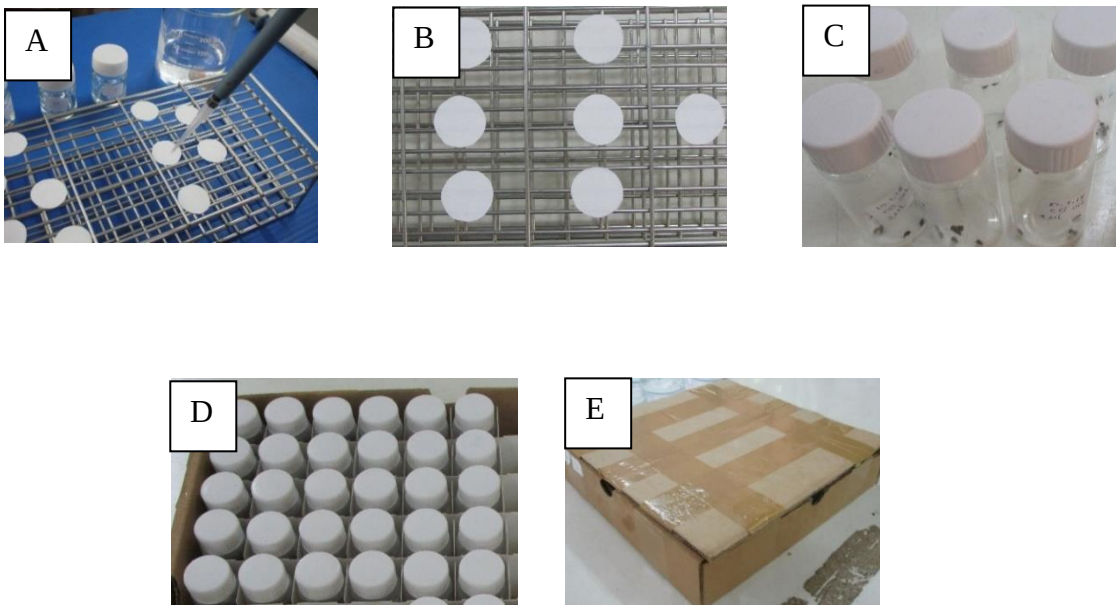


**Figure 8** Synergism in fumigant toxicity test: plant oil mixture impregnated on the filter paper (2 cm diameter) (A), filter papers were air dried about 2 minutes (B), filter paper were attached under the screw cap and screwed tightly the vial having five pairs of insects (C), the vials were placed inside the box (D), the box was kept under the laboratory conditions (E)

### 2.6 Application methods for controlling *Callosobruchus maculatus* on mung bean

The most effective ratio of plant oil and sesame oil obtained from the previous study was selected for this experiment. Three application methods including sack coating, seed dressing and fumigation were conducted.

#### 2.6.1 Sack coating application

Small jute bags (19 cm x 15 cm), plastic containers (30 cm x 20 cm x 12 cm), cleaned and un-infested mung bean seeds were used for this study. Different concentrations (0.5%, 1%, 2%, 3% and 5%) of clove oil and sesame oil mixture ratio (8:2) was diluted in acetone and jute bags were soaked in each solution about 30 sec (Figure 9A). Each jute bag was air-dried for one hour to evaporate the solvent (Figure 9B) and was filled with 500 g of mung bean seeds. After that, each bag was tied with

plastic rope and was placed in each plastic container. Ten pairs of *C. maculatus* adult were released in each plastic container and these containers were placed in the dark at the laboratory conditions (Figure 9D). Acetone was used for control treatment. The experiment was arranged in completely randomized design (CRD) with four replications. The number of *C. maculatus* adults reaching in the bag was counted after 1, 3, 5 and 7 days of exposure period. After one month, F1 progeny emergence and percentage of weight loss were recorded and infected seeds were removed from each bag. After that, 500 g of cleaned and un-infected mung bean seeds were filled again in each bag and the procedure for next one month and data collection were followed as mentioned above. The experiment duration was two months for storage condition. The percentage inhibition of infestation was calculated as follows;

$$\text{Percent inhibition of infestation} = \frac{\text{Number of insect cannot through to jute bag}}{\text{Total number of insect}} \times 100$$

Percentage weight loss was calculated using the following formula (Fekadu *et al*., 2012).

$$\text{Percent weight loss} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

All data were subjected to analysis of variance (one-way ANOVA) and significant differences among treatments means were compared at 0.05 significant level using Tukey's Test. All statistical analyses were run on SPSS program (version 23.0).

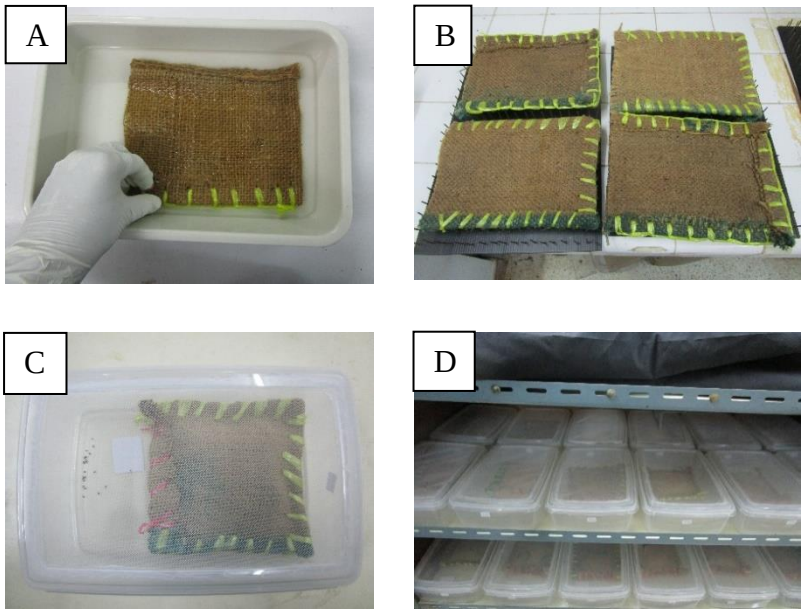


**Figure 9** Sack coating application: soaking the small jute bag in the plant oil mixture solution (A) air drying about one hour (B) small jute bag containing 500 g mung bean seeds was placed in the plastic box and released five pairs of insects (C) the boxes were kept in the dark (D)

### 2.6.2 Seed dressing application

In this application, cleaned and un-infested bean seeds, small jute bags (12 cm x 10 cm) and plastic container (17 cm x 12 cm x 7 cm) were used for this study and the seeds were heated in an oven at 65-75 ºC for 5 hours to kill the microorganisms or any other form of pests before use. The mixture of clove oil and sesame oil ratio (8:2) was dissolved in acetone at different concentrations (0.5%, 1.0%, 1.5%, 2.5% and 3.5%). A 500 ml conical flask containing 1,000 mung bean seeds and 4 ml oil solution were shaken manually for about three minutes until the seeds were uniformly coated with the oils (Talukder and Howse, 1994) (Figure 10A). The treated seeds were taken out from the flask and air-dried for forty-five minutes to complete evaporation of solvent (Figure 10B). Then 1,000 seeds were filled into the small jute bag (12 x 10 cm). Thereafter, five pairs of *C. maculatus* adults were released in this bag. These bags were put into the plastic containers (17 x 12 x 7 cm) and sealed with perforated plastic lids internally lined with transparent voile type fabric to allow ventilation and prevent other

insects from entering (Figure 10C). The same procedure was applied for acetone only and 10 ppm of chlorpyrifos solution which was negative and positive control respectively. Each treatment was replicated four times and arranged following a completely randomized design (CRD). All containers were kept in the dark under room temperature in the laboratory (Figure 10D). Female bruchids were allowed to lay eggs for 7 days after which all insects were sieved out.

The storage period of seeds was done for 6 months and data were collected every month on adult progeny production and percentage of seed damage. A seed was considered damaged if it had one or more exit holes. Weevil Perforation Index (WPI) was calculated using the formula below as given by Fatope *et al*. (1995).

$$\text{WPI} = \frac{\text{(\% treated seed perforated)}}{\text{(\% control seeds perforated + \% treated seeds perforated)}} \times 100$$

A WPI >50 indicates a negative grain protectant effect or an increase in infestation by the weevil, whereas a WPI <50 indicates a positive effect or a decrease in infestation.

Percentage of seed damage was calculated using the following formula (Boxall, 1986).

$$\text{Seed damage rate (\%)} = \frac{\text{Nd}}{\text{(Nd + Nu)}} \times 100$$

Where, Nd = number of damaged seeds

Nu = number of undamaged seeds

All data were subjected to analysis of variance (one-way ANOVA) and significant differences among treatments means were compared at 0.05 significant level using Tukey's Test. All statistical analyses were run on SPSS program (version 23.0).

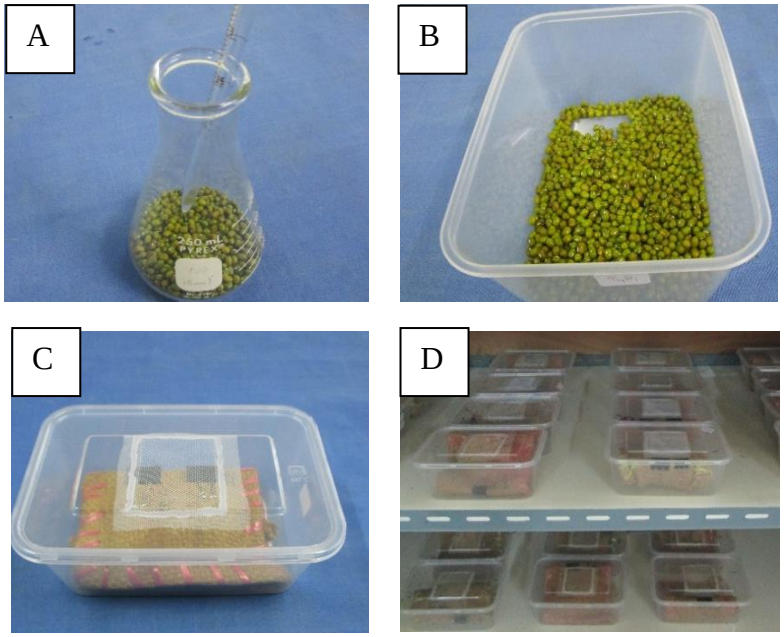


**Figure 10** Seed dressing application: dropping the plant oil mixture 4 ml on mung bean 1000 seeds and shook the conical flask (A) air dried the treated mung bean seeds about 45 minutes (B) five pairs of insects were released in the jute bag containing treated mung bean seeds and placed inside the plastic box (C), the boxes were kept in the dark (D)

### 2.6.3 Fumigation application on egg and adult stages

In this experiment, the emulsion of plant oil was prepared by adding clove oil and sesame oil mixture (8:2) at the concentrations of 1.5%, 3.0%, 4.5%, 6.0% and 7.5%, Tween -80 (1%) and distilled water were stirred (Figure 11A). Twenty-five pairs of *C. maculatus* adult were released into the 500 ml plastic container containing 150 g of cleaned and uninfested mung bean seeds for getting new eggs. After 24 h adult beetles were removed from the container and mung bean seeds bearing 1 to 3 eggs were selected. In fumigation method for eggs, mung bean (10-15) seeds having 25 eggs were placed into each petri dish (9 cm diameter) and small jute bag (12 cm x 10 cm) (Figure 11B). In fumigation method for adults, ten pairs of *C. maculatus* adult were released into each small jute bags which was filled 100 g of cleaned and uninfected mung bean seeds and the plastic cup (210 ml) that was empty seeds. To prevent moving out of insects, each bag was tied with plastic rope and the cup was covered with a muslin cloth

and tied with rubber band. An emulsion of plant oil (30 ml) was poured into the electric burner to produce gas. Tween- 80 (1%) emulsion without essential oil (30 ml) was used as control. The petri dish, jute bags and plastic cup were placed inside the airtight plastic cage (50 cm x 30 cm x 30 cm) which contained an electric burner (Figure 11C). Four replications of each treatment and control were set up. The electric burner was run 1 h and subsequently kept under the closed system for 72 h of fumigation exposure under laboratory condition. Completely randomized design was used for this experiment. After 72 hours of exposure, petri dish, jute bags and plastic cup were removed from airtight plastic cage and placed under laboratory condition. Adult mortality was checked after fumigation determination at 24, 48 and 72 h . Petri dishes and jute bags included with the eggs of *C. maculatus* were transferred under the laboratory conditions while petri dish was covered with the lid and jute bag was tied with plastic robe. After one month, mortality of eggs was calculated from the number of adult emergence from the eggs. Percent mortality was corrected by using Abbott's formula (Abbott, 1925).

The data will be analyzed with one-way ANOVA using statistical software SPSS for Windows®. Treatment means were compared and separated by Tukey's multiple comparison test at $P \leq 0.05$. The lethal concentration ($LC_{50}$ and $LC_{90}$) of plant oil mixture were calculated by using Probit analysis. All statistical analyses were run on SPSS program (version 23.0).

$$\text{Corrected mortality percentage} = \frac{\text{\% mortality of treated - \% mortality of control}}{\text{100 – \% mortality of control}} \times 100$$

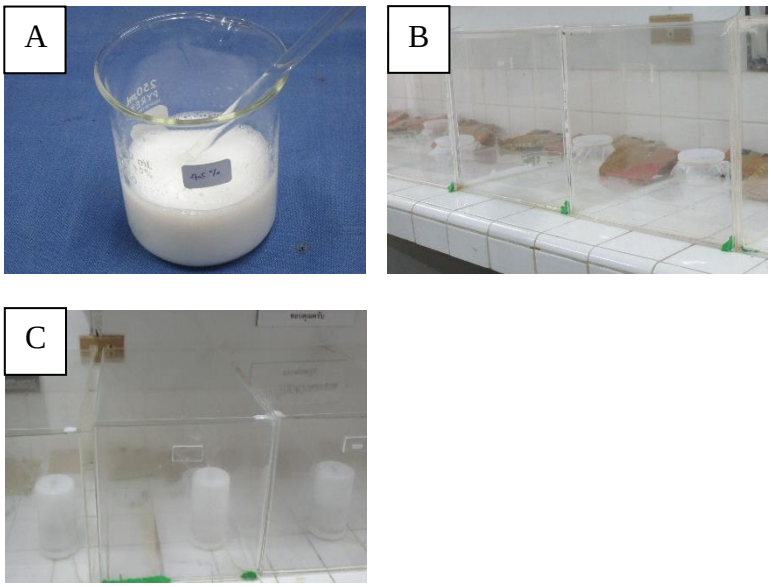


**Figure 11** Fumigation application: emulsion of plant oil mixture (A), *C. maculatus* eggs and adults are ready to be fumigant with each packages (B) plant oil mixture emulsion was done fumigation process by electric burner inside the airtight plastic container (C)

# CHAPTER 3
# RESULTS

## 3.1 Bioassay test by residual contact and fumigation methods

### 3.1.1 Residual contact toxicity test

The results of residual contact toxicity tests, i.e., insect mortality percentage, and, at different concentrations, on *C. maculatus* are shown in Table 2 and Table 3. Clove essential oil caused 96% mortality at concentration as low as 24 µl/ ml, and $LC_{50}$ value 16.05 µl/ml, after 24h exposure. This was followed by cinnamon, lengkuas and citronella oils with 84%, 64% and 50% of insect mortality, respectively. The $LC_{50}$ values of these three essential oils were 17.10 µl/ml, 21.98 µl/ml and 23.01 µl/ml after 24h, respectively. The mortality of insects by kaffir lime oil, concentration 24 µl/ml, after 24h exposure, was not significantly different from the control, while the $LC_{50}$ value was 35.81 µl/ ml. The five tested plant essential oils reached 100% mortality at the high concentration of 40 µl/ ml, and 72 h exposure.

### 3.1.2 Fumigant toxicity test

Fumigant toxicity of five plant essential oils results are shown in (Table 4 and Table 5). In the present study, clove essential oil showed 100 % mortality even at the lower concentration 400 µl/l and its $LC_{50}$ value was 291.65 µl/l after 24 h exposure. This was followed by lengkuas, cinnamon and citronella oils which gave 63.26%, 57.14% and 44.90% of insect mortality, respectively, and $LC_{50}$ values of these three essential oils were 346.60 µl/l, 350.40 µl/l and 487.30 µl/l after 24 h. Fumigant toxicity of kaffir lime oil was not significantly different as compared with untreated control at the concentration of 400 µl/l after 24 h exposure and $LC_{50}$ value was 567.50 µl/l after the same period. Fumigant toxicity of five plant essential oils exhibited 100% mortality at the high concentration of 600 µl/l and 72 h exposure period. In contrast, both residual contact toxicity and fumigant toxicity, insect mortality percentage of five plant essential oils increased with increasing concentration and exposure time. On the other hand, $LC_{50}$ values decreased with a rise of increasing exposure time.

**Table 2** $LC_{50}$ and 95 % confident limits values of the five plant essential oils against the pulse beetle, *Callosobruchus maculatus*, by residual contact toxicity test at 24, 48 and 72 h after treatment.

| Essential Oils | 24 h | | | 48 h | | | 72 h | | |
|---|---|---|---|---|---|---|---|---|---|
| | $LC_{50}$ (µl/ml) | 95 % confident limits | | $LC_{50}$ (µl/ml) | 95 % confident limits | | $LC_{50}$ (µl/ml) | 95 % confident limits | |
| | | Lower | Upper | | Lower | Upper | | Lower | Upper |
| Clove | 16.05 | 13.46 | 17.53 | 12.99 | 1.20 | 16.10 | 7.67 | 0.01 | 14.49 |
| Cinnamon | 17.10 | 13.23 | 19.59 | 13.12 | 3.072 | 15.65 | 11.08 | 0.19 | 15.51 |
| Lengkuas | 21.98 | 17.66 | 24.75 | 18.25 | 14.38 | 20.76 | 12.77 | 0.10 | 16.09 |
| Citronella | 23.01 | 15.28 | 27.55 | 20.97 | 18.32 | 22.45 | 17.07 | 14.66 | 18.83 |
| Kaffir Lime | 35.81 | 29.88 | 38.96 | 29.93 | 27.50 | 31.77 | 27.79 | 25.49 | 29.57 |

**Table 3** Residual contact toxicity of the five plant essential oils against the pulse beetle, *Callosobruchus maculatus*, after 24, 48 and 72 h exposure times.

| Essential Oils | Concentration (µl/ ml) | Mortality (Mean ± S.E., %) | | |
|---|---|---|---|---|
| | | 24 h | 48 h | 72 h |
| Clove | 8 | 36.00 ± 2.45c | 56.00 ± 5.10c | 70.00 ± 3.16[b] |
| | 16 | 68.00 ± 3.74b | 84.00 ± 2.45b | 94.00 ± 2.45a |
| | 24 | 96.00 ± 4.00a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | 32 | 100.00 ± 0.00a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | 40 | 100.00 ± 0.00a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | Control | 0.00 ± 0.00d | 0.00 ± 0.00d | 0.00 ± 0.00c |
| | F-test | ** | ** | ** |
| Cinnamon | 8 | 28.00 ± 3.74d | 48.00 ± 3.74c | 66.00 ± 5.10b |
| | 16 | 62.00 ± 3.74c | 80.00 ± 4.47b | 90.00 ± 4.47a |
| | 24 | 84.00 ± 4.00b | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | 32 | 100.00 ± 0.00a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | 40 | 100.00 ± 0.00a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | Control | 0.00 ± 0.00e | 0.00 ± 0.00d | 0.00 ± 0.00c |
| | F-test | ** | ** | ** |
| Lengkuas | 8 | 18.00 ± 3.74d | 30.00 ± 4.47d | 58.00 ± 2.00c |
| | 16 | 38.00 ± 3.74c | 58.00 ± 3.74c | 84.00 ± 2.45b |
| | 24 | 64.00 ± 2.45b | 82.00 ± 5.83b | 100.00 ± 0.00a |
| | 32 | 88.00 ± 3.74a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | 40 | 100.00 ± 0.00a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | Control | 0.00 ± 0.00e | 0.00 ± 0.00e | 0.00 ± 0.00d |
| | F-test | ** | ** | ** |
| Citronella | 8 | 0.00 ± 0.00e | 10.00 ± 3.16d | 22.00 ± 3.74c |
| | 16 | 18.00 ± 3.74d | 24.00 ± 2.45c | 54.00 ± 5.10b |
| | 24 | 50.00 ± 3.16c | 70.00 ± 3.16b | 92.00 ± 3.74a |
| | 32 | 78.00 ± 3.74b | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | 40 | 100.00 ± 0.00a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | Control | 0.00 ± 0.00e | 0.00 ± 0.00e | 0.00 ± 0.00d |
| | F-test | ** | ** | ** |
| Kaffir Lime | 8 | 0.00 ± 0.00c | 0.00 ± 0.00d | 6.00 ± 2.45de |
| | 16 | 0.00 ± 0.00c | 6.00 ± 2.45d | 14.00 ± 2.45d |
| | 24 | 8.00 ± 3.74c | 22.00 ± 3.74c | 30.00 ± 3.16c |
| | 32 | 30.00 ± 3.16b | 58.00 ± 3.74b | 72.00 ± 4.90b |
| | 40 | 68.00 ± 4.90a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | Control | 0.00 ± 0.00c | 0.00 ± 0.00d | 0.00 ± 0.00e |
| | F-test | ** | ** | ** |

Means followed by the same letter(s) in the same column of each essential oil are not significantly different ($P>0.05$) from each other using Turkey's Test.

** significantly at $P< 0.01$

**Table 4** $LC_{50}$ and 95% confident limits values of the five plant essential oils against the pulse beetle, *Callosobruchus maculatus*, by fumigation toxicity test at 24, 48 and 72 h after treatment.

| Essential Oils | 24 h | | | 48 h | | | 72 h | | |
|---|---|---|---|---|---|---|---|---|---|
| | $LC_{50}$ (µl/l air) | 95 % confident limits | | $LC_{50}$ (µl/l air) | 95 % confident limits | | $LC_{50}$ (µl/l air) | 95 % confident limits | |
| | | Lower | Upper | | Lower | Upper | | Lower | Upper |
| Clove | 297.80 | 266.73 | 313.54 | 221.69 | 210.67 | 231.54 | 136.20 | 94.23 | 164.25 |
| Lengkuas | 348.81 | 307.74 | 384.36 | 256.36 | 228.15 | 276.02 | 196.76 | 160.97 | 221.02 |
| Cinnamon | 388.04 | 349.91 | 424.89 | 261.40 | 236.66 | 278.78 | 212.71 | 183.23 | 234.27 |
| Citronella | 420.89 | 372.04 | 470.99 | 294.17 | 267.38 | 316.61 | 241.24 | 219.23 | 258.66 |
| Kaffir Lime | 567.51 | 512.69 | 630.43 | 443.57 | 407.95 | 485.72 | 358.58 | 330.32 | 381.29 |

**Table 5** Fumigant toxicity of five plant essential oils against adult of *Callosobruchus maculatus* during 24, 48 and 72 h exposure time.

| Essential Oils | Concentration (µl/l) | Mortality (Mean ± S.E., %) | | |
|---|---|---|---|---|
| | | 24 h | 48 h | 72 h |
| Clove | 100 | 14.00 ± 2.00c | 22.45 ± 2.50c | 45.83 ± 3.90b |
| | 200 | 24.00 ± 5.10c | 46.94 ± 3.82b | 89.58 ± 4.66a |
| | 300 | 64.00 ± 6.63b | 93.88 ± 2.50a | 100.00 ± 0.00a |
| | 400 | 100.00 ± 0.00a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | 600 | 100.00 ± 0.00a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | Control | 0.00 ± 0.00d | 2.00 ± 2.00d | 4.00 ± 2.45c |
| | F-test | ** | ** | ** |
| Lengkuas | 100 | 8.57 ± 2.92e | 16.67 ±3.30d | 41.67 ± 2.56c |
| | 200 | 22.45 ± 5.59d | 35.42 ± 3.90c | 72.92 ± 2.56b |
| | 300 | 44.90 ± 4.08c | 72.92 ± 5.31b | 91.67 ± 3.90a |
| | 400 | 63.26 ± 2.50b | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | 600 | 79.59 ± 3.23a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | Control | 2.00 ± 2.00e | 4.00 ± 2.45e | 4.00 ± 2.45d |
| | F-test | ** | ** | ** |
| Cinnamon | 100 | 6.52 ± 1.63d | 14.28 ± 2.50d | 29.78 ± 4.26c |
| | 200 | 18.36 ± 3.22d | 32.65 ± 2.50c | 61.70 ± 5.42b |
| | 300 | 34.69 ± 4.08c | 69.39 ± 3.22b | 87.23 ± 3.98a |
| | 400 | 57.14 ± 5.00b | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | 600 | 77.55 ± 3.82a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | Control | 2.00 ± 2.00d | 2.00 ± 2.00e | 6.00 ± 2.45d |
| | F-test | ** | ** | ** |
| Citronella | 100 | 4.90 ± 2.00e | 10.42 ± 2.55e | 21.27 ± 4.26d |
| | 200 | 16.33 ± 3.82de | 27.08 ± 3.29d | 44.68 ± 3.99c |
| | 300 | 30.61 ± 3.82cd | 56.25 ± 3.90c | 78.72 ± 3.36b |
| | 400 | 44.90 ± 2.50b | 79.17 ± 4.66b | 100.00 ± 0.00a |
| | 600 | 63.26 ± 5.20a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | Control | 2.00 ± 2.00e | 4.00 ± 2.45e | 6.00 ± 2.45e |
| | F-test | ** | ** | ** |
| Kaffir Lime | 100 | 0.00 ± 0.00c | 3.26 ± 2.00d | 8.57 ± 2.92d |
| | 200 | 0.00 ± 0.00c | 4.90 ± 2.00d | 16.37 ± 3.82d |
| | 300 | 10.00 ± 3.16c | 20.41 ± 3.81c | 34.70 ± 5.20c |
| | 400 | 26.00 ± 4.00b | 46.94 ± 5.95b | 67.35 ± 5.95b |
| | 600 | 52.00 ± 3.74a | 73.47 ± 5.20a | 100.00 ± 0.00a |
| | Control | 0.00 ± 0.00c | 2.00 ± 2.00d | 2.00 ± 2.00d |
| | F-test | ** | ** | ** |

Means followed by the same letter(s) in the same column of each essential oil are not significantly different ($P>0.05$) from each other using Turkey's Test.

** significantly at $P< 0.01$

### 3.2 Synergistic effect of sesame oil and clove oil

Mean percent mortality of tested *C. maculatus* after exposure to different mixed plant oil ratios applied as residual contact and fumigation methods are presented in Figure 12 and Figure 13. Insect mortality was absent after treated with sesame oil alone (ratio 0:10) at 24 h in both residual contact and fumigation. It was not significantly different percent mortality between sesame oil alone (ratio 0:10) and control of both applications (Figure 12 and Figure 13), suggesting that sesame oil did not have insecticidal activity. On the other hand, clove oil exhibited insecticidal activity because mean percent mortalities of insect reached 44.00±2.45% and 46.00±5.10% post-exposure with clove oil alone (ratio 10:0) by residual contact and fumigation methods, respectively. Synergistic effect was also present when the clove oil was mixed with the sesame oil at the ratio of (8:2). After 24 h of exposure, insect mortalities of that mixture were 48.00±5.83% and 62.00±3.74%, which were greater than 44.00±2.45 and 46.00±5.10 of the clove oil alone (ratio 10:0) in residual contact and fumigation tests, respectively .

Application method also affected mortality of *C. maculatus* adults. Clove oil applied by fumigation was more effective to kill this insect pest than that applied by residual contact method (Figure 14). Clove oil alone (ratio 10:0) applied by residual contact reached the maximum mean percent mortality of 89.56±4.73% at 72 h, whereas that applied by fumigation reached the maximum mean percent mortality of 100.00± 0.00% (Figure 14). In the same manner, insect mortality subjected by the residual contact to the mixture (ratio 8:2) for 24, 48 and 72 h were 48±5.83%, 91.56±4.12% and 100.00±0.00%, respectively, which were lower than 62.00±3.74%, 100.00±0.00% and 100.00±0.00% subjected by fumigation, respectively. In addition, mortality of *C. maculatus* adults increased with a rise of exposure time in all treatments of two application methods (Figure 14).

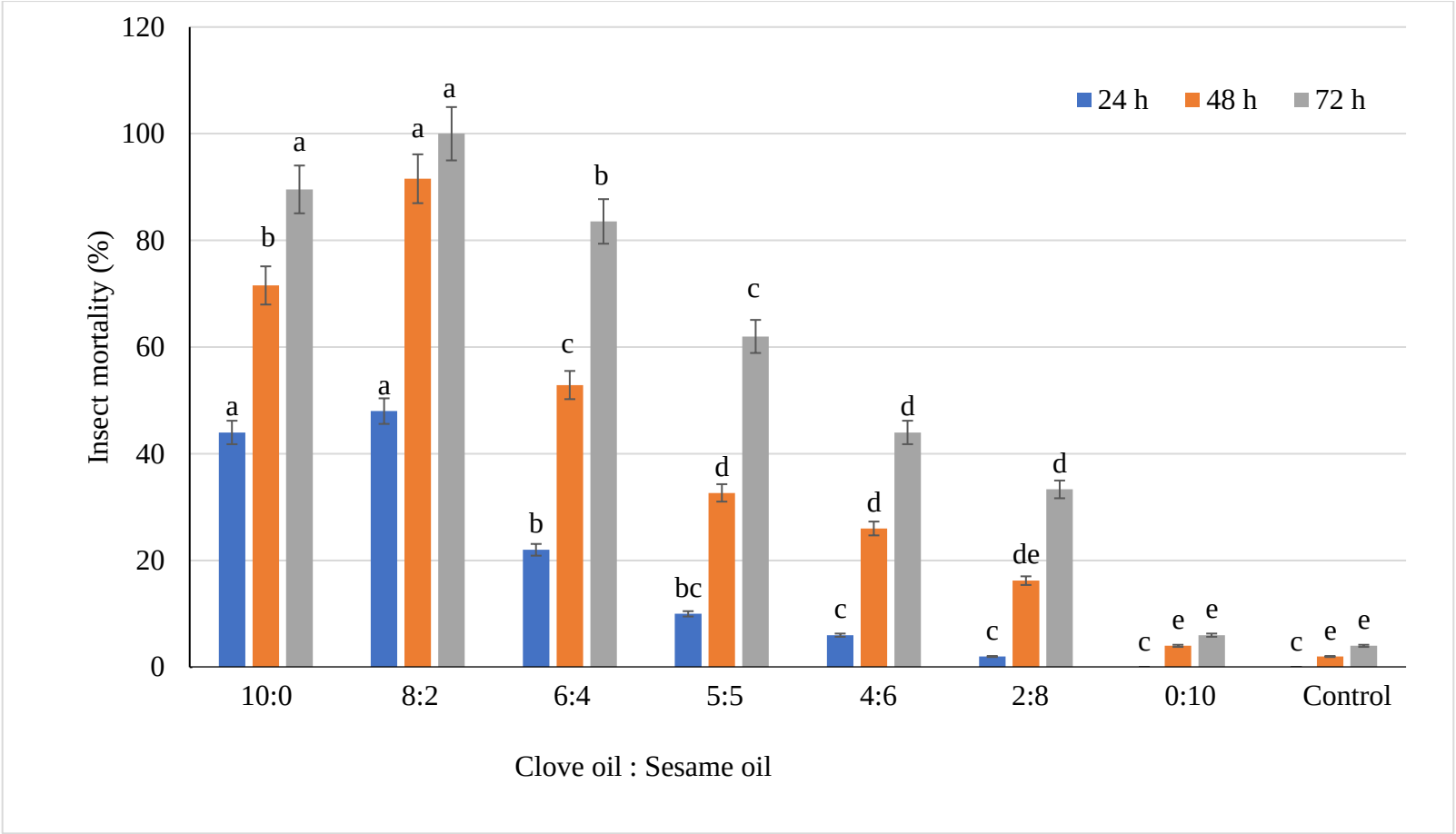


**Figure 12** Mortality of *Callosobruchus maculatus* adults, in residual contact toxicity tested with different ratio of clove oil and sesame oil. Bars with the same letter are not significantly different ($P > 0.05$) from each other using Tukey's Test.

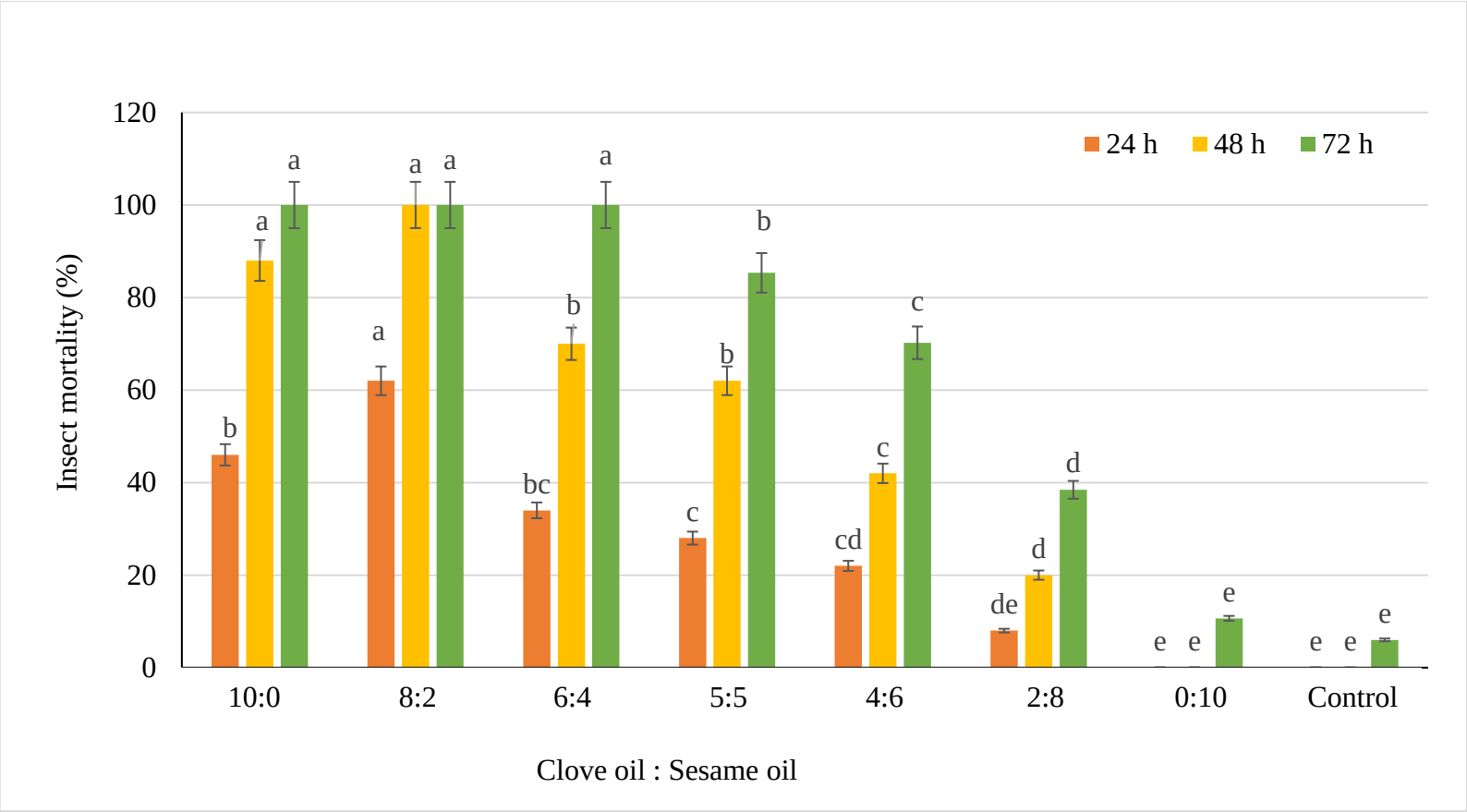


**Figure 13** Mortality of *Callosobruchus maculatus* adults, in fumigation toxicity tested with different ratio of clove oil and sesame oil. Bars with the same letter are not significantly different ($P > 0.05$) from each other using Tukey's Test.

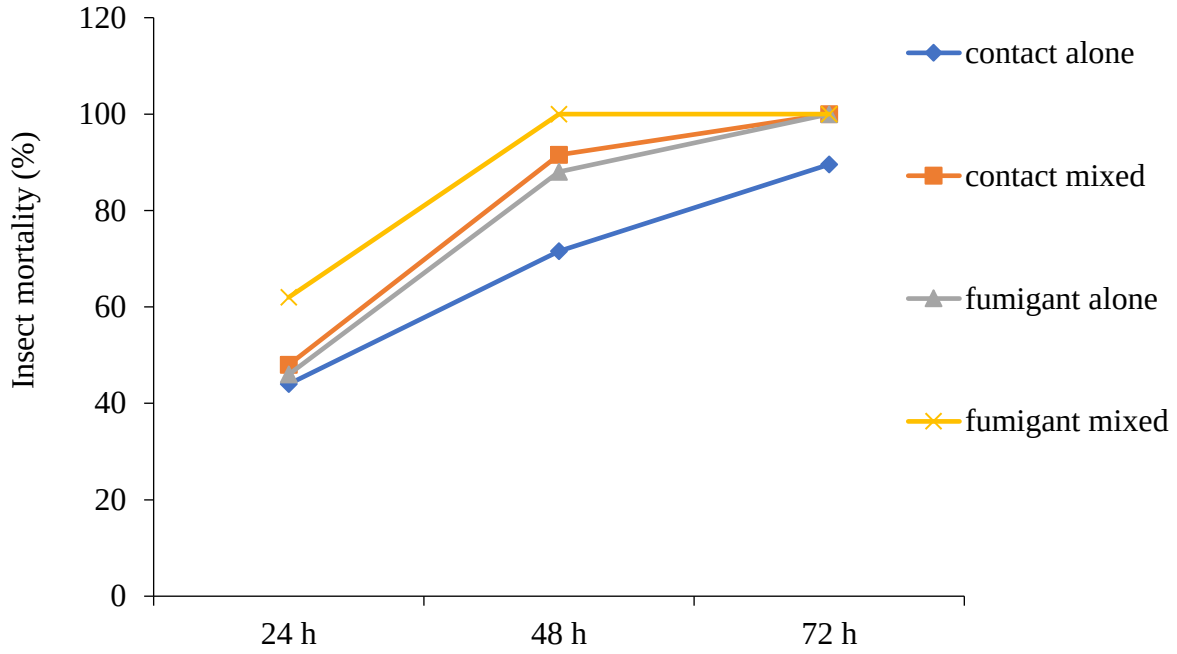


**Figure 14** Mortality of *Callosobruchus maculatus* adults after exposure to clove oil alone and the mixture of clove oil and sesame oil at the ratio of 8:2 by residual contact and fumigation method at 24, 48 and 72 h.

## 3.3 Application methods for controlling *Callosobruchus maculatus* on mung bean

### 3.3.1 Sack coating application

For 1st month of this experiment, movement inhibition percentage of *C. maculatus* through the jute bag coated with different concentrations of plant oil mixture of clove oil and sesame oil (8:2) at 1, 3, 5 and 7 days after releasing of *C. maculatus* adults are presented in Table 6. After 1 day of pulse beetles releasing, movement inhibition percentages of all concentrations reached over 50% and were significantly difference form the control, except 0.5% concentration of plant oil mixture. After 3 days of treatment, movement inhibition percentages of 67.58±2.73%, 61.06±2.58%, 54.56±1.94% were observed at 5.0%, 3.0% and 2.0% concentrations, respectively. After 5 days of treatment, the highest inhibition percentage of 66.64±2.93% was recorded at 5.0% concentration of plant oil mixture. The plant oil mixture showed less effective than 63.52±1.47% to inhibit *C. maculatus* after 7 days of treatment. Inhibition percentages of 36.05 ± 3.38%, 29.65 ± 3.52%, 26.44 ± 4.93% and 23.99 ± 2.02% were recorded at 0.5% concentration after 1, 3, 5 and 7 days, respectively, which were not significantly different form jute bag coating with acetone (negative control).

For 2nd month of this experiment, the results of movement inhibition percentage of *C. maculatus* at different concentrations of 0.5, 1.0, 2.0, 3.0 and 5% of plant oil mixture of clove oil and sesame oil (8:2) at 1, 3, 5, and 7 days after releasing of *C. maculatus* adults are presented in Table 7. It is evident that movement inhibition percentage of *C. maculatus* increased with increase of concentrations of the mixture but decreased with increase of exposure time. The highest concentration of 5.0% showed significantly higher movement inhibition percentage than other concentrations except concentration of 3.0%, with 62.39 ± 4.55%, 53.80 ± 2.10%, 47.97 ± 4.30% and 41.34 ± 3.65% at 1, 3, 5 and 7 days after treatment, respectively. The control exhibited the lowest movement inhibition percentage of 24.45±2.49%, 16.77±2.39%, 11.07±3.91% and 6.47±3.73% at the same time. There was no significant difference between concentration of 0.5%, 1% and control at 1 and 3 days after treatment. By 5 and 7 days, the movement inhibition percentage of *C. maculatus* at concentration of 0.5%, 1.0%, 2.0% and control was not significantly different.

After 1st month and 2nd month of storage, the results of F1 adult emergence and weight loss of mung bean in jute bag coated with 0.5%, 1.0%, 2.0%,

3.0% and 5.0% concentrations of the plant oil mixture of clove oil and sesame oil (8:2) are presented in Table 8. At 1st month of storage, all concentrations of the mixture of clove oil and sesame oil at the ratio of 8:2 significantly reduced progeny production compared to the control. Completely inhibited progeny production was observed from the concentration of 3.0% and 5.0%. There was a significant difference in weight loss between treatments. No weight loss was observed from mung bean seeds at concentration of 3.0% and 5.0%. On the other hand, the control seeds showed the highest weight loss of 3.41%. At 2nd month of storage, the highest mean number of F1 adult emergence of 490.50 ± 8.66 was recorded at 0.5% concentration which was not significantly different from adult emergence of 508.75 ± 7.04 in the control. F1 adult emergence was reduced from 476.75 ± 8.11 to 33.25 ± 4.44 at the concentrations of plant oil mixture ranged from 1.0% to 5.0%, respectively. The lowest weight loss of 0.27 ± 0.09% was recorded at the concentration of 5.0% which was not significantly different from the weight loss 0.89 ± 0.11% at the concentration of 3.0%. The highest weight loss 3.65±0.59% was observed at concentration 0.5% and this was not significantly different to the control.

**Table 6** Movement inhibition percentage of *Callosobruchus maculatus* across jute bag coated with different concentration of plan oil mixture of clove oil and sesame oil (8:2) after 1 month of storage

| | **Movement inhibition (means ± S.E., %)** | | | |
|---|---|---|---|---|
| **Concentrations (%)** | **Day 1** | **Day 3** | **Day 5** | **Day 7** |
| 0.5 | 36.05 ± 3.38d | 29.65 ± 3.52c | 26.44 ± 4.93c | 23.99 ± 2.02c |
| 1.0 | 53.08 ± 2.79c | 47.92 ± 3.55b | 45.00± 2.63b | 43.57 ± 2.50b |
| 2.0 | 59.26 ± 1.59bc | 54.56 ± 1.94ab | 51.63 ± 2.87ab | 50.91 ± 3.25ab |
| 3.0 | 65.33 ± 1.09ab | 61.06 ± 2.58a | 59.35 ± 2.44ab | 56.13 ± 2.32ab |
| 5.0 | 73.23 ± 2.39a | 67.58 ± 2.73a | 66.64 ± 2.93a | 63.52 ± 1.47a |
| Control (acetone) | 29.80 ± 2.46d | 25.31 ± 2.85c | 14.48 ± 5.58c | 7.84 ± 4.66c |
| F-test | ** | ** | ** | ** |

Means followed by the same letter(s) in the same column are not significantly different ($P>0.05$) by Turkey's test.

** significantly at $P<0.01$

**Table 7** Movement inhibition percentage of *Callosobruchus maculatus* across jute bag coated with different concentration of plan oil mixture of clove oil and sesame oil (8:2) after 2 month of storage

| Concentrations (%) | Movement inhibition (means ± S.E., %) | | | |
|---|---|---|---|---|
| | **Day 1** | **Day 3** | **Day 5** | **Day 7** |
| 0.5 | 29.08 ± 1.60cd | 21.70 ± 1.09bc | 15.39 ± 2.47c | 12.45 ± 4.35c |
| 1.0 | 32.28 ± 2.41cd | 26.34 ± 2.46bc | 19.24 ± 2.34bc | 14.30 ± 1.38bc |
| 2.0 | 37.71 ± 1.91bc | 29.71 ± 3.07b | 23.22 ± 3.48bc | 16.77 ± 2.39bc |
| 3.0 | 50.13 ± 3.25ab | 44.28 ± 2.46a | 35.78 ± 5.27ab | 28.62 ± 3.95ab |
| 5.0 | 62.39 ± 4.55a | 53.80 ± 2.10a | 47.97 ± 4.30a | 41.34 ± 3.65a |
| Control (acetone) | 24.45 ± 2.49d | 16.77 ± 2.39c | 11.07 ± 3.91c | 6.47 ± 3.73c |
| F-test | ** | ** | ** | ** |

Means followed by the same letter(s) in the same column are not significantly different ($P>0.05$) by Turkey's test.

** significantly at $P<0.01$

**Table 8** F1 adult emergence and weight losses of mung bean seeds in jute bag coated with plant oil mixture of clove oil and sesame oil (8:2) against *Callosobruchus maculatus*

| **Concentration (%)** | **1st month** | | **2nd month** | |
|---|---|---|---|---|
| | **F1 emergence (mean ± S.E.)** | **Weight loss (mean ± S.E., %)** | **F1 emergence (mean ± S.E.)** | **Weight loss (%) (mean ± S.E., %)** |
| 0.5 | 419.75 ± 11.56b | 2.99 ± 0.67ab | 490.50 ± 8.66a | 3.65 ± 0.59a |
| 1.0 | 229.25 ± 4.73c | 1.70 ± 0.27bc | 476.75 ± 8.11a | 3.58 ± 0.66a |
| 2.0 | 119.75 ± 7.42d | 0.72 ± 0.06c | 350.25 ± 7.49b | 2.60 ± 0.42ab |
| 3.0 | 0.00 ± 0.00e | 0.00 ± 0.00c | 121.50 ± 6.28c | 0.89 ± 0.11bc |
| 5.0 | 0.00 ± 0.00e | 0.00 ± 0.00c | 33.25 ± 4.44d | 0.27 ± 0.09c |
| Control (acetone) | 487.75 ± 9.42a | 3.41 ± 0.58a | 508.75 ± 7.04a | 3.81 ± 0.68a |
| F-test | ** | ** | ** | ** |

Means followed by the same letter(s) in the same column are not significantly different ($P>0.05$) by Turkey's test.

** significantly at $P< 0.01$

### 3.3.2 Seed dressing application

All concentrations of the mixture of clove oil and sesame oil at ratio of 8:2 and chlorpyrifos significantly reduced progeny production caused by *C. maculatus* compared to control (Table 9). The progeny production of *C. maculatus* decreased with increasing of concentration of tested oil but increased with increasing of storage period. The highest concentration of 3.5% of tested oil and chlorpyrifos completely suppressed the progeny production of *C. maculatus* during the whole storage period. The highest progeny production of *C. maculatus* was obtained in the control of 297.75±7.05, 549.00±6.65, 667.00±4.92, 471.75±7.05, 312.75±8.41 and 295.00 ± 8.85 at 1, 2, 3 ,4 ,5 and 6 months after storage, respectively.

It is evident that all concentrations of 0.5%, 1.0%, 1.5%, 2.5% and 3.5% of the mixture of clove oil and sesame oil significantly protected to the mung bean seeds from the damage of *C. maculatus* as shown in Table 10. Damage percentage of mung bean seed was depended on the storage time and concentration of plant oil mixture. While the concentration increased from 0.5% to 2.5% the seed damage decreased from 79.85±0.56% to 15.47±0.35% after 6 months of storage period. Throughout the one to six month storage period, there was no damage in mung bean seed which was treated with the highest 3.5% concentration of plant oil mixture and chlorpyrifos. All concentrations significantly reduced the seed damage percentage of mung beans seeds as compared with the control which suddenly increased the damage rate of 100.00±0.00 after 3 months storage period.

Table 11 presents the mean of weevil perforation index (WPI) of all concentrations treated on mung bean seeds, demonstrating that a positive protectant effect rates were ranged from between 0.00±0.00 to 44.40±0.17 as compared to 50.00±0.00 of control after 6 month storage. WPI value depended on the concentration of plant oil mixture and storage period. The lowest value of weevil perforation index or completely protectant rate (0.00±0.00) was recorded by the mung bean seed treated with 3.5% concentration of plant oil mixture and chlorpyrifos throughout the 1 to 6 months storage period.

**Table 9** Adult progeny production of *Callosobruchus maculatus* on mung bean seed treated with different concentration of plant oil mixture of clove oil and sesame oil (8:2) after storage for 6 months

| **Concentration (%)** | **Adult progeny production (mean ± S.E.)** | | | | | |
|---|---|---|---|---|---|---|
| | **1 month** | **2 months** | **3 months** | **4 months** | **5 months** | **6 months** |
| 0.5 | 79.25 ± 5.20b | 111.00 ± 3.85b | 128.00 ± 5.15b | 182.75 ± 2.84b | 215.50 ± 6.06b | 257.25 ± 4.03b |
| 1.0 | 53.75 ± 4.69c | 92.75 ± 5.36c | 111.25 ± 7.73b | 167.00 ± 5.70b | 195.75 ± 5.12b | 236.75 ± 4.40b |
| 1.5 | 26.00 ± 1.22d | 41.00 ± 2.16d | 65.25 ± 3.97c | 87.25 ± 2.66c | 102.50 ± 3.10c | 113.25 ± 5.69c |
| 2.5 | 8.50 ± 1.55de | 14.50 ± 0.96e | 24.75 ± 5.56d | 33.00 ± 3.39d | 57.75 ± 3.44d | 84.25 ± 3.97d |
| 3.5 | 0.00 ± 0.00e | 0.00 ± 0.00e | 0.00 ± 0.00e | 0.00 ± 0.00e | 0.00 ± 0.00e | 0.00 ± 0.00e |
| Chlorpyrifos | 0.00 ± 0.00e | 0.00 ± 0.00e | 0.00 ± 0.00e | 0.00 ± 0.00e | 0.00 ± 0.00e | 0.00 ± 0.00e |
| Control | 297.75 ± 7.05a | 549.00 ± 6.65a | 667.00 ± 4.92a | 471.75 ± 7.05a | 312.75 ± 8.41a | 295.00 ± 8.85a |
| F-test | ** | ** | ** | ** | ** | ** |

Means followed by the same letter(s) in the same column are not significantly different ($P$>0.05) by Turkey's test.

** significantly at $P$< 0.01

**Table 10** Seed damage caused by *Callosobruchus maculatus* on mung bean seed treated with different concentration of plant oil mixture of clove oil and sesame oil (8:2) after storage for 6 months

| Concentration (%) | Seed damage (mean ± S.E., %) | | | | | |
|---|---|---|---|---|---|---|
| | 1 month | 2 months | 3 months | 4 months | 5 months | 6 months |
| 0.5 | 7.58 ± 0.36b | 18.88 ± 0.84b | 27.05 ± 0.37b | 38.28 ± 0.28b | 51.55 ± 0.61b | 79.85 ± 0.56b |
| 1.0 | 5.50 ± 0.49c | 14.63 ± 0.46c | 24.88 ± 0.86b | 36.45 ± 0.57c | 49.58 ± 0.51b | 74.08 ± 1.27c |
| 1.5 | 2.53 ± 0.09d | 6.55 ± 0.38d | 11.20 ± 0.89c | 17.13 ± 0.38d | 25.20 ± 0.58c | 32.93 ± 0.57d |
| 2.5 | 0.85 ± 0.16e | 2.30 ± 0.23e | 4.55 ± 0.38d | 6.25 ± 0.50e | 11.98 ± 0.73d | 18.30 ± 0.49e |
| 3.5 | 0.00 ± 0.00e | 0.00 ± 0.00f | 0.00 ± 0.00e | 0.00 ± 0.00f | 0.00 ± 0.00e | 0.00 ± 0.00f |
| Chlorpyrifos | 0.00 ± 0.00e | 0.00 ± 0.00f | 0.00 ± 0.00e | 0.00 ± 0.00f | 0.00 ± 0.00e | 0.00 ± 0.00f |
| Control | 29.78 ± 0.71a | 71.48 ± 0.73a | 100.00 ± 0.00a | 100.00 ± 0.00a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| F-test | ** | ** | ** | ** | ** | ** |

Means followed by the same letter(s) in the same column are not significantly different ($P$>0.05) by Turkey's test.

** significantly at $P$< 0.01

**Table 11** Weevil perforation index by *Callosobruchus maculatus* on mung bean seed treated with different concentration of plant oil mixture of clove oil and sesame oil (8:2) after storage for 6 months

| Concentration (%) | Weevil perforation index (WPI) (mean ± S.E.) | | | | | |
|---|---|---|---|---|---|---|
| | **1 month** | **2 months** | **3 months** | **4 months** | **5 months** | **6 months** |
| 0.5 | 19.87 ± 0.76b | 21.10 ± 0.74b | 21.29 ± 0.23b | 27.68 ± 0.15b | 34.01 ± 0.26b | 44.40 ± 0.17b |
| 1.0 | 15.23 ± 1.16c | 17.18 ± 0.45c | 19.91 ± 0.55b | 26.71 ± 0.30b | 33.14 ± 0.23b | 43.31 ± 0.11c |
| 1.5 | 7.64 ± 0.24d | 8.50 ± 0.90d | 10.05 ± 0.72c | 14.62 ± 0.28c | 20.12 ± 0.37c | 24.77 ± 0.32d |
| 2.5 | 2.70 ± 0.96e | 3.16 ± 0.60e | 4.35 ± 0.35d | 6.21 ± 0.44d | 10.68 ± 0.58d | 15.47 ± 0.35e |
| 3.5 | 0.00 ± 0.00f | 0.00 ± 0.00f | 0.00 ± 0.00e | 0.00 ± 0.00e | 0.00 ± 0.00e | 0.00 ± 0.00f |
| Chlorpyrifos | 0.00 ± 0.00f | 0.00 ± 0.00f | 0.00 ± 0.00e | 0.00 ± 0.00e | 0.00 ± 0.00e | 0.00 ± 0.00f |
| Control | 50.00 ± 0.00a | 50.00 ± 0.00a | 50.00 ± 0.00a | 50.00 ± 0.00a | 50.00 ± 0.00a | 50.00 ± 0.00a |
| F-test | ** | ** | ** | ** | ** | ** |

Means followed by the same letter(s) in the same column are not significantly different ($P>0.05$) by Turkey's test.

** significantly at $P< 0.01$

### 3.3.3 Fumigation on egg and adult of *Callosobruchus maculatus*

Mean mortality percentage of *C. maculatus* egg treated with mixture of clove oil and sesame oil at ratio of (8:2) by fumigation with electric burner at 1 month are presented in Table 12. There was a significantly different between treatments both in jute bag and petri dish. Mortality percentage of *C. maculatus* increased with increasing concentration. The highest concentration of 7.5% cause the highest mortality of 58.00% and 81.00% for jute bag and petri dish, respectively. On the other hand, the lowest insect mortality was obtained from control with 6.00% and 5.00% for jute bag and petri dish and there was no significant difference between control and concentration of 1.5%.

The $LC_{50}$ and $LC_{90}$ values of mixture of clove oil and sesame oil at ratio 8:2 against *C. maculatus* egg by fumigation are presented in Table 13. The plant oil exhibited insecticidal activity against eggs of *C. maculatus* with $LC_{50}$ and $LC_{90}$ values of 7.81% and 23.13% for jute bag and 5.05% and 10.01% for petri dish, respectively.

Mean mortality percentage of *C. maculatus* adult treated with mixture of clove oil and sesame oil at ratio of (8:2) by fumigation with electric burner at 24, 48 and 72 h are presented in Table 14. When jute bag used for package, the highest concentration of 7.5% exhibited fumigant toxicity with the highest mortality percentage of 60.00 ± 4.08%, 82.50 ± 4.79% and 100.00±0.00% at 24, 48 and 72 h after fumigation, respectively. The lowest concentration at 1.5% showed the lowest mortality percentage of 0.00±0.00%, 12.50 ± 2.50% and 22.50±6.29% at 24, 48 and 72 h after fumigation, respectively, which was not significantly different as compared with the control. When plastic cup used for package, a complete mortality percentage 100.00±0.00% was observed at the highest concentration of 7.5% at 24, 48 and 72 h after fumigation which was followed by concentration of 6.0% providing complete mortality percentage 100.00±0.00% at 72 h after fumigation. The lowest concentration of 1.5% showed mortality percentage of 10.00±4.08% at 24 h and this was not significantly different form 2.50 ± 2.50% of the control. However, 48 and 72 h after fumigation, mortality percentage increased from 30.00±8.16% at 48 h to 45.00±6.45% at 72 h, which was significantly different form the control.

The $LC_{50}$ and $LC_{90}$ values of mixture of clove oil and sesame oil at ratio (8:2) against *C. maculatus* adult by fumigation are presented in Table 15. $LC_{50}$ and $LC_{90}$ values were 6.64% and 15.73% in jute bag and 4.97% and 6.55 % in plastic cup (24 h after fumigation), 5.46% and 10.26% in jute bag and 4.27% and 5.61% in plastic cup (48 h after fumigation) and 4.31% and 7.09% in jute bag and 3.64% and 5.08% in plastic cup (72 h after fumigation), respectively. The $LC_{50}$ and $LC_{90}$ values for adults were progressively decreased with increasing the time after fumigation (Table 15).

**Table 12** Mean mortality percentage of eggs of *Callosobruchus maculatus* treated with plant oil mixture at different concentrations by fumigation with electric burner at 1 month

| **Concentration (%)** | **Mortality (means± S.E., %)** | |
|---|---|---|
| | **Jute sack** | **Petri dish** |
| 1.5 | 10.00 ± 2.58cd | 16.00 ± 1.63cd |
| 3.0 | 23.00 ± 3.79bc | 31.00 ± 1.91c |
| 4.5 | 32.00 ± 1.63b | 48.00 ± 5.89b |
| 6.0 | 35.00 ± 3.79b | 69.00 ± 3.42a |
| 7.5 | 58.00 ± 2.58a | 81.00 ± 4.43a |
| Control | 6.00 ± 3.83d | 5.00 ± 3.00d |
| F-test | ** | ** |

Means followed by the same letter(s) in the same column are not significantly different ($P>0.05$) by Turkey's Test. ** significantly at $P<0.01$

**Table 13** $LC_{50}$ and $LC_{90}$ values of mixture of clove oil and sesame oil at the ratio (8:2) against eggs of *Callosobruchus maculatus* by fumigation with electric burner at 1 month

| **Type of package** | **LC 50 (%)** | **95 % confident limit** | | **LC90 (%)** | **95 % confident limit** | |
|---|---|---|---|---|---|---|
| | | **Lower** | **Upper** | | **Lower** | **Upper** |
| Jute bag | 7.81 | 6.45 | 10.05 | 23.13 | 14.78 | 128.77 |
| Petri dish | 5.05 | 4.39 | 5.57 | 10.01 | 8.71 | 12.71 |

**Table 14** Mean mortality percentage of *Callosobruchus maculatus* adult treated with plant oil mixture at different concentration by fumigation with electric burner at 24, 48 and 72 h

| Type of package | Concentration (%) | Mortality (% means± S.E) | | |
|---|---|---|---|---|
| | | 24 h | 48 h | 72 h |
| Jute sack | 1.5 | 0.00 ± 0.00d | 12.50 ± 2.50de | 22.50 ± 6.29d |
| | 3.0 | 12.50 ± 2.50cd | 30.00 ± 4.08cd | 47.50 ± 4.79c |
| | 4.5 | 25.00 ± 6.45bc | 40.00 ± 9.13bc | 62.50 ± 7.50bc |
| | 6.0 | 40.00 ± 4.08b | 55.00 ± 6.45b | 75.00 ± 6.45b |
| | 7.5 | 60.00 ± 4.08a | 82.50 ± 4.79a | 100.00 ± 0.00a |
| | Control | 0.00 ± 0.00d | 2.50 ± 2.50e | 5.00 ± 5.00d |
| | F-test | ** | ** | ** |
| Plastic cup | 1.5 | 10.00 ± 4.08cd | 30.00 ± 8.16c | 45.00 ± 6.45b |
| | 3.0 | 25.00 ± 6.45bc | 45.00 ± 6.45bc | 62.50 ± 4.79b |
| | 4.5 | 37.50 ± 4.79b | 62.50 ± 4.79b | 82.50 ± 4.79a |
| | 6.0 | 82.50 ± 4.08a | 97.50 ± 2.50a | 100.00 ± 0.00a |
| | 7.5 | 100.00 ± 0.00a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | Control | 2.50 ± 2.50d | 7.50 ± 2.50d | 10.00 ± 4.08c |
| | F-test | ** | ** | ** |

Means followed by the same letter(s) in the same column are not significantly different ($P$>0.05) according to Turkey's.

** significantly at $P$ <0.01

**Table 15** $LC_{50}$ and $LC_{90}$ values of mixture of clove oil and sesame oil at the ratio (8:2) against *Callosobruchus maculatus* adult by fumigation with electric burner at 24, 48 and 72 h

| Time after fumigation (h) | Type of package | $LC_{50}$ (%) | 95% confident limit | | $LC_{90}$ (%) | 95% confident limit | |
|---|---|---|---|---|---|---|---|
| | | | Lower | Upper | | Lower | Upper |
| 24 | Jute sack | 6.64 | 5.07 | 8.11 | 15.73 | 12.05 | 27.35 |
| | Plastic cup | 4.97 | 4.19 | 5.4 0 | 6.55 | 6.01 | 7.93 |
| 48 | Jute sack | 5.46 | 4.15 | 6.34 | 10.26 | 8.26 | 19.96 |
| | Plastic cup | 4.27 | 2.90 | 4.73 | 5.61 | 5.17 | 6.87 |
| 72 | Jute sack | 4.31 | 2.61 | 5.11 | 7.09 | 6.08 | 10.17 |
| | Plastic cup | 3.64 | 2.56 | 4.20 | 5.08 | 4.47 | 6.10 |

# CHAPTER 4
# DISCUSSION

## 4.1 Bioassay test by residual contact and fumigation method

### 4.1.1 Residual contact toxicity test

In residual contact toxicity experiments, clove oil had the most effective contact toxicity on *C. maculatus* adult. This was followed by cinnamon, lengkuas, and citronella oils, which had moderate toxicity effects on *C. maculatus* adult. As shown in Tables 2 and 3, the results of $LC_{50}$ values and insect mortality percentage, kaffir lime oil had the least contact toxicity on tested insects. However, all selected plant essential oils significantly affected the mortality of adults of *C. maculatus* as compared with the untreated control. The results of this study are similar to those of Mahfuz and Khalequzzaman (2007), who also reported the toxic effects of clove oil, cinnamon oil, cardamom oil, eucalyptus oil and neem oil on *C. maculatus*. The insecticidal toxicity followed in the order: clove> cinnamon> cardamom> neem> eucalyptus after 24 h and 48 h, respectively. In related research, clove oil and jojoba oil gave the highest mortality percentages of *C. maculatus* adults, followed by rosemary, eucalyptus and citronella oil (Abdullah *et al.*, 2017). Al Yousef (2015) reported that the mortality of *C. maculatus,* increased with the oil concentration and exposure duration after treated with clove oil. At the highest concentration of clove oil at 5 mg/l, insect mortality percentage increased from 73.33% two days after treatment to 96.66% four days after treatment. When the lowest concentration of 0.12 mg/l, the mortality percentage reached 16.66% and 63.33% after treatment for two days and four days, respectively.

### 4.1.2 Fumigant toxicity test

The results obtained from fumigant toxicity investigation indicated that the clove oil gave the highest efficacy with 100% insect mortality after 24 h of treatment and followed by lengkuas, cinnamon and citronella oils which were found 100% insect mortality after 48 h of exposure period. However, as the results of $LC_{50}$ values in Table 5, lengkuas essential oil at 254.05 µl/l was more toxic to tested insects than cinnamon oils at 260.60 µl/l after 48 h of treatment, whereas, the order of these two essential oils

was not the same with contact toxicity test. Following the results, kaffir lime oil had the lowest toxicity level in which $LC_{50}$ values were higher than other four plant oils and showed 100 % insect mortality rate after 72 h of exposure time with the highest dosage rate of 600 µl/l in treatment. Oliverira *et al.* (2017) reported that the essential oils of clove (*S. aromacticum*), cinnamon (*C. zeylanicam*), and the eugenol compound had the most promising for controlling the pulse beetle, *C. maculatus*, via fumigation. Abd El-Salam (2010) found that *C. maculatus* was more sensitive than *S. oryzae* to the clove (*S. aromacticum*) essential oil and eucalyptus (*E. globulus*) whereas the $LC_{95}$ values were 1.032 and 3.66 µl/ 50 ml air, respectively.

**4.2 Synergistic effect of sesame oil and clove oil**

In this study, the plant oil mixture of clove oil and sesame oils ratio (8:2) exhibited the higher mortality percentage of *C. maculatus* than the clove oil alone in contact and fumigation tests. Results indicated that sesame oil was a synergist of clove oil at the ratio of (8:2) and enhanced the efficacy of clove oil in both application methods. Sesame oil has synergistic activity and possesses important antioxidant components such as sesamin, sesamolin and sesamol. It is highly stable to oxidation as compared with other vegetable oils (Jan, 2001). Synergisms of insecticide are normally accepted to inhibit the detoxication process. For instance, synergists arise to prevent the detoxification of pyrethrins in insects (Metcalf, 1955), this may carry out the inhibition by the synergists of naturally detoxifying oxidation reactions (Sun and Johnson, 1960).The present results agree with Karso and Al-Mallah (2015) found that synergism of sesame oil at the mixture ratio of 1:2 (acetamprid:sesame oil) was controlled the larvae of *Trogoderma granarium*. The mixtures of clove and sesame oils increased the percent larval mortality in comparison to their use alone against the treated $4^{th}$ larval instar of the cotton leaf-worm *Spodoptera littoralis* (Mesbah *et al.,* 2006).

Current study, clove oil alone in fumigation method exhibited 100% mortality of tested insects and contact method showed 89.56% mortality of adult *C. maculatus* after 72 h of exposure. And also, in the mixture of clove oil : sesame oil (8:2), fumigation method reached the 100% mortality of insects at 48 h exposure,

however, contact method showed 100% mortality of insects after 72 h of exposure. It suggests that fumigation method was more toxic than residual contact method. It is supposed that mode of entry of such insecticide via inhalation was more toxic than via contact exposure. In fumigation method, the clove oil penetrated directly into insect body via respiratory system as volatile oil which more rapidly moved into insect body than contact method. Usha Rani and Rajasekharareddy (2010) reported that the contact exposure of plant extracts from *Sterculia foetida* delayed activity as compared to the fumigation exposure for controlling stored grain pests. Al Yousef (2015) revealed the effectiveness of clove oil against the cowpea seed beetle, *C. maculatus*, especially when using at high concentration (5 mg/l). Wuttiwong (2018) reported that clove oil was the most toxic to *Rhyzopertha dominica* and *Sitophilus zeamais* by both contact and fumigation methods. In addition, percent mortality of *C. maculatus* adults increased with a rise of exposure time in all treatments of two application methods. The present study corroborates with the finding of Abdullah *et al.* (2017) which reported the efficacy of some botanical oils against stored product pest, *C. maculatus.* In this result, percent mortality of insect increased in all botanical oils at increasing exposure time.

## 4.3 Application methods for controlling *Callosobruchus maculatus* on mung bean

### 4.3.1 Sack coating application

This experiment was carried out to observe the movement inhibition percentage of *C. maculatus*, F1 adult emergence and weight loss of mung beans seed packed in jute sack which was coated by the plant oil mixture of clove oil and sesame oil. As the results of Table 6 and Table 7, movement inhibition percentage of *C. maculatus* decreased (< 41.34±3.65%) for all concentrations at 7 days after a release of *C. maculatus* in the 2$^{nd}$ month of experiment. This demonstrates that the mixture of clove oil and sesame oil showed a low repellent action against *C. maculatus*. This results is similar to Wuttiwong (2018) who reported that clove oil showed low repellent activity against *R. dominica* and *S. zeamais.* Jumbo *et al.* (2014) revealed that clove oil showed the percentage of repellency values which is not different from repellency index value 1 (indicative of neutrality) and did not repel *A. obtectus* at tested dosage rate of

13.5, 43.6 and 141.0 µl/kg beans. Other researchers, Kafle and Shih (2013) revealed that clove oil did not exhibit repellent activity against the red imported fire ants *Solenopsis invicta.* Sesame oil also had no repellent activity against the *C. maculatus* (Ratnasekera and Rajapakse, 2009). According to the finding of researchers and the present results, this may be attributed to the mixture of clove oil and sesame oil showed low repellent activity against *C. maculatus*.

The number of F1 adult emergence was completely suppressed in 3.0% and 5.0% concentration after 1 month. On the other hand, 0.5% concentration showed F1 adult emergence 419.75±11.56 after 1 month. This suggests that *C. maculatus* was affected by residual contact toxicity of clove oil and sesame oil while they pass through the jute bag. This may be caused the adult mortality and oviposition deterrent to *C. maculatus*. Even through, F1 adult emergence steadily increased from 0.00±0.00% to 33.25±4.44 in the 5.0% concentration and from 419.75±11.56% to 490.50±8.66% in the 0.5% concentration after the 2$^{nd}$ month. Except 1.0% and 0.5% of concentration, all concentrations were significantly different from control treatment. This may be attributed to the residual contact toxicity by jute sack coating of clove oil and sesame oil was extended to 2 months. Wight loss of mung beans seeds was related to F1 adult emergence, and the lowest values was 0.27±0.09% in the 5.0% concentration after 2 months. The present study result was consistency with the finding of Pongsai (2008) who reported that the residual activity of 10% clove oil extended to 2 months against *S. zeamais*. However, this present study results were not followed by Wuttiwong (2018) who indicated that the residual toxicity of clove oil by sack coating decreased at 7 days after treatment. Raja *et al*. (2000) reported that when jute bags treated with different plant leaf extracts of *A. indica, V. negundo, C. collinus* and *J. curcas* for storage of cowpea seeds, the egg laying rates, adult progeny emergence and seed damage rate by *C. maculatus* were reduced.

### 4.3.2 Seed dressing application

This present study, five different concentrations of the mixture of clove oil and sesame oil significantly reduced adult progeny production up to 6 month storage

as compared with control treatment. Even the lowest concentration at 0.5% reduced adult progeny production of 79.25±5.20, 111.00±3.85, 128.00±5.15, 182.75±2.84, 215.50±6.06 and 257.25±4.04 after 1, 2, 3, 4, 5 and 6 months storage which was significantly different from of the control. The highest concentration of 3.5% as well as chlorpyrifos completely suppressed the adult progeny production throughout the 6 months storage. Furthermore, all concentrations, except 3.5%, adult progeny production steadily increased throughout the 1-6 month storage periods. This may be attributed to the plant oil mixture slowly decreased the residual toxicity effect for six month storage period and reduced the oviposition rate, egg hatchability and longevity of adult. Which is exhibited the antioxidant activity and synergistic activity of plant oil mixture by sesame oil. Because sesame oil possess antioxidant components such us sesamin, sesamolin and sesamol and these components may be delayed the degradation process of clove oil and showed synergistic effect. Sesame oil showed synergistic effect with clove oil at the ratio of (8:2) as mentioned previously. Abd El-Razik and Zayed (2014) indicated that sesame oil showed high synergistic effect with the spinosad at the ratio of spinosad: sesame (90:10) against *C. maculatus*. Wuttiwong (2018) reported that clove oil showed the action of killing, antifeeding and suppressing progeny production of *R. dominica* and *S. zeamais*. A reduction of progeny production might be attributed to less extent of oviposition, egg hatchability as well as a survival of larval and pupal stages. Present study results could be in harmony with Ojiako *et al*. (2018) found that the highest application rate of *Piper guineense* seed powder (10g/ 100g) exhibited a very low number of 16 adults *C. maculatus* emergence as compared with 272.70 adults in the control, throughout the 6 months of the experiment. Neupane *et al*. (2016) reported that at the concentration of 5 ml/kg mung bean seed, total number of *C. chinensis* adult emergence was significantly lower by 23.5 adults of sesame oil and by 15.25 adults of neem oil as compared with 2,896.00 adults of the control after 9 months storage period.

The present result is similar to Pereira (1983) who reported that neem oil at 3 ml/ kg cow pea seeds significantly reduced the adult emergence of *C. maculatus* after 3 months and this activity was retained up to 6 months storage.

Weevil Perforation Index (WPI) was recorded between 0.00-44.40 in all concentrations of mixture of clove oil and sesame oil after 6 months storage, that is less than 50 suggesting a positive protection ability. This study also observed that the seeds coated with 3.5% concentration showed no seed damage throughout the 1-6 months storage as the same effectiveness of positive control (chlorpyrifos). The highest seed damage percentage of 79.85±0.56% was observed in seed treated with 0.5% concentration after 6 months storage which is significantly reduced in a comparison with the control treatment. This may be attributed to the residual contact toxicity of plant oil mixture previously mentioned and it inhibited the egg hatching, thereby leading to reduction of adult progeny and seed damage. This study results was similar to the finding of Ojiako *et al*. (2018) who reported that the WPI values of 0.00 to 33.3% was observed in 1.0g/100 g cow pea seeds treated with actellic dust against *C. maculatus* throughout the 1-6 moth storage. This study results agreed with the finding of Wuttiwong (2018) who reported that clove oil exhibited highly effective on rice seed protection against *R. dominica* by reduction the progeny production, insect infestation and seed damage. Other researchers found that bioactivity of caster, neem, karanj, groundnut and mustard oils significantly reduced seed damage rate by *C. maculatus* on pigeon pea seed (Lolage and Patil, 1992). Rotimi and Ekperusi (2012) reported that the essentials oils of *Citrus lumonium, Citrus aurantifolia*, *Citrus paradisi* and *Citrus sinensis* had high effectiveness as biopesticide for protecting cowpea seeds from *C. macualtus* infection and damage. Sesame, corn, sunflower and groundnut oil reduced 70% oviposition of three bruchid species at the concentration of 10ml/kg cowpea seeds. The concentration of 5 ml/kg was the most effective against *C. maculatus.* A number of authors found that oil coating was effective to control *C. maculatus* (Messina and Renwick, 1983; Pandey *et al*., 1983; Pereira, 1983; Singh *et al*., 1978).

### 4.3.3 Fumigation on egg and adult of *Callosobruchus maculatus*

The results of this study demonstrated that lethal concentration of plant oil mixture depended on stage of insects and type of packages. Fumigant toxicity on *C. maculatus* adult in jute bag, the plant oil mixture showed the $LC_{50}$ values of 6.64%, 5.46% and 4.31% after 24, 48 and 72 h, respectively and the higher fumigant toxicity with the lowest $LC_{50}$ values of 3.64% was shown after 72 h while using the plastic cup for package. On the other hand, $LC_{50}$ values of plant oil mixture were 7.81% and 5.05% for fumigation on egg by packing jute bag and petri dish, respectively. Based on the results of $LC_{50}$ values, *C. maculatus* adult were more susceptible to plant oil mixture than egg and plant oil mixture showed low toxicity effect on tested insects packed by jute sack. This may be attributed to a low penetration of the plant oil mixture into jute bag and a high oxygen consumption adult as compared with eggs. This study results agree with Shojaaddini *et al*. (2008) who assessed the effect of *Carum copticum* essential oil on eggs, larvae, pupae, and adults of *P. interpunctella.* They reported that adult stage was more susceptible than other growth stages. Tarigan *et al*. (2016) reported that cinnamon oil exhibited the highest fumigant toxicity against egg, larva, and adult of *C. maculatus* with the $LC_{50}$ values of 0.01%, 0.132%, and 0.186%, respectively.

All different concentrations of plant oil mixture revealed fumigant toxicity effect against eggs and adults of *C. maculatus*, mortality percentage was depended on type of packing, concentration and time after fumigation. Both of egg and adult mortality percentages were significantly different from jute bag package to another package of petri dish and plastic cup. For the *C. maculatus* eggs, fumigant toxicity of plant oil mixture at 1.5-7.5% concentration exhibited the mortality percentage of eggs ranged from 16.00±1.63 to 81.00±4.43% in petri dish and from 10.00±2.58 to 58.00±2.58 in jute bag, respectively. For the *C. maculatus* adults, the highest concentration at 7.5% caused 100% mortality of adults after 24 h in the plastic cup. However, in jute sack package, 100% mortality was achieved after 72 h with jute bag. According to the results of this study, *C. maculatus* adult was more susceptible to

the plant oil mixture than egg stage and the fumigant toxicity effect on both stages was smaller in jute bag than in petri dish and plastic cup.

According to the present results, it is clear that egg of *C. maculatus* was more tolerant to plant oil mixture than adult and plant oil mixture showed a low penetration through jute bag. Although there was limited literature for this study, this may be contributed to a requirement of the higher concentration and exposure time to get the complete mortality of the both stages of tested insect. Ketoh *et al*. (2005) reported that the mortality of the eggs of stored product pests depended on the species and the plant oil. *Cymbopogon schoenanthus* essential oil concentration of 33.3 ml/l exhibited 100% mortality of C. *maculatus* eggs after 24 h exposure. Wuttiwong (2018) found that clove oil at the concentration of 7.5% and 10.0% showed the highest fumigant toxicity against *R. dominica* and *S. zeamais* after 5-7 day.

Even though fumigation process of plant oil against stored product pest has not clearly clarified, the route of oil action was largely in the vapor through the respiratory system (Tripathi *et al.,* 2009). Sesame oil alone did not show the fumigant toxicity, but it exhibited synergistic effect while mixing with clove oil and enhance the fumigant toxicity of plant oil mixture as mentioned in previous experiment study. Both eggs and adults of *C. maculatus* died when fumigated by the clove oil and sesame oil mixture. This may be due to interference in gaseous exchange in respiration or asphyxiation. This noted was supported by Tian *et al.* (2012) demonstrated that essential oil composition like eugenol inhibited respiration and ion-transport an increased membrane. Especially, monoterpenoid constituents are typically volatile and rather are lipophilic compounds group, which can rapidly penetrate into insects and easily interfere with their physiological functions (Lee *et al.,* 2003). The insecticidal activity of the essential oil could be linked to the synergistic effects of the individual components. Joint action studies have established that mixture of several components of essential oils potentiates their individual insecticidal activity (Don-Pedro, 1996).

# CHAPTER 5
# CONCLUSION AND RECOMMENDATIONS

Five plant essential oils of clove, cinnamon, lengkuas, citronella and kaffir lime were investigated for residual contact toxicity and fumigant toxicity against *C. maculatus* adults under laboratory conditions. Among the tested oils, clove oil showed the highest residual contact toxicity with the following order: clove>cinnamon>lengkuas> citronella >kaffir lime. Furthermore, clove oil possessed the highest fumigant toxicity among the tested oils, however, descending order of plant oils were clove>lengkuas>cinnamon> citronella>kaffir lime. Fumigant toxicity of lengkuas oil was more effective than cinnamon oil against *C. maculatus*. As the results of this investigation, clove oil was selected to study the synergistic action with sesame oil. Synergistic effect was presented when the clove oil was mixed with the sesame oil at the ratio of clove oil and sesame oil (8:2) in both residual contact and fumigant toxicity. This ratio of clove oil and sesame oil mixture (8:2) was selected for further investigation.

The effectiveness of application methods sack coating, seed dressing and fumigation with clove oil and sesame oil mixture were evaluated for controlling the *C. maculatus* in mung bean seed. Sack coating method showed low effectiveness because repellent action of movement inhibition percentage decrease in the 2$^{nd}$ month and increased the F1 adult emergence and weight loss of mung bean seeds. Seed dressing method showed a high effectiveness with the complete exhibition of adult emergence, no seed damage and none of WPI with positive protectant up to 6 months storage. Meanwhile, fumigation method showed moderate effectiveness against *C. maculatus*. Because it was found that the adults were susceptible to highest concentration 7.5% of plant oil mixture, however, eggs were tolerant and could not reach to complete mortality at 7.5% concentration. Moreover, plant oil mixture has low penetration through jute sack.

This study provided a scientific basis in applying botanical insecticides against *C. maculatus*. Furthermore, this plant oil mixture of clove oil and sesame oil could be exploited against insect infestation at smallholder farmer's level as this can be

more effective and easier to apply in the warehouse. This plant oil mixture is not a harmful effect to consumers because these two plant oils are commonly used in many medicinal preparations and during cooking of the foodstuff. Further studies should be done for the bioactivity of this plant oil mixture and their constituents against other stored-product insect pests before considering commercial application. There is a need to assess the cost-effectiveness and feasibility of using the plant oil mixture on large scale seed storage.

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# APPENDIX

The yields of five plant oils from steam distillation were calculated by the following formula.

$$\text{Percentage of yield} = \frac{\text{weight of oil extracted (ml)}}{\text{weight of plant material (kg)}} \times 100$$

**Appendix Table** Yield percentage of five plant oils extracted by steam distillation

| **Scientific name** | **Common name** | **Parts used** | **Percentage of yield [% Yield (v/w)]** |
|---|---|---|---|
| *Cinnamomum verum* | Cinnamon | bark | 0.82 |
| *Alpinia galanga* | Lengkuas | rhizome | 0.15 |
| *Syzygium aromaticum* | Clove | flower bud | 1.43 |
| *Cymbopogon nardus* | Citronella | leaf | 0.27 |
| *Citrus hystrix* | Kaffir Lime | peel | 1.73 |

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