Musca domestica L. Diets, Botanical Control of Muscid Flies (Muscidae) and Detection of *Escherichia coli* from Filth Flies in Local Markets of Hat Yai District, Songkhla Province

Warin Klakankhai

A Thesis Submitted in Fulfillment of the Requirements for the
Degree of Master of Science in Entomology
Prince of Songkla University
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ชื่อวิทยานิพนธ์ อาหารของหนอนแมลงวันบ้าน *Musca domestica* L. การควบคุมโดยใช้สารสกัดจากน้ำมันหอมระเหยเพื่อควบคุมแมลงวันกลุ่ม Muscid flies และการตรวจสอบหาเชื้อ *Escherichia coli* จากแมลงวันตอมสิ่งปฏิกูลในตลาดอำเภอหาดใหญ่จังหวัดสงขลา

ผู้เขียน นางสาววริน กล้าการขาย

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บทคัดย่อ

แมลงวันบ้าน, *Musca domestica* L. (Diptera: Muscidae) เป็นแมลงศัตรูสำคัญที่มีแหล่งที่อยู่อาศัยใกล้ชิดกับมนุษย์และเป็นแมลงที่มีความสำคัญทางการแพทย์ โดยเป็นพาหะนำโรคเชิงกล (mechanical vectors) ด้วยการนำเชื้อโรคติดไปกับส่วนต่างๆของร่างกายจากพื้นที่ไม่ถูกสุขอนามัยไปปนเปื้อนบนอาหารของมนุษย์และสัตว์เมื่อแมลงวันลงตอม จุดประสงค์ในการวิจัยในครั้งนี้ คือ 1) เปรียบเทียบน้ำหนัก และองค์ประกอบทางโภชนะของหนอนแมลงวันหลังจากเลี้ยงด้วยอาหาร 3 ชนิดภายใต้สภาพห้องปฏิบัติการ 2) ระบุประสิทธิภาพ และความเข้มข้นของน้ำมันหอมระเหยของสมุนไพรพื้นบ้านที่เหมาะสมในการกำจัดระยะหนอนและระยะตัวเต็มวัยของ *M. domestica* สายพันธุ์ห้องปฏิบัติการ และระยะตัวเต็มวัย *Stomoxys indicus* (Picard) ของพื้นที่ฟาร์ม 3) สำรวจแบคทีเรียก่อโรค *Escherichia coli* ที่ติดแมลงวันตอมสิ่งปฏิกูลจากตลาดท้องถิ่นของอำเภอหาดใหญ่ จังหวัดสงขลา

สำหรับจุดประสงค์ในการวิจัยแรก ได้ทำการทดลองโดยออกแบบการทดลองเป็นแบบสุ่มสมบูรณ์เพื่อเปรียบเทียบหนอนที่เลี้ยงในอาหารที่ 1ของเสียจากการประมง - กระดูกหัวของปลากะพงขาว, *Lates calcarifer* (Bloch) และอาหารที่ 2 อาหารแมวแบบเปียกกับอาหารมาตรฐานของ *M. Domestica* เป็นตัวควบคุมเชิงบวก สำหรับอาหารแต่ละชนิดทำซ้ำ 10 ซ้ำ หลังจากผ่านไป 5 วัน ของอาหารในแต่ละซ้ำทำการสุ่มเลือกหนอนระยะที่ 3 จำนวน 10 ตัวเพื่อการชั่งน้ำหนัก และสุ่มตัวหนอน 150 ตัว นำไปวิเคราะห์องค์ประกอบทางโภชนะ และเปรียบเทียบข้อมูลทางสถิติด้วยการวิเคราะห์ความแปรปรวน One-way ANOVA กับ post-hoc Tukey HSD (Honestly Significant Difference) พบว่า น้ำหนักเฉลี่ยของของหนอนที่เลี้ยงในอาหารที่ 1 (0.41 กรัม/ตัวหนอน) สูงกว่าอาหารที่ 2 (0.247 กรัม/ตัวหนอน) และอาหารมาตรฐานอย่างมีนัยสำคัญ

(0.253 ก./ตัวหนอน) นอกจากนี้ เปอร์เซ็นต์ไขมันหยาบของตัวหนอนในอาหารที่ 1 มีค่าสูงอย่างมีนัยสำคัญไม่แตกต่างกับตัวหนอนในอาหารมาตรฐาน แนะนำได้ว่าอาหารที่ 1 เป็นตัวเลือกเพิ่มเติมของอาหารเลี้ยงหนอน *M. domestica*

วัตถุประสงค์ที่สอง เพื่อศึกษาประสิทธิภาพและความเข้มข้นในฤทธิ์ฆ่าแมลงของน้ำมันหอมระเหยพื้นบ้านของไทย (กานพลู (*Syzygium aromaticum* (L.) Merr. & Perry), เทพทาโร (*Cinnamomum porrectum* Kosterm) และตะไคร้ต้น (*Litsea cubeba* Pers)) ต่อระยะหนอน และระยะตัวเต็มวัยของแมลงวันบ้าน *M. domestica* และระยะตัวเต็มวัยของแมลงวันคอกสัตว์ (*Stomoxys indicus*) ที่จับจากธรรมชาติ ทดสอบประสิทธิภาพการกำจัดระยะหนอนด้วย Dipping assays และทดสอบแมลงวันทั้งสองชนิดในระยะตัวเต็มวัยด้วยกรวยทดสอบ World Health Organization cone bioassay) ใช้เพื่อ ใช้สาร Cypermethrin เป็นชุดควบคุมเชิงบวกและเอทิลแอลกอฮอล์เป็นตัวทำละลายและใช้เป็นตัวควบคุมเชิงลบ ผลการทดลองพบว่าระยะหนอนของแมลงวันบ้าน (*M. domestica*) พบว่าน้ำมันหอมระเหยทั้ง 3 ชนิดที่ความเข้มข้น 10%v/v ให้เปอร์เซ็นต์การสลบ (KD) และอัตราการตายสูง ความเข้มข้นต่ำสุดของเทพทาโรที่ 6.134%v/v มีประสิทธิภาพในการกำจัดหนอนแมลงวันบ้านมากที่สุด สำหรับประสิทธิภาพการกำจัดระยะตัวเต็มวัยของ *M. domestica* คะไคร้ต้นที่ความเข้มข้น 5% v/v ให้เปอร์เซ็นต์การสลบ 100% อัตราการตาย 93.33% และค่า LC_{50} ที่ 3.82% สำหรับประสิทธิภาพการกำจัดตัวเต็มวัยของ *S. indicus* พบว่าน้ำมันกานพลูให้ค่า LC_{50} สูงสุดที่ 0.284%

การสำรวจแบคทีเรียก่อโรค *E. coli* ที่ติดบริเวณลำตัวของแมลงวันต่อสิ่งปฏิกูลจากตลาดในท้องถิ่นของอำเภอหาดใหญ่ จังหวัดสงขลา พบว่าแมลงวัน *M. domestica*, *M. autumnalis* และ *M. crassirostris* ซึ่งเป็นแมลงวันในวงศ์ Muscidae ให้ผลการทดสอบ *E. coli* เป็นบวก ดังนั้น การศึกษานี้จึงสรุปได้ว่าแมลงวันมีความสามารถในการเป็นพาหะนำโรคเชิงกลของเชื้อ *E. coli* เข้าสู่มนุษย์ได้

การค้นพบนี้ในการศึกษานี้ชี้ให้เห็นว่าระยะตัวหนอนของแมลงวันบ้านสามารถนำมาใช้ในการย่อยสลายขยะทางชีวภาพที่เกิดจากการทำประมงได้ น้ำมันจากเทพทาโรและน้ำมันจากตะไคร้ต้นสามารถใช้ในการควบคุมตัวหนอนและตัวเต็มวัย *M. domestica* ตามลำดับ ในขณะที่น้ำมันกานพลูมีประสิทธิภาพสูงสุดในการควบคุมตัวเต็มวัยแมลงวันคอกสัตว์ *S. indicus* แมลงวันต่อมสิ่งปฏิกูลในตลาดท้องถิ่นของอำเภอหาดใหญ่ จังหวัดสงขลา พบการปนเปื้อนเชื้อ *E. coli* แม้จะยังไม่มีการแยกสายพันธุ์ของแบคทีเรีย

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ABSTRACT

The house fly, *Musca domestica* L. (Diptera: Muscidae) is an important insect pest that lives close to humans and can be medically significant as mechanical vectors of different pathogens from unsanitary places to human food. Objectives of this study were to 1) compare larval weight rate and larval nutritionally component of house fly after feeding three different types of larval diet under laboratory conditions 2) determine the insecticidal efficacy and optimal discriminating lethal concentration of Thai essential oils against laboratory strains of *M. domestica* (larval and adult stages) and adult field population of *Stomoxys indicus* (Picard), and 3) survey the pathogenic bacteria, *Escherichia coli* which infected in the filth flies from local markets of Hat Yai, Songkhla Province.

For the primary objective, a completely randomized design were performed to compare treatments which included Diet 1: fishery waste - head bone of sea bass, *Lates calcarifer* (Bloch) and Diet 2: wet commercial cat food to standard Diet of *M. musca domestica* as a positive control with 10 repetitions for each diet. After 5-d period in each replicate, ten 3rd instar larvae were randomly selected for weight measurement and 150 dried larvae were used for analysis of nutritional composition. By using One-way ANOVA with post-hoc Tukey HSD (Honestly Significant Difference) Test, the mean weights of larvae feeding in Diet 1 (0.41 g/larva) was significantly higher than Diet 2 (0.247 g/larva) and standard Diet (0.253 g/larva). Additionally, percentage of crude fat of larvae in Diet 1 was significant high as larvae in standard Diet, suggested that Diet 1 was an additional option *M. domestica* diet.

The secondary objective was to determine the insecticidal efficacy of native Thai essential oils (clove (*Syzygium aromaticum* (L.) Merr. & Perry), citronella laurel (*Cinnamomum porrectum* Kosterm), and pheasant pepper tree (*Litsea cubeba* Pers)) against larvae and adults of *M. domestica*, and wild-caught adult of stable fly, *Stomoxys indicus*. Dipping assays and the World Health Organization cone bioassay system were performed for the larvicidal and adulticidal activities. Cypermethrin and ethyl alcohol were used for the positive and negative control, respectively. Result of larvicidal bioassay showed that 10%v/v of three essential oils gave high percentage of knockdown (KD) and mortality. The lowest concentration of citronella laurel at 6.134%v/v produced the most effective larvicidal activity. For the adulticidal activity of *M. domestica*, 5% v/v of pheasant pepper tree gave the highest in 100% KD, 93.33% mortality, and LC_{50} of 3.82%. For adulticide activity on *S. indicus*, clove oil gave the highest LC_{50} value at 0.284%.

A survey of the pathogenic bacteria, *E. coli* infected in filth flies from local markets in Hat Yai District, Songkhla Province exhibited the positive result for *M. domestica*, *M. autumnalis*, and *M. crassirostris* in family Muscidae. So, this study confirmed that muscid flies are a mechanical transmitter of coliform bacteria and *E. coli* to humans.

These findings in this study suggested that larvae of house flies could be used for biodegradation of fishery waste. Citronella laurel and pheasant pepper tree oil could be used for larval and adult *M. domestica* control, respectively, while clove oil was the most efficacy to control adult *S. indicus*. Muscid flies in local market of Hat Yai District, Songkhla Province were contaminated with *E. coli* despite having no differentiating bacterial species.

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LIST OF ABBREVIATIONS

GIS = Geographic Information System

LC_{50} = Median letal concentrantion

LT_{50} = Median letal time

GPS = Global Positioning System

WHO = World Health Organization

% RH = Relative humidity

SEM = Standard error of mean

CHAPTER I

INTRODUCTION

1.1 Research background

Food security is a multi-dimensional phenomenon including national and international political actions which requires the adoption of single and basic indicators for policy analysis in each country throughout the world. Since livestock production and aquaculture are the large components of agriculture in southern Thailand. Meat and seafood are delivered by platform operational in the cities across the country through the meat selling market. These markets are commonly found in Southeast Asian countries and Thailand and could be a source of food for people and also non-target dipteran insect vector of pathogens.

Agricultural waste, especially the waste from fishery waste is generating primary waste management challenges for cities along the Gulf of Thailand and the Andaman Sea coast which negative impact was highly contributing to the environment and economical society (Papargyropoulou et al., 2014). Fishery waste is found in over 20 million tons or 25% of fisheries around the world. These quantities include wastes from fish processing and aquacultures (Kim and Mendis, 2006; Rustad et al., 2011). This waste is different from other organic wastes as fish waste contains fatty acids, polyunsaturated (PUFAs). Therefore, the strategy of using fish waste as a food precursor is optimal. Waste treatment facilities are impractical waste to invest in recycling, waste prevention programs, and creating awareness. The fly larvae have the potential to reduce agricultural waste on livestock and aquaculture facilities and produce an animal-grade feedstuff high in protein and nutritional components. The nutritional content in the larval stage of a fly is mainly dependent on its diet (Stamer, 2015).

Numerous patents for house fly production exist in China, but performances have not been published and they are often not translated into English (Kenis et al. 2018). Moreover, information on house fly rearing is limited and the use

of house fly larvae for beneficial purposes is the narrow view in Thailand. Most extensively studies of using fly larvae for processing of organic waste on black soldier fly (*Hermetia illucens* L.) (Burana and Jamjanya 2011, Timcharoensombut et al. 2018, Jaichansukkit et al. 2020). Only one literature was documented nationally comparing shrimp heads and crab shell wastes mixed with rape fruit for house fly rearing for chicken feed (ORDPB 2013).

The house fly, *Musca domestica* L., (Diptera: Muscidae) is synanthropic fly species found worldwide and is medically significant as mechanical vector of different pathogens from unsanitary locations to food, resulting in diseases in humans. It has been reported to transmit such as *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus coagulase*, *Vibrio cholerae*, *Salmonella* spp., *Shigella* spp. and *Campylobacter* spp, and so on (Chaiwong et al., 2014). Most flies have occurred periodically, and the density of flies is somehow related to the increase of human diseases, especially dysentery. Recently acute diarrheal diseases were reported and accounted for 26% of the digestive diseases (foodborne infections in humans) in the 2010 Thai nationwide data with high expenditure (Treeprasertsuk et al. 2017). The strong reason for this phenomenon is that the good adaptation of disease pathogen as well as the high potential of insect vectors to survive from the control program.

This research purposes were to compare the weight rate and nutritional component of the house fly larval from standard diet, fishery waste diet, and wet cat food diet under laboratory conditions. Than to determine the insecticidal efficacy and optimal discriminating lethal concentration of Thai essential oils against laboratory larvae and adult *M. domestica* and adult field population of *Stomoxys indicus* (Picard). This could be served and promoted food security and less chemical residual than the synthetic compounds. Furthermore, the flies from local markets of Hat Yai District, Songkhla Province was surveyed the pathogenic bacteria, *Escherichia coli*.

1.2 Literature review

1.2.1 House fly (*Musca domestica*)

House fly is a member of Family Muscidae in subfamily Muscinae, normally lives in close association with people around the world. This insect feed on human foodstuffs and wastes where they can pick up and transport various disease agents. The house fly has a complete metamorphosis with distinct egg, larval or maggot, pupal, and adult stages. Depending on the temperature, the egg takes from 6 to 42 days to develop into the adult stage. The length of life is usually around 2–3 weeks but in the cooler temperature, adults fly may be until three months. Female flies usually laid eggs in masses on organic material such as manure and garbage. Hatching occurs within 2-3 hours. The larvae burrow into the breeding material and they can survive only where sufficient fresh air is available. When the breeding material is very wet, the larvae can live only on the surface, whereas in drier materials they may penetrate to a depth of several centimeters. The most larvae of this species are slender, white color, legless maggots that develop rapidly, passing through three instars. The time required for development diverges from at least three days to several weeks, depending on the species including the temperature, type, and quantity of the medium available. Before the larvae developed into pupal stage, the 3rd larvae migrate to a drier place and hided under objects offer protection. the puparium was a capsule-like case, within which the transformation from larva to adult stage. This stage usually takes time around 2–10 days, at the end of which the fly pushes open the top of the case find the way out and come up to the surface. The adult fly is grey, 6–9mm long and has four dark stripes running lengthwise on the back. Soon after emergence, the fly spreads wings, and the body dries and hardens as presented in Figure 1(Keiding. 1986). A few days go by before the adult is capable of reproduction. Under natural conditions, an adult female rarely lays eggs more than five times, and hardly lays more than 120–130 eggs on each occasion.

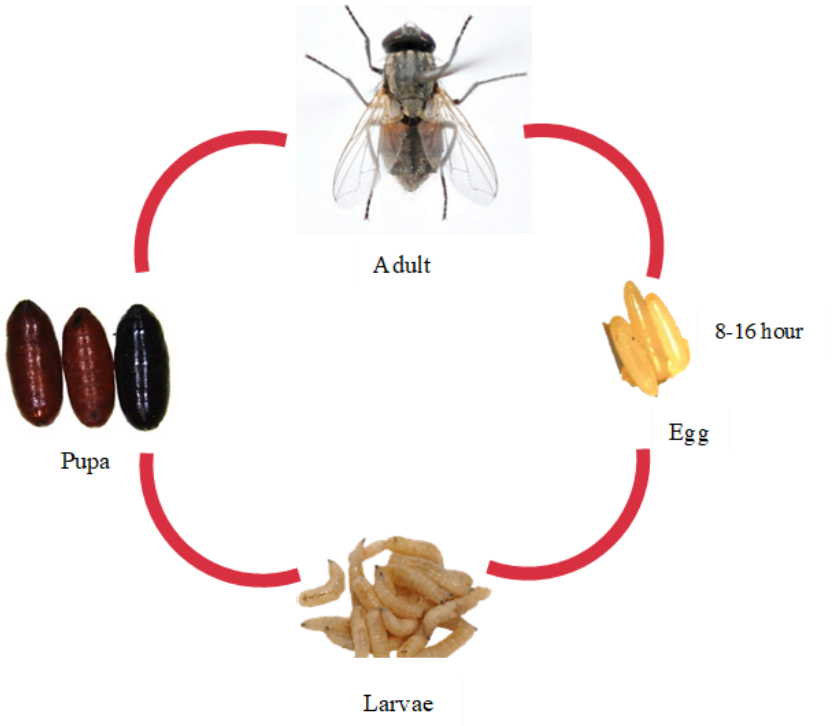


Figure 1 The life cycle of the house fly, *Musca domestica* Linnaeus (Warin, 2021).

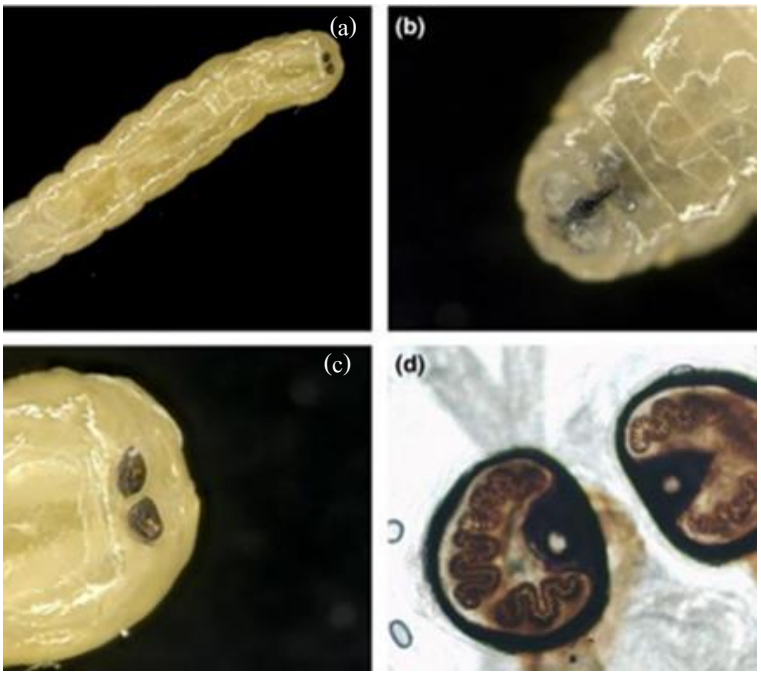


Figure 2 The whole larva of *Musca domestica* (a), 9 mm body length; (b) pair of hooks on the anterior segment; (c) respiratory stigmata of the larva; (d) microscopic detail of the respiratory stigmata. (Simon et al. 2018).

1.2.2. Stable flies (*Stomoxys* spp.)

The stable fly (*Stomoxys* spp.) was a filth flies species of vicious blood-sucking fly belonging to the Family Muscidae in subfamily Stomoxyinae, contains with eighteen species worldwide (Zumpt, 1973). Stable flies are about the same size, color, and dark longitudinal lines on the thorax as house flies but different in mouthpart (Figure 3). Adult both sex of stable fly are obligate blood feeder and primarily attack cattle for a blood meal. When the stable fly attack, that can cause pain, irritation, and annoyance to the livestock. In addition, the feeding cause affects to lose the blood, weight and transferred important disease suck as Trypanosomiasis and Anaplasmosis. *Stomoxys calcitrans* L. was a species that can be found worldwide. Masmeatathip et al. (2006) were the first report about morphological studies of *Stomoxys* spp. by using Vavoua traps and sweeping net in the Central part of Thailand. The resulted show that the 4 *Stomoxys* species were found, *S. calcitrans* (L.) was the most abundant and commonly found in all locations, followed by *S. sitiens* Rondani, *S. indica* Picard, and *S. bengalensis* Picard, respectively. In 2012, Keawrayup et al. revealed a study of species diversity of *Stomoxys* spp. and diurnal variations of activity of *S. indicus* and *S. calcitrans* in Wang Nam Khiao District, Nakhon Ratchasima Province, the Northeast part of Thailand. The most abundant species be in the collection farms were *S. indicus* (50.2%) and *S. calcitrans* (49.5%) followed by *S. sitiens* and *S. uruma*. The greatest higher number of flies was found in the rainy season. The latest study was reported in the Southern part of Thailand by Lorn et al. (2020). The result showed that the most common species was *S. calcitrans* (L.) with 2,512 specimens (87.43%), followed by *S. indicus* with 306 specimens (10.65%), *S. sitiens* with 44 specimens (1.53%), and *S. uruma* with 11 specimens (0.39%).

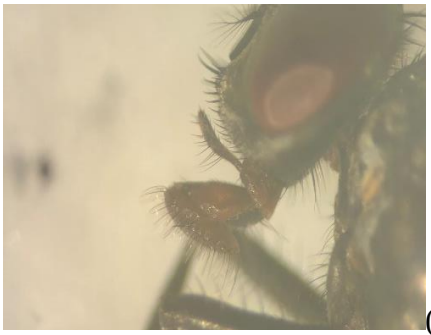

(a)

(b)

Figure 3 The mouthpart of *Stomoxys calcitrans* (a; piercing sucking type) and *Musca domestica* (b; sponging type) (Warin, 2021).

1.2.3 Prevalence of house flies in Thailand

There is a lack of study of *M. domestica* in Thailand and most studies present in the North, Northeast and Central part of Thailand, less study in southern Thailand. The first study on the population dynamics of the wild house fly, *M. domestica* population is found in northern Thailand. Ngoen-klan et al. (2011) revealed the study on investigation the climatic and physical factors affecting the population trend of the house fly, *M. domestica* in Chiang Mai Province, northern Thailand by using the Geographic Information System (GIS). Fresh beef viscera bait was used for luring *M. domestica* in 18 study sites. Out of 63,158 flies were captured, only 1.3% was *M. domestica* and the rest was *Chrysomya megacephala*. A significantly higher number of females than males was found in both species. Fly populations can be collected throughout most of the year with a peak in late summer, which shows a positive relation to temperature but a negative correlation with relative humidity. The density of house fly was associated with altitude and land use types.

Then, in 2014 the prevalence of the *M. domestica* was reported in Northeast Thailand by Chaiwong et al. (2014b) regarding determining five types of human habitations including fresh-food markets, garbage piles, restaurants, school cafeterias and paddy fields, in the Muang Ubon Ratchathani and Warinchamrap Districts of Ubon Ratchathani Province. Flies collections were conducted using a reconstructable funnel trap, containing 1 day-tainted beef offal as bait. A 1,349 specimens of *M. domestica* were collected from a total of 7,750 flies (744 specimens

at Muang Ubon Ratchathani and 605 specimens at Warinchamrap). The highest number was found in restaurants at Muang Ubon Ratchathani (391 specimens) and in fresh-food markets at Warinchamrap (296 specimens).

A recent report presents by Klong-klaew et al. (2020), study about the daily and seasonal variation of muscid flies (Diptera: Muscidae) in Chiang Mai Province, Northern Thailand. Sampling specimen by using semiautomatic traps and 1-day old beef offal as bait, twice per month for one year. A total of 3,419 specimens were caught, the most of sample was *M. domestica* at 1,329 specimens that as a 38.9% followed by *Hydrotaea spinigera* (770 specimens; 22.5%) and *Musca ventrose* (740 specimens; 21.7%). Also, this study reported peak activity time during the day of captured flies: *H. spinigera* peak at 6.00 to 9.00 hour in the early morning, *M. domestica* peak at 9.00 to 12.00 hour in the last morning and *M. ventrosa* peak at 12.00 to 15.00 hour in the afternoon.

1.2.4 Bacterial pathogen detection

Musca domestica is the potential carrier of more than 65 animal and human intestinal diseases and it was responsible for protozoan (amoebic dysentery), bacterial (salmonellosis, cholera, and shigellosis) and helminthic (hookworms, roundworms, pinworms, and tapeworms) infections as well as viral and rickettsia infections. Sukontason et al. (2000) were initially determined the potential of the *M. domestica* in order to be a mechanical carrier of bacteria in urban areas of Chiang Mai Province, northern Thailand. Sixty-one *M. domestica* specimens were found to carry bacteria. Each specimens harbored 1-5 bacteria. No significant difference between the sexes of flies for carrying bacteria was found. A total of 21 bacteria was isolated, of which the most common were coagulase-negative staphylococci (n = 38), followed by *Viridans streptococci* (n = 9). After that, Sukontason et al. (2007) reported a study an evaluation between the oriental latrine fly, *C. megacephala*, and the common house fly, *M. domestica*, for efficiency be as the potential carriers of bacteria in urban areas of Chiang Mai Province, the northern part of Thailand. The most common bacterium isolated from *M. domestica* was coagulase-negative staphylococci (n=57) followed by *Viridans streptococci* (n=10) and *E. coli* (n=10), though that *C. megacephala* was non-

fermentative gram-negative bacilli (n=59) followed by coagulase-negative staphylococci (n=54).

Chaiwong et al. (2014a) tested the antimicrobial activity against *Enterococcus* spp. isolated from *C. megacephala* and *M. domestica* by standard disk diffusion and minimal inhibitory concentration (MIC), and this study was to determine the potential of *M. domestica* and *C. megacephala* to transferred multi-drug resistant enterococcus to humans. Seven hundred specimens were collected from restaurants, fresh-food markets, garbage piles, rice paddy fields, and school cafeterias in Muang Ubon Ratchathani and Warinchamrap in Ubon Ratchathani Province. The study revealed that the potential of *M. domestica* and *C. megacephala* to carry *Enterococcus* spp Also in the Northeast Thailand, Chaiwong et al. (2014b) reported that *M. domestica* could be a potential mechanical vector of bacterial pathogens associated with human habitations year-round in this region of Northeast Thailand. The potential of these flies to carry pathogenic bacteria was examined. Bacteria were isolated from 439 *M. domestica* collected using a sweep net. Human pathogenic enteric bacteria isolated were *Escherichia coli* O157: H7, *Salmonella* sp., *Shigella* sp., *Salmonella typhi*, *Bacillus* sp., and *Enterococcus* sp.

Fukuda et al. (2017) described the role of *M. domestica* in spreading the bacterium *Stenotrophomonas maltophilia*. This is a bacterium capable of surviving in a wide variety of environments and is considered to be among the antimicrobial-resistant bacteria of greatest public health concern in hospital settings. A fly from the 15 locations (9 animal farms and 6 urban areas) in Thailand was collected. The results suggest that *M. domestica* ingest and host *S. maltophilia* from several different environmental sources. This finding suggested that *M. domestica* may facilitate the spread of antimicrobial-resistant *S. maltophilia* from environmental sources to humans.

1.2.5 *Escherichia coli*

Escherichia coli is a non-spore-forming, Gram-negative bacterium, usually motile by peritrichous flagella. The pathogenic *E. coli* strain may be responsible for foodborne infections in humans (Andrea Vásquez-García et al, 2019). Cohen et al. (1991) reported the effect of control of *M. domestica* on the incidence of diarrhea and

shigellosis at two military field bases at where?. In early summer, 1988, intensive fly control measures (mainly bait and trap strategy) were introduced. Alongside, clinic visits were dropped by 42% for diarrhoeal diseases and by 85% for shigellosis. These findings indicate that *M. domestica* acting as mechanical vectors, transmit *Shigella* (and possibly enterotoxigenic *E. coli*) diarrhoeal infections.

The persistence of *E. coli* in artificially fed larvae (Rochon et al. 2004) and pupae (Rochon et al. 2005) for up to 48 hours after ingestion by house flies, *M. domestica* and stable flies, *Stomoxys calcitrans* were evaluated. The *E. coli* load in *M. domestica* larvae and pupae increased when larvae were fed a low concentration of bacteria, but it declined when larvae were fed a high concentration of bacteria. These observations suggest that *M. domestica* larvae digest *E. coli* and use it as a food source but stable fly larvae do not. However, pupae of both species have the potential to act as reservoirs for *E. coli*. Petridis et al. (2006) reported that *M. domestica* gut may provide a favorable environment for the evolution and emergence of pathogenic bacterial strains (*E. coli*) through the gaining of antibiotic resistance genes or virulence factors.

1.2.6 Fishery production in Thailand

Aquaculture in Thailand plays an important role in food security and the economy. Freshwater aquaculture is mainly for domestic consumption globally. In 2003, the Food and Agriculture Organization (FAO) of the United Nations reported that Thailand has a total production from freshwater and brackish water aquaculture of approximately 0.32 and 0.45 million tones, respectively. Nile tilapia (Oreochromis niloticus), hybrid catfish (Clarias macrocephalus X C. gariepinus), and silver barb (Barbodes gonionotus) were the main freshwater species cultured.

In 2017, also FAO reported that fishery production was placed at 2.4 million tons and showed total marine waters and inland waters was 1.3 and 0.2 million tons, respectively. Fish and fishery products remain an important commodity as the main export products of Thailand's trade. The main export destinations of Thailand are the United States, Japan, and the European Union (FAO, 2017). There is no report for waste from fishery production (leftover) in Thailand but only the waste of

water which is used for aquaculture processing (Pollution Control Department: https://www.pcd.go.th/publication/4279/).

1.2.7 Benefit from house fly

Wang et al. (2013) studied zero waste swine manure management using *M. domestica* larvae. The bioconversion process produced a fresh larvae yield of 95-120 kg m^3 of fresh raw manure. This procedure presented an alternative animal foodstuff (giving 56.9 and 23.8% protein and total fat as dry matter, respectively), in addition captured nutrients for agricultural re-utilization. Bioconversion was reduced scent emanation (characterized by 3-methylindole) and reduced the *E. coli* index by 94.5 and 92.0%, respectively. Yearly profit under this interval assessment ranged from US$33.4-46.1 per m^3. It has been determined that the conversion bioconversion technology of the swine manure larvae with subsequent production of value-added biological products could be an encouraging avenue when considering a waste reduction program products in an comprehensive animal production system.

Ezewudo et al. (2015) researched about production and utilization of *M. domestica* maggots in the diet of *Oreochromis niloticus* L. fingerlings. The maggot meal was processed by oven-drying and grinding into powdery form. The fingerling dites were prepared into seven compounded isonitrogenous diets by mixed fishmeal with maggot meal at difference percent: 20%, 30%, 40%, 50%, 60% 70%, and 80% of maggot meal. Fishmeal (0% of maggot meal) was used as a control diet. The results showed the highest mean weight gain, relative growth rate and specific growth rate at 50% maggot meal diet and highest protein efficiency ratio at 60% maggot meal diet. *Oreochromis niloticus* fingerlings showed a completely 100% of survival rate in all maggot fish meal diets. These results could be represented that *M. domestica* maggot possibly used to be an ingredient for diets in the fish farming industry.

1.2.8 Fly control

Mechanical control

Hung et al. (2015) hypothesized that odors from the sugary excreta produced, honeydew by sucking insects feed on economic crops, or molds that were growing on honeydew, might attract house flies, therefore increasing the risk of food crop contamination. House flies were attracted by the combinations following between plant, pest, and honeydew: citrus mealybug on grapefruit leaves, whitefly on navel orange, squash fruit, and pea aphid on faba bean plants, and cottony cushion scale on mandarin orange leaves and combined citrus mealybug. The fungi related to honeydew's field-collected were identified and isolated as possible emitters of volatiles attractive to house flies for further study such as *Aureobasidium pullulans*, and *Cladosporium cladosporioides*. Both of fungal species were grown in potato dextrose enrichment broth and adult house fly attracted to volatiles from those fungal cultures was evaluated. Adults house fly were attracted to scent from *A. pullulans* cultures but not to those of *C. cladosporioides*. Identification of specific honeydew scent that are attractive to house flies could be beneficial for the development of improved house fly baits for the management of this fly species.

In Thailand, Upakut et al. (2017) researched the behavioral response of house fly, *M. domestica* L. (Diptera: Muscidae) to natural products in Northern, Chiang Mai Province. *Musca domestica* response test by using a dual-choice wind tunnel (T-box). T-box used to compare the three most attractive products for house fly (First - fresh beef viscera, second - ripe banana, third - fresh beef liver) against each other. The results show the most attractive product that house flies preferred was the fresh beef viscera when compared with the other products. That information of this result could confirm fresh beef viscera as an optimum natural product for attracting adult house fly.

Biological control

The bacterial *Bacillus thuringiensis* (*Bt*) has been reported to use as a microbial *M. domestica* control agent in one of the natural maggot breeding methods, namely poultry feces. Two commercial constructions, wettable powder, and a liquid concentrate were examined against *M. domestica* sorbens in synthetic fly breeding media and chicken feces. The conclude was found to favor maggot breeding when associated with an artificial one. Both formulations resulted in a significant reduction in maggot numbers, pupal development, and adult emergence. Contaminated feces produce from verbally fed chickens were found considerably toxic to the breeding

maggots, reaching maximum larvicidal activity till the 4th day post-feeding feces, and continued in less activity till the 6th day. Adding. chicken food in poultry mass breeding was suggested for *M. domestica* control (Labib and Rady 2001).

The efficacy of the pupal parasitoid *Spalangia cameroni* Perkins as a biological control against house flies *M. domestica* and stable flies *Stomoxys calcitrans* was tested by Skovgård and Nachman (2007) in one dairy cattle and two pig installations in Denmark. *Spalangia cameroni* was released in April through to September–October in 1999 and 2000, result showthat the population of house flies dropped below the nuisance level.

The pathogenic potential of five indigenous Beauveria species was assessed against *M. domestica* adults and larvae in laboratory bioassays test. An isolates Beauveria, *Beauveria bassiana* HQ917687 exhibited the highest virulence to larvae and adults of *M. domestica* mortality with 72.3 and 100 % respectively. The results obtained in this reported depict the significance of appropriate strain selection and process parameter optimization to facilitate the mass production of biocontrol agents (Mishra and Malik 2012). Furthermore, other entomopathogenic fungi have been reported such as *Beauveria bassiana* and *Metarhizium anisopliae* used to control *M. domestica* population (Acharya et al. 2014)

Insecticide

In 1953, an insecticide dieldrin-treated portable frame tied with string has been developed for improving the chance for *M. domestica* to contact the insecticide residue. The residual effect for two-and-a-half months was seen. As the frame method is cheap and effective for *M. domestica* control, it can be introduced into any community which has unsatisfactory sanitation and where the house fly is still susceptible to insecticide (Chow and Thevasagayam 1953). Phosphoric esters (Pino 1955) and insecticide-treated cords (Kilpatrick and Schoof 1956) were evaluated in the control of the house fly.

North America, pyrethroid insecticide effectiveness could be affected by exposure to direct sunlight and the rate of photodegradation. Insecticide-treated netting (ITN) was obtaining new applications in economic crop and livestock production systems. Baseline research using long-duration no-choice assays has been carried out to gauge the usefulness of ITN treated with beta-cyfluthrin, lambda-

cyhalothrin, and bifenthrin. After 12 weeks in direct daylight, ITN treated with beta-cyfluthrin was still highly insecticidal to house flies, producing 100% mortality in petri dish assays. However, daylight reduced the insecticidal activity of lambda-cyhalothrin, with 50% of house flies surviving after exposure that had been employed for long 10 weeks. The insecticidal activity was greatly decreased on bifenthrin-treated netting, with 50% of house flies surviving in that test with netting applied for only 3 weeks. Along with sensible choice of the pyrethroid applied, considered netting could be an important component of livestock integrated pest management programs concentrated on sustainable practices (Peck et al. 2014).

Plant-derived pesticides

The essential oil of *Cymbopogon citratus* and its major components were evaluated for control of the *M. domestica* fly field population. Assay of oil against house fly larvae and pupae through contact toxicity assay showed median lethal concentration (LC_{50}) value of 0.41 ml/cm^2 and percentage inhibition rate (PIR) of 77.3 %, respectively. Fumigation assay was comparatively more effective with LC_{50} of 48.6 ml/L against house fly larvae, and a PIR value of 100 % against house fly pupae. The study demonstrates the potentiality of *C. citratus* oil as an excellent insecticide for house fly control, and the results open up the opportunity of oil/monoterpenes being developed into an eco-friendly, economical, and acceptable product (Kumar et al. 2013). A year later, a PEG-Mentha oil nanoparticle was characterized for house fly control (Kumar et al. 2014).

Sinthusiri and Soonwera (2014) demonstrated the ovicidal and oviposition prevention of seven herbal essential oils derived from *Mentha piperita*, *Cymbopogon citratus*, *Citrus sinensis*, *Eucalyptus glubulus*, *Lavandula angustifolia*, *Zingiber cussumunar*, and *Illicium verum* against the gravid female of *M. domestica* under laboratory conditions and associated with commercial insecticide (10% w/v cypermethrin). Sinthusiri and Soonwera were evaluated at three concentrations suck as 1.0, 5.0, and 10.0%. High concentrations of herbal essential oils showed high percent effective repellency (ER). The 10% *I. verum* oil caused completed oviposition deterrence (100% ER, oviposition activity index (OAI) = -1 and this oil at same high concentration gave the maximum inhibiting rate at 97.3% (LC_{50} value of 6.85%). On

the contrary, cypermethrin 10% w/v showed complete oviposition deterrence (100% ER, OAI = -1.0) and ovicidal activity (100% inhibiting rate). These results showed that *I. verum* oil has a high potential for oviposition deterrence and ovicide *M. domestica* control.

Insecticide susceptibility test

The development of chemical control of *M. domestica* on Danish farms from 1945 to 1972 is summarized. Pesticides are heavily influenced by consecutive development of resistance and failure to control by other insecticides after another. The chlorinated hydrocarbons used as residual sprays were unsuccessful from 1947 to 1951. Organophosphorus compounds (OPC) have been widely used since 1953, starting as strips impregnated with parathion and residual sprays with diazinon. The first resistance to Organophosphorus compounds was found in 1955, diazinon was canceled in 1957-1959 and the parathion belt failed in the early 1960s. Trichlorfon paint-on baits were widely used from 1958 to 64 and there was no effective resistance appearing until 1967, caused by the selective pressure of fenthion and dimethoate used as the residual sprays. At present, everywhere in Denmark there occurs a high resistance to the contact effect of trichlorfon exposure. However, trichlorfon baits can also kill many flies. Residual sprays containing fenthion, ronnel, and fenitrothion were used to some scope from 1960 to 1970, but increased resistance reduced the residual effect developed in 2--3 years. Dimethoate was used on most farms from 1965 to 1972. It was very effective in the earliest years and resistance slowly escalated until 1971 and 1972, when severe dimethoate resistance became common on Danish farms. This was associated with high resistance to other Organophosphorus compounds for fly control, for example, fenthion, fenitrothion, bromophos, and tetrachlorvinphos, and to carbamates. As a result, there were no available effective residual sprays for general. In the years 1971 to 1972, synergized pyrethroids have been tried with frequent treatments. However, the method is often expensive, and there are serious problems resistance has occurred on a few farms. In this situation preventive and sanitary measures to eliminate or reduce the reproduction of fly breeding in manure would be becoming decisive again, but difficult to implement due to lack of farm labor. Danish’s extreme situation is compared to other situation and probable reasons for differences in resistance and control issues are

discussed, along with the possibility of strategies to reduce development resistance (Keiding 1975).

The first evidence for studying the insecticide susceptibility of *M. domestica* in Thailand was reported by Sullivan et al. (1972). This study was examined the insecticide susceptibility of *M. domestica* vicina Macquart. The study was conducted during 1969-1970 in eight provinces of Thailand-Phra Nakhon (Bangkok), Chon Buri, Nakhon Ratchasima, Trat, Samut Songkhram, Nakhon Sawan, Udonthani and Chiang Rai. F1 generation female flies were tested for tolerance to DDT, lindane, and malathion using an insecticide-treated jar assay. Lindane resistance was found to be widespread. In most areas, house fly populations were susceptible to DDT and malathion.

The pyrethroid insecticide susceptibility of *M. domestica* was studied in Chiang Mai Province. The field strains were collected from Muang District, two agricultural areas of Hang Dong District and San Kamphaeng District. These strains displayed susceptibility to both permethrin and deltamethrin insecticides (Sukontason et al. 2005). The Modification of carboxylesterase activity was observed to determine malathion and diazinon, organophosphate (OP) insecticides resistance in *M. domestica* (Taskin et al. 2004).

Six insecticides suck as deltamethrin, cypermethrin, thiamethoxam, permethrin, fipronil, and chlorfenapyr susceptibility of the *M. domestica* field populations were reported in Russia. The field population was collected from livestock from the Tyumen region. According to the resistance ratio, the result showed the highest susceptibility of field population *M. domestica* to fipronil (0.80) followed by thiamethoxam (1.12), chlorfenapyr (1.28), permethrin (1.25), deltamethrin (3.07) and cypermethrin (3.66), respectively (Levchenko et al. 2019).

Lethal toxicity of the botanical insecticides

Botanical insecticide or plant-based products are produced commercially and widely used on the market, especially plant essential oils from several botanical sources, many of which are members of the family Lamiaceae. The oils generally consist of complex mixtures of monoterpenes, sesquiterpenes, and biogenetically related phenols. Examples include eugenol, the main constituent from

clove oil (*Syzygium aromaticum*) and eucalyptus oil (*Eucalyptus globulus*), 1,8-cineole from rosemary (*Rosmarinus officinale*); menthol from various species of mint (*Mentha* species); and thymol from garden thyme (*Thymus vulgaris*) (Koul et al. 2008). These results have stimulate the development of essential oil as a based herbicides for agricultural, insecticides, fungicide, and industrial applications and the consumer market, using rosemary oil, clove oil, and thyme oil as active ingredients.

In previous studies, the toxicity of essential oil against *M. domestica* has been documented (Coats et al. 1991; Rice and Coats 1994a, b). The insecticidal activity of essential oil against a variety of insects via topical and fumigant application at 24 h was demonstrated. In addition to 33 tested topically assays with *M. domestica*, thymol and pulegone that found were the most active with concentrations required to kill 50% of the insects (LC_{50}) of 29 and 39 μg/fly, respectively, while the best fumigants were citronellal, menthol and l-fenchone with LC_{50} of 2, 3.6, and 3.8 mg/dm3 (Rice and Coats 1994a, b). The fumigant toxicity of 34 essential oils against *M. domestica* has been analyzed in a 24-h exposure period, and *Mentha pulegium* essential oil proved to be the most effective fumigant insecticide in milligram / cubic decimeter (LC_{50}=4.7 mg/dm^3) (Pavela 2008). More recently, Palacios et al. (2009) have determined the LC_{50} of essential oils from edible plants, *Citrus sinensis* oil was the most effective insecticide (LC_{50}=3.9 mg/dm^3), followed by essential oils from *Citrus aurantium* (LC_{50}=4.8 mg/dm^3), and *Eucalyptus cinerea* (LC_{50}=5.5 mg/dm^3) (Palacios et al. 2009). *Cinnamomum porrectum* (Roxb.) Kosterm commonly known as citronella laurel, safrole laurel, and Thep-Ta-Ro (in Thai) is found in Asian countries and used in traditional Thai medicine (Nuiden et al. 2019). Similar to *Litsea cubeba* commonly known as pheasant pepper tree is a native medicinal tree in Thailand. The essential oil from those plants is well known as mosquitoes repellent but has no official evidence for an insecticidal activity to another insect vector.

The determination of specific foundation discriminating concentrations of natural or synthetic active ingredients used for vector control is an important starting point of vector populations for detection of susceptibility or resistance for performance standardized monitoring (WHO 1981). Discriminating concentrations were developed using serial concentrations of six widely used active ingredients to control insect

vectors in the study area. This was achieved using an insecticide-susceptible laboratory strain of *insect vector* that were selected from ‘population’ that were never subjected to insecticidal pressure and where resistant individuals would be extremely rare.

These present studies are interested in finding more effective essential oils to achieve effective control with low concentration in a short (30 min) period to find the possibility to use essential oils from medicinal plants that grow in a local region of Thailand, clove (*Syzygium aromaticum*), citronella laurel (*Cinnamomum porrectum*) and phasent pepper tree (*Litsea cubeba*), as an insecticidal compound against *M. domestica* and *S. indicus*.

CHAPTER II

MATERIALS AND METHODS

2.1 Part 1 Mass rearing of house flies, Musca domestica under laboratory condition with three diets for maggot feed

2.1.1 House fly colony

The 500 pupae of a laboratory colony of *M. domestica* were obtained from an established colony at the National Institute of Health, Ministry of Public Health, Thailand in 2017 and colonized in the insectary of Agricultural Innovation and Management Division, Faculty of Natural Resources, Prince of Songkla University. The pupae were first transferred to an insect cage, size 30×30×30 cm and developed into adults in three-four days. For this objective, an experiment was conducted from July 2020 to February 2021. Adults are fed with a mixture of carbohydrates (sugar), proteins (milk powder), and water ad libitum (Čičková et al. 2012, Hogsette 1992). Males and females were held together in the same cages and allowed to mate naturally. Flies were reared at ambient temperature (30–33 °C) and 59–66% relative humidity. From approximately 6 days after emergence, the oviposition substrate was prepared by containing a mixture of the 20 g commercial cat food (protein 7.0%, fat 2.0%, fiber 1.0%, moisture 87%, Whiskas®, Thailand) and 20 g rice husk (local market in Hat Yai District, Songkhla Province) in a plastic cup, size 11×11×5 cm. Female flies were allowed to oviposit for 24 hours. The eggs were collected and transferred to a plastic tray (21 cm × 33 cm × 8.5 cm) which containeda larval diet. Newly hatched and all larvae (every 12 - 15 hours) were used for the experiment. To preserve the colonization, the pupal stage was collected and placed into nylon airy fabric mesh cages for adult emergence and continued the next generation.

2.1.2 Preparation of larval diets

Plastic trays (larval feed container) were randomly allocated to the three types of larval diet: as control (milk powder standard media), Diet 1 (fishery waste media), and Diet 2 (wet cat food media) (Table 1). Based on guideline of the Ministry of Public Health, Thailand, Control (milk powder standard media) was prepared with 60 g of milk powder, 30 g of sugar, 20 g of yeast, 100 g of rice husk, and 1 L of tap water. After transferring larvae into the plastic container, 400 g of rice husk was used for covering on top. Diet 1 (Fishery waste media) was mixture of 300 g of sea bass fish waste and milk powder. The sea bass fish waste, which was discarded from processing at an aquaculture farm, Songkhla Province, was prepared by the homogenizing head bone of sea bass fish, L. calcarifer The Diet 2 (cat food media) was prepared by mixing 255 g of wet cat food (Whiskas wet food for cats 7+ years with Mackerel Whiskas®, Thailand) with 400 g of rice husk in 500 ml of tap water. All larval diets were provided at 1 Kg to each container over a 5-d period. The tap water was sprayed into the containers if the diet was too dry. The container was covered with a nylon airy fabric mesh to transparent and avoid other flies. After 2 days, the ten larvae of 3rd instar larvae were randomly selected before the prepupal stage per replication, 10 repetitions for each diet. The sample of 150 larvae were harvested and sent to the Aquatic Science and Innovative Management Division, Faculty of Natural Resources, Prince of Songkla University, for analysis of nutritional composition.

Table 1 Larval feed component (per container) for *Musca domestica* rearing.

Type of diet for maggot feed		Component (%)		Weight (Gram)	Price (Bath)
Control	milk	protein	4.20	60	13.22
		sugar	29.23		
		fat	9.74		
		sodium	56.83		
	yeast	*Saccharomyces cerevisiae*		20	8
	sugar			30	0.66
	Total			110	21.92
Diet 1	fish waste			60	Free
	yeast	*Saccharomyces cerevisiae*		20	8
	sugar			30	0.66
	total			110	8.66
Diet 2	wet cat food	protein	7.00	255	39.99
		fat	2.00		
		fiber	1.00		
		moisture	87.00		

2.1.3 Analysis of Nutritional composition

The nutritional profile of the *M. domestica* was characterized following the Association of Official Analytical Chemists (AOAC) standard procedures (AOAC, 1990):

Protein analysis:

The percent of protein was calculated as:

% Protein = $[1.4 * (V_1-V_2) * N * 6.25] / w$

V_1 = Volume of standard acid to use sample titration (ml)

V_2 = Standard acid titrated volume with blank (ml)

N = Hydrochloric acid concentration in the norm

w = Sample weight (g)

Fat analysis

The percent fat was calculated as:

% Fat = [(b-a) / w] * 100

a = Weight of aluminum cup before analysis (g)

b = Weight of aluminum cup after analysis (g)

w = Sample weight (g)

Moisture Analysis

The percent moisture was calculated as:

% Moisture = [(a-b) / w] * 100

a = Weight of sample and crucible before drying (g).

b = Weight of sample and crucible after drying (g).

w = Sample weight (g)

Ash analysis

The percent ash was calculated as:

% Ash = [(b-a) / w] x 100

a = Weight of tiled cup

b = Weight of tiled cup and sample after firing (g)

w = Sample weight (g)

2.1.4 Statistical analyses

The mean values of fresh larval weights and nutrient values were tested for normality of which was the Shapiro-Wilk test and were compared using an ANOVA at 5% of probability. The means were separated using Tukey's significant difference test when the ANOVA statistic was significant ($P<0.05$). The analysis was performed using the SPSS 10.0 for Windows software (SPSS Inc., Chicago, IL, USA). P values of less than 0.05 were considered significant.

2.2 Part 2 Botanical essential oils bioassays

2.2.1 Test populations

2.2.1.1 Laboratory strain of *Musca domestica*

Mass rearing of a laboratory strain of *Musca domestica* was described in the method of 2.1.1. For larvicidal bioassay, twenty of third instar larvae were used for testing in each concentration of three commercial essential oils with five replicates. For adulticidal bioassay, thirty adult male and female *M. domestica* (3–6 days after emergence) were prepared in a fly cup and starved for 24 hours before the biological assay test. Five adult flies were introduced in each of the WHO cone with 6 replications. The physiological contact arises for 30 minutes and transferred into a new fly holding cup.

2.2.1.2 Wild-caught *Stomoxys indicus*

Wild-caught adult stable flies were collected from Animal Science farm, Faculty of Natural Resources, Prince of Songkla University, Hat Yai Campus, Songkhla, Thailand. Since this species was deficient in standard laboratory colony. Both sexes of stable flies were captured by the sweeping net and transferred to an insect cage, size 30×30×30 cm in the insectary. The drop of honey and cotton swooped with distilled water were served for energy sources. After 24 hours, both of sex were species identification using standard morphological characters based on the taxonomic keys (Zumpt et al. 1973; Tumrasvin et al. 1978; Changbunjong et al., 2012; Baldacchino et al., 2013, Malaithong et al 2019) under the stereomicroscope in the laboratory. Thirty flies were used to test in each concentration in all essential oils.

2.2.2 Essential oils and Chemical composition analysis

Three essential oils were selected based on bibliography (Kumar et al. 2012), including clove, *Syzygium aromaticum* (L.) Merr. & Perry, citronella laurel,

Cinnamomum porrectum Kosterm., and pheasant pepper tree, *Litsea cubeba* Pers and purchased from a local company (Thai-China Flavors and Fragrances Industry co. Lto, Bangkok), except *C. porrectum* oil from Wang Theptaro - Wooden Dragon, Woodcraft products, Trang Province, Southern Thailand. Analysis of the essential oil composition was performed by using a gas chromatography/mass spectroscopy (GC/MS) detector based on a standard protocol of Pharmaceutical Laboratory Service Center (Office of Scientific Instrument and Testing: OSIT), Prince of Songkla University, Songkhla Province, Thailand. The identification of compounds in the chromatographic profiles was accomplished by comparison of the mass spectra with a library database and confirmed by comparison of retention indices with those of authentic standards or with values from the literature. The percentage composition of each essential oil was computed by the area normalization method, from GC peak areas calculated as the mean value of two injections from each oil. The technical grade of cypermethrin ((RS)-α-cyano-3-phenoxybenzyl (1RS, 3RS; 1RS, 3SR)-3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropanecarboxylate, 93.52% purity) was used as the standard synthetic pyrethroid insecticide, a positive control for all test, and obtained from Sherwood Chemicals Public Company Limited, Suan Luang, Bangkok.

2.2.3 Larvicidal bioassays

Three commercial essential oils were diluted with 99% ethyl alcohol as a solvent in three concentrations at 1%, 5%, and 10% (Table 2). The 99% ethyl alcohol was sued as negative control while cypermethrin served as a positive control. The solutions were stored at 4 °C before testing. The larvicidal bioassay was evaluated by using the dipping method (Soonwera, 2015). Twenty-third instar larvae were used for testing in each concentration of three commercial essential oils with five replicates. To perform the testing, the larvae were dipped in a petri dish (100×15 cm) containing 10 mL solution for 60 seconds and consequently transferred to a plastic cup size 11×6 cm with the larval medium. The larval medium was prepared with 0.6 g of milk powder, 0.3 g of sugar, 0.2 g of yeast, 5 g of rice husk, and 10 ml of tap water. Larval mortality was checked by using a paintbrush (size 0) to lightly touch the larvae. The larvae which

have no response were considered dead and these data were recorded after the exposure at 1, 6, 12, and 24 hours.

2.2.4 Adulticidal activity test by WHO cones test

Preliminary testing began by selecting the initial series of baseline concentrations by three concentrations (1, 3, and 5%v/v) of each essential oil (Table 2). The recommended concentration of cypermethrin (0.25%v/v) (93.52%purity) for residual treatment of resting-site for fly control (ODOC5, 2018) was prepared for positive control. The baseline concentration of each test compound was diluted for the preliminary experiment. The lowest concentration that gave complete mortality was subsequently used to make six, 2-fold dilution concentrations, six concentrations in each essential oil (0.5, 1, 2, 3, 4, 5%v/v) for laboratory *M. domestica*, and six concentrations (0.5, 1, 1.5, 2, 2.5 and 3%) for *S. indicus* field population.

The impregnated filter paper was prepared before the bioassay. Filter papers (Whatman® no. 1), 10×10 cm in size was individually impregnated with one of the following test concentrations. The filter paper was treated separately with 1 mL of test solution. A hand-held 5 mL pipette uniformly applied the 1 mL insecticide solution on the paper. Negative control papers were arranged in the same manner as absolute alcohol only. Positive control papers were arranged in the same three concentrations (1%, 3% and 5%v/v) of cypermethrin. Control papers were impregnated with absolute alcohol alone. All treated papers were air-dried for at least 24 hours before use in assays.

The tarsal contact test of *M. domestica* and *S. indicus* to essential oil was performed according to World Health Organization (WHO) cone test kits procedure (WHO 2006) with slight modifications according to Tainchum et al., (2018). Six WHO cones were placed on each impregnated filter paper. Five adults of both sexes of tested flies (*M. domestica* or *S. indicus*) were introduced to each cone and the flies were allowed to physiological contact occur in 30 min, then tested flies were transferred to a clean fly cup. Honey cotton pads were provided on the fly's cup. A number of knockdowns (KD) and mortality of fly was observed immediately after 60 min and

following 24 hours, respectively. Six replicates were derived for each experimental trial.

Table 2 The percent concentration of three essential oils for larvicidal and adulticidal bioassay of *Musca domestica* and adulticidal bioassay of *Stomoxys indicus*.

Essential oils	Concentration (% v/v)		
	Larvae *M. domestica*	Adult *M. domestica*	Adult *S. indicus*
Clove	1	0.5	0.1
		1	0.2
	5	2	0.3
		3	0.4
	10	4	0.5
		5	1
Citronella laurel	1	0.5	0.5
		1	1
	5	2	1.5
		3	2
	10	4	2.5
		5	3
Pheasant pepper tree	1	0.5	0.5
		1	1
	5	2	1.5
		3	2
	10	4	2.5
		5	3

2.2.5 Data analysis

An insecticide-susceptible laboratory population of *M. domestica* and wild-caught *S. indicus* was used to analyze. If percent mortality in control is between 5 and 20%, the final mortality of treatment is adjusted (corrected) to reflect natural death (Abbott, 1925). Mean numbers of knockdown and mortality of fly from different treatments were tested for normality of which was the Shapiro-Wilk test and were compared using an ANOVA at 5% of probability. The means were separated using Tukey's significant difference test if the ANOVA statistic was significant (P<0.05).

The mean mortality data of the six replicates per concentration (4–6 concentrations per essential oil or insecticide compound) were used to analyze the concentration–mortality response and to obtain a LT_{50} (median lethal time) and LC_{50} (median lethal concentration) for larvicidal activity in *M. domestica*.

For adulticide assay, a series of concentration-response assays to establish the lethal concentrations required to kill 50% and 99% of the test population per active ingredient. The lethal concentrations achieving 50% and 99% mortality (LC_{50} and LC_{99}, respectively with associated 95% fiducial limits) were calculated from the baseline data using maximum likelihood estimates and log-probit regression analysis. The final diagnostic concentration was double the LC_{99} derived from the susceptible test population for *M. domestica* (WHO, 2016), and *S. indicus*, field population.

2.3 Part 3 Survey the pathogenic bacteria, *Escherichia coli* infected in filth flies from three fresh markets in Songkhla

2.3.1 Study site

Based on a current high human population, three fresh selling markets in Songkhla Provinces, including Koh Mee Market, Kho Hong, Hat Yai District (7°03'10.4"N 100°30'21.2"E), Khlong Rian Market, Kho Hong, Hat Yai District (7°00'02.3"N 100°29'23.2"E), Fresh food market in Khlong La, Khlong La, Khlong Hoi Khong District (6°55'23.4"N 100°24'46.0"E), were selected as collection sites for survey the pathogenic bacteria-infected in the filth flies. There were a wide variety of products selling in the market including meat, fish vegetables and take away food. The fly collection was set in the same route for each time and the collection was finished when accessed sufficient the fly number (15-20 flies). The collection started from Faculty of Natural Resources, Prince of Songkla University to the first market (Koh Mee market) takes time about 12 minutes with 6.3 kilometers apart. Koh Mee Market is a market located in the village community of Islamic people. Most of the vendors and customers was Muslims. So, mostly a meat variety selling in the market was seafood chicken (less pork) and take away food. Then, the second market (Khlong Rian

Market) takes time about 15 minutes with 7.3 kilometers apart from the first market. Khlong Rian Market was the main fresh market of rural village and has a variety of meats. The last selling market, the fresh food market in Khlong La was 17.8 kilometers apart from the second market and takes about 24 minutes to travel. That market was the main market in the village of Khlong Hoi Khong District, close to a military station which has a variety of fresh meat, vegetable and a lot of types of takeaway food. GPS coordinate of each fresh selling market was recorded, and locations were displayed by GIS mapping procedure (Figure 4 Three fresh selling markets (red marks) for filth flies collection in Hat Yai District, Songkhla Province (printed by Geo-Informatics Center for Natural Resources and Environment, Prince of Songkla University, Thailand).).

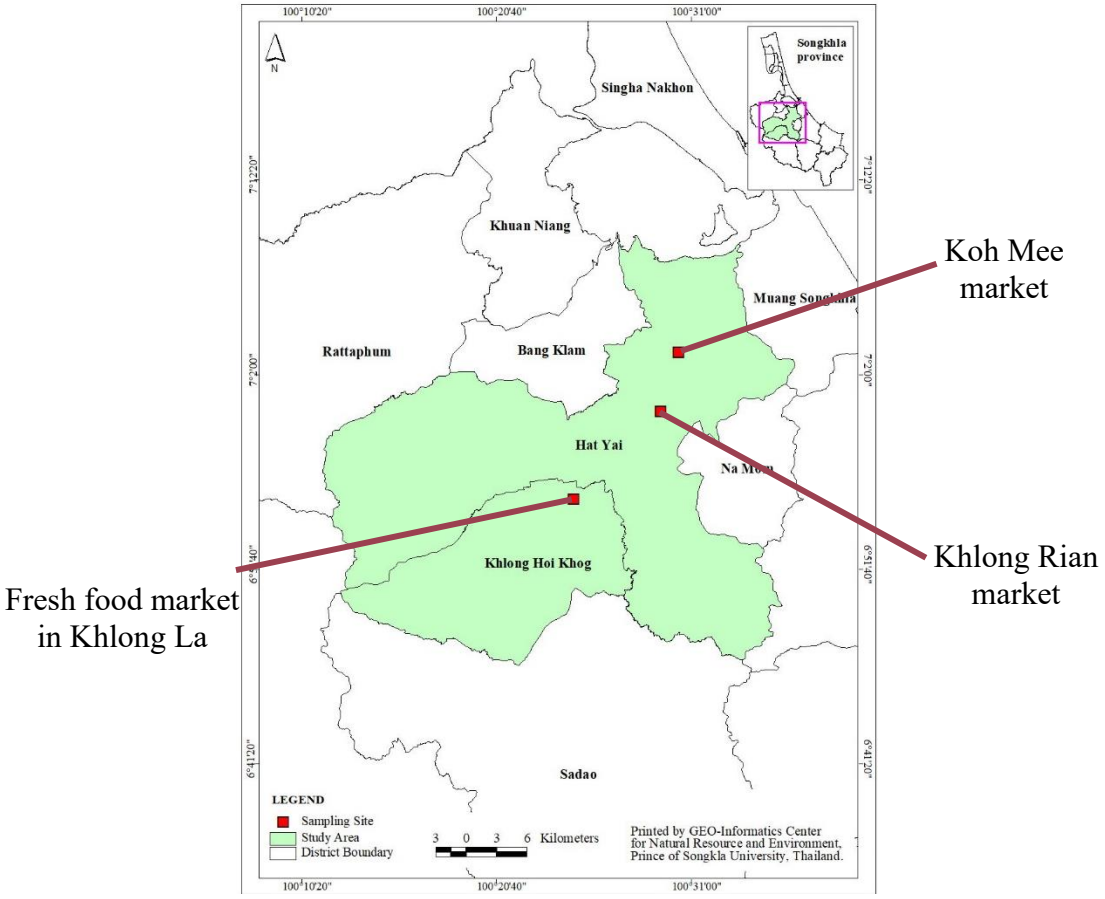


Figure 4 Three fresh selling markets (red marks) for filth flies collection in Hat Yai District, Songkhla Province (printed by Geo-Informatics Center for Natural Resources and Environment, Prince of Songkla University, Thailand).

2.3.2 Fly collection

Adult filth flies were collected three times (same route) around each fresh selling market by using a sweeping net (Figure 5a-d). Each time of collection, fifteen to twenty adult flies were captured for the top of the afternoon before the market opened (Error! Reference source not found.a-b). Specimens were kept in the plastic cup c overed by a white net, provided with a cotton ball soaked in distilled water and honey drop for the source of energy. All fly was kept alive in the insectary at 30–33°C and 59–66% relative humidity and morphologically identified into Family taxonomy levels (Muscidae, Calliphoridae, Sarcophagidae, Drosophilidae). Only a member of the Family Muscidae was identified to species level by using a morphological key according to Tumrasvin and Shinonaga (1977). For the *Escherichia coli* detection, adult flies were fed with 10% sugar solution by placing a soaked cotton ball above the holding clean cup and kept it 24 hours before the detection assay.


(a)

(b)

(c)

(d)

Figure 5 The fly collection for *Escherichia coli* detection in Songkhla Province: fresh selling market area (a and b), and adult flies around each fresh selling market captured by sweeping net (c) and transferred to plastic cup (d).

2.3.3 *Escherichia coli* detection

To perform the experiments, adult flies were anesthetized by exposing them to 95%alcohol cotton in a plastic zip bag for a few minutes. The test kit microbial namely Compact Dry EC was used for *E. coli* detection (Figure 6) This test kit was purchased from B Smart Science company limited, Muang Nonthaburi, Nonthaburi Province. The detection procedure was followed the standard method according to the description of the test kit manual. Each species (or Family) of fly specimen was separately put into the zip lock bag and grind flies thoroughly. Solution number one was poured into the zip lock bag and vigorously shaken at least 25 times. Use a syringe to pick up solution number one from the zip lock bag at 1 ml into solution bottle number two and shake the solution bottle vigorously at least 25 times. Use a new syringe to suck up the solution from solution bottle number two at 1 ml and pour it into the middle of the Compact Dry Plate. Allow the sample to automatically spread across the plate for a few seconds. Close the lid, place the Compact Dry EC upside down and put the new zip lock bag. Inoculate at room temperature (or around 33-37 °C) for 20-24 hours. After 20-24 hours of incubation, the results were examined by noticing that if there was microbial contamination, a colored dot would appear. If the plate showed a purple-red color, can be interpreted as fly samples contaminated by coliform bacteria. And if the plate show blue color, can be interpreted as fly samples contaminated by *E. coli*. The number of dots in each color per plate was recorded.

Figure 6 The Compact Dry EC test kit purchased from B Smart Science company limited, Muang Nonthaburi, Nonthaburi Province. (https://www.bsmartsci.com/)

2.3.4 Data analysis

The mean number of each fly group in all of the fresh markets where found purple red color dots in the plate were analyzed by Microsoft Excel version 2010. If the compact dry plate showed red/purple color dots were present in the table at coliform bacteria column or if showed the blue color dots in the compact dry plate, was present in the table with the *E. coil* column.

CHAPTER III

RESULTS

3.1 Part 1 Mass rearing of house flies, *Musca domestica* under laboratory condition with three diets for maggot feed

3.1.1 Effect of different diets on *M. domestica* larva nutritional composition

A sampling of fresh larvae from three larval diets composed; control (milk powder standard media), Diet 1 (fishery waste media), and Diet 2 (wet cat food) demonstrated the impact of larval weight. The mean weight of the 3rd instar larvae of *M. domestica* (g/larva) among rearing diets was illustrated in Figure 7. At Diet 1, there was a significantly higher mean weight of house fly larvae (0.41 g/larva) than that of diet 2 (0.247 g/larva) and control (0.253 g/larva) ($F = 68.67$, $df = 2$, $P < 0.05$) (Figure 7, Table 3)

The results of the nutritional analysis of house fly larvae are presented in Table 3. The nutritional components (crude protein, crude fat, moister content, and ash) of 3rd instar larvae were significantly different among three types of larval diet (F, $df = 2$, $P < 0.01$). House fly larvae feeding on Diet 1 had a significantly fewer protein content at 39.59%, compared to control and Diet 2 at 50.78% and 56.48%, respectively

(F, *df* = 2, P <0.01). Diet 1 had the highest percent fat (23.50%) but there was not significantly different from the control, except Diet 2 (F, *df* = 2, P < 0.01). The percent moisture in larvae feeding on Diet 2 (75.87%) was significantly lower than control (80.96%) and Diet 1 (79.66%) while there were no significant differences of the percent moisture between control and Diet 1 (F, *df* = 2, P < 0.01).

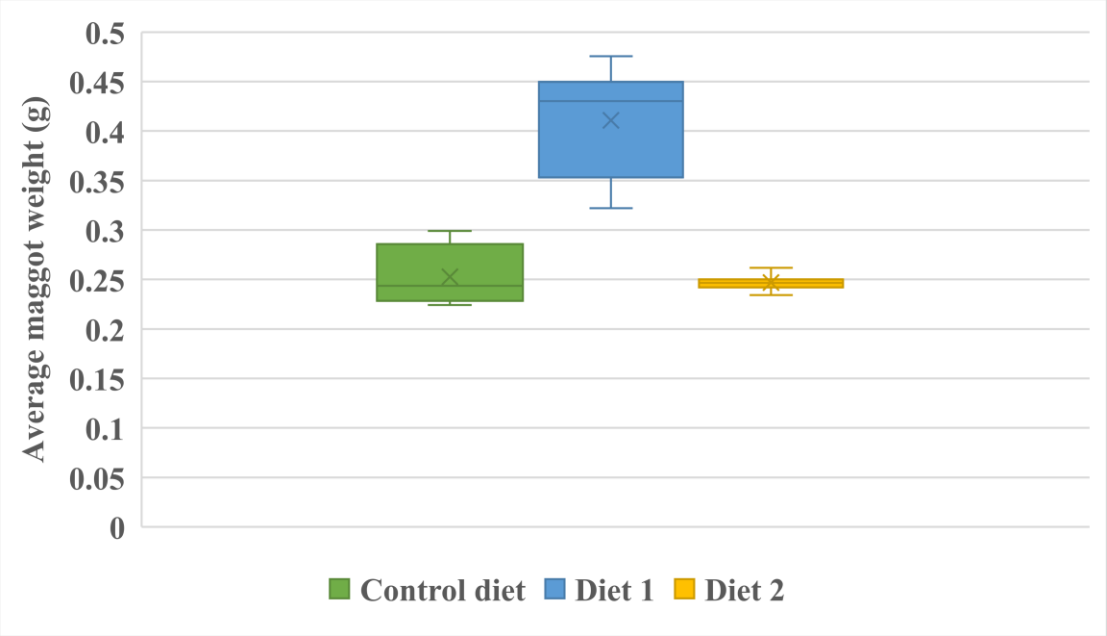


Figure 7 Mean weight of *Musca domestica* larvae (g/larva) (n=10) between rearing the larval in different types of Diet: control (milk powder standard media), Diet 1 (fishery waste media) and Diet 2 (wet cat food media)

Table 3 Mean flesh weight of the 3rd instar larvae of *M. domestica* (g/larva) and the percentage of nutritional composition of larval dry matters after 5-day feeding on different diets (SEM=Standard error of mean).

Larval Diet	Weight (g/larva)	% Crude protein	% Crude fat	% Moisture content	% Ash
Control	0.25268 (8.11E-04)[b]	50.78 (0.92)[a]	19.67 (0.33)[a]	80.96 (1.32)[a]	1.82 (0.11)[a]
Diet 1	0.41076 (2.91E-03)[a]	39.59 (2.20)[b]	23.50 (0.97)[a]	79.66 (0.87)[ab]	1.37 (0.36)[b]
Diet 2	0.24676 (5.86E-05)[b]	56.48 (0.85)[a]	7.42 (1.27)[b]	75.87 (0.45)[b]	1.39 (0.06)[b]
F-test	0.6867				
P value	0.05*	0.01**	0.01**	0.01**	0.01**

[a,b] different superscripts in the column indicated significant difference among treatments by One way-ANOVA with Tukey's multiple comparison test.

* Statistically highly significant as $P < 0.05$

** Statistically highly significant as $P < 0.01$

3.2 Part 2 Botanical essential oils susceptibility

3.2.1 Chemical composition

For the chemical constituents found in each essential oil, there were three main components, including terpenoid, phenylpropanoid, heptanone, and other compounds (Table 4). The main constituents of the clove oil, *Syzygium aromaticum* were phenylpropanoids which consisted of eugenol (57.54%), benzyl alcohol (24.15%) and caryophyllene (13.44%). For the main chemical composition identified in citronella laurel oil, *Cinnamomum porrectum* were phenylpropanoids, including safrole and methyl eugenol at 90.03% and 2.91%, respectively and other compounds were benzene and 1,2,3-trimethoxy-5-(2-propenyl) at 7.06%. Among 20 compounds of pheasant pepper tree oil, *Litsea cubeba*, 58.73% of terpenoids (citral: 31.14 %, 2,6-Octadienal, 3,7-dimethyl-(Z)-: 27.59% and D-Limonene: 16.22%) were mostly found in this plant.

Table 4 Chemical composition of clove (*Syzygium aromaticum*), citronella laurel (*Cinnamomum porrectum*) and phasent pepper tree (*Litsea cubeba*) by Pharmaceutical Laboratory Service Center (Office of Scientific Instrument and Testing: OSIT).

Chemical composition	Essential oils								
	Syzygium aromaticum			*Cinnamomum porrectum*			*Litsea cubeba*		
	Compound Name	Component RT	% of Total	Compound Name	Component RT	% of Total	Compound Name	Component RT	% of Total
Terpenoid	Caryophyllene	26.1785	13.44				(1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene	11.9827	2.66
	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	28.5223	1.45				2,2-dimethyl-3-methylene-bicyclo [2.2.1] heptane	12.4663	0.63
	(-)-5-Oxatricyclo [8.2.0.0(4,6)] dodecane,,12-trimethyl-9-methylene-	30.053	0.79				4(10)-Thujene	13.2697	1.3
							2(10)-Pinene	13.3776	1.82
							D-Limonene	15.0457	16.22
							Eucalyptol (1,8-Cineole)	15.1241	2
							Linalool	17.1752	2.93
							Citronellal	18.7561	1.36
							Isoneral	19.083	0.65
							Terpinen-4-ol	19.5331	0.4

Table 4 (continued)

Chemical composition	Essential oils								
	Syzygium aromaticum			*Cinnamomum porrectum*			*Litsea cubeba*		
	Compound Name	Component RT	% of Total	Compound Name	Component RT	% of Total	Compound Name	Component RT	% of Total
							Isogeranial	19.598	1.34
							3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, (S)-	19.9048	0.96
							2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	20.9302	1.09
							2,6-Octadienal, 3,7-dimethyl-, (Z)-	21.3738	27.59
							trans-Geraniol	21.6426	1.47
							Citral	22.189	31.14
							Copaene	24.9907	0.36
							Caryophyllene	26.1351	2.04
Phenylpropanoid	Eugenol	24.6108	57.54	Safrole	22.8591	90.03			
				Methyleugenol	25.5378	2.91			
Heptenone							5-Hepten-2-one, 6-methyl-	13.6602	2.39
							Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	13.8065	1.66

Table 4 (continued)

Chemical composition	Essential oils								
	Syzygium aromaticum			*Cinnamomum porrectum*			*Litsea cubeba*		
	Compound Name	Component RT	% of Total	Compound Name	Component RT	% of Total	Compound Name	Component RT	% of Total
other compounds	Benzyl alcohol	15.2716	24.15	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	29.1875	7.06			
	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	26.9825	2.63						

3.2.2 Larvicidal bioassays of *M. domestica*

The three different percentage concentrations of each essential oil in this study were established according to Morey et al. (2012) and Soowera (2015) (Table 5).

The larvicidal activity (percent knockdown and mortality) of clove oils, citronella laurel, and pheasant pepper tree of each three concentration and positive control (cypermethrin) against the 3rd instar larvae of laboratory *M. domestica* was shown in Table 5. Mortality and knock-down (KD) rate at 100% was seen in *M. domestica* larvae after testing with cypermethrin, the positive control.

Percent knockdown and mortality in *M. domestica* larvae was higher, relative to a higher percent concentration of essential oil. In the larval dipping test in at 1% concentration of essential oils, the highest knockdown and mortality rate of *M. domestica* larvae were observed in citronella laurel oil with 50.00±5.24% and 84.00±4.30%, respectively. At the highest concentration of 10%, the highest knockdown and mortality rates were observed in all trials. Above 90% of knockdown and mortality rate were seen after *M. domestica* larvae exposed to 10% of clove and citronella laurel oils (Table 5).

The lethal concentration value and lethal time value of larva *M. domestica* were shown in Table 6 and Table 7, respectively. At 10% concentration of essential oils, citronella laurel oil exhibited the highest larvicidal effect against larvae with LC_{50} values of 1.667% and LT_{50} values of 1.74 min, followed by clove oil and pheasant pepper tree oil with LC_{50} values of 22.541 and 2.594, respectively (Table 5 and Table 6). The final discriminating concentration (LC_{99} x 2) showed the lowest concentration affected to larvae of *M. domestica* at 6.134% in citronella laurel, followed by pheasant pepper tree oil at 10.398% and the highest discriminating showed in clove oil at 13.038% (Table 6).

Table 5 Mean percent of knockdown and mortality (SE) of larva and adult *Musca domestica* laboratory colony exposure to a serial concentration of three essential oils.

Test compound	Larvae *Musca domestica*				Adult *Musca domestica*			
	Concentration (%)	N	Mean ± SE		Concentration (%)	N	Mean ± SE	
			%Knockdown	%Mortality			%Knockdown	%Mortality
Clove	1	100	5 ± 1.58	38 ± 3.39	0.5	30	0	0
					1	29	0	7.50 ± 4.79
	5	100	16 ± 2.92	49 ± 6.78	2	30	100 ± 0.00	33.33 ± 4.22
					3	30	16.67 ± 8.03	36.67 ± 9.55
	10	100	90 ± 2.24	94 ± 1.87	4	30	23.33 ± 6.15	50.00 ± 11.25
					5	30	40.00 ± 8.94	63.33 ± 6.15
Citronella laurel	1	100	50 ± 5.24	84 ± 4.30	0.5	29	0	0
					1	28	5.56 ± 5.56	28.98 ± 8.37
	5	100	79 ± 3.32	97 ± 2.00	2	30	6.67 ± 6.67	3.33 ± 3.33
					3	28	20.83 ± 7.35	35.00 ± 6.06
	10	100	94 ± 2.45	99 ± 1.00	4	30	73.33 ± 6.67	100 ± 0.00
					5	30	26.67 ± 9.89	33.33 ± 11.16
Pheasant pepper tree	1	100	4 ± 1.87	24 ± 9.54	0.5	30	6.67 ± 6.67	13.33 ± 9.89
					1	30	10.00 ± 4.47	33.33 ± 6.67
	5	100	39 ± 4.85	57 ± 9.95	2	30	73.33 ± 9.89	53.33 ± 6.67
					3	30	20.00 ± 7.30	43.33 ± 9.55
	10	100	82 ± 4.36	98 ± 1.22	4	30	66.67 ± 11.16	90.00 ± 6.83
					5	30	100 ± 0.00	93.33 ± 4.21
Negative control: Alcohol		100	0	0		30	0	0
Positive control: Cypermethrin		100	100 ± 0.00	100 ± 0.00		30	100 ± 0.00	100 ± 0.00

Table 6 The percent lethal concentration value to 50% (LC_{50}) and 95% (LC_{95}) mortality of laboratory larva *Musca domestica* exposure to a serial concentration of three test compounds.

Essential oils	N	LC_{50} (95% FL*) (%)	LC_{95} (95% FL*) (%)	Slope ± SE	χ^2	*P* – value[+]	Final discriminating concentration[‡]
Clove	100	2.541	4.947	5.685 ± 0.541	23.531	0.000	13.038
Citronella laurel	100	1.667	2.565	8.782 ± 0.810	30.763	0.000	6.134
Pheasant pepper tree	100	2.594	4.241	7.706 ± 0.734	12.097	0.002	10.398

*FL = 95% fiducial limits.

[+]Chi-square goodness-of-fit test.

[‡]2 x probit-derived $\%LC_{99}$

Table 7 The percent lethal time value to 50% (LT_{50}) (min) and 95% (LT_{95}) and percent mortality at 24 hours of laboratory larva *Musca domestica* exposure to a serial concentration of three test compounds.

Essential oils	Concentration (%)	No. tested	KT_{50} (min)	95% CL	KT_{95} (min)	95% CL
Clove	1	100	292.74	4.022 - 6.782	1,449.96	14.010 - 67.927
	5	100	236.82	3.208 - 5.645	2,227.62	17.667 - 176.490
	10	100	0.06	-	442.08	-
Citronella laurel	1	100	68.88	0.741 - 1.457	820.68	7.993 - 43.170
	5	100	19.44	0.069 - 0.583	206.58	2.569 - 6.554
	10	100	1.74	-	75.06	-
Pheasant pepper tree	1	100	564.06	6.051 - 29.049	5240.88	28.508 - $1.750E^{3}$
	5	100	123.66	1.175 - 3.174	1.398	34.443 - $5.984E^{6}$
	10	100	19.02	0.073 - 0.560	154.68	2.008 - 4.092

3.2.3 Adulticidal bioassay of *Musca domestica*

The adulticidal activity of clove oils, citronella laurel, and pheasant pepper tree with six concentrations and positive control (cypermethrin) against both sex of laboratory strain of *M. domestica* was done by using the WHO cone test. The percent knockdown and mortality of susceptibility adult *M. domestica* to three essential oils and two types of control following 60 min and 24 hours after exposure was presented in Table 9. The completed knockdown rate (100%) of adults *M. domestica* was found at 2% of clove oil and 5% of pheasant pepper tree oil. Whereas 4% concentration of citronella laurel oil showed the highest percent knockdown at 73.33±6.67% and completely killed adult *M. domestica*. The mortality rate derived from 4% and 5% concentration of clove oil showed percent mortality of adult *M. domestica* above 50%, compared to pheasant pepper tree oil showing percent mortality above 90% (Table 8).

The regression equation, the LC_{50} and LC_{95} values were calculated from log-concentration probit mortality from each essential oil (Table 9). In the regression equation, the LC_{50} and LC_{95} values were calculated at 3.721% (3.058% - 4.881%) and 16.456% (10.143% - 43.195%) for clove oil, 2.363% (0.992% - 5.223%) and 15.322% (6.201% - 2.832E^4%) for citronella laurel, and 1.756% (0.569% - 3.630%) and 19.337% (6.591% - 4.431 E^4%) for pheasant pepper tree. The final discriminating concentration (LC_{99} x 2) showed the lowest concentration at 30.644% of citronella laurel affected to adults of *M. domestica*, followed by pheasant pepper tree oil at 38.674% and the highest discriminating showed in clove oil at 60.938% (Table 9).

Table 8 Percent knockdown and mortality of laboratory adult *Musca domestica* exposure to a serial concentration of five test compounds, three essential oils recorded at 60 min (1 hour) and 24 hours after contact test compounds.

Test compound	Concentration (%)	N	No. knockdown (%)	No. mortality (%)
Clove	0.5	30	0	0
	1	29	0	6.89
	2	30	100	33.33
	3	30	16.67	36.67
	4	30	23.33	50
	5	30	40.00	63.33
Citronella laurel	0.5	29	0	0
	1	28	3.57	28.57
	2	30	6.67	3.33
	3	28	21.43	35.71
	4	30	73.33	100
	5	30	26.67	33.33
Pheasant pepper tree	0.5	30	6.67	13.33
	1	30	10.00	33.33
	2	30	73.33	53.33
	3	30	20.00	43.33
	4	30	66.67	90.00
	5	30	100.00	93.33
Negative control: Alcohol		30	0	0
Positive control: Cypermethrin		30	100	100

Table 9 Final percent discriminating concentration of three essential oils (clove, citronella laurel, and pheasant pepper tree) determined from laboratory adult *Musca domestica.*

Essential oils	N	LC_{50} (95% FL*) (%v/v)	LC_{95} (95% FL*) (%v/v)	Slope ± SE	χ^2	*P* - value[+]	Final discriminating concentration[‡]
Clove	179	3.721 (3.058 - 4.881)	16.456 (10.143 - 43.195)	2.547 ± 0.450	1.955	0.744	60.938
Citronella laurel	175	2.363 (0.992 - 5.223)	15.322 (6.201 - 2.832E4)	2.865 ± 0.409	16.313	0.003	30.644
Pheasant pepper tree	180	1.756 (0.569 - 3.630)	19.337 (6.591 - 4.431 E4)	2.233 ± 0.326	14.466	0.006	38.674

*FL = 95% fiducial limits.

[+]Chi-square goodness-of-fit test.

[‡]2 x probit-derived $\%LC_{99}$

3.2.4 Adulticidal bioassays of *Stomoxys indicus*

The adulticidal activity of *S. indicus* field population exposure to clove oils, citronella laurel, and pheasant pepper tree with six concentrations and positive control (cypermethrin) by using the WHO cone test. The percent knockdown and mortality of adult *S. indicus* to three essential oils and two types of control following 60 min and 24 hours after exposure was presented in Table 10. Based on using six concentrations (0.5, 1, 1.5, 2, 2.5, and 3) of essential oils, clove oil at 1 % showed completely 100% in both percent knockdown and mortality. The result of *S. indicus* responding to pheasant pepper tree at 2%, 2.5%, and 3% completely showed 100% knockdown and 100% mortality. Citronella laurel oil at 3% showed the highest percent knockdown and mortality of 86.67±6.67% and 90.00±6.83%, respectively.

The regression equation, the LC_{50} (%v/v) and LC_{95} (%v/v) values were calculated from log-concentration probit mortality from each essential oil (Table 11). The LC_{50} and LC_{95} value were calculated as 0.284% (0.136%-0.447%) and 0.547% (0.379%-13.573%) for clove oil, 1.683% (1.447%-1.905%) and 4.229% (3.395%-6.104%) for citronella laurel and 1.180% (1.018%-1.335%) and 2.746% (2.310-3.564) for pheasant pepper tree. The final discriminating concentration (LC_{99} x 2) showed the highest concentration at 1.434% in clove oil could be affected to field population of adults *S. indicus* catch from animal Science farm, followed by pheasant pepper tree oil at 7.794% and the lowest discriminating showed in citronella laurel at 12.39%.

Table 10 Percent knockdown and mortality of *Stomoxys indicus* field population exposure to a serial concentration of six test compounds.

Test compound	Concentration (%)	N	No. knockdown (%)	No. mortality (%)	Mean % ± SE	
					Knockdown	Mortality
Clove	0.1	30	0.00	3.33	0	3.33 ± 3.33
	0.2	30	3.33	16.67	3.33 ± 3.33	16.67 ± 6.15
	0.3	30	20.00	43.33	20.00 ± 5.16	43.33 ± 6.15
	0.4	30	10.00	80.00	10.00 ± 4.47	80.00 ± 5.16
	0.5	30	16.67	100.00	16.67 ± 8.03	100.00 ± 0
	1	30	100.00	100.00	100.00 ± 0	100.00 ± 0
Citronella laurel	0.5	29	0.00	3.45	0	3.33 ± 3.33
	1	29	6.90	13.79	6.67 ± 4.21	13.33 ± 6.67
	1.5	30	56.67	43.33	56.67 ± 9.55	43.33 ± 6.15
	2	29	79.31	58.62	78.33 ± 11.08	57.50 ± 11.53
	2.5	30	63.33	73.33	63.33 ± 9.55	73.33 ± 8.43
	3	30	86.67	90.00	86.67 ± 6.67	90.00 ± 6.83

Table 10 (continued)

Test compound	Concentration (%)	N	No. knockdown (%)	No. mortality (%)	Mean % ± SE	
					Knockdown	Mortality
Pheasant pepper tree	0.5	30	16.67	6.67	16.67 ± 8.03	6.67 ± 4.22
	1	29	41.38	37.93	41.67 ± 9.10	37.50 ± 5.74
	1.5	28	71.43	64.29	71.67 ± 3.80	65.00 ± 6.06
	2	30	100.00	76.67	100.00 ± 0	76.67 ± 6.15
	2.5	29	100.00	96.55	100.00 ± 0	96.67 ± 3.33
	3	30	100.00	100.00	100.00 ± 0	100.00 ± 0
Negative control: Alcohol		30	0	0	0	0
Positive control: Cypermethrin		30	100	100	100	100

Table 11 Final percent discriminating concentration of three essential oils determined from field population *Stomoxys indicus* from Animal Science farm.

Essential oils	N	LC_{50} (95% FL*) (%v/v)	LC_{95} (95% FL*) (%v/v)	Slope ± SE	χ^2	*P* - value[+]	Final discriminating concentration[‡]
Clove	150	0.284 (0.136 – 0.447)	0.547 (0.379 – 13.573)	5.786 ± 0.827	10.226	0.017	1.434
Citronella laurel	177	1.683 (1.447 – 1.905)	4.229 (3.395 – 6.104)	4.111 ± 0.584	1.929	0.749	12.39
Pheasant pepper tree	176	1.180 (1.018- 1.335)	2.746 (2.310 – 3.564)	4.484 ± 0.571	3.649	0.456	7.794

[*]FL = 95% fiducial limits.

[+]Chi-square goodness-of-fit test.

[‡]2 x probit-derived $\%LC_{99}$

3.3 Part 3 Survey the pathogenic bacteria, *Escherichia coli* infected in the flies.

In the fresh selling, specimens of adult fly were classified into 3 families of Muscidae-*Musca domestica*, *Musca autumnali* and *Musca crassirostris*-, family Calliphoridae, family Drosophilidae and others fly were recorded (Table 12).

The *E. coli* detection by test kit in *M. domestica* samples from Khlong Rian Market, where located nearby the Prince of Songkla University at 3.2 kilometers apart, showed the positive result *E. coli* by the total density of 578 purple-red dots/ 59 flies with a mean number of positive dots of 192.67 followed by *Musca cracirostris* and *Musca autumnalis* with 173 and 163 of total positive dots, respectively. For blow fly, fruit fly and other flies, there are a few numbers from this study. Blow flies were able to catch one or two times from the entire collection, but all catches were found to be contaminated with *E. coli*.

The second fresh selling market, Koh Mee Market, fresh meat such as fish, seafood, pork, chicken, and beef were observed and well known an the meat selling market. The most abundance was *M. domestica* and were found to be contaminated with *E. coli*, according to a positive detection at 211.67 dots in average from three replications, followed by *M. autumnalis*, and *M. cracirostris* at 167 dots in average from two samplings. Interestingly, fruit fly (family Drosophilidae) did not show any positive result with *E. coli* contamination.

For Khlong La market, the last selling fresh market that selling a fresh product, a lot of all ready to eat and take away food. *Musca domestica* and blow flies were entirely sampled from the three collections. *Musca domestica* presented a total of 532 purple-red dots and gave a mean of 177.33 dots. Small numbers of fly in family Calliphoridae showed the positive result of mean at 44.67 dots, while *M. autumnalis* and *M. cracirostris* from two collections gave a total dot of *E. coli* with the mean of 229 and 114.5, respectively. There was no *E. coli* detected in family Drosophilidae (fruit flies) which were once collected from the market.

Table 12 Detection of *Escherichia coli* in the compact dry plate of three markets that were selected for survey pathogenic bacteria-infected in the 205 flies.

	Time of sampling	*Musca domestica*		*Musca autumnalis*		*Musca cracirostris*		Bowl fly		Other flies		Fruit fly	
		E. coli	Coliform bacteria	*E. coli*	Coliform bacteria	*E. coli*	Coliform bacteria	*E. coli*	Coliform bacteria	*E. coli*	Coliform bacteria	*E. coli*	Coliform bacteria
Khlong Rian Market	1	228	+	76	+	156	+	42	+	152	+	-	-
	2	164	+	72	+	no	no	no	no	no	no	74	+
	3	186	+	15	+	17	+	no	no	-	-	-	-
	Total	578		163		173		42		152		74	
	Mean	192.67		54.33		86.5		42		152		74	
Koh Mee Market	1	276	+	188	+	121	+	129	+	no	no	-	-
	2	161	+	146	+	no	no	no	no	no	no	no	no
	3	198	+	no	no	213	+	no	no	39	+	no	no
	Total	635		334		334		129		39			
	Mean	211.67		167		167		129		39			
Fresh food market in Khlong La	1	196	+	no	no	22	+	46	+	250	+	no	no
	2	182	+	209	+	no	no	37	+	no	no	-	-
	3	154	+	105	+	207	+	51	+	no	no	-	-
	Total	532		314		229		134		250			
	Mean	177.33		157		114.5		44.67		250			

* P = present to founded pathogenic bacteria, + = positive, - = negative and on = no sample

CHAPTER IV

DISCUSSION

4.1 Nutritional composition of larval *Musca domestica*

The house flies larval weight rate was measured from three different types of larval diet under laboratory conditions. All diets had a significant effect on mean larval weight. An equivalent weight rate was seen in control diet and Diet 2 (milk powder standard media and wet cat food media), that was possibly due to the effect of the initial diet are content with additional protein, sugar, and fat by the commercial process. However, the weight from larvae reared at fresh fishery waste diet was the highest. Related results were reported by Hogsette, 1992 that fly larvae reared at a high protein diet was heavier than larvae reared at a low protein diet. Besides, Nash and Chapman (2014) showed that carbohydrates also had a significant effect on weight. Green et al., (2003) show that the high protein content of the wheat bran diet affected pupal weight and adults.

The results of the nutritional composition of house fly larvae obtained in this study remained promising with the previous reports, observed by Fasakin et al 2003, Dordevic et al 2008 and Hwangbo et al 2009 which was fed by chicken manure. The virtual protein, fat, moisture, and ash contents of larvae ranges from 39.59-56.48%, 7.42-23.50%, 75.87-80.96%, and 1.39-1.82, accordingly. The nutrient composition of the well-cultivated larvae obtained in the present study turned out to be slightly lower than the 43.45% (Fasakin et al 2003), 59.48% (Dordevic et al 2008), and 63.99% (Hwangbo et al 2009) protein content. This was agreed with the finding of fat content, 14.30% (Fasakin et al 2003), 6.66% (Dordevic et al 2008), and 24.31% (Hwangbo et al 2009). While moisture content in the current finding was extremely higher than previous observations, 8.25% (Fasakin et al 2003) and 5.28 % (Hwangbo et al 2009). Besides, low ash content was found in 5.96% (Dordevic et al 2008), but greater than 0.36% by Fasakin et al (2003), and 2.01% by Hwangbo et al (2009).

This study was not designed to evaluate the life histories of house fly used for biodegradation as there was no comparison between manure, garbage, and food scraps. As that information was reported elsewhere, e.g., the result of duration of larvae development 3-5 days, the weight of pupae 0.005- 0.021 g/pupa, and adult female fecundity 200-400 eggs/female (Bernard et al 1998, Hogsette and Farkas 2000, Pastor et al 2011). Cickova et al. (2015) reported that the reproduction of house fly for the biodegradation of organic waste is great. Under the laboratory conditions, the maximum lifetime female reproduction has been assessed to reach 729 eggs at 25 °C (Fletcher et al, 1990).

The present study findings showed only the baseline results of larval diet on nutrient composition of larvae. A further study is planned to evaluate the possibility to replace costly protein sources in fish diets with larval meals which have shown promising results (Idowu and Afolayan 2013; Ajani et al 2004; Sogbesan 2014). Ajani et al (2004) reported that the complete replacement of fishmeal by a larval meal in diets of Nile tilapia, Oreochromis niloticus enhanced growth and correspondingly led to an 18 to 28% of cost reduction. Similarly, Gao et al. (2019) report that the tilapia fed with dried house fly larvae presented a greater final weight and protein content than those fed with commercial feed. Nevertheless, the protein content obtained approves the applicability of additive protein in animal feed.

4.2 Insecticidal activity of essential oils

4.2.1 Chemical compositions of essential oil

The main chemical compositions of clove oil from this study were eugenol, eugenol acetate, and beta-caryophyllene, similar to the previous report such as Kafle et al. (2013) who revealed the study on toxicity and repellency of compounds from clove to red imported fire ants. In addition, the report of Chaieb et al. (2007) also showed the chemical components with a high percent concentration of eugenol (88.58%), eugenyl acetate (5.62%), and -caryophyllene (1.39%) isolated by hydro-distillation using GC-MS analysis.

Cinnamomum porrectum (Roxb.) Kosterm was an important plant that produced natural essential oil. *Cinnamomum porrectum* commonly as known as Thep-Ta-Ro and safrole, that major compound found in *C. porrectum* were used in traditional Thai medicine. Thep-Ta-Ro is the most scattered throughout in southern part of Thailand (Saetan et al. 2018). In any reported study about chemical composition isolated by hydro-distilled from leaves, safrole was identified as the major compound of this plant (Sukcharoen et al. 2017 and Subki et al. 2013).

And the last essential oil in this study, *Litsea cubeba* Persoon, the aromatic litsea or may chang, is a plant of the family Lauraceae which smells like citronella oil. This plant has been known as an insect repellent, especially for household insect pests. *Litsea cubeba* is a native plant in Asian countries- China, Indonesia, Taiwan and other parts of Southeast Asia. The chemical components of *L. cubeba* were reported by Chen et al. (2012). The main components from fruit oil of *L. cubeba* were geranial (37.16%), neral (28.29%), and *d*-limonene (22.09%), similar to this study result. Moreover, the report of Wang and Liu (2010) revealed that the chemical compositions of essential oil from different parts of *L. cubeba* have different major components such as from roots and fruits (citral B), from stems, leaves, and alabastra (β-phellandrene), and flowers (β-terpinene).

4.2.2 Bioassay of essential oils against *Musca domestica*

The larvicidal and adulticidal susceptibility of essential oils against laboratory *M. domestica* showed a percent lethal concentration of clove oil in both larvicidal and adulticidal activity was lower than citronella laurel and pheasant pepper tree. Our results suggested that clove oil gave the highest mortality rate of *M. domestica*, compared to other essential oil with the same concentration. In the previous research, Soonwera et al. (2014a) also report the highest insecticidal activity by clove oil at 1.20-10.55% of LC_{50} value that also more than the LC_{50} value of lemongrass oils with the same concentration. Moreover, clove oil (*Syzygium aromaticum* L.) also showed the LT_{50} values and LC_{50} values of *S. aromaticum* oil exhibited the highest larvicidal effect against house fly larvae with 1623 min and 9.83%, respectively. followed by *Cymbopogon nardus* oil and *Cananga odorata* oil with LT_{50} values of 38.99 and

3,124.8 min and LC_{50} values of 13.60 and 29.36%, respectively. the highest larvicidal activity and oviposition deterrent activity with LC_{50} value deterrent activity was shown by *S. aromaticum* oil with LC_{50} values of 9.83% and 100% (Soonwera, 2015b).

4.2.3 Bioassay of essential oils susceptibility of *Stomoxys* spp.

The result from the botanical essential oil bioassay part in this study could be indicated that clove oil showed the highest effectiveness to adult *S. indicus* according to mortality rate compared to other essential oils. The result of the botanical insecticidal bioassay test to *S. indicus* is the first report in southern Thailand. One of the previously report on the concentration of citronella oil and synthetic DEET for *S. indicus* control in animal farm showed that LC_{50} value of citronella oil was lower than synthetic DEET by 0.72% and 3.15%, respectively (Thongeiab, 2020). The same area also found the report of bioassay test on different species of *S. calcitrans* with clove oil and citronella laurel (Lorn, 2019). The results showed that the highest percent knockdown and mortality of clove oil was 2.5% concentration at 70% and 50%, respectively.

4.3 Survey the pathogenic bacteria infected in the flies

The result of exploration confirmed that the population of flies species- *M. domestica*, *M. autumnalis*, and *M. crassirostris* around a fresh market which surveys to examine for contamination of *E. coli* were positive in all the observed markets. Related results were reported by Butler et al., 2010; wild-caught *M. domestica* collected near the rear entrances and dumpsters of the restaurants in Florida could be carried bacteria as a mechanical transmitter. The bacteria identification has been associated with including *Bacillus cereus, B. thuringiensis, E. coli* 0157:H7, *Shigella dysenteriae, Staphylococcus saprophyticus*, and *Staphylococcus xylosus*. Furthermore, Vasan et al. (2008) report that house fly could be a potential vector or carrier the pathogenic bacteria such as *Citrobacter freundii, E. coli* and *S. paratyphi* from around the government hospital in India. Another evidence from Szalanski (2004) shows that *E. coli* O157:H7

was carried by house flies and black dump flies associated with poultry in the United States. Moreover, in Thailand was research about efficiency as a carrier of bacteria by house fly, *M. domestica*, and the Oriental latrine fly, *C. megacephala* (Sukontason et al., 2007). The result shows that 42 species of bacteria were isolated from both house fly and oriental latrine fly.

In addition, there was reported by Talley et al. (2009) that house flies could be a carrier of the pathogens to spinach leaves. The result showed that house flies containing *E. coli* O157:H7 tagged with a green fluorescent protein (GFP) could transfer the microbes into the spinach plants under laboratory conditions. So, those house flies could be a capable mechanical vector of transmission, serious infections pathogens which can cause disease to humans by contamination of fresh food production.

CHAPTER V

CONCLUSION

This study provides knowledge on the ability of *M. domestica* larvae to develop in standard media (milk powder media), fishery waste media, and cat food media. The *M. domestica* larvae which reared by fishery waste media showed the highest mean weight and highest percent fat more than other substances Diet. The nutritional composition of larvae feed of fishery waste, therefore making the *M. domestica* larvae is an ideal alternative to waste management in animal leftovers from the fishing industry.

The citronella laurel oil was most effective for larvicidal and adulticidal effects to *M. domestica*. The larvicidal bioassays test showed the lowest establishing discriminating concentration of citronella laurel at 6.134%. Moreover, the result of the adulticidal bioassays test with citronella laurel also showed the lowest establishing discriminating concentration more than all of the essential oils. This study demonstrated thathigh effectiveness to control *S. indicus* and was the lowest establishing discriminating concentration is clove oil. Therefore plant oils may be the best alternative to insecticides because they are safe for the environment and human beings. In the present study, botanical oil was evaluated against muscid fly pests under laboratory conditions. In near future, the isolation of pure compounds from the most bioactive fraction and their activity against fly should be undertaken. Further study in field-scale applications of both larvicidal and adulticidal effects to prevent and control flies is needed.

Adult flies in the study sites were confirmed to be contaminated with *E. coli*. This information can be used to be a caution to well cleaned and well-prepared food before consumption. Fly prevention and control, especially sanitation methods in the market should be introduced.

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List of Proceeding, and presentations

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