Efficiency of Plant Oils to Control Lesser Grain Borer [*Rhyzopertha dominica* (Fabricius)] and Maize Weevil (*Sitophilus zeamais* Motschulsky) in Rough Rice

Kanok-on Wuttiwong

A Thesis Submitted in Partial Fulfillment of the Requirement for the Degree of
Doctor of Philosophy in Tropical Agriculture Resource Management
Prince of Songkla University
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Author	Miss Kanok-on Wuttiwong
Major Program	Tropical Agriculture Resource Management

Major Advisor

……..

(Assoc.Prof.Dr.Aran Ngampongsai)

Co-advisor

……

(Asst.Prof.Dr.Yaowaluk Chanbang)

Examining Committee :

……Chairperson

(Asst.Prof.Dr.Patcharaporn Vanichpakorn)

…….…...Committee

(Assoc.Prof.Dr.Aran Ngampongsai)

…….……..…..Committee

(Asst.Prof.Dr.Yaowaluk Chanbang)

…….…...…Committee

(Asst.Prof.Dr.Wisut Sittichaya)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Doctor of Philosophy Degree in Tropical Agriculture Resource Management

……..…..……..……..

(Prof. Dr. Damrongsak Faroongsarng)

Dean of Graduate School

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

……...…….Signature
(Assoc.Prof.Dr.Aran Ngampongsai)
Major Advisor

……...…….Signature
(Miss Kanok-on Wuttiwong)
Candidate

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

……..…….Signature

(Miss Kanok-on Wuttiwong)

Candidate

ชื่อวิทยานิพนธ์ ประสิทธิภาพของน้ำมันจากพืชต่อการควบคุมมอดข้าวเปลือก [*Rhyzopertha dominica* (Fabricius)] และด้วงงวงข้าวโพด (*Sitophilus zeamais* Motschulsky) ในข้าวเปลือก

ผู้เขียน นางสาวกนกอร วุฒิวงศ์

หลักสูตร การจัดการทรัพยากรเกษตรเขตร้อน

ปีการศึกษา 2561

บทคัดย่อ

มอดข้าวเปลือก [*Rhyzopertha dominica* (Fabricius)] และด้วงงวงข้าวโพด (*Sitophilus zeamais* Motschulsky) เป็นศัตรูที่สำคัญสร้างความเสียหายต่อคุณภาพและปริมาณระหว่างการเก็บรักษาข้าวเปลือก ในการควบคุมแมลงดังกล่าวนิยมใช้สารเคมีทั้งการคลุกเมล็ดและการรม ก่อให้เกิดผลกระทบตามมา เช่น การสร้างความต้านทานต่อสารเคมีของแมลงและเกิดพิษต่อสิ่งมีชีวิตนอกเป้าหมาย ดังนั้นการศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อคัดเลือกน้ำมันจากพืชที่มีประสิทธิภาพและวิธีการใช้ที่เหมาะสมในห้องปฏิบัติการ เพื่อนำไปประยุกใช้ควบคุมมอดข้าวเปลือกและด้วงงวงข้าวโพดในโรงเก็บเมล็ดพันธุ์ข้าว

สกัดน้ำมันจากพืช 6 ชนิด ได้แก่ ขมิ้นชัน (*Curcuma longa*) พริกไทยดำ (*Piper nigrum*) กานพลู (*Syzygium aromaticum*) ตะไคร้หอม (*Cymbopogon nadus*) ด้วยวิธีกลั่นด้วยไอน้ำ (water distillation method) ผักเสี้ยนผี (*Cleome viscose*) และสะเดาช้าง (*Azadirachta excelsa*) ด้วยวิธีแช่ยุ่ย (maceration) ด้วยตัวทำละลาย *n*-hexane นำน้ำมันที่สกัดได้ไปทดสอบฤทธิ์การไล่ ความเป็นพิษ และฤทธิ์ยับยั้งการกินอาหารของตัวเต็มวัยมอดข้าวเปลือกและด้วงงวง

ข้าวโพดในห้องปฏิบัติการ คัดเลือกน้ำมันที่มีพิษต่อแมลงดังกล่าวสูงสุดไปวิเคราะห์องค์ประกอบทางเคมีและทดสอบวิธีการใช้โดยการคลุกเมล็ด รมควัน และชุบกระสอบ และผลต่อการงอกของเมล็ดข้าวเปลือกเปรียบเทียบกับสารฆ่าแมลงคลอไพรีฟอสและสารฟอสฟีนในห้องปฏิบัติการและโรงเก็บเมล็ดพันธุ์ข้าว พร้อมทั้งวิเคราะห์ต้นทุนเพื่อเปรียบเทียบค่าใช้จ่ายระหว่างการใช้น้ำมันจากพืชและสารฆ่าแมลงดังกล่าวในสภาพโรงเก็บเมล็ดพันธุ์ ผลการศึกษาพบว่า น้ำมันพริกไทยดำออกฤทธิ์ไล่แมลงได้ดีที่สุด โดยไล่แมลงทั้ง 2 ชนิดดังกล่าวได้ 100% ที่ความเข้มข้น 0.47 μL/cm^2 ที่ 48 ชั่วโมง น้ำมันกานพลูมีพิษต่อมอดข้าวเปลือกและด้วงงวงข้าวโพดมากที่สุดเนื่องจากมีค่า LC_{50} ต่ำสุด ค่า LC_{50} โดยการสัมผัส ที่ 72 ชั่วโมง เท่ากับ 6.13 μL/L และ 3.52 μL/L ตามลำดับ และค่า LC_{50} โดยการรมที่ 48 ชั่วโมง เท่ากับ 92.95 μL/L และ 77.63 μL/L ตามลำดับ นอกจากนี้น้ำมันกานพลูยังออกฤทธิ์ยับยั้งการกินอาหารของมอดข้าวเปลือกและด้วงงวงข้าวโพดได้ดีที่สุด โดยยับยั้งได้สูงถึง 97.12% และ 95.91% ตามลำดับ พบองค์ประกอบสารที่สำคัญในน้ำมันกานพลู ได้แก่ eugenol 65.83%, β–caryophyllene 13.54% และ eugenol acetate 8.29% ตามลำดับ

การใช้น้ำมันกานพลูคลุกเมล็ดและรมควันให้ผลไม่แตกต่างกัน เนื่องจากที่เวลา 7 วันหลังการทดสอบที่ความเข้มข้นเดียวกัน คือ 200 μL/L ด้วยการคลุกเมล็ดทำให้มอดข้าวเปลือกและด้วงงวงข้าวโพดตาย 99.33% และ 95.33% ตามลำดับ และด้วยการรมทำให้มอดข้าวเปลือกและด้วงงวงข้าวโพดตาย 98.67% และ 100% ตามลำดับ ส่วนการชุบกระสอบให้ผลน้อยที่สุดเนื่องจากป้องกันการเคลื่อนย้ายผ่านกระสอบของมอดข้าวเปลือกและด้วงงวงข้าวโพดได้เพียง 38.33% และ 46.67% ตามลำดับ ที่เวลาเดียวกันเมื่อคลุกเมล็ดด้วยสารคลอไพริฟอส ทำให้มอดข้าวเปลือกและด้วงงวงข้าวโพดตาย 66.00% และ 100% ตามลำดับ และเมื่อรมด้วยสารฟอสฟีน

ทำให้มอดข้าวเปลือกและด้วงงวงข้าวโพดตาย 62.00% และ 98.67% ตามลำดับ ชี้ให้เห็นว่าสารฆ่าแมลงทั้ง 2 ชนิดดังกล่าวให้ประสิทธิภาพต่ำกว่าน้ำมันกานพลูในการควบคุมมอดข้าวเปลือก แต่ยังคงประสิทธิภาพในการควบคุมด้วงงวงข้าวโพดไม่แตกต่างกัน

ส่วนการทดสอบในโรงเก็บเมล็ดพันธุ์ที่ศูนย์เมล็ดพันธุ์ข้าวสุราษฎร์ธานี (SRSC) และ ศูนย์เมล็ดพันธุ์ข้าวพัทลุง (PRSC) เป็นระยะเวลา 6 เดือน (เมษายน – กันยายน 2559) การคลุกเมล็ดร่วมกับการรมด้วยน้ำมันกานพลูมีประสิทธิภาพสูงสุดในการควบคุมมอดข้าวเปลือก สามารถควบคุมการเข้าทำลายได้ 85.96% และ 90.31% เทียบกับชุดควบคุม ในโรงเก็บ SRSC และ PRSC ตามลำดับ ในขณะที่การคลุกเมล็ดด้วยคลอไพริฟอสและรมด้วยฟอสฟีน ควบคุมมอดข้าวเปลือกได้เพียง 40.09% และ 40.92% ในโรงเก็บ SRSC และ PRSC ตามลำดับ น้ำมันกานพลูและสารฆ่าแมลงทั้ง 2 ชนิดดังกล่าวไม่มีผลต่อการงอกของเมล็ด หลังเก็บรักษาเป็นระยะเวลานาน 6 เดือน อย่างไรก็ตามต้นทุนการใช้น้ำมันกานพลูสูงกว่าการใช้สารเคมีมาก การคลุกเมล็ดด้วยคลอไพริฟอสร่วมกับการรมด้วยฟอสฟีน มีต้นทุน 6.90 บาท/ตัน และ 7.50 บาท/ตัน ตามลำดับ ในขณะที่การคลุกและรมด้วยน้ำมันกานพลูมีต้นทุนสูง เท่ากับ 2,568.00 บาท/ตัน และ 1,945.97 บาท/ตัน ตามลำดับ จากผลการศึกษาในครั้งนี้สรุปได้ว่าสารคลอไพริฟอสและฟอสฟีนยังสามารถใช้ควบคุมด้วงงวงข้าวโพดได้อย่างมีประสิทธิภาพ แต่มีประสิทธิภาพต่ำในการควบ คุมมอดข้าวเปลือก ดังนั้นน้ำมันกานพลูจึงเป็นทางเลือกหนึ่งที่จะนำมาใช้ควบคุมมอดข้าวเปลือกได้ หากสามารถลดต้นทุนของน้ำมันกานพลูลงได้

Thesis Title Efficiency of Plant Oil to Control Lesser Grain Borer [*Rhyzopertha dominica* (Fabricius)] and Maize Weevil (*Sitophilus zeamais* Motschulsky) in Rough Rice

Author Miss Kanok-on Wuttiwong

Major Program Tropical Agriculture Resource Management

ABSTRACT

Lesser grain borer (*Rhyzopertha dominica* (Fabricius)] and maize weevil (*Sitophilus zeamais* Motschulsky) are major insect pests leading to qualitative and quantitative damages on rough rice during storage. Chemical seed treatment and fumigation have been used for controlling these insect pests, resulting in adverse effects such as resistant to insecticides and toxic to non-target organisms. The objectives of this study were to screen plant oils and find their appropriate application in a laboratory in order to apply for controlling these pests under warehouse conditions.

Six plant oils were extracted from turmeric (*Curcuma longa*), black pepper (*Piper nigrum*), clove (*Syzygium aromaticum*), citronella (*Cymbopogon nadus*) by water distillation, wild spider flower (*Cleome viscose*) and thiam (*Azadirachta excels*a) by maceration with *n*-hexane. Their repelling, killing and anti-feeding activities were evaluated against *R. dominica* and *S. zeamais* adults in laboratory. The most toxic plant oil was selected to further study for chemical composition analysis, application methods by seed treatment, fumigation and sack coating as well as effect on seed germination as compared to chlorpyrifos and phosphine under laboratory and warehouse conditions. Cost of plant oil application was also compared with chlorpyrifos and phosphine under warehouse conditions. Black pepper oil was the most effective to repel both insect species with 100% repellency at the concentration of 0.47 µL/cm^2 after 48 h of treatment. Clove oil was the most toxic due to the lowest LC_{50} value. Dermal LC_{50} at 72 h were 6.13 µL/L and 3.52 µL/L and inhalation LC_{50} at 48 h were 92.95 µL/L air and 77.63 µL/L air for *R. dominica* and *S. zeamais*, respectively. In addition, clove oil inhibited feeding action with 97.12% and 95.91%

of inhibition against *R. dominica* and *S. zeamais*, respectively. Major chemical components in clove oil included eugenol with 65.83%, *β*–caryophyllene with 13.54% and eugenol acetate 8.29%, respectively.

Effectiveness of clove oil was not different between applications by seed coating and fumigation. At 200 uL/L after 7 d of application, the mortality of *R. dominica* and *S. zeamais* were 99.33% and 95.33% for seed coating, and were 98.67% and 100% for fumigation, respectively. Sack coating method was the least effective with the penetration inhibition through the sack of 38.33% and 46.67% for *R. dominica* and *S. zeamais*, respectively. At the same time of application, the mortality of *R. dominica* and *S. zeamais* were 66.00% and 100%, after seed coating with chlorpyrifos, and were 62.00% and 98.67% after fumigation with phosphine, respectively. It suggests that these two insecticides were lower effective to kill *R. dominica* than clove oil, but all of them were still effective to kill *S. zeamais.*

Under warehouse conditions for six months at Suratthani Rice Seed Center (SRSC) and Phatthalung Rice Seed Center (PRSC), the clove oil application by seed coating combined with fumigation was the most effective to control *R. dominica* with 85.96% and 90.31% as compared to control in SRSC and PRSC, respectively. The application by seeds coating with chlorpyrifos and fumigation with phosphine could control *R. dominica* 40.09% and 40.92% in SRSC and PRSC, respectively. Clove oil and these two insecticides had no affect rice seed germination after six months of storage. However, cost of clove oil application was higher than that of chemical application. Costs of seed coating with chlorpyrifos and fumigation with phosphine were 6.90 Bath/ton and 7.50 Bath/ton, whereas those of seed coating with clove oil and fumigation with clove oil were 2,568.00 Baht/ton and 1,945.97 Baht /ton, respectively. In conclusions, chlorpyrifos and phosphine were low effective to control *R. dominica*, but still effective to control *S. zeamais*. Therefore, clove oil is an alternative method for controlling *R. dominica* under a reasonable cost of application.

ACKNOWLEDGMENTS

Firstly, I would like to express appreciation my sincere gratitude and heartfelt thanks to my advisor, Associate Professor Dr. Aran Ngampongsai, who have valuable constructive comments, suggestions, persistent encouragement and unending benevolence during the course of my study and thesis work and to my co-advisor, Assistant Professor Dr. Yaowaluk Chanbang for suggestions, technical skills of rearing stored-insect pests and recommendations.

I am very grateful my examing committees, Assistant Professor Dr. Patcharaporn Vanichpakorn from Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat province and Assistant Professor Dr. Wisut Sittichaya from Prince of Songkla University, Songkhla province, who gave valuable suggestions, and helpful comments for improving this thesis.

I am much thankful to the Agricultural Research Development Agency (Public organization) for providing financial support throughout this study and this research will not be successful unless supported by Graduate School and the Center of Excellence in Agricultural and Natural Resources Biotechnology (CoE-ANRB), Prince of Songkla University, Thailand. I am thankful to the Department of Pest Mangement and Tropical Agricultural Management Program for supporting me in facilities.

I would like to deeply thank the Department of Rice, Ministry of Agriculture and Co-operative for giving me the prospect to study Ph.D. program. I thank the Surat Thani rice seed center and Patthalung rice seed center for helping and supporting the facility of the field research place. Special thanks go to all my junior and senior friends in entomological laboratory, Panissa Thummasavat, Kanokkarn Taluengphol, Chayaporn Tapdontree, for friendship, help, and encouragement during my graduate study.

Finally, I profoundly wish to express my gratitude to my family, my beloved parents, young brothers and my love, who always affectionate encouragement and support everything to me throughout study and thesis work.

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LIST OF ABBREVIATIONS

µL = microliter

EC_{50} = Effective concentration 50%

LC_{50} = Letal concentration 50%

LC_{90} = Letal concentration 90%

FDI = Feeding deterrent index

PR = percentage repellent

cm = centimeter

RC_{50} = repellent concentration 50%

RC_{90} = repellent concentration 90%

SRSC = Suratthani Rice Seed Center

PRSC = Phattalung Rice Seed Center

CHAPTER 1

INTRODUCTION

1.1 Statement of the problem

Rice is the most economically important crops of Thailand. Rice planting areas in Thailand by 2013/2014 were approximately 77.27 million Rai, producing paddy yield about 36.76 million tons of which 10.30 million tons were exported (Office of Agricultural Economics, 2016). Quality of rice grains and their products in storage are affected by two main important factors including physical factors (temperature and humidity) and biological factors (insects, mites, fungi, birds, and rodents) (Sukprakarn *et al*., 1996; Nakakita *et al*., 1991). The insect pests are considered a serious problem infesting on stored rice worldwide (Snelson, 1987). There were approximately more than 600 species of beetles, 70 species of moths and about 355 species of mites attacking stored grains and stored products, resulting in qualitative and quantitative yield loss (Maniruzzaman, 1981; Rajendran and Sriranjini, 2008). Annual post-harvest due to from insect damage, microbial deterioration and other factors were estimated to be 10–25% of global production and the widespread use of synthetic insecticides has led to the development of resistant strains to pesticides (Mohan and Fields, 2002). In Southeast Asia, post-harvest losses of grains have been estimated ranging from 10-30%, caused mainly losses by improper drying and insect infestation during crop storage and distribution of cereals (Hayashi *et al*., 2004)

Thailand is located in the tropical zone under relatively high humidity and temperature as compared to the temperate zone. Therefore, stored products are seriously infested by several insects, mites, and diseases. The surveying of insect damage in rice store of farmers from 16 provinces showed that the percentage of damage toward insects increased by 1.73% after storage rough rice in the barn for 6-7 months. The key insect pests causing losses of rough rice in storage were classified

into 3 orders: Coleoptera (8 families), Lepidoptera (2 families) and Psocoptera (1 family) (Hayashi *et al*., 2004).

The Coleoptera is a large group and dominant insect pest of rice in storage. *Rhyzopertha dominica* (Fabricius) and *Sitophilus zeamais* Motschulsky are major pests infesting rough rice and mill rice in storage (Visarathanonth *et al*., 2005; Hayashi *et al*., 2004). Female adult of *R. dominica* laid their eggs outside the grain or in the fine powdered grain and then larva punctured inside, living and feeding in the kernel. Female of *S. zeamais* drilled a hole, laid eggs inside the kernel and enveloped by secretes mucilaginous plug. Both larval and pupal stages of these 2 species fed and developed to adult inside the rice kernel on milled and rough rice in storage (Koehler and Pereira, 2012).

The control of these insect pests is the main factor that dependent on the related of synthetic insecticides (Hasan and Reichmuth, 2004). Methyl bromide and phosphine fumigants are widely used for disinfestations of stored food, foodstuffs and other agricultural commodities under storage conditions. They have broad activities, penetrate deeply into the grains and leave a few residues (Mueller, 1990). However, fumigants have serious drawbacks, such as the environmental pollution, insect resistance and harmful effect on workers (Michaelraj and Sharma, 2006). The use of methyl bromide has been highly restricted because of its ozone-depleting potential, which leads to harmful effects of radiation on the organisms living on the earth and subsequently causes cancer skin. Under the Montreal protocol, the world has decided to restrict the use of methyl bromide in 2005 in developed countries and 2015 in developing countries (World Meteorological Organization, 1995). Therefore, phosphine is the remained best option for fumigation in the future. Many stored grain pests have developed resistance to phosphine (Bell and Wilson, 1995; Sayaboc *et al*., 1998), such as *Trogoderma granarium* Evert, *R. dominica* and *Oryzaephilus surinamensis* (Linnaeus) (Ignatowicz, 1999; Zeng, 1999). Visarathanonth *et al*. (1994) reported that in 1991 rice weevil developed resistance to phosphine in Chiang Rai,

Sakon Nakhon and Suphanburi provinces, particularly in Chiang Rai province significantly showed resistance to phosphine approximately 3 times as compared to previous time. In addition, rice weevil developed resistance to other insecticides such as malathion, chlorpyrifos, and deltamethrin. These insecticides have been widely used to control stored-product insects and could be directly sprayed to the wall, fall and ceiling of barn and warehouse. Insecticides can also be sprayed on bags or directly to grains and seeds or may be mixed with seeds; so far stored-product insects have developed resistance to insecticides throughout the world.

Oil from the extracted plant; both essential oils and fixed oils contained secondary metabolites known to have several biological activities against different insect species (Gonzales-Coloma *et al*., 2002; Huang and Ho, 1998). The uses of plant oil extracts in pests control become an important alternative application to protect insect pests in rice storage (Arnanson *et al*., 1987). These secondary compounds derived from some parts of plants, such as leaves, seeds, bark, and root (Bakkali *et al*., 2008). Extracted oils are composed of bioactive chemicals which were safe to mammals, easily obtained, eliminated the risk associated with hand mixing of insecticide and toxic to insect pests in rice storage. (Hamed *et al*., 2012). Several biological actions of plant oils have been investigated for controlling insect pests in the stored product, such as antifeedant (Hough-Goldstein, 1990), repellent (Pugazhvendan *et al*., 2012), contact toxicity (Rani, 2012) and fumigant action (Michaelraj and Sharma, 2006).

Currently, the synthetic insecticides are widely used for management stored-insects pest at the storage facilities and their present times, there causes harmful effect to human, environmental and other animals. These research focuses are considered for finding and development efficacy plant extracted oils, including determines appropriate methods combine used with oil plant extract to control *R. dominica* and *S. zeamais* that their major pests of rough rice during stored in the warehouse. The use of plant extracts has an advantage because their have high

volatility, ability to kill in a broad spectrum and non-residue stability. In addition, use of plant products is a high opportunity to reduce and replace the application of synthetic insecticides (Chlorpyrifos and phosphine) resulted in less harmful to the consumers and environmentally safe and IPM management leading to develop into sustainable agriculture including organic farming in the future.

1.2 Literature Reviews

1.2.1 Importance of insect pests in rice storage

Grains, such as wheat, rice, corn, and legumes serve a large part of the diet of the world's population and feed of animal. These products are stored as dry seeds and formed the only real reserve of food in the warehouse for a long period and may be cause loss of quantity and quality (Snelson, 1987; Chankeawmanee, 2004). The stored grains are subjected to attack by a variety of insects, mites, fungi, birds, and rodents. Insects in storage are a major problem because they easily spread and rapidly increase population (Snelson, 1987). Crop loss due to insect infestation reached about 5-10% but can be above 30% in developing countries. In 1970 in ASEAN countries, the damage of paddy rice after harvested was reported to be about 25%. (Chankeawmanee, 2004).

1.2.2 Lesser grain borer [*Rhyzopertha dominica* (Fabricius); Coleoptera : Bostrichidae]

General information

The lesser grain beetle, *Rhyzopertha dominica* is a very small beetle. The beetle is in a family Bostrichidae of order Coleoptera (Potter, 1935). The most species of this family infest wood, bamboo, and similar cellulose materials and this species could be found in the warehouse (Hayashi *et al*., 2004). The *R. dominica* is a

serious and a primary pest of stored commodities and attacks a wide variety of stored foods including cereals, seeds, and dried fruits; almost all grain, wheat, barley, maize, sorghum and rice (including grain and seed) (Potter, 1935; Plant Biosecurity, 2009). Both larval and adult stages of *R. dominica* are feeding on stored grains and seeds, which clearly produce their damage symptoms of dust around the sacks (Nounwat *et al*., 2005). They produced 90% damage to grain after 5 months in storage (Nukenine *et al*., 2002). Larva and adult fed on rice and adults laid their eggs inside rice kernels, where the larvae could develop to the adult stage (Lee *et al*., 2001). *R. dominica* occurs mostly in tropical and sub-tropical regions of the world, but it has also been found in some warm and temperate zone (Potter, 1935; Haines, 1991). The beetle is characterized as both an external and internal feeder and of both whole kernels stored grain and cereal products. The adults and larvae bore into undamaged kernels, reducing them to the hollow husk. The result of damage can severely reduce grain quality (Koehler and Pereira, 2012).

Biology and life cycle of lesser grain borer

The life cycle of *R. dominica* undergoes complete metamorphosis includes four stages of egg, larvae, pupae, and adult. The female gonads, the processes of vitellogenesis, and establishing of the egg envelopes are similar to other insects described for Pterygota. (Szklarzewicz *et al*., 1992). Larval development occurs more rapidly in whole grains than on flours and first instars larvae penetrate into grains as soon as they hatch from eggs which are laid outside grains (Haines, 1991; Elek, 1994). Both the adults and larvae are capable of boring into and feed on paddy and rice grain and cereals kernel (Elek, 1994).

R. dominica females are laid singly eggs or in clusters which able up to about 20 amongst debris or frass outside grains which may have been damaged by adult feeding (Mason, 2003; Potter, 1935). The female drops eggs loosely into the grain or lays each individually in cracks of grain or seed. The egg production higher

when has been observed at high temperatures (Howe, 1950). Under optimum environmental conditions as 28–32 °C and 70–80% RH (Astuti *et al.,* 2013). The number of eggs lays per female average from 200 to 500 during her lifetime (Howe, 1950; Osuji, 1982). The eggs are shaped like a pear, freshly laid eggs are white and shining, and they change color are pinkish and opaque before hatching as larvae developed inside the eggshell (Potter, 1935; Vardeman *et al*., 2007b). The average time period of the egg stage is 5-6 days during summer, 7-11 days during autumn and much longer time in winter (Vardeman *et al*., 2007b).

The neonate emerging out from the eggs laid near the grain straightaway enters into the grain (Birch, 1945). They are quite active, the body usually C-shaped and creamy white in color (Potter, 1935). The first instar larvae only prefer to penetrate in the grain kernel and feeding on the part of germ rather than the endosperm part of the grains (Mahroof and Phillips, 2006; Edde, 2012) because the second stage larvae, toward its curved shape that cannot penetrate into the grain (Vardeman *et al*., 2007b). They are four larval instars (Mason, 2003) and the first and second larvae stages of *R. dominica* are mostly mobile and later instars become immobile (Potter, 1935; Guedes *et al.,* 1996). The *R. dominica* larvae growth are faster in the whole grains than derived products of grains such as flour, frass and that normally takes about 30–46 days at 25ºC and 27–31 days at 28ºC before developed to pupa (Majeed et al., 2015). Larva feed and develop inside a grain kernel resulting eventually cause damage to germplasm and endosperm and lead to grain weight loss and grain quality loss (Mason, 2003; Chanbang *et al.*, 2008).

The lifespan of pupal stages is about 8 days at 25ºC and 5–6 days at 28ºC (CSIRO-SGRL; Mason, 2003; Hodges, 1986). The length of the pupa is about 3.9 mm, and having white to brownish color (Potter, 1935; Nguyen, 2006). Pupation takes place inside the hollow shell of the seed or in the “flour” that accumulates with infested grain. *R. dominica* able to develop on grains of a very low moisture content (about 9%) When the end completion of the pupal stage, newly emerged adult that

chewed its out through the outer grain layers. (Birch, 1945; Haines *et al*., 1991; Koehler and Pereira, 2012).

Diagnosis characters

The *R. dominica* adult is usually 2–3 mm in length and cylindrically shaped (Vardeman *et al*., 2007b). The body color after emerges 7–10 days is yellowish brown and darkens slowly when the past of time and becomes reddish-brown (Majeed *et al*., 2015). The head of the beetle is tucked underneath the prothorax and is not visible from above (Vardeman *et al*., 2007b). The adult usually remains within grains kernel for a few days after comes outside when its cuticle hardens. *R. dominica* normally completes lifespan of egg to adults in about 25 days at 34°C and 68 ± 5% RH and about 60 days at 25 °C and 75± 5% RH (Howe, 1950; Edde, 2012). The adult beetle is longevity up to 4-8 months (CSIRO-SGRL, Mason 2003). In addition, the adults mate soon after emergence but oviposition begins approximately 15 days and can last up to 4 months. Female of the beetles survive for several days after oviposition ceases (Mason, 2003). Moreover, after mating only that *R. dominica* females produce large amounts of frass consisting of chews but undigested grains (Hodges, 1986). The life cycle of *R. dominica* is shown in Figure 1.

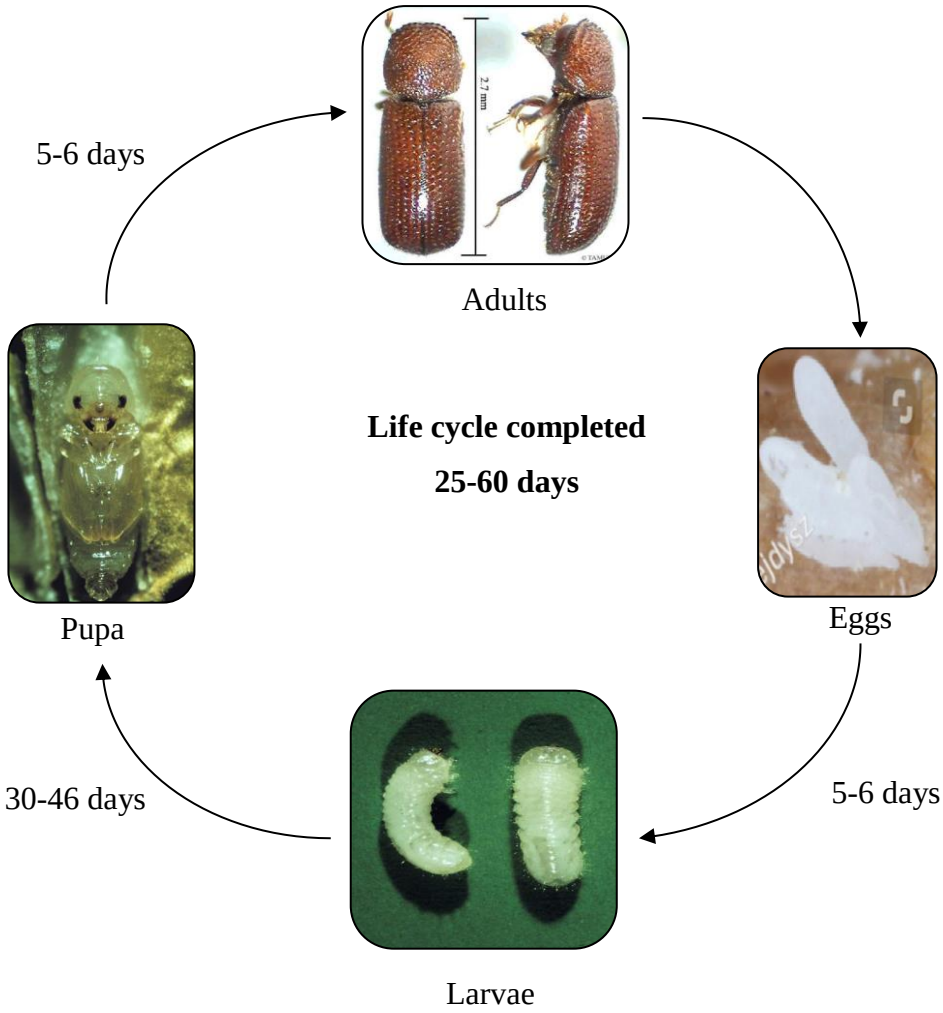

Figure 1 Life cycle of *Rhyzopertha dominica* (Fabricius)
Source: PaDIL (http://www.ars.usda.gov) and https://www.shutterstock.com

Damage of lesser grain borer

There have been several reports of a small population of *R. dominica* on cereals in the field, but the infestation is mostly post-harvest (Majeed *et al*., 2015). The grains very considerable weight loss occurs as a result of the heavy attack and further damage may be done by the insect boring into the small tunnels of the grains in the store (Birch, 1945). Grain infestations may be resulted from residual insect populations between the storage processes and mixed with infested and un-infested

grains or individuals from external sources (Fields and Phillips, 1994; Hagstrum, 2001). *R. dominica* adult is a strong flyer (Mason, 2003) which can spread at least one mile away from release areas (Jia *et al*., 2008). Their adult migrated into stored seed or grain by wind from infested storage to new storage (Vardeman *et al*., 2007a). After adults of *R. dominica* alight on the sack surface, it gradually drills into a sack and moves through into the grain (Vardeman *et al*., 2007a; Hagstrum, 2001). The behavior of *R. dominica* could move down into the grain mass to a depth of 12 m, which is deeper than other grain beetles (Flinn *et al*., 2010). Correspondingly, the beetle moves upward into the grain mass with residual infestations at the bottom. The ability of *R. dominica* to move deep within the grain mass and the ambiguous feeding within the grain kernels make the early to detection of infestations difficult.

Moreover, the grains infested by *R. dominica* have an attribute sweetish odor, which is because the male adult produced aggregation pheromones (Khorramshahi and Burkholder, 1981). The activities of adult feeding produced large amounts of frass that most of which consists of endosperm mixed with a part of the flour. The frass contains with larvae exuviae, feces, fragments of immature stage of insects, and other, which could affect the quality of the grain (Sanchez-Marinez *et al*., 1997). It is found that commodities infested by *R. dominica* rarely become mold and may cause an increase of 5-6% of food moisture (Birch, 1945).

1.2.3 Maize weevil (*Sitophilus zeamais* Motschulsky; Coleoptera: Curculionidae)

General information

S. zeamais (Maize weevil) is a serious pest of grain and seeds during pre-harvest and storage. It is a species in the family Curculionidae and order Coleoptera. It is a major pest of stored rice including can infest undamaged grain and feed directly on grain, maize, wheat, peas, cotton seed and other stored product, especially rice and maize. However, Sukprakarn *et al*. (1984) reported that *S. zeamais*

was mostly abundance in rough rice, (paddy rice), milled rice, corn, oat, sorghum and barley in Thailand. The adult of maize weevil feeds into the grain and develops immature stages within the grain kernel. Therefore, its infestation affects the decline in the grain quality and produced a lot of dust mixed with frass (Longstaff, 1981). *S. zeamais* could indirectly effect which producing heat by their in infestation grain (Longstaff, 1981). Its produced more than 22% damage to grain after 6 months storage (Sukprakarn *et al*., 1996). Generally, *S. zeamais* infested to pre-harvesting crop when the high moisture contents exceed 20% (Longstaff, 1981).

Biology and life cycle of maize weevil

The adults mate soon after emergence 7 days and female start to lay eggs individual inside the paddy rice, up to 300-400 eggs during their lifetime that about 4-12 mouths depend on the grain quality being infested (Lyon, nd.). The maximum of fecundity in daily rate were 6.7 eggs per female and optimal condition was produced number of progeny at 30^{o}C and 75% RH (Throne and Cline, 1994). The female of weevil drills a hole into the grain kernel lays a single egg in the cavity of grain, and then the female adult secretes a mucilaginous plug for enclosing them egg as the ovipositor is withdrawn. The egg plug rapidly hardens, raised slightly above the seed surface, which provides the only external premise for indicating the grain kernel is infested. The eggs were laid deposit in the endosperm, but 28% were laid around in the germ (Throne and Cline, 1994). The most of the eggs are deposited at the end farthest from the embryo in wheat grain. The egg may be laid more than one egg in a single grain kernel, but the development of more than one larva to maturity is rare, because of cannibalism behavior (Longstaff, 1981). Oviposition of *S. zeamais* takes place under a temperature of 15–35 °C and moisture content at 12% (Howe, 1952). The eggs hatched from grain within 3-6 days.

The larval of *S. zeamais* are four instars which remain within the grain. Immediately of hatching, the first instar fed borrowing through into grain tissue. The

white larva fed and developed inside the grain. The end instar used a mixture of frass and secreted mucilaginous plug to enclose of the hole for form pupal (Longstaff, 1981). The pupae development inside the grain and pupal stage ranged 3-7 day.

The adult of *S. zea*mais has developed; it remains within the grains for several days before emerging as adult beetles by biting a circular exit hole through the grain (Longstaff, 1981). The maize weevil adult is small insect and body length of 3–3.5 mm. The maize weevil and rice weevil are similar in appearance except body longer. The body color of *S. zeamais* is brown and it has four reddish-brown spots on the wings (elytra) and more clearly marking sport defined. The reddish-brown spot of *S. zeamais* adult is almost black and large. It has snout as long and thin, antennae are elbowed type. It is developed hide-wing able to flies readily as far away and strong (Halstead, 1963). The *S. zeamais* can be distinguished from *S. oryzae* by characters of male aedeagus and female genitalia (Hayashi *et al*., 2004). The male aedeagus of *S. zeamais* has two longitudinal grooves, while *S. oryzae* has a smooth surface and equally convex (Hayashi *et al*., 2004). In addition, a female genitalia of *S. zeamais* has the Y-shaped on the lateral lobes of apical regions, while *S. oryzae* has round and blunt (Halstead, 1963). The life cycle of *S. zeamais* is complete metamorphosis and averaged developing time 36 days (ranged 33-45 days) at 27 ± 1^{o}C, and 69 ± 3% RH (Sharifi and Mills, 1971). After emergence, females moved to above the food surface and released sex pheromone to attract males for mating (Mason, 2003). The life cycle of *S. zeamais* is shown in Figure 2.

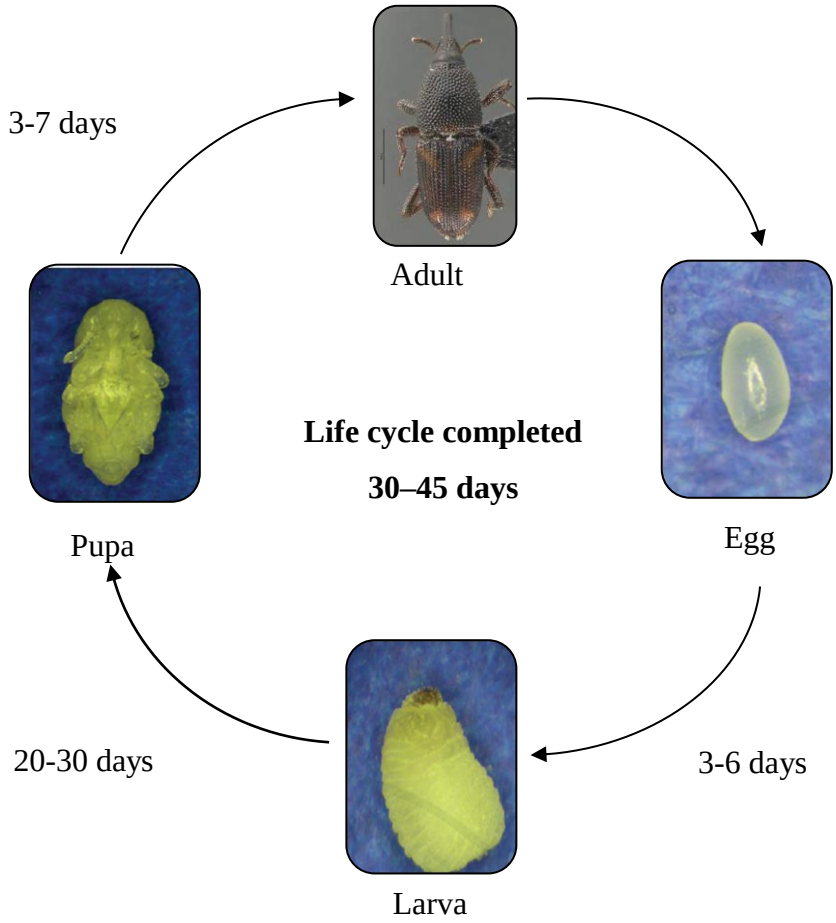

Figure 2 Life cycle of *Sitophilus zeamais* Motschulsky
Source: Suthisut (2011)

Damage of maize weevil

Infestation by maize weevil begins in the field, but most damage occurs during storage (Rees, 1996). The *S. zeamais* feed on single grains, leaving only the hulls, and a severe interference that can be reduced to a stored grain mass, flour-like, and powdery frass. The infested grains present holes that adults have emerged. Grain which floats on the surface of water often indicates that larvae destroy inside the grain kernel (Rajendran, 2005). The larval stages feed on the internal portions of whole grains, make early and difficult detection of infestations. It does not only reduce the quality of the grain but also produce large amounts of heat, grain dust and frass (Longstaff, 1981; Lyon, nd.). Seed stored was infested that resulted in seed

weight loss, reductions in nutritional value, lower germination percentage and lower commercial value (Yuya *et al*., 2009). The commercial value of the infested grain is reduced by contaminating with uric acid, fragments of insect body, and other toxic substances (Borikar and Tayde, 1979; Gupta *et al*., 2000). It also attracts the grains to be attacked by storage fungi (Subramanyam *et al*., 1992).

Rice and maize weevils are extensively distributed in tropical and sub-tropical regions. The *S. zeamais* infested on the grain while still in the field depending on the temperature (Demissie *et al*., 2008). In the field, the flight period curve of *S. zeamais* adult was bell-shaped and very little occurred in the night. Whereas in the warehouse, flight periodicity curve was flat-topped and flight activity was prolonged time during the night but they can not flight often (Taylor, 1971). The population of weevil is the most insidious due to owing largely to the ability to fly away and rapidly distributions throughout a storage. (Sukprakarn *et al*., 1996; Nukenine *et al*., 2002). The percentage of weight loss on milled rice due to *S. zeamais* infestation was 14.8% within 3 months of storage (Sidik and Pranata, 1988) and increasing up to 90% damage after 5 months of storage (Nukenine *et al*., 2002).

1.2.4 Chemical control of insect pests in rice storage

Normally, the control of *R. dominica* and *S. zeamais* are primary treatment dependent upon the repeated application of synthetic insecticides (Hasan and Reichmuth, 2004). Chemical control with synthetic insecticides (organophosphates and pyrethroids) and fumigants (phosphine) is a common practice which has been used for many years to control pests of stored grains (Salem *et al*., 2007). However, the potential adverse effects of synthetic insecticides have been raised in several ways such as toxic to consumers over insecticide residues in foods, the occurrence of insecticide-resistance of insect strains, the ecological consequences, the increased of the cost of application and the precautions necessary to work with

traditional chemical insecticides, which call for new approaches to control stored-product insect pests (Aslam *et al*., 2002 and Fields, 2006).

Methyl bromide and phosphine fumigations have been used for decades to control stored insect pests (Islam *et al*., 2009) and belonged to the most effective application to protect stored food, foodstuffs, and other agricultural commodities. The fumigants are effective to kill pest in broader activities and easily penetrate into the grains (Mueller, 1990; Emekci and White, 2002). According to the Montreal Protocol in 2000, methyl bromide was decided to restrict use in 2005 in developed countries and phased out by 2015 in developing countries; it was proven that this cause ozone depletion potential in the Stratosphere (World Meteorological Organization, 1995). The growers are disused methyl bromide as a post-harvest fumigant because of ozone-depleting nature, whereas phosphine, which repeated use as it disrupts the ecological system resulting to the development of insect resistance (Ignatowicz, 1999; Zeng, 1999). Resistance will probably cause to discontinuation of phosphine use as a fumigant in several countries in the near future (Mueller, 1990). Therefore, other fumigant options should be investigated to replace methyl bromide and phosphine in the future.

1.2.5 Natural products from the plant for controlling stored-product insects

Plant extracts and essential oils have recently attracted particular attention as alternative pest control agents due to their reduction in losses of agricultural produce, low toxicity to warm-blooded mammals, reduce environmental pollution and their high volatility (Shaaya *et al*., 1997; Li and Zou, 2001, Park *et al*., 2003). Essential oil constituents are monoterpenoids, which are secondary plant chemicals and considered to be of little metabolic importance. The toxicity of a large number of essential oils and their compositions has been evaluated against a number of stored-product insects. They contained a rich source of bioactive metabolites which

show antifeedant, repellent and toxic effects to insects (Rajendran and Sriranjini, 2008).

Both crude extracts and essential oils of many plant species exhibit bio-insecticide activities against stored-product insects. Liu and Ho (1999) revealed that the essential oil extracted from *Evodia rutaecarpa* Hook f. et Thomas, had the contact and fumigant toxicity, as well as repellent and minor feeding deterrent activities with both adults of *T. castaneum* and *S. zeamais* and larvae of *T. castaneum*. In recent years, the results of several studies showed the repellent and antifeedant actions of potential oils from plants to control stored insect pests, mites, aphids and mosquitoes (Takabayashi and Dicke, 1996).

The oil of the neem seeds (*Azadirachta indica*) contains azadirachtin (AZA) and other potent bioactive compounds, which are effective to control pests of stored grains (Boeke *et al*., 2004). The bioactivity of neem is attributed to various compounds, especially to its main component, the tetranortriterpenoid azadirachtin, which acts as an insecticide in the deterrence of the feeding and oviposition, as a repellent and in the growth inhibition of insects (Morgan, 2009). Liu and Ho (1999) studied the bioactivity of the essential oil extracted from *Evodia rutaecarpa* Hook f. et Thomas against the grain storage insects, *S. zeamais* Motsch. and *T. castaneum* (Herbst). The toxic, repellent and feeding deterrent activities were recorded for the essential oil extracted from *E. rutaecarpa* against *S. zeamais* adults and *T. castaneum* larvae and adults. Phetwaikul *et al.* (2009) investigated a potential of essential oil from the pomelo peel for controlling maize weevil. The essential oils at the concentration of 121 µl completely inhibited survival of adult maize weevil, oviposition, and eclosion after 48 hours of exposure in a bottle size of 250 cc with 25 g brown rice.

Suthisut *et al.* (2011) studied the fumigant toxicity of essential oils from three Thai plants (Zingiberaceae), including *Alpinia conchigera*, *Zingiber zerumbet*, and *Curcuma zedoari* against *S. zeamais* and *T. castaneum*. It was found that *A. conchigera* oil had highly toxic to *S. zeamais* and *T. castaneum*, while the other two plant oils had low toxicity. Furthermore, adults of *S. zeamais and T. castaneum* were more susceptibility to *A. conchigera* oil than their eggs, larvae or pupae. *S. zeamais* adult (LC_{50} 85 mL/L in air) was slightly more tolerant to *A. conchigera* oils than *T. castaneum* (LC_{50} 73 mL/L in air) after 48 h of treatment. Synthetic essential oils were more toxic to both insects than the extracted essential oils. *Z. zerumbet* oil (LC_{50} 26 mL/L in air) and *C. zedoaria* oil (LC_{50} 25 mL/L in air) were higher toxic to adults of *A. calandrae* than *A. conchigera* oils (LC_{50} 37 mL/L in air).

The essential oils from cinnamaldehyde, α-pinene, anethole, clove extract (*Syzygium aromaticum*) and star anise *(Illicium verum*) have efficiency for fumigation and antifeedant actions to red flour beetle *(T. castaneum*) and maize weevil *(S. zeamais*) (Ho *et al*.,1997; Huang and Ho, 1998). Bouda *et al.* (2001) studied the influencing of volatile oils from the leaves of *Ageratum conyzoides*, *Lantana camara* and *Chromolaena odorata* to the mortality of *S. zeamais*. The results showed that the *A. conyzoides* gave the best insecticidal activity with the lowest LD_{50} of 0.09% within 24 hours. Kanyarat *et al.* (2013) investigated the repellent activity of volatile oils from 10 plant species to maize weevil. The coriander seed, lemon grass, kaffir lime, and pepper were extracted the volatile oils by hydro-distillation. Sweet basil, betel vine, Indian coral tree, wild betel, celery, and garlic were achieved by simultaneous distillation extraction. All plant oils at the highest rate of 8 μL/cm^2 showed the potent repellent of 80.1-100% classified as level 5 (determined the level of maize weevil 0-5 class). Plant oils from lemon grass, kaffir lime, pepper and betel vine at 1, 2, 4 and 8 μL/cm^2 had repelled as level 5 followed by those from coriander seed and garlic at all concentrations (1, 2, 4 and 8 μL/cm^2).

Xie *et al*. (2010) studied fumigation toxicity of horseradish essential oil from *Armoracia rustican* against *S. zeamais* and *R. dominica.* The results showed that at 3 ppm concentration of horseradish oil killed all adult of *S. zeamais* and *R. dominica* on the maize, wheat and paddy at 72 hours of exposure at 25^{o}C. At 24 ppm of horseradish oil, the mortality of *S. zeamais* in maize, wheat, and paddy was 100%, 100%, and 98%, respectively, after exposure for 72 hours at 25^{o}C. While, the mortality of *R. dominica* was 100%, 93%, and 86%, respectively, under the above conditions.

Jemâa *et al*. (2012) studied on insecticide activities of essential oils from the leaves of *Laurus nobilis* L., Tunisia, Algeria and Morocco for repellent and toxic activities against *R. dominica* and *T. castaneum.* Fumigant activity of *L. nobilis* essential oil from Morocco was more effective to mortality than Tunisian and Algerian oils. Their LC_{50} values were 68, 99 and 113 ml/l air for *R. dominica* and 172, 194 and 217 ml/l air *for T. castaneum*, respectively. The oils were more toxic to *R. dominica* than to *T. castaneum*, both when calculated of LC_{50} or LT_{50}. Also, probit analysis showed that both insects were more susceptible to Moroccan oil than to Algerian or Tunisian oil. LT_{50} ranged 14 to 20 h for *R. dominica*, and ranged 43 to 56 h for *T. castaneum*. Huang and Ho (1998) reported that essential oils from cinnamaldehyde, α-pinene, anethole, clove extract (*Syzygium aromaticum*) and star anise (*Illicium verum*) were effective as an antifeedant against red flour beetle (*T. castaneum*) and maize weevil (*S. zeamais*).

Thesis objectives

1. To evaluate the repelling, anti-feeding and killing activities of six plant oils against *R. dominica* and *S. zeamais* in rough rice in laboratory and chemical composition analysis of the most effective plant oil

2. To assess the application methods by seed treatment, fumigation and sack coating of the most effective plant oil against *R. dominica* and *S. zeamais* in the laboratory.

3. To evaluate the efficiency of the most effective oil for controlling *R. dominica* and *S. zeamais* and their effect on seed germination in rough rice under storage conditions.

4. To compare application costs between the most effective oil and synthetic insecticide for controlling *R. dominica* and *S. zeamais* under storage conditions

Outcomes of the research

1. Know the quantity, chemical composition and major compound of plant oils possessing the potential to prevent stored rice pests.

2. Know the efficiency of oils extracted from plants that are able to manage stored insects on paddy rice.

3. Have alternative application methods using plant oils to control insect pests during storage of seeds to reduce the use of chemicals and contribute to the development of organic crop production for sustainable agricultural development.

CHAPTER 2

Research Methodology

1. Insect rearing

The adults of *Rhyzopertha dominica* and *Sitophilus zeamais* were collected from Surat Thani rice seed center, Surat Thani province, Thailand. They were reared separately in a cylindrical plastic container (7.5 cm diameter and 13 cm height), containing paddy rice with moisture content at 15% mixed with barley in a ratio of 2:1. The container was covered with muslin cloth and closed firmly with a rubber band to prevent the escape of insects (Figure 3). Prior to use for insect rearing, paddy rice was subjected to cool condition by keeping in a freezer at a temperature of 4-6 ºC for 14 days to killing other insects contaminated in the grains. Hundred unsexual adults of *R. dominica* and *S. zeamais* were released in each plastic container and kept under conditions of completely dark, the temperature at 30 ± 3 °C and 76 ± 5 % RH. All insect adults were sieved out of the container after 7 days and they were moved to a new container and fed with a new food. Egg-infested grains were kept in the dark. After adult emergence, only 0-14 days old adults of both insect species were used for all experiments according to Shayesteh and Ashouri (2010).

Figure 3 Cylindrical plastic containers filled with paddy rice mixed with barley (2:1) for insect rearing.

2. Plant oil extraction

Six plant species and their parts used for extraction in this study are listed in Table 1. All dried plant samples were purchased from an herbal shop in Hat Yai, Songkhla, Thailand, except thiam seeds which were naturally collected from agroforestry in Songkhla province. Fresh thiam seed kernels were exposed to sunlight for 2-3 days, subsequently ground through a blender prior to maceration with *n*-hexane for 7 days (Figure 5B). The maceration process was repeated 5 times. Crude oil extracts were filtered before *n*-hexane removal by a rotary evaporator (Figure 5C). This crude oil was used for the experiments. Wide spider flower (Figure 4D) was also extracted as the same method with thiam seed (Figure 4E). Other four plant species, including turmeric (*Curcuma longa*) (Figure 4A), black pepper (*Piper nigrum*) (Figure 4B), clove (*Syzygium aromaticum*) (Figure 4C) and citronella (*Cymbopogon nadus*) (Figure4D) were extracted by water distillation apparatus (Figure 5A). One-hundred grams of air-dried samples were placed in the flask containing (1,000 mL) mixed with 500 mL of sterile water. The distillation process was undertaken for 12 hours and water was eliminated by using sodium sulfate anhydrous. Oils obtained from these two processes were stored in a refrigerator at 10–12 ºC until use for the experiments. In addition, the yield of oil extraction was calculated as a percentage of yield (% yield) (Table 1) according to the following formula.

$$\text{Percentage of yield (\%yield)} = \frac{\text{weight of oils extracted (mL)}}{\text{weight of plant material}} \times 100$$

Table 1 Plant species, plant parts and extraction methods used for the experiments.

Common name (Scientific name)	**Plant part extraction**	**Method of extraction**
Turmeric (*Curcuma longa*)	Dried rhizomes	Water distillation
Black pepper (*Piper nigrum*)	Dried fruit	Water distillation
Clove (*Syzygium aromaticum*)	Dried flower bud	Water distillation
Citronella (*Cymbopogon nadus*)	Dried leaf and stem	Water distillation
Wild spider flower (*Cleome viscose*)	Dried stem and flower	Maceration
Thiam (*Azadiracta excelsa*)	Dried seed kernel	Maceration

Figure 4 Plant species; turmeric rhizome (A), black pepper fruit (B) clove flower bud (C) citronella leaf and stem (D), wide spider flower stem and flower (E) and thiam seed (F).

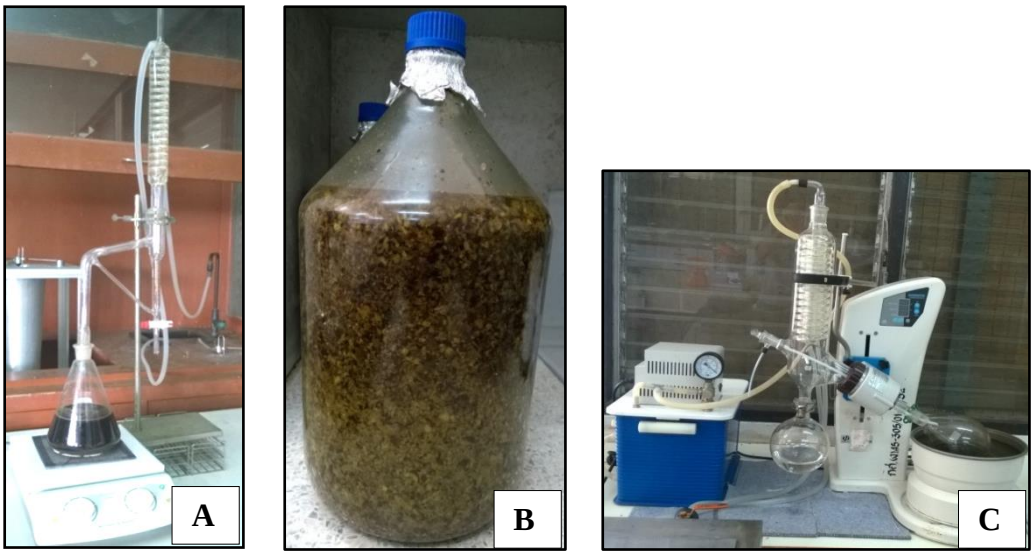

Figure 5 Extraction of plant oils using water distillation apparatus (A) and maceration method (B) before solvent removal by a rotary evaporator (C).

3. Bioactivity tests of six plant oils against *Rhyzopertha dominica* and *Sitophilus zeamais* in laboratory and chemical composition analysis of the most effective plant oil

3.1. Bioactivity tests of six plant oils

1. Repellent test

The repellent action of all plant oils mentioned above was tested against *R. dominica* and *S. zeamais* by petri-dish choice bioassay described by McDonal *et al*. (1970); Ko *et al*. (2009) and Sagheer *et al*. (2014). This technique can be used for repellent pre-screening test of plant extracts under closely stored conditions and short time of investigation (Pretheep *et al*., 2004). Each plant oil extract was dissolved in acetone achieving difference concentrations (0.1, 1, 5, 10, 15, 20 and 40 μL equivalent to 0.003, 0.03, 0.16, 0.31, 0.47, 0.63 and 1.26 μL/cm^2, respectively). Filter-paper (9 cm diameter, the surface area of 63.6 cm^2) was cut into

two equal halves (Figure 6A). One half of each disc was treated with 500 μL of oil plant extract solution as uniform as possible by using a micro pipette. The other half of the filter-paper (control) was treated with 500 μl of acetone only (Figure 6B). The oil treated and acetone treated filter-papers were air-dried for 10 minutes to evaporate the remaining solvent completely.

Oil treated and acetone treated half-discs were carefully then attached lengthwise, edge-to-edge with adhesive tape and were placed in the bottom of a glass petri dish (Figure 6C). Ten unsexed adults of *R. dominica* and *S. zeamais* were released at the center of each filter-paper circle and above petri dish edge was coated with vaseline for preventing insect escape before covering a petri dish lid and kept in the dark (Figure 6D). Five replications were done for each concentration of the plant oils extracts. The number of insects presented on treated and untreated halves were recorded after 1, 2, 3, 4, 5, 6, 12, 24 and 48 hours. After 48 hours, individuals number of insects presented on the treated part of the filter paper which compared with the untreated part. The percentage repellency (%PR) was calculated according to Nerio *et al*. (2009) as the following formula:

$$PR\ (\%) = \frac{Nc\text{-}Nt}{Nc+Nt} \times 100$$

Nc: the number of insects on the control half

Nt: the number of insects on the treated half

Accumulative percentages are classified into 6 repellent levels following by Juliana and Su (1983):

Class 0 PR < 0.1 %	Class III 40.1 - 60 %
Class I 0.1 - 20 %	Class IV 60.1 - 80 %
Class II 20.1 - 40 %	Class V 80.1 - 100 %

Probit analysis was also used to calculate the median repellent concentration EC_{50} and EC_{90} (effective concentration that repels 50% and 90% of the exposed insects). In addition, effective time for 50% (ET_{50}) repellency was calculated

for both of insects. The repellent percentages were performed for analysis of variance (ANOVA) and significant differences of means among treatments were compared by Tukey's multiple range tests.

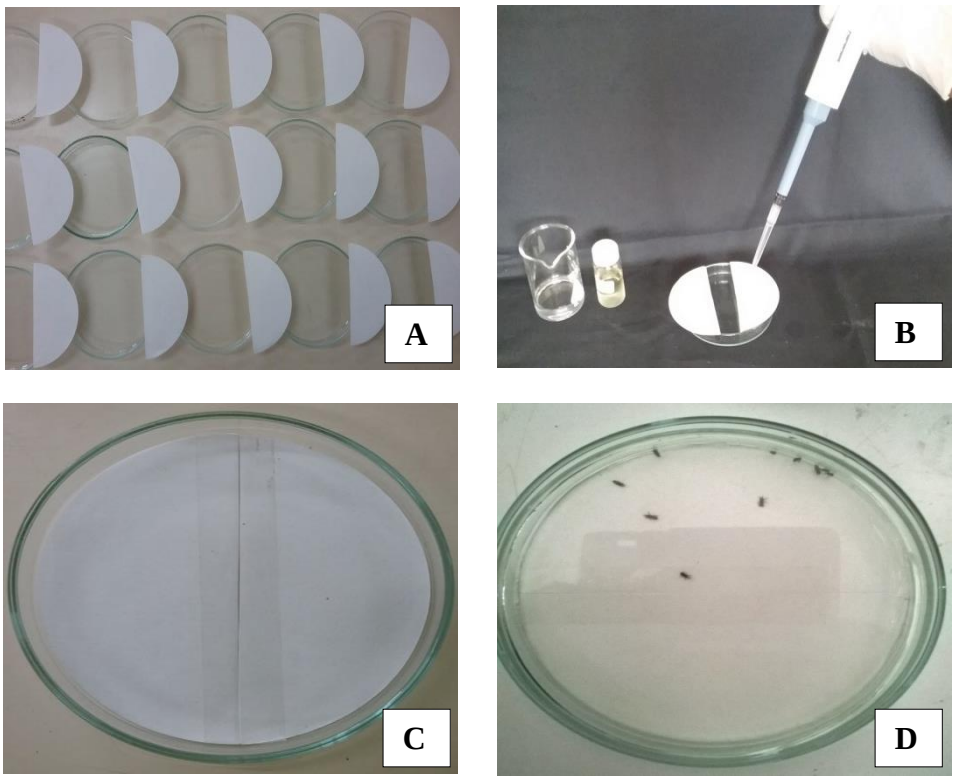

Figure 6 Halves cut of filter paper used for repellency test (A) application of tested oil solutions on the filter paper (B); attachment lengthwise of treated half and control half (C) and tested insects moving to edge of petri dish after releasing ten adults at the center of a petri dish (D).

2. Contact toxicity test

The insecticidal contact activity of plant oils was determined by impregnated filter paper application. A serial dilution of each oil dissolved in acetone was prepared and 1 ml of oil solution was subsequently applied on each of filter paper (9 cm diameter) by using a micro pipette in a comparison with acetone as the control (Figure 7A). Filter papers were treated with different concentrations of oil solutions at 0, 10, 40, 80, 120, 160 and 200 μL (equivalent to 0, 0.16, 0.63, 1.26, 1.89, 2.52, and 3.15 μL/cm^2). After the remaining acetone had completely evaporated for 10 minutes, ten unsexed adults of tested insects were placed in a glass petri dish (9 cm diameter) and above petri dish was coated with vaseline for preventing insect escape (Figure 7B). The experiment was arranged by using a completely randomized design (CRD) and all treatments were replicated five times. After 12, 24, 48 and 72 hours, insect mortality was recorded. Death of insect was recorded with absence movement of antennae and legs (Figure 7C).

Corrected mortality percentage was calculated by using Abbott's formula (Abbott, 1925) as shown below. Probit analysis was performed to calculate the lethal concentration for 50% (LC_{50}) and 90% (LC_{90}). The percentages of mortality at 12, 24, 48 and 72 hours were performed for analysis of variance (ANOVA) and significant differences of means among treatments were compared by Tukey's multiple range tests.

% Corrected Mortality

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

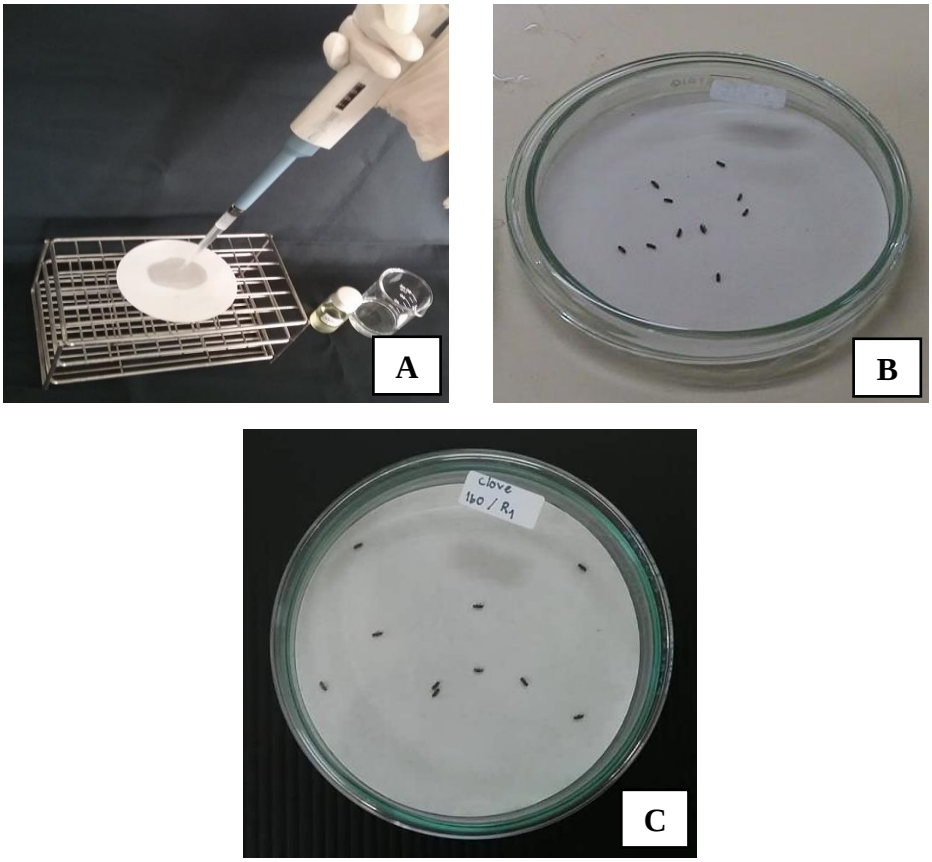

Figure 7 Contact toxicity test by application of tested oil solution on filter paper (A); placing tested insects on the center of filter paper prior to keep under room temperature (B) and recorded the death of insects with the absence of antennae and leg movement (C).

3. Fumigant toxicity test

Fumigant test method used in this bioassay was described by Suttisut (2011). Filter-paper was cut into 2 cm diameter pieces (Figure 8A) and each filter-paper was treated with the different oil concentrations of 0, 1.5, 3, 6, 9, 12 and 20 μL equivalents to 0, 75, 150, 300, 450, 600 and 800 μL/L air (Figure 8B). A serial dilution of each oil dissolved in acetone was prepared and 100 µl of oil solution was subsequently applied on each of the filter paper. After air-drying for 2 minutes, the treated filter paper was then attached to the under-surface of the screw cap of a glass vial (20 mL). Acetone solution alone was used as the control. After ten unsexed adults of *R. dominica* and *S. zeamais* had placed in each vial without foods and above glass vial was coated with vaseline for preventing insect escape, the caps were tightly screwed (Figure 8C). The vials were placed in the box and kept under room temperature in the laboratory (Figure 8D and 8E). Each concentration and control was replicated five times. Mortality was checked for 12, 24, 48 and 72 hours after fumigation. An absent movement of the antennae and legs of tested insects was considered to be dead insects.

The corrected mortality percentage was calculated by using Abbott's formula (Abbott 1925) as shown below. Probit analysis was performed to calculate the lethal concentration for 50% (LC_{50}) and 90% (LC_{90}) to insect mortality. Analysis of variance was done for the percentages of mortality at 12, 24, 48 and 72 hours. Significant differences of means among treatments were compared by Tukey's multiple range tests.

% Corrected Mortality

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

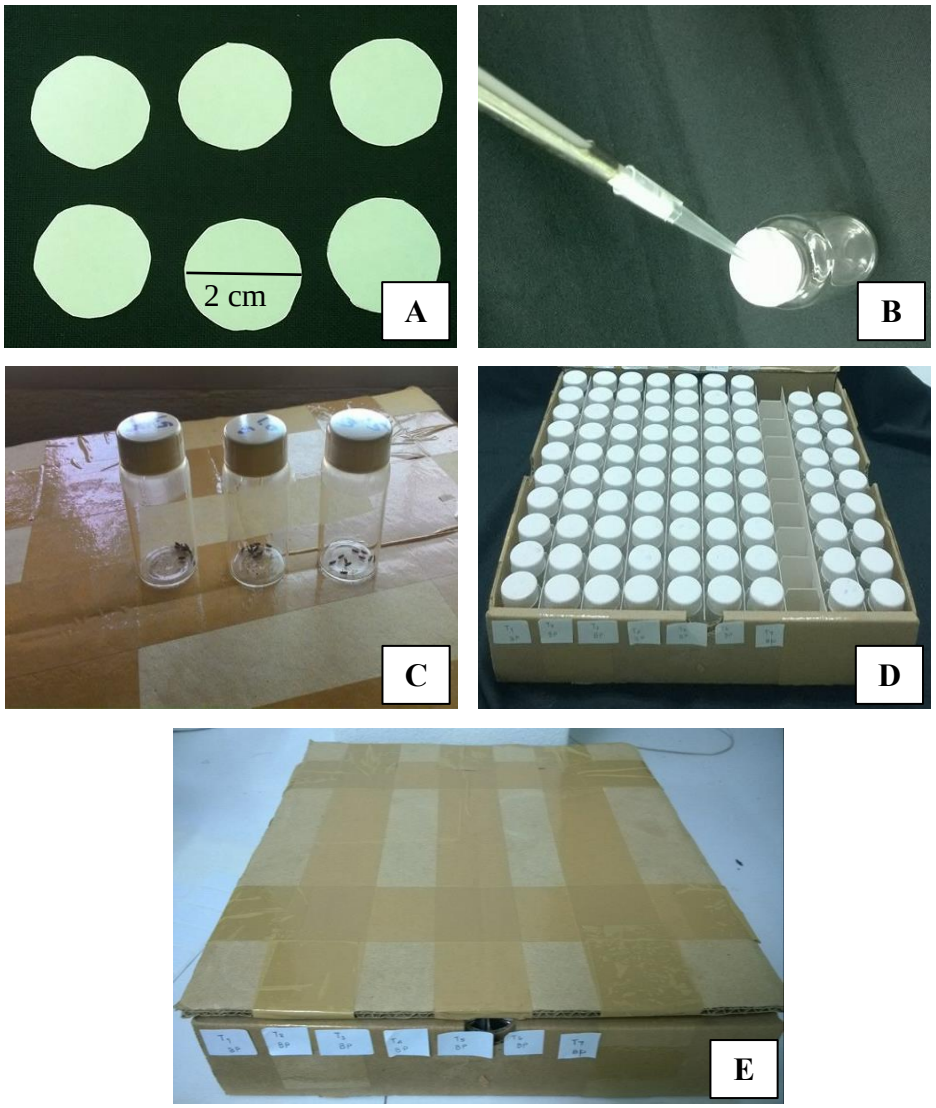

Figure 8 Fumigant toxicity test of the application of plant oil solutions on a 2-cm diameter filter paper (A, B) prior to place insects into the vial (C) and sealing the vial cap with parafilm (D) keeping tested vials in the box under room temperature (E).

4. Toxicity test by ingestion

Flour disks are prepared according to the method of Xie *et al*. (1996) with some modifications (Huang and Ho, 1998). In this study, feeding deterrence was adopted by the no-choice test. They were made of 200 μL of a stirred suspension of wheat flour in water (mixing 20 g of wheat flour in 50 mL of water) (Huang *et al*., 2000; Xie, 1996). Two hundred microliter (200 μL) of wheat flour suspension have then taken onto a plastic sheet by micro-pipet (Figure 9A). Flour disks on the plastic sheet were allowed to dry for 24 hours at room temperature and subsequently dried in an oven at 60°C for 1 hour (Figure 9B). Each plant oil sample was diluted in acetone to attain different concentrations of 0, 1, 3, 5, 10, 15 and 30%. A 5 μL of each concentration was dropped onto the surface each flour disks (Figure 9C). Acetone was used as the control.

A completely randomized design was arranged for the experiment. Treatment comprised different concentrations of tested oils in a comparison with acetone as the control. Each treatment was replicated five times. After dropping acetone-dissolved oils on the flour disks, the solvent was allowed to evaporate for 1 hour under room temperature. Two flour disks of each treatment were weighed (Figure 9D) and placed in each petri dish (9 cm diameter, 1.5 cm height). All adults of *R. dominica* and *S. zeamais* were starved for 24 h before use in the experiment. A group of 20 unsexed adults of both species was separately released in each petri dish and above petri dish was coated with vaseline for preventing insect escape. After three days, the flour disks were weighed again and insect mortality was recorded. Death of insect was considered to the absence of movement or response to feeding with a blunt probe (Figure 9E).

The corrected mortality percentage of insect was calculated by using Abbott's formula (Abbott 1925). Probit analysis was performed to calculate the oral lethal concentration for 50% (LC_{50}) and 90% (LC_{90}). ANOVA was performed for the percentages of mortality after 3 days and significant differences of means among

treatments were compared by Tukey's multiple range tests. Feeding deterrent action was calculated by using the formula described by Isman *et al*. (1990) and modified in calculating the feeding deterrence index (FDI) (Huang and Ho, 1998) as follows:

$$\text{FDI (\%)} = \frac{C - T}{C} \times 100$$

where C=the consumption of control disks and T= the consumption of treated disks, as the control and treated disks placed in separate vials. The following criteria were adopted to categorize the deterrent action of the tested oils as follows:

FDI% < 20% No feeding deterrence
50% > FDI% ≥ 20% Weak feeding deterrence
70% > FDI% ≥ 50% Moderate feeding deterrence
FDI% ≥ 70% Strong feeding deterrence

The percentages of feeding deterrence index were performed for analysis of variance and significant differences between treatments were compared by Tukey's multiple range tests.

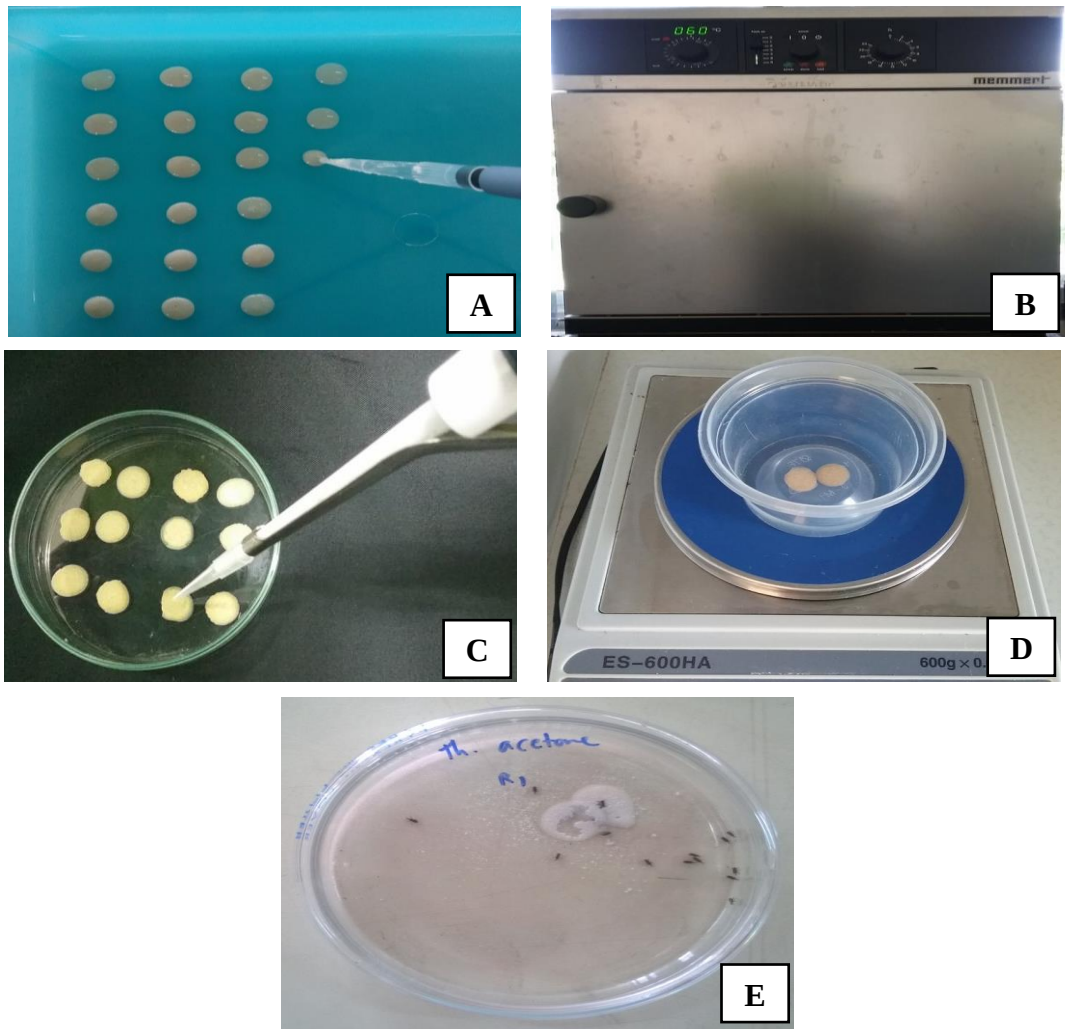

Figure 9 Toxicity of ingestion test application of plant oil solutions on wheat flour suspension were prepared onto a plastic sheet (A), wheat flour disks were dried in an oven at 60°C for 1 hour (B), dropping the solution on the surface flour disks (C), and the wheat flour disks were weight (D), before releasing the insects into petri-dish and keeping under room temperature (E).

3.2. Chemical composition analysis

Bioactivity of all plant oils obtained from the extraction previously described were tested against *R. dominica* and *S. zeamais.* The most effective oil in killing action was selected for analysis of chemical composition. Major compounds containing in plant oils were analyzed by using GC-MS (Gas Chromatography-Mass Spectrometry, 7890B GC-5977 A MSD, Agilent, USA) at the Scientific Equipment Center, Prince of Songkla University, Thailand (Figure 10) with the GC-MS conditions as shown in Table 2.

Table 2 GC-MS conditions for analysis chemical component of plant oils.

GC-MS conditions	
Injection source	Manual
Inlet	Split mode (split ratio of 7:1)
Column	A column of capillary HP-5MS Ultra inert (length of 30 m, film width of 0.25 μm and ID. 0.25 mm.)
Carrier gas	Helium in constant flow mode of 1.2 mL/min.
Oven temperature program	60 °C (3 min) 4°C to 220°C (/min) 10°C to 250°C (/min) Hold for 14 min
Injection volume	1 μL
Injector and transfer line temperature	250 °C

Figure 10 Gas Chromatography-Mass Spectrometry (GC-MS 7890B GC-5977 A MSD, Agilent, USA) used for chemical composition and quantification.

4. Assessment of application methods of the most effective plant oil against *Rhyzopertha dominica* and *Sitophilus zeamais* in laboratory

The most effective plant oil obtained from the previous study was selected for further experiments. Three application methods including seed coating, fumigation and sack coating were conducted in the laboratory to select the most effective method for application in rice storage.

4.1 Seed coating application

The extract of clove oil was made from 100 g dried-flower buds of clove in 500 mL of distilled water for 24 hrs. The essential oil was stored in a refrigerator at 10-12 ºC until being used in the experiments. The plant oil was diluted in distilled water mixed with red food coloring (2 g/L) at different concentrations (10, 30, 50, 100, 150, and 300 μl/L), 3 drops of Polysorbate (Tween-80) were then added

simultaneously (Figure 11A**)**. Cleaned rough rice (3,000 g) was placed in a plastic container, then was put in a freezer at temperature 4-6 ºC. It was removed from the fridge for up to 48 hours before using. Sample seeds were placed into 250 ml conical flasks, each containing 100 g of rice seeds. The emulsion at different concentrations in the amount of 3 ml was pipetted by hand into each flask (Figure 11B). The flask was then shaken vigorously for 15 minutes to ensure that the seeds were thoroughly coated (Figure 11B, 11C). The stirred samples were placed in a separate 400 ml plastic cup with a lid and stored at the laboratory (Figure 11D). The same procedure was applied for a 0.4% chlorpyrifos solution (4 ml of Chlorpyrifos was dissolved in 1 L of distilled water), and distilled water which served as positive and negative control, respectively. A completely random design (CRD) with three replicates per treatment was used. Fifty unsexed adults of *R. dominica* and *S. zeamais* were released in each treatment. The top of the cup was covered with a piece of muslin cloth and a rubber band was tied around. All containers were then kept in the dark under room temperature (Figure 11E).

Mortality counts were recorded on days 1, 3, 5, 7, 14, and 21 after application. The corrected mortality percentage was calculated by using Abbott's formula (Abbott, 1925).

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

After the 21-day mortality count, all remaining adults were removed. The number of *R. dominica* and *S. zeamais* adults that successfully emerged was recorded daily until the emergence of insects was no longer present. Then, the adults were removed from the containers to prevent breeding and egg-laying in the future. The number of F1 adults or reproduction inhibition rate (%IR) was computed according to Tapondjou *et al*. (2002) as shown in the equation below.

Reproduction inhibition rate (%) = [(Cn-Tn)/Cn]*100

where: C = Number of emerged adults in control.

T = Number of emerged adults in treatment.

Each sample has weighed the weight of contents which included whole and damaged seeds, and frass material. The data obtained including the percentages of mortality, reproduction inhibition rate (%IR), and percent weight loss and frass were subjected to the analysis of variance (ANOVA). Means of treatments were compared by using Tukey's multiple range tests. The lethal concentration (LC_{50} and LC_{90}) was calculated using Probit analysis.

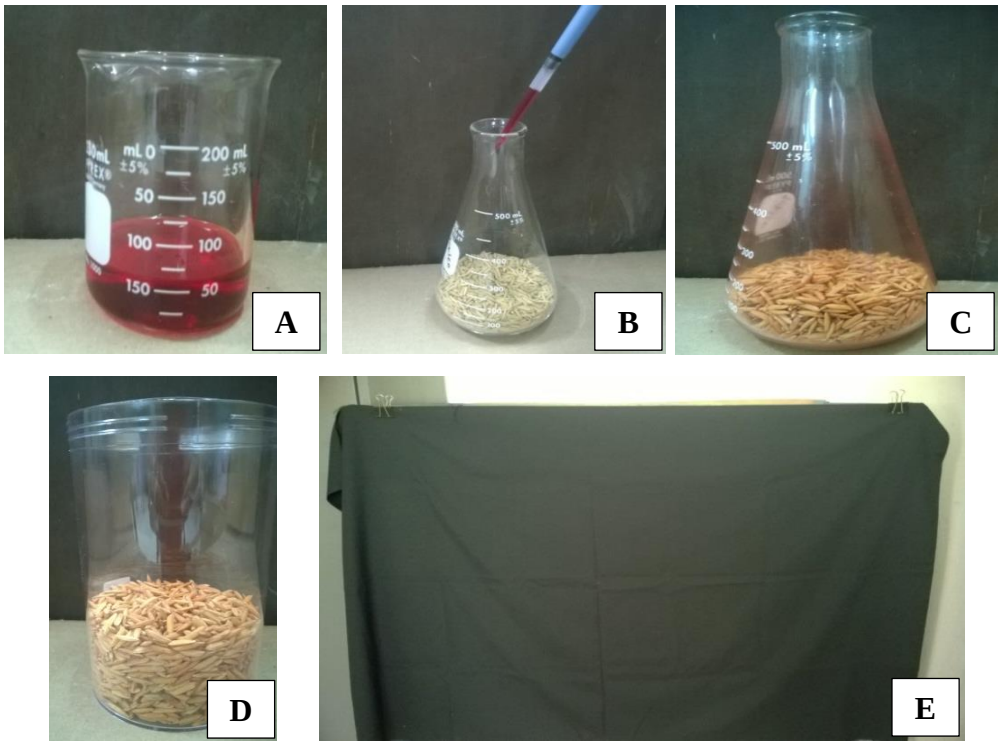

Figure 11 Process of seed coating by mixing clove oil together with red food coloring solutions (A), dropping the mixed solutions onto rice grains in a flask (B); after handed-shaking for about 15 minutes (C); placing the treated grained in a bigger (D), and keeping under dark condition (E).

4.2 Fumigation application

In fumigation assay, the clove oil was dissolved in 70% ethanol and used to establish five different concentrations by serial dilutions of 10, 7.5, 5, 2.5 and 1.25% of oils (Figure 12A). The cleaned rough rice was placed in four jute bags (15 × 20 cm in diameter), each containing 500 g of rice seed. Fifty unsexed adults of each *R. dominica* and *S. zeamais* were transferred to each bag, and then tied with plastic rope tightly to prevent the escape of insects. A 40 ml of each oil dilution was poured into an electric burner to produce gas (Figure 12B). A positive and negative control consisting the fumigation of phosphine (1 tablet) and 70% ethanol without essential oil component (40 ml) were used. Three replicates of each control and treatment were set up. The sealed bag was put separately inside a plastic cage (50×70×50 cm^3) which contained a 100 ml-electric oil essential burner (Figure 12C). After switching on, the equipment was monitored for two hours, subsequently kept under the closed system for 7 days of fumigation duration under room temperature (28±5ºC). CRD was used for the experiment. Insect mortality was checked after fumigation determination at 1, 3, 5 and 7 days. Percentage insect mortality was calculated using Abbott's correction formula (Abbott, 1925)

% Corrected Mortality

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

The data obtained were subjected to the analysis of variance (ANOVA). Means of treatments were compared by using Tukey's multiple range tests. The lethal concentration (LC_{50} and LC_{90}) and the effective time of 50% mortality of insects were calculated using Probit analysis.

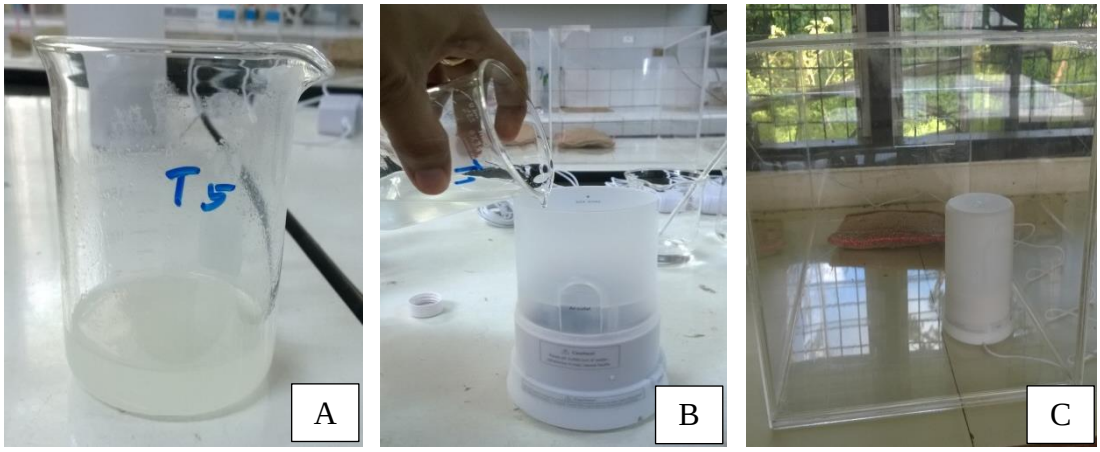

Figure 12 Fumigation applications by preparing solutions of clove oil dissolved in 70% ethanol (A); before pouring them into an electric burner (B); followed by a fumigation process in the plastic cage which contained tested insects dwelling in the jute sack (C).

4.3 Sack coating application

Treatments comprised various concentrations of 0, 1, 3, 5, 9, 12 and 20% of clove oil dissolved in acetone for each sack coating as compared to acetone as the control. Each treatment was replicated three times. The cup-test method described by Gerhardt and Lindgren (1954) and Pongsai (2008). A volume of 5 ml of each test concentration was dropped on all over the jute sheet which cut into 100 cm^2. Two transparent plastic cups (7.5 × 14.5 × 28 cm) with the bottom cut out were glued together, a piece of applied jute sheet was held between holes in the bottom (Figure 13 A). The treatments were arranged in a completely random design (CRD) with three replicates per concentration. For each treatment, 20 unsexed of each *R. dominica* and *S. zeamais* adults were released in a rearing cup, while cleaned rough rice (10 g) was placed in the other cup, then both cups were capped (Figure 13 B and 13C). All tested cups were kept in the dark at room temperature for 60 days (Figure 13D). The number of adults reaching rearing cup was counted at 1, 3, 5 and 7 days. The residual toxicity

of clove oil was observed 8, 24, 21, 28, 35 and 60 days after application. The percentage inhibition of infestation was calculated as follows

Percent inhibition of infestation

$$= \frac{\text{Number of insects can not through to jute sheet}}{\text{Total number of insect}} \times 100$$

The data obtained were subjected to the analysis of variance (ANOVA). Means of treatments were compared by using Tukey's multiple range tests. The lethal concentration (LC_{50} and LC_{90}) was calculated using Probit analysis.

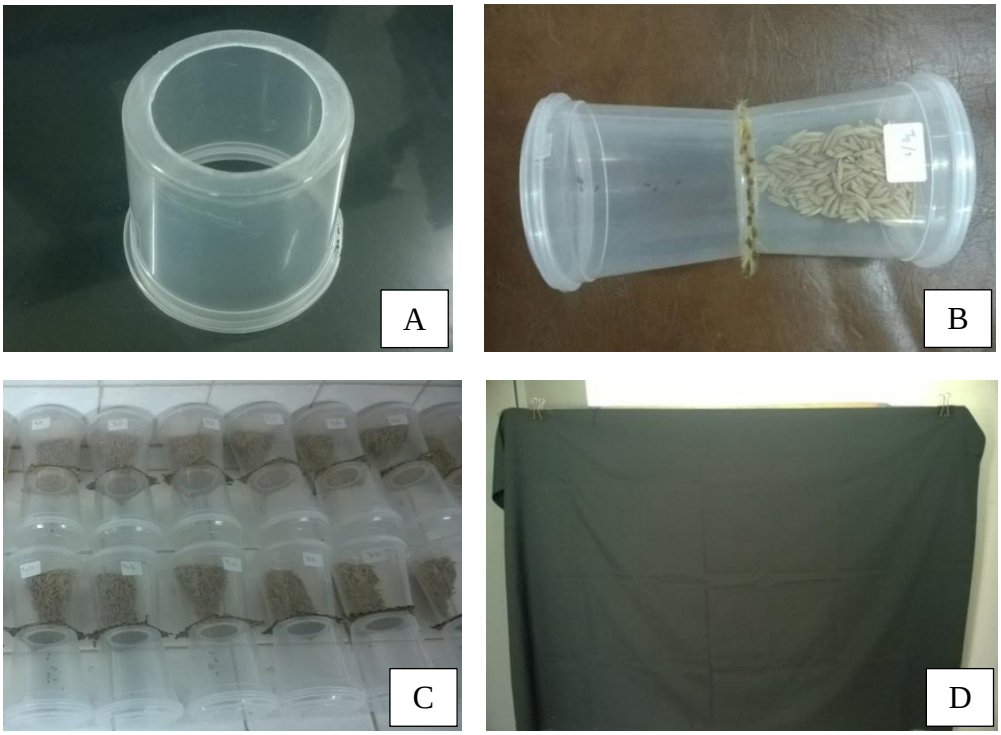

Figure 13 Sack coating application by cup test, drilling hold of a cup (A), coating clove oil solutions on jute sheets and sticking them between the first cup (containing adults of insect) and the second cup (containing 10 g of rough rice) (B), placing in the plastic cup for 60 days (C) and keeping in darkness under room temperature (D).

5. Evaluation of clove oil for controlling *Rhyzopertha dominica* and *Shitophilus zeamais* and their effect on seed germination effect in rough rice under storage conditions

Clove oil which was the most effective to control *R. dominica* and *S. zeamais* were selected to investigate in this experiment under rice seed storage condition. Two application methods of seed coating and fumigation were used due to their highly effective control of both *R. dominica* and *S. zeamais* in the previous study. The positive control was done by seed coating with chlorpyrifos and seed fumigation with phosphine. The non-treated seed was also done as a negative control.

The studies were conducted in warehouses at Suratthani Rice Seed Center and Phatthalung Rice Seed Center, representing the upper south and the lower south of Thailand, respectively. The duration of the study was during March to September 2016. The randomized complete block (RCB) was designed for the experiment with six treatments as shown in Table 3. Each treatment was replicated three times.

Table 3 Various method applications of treatments used in the study.

Treatments	Method applications
T1	Seed coating with chlorpyrifos + fumigation with PH_3
T2	Seed coating with clove oil fumigation with PH_3
T3	Seed coating and fumigation with clove oil
T4	Fumigation with clove oil
T5	Seed coating with chlorpyrifos and + Fumigation with clove oil
T6	Control (non-treated seeds)

The rice seeds (Pathum Thani1) containing 10-11% moisture content were collected from Suratthani Rice Seed Center. Ten kilograms of rough rice were thoroughly coated with chlorpyrifos at rate 0.03 ml kg^{-1} seed. Other ten kilograms were coated with clove oil at the rate of 1.2 ml kg^{-1} dissolved in 7 ml of water mixed with red food dye. The rice seed and the extracted oil were thoroughly mixed in a plastic container for 15 to 20 minutes (Figure 14A). The seed coating was done just once throughout the study. The coated seeds were packed into 6-kg sacks before placing them on a wooden pallet (Figure 14B). Fumigation was done by placing two burners containing 10% clove oil dissolved in 70% alcohol in the corner of a plastic cage (40×90×70 cm), presenting a jute sack of 5 kg rice (Figure 14C). The burner was run two hours and the fumigation duration subsequently extended for before moving out of the cage. The fumigation with clove oil was additionally repeated at 2, 4 and 6 months. Phosphine (1 Tablet/5 kg) fumigation was done monthly after seed sampling for six months according to the modified method developed by Chankeawmanee (2004). All sack samples were kept with other stockpiles of rice seed in a warehouse. Temperature and relative humidity were recorded daily. Five hundred grams of rice grain in each sack of all treatments were monthly sampled using a sampling spear for six months (Figure 14D) for analysis of insect incidence, moisture content, and germination of seeds.

Figure 14 Seed coating and fumigation of rice grains under warehouse storage condition; handed seed coating with solution of the clove and red food dye in plastic bowl (A), treated rice seeds filled into jute sacks placing on wooden pallet (B), fumigation of clove oil in a plastic cage (C), and the rice sampling by a sampling spear (D).

A total number of *R. dominica* and *S. zeamais* was counted and removed (Figure 15A). A sample of 500 g of rough rice was divided into two equal parts prior to measuring moisture content through grain moisture meter (SB 900) (Figure 15B). Seeds were randomly sampled using counting board with 100 holes for a germination test (Figure 15C). The seed germination test was evaluated by top of paper (TP) method which according to the standard method of ISTA (2018). The selected seeds were placed on moisturized tissue paper inside a waterproof plastic container which kept in the laboratory at prevailing temperature and relative humidity.

The germination test was replicated four times during the experiment. Seed germination was weekly checked and germinated seeds were recorded after seven days (Figure 15D). Percentages of moisture content and seed germination were analyzed for ANOVA using statistical package SPSS (version 16.0). Significant differences among the means of different treatments were compared by using Tukey's multiple range tests.

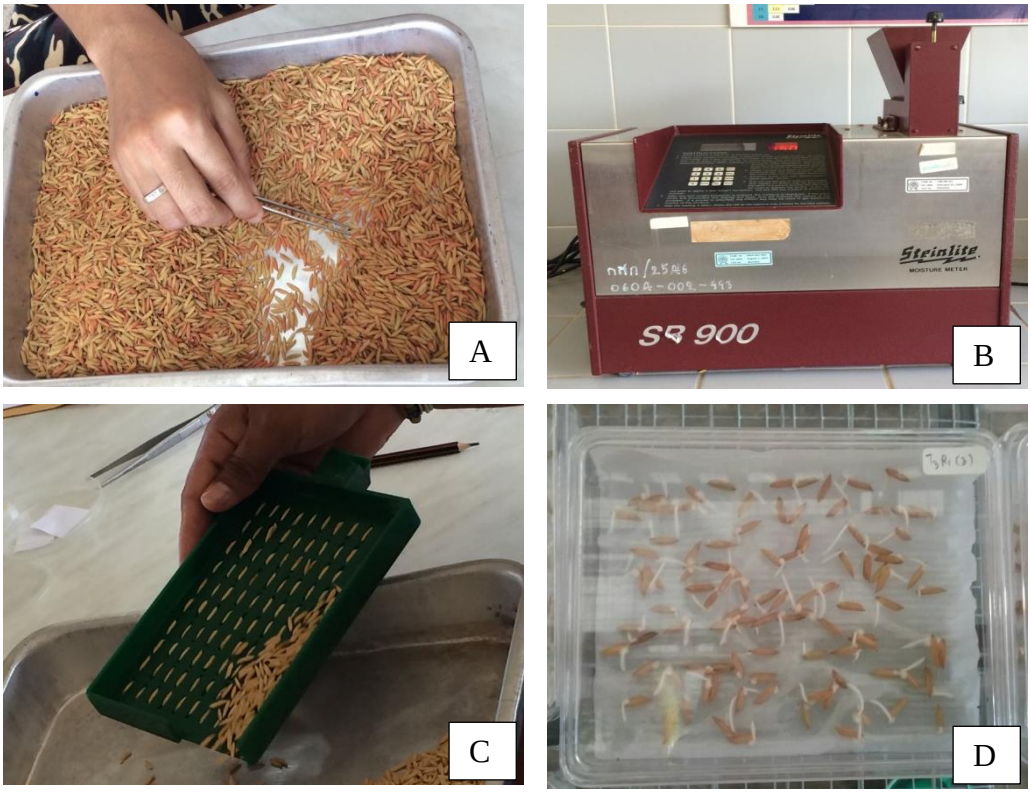

Figure 15 Counting number of insect (A), measuring moisture content through grain moisture meter (B) and test of seed germination (C and D).

6. Cost comparison between clove oil and synthetic insecticide applications for controlling *R. dominica* and *S. zeamais* under storage conditions

Currently, *R. dominica* and *S. zeamais* cause serious losses in quality of stored products and considerable economic losses to smallholder farmer in Thailand. Control of these storage pests relies heavily on the use of commercial pesticides which is obviously harmful to humans, other living organisms, and the environment. In addition to being hazardous to users, the synthetic insecticides including fumigants cause several problems such as the insect resistance selection, environmental contamination, and increasing costs of the application. Due to the high cost of insecticide seed treatments and the harmful effects of chemicals have been realized, recently, plant oils and their components which are less expensive and less hazardous in comparison to the chemical insecticides have been proved to be highly effective against storage pests (Akter and Jahan, 2013).

The study undertaken for this part considered the determinants of application costs of synthetic chemical insecticides in compared to plant oil products. The production costs of the combination of seed coating and fumigation were shown in Table 3. The costs of plant oils application to protect the rough rice were also computed. The current price of clove oil (*S. aromaticum*) and chemical substances (Chlorpyrifos, 40% EC and fumigation Aluminium phosphide) are also presented in table 4.

Data including prices of plant oil and insecticides used for both seed coating and fumigation, amount of plant oil and insecticides used for each application and number of the application during a study period of six months were recorded for a comparable cost of plant oil and synthetic insecticide applications.

Table 4 The commercial production costs of insecticide and clove oil in each treatment used in the study.

Products	**Costs (Baht)**	**Unit**
Chlorpyrifos 40% EC	230	Liter
Aluminium phosphine	2.50	Tablet
S. aromaticum oil	2,160	Liter

In addition, the development of Thai organic rice is also interesting for increasing economic value in both domestic and global markets. Insecticides are not allowed to use in organic rice production, particularly during storage which is difficult to manage insects. Therefore, the plant extract is a good option to control insect pests. The results of this study demonstrate that clove oil was the most effective for controlling stored-product insects, although its application was more expensive than insecticide application. Therefore, in promoting the organic rice market, it is necessary to consider the price of organic rice as compared with inorganic rice in the domestic market. The production of organic rice should be realized economic-value which will contribute to the development of organic rice markets in the future.

CHAPTER 3

Results and Discussion

1. Plant oil extraction

Yield percentages of different plant oils extracted from two methods, water distillation, and maceration are shown in Table 5. There was evidently different yield production between water distillation and maceration. Oil percentages of water distillation ranged from 0.85-2.18%, whereas those of maceration were 7.31% and 13.33%. It was probably due to a short duration of water distillation extraction for 12 hours, whereas a long period of 7 days was taken for maceration extraction. In addition, the later process was repeated five times. Different oil colors were observed between water distillation and maceration. The colors of the four plant oils from water distillation were transparent, whereas the macerated plant oils seemed to be turbid (Figure 16).

Table 5 Yield percentages of plant oils extracted by water distillation and maceration.

Scientific name	**Extraction method**	**Percentage of yield [%Yield (v/w)]**
Curcuma longa	Water distillation	1.32
Piper nigrum	Water distillation	0.85
Syzygium aromaticum	Water distillation	2.18
Cymbopogon nadus	Water distillation	0.95
Cleome viscose	Maceration	7.31
Azadirachta excelsa	Maceration	13.33

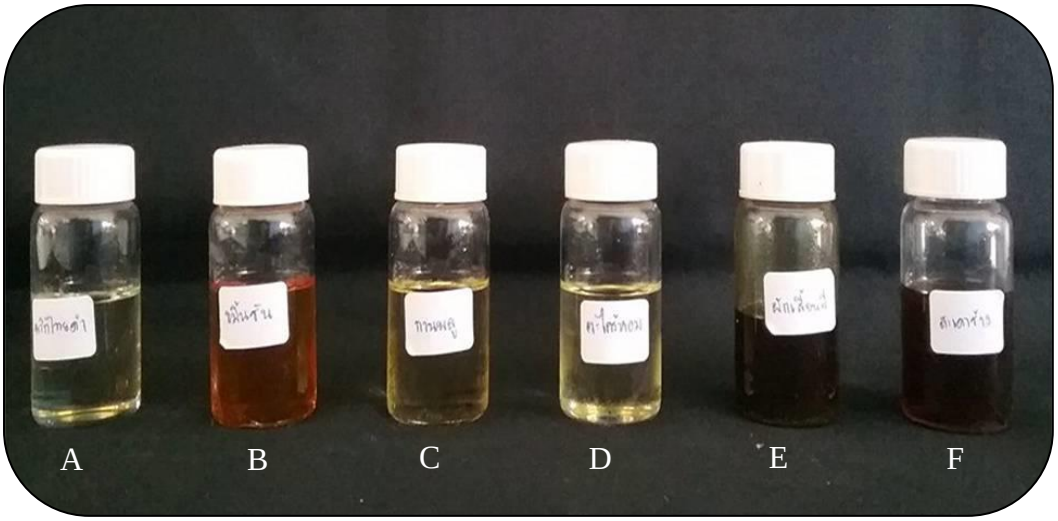

Figure 16 Colors of plant oil extract of *Piper nigrum* (A) *Curcuma longa* (B) *Syzygium aromaticum* (C) *Cymgopogon citratus* (D) *Cleome viscose* (E) and *Azadirachta excelsa* (F)

2 Bioactivity tests of six plant oils against *Rhyzopertha dominica* and *Sitophilus zeamais* in laboratory and chemical composition analysis of the most effective plant oil

2.1. Bioassays test of six plant oils

Different plant oils showed different actions in terms of repellent activity and toxicity through contact, stomach, and inhalation against *R. dominica* and *S. zeamais* in the laboratory. Details are showed and discussed as follows:

1 Repellency bioassay test

Repellent activity of various plant oils against *R. dominica* and *S. zeamais* depended on the kind of plant oils, concentrations and (Table 6) and exposure time (Figure 17). *P. nigrum* was the most effective to repel both of those

species with the highest repellent percentage ranged from 60–100% in various concentrations for *R. dominica* and from 76–100% for *S. zeamais* after 48 hours of treatment (Table 6). The lowest repellent percentage of *C. viscose* was recorded ranged from 8–64% and 20–68% for *R. dominica* and *S. zeamais*, respectively (Table 6). According to ET_{50} at different concentrations, four plant oils of *P. nigrum, C. longa, C. nadus* and *S. aromaticum* showed highly repellent action to *R. dominica*, whereas all plant oils except oil of *C. viscose* were likely effective to repel *S. zeamais* (Figure 17). Interestingly, oil from *A. excelsa* showed significantly repel to *S. zeamais* as compared to *R. dominica* (Table 7, Figure 17).

In terms of plant oil concentrations as illustrated in Table 6, repellent percentages increased with increase in concentration. At 48 hours after treatment, repellent percentages were statistically different from plant oils at the concentrations of 0.16 – 1.26 µL/cm^2. The longer period of exposure to plant oil the larger repellent percentages was recorded (Figure 18). Performance of repellent action can be demonstrated based on ET_{50} at different concentrations of plant oils as shown in Figure 17. Plant oils of *P. nigrum, C. nadus, C. longa* and *S. aromaticum* evidently showed repellent action to *R. dominica* at 0.16 µL/cm^2, whereas those of *P. nigrum* and *A. excelsa* exhibited a high repellent action to S. *zeamais* at 0.03 µL/cm^2 (Table 6). In addition, *C. viscose* oil showed low repellent activity against both *R. dominica* and S. *zeamais* at all concentrations. However, *C. viscose* oil had a RC_{50} value that can be used as a repellent to certain insects at the highest concentration (Table 7).

The result obtained from this study showed that *P. nigrum* oils mostly repelled both *R. dominica* and *S. zeamais,* whereas *C. viscose* oils had less repellent action to both insect species. This may be attributed to their chemical constitutes containing in *P. nigrum*, particularly piperine which was as a major compound found in plants family piperaceae such as *P. nigrum* and *P. longum* (Reshmi *et al*., 2010; Bhardwaj *et al*., 2002). It is an alkaloid in terpenes group which exhibited repellent and neurotoxic actions to insects (Sagheer *et al*., 2014). Many documents have been

reported on the repellent activity of black pepper against stored-product insects. Shayesteh and Ashouri (2010) found that black pepper oil at 2.5% w/w effectively repelled *S. granaries, T. castaneum* and *R. dominica,* respectively. Kanyarat *et al*. (2013) revealed that black pepper oil at concentrations of 1, 2, 4 and 8 μl/cm^2 could repel 80.1-100% maize weevil. Ishii *et al*. (2010) evaluated the repellent activity of *P. nigrum* against adult of *S. zeamais* and showed moderate repellent against *S. zeamais* at 20 and 50 mg/ml after 6 hours exposure. Khani *et al*. (2011) reported different repellent activities to *S. oryzae* after *P. nigrum* extraction with different solvents. Petroleum ether extracts showed markedly repellent action with 92% as compared to chloroform extracts with 75.2% after 24 hours of exposure.

Besides black pepper, other plant species have been reported to repel *R. dominica* and *S. zeamais* and other stored product insects. Viglianco *et al*. (2007) reported that hexane and ethanolic extracts from *Solanum argentinum* showed repellent action against *S. zeamais.* Shah *et al*. (2015) reported that plant extracts from *Mentha longifolia, Momordica charantia*, *Luffa aegyptiaca*, *Carum copticum* and *C. longa* showed repellent activity to *R. dominica* with 100%, 90%, 80%, 76.67% and 66.67% repellency, respectively after exposure at the concentration of 75% for 10 days. Ko *et al*. (2009) reported that the leaf essential oil from *Melaleuca cajuputi* could completely repel *S. zeamais* and *T. castaneum*.

In addition, Talukder and Howse (1993, 1994) revealed that methanol, acetone and ethanol extracts from *Aphanamixis polystachya* showed a repellent effect against *S. oryzae.* Chander *et al*. (2000) reported that *C. longa* extracts could repel *S zeamais, T. castanium* and *Oryzaephilus surinamensis* for 3 months. Mona *et al* (2009) showed the results of high repellency (>70%) activity against *T. catanium* of garlic extracts. Nevertheless, insecticidal and fungicidal activities of clove oil were documented (Han, 2006; Lou, 2006; Shang, 2007). The repellent action of clove oil was revealed by several researchers. Gharsan (2015) reported that clove oil was the most potent repellent against the *Trogoderma granarium*. Eamsobhana *et al*. (2009)

reported that clove oil had highly repellent activity against the chigger (*Leptotrombidium imphalu*) with 100% repellency at the 5% concentration. Zhang *et al*. (2013) found clove oil was also effective to repel the wasps *Vespula pensylvanica* and paper wasps mainly *Polistes dominulus*.

Table 6 Repellent percentages of different plant oils at various concentrations to *Rhyzopertha dominica* and *Sitophilus zeamais* at 48 hours of treatment.

Insects	Plant oils	Accumulative repellency percentages (%Mean±SE)[1/]						
		0.003µl/cm^2	**0.03 µl/cm^2**	**0.16 µl/cm^2**	**0.31 µl/cm^2**	**0.47 µl/cm^2**	**0.63 µl/cm^2**	**1.26 µl/cm^2**
R. dominica	*C. longa*	54.00±6.00 (III)[3/]	60.00±16.73 (II)	72.00±13.32a[2/] (IV)	76.00±14.70ab (IV)	84.00±7.48a (V)	84.00±7.48ab (V)	96.00±4.00a (V)
	P. nigrum	60.00±14.14 (III)	64.00±16.00 (IV)	92.00±4.90a (V)	96.00±4.00a (V)	100.00±0.00a (V)	100.00±0.00a (V)	100.00±0.00a (V)
	S. aromaticum	44.00±4.00 (III)	48.00±10.20 (III)	64.00±4.00ab (IV)	72.00±10.20ab (IV)	84.00±4.00a (V)	92.00±4.90ab (V)	92.00±4.90ab (V)
	C. nadus	52.00±18.54 (III)	64.00±11.66 (IV)	76.00±7.48a (IV)	80.00±8.94ab (IV)	82.00±6.63a (V)	88.00±8.00ab (V)	92.00±4.90ab (V)
	A. excelsa	24.00±11.66 (I)	40.00±17.89 (II)	44.00±13.27ab (III)	52.00±8.00ab (III)	68.00±13.56ab (IV)	80.00±6.32ab (IV)	84.00±4.00ab (V)
	C. viscose	8.00±14.17 (I)	12.00±10.72 (I)	12.00±14.97b (I)	32.00±10.59b (II)	40.00±14.14b (III)	52.00±12.14b (III)	64.00±13.27b (IV)
	F-test	ns	ns	**	*	**	**	**
S. zeamais	*C. longa*	32.00±10.20 (II)	52.00±10.20 (III)	76.00±7.48ab (IV)	84.00±7.48ab (V)	88.00±8.00ab (V)	88.00±8.00ab (V)	96.00±4.00a (V)
	P. nigrum	76.00±11.66 (IV)	84.00±11.66 (V)	96.00±4.00a (V)	96.00±4.00a (V)	100.00±0.00a (V)	100.00±0.00a (V)	100.00±0.00a (V)
	S. aromatic	32.00±18.55 (II)	44.00±10.39 (III)	68.00±10.20ab (IV)	72.00±8.54ab (IV)	84.00±4.00ab (V)	84.00±8.94a (V)	92.00±4.90a (V)
	C. nadus	40.00±10.95 (II)	48.00±16.25 (III)	68.00±12.00ab (IV)	80.00±6.32ab (IV)	84.00±11.66ab (V)	92.00±4.90ab (V)	100.00±0.00a (V)
	A. excelsa	76.00±4.00 (IV)	84.00±7.48 (V)	88.00±4.90a (V)	96.00±4.00a (V)	92.00±8.00a (V)	96.00±4.00a (V)	100.00±0.00a (V)
	C. viscose	20.00±8.97 (I)	32.00±8.00 (I)	40.00±8.94b (III)	48.00±8.00b (III)	52.00±13.56b (III)	60.00±6.32b (III)	68.00±4.90b (IV)
	F-test	ns	ns	**	*	*	**	**

[1/] average from 5 replications, [2/] means within a column followed by the same letters are not significantly different (P > 0.05) by Turkey's multiple range tests, * significantly at P < 0.05, ** significantly at P < 0.01, [3/] repellency class (V) = 80.1-100 %, (IV) = 60.1-80 %, (III) = 40.1-60 %, (II) = 20.1-40 %, (I) = 0.1-20 %, (0) = PR < 0

Table 7 Repellency concentration 50% (RC_{50}) and repellency concentration 90% (RC_{90}) of different plant oils exposed to *Rhyzopertha dominica* and *Sitophilus zeamais* at 48 hours.

Insects	**Plant oils**	RC_{50} ($\mu l/cm^2$)	95% confident limit		RC_{90} ($\mu l/cm^2$)	95% confident limit	
			Lower	Upper		Lower	Upper
R. dominica	*C. longa*	0.004	0.000	0.018	2.198	0.566	8.485
	P. nigrum	0.003	0.000	0.012	0.091	0.030	0.596
	S. aromaticum	0.015	0.001	0.048	1.982	0.507	6.944
	C. nadus	0.004	0.000	0.009	1.787	0.857	5.507
	A. excelsa	0.085	0.019	0.234	10.024	1.878	18.135
	C. viscose	0.912	0.345	3.397	44.152	15.222	64.882
S. zeamais	*C. longa*	0.003	0.000	0.008	0.038	0.015	0.166
	P. nigrum	0.001	0.000	0.002	0.031	0.011	0.089
	S. aromaticum	0.027	0.008	0.056	1.511	0.607	8.106
	C. nadus	0.016	0.002	0.046	0.869	0.289	10.763
	A. excelsa	0.001	0.000	0.002	0.084	0.023	0.369
	C. viscose	0.273	0.174	0.457	118.258	89.89	139.014

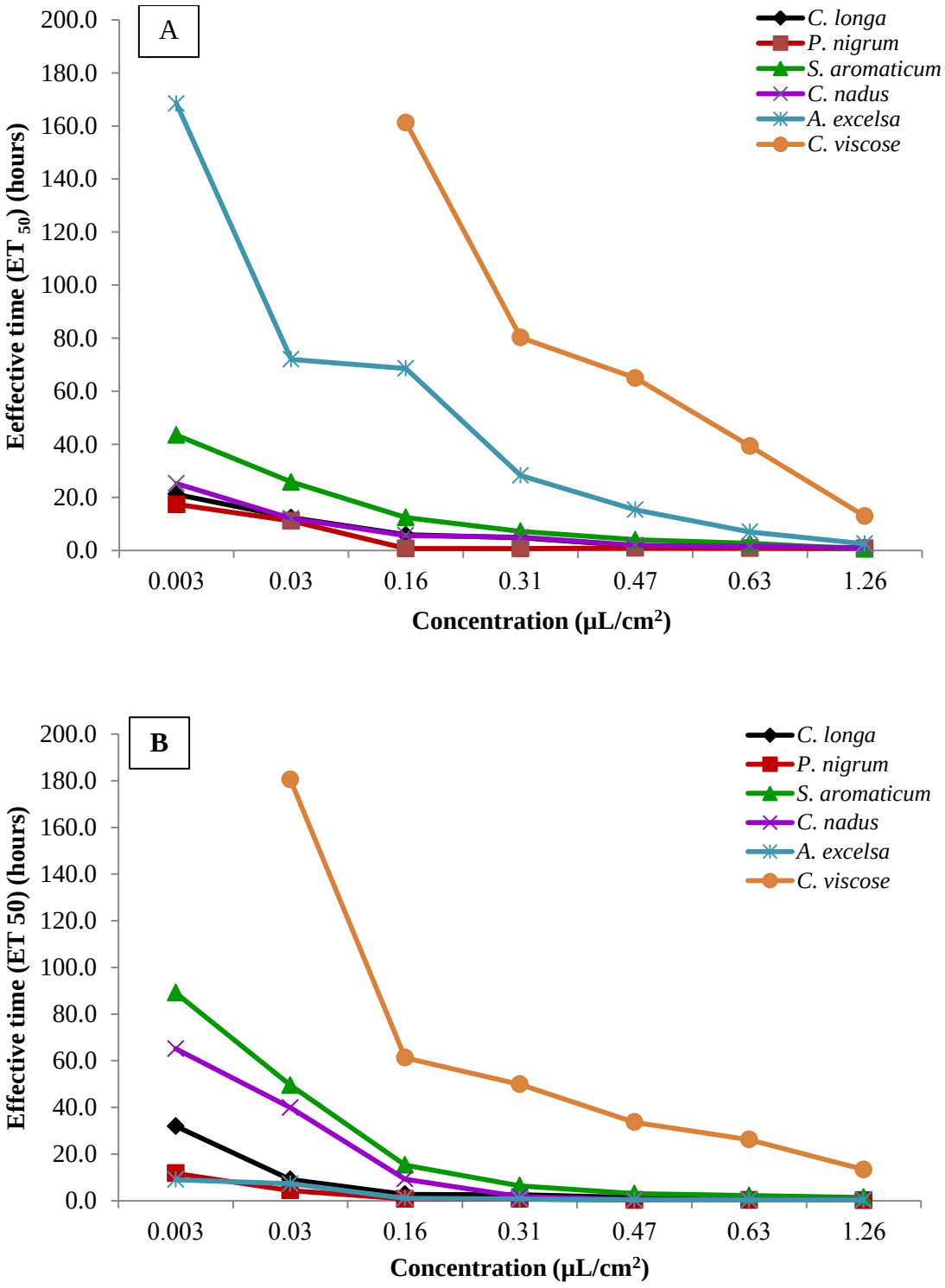

Figure 17 Effective times 50% (ET_{50}) of six plant oils in various concentrations to repel (A) *Rhyzopertha dominica* and (B) *Sitophilus zeamais*.

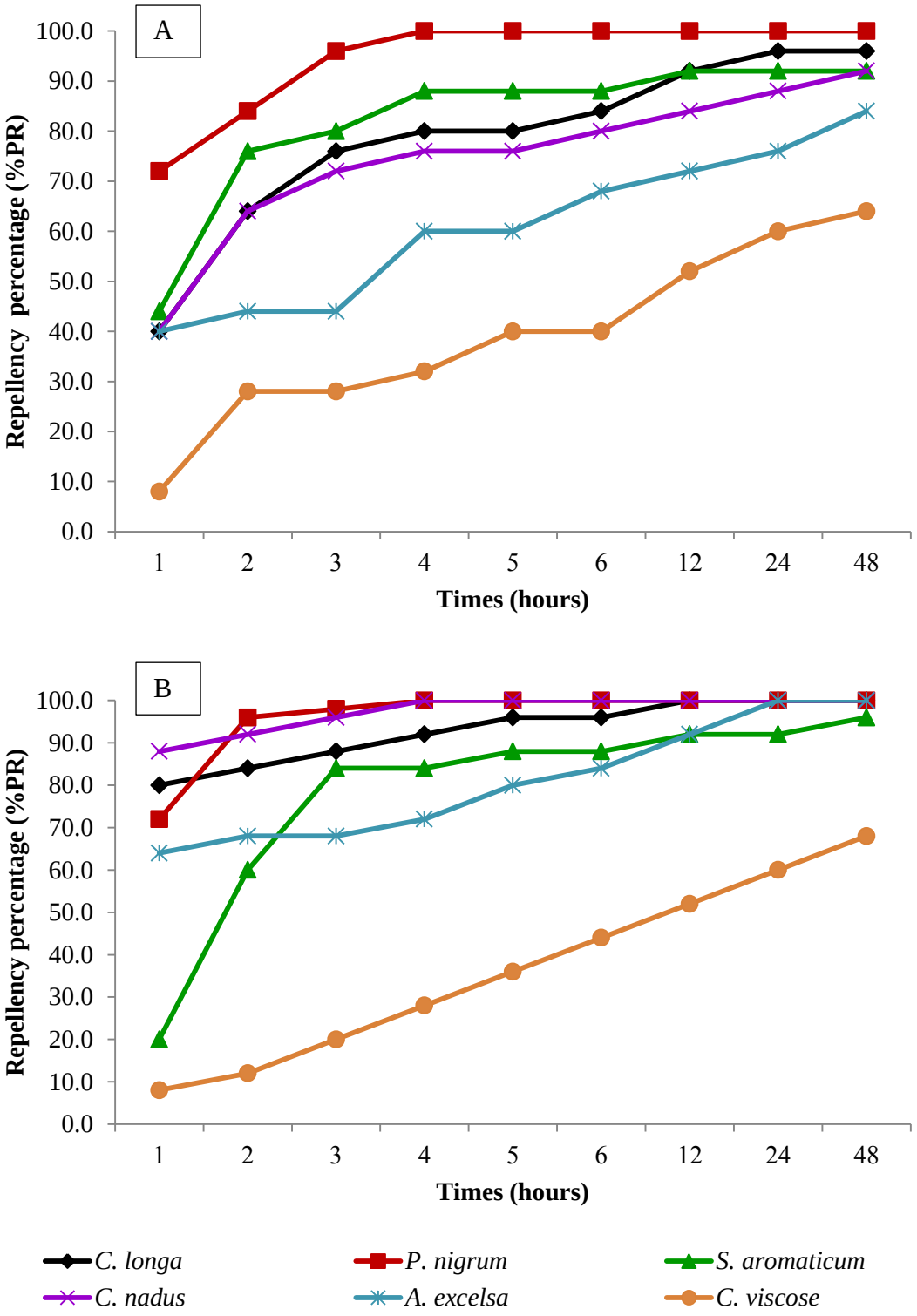

Figure 18 Repellency percentage of six plant oils in various times against (A) *Rhyzopertha dominica* and (B) *Sitophilus zeamais* at the concentration of 1.26 µL/cm^2.

2. Contact toxicity bioassay

Six plant oils used in this study were toxic to *R. dominica* and *S. zeamais.* Toxicity depended on plant species, concentration and time of exposure. The results given in Table 7 showed that *S. aromaticum* oil was the most toxic by contact to *R. dominica* and *S. zeamais*, while oils of *A. excelsa* and *C. viscose* showed low contact toxicity. Percent mortality percentage of adults of different plant oils were significantly different ($p<0.05$) in all concentrations at all times of exposure (Table 8-11).

Mortality percentage of *S. zeamais* was more susceptible to all plant oils than *R. dominica* due to lower LC_{50} and higher mortality of *S. zeamais* (Table 12). Interestingly, clove oil caused 100% mortality of *R. dominica* after 24 hours at the highest concentration (200 μl/L), whereas 98% mortality of *S. zeamais* (Table 9). According to 72 hours of period time exposure, clove oil exhibited complete mortality of *S. zeamais* at 80 μl/L of treatment as compared to *R. dominica* mortality, while caused 100% mortality of *R. dominica* at 120 μl/L (Table 11). As considered to LC_{50} values of clove oils was lower in *S. zeamais* (3.52 μl/L) as compared to *R. dominica* (6.13 μl/L) throughout the entire periods of study (Table 12). Based on the results of contact toxicity to two storage insect pests of this study, toxicity category can be classified as low, moderate, and high toxicity. *S. aromaticum* and *C. nadus* were classified in high toxicity. *C. longa* and *P. nigrum* were classified in moderate toxicity, and *A. exelsa* and *C. viscose* were classified in low toxicity, respectively. The results obtained from this study indicated that clove oil was highly effective to kill both *R. dominica* and *S. zeamais*, but it showed most effective to control *S. zeamais*. This may be attributed to the different behavior of these two insect species. *R. dominica* walked slowly on filter-paper leading to the organs less contact opportunity with oil than *S. zeamais* which moved rapidly on filter-paper resulting in an organ such as legs, antenna more frequency contact with the oil.

Mortality percentage of both stored-insect species may be attributed in the mode of action which was the characteristics of the chemical compounds present in the composition of clove oil, especially eugenol which was the main compounds in clove oil (Huang *et al*., 2000). The several compounds such as 1,8-cineole, eugenol, methyl-eugenol, α-pinene were highly toxic to stored insect pests. Jairoce *et al*. (2016) showed that the results of eugenol from clove caused mortality of 100% for *S. zeamais* and *Acanthoscelides obtectus* under laboratory conditions at 48 h after treatment with the concentrations of 17.9 and 35 μl/g. The toxicity of eugenol was investigated high toxic to *T. castaneum* of 87.5% at the dose of 0.2 μl after 4 hours (Liska *et al*., 2010). Moreover, eugenol also inhibited the development of eggs and immature stages inside grain kernels (Obeng-Ofori and Reichmuth, 1997). Hang *et al*. (2002) reported that eugenol, iso-eugenol, and methyl-eugenol was mortality to *S. zeamais* and *T. castaneum* and had LD_{95} ranging from 47–116 μg/mg.

In addition, the plant oil extraction from clove was produced insecticide potential for several pest controls. For example, Sighamony *et al*. (1986) reported that the clove oil was toxic to *S. oryzae* and *R. dominica* and gave complete mortality at doses of 25–100 ppm after 15 days of contact test. Ho *et al*. (1994) demonstrated that the clove extract with hexane caused 90% mortality of *S. zeamais* but had no effect on *T. castaneum* at 100 g/100 ml [100% (w/v)]. Kerdchoechuen *et al*. (2010) found that clove oil extract was the most toxic to maize weevil on filter paper at the lowest concentration of 30 ml for 2 hours to obtain 100% mortality.

In addition, the several oils were tested against *R. dominica* and *S. zeamais*, but there were differences in plant extract efficacy at the concentration under different experimental conditions. Tapondjou *et al*. (2005) demonstrated that maize weevil was more susceptible to eucalyptus oil than cupressus oil due to the lower LD_{50} values of 0.36 μl/cm^2 on filter paper discs test. Huang and Ho (1998) reported adults of *S. zeamais* and *T. castaneum* were susceptible toxicity to *Elletaria cardamomum* oil at the LD_{50} of 56 and 52 µg·mg^1 insect after 7 days. Tripatri *et al*.

(2002) demonstrated that the *C. longa* leaf oil was highly toxic to adults of *R. dominica* in contact action with LD_{50} of 36.71 μg/mg weight of insect. El-Guedoui (2003) reported that the essential oil from *Thymus fontanesii* at 0.69 mg/cm^2 caused 100% mortality of *R. dominica* by contact method than inhalation method (40.9% kill at 1.44 mg/cm^3). Owabali *et al*. (2009) showed *S. zeamais* was susceptible toxicity to ginger due to low LD_{50} values of 0.7 μl cm^2.

However, several researchers evaluated some plant extracts are toxic to stored insect by contact activity. The essential oils from *Litsea cubeba* were more susceptible toxic to *Alphitobius diaperinus* in 6th instars than *Allium sativum* after 24 hours of treatment by the contact toxicity (Wang *et al*., 2014). Kim *et al*. (2003) reported *Cinnamomum sieboldii* oil caused 100% mortality of *S. oryzae* and *Callosobruchus chinensis* at 3.5 mg/cm^2 after 2 days of treatment.

Table 8 Mortality percentage of *Rhyzopertha dominica* and *Sitophilus zeamais* adults after treated with different concentrations of six plant oils by residual contact method for 12 hours.

Insects	Plant oils	Percentage mortality (Mean±SE)[1/]							
		10*µ*l/L	**40 *µ*l/L**	**80 *µ*l/L**	**120 *µ*l/L**	**160 *µ*l/L**	**200 *µ*l/L**	**acetone**	**water**
R. dominica	*C. longa*	6.00±2.45ab[2/]	14.00±2.45bc	20.00±3.16b	22.00±3.74cd	42.00±3.74b	50.00±3.16bc	0.00±0.00	0.00±0.00
	P. nigrum	4.00±2.45ab	16.00±5.10bc	24.00±5.10b	34.00±5.10bc	38.00±3.74b	50.00±5.48bc	0.00±0.00	0.00±0.00
	S. aromaticum	16.00±5.10a	34.00±2.45a	58.00±3.74a	62.00±5.83a	78.00±3.74a	86.00±2.45a	0.00±0.00	0.00±0.00
	C. nadus	10.00±3.16ab	24.00±4.00ab	46.00±5.10a	50.00±3.16ab	74.00±5.10a	74.00±2.45a	0.00±0.00	0.00±0.00
	A. excelsa	2.00±2.00b	4.00±2.45c	8.00±2.00b	12.00±2.00d	18.00±3.74c	28.00±3.74c	0.00±0.00	0.00±0.00
	C. viscose	2.00±2.00b	6.00±2.45c	12.00±2.00b	12.00±2.00d	16.00±2.45c	24.00±2.45c	0.00±0.00	0.00±0.00
	F-test	*	**	**	**	**	**	ns	ns
S. zeamais	*C. longa*	6.00±2.45b	18.00±6.63bc	28.00±4.90bc	32.00±3.74cd	44.00±2.45bc	48.00±3.74cd	0.00±0.00	0.00±0.00
	P. nigrum	4.00±2.45b	30.00±4.47b	30.00±3.16b	40.00±3.16bc	44.00±5.10bc	54.00±5.10bc	0.00±0.00	2.00±2.00
	S. aromaticum	24.00±2.45a	56.00±5.10a	62.00±3.74a	70.00±7.07a	82.00±5.83a	90.00±4.47a	2.00±2.00	0.00±0.00
	C. nadus	2.00±2.00b	16.00±5.09bc	54.00±5.10a	58.00±3.74ab	62.00±3.74b	72.00±3.74	0.00±0.00	0.00±0.00
	A. excelsa	4.00±2.45b	10.00±3.16bc	12.00±2.00cd	16.00±2.45de	26.00±5.10cd	36.00±5.10cd	0.00±0.00	0.00±0.00
	C. viscose	2.00±2.00b	6.00±2.45c	6.00±2.45d	10.00±3.16e	14.00±2.45d	30.00±3.16d	0.00±0.00	0.00±0.00
	F-test	**	**	**	**	**	**	ns	ns

[1/] average from 5 replications, [2/] means within a column followed by the same letters are not significantly different (P > 0.05) by Turkey's multiple range tests, * significantly at P < 0.05, ** significantly at P < 0.01, ns: non-significantly at P>0.05.

Table 9 Mortality percentage of *Rhyzopertha dominica* and *Sitophilus zeamais* adults after treated with different concentrations of six plant oils by residual contact method for 24 hours.

Insects	Plant oils	Percentage of mortality (Mean±SE)[1/]							
		10 µl/L	**40 µl/L**	**80 µl/L**	**120 µl/L**	**160 µl/L**	**200 µl/L**	**acetone**	**water**
R. dominica	*C. longa*	18.00±3.74abc[2/]	38.00±3.74ab	42.00±3.74b	50.00±5.48b	68.00±3.74b	74.00±5.10b	0.00±0.00	0.00±0.00
	P. nigrum	12.00±3.74bc	30.00±3.74b	44.00±5.10b	54.00±5.10b	60.00±4.47b	78.00±5.83b	0.00±0.00	0.00±0.00
	S. aromaticum	32.00±5.83a	54.00±4.00a	72.00±3.74a	84.00±4.00a	96.00±2.45a	100.00±0.00a	0.00±0.00	0.00±0.00
	C. nadus	26.00±4.00ab	42.00±3.74ab	68.00±5.83a	74.00±4.00a	88.00±3.74a	90.00±3.16ab	0.00±0.00	0.00±0.00
	A. excelsa	6.00±2.45c	8.00±3.74c	14.00±5.10c	20.00±3.16c	40.00±3.16c	48.00±3.74c	0.00±0.00	0.00±0.00
	C. viscose	10.00±3.16bc	12.00±2.00c	18.00±3.74c	20.00±3.16c	28.00±3.74c	46.00±4.00c	2.00±2.00	0.00±0.00
	F-test	**	**	**	**	**	**	ns	ns
S. zeamais	*C. longa*	22.00±3.74b	36.00±4.00c	50.00±7.07b	56.00±5.10b	66.00±4.00b	68.00±3.74cd	0.00±0.00	2.00±2.00
	P. nigrum	14.00±5.10b	40.00±3.16bc	56.00±7.07b	58.00±5.83b	66.00±5.01b	72.00±5.83bc	2.00±2.00	2.00±2.00
	S. aromaticum	44.00±5.10a	66.00±5.10a	76.00±5.03a	86.00±5.06a	86.00±4.00a	98.00±2.00a	2.00±2.00	2.00±2.00
	C. nadus	16.00±5.10b	52.00±3.74ab	64.00±4.00ab	70.00±3.16ab	74.00±2.45ab	86.00±2.45ab	0.00±0.00	0.00±0.00
	A. excelsa	6.00±2.45b	14.00±2.45d	20.00±3.16c	22.00±5.83c	36.00±2.00c	50.00±3.16e	2.00±2.00	2.00±2.00
	C. viscose	8.00±3.74b	18.00±2.00d	22.00±2.00c	26.00±2.45c	40.00±4.47c	54.00±2.45de	4.00±2.45	2.00±2.00
	F-test	*	**	**	**	**	**	ns	ns

[1/] average from 5 replications, [2/] means within a column followed by the same letters are not significantly different (P > 0.05) by Turkey's multiple range tests, * significantly at P < 0.05, ** significantly at P < 0.01, ns: non-significantly at P>0.05.

Table 10 Mortality percentage of *Rhyzopertha dominica* and *Sitophilus zeamais* adults after treated with different concentrations of six plant oils by residual contact method for 48 hours.

Insects	Plant oils	Percentage of mortality (Mean±SE)[1/]							
		10 µl/L	**40 µl/L**	**80 µl/L**	**120 µl/L**	**160 µl/L**	**200 µl/L**	**acetone**	**water**
R. dominica	*C. longa*	28.00±4.90bc[2/]	52.00±5.83b	66.00±4.00bc	70.00±5.48b	86.00±2.45ab	90.00±3.16a	2.00±2.00	2.00±2.00
	P. nigrum	32.00±3.74ab	52.00±5.83b	62.00±5.83c	68.00±4.90b	78.00±3.74b	86.00±2.45a	0.00±0.00	0.00±0.00
	S. aromaticum	52.00±3.74a	76.00±6.78a	90.00±4.47a	96.00±2.45a	100.00±0.00a	100.00±0.00a	2.00±2.00	2.00±2.00
	C. nadus	32.00±4.90ab	68.00±5.83ab	82.00±4.90ab	90.00±3.16a	98.00±2.00a	100.00±0.00a	0.00±0.00	0.00±0.00
	A. excelsa	12.00±2.00c	14.00±2.45c	20.00±3.16d	24.00±4.00c	52.00±3.74c	62.00±3.74b	0.00±0.00	0.00±0.00
	C. viscose	14.00±5.10c	18.00±3.74c	24.00±2.45d	30.00±3.16c	48.00±6.63c	62.00±6.63b	2.00±2.00	2.00±2.00
	F-test	**	**	**	**	**	*	ns	ns
S. zeamais	*C. longa*	32.00±3.74b	62.00±7.35b	70.00±5.58ab	76.00±6.00a	80.00±3.16bc	96.00±2.45a	4.00±2.45	4.00±2.45
	P. nigrum	38.00±3.74b	58.00±3.74b	66.00±2.45b	72.00±4.90a	74.00±4.00c	88.00±3.74ab	6.00±2.45	4.00±2.45
	S. aromaticum	62.00±5.83a	86.00±5.10a	90.00±3.16a	92.00±3.74a	96.00±2.45a	100.00±0.00a	4.00±4.25	2.00±2.00
	C. nadus	48.00±3.74ab	74.00±5.10ab	78.00±3.74ab	86.00±4.00a	90.00±3.16ab	96.00±2.45a	0.00±0.00	0.00±0.00
	A. excelsa	10.00±3.16c	16.00±2.45c	22.00±5.83c	40.00±8.37b	56.00±4.00d	60.00±6.32c	4.00±2.45	2.00±2.00
	C. viscose	10.00±3.16c	22.00±2.00c	32.00±3.74c	42.00±3.74b	50.00±3.16d	74.00±5.10bc	4.00±2.45	4.00±2.45
	F-test	**	**	**	*	**	**	ns	ns

[1/] average from 5 replications, [2/] means within a column followed by the same letters are not significantly different (P > 0.05) by Turkey's multiple range tests, * significantly at P < 0.05, ** significantly at P < 0.01, ns: non-significantly at P>0.05.

Table 11 Mortality percentage of *Rhyzopertha dominica* and *Sitophilus zeamais* adults after treated with different concentrations of six plant oils by residual contact method for 72 hours.

Insects	Plant oils	Percentage of mortality (Mean±SE)[1/]							
		10 µl/L	**40 µl/L**	**80 µl/L**	**120 µl/L**	**160 µl/L**	**200 µl/L**	**acetone**	**water**
R. dominica	*C. longa*	42.00±3.74b[2/]	72.00±3.74b	78.00±4.90ab	86.00±4.00a	92.00±3.74a	96.00±2.45a	2.00±2.00	2.00±2.00
	P. nigrum	42.00±3.74b	68.00±5.83b	72.00±5.83b	84.00±4.00a	90.00±3.16a	96.00±2.45a	4.00±2.45	2.00±2.00
	S. aromaticum	68.00±2.00a	94.00±4.00a	98.00±2.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	2.00±2.00	2.00±2.00
	C. nadus	52.00±3.74b	86.00±5.10ab	94.00±4.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	6.00±2.45	4.00±2.45
	A. excelsa	22.00±2.00c	24.00±2.45c	32.00±5.83c	44.00±4.00b	62.00±3.74b	76.00±4.00b	0.00±0,00	0.00±0.00
	C. viscose	12.67±3.97c	25.11±4.79c	31.33±5.80c	38.00±6.91b	59.33±5.28b	74.22±8.31b	2.00±2.00	0.00±0.00
	F-test	**	**	**	*	*	*	ns	ns
S. zeamais	*C. longa*	50.00±3.16b	72.00±4.90b	80.00±3.16b	88.00±5.83ab	92.00±3.74a	100.00±0.00a	6.00±2.45	4.00±2.45
	P. nigrum	54.00±5.10b	74.00±2.45b	86.00±5.10ab	90.00±3.16a	90.00±3.16a	98.00±2.00a	6.00±2.45	6.00±2.45
	S. aromaticum	82.00±5.83a	94.00±2.45a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	8.00±3.74	6.00±2.45
	C. nadus	68.00±3.74ab	86.00±5.10ab	90.00±3.16ab	96.00±2.45a	98.00±2.00a	100.00±0.00a	10.00±4.47	8.00±2.45
	A. excelsa	18.00±3.74c	28.003.74c	42.00±4.90c	60.00±8.37c	68.00±3.74b	82.00±3.74b	6.00±4.00	6.00±2.45
	C. viscose	18.00±2.00c	32.00±3.74c	58.00±3.73c	64.00±6.78bc	68.00±3.74b	82.00±3.74b	6.00±4.00	4.00±2.45
	F-test	**	**	**	**	*	*	ns	ns

[1/] average from 5 replications, [2/] means within a column followed by the same letters are not significantly different (P > 0.05) by Turkey's multiple range tests, * significantly at P < 0.05, ** significantly at P < 0.01, ns: non-significantly at P>0.05.

Table 12 LC_{50} and LC_{90} of six plant oil extracts against of *Rhyzopertha dominica* and *Shitophilus zeamais* by residual contact method at 12, 24, 48 and 72 hours.

Insect	Plant oils	12 hour		24 hour		48 hour		72 hour	
		LC_{50} (μl/L)	**LC_{90} (μl/L)**	**LC_{50} (μl/L)**	**LC_{90} (μl/L)**	**LC_{50} (μl/L)**	**LC_{90} (μl/L)**	**LC_{50} (μl/L)**	**LC_{90} (μl/L)**
R. dominica	*C. longa*	291.86	-	80.03	-	34.72	308	15.98	146.8
	P. nigrum	242.57	-	88.51	755.94	32.69	520.03	17.78	184.95
	S. aromaticum	57.53	389.53	25.42	144.15	11.39	63.68	6.13	26.7
	C. nadus	90.94	434.91	46.43	265.31	20.18	98.28	10.64	44.76
	A. excelsa	785.56	-	324.93	-	220.45	-	107.64	-
	C. viscose	-	-	623.96	-	226.44	-	122.59	-
S. zeamais	*C. longa*	242.76	-	73.89	-	28.14	268.77	13.32	128.91
	P. nigrum	183.39	-	74.71	753.307	29.65	589.13	11.08	125.66
	S. aromaticum	37.72	328.31	16.59	178.253	6.48	68.06	3.52	19.21
	C. nadus	98.37	417.64	45.03	395.226	11.83	155.50	5.99	57.69
	A. excelsa	517.26	-	309.03	-	177.57	-	87.95	595.28
	C. viscose	987.61	-	278.84	-	143.58	964.05	73.74	511.92

3. Fumigant toxicity bioassay test

Results of percent mortality after fumigant toxicity test of six plant oils on adults of *R. dominica* and *S. zeamais* are shown in Table 13-16. The mortality percentage increased with a rise of oil concentrations and exposure times. Their mortality percentage of these two species were significantly different ($p<0.05$) among treatments, except at 72 hours against *R. dominica* at the highest concentration (800 μl/L air) (Table 16). Moreover, it was obviously found that *S. aromaticum* oil was the most effective as a fumigant, while *C. nadus*, *C. longa*, and *P. nigrum* oils exhibited moderate toxicity, and *A. excelsa* and *C. viscose* oils were low toxic to both *R. dominica* and *S. zeamais* adults.

Percent mortality of *R. dominica* and *S. zeamais* were more susceptible to clove oil due to lower LC_{50} and higher mortality (Table 17). Clove oil exhibited highly percent mortality of these two species that reached 100% mortality after 48 hours at the highest concentration (Table 15). At 72 hours of fumigation time, clove oil caused 100% mortality of *S. zeamais* at 600 μl/L air of treatment as compared to *R. dominica* mortality (Table 16). The LC_{50} and LC_{90} values of clove oils were lower in *S. zeamais* than those in *R. dominica* resulting higher toxicity achieved to *S. zeamais* throughout the entire periods of study (Table 17). This indicates that R. *dominica* was more tolerant to clove oil than *S. zeamais*.

This results agreed with the study of Mahfuz and Khalequzzaman (2007) reported clove oil as most effective in inducing mortality *Callosobruchus maculatus* and LD_{50} was 92.81 and 69.63 μg/ cm after 24 and 48 hours of exposure, respectively. Miyazawa *et al*. (2012) showed results that fumigant toxicity at 1 mL/L air caused 79%, 61% and 100% mortality of *R. dominica*, *Oryzaephilus surinamensis*, and *Callosobruchus chinensis*, respectively, 24 hours after treatment with eugenol compound from clove only. Ogendo *et al*. (2008) reported the eugenol compound

caused 79, 61 and 100% mortality of *R. dominica*, *O. surinamensis* and *C. chinensis*, respectively at 1 µl/L air after 24 hours of treatment.

Monoterpenoids were reported earlier as fumigants and contact toxicants on various insect pests including stored-product insects (Rice and Coats, 1994; Tsao *et al*., 1995). Many researchers have demonstrated differential susceptibility of stored-product species to the plant oils extract. The essential oil of *Coriandrum sativum* seeds caused 100% and 25% mortality of *T. confusum* and *C. maculatus* at 43 μL/L air after 24 hours of exposure. It indicated that *C. maculatus* was more susceptible to *C. sativum* (LC_{50} = 1.34 μL/L air) than *T. confusum* (LC_{50} = 318.02 μL/L air (Khani and Rahdari, 2012). Kim *et al*. (2012) evaluated the fumigant effect of essential oil from *Brassica juncea* and *Cinnamomum cassica* and their caused 84.2% and 98% mortality of *C. chinensis* and *S. oryzae*. Rozman *et al*. (2007) reported that the mortality of *R. dominica* after fumigation with linalool was highly effective and caused 100% mortality at 0.1 mL/720 mL of volume.Moreover, several researchers of some plant extracts for fumigant tested against other insect pests. For example, Pumnuan and Insung (2013) presented that essential oils from eight essential oils including clove (*S. aromaticum*) were found that highly toxic to the aphid mortality more than 60% at 24 hours after fumigation. Brito (2014) reported that the fumigant effect of *Ocimum basilicum* oil caused 100% mortality of *C. maculatus* as compared with *Croton pulegiodorus* oil.

Table 13 Mortality percentage of *Rhyzopertha dominica* and *Sitophilus zeamais* adults after treated with different concentrations of six plant oils by fumigation method for 12 hours.

Insects	Plant oils	Percentage of mortality (Mean±SE)[1/]							
		75 µl/L air	**150 µl/L air**	**300 µl/L air**	**450 µl/L air**	**600 µl/L air**	**800 µl/L air**	**acetone**	**water**
	C. longa	10.00±3.16ab[2/]	26.00±5.10ab	26.00±5.10bc	36.00±5.10b	56.00±4.00b	70.00±4.47bc	0.00±0.00	0.00±0.00
	P. nigrum	8.00±3.74c	16.00±5.10b	36.00±2.45b	38.00±3.74b	64.00±5.10ab	68.00±3.74c	0.00±0.00	0.00±0.00
	S. aromaticum	24.00±2.45a	44.00±6.00a	64.00±7.48a	76.00±5.10a	86.00±8.72a	96.00±2.45a	0.00±0.00	0.00±0.00
R. dominica	*C. nadus*	10.00±5.48ab	18.00±3.74b	28.00±3.74bc	62.00±4.90a	68.00±5.83ab	86.00±.00ab	0.00±0.00	0.00±0.00
	A. excelsa	8.00±2.00c	12.00±3.74b	18.00±3.74bc	28.00±3.74b	48.00±3.74b	56.00±5.10c	0.00±0.00	0.00±0.00
	C. viscose	6.00±4.00c	10.00±3.16b	12.00±2.00c	20.00±3.16b	44.00±5.10b	60.00±3.16c	0.00±0.00	0.00±0.00
	F-test	*	**	**	**	*	**	ns	ns
	C. longa	16.00±2.45ab	30.00±4.47b	38.00±7.35b	46.00±2.45bc	48.00±3.74cd	62.00±3.74bc	0.00±0.00	0.00±0.00
	P. nigrum	12.00±3.74bc	20.00±3.16b	22.00±2.00b	30.00±3.16d	36.00±4.00d	60.00±4.47c	2.00±2.00	2.00±2.00
	S. aromaticum	22.00±2.00a	54.00±4.00a	76.00±6.78a	88.00±5.83a	92.00±2.00a	92.00±3.74a	0.00±0.00	0.00±0.00
S. zeamais	*C. nadus*	18.00±2.00ab	32.00±8.83b	42.00±3.74b	50.00±3.16b	70.00±3.16b	80.00±3.16ab	0.00±0.00	2.00±2.00
	A. excelsa	8.00±2.00bc	12.00±3.74b	20.00±3.16b	30.00±3.16d	52.00±4.90c	66.00±5.10bc	0.00±0.00	0.00±0.00
	C. viscose	4.00±2.45c	12.00±2.00b	20.00±3.16b	32.00±2.00cd	54.00±2.45bc	60.00±3.16c	0.00±0.00	0.00±0.00
	F-test	**	**	**	**	**	**	ns	ns

[1/] average from 5 replications, [2/] means within a column followed by the same letters are not significantly different (P > 0.05) by Turkey's multiple range tests, * significantly at P < 0.05, ** significantly at P < 0.01, ns: non-significantly at P>0.05.

Table 14 Mortality percentage of *Rhyzopertha dominica* and *Sitophilus zeamais* adults after treated with different concentrations of six plant oils by fumigation method for 24 hours.

Insects	Plant oils	Percentage of mortality (Mean±SE)[1/]							
		75 µl/L air	**150 µl/L air**	**300 µl/L air**	**450 µl/L air**	**600 µl/L air**	**800 µl/L air**	**acetone**	**water**
R. dominica	*C. longa*	14.00±5.10b[2/]	34.00±2.45b	38.00±4.90bc	54.00±2.45bc	60.00±3.16c	66.00±5.10c	0.00±0.00	0.00±0.00
	P. nigrum	12.00±4.90b	22.00±3.74bc	48.00±3.74b	48.00±5.83c	68.00±5.83bc	78.00±3.74bc	0.00±0.00	0.00±0.00
	S. aromaticum	32.00±2.00a	52.00±5.83a	68.00±6.63a	90.00±3.16a	92.00±5.58	98.00±2.00a	0.00±0.00	0.00±0.00
	C. nadus	16.00±2.45ab	28.00±3.74bc	42.00±3.74b	74.00±5.10ab	82.00±3.74ab	90.00±3.16ab	0.00±0.00	0.00±0.00
	A. excelsa	10.00±3.16b	18.00±3.74bc	22.00±3.74cd	34.00±5.10c	52.00±3.74c	70.00±3.16c	2.00±2.00	2.00±2.00
	C. viscose	10.00±4.47b	14.00±2.45c	18.00±3.74d	34.00±5.10c	52.00±5.83c	64.00±4.00c	0.00±0.00	2.00±2.00
	F-test	**	**	**	**	*	**	ns	ns
S. zeamais	*C. longa*	22.00±3.74ab	40.00±4.47b	44.00±6.78bc	54.00±5.10bc	62.00±3.74c	80.00±5.48bc	0.00±0.00	0.00±0.00
	P. nigrum	18.00±3.74ab	30.00±3.16bc	36.00±4.00bcd	40.00±3.16c	58.00±3.74c	72.00±3.74bc	2.00±2.00	2.00±2.00
	S. aromaticum	26.00±4.00a	62.00±5.83a	84.00±5.10a	94.00±4.00a	96.00±2.45a	98.00±2.00a	2.00±2.00	0.00±0.00
	C. nadus	26.00±2.45a	42.00±5.83ab	50.00±3.16b	62.00±5.83b	80.00±3.16b	84.00±2.45ab	0.00±0.00	2.00±2.00
	A. excelsa	14.00±2.45ab	16.00±2.45c	24.00±2.45d	38.00±3.74c	58.00±3.74c	74.00±2.45bc	2.00±2.00	0.00±0.00
	C. viscose	10.00±1.63b	16.00±5.10c	26.00±4.00cd	42.00±4.90bc	60.00±3.16c	66.00±2.45c	0.00±0.00	0.00±0.00
	F-test	*	**	**	**	**	**	ns	ns

[1/] average from 5 replications, [2/] means within a column followed by the same letters are not significantly different (P > 0.05) by Turkey's multiple range tests, * significantly at P < 0.05, ** significantly at P < 0.01, ns: non-significantly at P>0.05.

Table 15 Mortality percentage of *Rhyzopertha dominica* and *Sitophilus zeamais* adults after treated with different concentrations of six plant oils by fumigation method for 48 hours.

Insects	Plant oils	Percentage of mortality (Mean±SE)[1/]							
		75 µl/L air	**150 µl/L air**	**300 µl/L air**	**450 µl/L air)**	**600 µl/L air**	**800 µl/L air**	**acetone**	**water**
R. dominica	*C. longa*	22.00±3.74ab[2/]	48.00±3.74ab	58.00±3.74b	62.00±5.83cd	78.00±4.90bc	86.00±4.00ab	2.00±2.00	2.00±2.00
	P. nigrum	16.00±4.00b	38.00±3.74bc	56.00±2.45b	64.00±5.10bc	78.00±3.74bc	84.00±4.00ab	0.00±0.00	2.00±2.00
	S. aromaticum	36.00±4.00a	62.00±5.83a	90.00±6.32a	96.00±2.45a	98.00±2.00a	100.00±0.00a	4.00±2.45	2.00±2.00
	C. nadus	22.00±2.00ab	34.00±2.45bcd	56.00±2.45b	84.00±5.10ab	90.00±3.16ab	96.00±2.45a	4.00±2.45	2.00±2.00
	A. excelsa	16.00±4.00b	26.00±4.00cd	34.00±5.10c	42.00±4.90d	56.00±2.45d	78.00±4.90b	2.00±2.00	2.00±2.00
	C. viscose	16.00±2.45b	18.00±2.00d	28.00±3.74c	44.00±4.00cd	64.00±5.10cd	76.00±4.00b	2.00±2.00	4.00±2.45
	F-test	*	**	**	**	*	*	ns	ns
S. zeamais	*C. longa*	28.00±3.74ab	48.00±3.74bc	60.00±4.47b	76.00±2.45b	80.00±5.48bc	92.00±2.00ab	4.00±2.45	4.00±2.45
	P. nigrum	20.00±3.16b	36.00±4.00cd	48.00±3.74bc	52.00±5.83cd	78.00±3.74bcd	84.00±4.00bc	4.00±2.45	4.00±2.45
	S. aromaticum	42.00±3.74a	72.00±5.83a	90.00±4.47a	96.00±2.45a	98.00±2.00a	100.00±0.00a	2.00±2.00	4.00±2.45
	C. nadus	32.00±2.00ab	54.00±2.45b	64.00±2.45b	70.00±5.48bc	92.00±2.00ab	94.00±2.45ab	2.00±2.00	2.00±2.00
	A. excelsa	20.00±3.16b	26.00±2.45d	38.00±5.83c	50.00±3.16d	64.00±2.45cd	80.00±3.16c	4.00±2.45	2.00±2.00
	C. viscose	20.00±3.16b	28.00±2.00d	42.00±4.47c	52.00±4.90cd	70.00±3.16d	84.00±2.45bc	4.00±2.45	2.00±2.00
	F-test	**	**	**	**	**	**	ns	ns

[1/] average from 5 replications, [2/] means within a column followed by the same letters are not significantly different (P > 0.05) by Turkey's multiple range tests, * significantly at P < 0.05, ** significantly at P < 0.01, ns: non-significantly at P>0.05.

Table 16 Mortality percentage of *Rhyzopertha dominica* and *Sitophilus zeamais* adults after treated with different concentrations of six plant oils by fumigation method for 72 hours.

Insects	Plant oils	Percentage of mortality (Mean±SE)[1/]							
		75 µl/L air	**150 µl/L air**	**300 µl/L air**	**450 µl/L air**	**600 µl/L air**	**800 µl/L air**	**acetone**	**water**
R. dominica	*C. longa*	36.00±2.45ab[2/]	56.00±4.00ab	66.00±4.00b	72.00±3.74bc	88.00±3.74ab	94.00±4.00	2.00±2.00	2.00±2.00
	P. nigrum	30.00±3.16b	46.00±5.10b	62.00±2.00bc	72.00±3.74bc	82.00±6.63ab	92.00±3.74	4.00±2.45	2.00±2.00
	S. aromaticum	44.00±4.00a	70.00±4.47a	92.00±4.90a	98.00±2.00a	98.00±2.00a	100.00±0.00	4.00±2.45	4.00±2.45
	C. nadus	34.00±2.45ab	54.00±2.45ab	60.00±3.16bc	88.00±5.83ab	94.00±2.45a	98.00±2.00	4.00±2.45	4.00±2.45
	A. excelsa	32.00±2.00ab	46.00±2.45b	50.00±5.48bc	52.00±5.83d	70.00±3.16c	86.00±5.10	4.00±2.45	4.00±2.45
	C. viscose	34.00±2.45ab	42.00±3.74b	46.00±5.10c	54.00±2.45cd	72.00±2.00bc	86.00±5.10	2.00±2.00	4.00±2.45
	F-test	*	**	**	**	**	ns	ns	ns
S. zeamais	*C. longa*	38.00±3.74b	58.00±3.74bc	66.00±4.00bc	80.00±3.16b	86.00±2.45bc	96.00±2.45ab	4.00±2.45	4.00±2.45
	P. nigrum	34.00±2.45b	52.00±2.00bc	60.00±4.47bcd	68.00±3.74bc	86.00±2.45bc	94.00±2.45ab	4.00±2.45	4.00±2.45
	S. aromaticum	52.00±2.00a	78.00±5.83a	94.00±4.00a	98.00±2.00a	100.00±0.00a	100.00±0.00a	4.00±2.45	4.00±2.45
	C. nadus	44.00±2.45ab	62.00±2.00ab	70.00±3.16b	78.00±3.74bc	96.00±2.45ab	98.00±2.00ab	4.00±2.45	4.00±2.45
	A. excelsa	32.00±3.74b	46.00±5.10bc	50.00±3.16d	64.00±5.10c	70.00±3.16d	86.00±2.45b	4.00±2.45	4.00±2.45
	C. viscose	34.00±2.45b	44.00±2.45c	52.00±2.00cd	64.00±2.45c	76.00±2.45cd	90.00±3.16ab	4.00±2.45	4.00±2.45
	F-test	**	**	**	**	**	**	ns	ns

[1/] average from 5 replications, [2/] means within a column followed by the same letters are not significantly different (P > 0.05) by Turkey's multiple range tests, * significantly at P < 0.05, ** significantly at P < 0.01, ns: non-significantly at P>0.05.

Table 17 LC_{50} and LC_{90} of six plant oil extracts against of *Rhyzopertha dominica* and *Shitophilus zeamais* by fumigation method at 12, 24, 48 and 72 hours**.**

Insect	Plant oil extracts	12 hour		24 hour		48 hour		72 hour	
		LC_{50} (μl/L air)	**LC_{90} (μl/L air)**	**LC_{50} (μl/L air)**	**LC_{90} (μl/L air)**	**LC_{50} (μl/L air)**	**LC_{90} (μl/L air)**	**LC_{50} (μl/L air)**	**LC_{90} (μl/L air)**
R. dominica	*C. longa*	524.78	-	395.42	-	217.29	-	147.95	869.13
	P. nigrum	477.69	-	358.00	-	245.23	-	178.60	990.61
	S. aromaticum	178.67	736.03	137.78	526.11	111.75	312.07	92.95	272.67
	C. nadus	361.69	-	258.36	949.21	209.81	666.03	139.37	575.82
	A. excelsa	776.03	-	600.52	-	453.20	-	245.85	-
	C. viscose	815.51	-	659.66	-	454.81	-	251.80	-
S. zeamais	*C. longa*	503.64	-	284.63	-	175.13	896.47	135.06	766.69
	P. nigrum	938.96	-	464.80	-	296.00	-	168.73	714.15
	S. aromaticum	148.45	553.74	127.74	383.06	92.99	289.47	77.63	228.36
	C. nadus	322.38	-	221.29	-	154.38	810.53	113.38	603.87
	A. excelsa	631.41	-	526.27	-	390.57	-	225.49	-
	C. viscose	635.85	-	517.77	-	346.78	-	209.54	-

4. Toxicity test by ingestion

Six plants oil used in this study were toxic to *R. dominica* and *S. zeamais* by feeding deterrent method as shown in Table 18. Feeding toxicity of various plant oils to *R. dominica* and *S. zeamais* depended on kind of plant and concentrations after 3 days of feeding (Table 18). Mortality percentage of two insects treated with oils different plant were significantly different ($p<0.05$) in all concentrations at all times of exposure (Table 18). The result of percent mortality revealed that clove oil at all concentrations was the most effective to those insect species with the highest mortality ranged from 38–99% for *R. dominica* and 30–96% for *S. zeamais* as compared with control and acetone after 3 days of feeding (Table 18).

Mortality percentage of *R. dominica* was significantly higher toxic to clove oil than *S. zeamais* at all concentrations. As considered to LC_{50} and LC_{90}, *R. dominica* was more susceptible to clove oil than *S. zeamais* at 3 days of treatment (Table 19) due to higher mortality of *R. dominica* (Table 18). Moreover, *S. aromaticum* oil showed high toxicity as a feeding to both of insects, whereas *C. longa*, *C. nadus*, *A. exelsa* and *C. viscose* oils exhibited moderately toxicity, and *P. nigrum* oil was low toxic to both *R. dominica* and *S. zeamais*. Interestingly, *C. longa* oil caused over 99% mortality of *R. dominica* and *S. zeamais* at the highest concentration.

The result of feeding deterrent activities of various plant oils showed as percent FDI of *R. dominica* and *S. zeamais* feeding on flour disk with different concentrations in a comparison with control are presented in Table 20. FDI percentage was significantly different ($P<0.05$) as compared to the control in both of insect species. Effect of *S. aromaticum* oil on the feeding deterrence index exhibited the most effective to feeding deterrence both of those two species with the highest FDI percentage ranged from 51 – 97% and 45 – 95% for *R. dominica* and *S. zeamais*,

respectively as compared with other plants (Table 20). The overall effect of concentrations on the feeding deterrence index to both *R. dominica* and *S. zeamais* were also increased with respect to the concentration of clove oil increased (Table 20). In addition, plant oil of *P. nigrum* and *C. nadus* exhibited stronger feeding deterrent effects to both of insect as compared to other plant oils (Table 20). Interestingly, plant oil of *C. vicose* showed significantly effective for mortality and feeding deterrent activity to *R. dominica* as compared to *S. zeamais*, while effective mortality and feeding deterrent index of *S. zeamais* showed susceptible to plant oil of *A. excelsa* as compared *R. dominica* (Table 18 and 20).

The results obtained from this study indicated that adults of *R. dominica* were more susceptible to all plant oil extracts than *S. zeamais* by increasing mortality, and rising of feeding deterrent rate. Particularly, clove oil at all concentrations was more effective to control and deterrent of feeding both *R. dominica* and *S. zeamais.* In addition, *R. dominica* and *S. zeamais* exhibited low tolerant to *P. nigrum* and *C. nadus* oils. However, plant oil of *C. vicose* and *A. excels* showed likely effective for feeding deterrence to *S. zeamais* rather than *R. dominica* (Tabel 19).

Several studies with oil extracted from plants exhibited insecticidal effect against stored-product insects. For example, Huang and Ho (1994) found that clove oil was effective mortality to an adult of *S. zeamais* by fumigant and feeding deterrence methods. Valladares *et al.* (2003) indicated the feeding deterrence efficacy of ethanol extract of *Melia azedarach* leaves against *S. oryzae* and found the oil extracted from *M. azedarach* that caused inhibition feeding activities with an antifeedant index of 100%. In addition, Viglianco *et al.* (2008) mentioned that the *S. oryzae* more susceptible in the feeding effect of *Aloysia polystachia* than *Solanum argentinum* and *Tillandsia recurvata*. Huang and Ho (1998) reported cinnamaldehye which is a compound of *Ctenium aromaticum*, reduced feeding of *S. zeamais* at 7 mg/g of concentration. Akhtar *et al*. (2015) showed most potent of *A. indica* oil

against *T. castaneum*, *T. granarium* and *R. dominica* with maximum reduction in weight loss of 0.56, 1.02, 1.69% and feeding deterrence index of 75.44, 54.57 and 39.21%, respectively. Abbasipour *et al*. (2011) reported that feeding deterrent index (FDI) percentage of *T. castaneum* with *Datura stramonium* extracted achieved 34.93% and raised up 97.21% at ranged concentrations from 947-3,007 mg/L. Jaya *et al*. (2012) determined in feeding deterrent effect of *Coleus aromaticus* in controlling *T. castaneum* and obtained 56.39%, 72.31 % and 100% FDI at concentrations of 250, 500 and 1,000 ppm, respectively. Ko *et al*. (2010) evaluated the antifeedant effect of *Litsea salicifolia* against *T. castaneum* and *S. zeamais*. The result indicated that *T. castaneum* was more susceptible than *S. zeamais* at all concentrations and presented the highest FDI (75.44%) in *T. castaneum*.

In addition, many studies for clove extract inhibited feeding to other insect pests. Indrayani *et al*. (2016) reported that the crude extract of *S. aromaticum* caused 100% mortality of termite and reduced 0.3% consumption rates at 0.1% of concentration. Priyanka and Srivastava (2012) evaluated toxicity and insect growth regulator activities to third instar larvae of *Spodoptera litura* of plant oils and found that clove oil was the most effective mortality of 93.33%, followed by ratan jot oil (73.33%) and black pepper oil (43.33%), respectively at 2% concentrations and clove caused reduction in growth of the larvae over control by -15.15 and -11.11% at 1 and 2%, respectively.

Table 18 Mortality percentage of *Rhyzopertha dominica* and *Sitophilus zeamais* adults after treated with different concentrations of six plant oils by feeding deterrent method at 3 days after treatment.

Insects	Plant oils	Mortality percentage (Mean±SE)[1/]							
		0%	1%	3%	5%	10%	15%	30%	acetone
R. dominica	*C. longa*	2.00±1.22	25.00±1.58b[2/]	50.00±2.58ab	67.00±3.74b	80.00±2.24b	84.00±2.45ab	95.00±2.24ab	0.00±0.00
	P. nigrum	2.00±1.22	13.00±2.00cd	21.00±2.92c	48.00±2.00c	56.00±1.87c	66.00±2.92c	75.00±2.24d	1.00±1.00
	S. aromaticum	4.00±1.87	38.00±3.74a	62.00±5.83a	81.00±3.32a	93.00±2.55a	97.00±2.00a	99.00±1.00a	3.00±2.74
	C. nadus	2.00±1.22	9.00±1.87d	24.00±3.32c	41.00±1.87c	64.00±3.67c	79.00±4.30bc	82.00±2.55cd	1.00±1.22
	A. excelsa	2.00±1.22	13.00±2.45cd	22.00±4.36c	50.00±1.58c	54.00±1.87c	69.00±5.34c	81.00±1.87cd	2.00±1.22
	C. viscose	2.00±1.22	22.00±2.55bc	37.00±2.55bc	51.00±1.87c	60.00±3.54c	76.00±3.16bc	88.00±2.55bc	2.00±1.22
	F-test	ns	*	**	**	**	**	**	ns
S. zeamais	*C. longa*	7.00±2.00	16.00±2.45bc	43.00±1.2ab	63.00±3.39ab	79.00±4.00ab	82.00±3.00ab	90.00±2.24ab	6.00±1.87
	P. nigrum	3.00±1.22	7.00±2.00c	12.00±3.00d	37.00±5.15d	52.00±2.45d	59.00±2.45c	70.00±2.24c	3.00±1.22
	S. aromaticum	4.00±1.00	30.00±1.58a	56.00±2.92a	76.00±1.87a	87.00±2.00a	91.00±2.92a	96.00±1.87a	4.00±1.87
	C. nadus	5.00±1.00	15.00±1.58bc	27.00±4.64c	46.00±2.45cd	67.00±2.554bc	81.00±4.30ab	86.00±1.87ab	5.00±1.22
	A. excelsa	5.00±1.20	17.00±2.55b	31.00±2.45bc	53.00±2.00bc	63.00±2.55cd	75.00±4.18abc	85.00±3.87b	5.00±1.58
	C. viscose	6.00±1.78	19.00±2.45b	32.00±4.06bc	47.00±4.64cd	57.00±3.39cd	73.00±5.39bc	84.00±1.87b	6.00±1.00
	F-test	ns	**	**	**	*	**	**	ns

[1/] average from 5 replications, [2/] means within a column followed by the same letters are not significantly different ($P > 0.05$) by Turkey's multiple range tests, * significantly at $P < 0.05$, ** significantly at $P < 0.01$, ns: non-significantly at $P>0.05$.

Table 19 LC_{50} and LC_{90} of six plant oil extracts against of *Rhyzopertha dominica* and *Shitophilus zeamais* by feeding deterrent method at 3 days after treatment.

Insects	Plant oils	LC_{50} (%)	95% confident limit		LC_{90} (%)	95% confident limit	
			Lower	Upper		Lower	Upper
R. dominica	*C. longa*	2.82	2.26	3.41	19.85	15.36	27.81
	P. nigrum	8.08	5.66	11.95	74.31	37.99	271.26
	S. aromaticum	1.74	1.39	2.10	8.56	7.03	10.95
	C. nadus	6.83	5.86	7.94	37.65	28.89	53.27
	A. excelsa	7.34	5.19	10.50	57.38	31.55	174.59
	C. viscose	4.91	4.01	5.94	46.45	32.47	76.39
S. zeamais	*C. longa*	4.39	3.72	5.12	23.95	18.97	32.33
	P. nigrum	11.85	8.31	18.89	74.70	38.17	303.23
	S. aromaticum	2.31	1.85	2.79	13.73	11.00	18.24
	C. nadus	6.24	5.35	7.25	34.22	26.45	47.86
	A. excelsa	6.07	5.10	7.19	43.38	31.76	66.01
	C. viscose	6.66	5.55	7.99	55.64	38.90	91.17

Table 20 Percent of feeding deterrence index (FDI) of six plant oil extracts of *Rhyzopertha dominica* and *Shitophilus zeamais* at 3 days after treatment.

Insects	Plant oils	Percentage feeing deterrent index (FDI) (Mean±SE)[1/]						
		0%	1%	3%	5%	10%	15%	30%
R. dominica	*C. longa*	0.65±1.10(I)[3/]	45.65±2.55a(II)	63.16±3.78a[2/](III)	72.51±1.09a(IV)	84.90±1.41a(IV)	90.02±1.84a(IV)	95.44±1.57a(IV)
	P. nigrum	0.53±1.63(I)	10.71±2.50d	15.08±2.19d(I)	46.01±3.60cd(II)	60.89±3.51b(III)	62.68±3.54bc(III)	71.79±4.06bc(IV)
	S. aromaticum	1.14±1.40(I)	51.66±4.25a(III)	68.91±2.18a(III)	76.10±1.25a(IV)	91.78±1.72a(IV)	95.54±1.40a(IV)	97.12±1.39a(IV)
	C. nadus	0.64±1.43(I)	20.37±2.03cd(II)	29.17±2.40c(II)	38.00±1.87d(II)	43.46±3.12c(II)	53.72±2.20c(III)	64.26±1.73c(III)
	A. excelsa	0.27±1.49(I)	30.79±5.66bc(II)	43.12±2.88b(II)	51.35±3.82bc(III)	55.71±2.69b(III)	59.76±2.61c(III)	67.97±1.68c(III)
	C. viscose	0.23±1.10(I)	41.86±2.97ab(II)	48.10±1.67b(II)	57.31±1.74b(III)	64.62±1.95b(III)	72.10±3.98b(IV)	81.03±2.81b(IV)
	F-test	ns	**	**	**	**	**	**
S. zeamais	*C. longa*	0.52±0.64(I)	40.84±1.98a(II)	53.86±1.90ab(III)	70.85±6.10a(IV)	83.13±1.11a(IV)	84.84±1.68ab(IV)	90.51±1.83ab(IV)
	P. nigrum	0.27±1.39(I)	6.10±1.23c(I)	11.26±2.08d(I)	32.94±3.25d(II)	37.92±2.96c(II)	50.13±8.82d(III)	52.67±6.52e(III)
	S. aromaticum	0.05±2.89(I)	42.72±2.87a(II)	64.87±2.12a(III)	72.22±1.85a(IV)	90.73±2.71a(IV)	94.50±3.07a(IV)	95.91±2.80a(IV)
	C. nadus	0.75±1.74(I)	21.07±4.45b(II)	31.67±5.44c(II)	40.26±3.12cd(II)	44.85±3.58c(II)	56.80±1.27cd(III)	65.08±1.40de(III)
	A. excelsa	1.17±1.23(I)	38.57±1.81a(II)	50.41±2.62b(III)	58.07±1.73ab(III)	63.27±4.43b(III)	70.29±2.10bc(IV)	79.65±1.38bc(IV)
	C. viscose	0.76±1.76(I)	39.14±2.56a(II)	45.98±2.17b(II)	52.35±1.79bc(III)	60.60±3.47b(III)	66.84±1.99cd(III)	77.45±1.75cd(IV)
	F-test	ns	**	**	**	**	**	**

[1/] average from 5 replications, [2/] means within a column followed by the same letters are not significantly different (P > 0.05) by Turkey's multiple range tests, * significantly at P < 0.05, ** significantly at P < 0.01, ns: non-significantly at P>0.05 and FDI class**,** I: FDI% < 20% No feeding deterrence, II: 50% > FDI% ≥ 20% Weak feeding deterrence, III: 70% > FDI% ≥ 50% Moderate feeding deterrence, IV: FDI% ≥ 70% Strong feeding deterrence.

2.2. Chemical composition analysis of the most effective plant oil

Oil of *S. aromaticum* was selected for analysis of chemical composition by using Gas chromatography-Mass spectrometry (GC-MS) technique. This was due to its high toxicity to *R. dominica* and *S. zeamais* according to contact, fumigant, and antifeedant bioassay. Eugenol was the major compound presented in a high amount of 65.83% with the retention time of 21.10 minutes. Two minor compounds were *β*–caryophyllene and eugenol acetate, consisting of 13.54% (RT 22.10 min) and 8.29% (RT 25.25 min), respectively (Table 21). Compounds comprising of <4.0 and > 1% included humulene (3.32%), naphthalene (2.07%) and *α*-caryophyllene (1.59%), whereas the remaining compounds were considered to trace element of clove oil (Table 21). However, several factors related to the different constituent quantity of clove oil such as the part of the clove extracted, extraction method, cultivation, growing condition, genetics, and climate, etc (Alma, *et al*. 2007).

There were many biologically active compounds presenting in clove oil (Kong, 2004). Eugenol was the key composition with different amounts of content ranged from 59.3–89.0% in previous studies. Eugenol acetate and *β*–caryophyllene were reported as a minor composition ranged from 4.2–15.8% and 7.5–24.9%, respectively (Akhtar *et al*., 2008, Hector and Simon, 2004; Fichi *et al.*, 2007, Jirovetz *et al*., 2006). Milind and Deepa (2011) found the eugenol constituent in clove bud ranged 72.08–82.36% and the second major component are eugenol acetate, *β*-caryophyllene, α-humulene ranged 8.6–21.3%, 2.76–8.64% and 0.34–1.04% respectively. Park *et al*. (2005) revealed the mostly component in clove oil that eugenol and *β*–caryophyllene of 86.1% and 11.1%, respectively. The same result with Santoro *et al*. (2007) found that the eugenol and *β*–caryophyllene were mostly of components in clove oils as 86.34% and 8.20%, respectively. Moreover, other components of clove oil found as the minor compound of the clove oil in this study were not reported in the previous study. There was a small number of other compounds found in our study (Table 3) and previous studies such as α-humulene, *β*-

pinene, limonene, farnesol, benzaldehyde, 2-heptanone and ethyl hexanoate (Cortés-Rojas *et al*., 2014).

Although mode of action of clove oil was not clearly documented, but eugenol presenting as the major component in clove oil was reported to irritate skin and eye, respiratory system and effect to nervous system as well as inhibit ingestion resulting in death of insects (Dobroriz *et al*, 2004). Tian *et al*. (2012) demonstrated that essential oil compositions like eugenol inhibited respiration and ion-transport an increased membrane. Also, clove oil toxicity to insects was highly variable.Some orders of insect were quite susceptible and some others were quite tolerant depending on species and formulation of clove (Ho *et al*., 1994).

Table 21 Retention time and chemical composition of essential oils from the flower of *Syzygium aromaticum* at Trai-buri herbal shop in Hat Yai, Songkhla, Thailand

Compound	Retention time (min)	% composition
Benzaldehyde	6.39	0.04
2-haptanal acetate	8.96	0.06
chavicol	16.3	1.07
α–cubebene	19.37	0.55
eugenol	21.10	65.83
benzaldehyde, 4-hydroxy-3-methoxy-	21.51	0.27
benzene, 1,2-dimethoxy-4-(2-propenyl)	21.60	0.33
β-caryophyllene	22.10	13.54
α-humulene	22.93	3.32
δ–cadinene	23.42	0.58
δ–selinene	23.79	0.16
α–muurolene	24.18	0.28
α–farnesene	24.42	0.31
naphthalene	24.89	2.07
eugenol acetate	25.25	8.29
calacorene	25.45	0.17
4(1H)-Azulenone, octahydro-1-methylene-	25.74	0.71
α-caryophyllene	26.63	1.59
10-cubinol	27.85	0.47
tau.-Muurolol	28.24	0.36

3. Assessment of application methods of the most effective plant oil against *R. dominica* and *S. zeamais* in laboratory

Three application methods of seed coating, fumigation and sack coating of *S. aromaticum* oil which was the most effective against *R. dominica* and *S. zeamais* in the previous experiments were tested for this experiment.

3.1 Seed coating application

Results of the adult mortality percentag using seed coating as shown in Table 22 revealed that all clove oil concentrations were toxic to *R. dominica* and *S. zeamais*. Their mortalities were mostly significantly different ($P<0.01$) among treatments. Mortality percentage of *R. dominica* and *S. zeamais* increased with a rise in concentration and exposure time (Table 22). *R. dominica* was more susceptible to clove oil than *S. zeamais* due to lower LC_{50} and LC_{90} (Table 23) and higher mortality of *R. dominica* (Table 22). In opposite to the synthetic insecticide chlorpyrifos, *S. zeamais* was more susceptible to chlorpyrifos than *R. dominica* (Table 22). Samson and Parher (1989) reported that the *S. zeamais* was most susceptible to chlorpyrifos and fenitrothion, whereas the resistance of *R. dominica* has been previously recorded in the United States (Beeman and Wright, 1990). In addition, *S. zeamais* exhibited low resistance to chlorpyrifos and fenitrothion (Ribeiro *et al.*, 2003), and was more susceptible to chlorpyrifos than *S. oryzae* (Arthur, 1994).

Mortality percentage of *S. zeamais* was significantly higher in rough rice seeds coated with chlorpyrifos as compared to seeds treated with *S. aromaticum* oil at all concentrations and times after treatment. On the other hand, clove oil exhibited significantly higher *R. dominica* mortality at 100 μl/L of treatment as compared to chlorpyrifos during the assessed period of time (Table 22). It indicates that seed coating with clove oil at 100 μl/L was more effective to control *R. dominica* than chlorpyrifos. This may be attributed to their chemical constitutes containing in

clove oil, particularly eugenol which was the key component in clove oil. Eugenol was highly toxic and anti-oviposition to *Zabrotes subfasciatus* (Paranhos *et al*., 2006). A high susceptible to plant oils of stored insects was reported. *R. dominica*, *S. oryzae* and *T. castaneum* were susceptible to essential oil extracted of *C. longa*. Qari (Tripathi *et al.*, 2002). Oils of *Z. officinale* and *O. majorana* caused 100% mortality of *R. domininca*, whereas 88.330% mortality of that was achieved after 4 days of application with chlorpyrifos at the recommended concentration in wheat grains (Abdel-Fattah, 2017).

Recently, some of stored product insect pests were found to resist to several insecticides such as malathion, fenitrothion, chlorpyrifos, pyrethrins and phosphine (DARP, 2003; Rajashekar *et al*., 2012). Groot (2004) mentioned that chlorpyrifos was effective against a wide range of stored insect pests, except *R. dominica*. All resistant *R. dominica* populations to organophosphate showed higher acetylcholinesterase activity and susceptible to pyrethrin (Guedes *et al*., 1997). These were consistent with this study result that *R. domoinica* was more tolerant to chlorpyrifos than *S. zeamais* (Table 22).

Results of feeding deterrence expressed as weight loss and frass arising from an adult of *R. dominica* and *S. zeamais* feeding on rough rice seeds coated with different concentrations of *S. aromaticum* oil in a comparison with chlorpyrifos and control are presented in Figure 19. Weight loss and frass production were significantly (p<0.05) higher in control than in both *S. aromaticum* oil and chlorpyrifos after 21 days of treatment. Mastapha (2000) noted no significantly effective of the coating cowpea seed with neem oil and pirimiphos-methyl to reducing oviposition, emergence and seed weight loss from an adult of *Callosobruchus maculatus*. These losses of seeds coated with clove oil were greater in *S. zeamais* than in *R. dominica*. This was attributed to more susceptible of *R. dominica* to clove oil than *S. zeamais*. This was opposite to the result of seeds coated with chlorpyrifos (Figure 19). Wheat grains coated with 5.0% neem powder reduced grain damage by

R. domonica 2.55%, 3.15% and 7.13% as compared to control after 32, 64 and 96 days of treatment (Patel *et al*., 1993). Eucalyptus oil was also reported to be highly effective to reduce grain damage of 6.37% and weight loss of 3.48% as compared to control of those values of 28.58% and 18.39%, respectively in wheat after 120 days of grain coating (Singh *et al*., 2016).

Figure 20 presents the progeny emergence rate of *R. dominica* and *S. zeamais* in F_1 generation on rough rice seeds treated with different concentrations of *S. aromaticum* oil and chlorpyrifos after 21 days of treatment. The results showed that emerged adults were significantly different ($p<0.05$) among the treatments. Clove oil inhibited adult emergence both *R. dominica* and *S. zeamais*. In particular, *R. dominica* was markedly inhibited rather than *S. zeamais*. In contrast to chlorpyrifos, the adult emergence of *S. zeamais* was highly inhibited, whereas that of *R. dominica* was less effective (Figure 20). The lowest tested concentrations (10 µl/L) inhibited the emergence of the F_1 progeny of *R. dominica* by 30.51%, whereas F_1 progeny of *S. zeamais* by 25.76%. A 96.30 % reduction in the F_1 progeny of *R. dominica*, and 93.64% reduction in the F_1 progeny of *S. zeamais*, were observed at a concentration of 200 µl/L. In case of the insecticide seed treatment, chlorpyrifos (40% w/v) completely prevented the emergence of *S. zeamais* adults, whereas it reduced the progeny of *R. dominica* by 24.44%.

This results agreed with the study of Sharma and Meshram (2006) finding that *S. aromaticum* oil at the concentration of 25–250 ppm inhibited F1 progeny from 50.42–72.50%. Ho *et al*. (1997) investigated seed coating with the oil from fresh garlic (*Allium sativum*) and found that *T. castaneum* and *S. zeamais* failed to lay their eggs at the concentrations of >2,000ppm in rice and F1 progeny production was inhibited at the concentration of > 5,000 ppm in wheat, respectively. The emergence of *R. dominica* significantly decreased after treated with 1 ml/100 g rice of clove oil extracted by hexane and methanol (Ho *et al*., 1994). Rahman and Talukder (2006) reported that black gram seed coating with 3% nishinda, eucalyptus,

and bankalmi oils showed good protection against *C. maculatus* by reducing insect oviposition, F1 adult emergence, and grain infestation rates. The oil treatment did not show adverse effects on seed germination even after three months of treatment.

Clove oil as seed coating showed evidently more effective to control *R. dominica* than *S. zeamais.* This may be attributed to different movement behavior of these two insect species. *R. dominica* moved slowly in a downward direction to the bottom of container leading to higher contact opportunity with oil more than *S. zeamais* which was rushed rapidly to the top surface of grains resulting in less contact to the oil. Even the mechanism of plant oil as seed coating against stored insect has not clearly clarified, but the results of our studies demonstrate that clove oil possessed actions of killing, antifeeding and suppressing progeny production. A reduction of progeny production might be attributed to a less extent of oviposition, egg hatchability as well as a survival of larval and pupal stages.

The results obtained from this study clearly indicated that rice seeds coated with chlorpyrifos remained highly effective to control *S. zeamais* by increasing mortality, reducing seed damage and completely suppressing F1 progeny emergence. On the other hand, *R. dominica* showed more tolerant to chlorpyrifos than *S. zeamais* resulting in a low effective to kill *R. dominica*, to protect seed damages and to inhibit progeny emergence as compared to *S. zeamais*. However, clove oil exhibited highly effective on seed rice protection against *R. dominica* due to low insect infestation, low seed damage and low progeny production. Hence, seed coating with clove oil is an alternative method for a good rice seed protection from *R. dominica*. However, this method should be further studied in terms of reasonable application such as economic consideration.

Table 22 Accumulative mortality percentages of *Rhyzopertha dominica* and *Sitophilus zeamais* after seed coating with different concentrations of *Syzygium aromaticum* oil for 1, 3, 5, 7, 14 and 21 days.

Insect species	Concentrations (µL/L)	% Mortality (Mean±SE)[1/]					
		1d	3d	5d	7d	14d	21d
R. dominica	10	5.33±0.67c[2/]	12.67±0.67cd	18.00±3.06de	24.00±5.03cd	36.67±6.70e	40.00±6.11d
	30	8.00±1.15c	18.67±0.6c	23.33±2.40d	36.67±6.96c	64.00±6.43d	67.33±5.70c
	50	11.33±0.67c	23.33±1.76c	46.67±5.46c	64.67±8.11b	77.33±6.36cd	78.67±6.36bc
	100	33.33±1.76b	56.00±5.29b	76.67±5.33ab	89.33±2.91a	95.33±2.91abc	97.33±2.67ab
	150	50.00±2.31a	70.67±1.33a	84.67±2.91a	92.00±1.15a	97.33±0.67ab	99.33±0.67a
	200	57.33±3.06a	81.33±3.33a	92.67±2.91a	99.33±0.67a	100.00±0.00a	100.00±0.00a
	0.4% chlorpyrifos	28.00±6.43a	56.67±4.37b	63.33±5.70bc	66.00±5.03b	80.00±2.31bcd	84.00±3.06abc
	water (control)	0.00±0.00c	0.00±0.00d	2.00±1.15e	3.33±0.67d	6.00±0.67f	6.67±0.67e
	F-test	*	**	**	**	**	**
S. zeamais	10	2.00±1.15d	5.33±1.33e	10.67±1.76ef	14.67±0.67ef	22.67±1.33e	25.33±2.40e
	30	6.67±2.40d	14.00±4.00de	20.00±2.31de	20.00±2.31de	26.00±2.31e	30.67±3.71e
	50	10.00±1.15d	20.67±3.33d	28.67±3.53d	30.67±3.53d	45.33±1.76d	51.33±1.76d
	100	32.67±4.37c	47.33±2.91c	61.33±4.06c	65.33±5.21c	70.67±4.06c	72.00±3.46c
	150	49.33±1.76b	62.00±1.15b	75.33±2.40b	80.67±4.81b	84.00±4.16b	84.67±3.53c
	200	49.33±2.40b	72.67±4.37b	90.00±4.00a	95.33±2.91ab	97.00±2.67a	97.33±2.67ab
	0.4% chlorpyrifos	96.00±3.06a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a
	0 (control)	1.33±1.33d	3.33±0.67e	3.33±0.67f	3.33±0.67f	4.67±0.67	5.33±0.67f
	F-test	**	**	**	**	**	**

[1/] average from 5 replications, [2/] means within a column followed by the same letters are not significantly different (P>0.05) by Turkey's multiple range tests, * significantly at P < 0.05, ** significantly at P < 0.01.

Table 23 LC_{50} and LC_{90} of *Syzygium aromaticum* oil against of *Rhyzopertha dominica* and *Sitophilus zeamais* after rice seed coating for 1, 3, 5, 7, 14 and 21 days.

Time after seed coating (Days)	Insect species	LC_{50} (µL/L)	95% confident limit		LC_{90} (µL/L)	95% confident limit	
			Lower	Upper		Lower	Upper
1	*R. dominica*	171.31	114.88	372.01	993.25	430.52	1959.20
	S. zeamais	181.20	154.53	223.05	739.27	518.07	1250.91
	T-test	*[1/]			ns		
3	*R. dominica*	80.42	49.44	143.27	447.42	216.68	834.51
	S. zeamais	113.66	100.09	130.93	445.51	343.75	799.91
	T-test	ns			**		
5	*R. dominica*	48.51	29.85	72.45	214.17	128.26	632.01
	S. zeamais	73.57	50.95	113.08	286.59	175.34	633.86
	T-test	ns			ns		
7	*R. dominica*	31.96	19.06	46.41	124.43	80.53	282.53
	S. zeamais	63.62	34.26	106.8	246.25	131.83	576.03
	T-test	ns			ns		
14	*R. dominica*	19.03	15.65	22.4	78.29	65.67	97.19
	S. zeamais	48.91	24.02	85.31	225.46	118.04	442.06
	T-test	ns			ns		
21	*R. dominica*	17.39	10.66	24.06	67.82	48.37	85.64
	S. zeamais	43.86	22.48	72.12	219.77	118.80	364.87
	T-test	ns			ns		

[1/] * = significantly at P<0.05, ns: non-significantly at P>0.05.

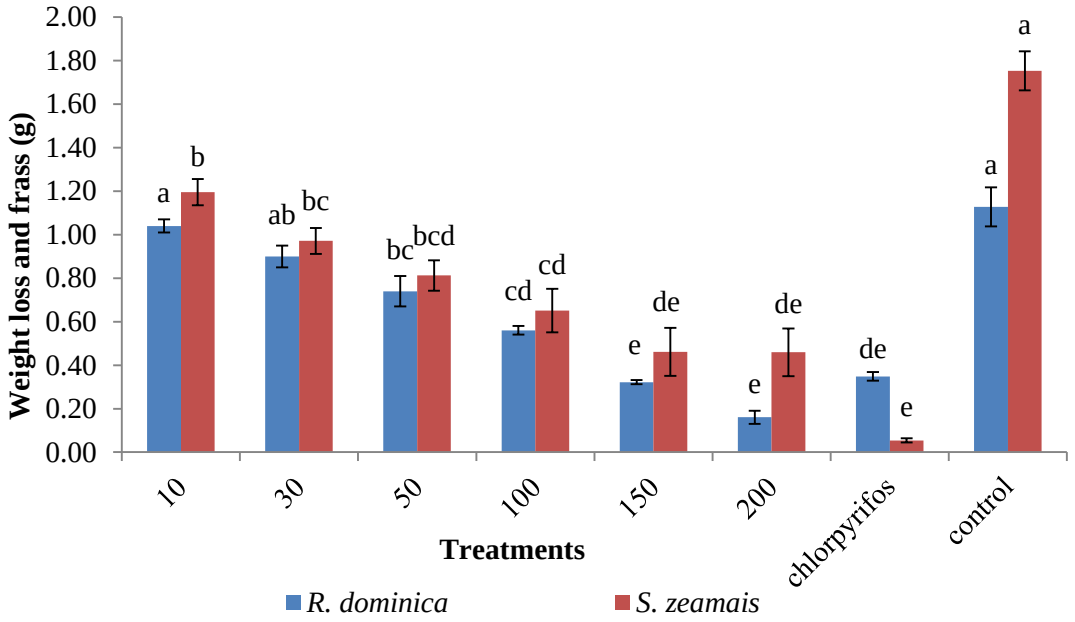

Figure 19 Weight loss and frass production by *Rhyzopertha dominica* and *Sitophilus zeamais* after seed coating with different concentrations of *Syzygium aromaticum* oil as compared to chlorpyrifos and control.

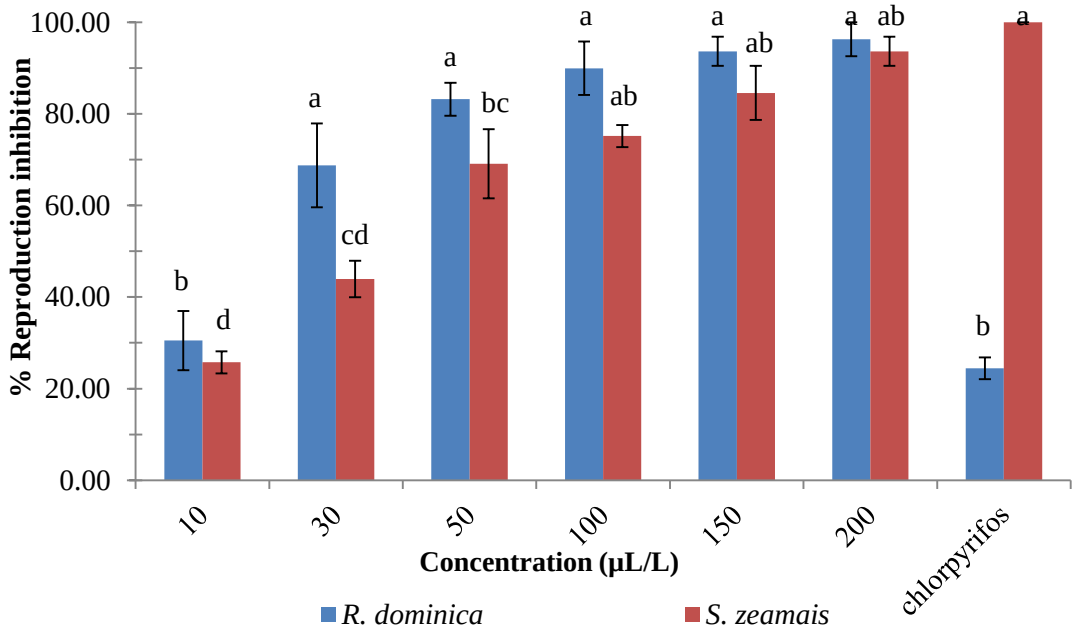

Figure 20 Reproduction inhibition rate of the F1 progeny of *Rhyzopertha dominica* and *Sitophilus zeamais* after treated with different concentrations of *Syzygium aromaticum* oil and chlorpyrifos.

3.2 Fumigation application

Mortality of *R. dominica* and *S. zeamais* after fumigation with clove oil at different concentrations as compared to phosphine and control are summarized in Table 24. Mortality percentages of these two species were a significant difference (p<0.01) among treatments. The mortality increased with an increase of clove oil concentrations and times after fumigation. Even there was no significant difference in LC_{50} and LC_{90} between those two insect species (Table 25), however, *R. dominica* was more tolerant to clove oil and phosphine than *S. zeamais* (Table 24). The mortality of *R. dominica* ranged from 29.33±2.91-62.00±3.06%, whereas that of *S. zeamais* ranged from 66.67±1.76-98.67±1.33% after phosphine fumigation for 1, 3, 5 and 7 days. This may be attributed to the different behavioral movement between these both insect species. The *S. zeamais* moved rapidly in container leading to high O_2 consumption as well as a high uptake of clove oil and phosphine as fumigants via spiracle and tracheal system. This phenomena possibly exhibited higher mortality in *S. zeamais* than *R. dominica* which moved slowly resulting in a low uptake of clove oil and phosphine into insect body. The mechanisms of phosphine resistance in insect included less uptake the gas in respiratory, lower respiration rate and phosphine detoxification (Chaudhry and Price 1992; Pimentel *et al*., 2007).

As compared to phosphine it suggests that clove oil at the concentration of > 5% was more toxic to *R. dominica* than phosphine, while that of >10% showed larger toxicity to *S. zeamais* than phosphine. Due to the low efficiency of phosphine to kill *R. dominica* (<62%), clove oil would be an alternative choice fumigation to control this pest species. However, phosphine still remained highly effective to kill *S. zeamais* (>98.67%). Although phosphine has been an only important fumigant used for controlling stored insect pest because of its high evaporation, low cost and easy application but insects have developed to resist this chemical, particularly *R. dominica* (Borah and Chahal, 1979; Tyler *et al*., 1983; Ahmed and Ahmed, 2000; Chaudhry, M.Q., 2000; Donahaye, 2000; Opit *et al*.,

2012). In addition, Rajendran (1998) reported different extent of phosphine resistance in descending order of different insect species as follows: *T. castameum* (100%) > *R. dominica* (95 %) > *Oryzaephtlus sunnamensis* (92 %) > *S. oryzae* (72%). Pimente *et al*. (2008) revealed that resistance to phosphine was higher in *R. dominica* and *O. surinamensis* than in *S. zeamais* and *T. castaneum* in Brazil.

Respiratory inhibition by disrupting the mitochondrial electron transport chain was the mode of action of phosphine (Chaudhry, 1997). Resistance development of stored insect pests to phosphine was according to repeated fumigations at the same dose of phosphine (Zeng, 1999). The relation between phosphine resistance and reduced respiration rate was observed suggesting that phosphine resistance was associated with reduced phosphine uptake into the insects (Pimentel *et al*., 2007).

Insecticidal activity depended on insect species, the concentration, and kind of plant oils as well as time after treatment. Miyazawa and Hisama (2001) reported that eugenol at 1 mL/L air caused 98%, 99% and 100% mortality of R. *dominica*, *Oryzaephilus surinamensis*, and *C. chinensis*, respectively at 24 h after fumigation. Hamza *et al*. (2016) reported that mortality of *S. granarius* after fumigation with thuja, eucalyptus and peppermint oils achieved 91.2%, 95.0% and 91.2% at 24 h and raised up 100% after 72 h. Forouzan *et al*. (2013) revealed that *Citrus reticulate* oil showed a good killing action against *R. dominica* after fumigation at different exposure times. Kim *et al*. (2003) found that the mortality of *S. oryzae* increased up to 100% after fumigation with cinnamon oil at a dosage of 0.7 mg/cm^2 after 24 h.

Even the mechanism of plant oil as fumigation against stored insect has not clearly clarified, but the route of oil action was largely in the vapor through the respiratory system (Tripathi *et al*., 2009). Both insects death when fumed by the clove oil may be attributed due to interference in gaseous exchange in respiration or

asphyxiation. This noted was supported by Tian *et al*. (2012) demonstrated that essential oil compositions like eugenol inhibited respiration and ion-transport an increased membrane. Especially, monoterpenoid constituents are typically volatile and rather are lipophilic compounds group, which can rapidly penetrate into insects and easily interfere with their physiological functions (Lee *et al*. 2002). Ryan and Byrne (1988) and Houghton *et al*. (2006) indicated that the toxicity effects of plant oil extracts which monoterpenoid composition may be attributed to the reversible competitive inhibition of acetylcholinesterase enzyme (AChE) activity by the occupation of the hydrophobic site, which the center of enzyme's active. Additionally, it is AChE inhibition, the monoterpenes may be acted on other sites target such as cytochrome P450-dependent mono-oxygenases (Lee *et al*., 2001; Ketoh *et al*., 2002).

Our studied results showed that the fumigation with phosphine for control to *S. zeamais* on rice seed still remained highly effective to kill this insect, whereas *R. dominica* showed tolerance to phosphine resulting in less mortality percentage when comparing with clove oil. However, the fumigation with > 5% clove oil showed good effective to control of *R. dominica.* In addition, >10% clove oil showed closely effective control of phosphine against *S. zeamais*. Therefore, the clove oil would be an alternative fumigant to control these pest species in order to reduce phosphine application. However, the cost of clove oil fumigation should be included for making a decision before its application to the control these pests in the future.

Table 24 Mortality percentage of *Rhyzopertha dominica* and *Sitophilus zeamais* after fumigation with different concentrations of *Syzygium aromaticum* oil for 1, 3, 5 and 7 days.

Insects	Concentrations (v/v)	Duration times (Days) (Mean±SE)[1/]			
		1	3	5	7
R. dominica	0.50%	4.67±1.67ef[2/]	10.00±2.31ef	18.67±2.67d	22.00±4.16e
	1.25%	15.33±4.67def	22.00±3.06de	28.67±3.71d	50.00±7.21d
	2.50%	20.00±3.46de	26.67±1.76d	42.00±2.00c	66.00±2.00cd
	5.00%	36.00±4.16bc	54.00±2.00bc	70.00±3.46b	80.00±2.31bc
	7.50%	48.67±4.67ab	60.00±5.03b	82.67±1.33a	91.33±1.33ab
	10.00%	53.33±1.76a	76.67±1.76a	90.00±2.31a	98.67±1.33a
	phosphine	29.33±2.91cd	43.33±4.06c	50.67±2.91c	62.00±3.06d
	ethanol 70%(control)	0.67±0.67f	2.00±1.15f	2.67±0.67e	5.33±0.67f
	F-test	**	**	**	**
S. zeamais	0.50%	1.33±1.33d	10.67±2.91c	24.67±5.21c	44.67±5.21c
	1.25%	8.67±1.67cd	28.67±1.33b	46.67±1.76b	66.67±1.76b
	2.50%	22.67±4.84c	35.33±5.81b	51.33±3.71b	77.33±4.67b
	5.00%	42.67±4.67c	66.67±4.06a	80.00±2.31a	92.00±2.00a
	7.50%	66.00±1.15a	76.67±3.85a	84.67±1.15a	93.00±1.76a
	10.00%	76.67±4.06a	80.67±3.33a	91.33±1.33a	100.00±0.00a
	phosphine	66.67±1.76a	78.00±3.06a	91.33±1.33a	98.67±1.33a
	ethanol 70%(control)	0.00±0.00d	2.67±0.67c	4.00±1.21d	4.67±0.67d
	F-test	**	**	**	**

[1/] average from 5 replications, [2/] means within a column followed by the same letters are not significantly different (P > 0.05) by Turkey's multiple range tests, * significantly at P < 0.05, ** significantly at P < 0.01.

Table 25 LC_{50} and LC_{90} of *Syzygium aromaticum* oil against of *Rhyzopertha dominica* and *Sitophilus zeamais* after fumigation for 1, 3, 5 and 7 days.

Time after fumigation (Days)	Insects species	LC_{50} (%)	95% confident limit		LC_{90} (%)	95% confident limit	
			Lower	Upper		Lower	Upper
1	*R. dominica*	8.81	7.03	11.91	75.41	43.61	173.31
	S. zeamais	8.57	5.83	17.19	52.63	23.37	169.65
	T-test	ns			ns		
3	*R. dominica*	4.66	3.97	5.57	30.07	21.19	48.84
	S. zeamais	3.92	2.47	6.74	22.86	11.24	34.86
	T-test	ns			ns		
5	*R. dominica*	2.50	1.76	3.43	13.33	8.39	30.18
	S. zeamais	2.33	1.59	3.26	10.56	6.75	23.67
	T-test	ns			ns		
7	*R. dominica*	1.52	1.28	1.75	6.88	5.67	8.76
	S. zeamais	1.51	1.03	2.06	6.01	4.16	10.94
	T-test	ns			ns		

1/ ns = ns: non-significantly at P>0.05.

3.3 Sack coating application

Percent movement inhibition of *R. dominica* and *S. zeamais* across jute sheets coated with different concentrations of clove oil after treatment for 1 day to 60 days was shown in Table 26 and Table 27. Movement inhibition depended on time after treatment, clove oil concentration and insect species. Percent inhibition significantly increased with an increase of clove oil concentration, but gradually decreased with time after treatment. Sack coated with clove oil was more effective to inhibit the movement across a sack of *S. zeamais* than *R. dominica.*

The experimental results on *R. dominica* showed that percent inhibition reached over 50% in all tested concentrations of clove oil at the first day after treatment and subsequently decreased with increasing time, from 1 to 7 days. At 3 days after adult introduction, infestation level of 60.00±2.89%, 63.33±4.41%, and 70.00±2.89% were observed at clove oil concentration of 9.0, 12.0, and 20.0%, respectively. After 5 days of treatment, the largest inhibition of 53.33±1.67% was noted at the 20% concentration of clove oil. The jute sheet coated with plant oil showed less effective (<38.33±6.01%) to inhibit *R. dominica* at 7 days after treatment. This was similar to Jute sheets coating with acetone (control) which was less effective inhibition *R. dominica* of 36.67±1.67%, 26.67±1.67%, 13.33±1.67% and 6.67±1.67% at 1, 3, 5, 7 days after adult introduction, respectively (Table 26).

A rise of clove oil concentration significantly ($p < 0.01$) reduced infestation of *S. zeamais* on rough rice. However, the inhibition decreased with time of storage (Table 26). *S. zeamais* infestation was inhibited over 50% at all concentrations recorded on the first day. At 3 days, all concentrations still remained over 50% inhibition except the lowest concentration of 1.0 %, inhibiting 45.00±2.89%. At 5 days, the clove oil at high concentrations of 20% and 12% showed moderate inhibition of 70.00±2.89% and 60.00±2.89% against *S. zeamais,* respectively. However, it was low effective to reduce infestation of *S. zeamais* with

low percent inhibition ranging from 21.67±1.67% to 46.67±6.01% in all concentrations at 7 days (Table 26).

The results of residual toxicity by sack coating of the clove oil at different concentrations to *R. dominica* and *S. zeamais* were significantly different ($P < 0.05$), except at 60 days against *R. dominica* (Table 26 and Table 27). All concentrations of this oil showed infestation inhibition of less than 50% to both species. As considered to EC_{50} of infestation inhibition versus time of sack coating, the EC_{50} values were lower in *S. zeamais* than in *R. dominica* throughout the entire period of study (Figure 21). This indicates that *R. dominica* was more tolerant to clove oil than *S. zeamais* for penetration through the jute sacks. This result was confirmed by ET_{50} values versus concentration as shown in Figure 22. At the same oil concentration, *R. dominica* needed more time to inhibit infestation as compared to *S. zeamais*.

According to the findings of this study, clove oil application by sack coating cannot be used for protection infestation of those two insect species in paddy rice because of its low inhibition penetration of insects (<50% inhibition) at 7 days after treatment (Table 26). It demonstrates that clove oil possessed low repellent activity against *R. dominica* and *S. zeamais.* However, this result was opposite to the study reported by Pongsai (2008) that residual activity of 10% clove oil extended for 2 months against *S. zeamaiz*. Our result this studied is consistent with previously studied of clove oil in repellent bioassay showed that it was a moderate efficiency to repellent both species at all concentration compared with other oil.

An increased time of clove oil by sack coating tended to decrease the prevention of insect penetration. One possible explanation is that some compounds of clove oil are non persistence on sack due to degradation by high temperature or relative humidity (Licciardello *et al*., nd). The essential oils from the plant were volatile compounds (Papachristos and Stamopoulos, 2002; Keita *et al.,* 2000). They

tended to have short-time repellent due to a high volatilization and readily lost because of a small molecule weight of eugenol as 164.20 g (ChemBlink, 2009; Wong *et al*. 2005). In addition, Mullen and Mowery (2000) mentioned that the most insects entered into products through openings caused by sewing, folding, or damage, not by chewing through packaging. However, adults of some insect species could pass through holes less than 1 mm diameter, and their larvae could enter through smaller holes of packaging (Cline and Highland, 1981). Therefore, the ability of chemical component barriers to prevent insects from invading was more important than the prevention of penetration (Hou *et al*., 2004).

Sack coating with plant oils to prevent infestation of stored insects have been extensively studied. Whalon and Malloy (1998) used a mixture of plant extracts from eucalyptus, orange peel, cinnamon, neem, turmeric, and sweet flag with lacquers to apply on food packages and confirmed that the mixture repelled Indian meal moths from invading to the packages, over an 8-week period. Muhammad *et al*. (2005) indicated that neem oil had a strong insecticidal effect on stored grain beetles at 5% to 20% of oil applied on packing bags. Wong *et al*. (2005) reported that the cartons packaging coated with citronella oil of 0.2 g/m^2 reduced beetle infestation to the carton approximately 50% in a comparison with control, and the repellent effect persisted for at least 16 weeks. Pongsai (2008) evaluated three plant oils coated on jute sheet and found that sweet basil was the most effective to protect maize weevil as compared to holy basil and clove oil. Clove oil had the most repellent activity to *E. kuehniella* and prevented its infestation foodstuffs packages, whereas it did not effectively prevent larvae of *S. cerealella* (Allahvaisi *et al*., 2010).

The development packaging that provided adequately for prevention of stored insect pest is limited because of lack of scientist studies. Even, our study showed clearly indicated that clove oil not-remained effective to the protection of those insect species due to some composition of oil destroyed. Therefore, the future studies should be an investigation and consider active oil extract, concentrations, the

persistence of chemical component in packaging, including mechanism and target insects. In addition, the application of coating with plant extract must be considered the disadvantage of sack impregnation with plant extract or chemical insecticide which effect sack cankered rapidly in the long-term period (Navarro and Finkelman, 2014).

Table 26 Percent movement inhibition of *Rhyzopertha dominica* and *Sitophilus zeamais* across jute sheets coated with different concentrations of *Syzygium aromaticum* oil at 1, 3, 5 and 7 days.

Insects	Concentrations (%)	Duration times (Days) (Mean±SE)[1/]			
		1	3	5	7
R. dominica	1.0	50.00±2.29c[2/]	40.00±2.89c	30.00±2.89c	13.33±1.67cd
	3.0	61.67±3.33bc	45.00±5.77bc	30.00±2.89c	20.00±2.89bcd
	5.0	65.00±2.89b	45.00±5.77bc	36.67±3.33cd	23.33±1.67abc
	9.0	68.33±1.67b	60.00±2.89ab	40.00±5.00abc	30.00±2.89ab
	12.0	71.67±3.33ab	63.33±4.41ab	48.33±1.67ab	31.67±4.41ab
	20.0	83.33±1.67a	70.00±2.89a	53.33±1.67a	38.33±6.01a
	Control (acetone)	36.67±1.67d	26.67±1.67c	13.33±1.67d	6.67±1.67d
	F-test	**	**	**	**
S. zeamais	1.0	53.33±3.33c	45.00±2.89b	31.67±1.67d	21.67±1.67bc
	3.0	65.00±2.89bc	53.33±1.67b	36.67±6.01cd	31.67±3.33ab
	5.0	75.00±4.41ab	53.33±1.67b	48.33±1.67bc	36.67±4.41ab
	9.0	76.67±4.41ab	61.67±6.01ab	48.33±1.67bc	38.33±1.67ab
	12.0	78.33±1.67ab	71.67±3.33a	60.00±2.89ab	43.33±4.41a
	20.0	86.67±1.67a	76.67±1.67a	70.00±2.89a	46.67±6.01a
	Control (acetone)	33.33±4.41d	25.00±2.89c	13.33±1.67e	5.00±2.89c
	F-test	**	**	**	**

[1/] average from 5 replications, [2/] means within a column followed by the same letters are not significantly different (P> 0.05) by Turkey's multiple range tests, * significantly at P < 0.05, ** significantly at P < 0.01.

Table 27 Percent inhibition of infestation into jute sheets of *Rhyzopertha dominica* and *Sitophilus zeamais* across jute sheets coated with different concentrations of *Syzygium aromaticum* oil at 8, 14, 21, 28, 35 and 60 days.

Insects	Concentrations (%)	Duration times (Days) (Mean±SE)[1/]					
		8	14	21	28	35	60
R. dominica	1.0	20.00±2.89bc[2/]	10.00±2.89abc	8.33±1.67dc	6.67±4.41ab	3.33±1.67bc	1.67±1.67
	3.0	20.00±2.89bc	13.33±7.26abc	8.33±3.33cd	8.33±3.33ab	5.00±2.89abc	3.33±3.33
	5.0	23.33±1.67ab	21.67±1.67abc	10.00±5.00bcd	10.00±5.00ab	10.00±2.89ab	5.00±2.89
	9.0	30.00±2.89ab	26.67±1.67abc	18.33±1.67abc	16.67±1.67ab	13.33±1.67a	5.00±2.89
	12.0	31.67±4.41ab	31.67±4.41ab	21.67±1.67ab	16.67±1.67ab	13.33±1.67a	6.67±1.67
	20.0	38.33±6.01a	33.33±3.33a	25.00±2.89a	21.67±4.41a	13.33±1.67a	10.00±2.89
	Control (acetone)	6.67±1.67c	6.67±1.67bc	5.00±2.89d	3.33±1.67b	1.67±1.67bc	0.00±0.00
	F-test	**	*	**	*	**	ns
S. zeamais	1.0	23.33±1.67ab	21.67±1.67b	16.67±4.42bcd	11.67±1.67ab	8.33±1.67abc	1.67±1.67ab
	3.0	26.67±3.33ab	21.67±4.41b	18.33±2.89bc	13.33±1.67ab	11.67±1.67abc	3.33±1.67ab
	5.0	30.00±2.89a	23.33±1.67b	21.67±1.67ab	15.00±2.89ab	13.33±1.67abc	3.33±1.67ab
	9.0	38.33±4.41a	30.00±2.89ab	21.67±1.67ab	16.67±1.67ab	13.33±1.67abc	6.67±1.67ab
	12.0	40.00±2.89a	35.00±2.89ab	23.33±2.89ab	20.00±2.89a	15.00±2.89ab	8.33±4.41ab
	20.0	41.67±3.33a	38.33±1.67a	33.33±4.41a	25.00±5.77a	16.67±3.33a	11.67±1.67a
	Control (acetone)	8.33±4.41b	6.67±4.41c	5.00±2.89cd	5.00±2.89b	1.67±1.67bc	0.00±0.00b
	F-test	**	**	**	**	*	*

[1/] average from 5 replications, [2/] means within a column followed by the same letters are not significantly different (P > 0.05) by Turkey's multiple range tests, * significantly at P < 0.05, ** significantly at P < 0.01, ns: non-significantly at P>0.05.

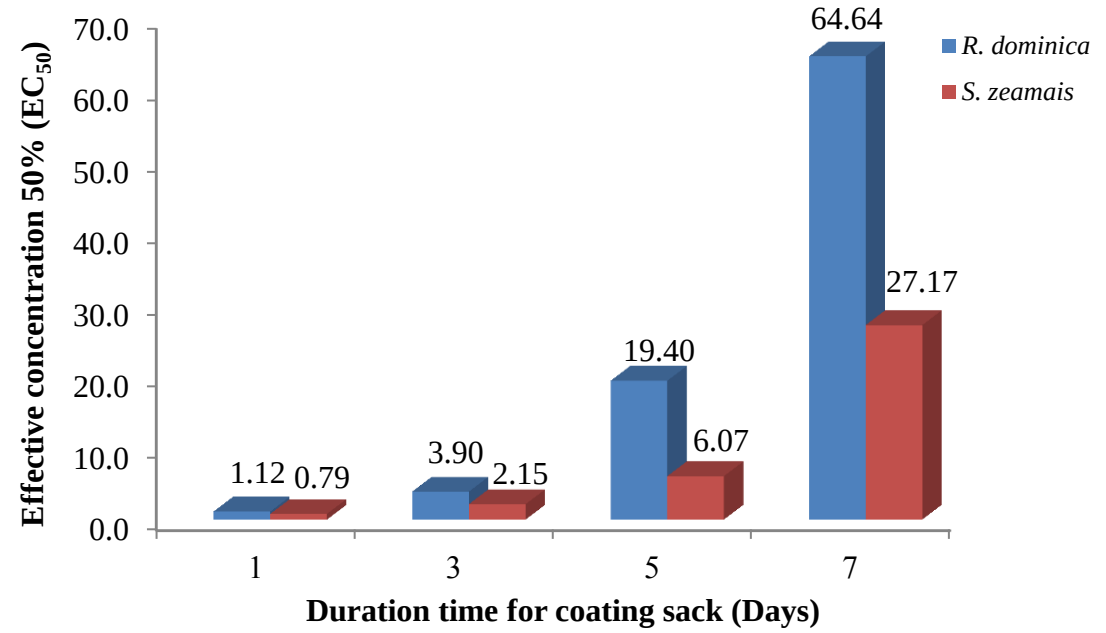

Figure 21 EC_{50} for movement inhibition across a sack of *Rhyzopertha dominica* and *Sitophilus zeamais* after different times of sack coating with *Syzygium aromaticum* oil for 1, 3, 5 and 7 days.

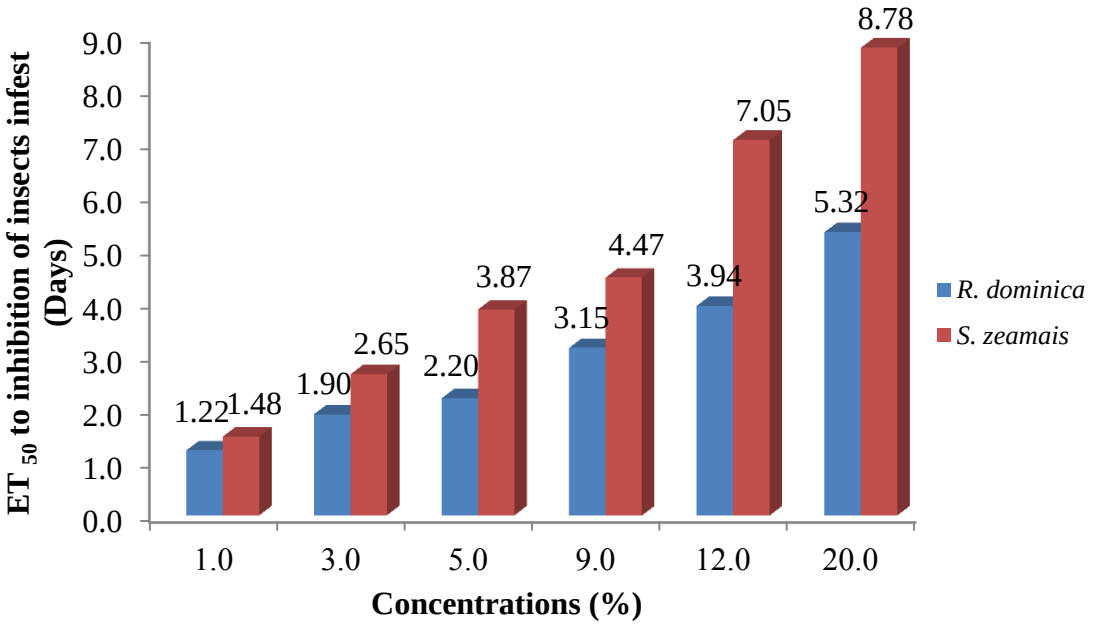

Figure 22 ET_{50} for movement inhibition across a sack of *Rhyzopertha dominica* and *Sitophilus zeamais* after coating with different concentrations of *Syzygium aromaticum* oil.

5. Evaluation of clove oil for controlling *Rhyzopertha dominica* and *Shitophilus zeamais* and seed germination effect in rough rice under storage conditions.

Averages a total number of *R. dominica* and *S. zeamais* and control efficiency in different applications of clove oil and synthetic insecticides under warehouse conditions at SRSC and PRSC were shown in Table 28. *R. dominica* was more abundant than *S. zeamais* in the present study. The population level of *S. zaemais* was very low in the control treatment averaging 2.0 and 10.0 adults/month at SRSC and PRSC, respectively. Therefore, the results of this study can not be applied for controlling this species under warehouse condition. Average numbers of *R. dominica* in all treatments were significantly ($P<0.01$) lower than the control both in SRSC and PRSC. A combination application of seeds coating and fumigation with clove oil was the most effective method with the highest control efficiency of 85.96% and 90.31% at SRSC and PRSC, respectively (Table 28). Consistent with the study of the application of synthetic insecticide as seed coating with chlorpyrifos together with fumigation with phosphine was the least effective with the lowest control efficiency of 40.09% and 40.92% at SRSC and PRSC, respectively (Table 28). It indicates that a conventional practice with combination seed dressing with chlorpyrifos and fumigation with phosphine was seemly poor effective against *R. dominica*. Interestingly, control efficiency percentages of combination treatments with clove oil ranged from 70.92–90.31%, higher than those of chlorpyrifos and phosphine combination. Possibly, clove oil will be an alternative way for an application to control this insect pest in rice storage. However, cost of its application should be further evaluated.

This result is in agreement with the study of Tembo and Murfitt (1995) conducted using the oil of groundnut, rape seed and sunflower at 10 ml/kg and tested alone and combination with pirimiphos-methyl against *Sitophilus granarius* in wheat grain. Those results showed that treatment in oil alone and combined with pirimiphos-methyl were effectively significant to mortality of insects compared with controls

(untreated grain) after 90 days of storage. Moreover, Fallatah (2003) mentioned that vapors of plant oils of cardamom, cinnamon, eucalyptus, coriander and basil inhibited egg oviposition and protected of the potato tubers from an infestation of *P. operculella* moths up to 2 weeks during the storage period. Furthermore, the management of the warehouse before rice stored such as cleaning the warehouse, eliminate of insect still in the warehouse which is one important factor related to insect increasing during rice storage, etc.

There were significant differences in the number of *R. dominica* among the treatments ($P<0.05$) during the entire periods of study from April to September 2016 at SRSC and PRSC (Figure 23 and Figure 24). Population number of *R. dominica* in the control gradually increased from April to July 2016, thereafter the population markedly decreased. The insect population in all treatments continuously decreased from the beginning of April 2016 to the end of the experiment in July 2016 (Figure 23 and Figure 24). It demonstrates that all treated applications did not sharply reduce the *R. dominica* population, but they needed some period of times to gradually decreased the population. Mostly, there was no a significant difference among treatments in *R. dominica* population, however, the combined application of seeds coating and fumigation with clove oil was the most effective method, whereas seed dressing with chlorpyrifos and fumigation with phosphine was the least effective. Similar results have been reported of Bengston *et al*. (1980) reported that chlorpyrifos-methyl was effective against a wide range of stored-insect pests except for lesser grain borer.

In a warehouse at SRSC, the results in Figure 23 showed the efficacy of different applications of *S. aromaticum* oil, chlorpyrifos, and phosphine as a seed treatment and fumigation against *R. dominica.* There was a significant difference ($P<0.05$) in *R. dominica* numbers among treatments. During the first to six months of experiment, the combined application of seed coating and fumigation with *S. aromaticum* oil significantly reduced those numbers from 20.67±3.48 adult to

3.67±2.73 adult as compared with the control treatment which reduced from 80.33±21.07 adult to 57.33±13.38 adult, respectively (Figure 23). Descending order of treated applications were seed coating and fumigation with *S. aromaticum* oil > seed coating with *S. aromaticum* oil + PH_3 fumigation > fumigation with *S. aromaticum* oil > seed coating with chlorpyrifos + PH_3 fumigation.

Similar results of *R. dominica* numbers in different treatments were recorded in the PRSC warehouse (Figure 24). However, this value was larger in the SRSC than in the PRSC, the population density of *R. dominica* was not significantly different between the SRSC and the PRSC (Figure 25). This may be attributed to the difference in average temperature, relative humidity and moval of seed rice during the period of study between both warehouses. A low average temperature ranged from 27.00–29.50 ºC and a high relative humidity ranged from 72.00–81.00% at the SRSC were possibly suitable for increasing insect population as compared to a high average temperature ranged from 30.12–32.36 ºC and a low relative humidity ranged from 67.85–75.75% at the PRSC.

It has been widely known that the temperature and relative humidity are positively correlated with populations of insects. The average temperature in the warehouse at Suratthani Rice Seed Center varied from a low level of 27.30ºC in July to a high level of 29.50ºC in April (Figure 23). Similarly, the average maximum and minimum temperatures of 32.36 ºC and 30.12 ºC were recorded in April and July, respectively at Phatthalung Rice Seed Center (Figure 24). For SRSC, the average relative humidity recorded during the sampled period showed a gradual increase from 72% in April to 81% in September (Figure 23). This pattern was different from that observed at PRSC which the relative humidity gradually increased from 67.85% to 75.5% during April to July 16, and gradually decreased to 70.18% in August, 70.15% in September, respectively (Figure 24). Wang *et al*. (2009) reported that most stored insect could be completely developed at the range of temperature from 20–40 ºC and it had the ability to produce egg and their hatchability at a temperature of 27 ºC. In

addition, Astuti *et al.* (2013) reported that the optimal relative humidity for *R. dominica* development ranged from 70–80% RH. This supports our study results that the number of *R. dominica* at SRSC was greater than at PRSC (Figure 25). Astuai, *et al*. (2013) noted that not only for quality and quantity of foods which are important factors for insect infestation but also temperature and relative humidity are also important factors in the warehouse affecting the growth and development of *R. dominica*. They found that the highest number of 39.67 adults in *R. dominica* adults were recorded at 32 ºC and 80% relative humidity, whereas the lowest one of 6.67 adults was recorded at 20 ºC and 60% relative humidity. However, they could not survive at 40 ºC and relative humidity ranged from 60-80% and died at 7 days after incubations.

All the storage period in this study, temperature and relative humidity may affect the number of *R. dominica* in both Phattalung and Suratthani Rice Seed Center. The results in Figure 23 showed that the number of *R. dominica* in untreated samples varied greatly at different temperature and relative humidity from 80.33 adults to 57.33 adults in SRSC. At 27.30ºC and 80.00% RH, the insect number reached 87.67 adults in July, whereas the lowest one of 57.33 adults was recorded at 27.00ºC and 81.00% RH (Figure 23). In Phatthalung Rice Seed Center, likewise, a number of *R. dominica* varied from 65.00 adults to 50.00 adults which reached the highest number of 80.33 adults at 30.12ºC and 75.75% RH in July and the lowest number of 50.00 adults at 30.47ºC and 70.15% RH in September (Figure 24). In addition, in this study both SRSC and PRSC during the storage period of rice seeds, althought the temperature and relative humidity factors that directly affected to the number of insect pests, but seed rice was moved in or out throughout during storage period that it is one of the factors contributing for rising or declining of stored insect pest populations.

Moreover, Rees (2004) and Hill (2002) mentioned that *R dominica* reach the optimum development at 34ºC. Hagstrum and Subramanyam (2006)

revealed that the development from egg to adult of *R. dominica* vary from 58.8 days at 25 ºC and 31.1 days at 35 ºC and could not develop to adults at low temperature (<25 ºC) and at above 34 ºC. Navarro *et al*. (2002) showed that optimum temperature for development of *R. dominica* occurred ranged from 33–35 ºC. Kumawat (2007) found that *R. dominica* achieved the highest growth and fecundity at 30±1 ºC and 75±5% relative humidity on wheat. However, the number of stored-insect pests in the warehouse may depend on the factors of rice quality, temperature and relative humidity (Astuai, *et al*., 2013). The removal of rice seeds from the warehouse both at the SRSC and PRSC in August and September 2016 may be contributing to reducing the *R. dominica* population (Table 20 and 21).

The moisture contents of rough rice seeds in each treatment stored at the SRSC and the PRSC during the periods of study from April to September 2016 were shown in Table 27 and Table 28, respectively. Mostly, there was not significantly different in seed moisture contents among treatments, except in June, August and September in the SRSC (Table 27). Seed moisture contents in the SRSC ranged from 11.27±0.13–13.70±0.07%, higher than those in the PRSC ranged from 10.53±0.28–12.77±0.15% (Table 27 and Table 28). It suggests that an average high relative humidity during the period of study at the SRSC (72.00–81.00%) evidently affected the high moisture content of rice seeds as compared to the PRSC (67.85–75.75%).

Seed germination of rough rice after treated with clove oil and synthetic insecticides, chlorpyrifos and phosphine, for six months from April to September 2016 was shown in Table 29 and Table 30. Seed germination decreased gradually with an increase in storage time from 98.00±0.25% to 83.50±1.76% at SRSC and from 99.42±0.17% to 88.42±1.60% at PRSC, respectively. A significant difference ($p<0.05$) in percent seed germination among treatments was recorded in the first month of storage at the PRSC, whereas that was recorded in last two months of storage in the SRSC. It indicates that seed germination of rough rice was not affected

by seed coating and fumigation of clove oil and synthetic insecticides, chlorpyrifos and phosphine, under six months of storage.

This study results indicated that the application of clove oil by seed coating and fumigation were effective to reduce population and reproduction of *R. dominica* with no effect on seed germination under six months of storage in the warehouse. Other plant extracts were documented against insect control and their influence on seed viability. Many plant extracts exhibited high effective to control insects without effect on seed viability. Keira *et al.* (2001) showed that seed coating with basil oil extracts in powder form provided completely protection damage from *Callosobruchus maculatus*, and did not affect seed germination. Aliyu (1995) reported that the efficiency of pepper powder at various concentrations reduced oviposition and damage of *C. maculatus*, but seed viability and quality were not affected. Chakradhar *et al*. (2010) indicated that the extracts of *Eicchornia crassipes* was effective to control *S. zeamais* in maize grain when used as surface coating method (100%, $P = 0.05$) and also showed significantly reduced the development of F1 progeny ($97 \pm 0.7\%$) and no effect on seed germination of maize.

Pandey *et al*. (1985) reported that formulations of neem extracts oils, powder, cake, leaves and flowers, and babul gum at 0.1, 1.0, 5.0, 1.0, 0.5 and 1.0% of concentrations were effective against *C. cephalonica* and reduced fecundity, delayed development and emergence of adult in wheat storage with no effect to seed germination. Kumawat and Naga (2013) revealed that neem oil was effective to inhibit adult emergence of *R. dominica* (4.7, 0.0 and 0.0 adults) and showed low grain damage (15.7, 9.3 and 0.0%) and weight loss (4.9, 7.3, 0.0%) at concentrations of 0.1, 0.5 and 1.0 %, respectively, after 90 days of treatment. In addition, adverse effect on the seed viability was not observed when plant oil was applied after 270 days.

Haider *et al*. (2015) investigated the fumigation action of *Tanacetum nubigenum* essential oil against *T. castaneum* and found that it was more effective as

repellent and killing actions with LC_{50} of 13.23 and 8.32 µl/25 L air at 24 and 48 hours of exposure, respectively. There was no side effect on the germination rate of grain (<85%) after 6 months of fumigation. Singh *et al.* (2014) reported that the essential oils of *H. suaveolens* and *A. conyzoides* showed 100% mortality to *T. castaneum* at 250 ppm while *C. aromaticus* at 350 ppm with no adverse effect on seed germination.

Although the mechanism of oil protection seeds is not clear completely, it effected to laying egg and development of larvae on the seed surface. Moreover, their caused mortality of larva before penetrated into the seed, due to female able to lay egg on the seed surface, but the hatching of the larvae is prevented by the oil or could be having not developed normally, moreover mode of action of oil fumigation affected toxic by penetrating to insect body via respiratory system. This study indicated the clove oil application combined together with coating and fumigation method which showed most effective prevention to *R. domina* more than synthetic insecticide including chlorpyrifos as seed coating and phosphine fumigant in 6 months in storage. Although, the oil led to a moisture content of seed slightly up and reduced the percentage of seed germination, the seed germination rate as >80% followed by the department of rice requirement. On the other hand, Nochai and Srichuwong (2007) reported that the seed rice soaked clove oil 40 ml. mixing with a spore suspension of *Fusarium moniliform* (2×10^7 spore/ml) which increased the percent of seed germination by up to 99% and non-significantly when compared with control.

Moreover, the clove oil coating seed combined with PH_3 fumigation indicated not significant different effective against *R. dominca* including moisture content and seed germination when compared with the oil coating and fumigation. In addition, similar to study of control insect in the warehouse of Jilani and Saxena (1988) reported that neem oil alone and combined with Phostoxin fumigant was significantly less prevented *T. castaneum* than untreated control of stored for 8 months in

the Philippines. Moreover, fumigation with ‘Phostoxin’ was effective only for a period of about 2 months against *R. dominica*, and for up to 6 months against other pest species. Zehrer (1984) reported white cowpea coating with 0.5% neem oil protected to *C. maculatus* up to 6 months in storage and weight loss of 18% after 10 months of storage in Togo. However, the use of synthetic insecticide for controlling *R. domininca* including seed coating and fumigation found that this insect high population and tolerance to insecticide but good effective to control *S. zeamais* and not increase the moisture content of seed and reduce seed germination in 6 months after storage. In contrast with Zehrer (1984) reported that the fumigation with aluminum phosphide in cowpeas initially killed all stored insect pests, but re-infestation occurred after 6 months and destroyed all cowpea within 4 months. Therefore, the research for grains protection with application botanical extracts combined together with synthetic insecticide could be necessary for increasing effective prevention of grain to storage pest and inhibition insect development resistance to insecticide rapidly.

Table 28 Average total numbers of *Rhyzopertha dominica* and *Sitophilus zeamais* and control efficiency of different treatments under warehouse conditions at Suratthani Rice Seed Center (SRSC) and Phatthalung Rice Seed Center (PRSC) during April to September 2016.

Treatment	Average number of insects[1/] (control efficiency (%) as compared to the control)			
	SRSC		PRSC	
	R. dominica	*S. zeamais*	*R. dominica*	*S. zeamais*
Seed coating with chlorpyrifos and PH_3 fumigation	276.00±54.37ab[2/] (40.09%)	0.00	237.67±7.51b (40.92%)	6.00
Seed coating with *S. aromaticum* oil and PH_3 fumigation	90.33±34.56 b (80.39%)	0.00	64.67±6.51cd (83.92%)	0.00
Seed coating and fumigation with *S. aromaticum* oil	64.67±18.00b (85.96%)	0.00	39.00±9.54d (90.31%)	0.00
S. aromaticum oil fumigation only	120.33±26.08 b (73.88%)	0.00	71.00±18.36cd (82.35%)	4.00
Seed coating with chlorpyriphos and *S. aromaticum* oil fumigation	127.00±26.50 (72.43%)b	0.00	117.00± 13.23c (70.92%)	6.00
control	460.67± 157.54a (0.00%)	2.00	402.33± 64.31a (0.00%)	10.00
F-test	**		**	

[1/] Averaged number of *Rhyzopertha dominica* and *Sitophilus oryzae* from 6 months, [2/] Means within a column followed by the different letters are not significantly different (P >0.05) by Turkey's multiple range tests, ** significantly at P < 0.01.

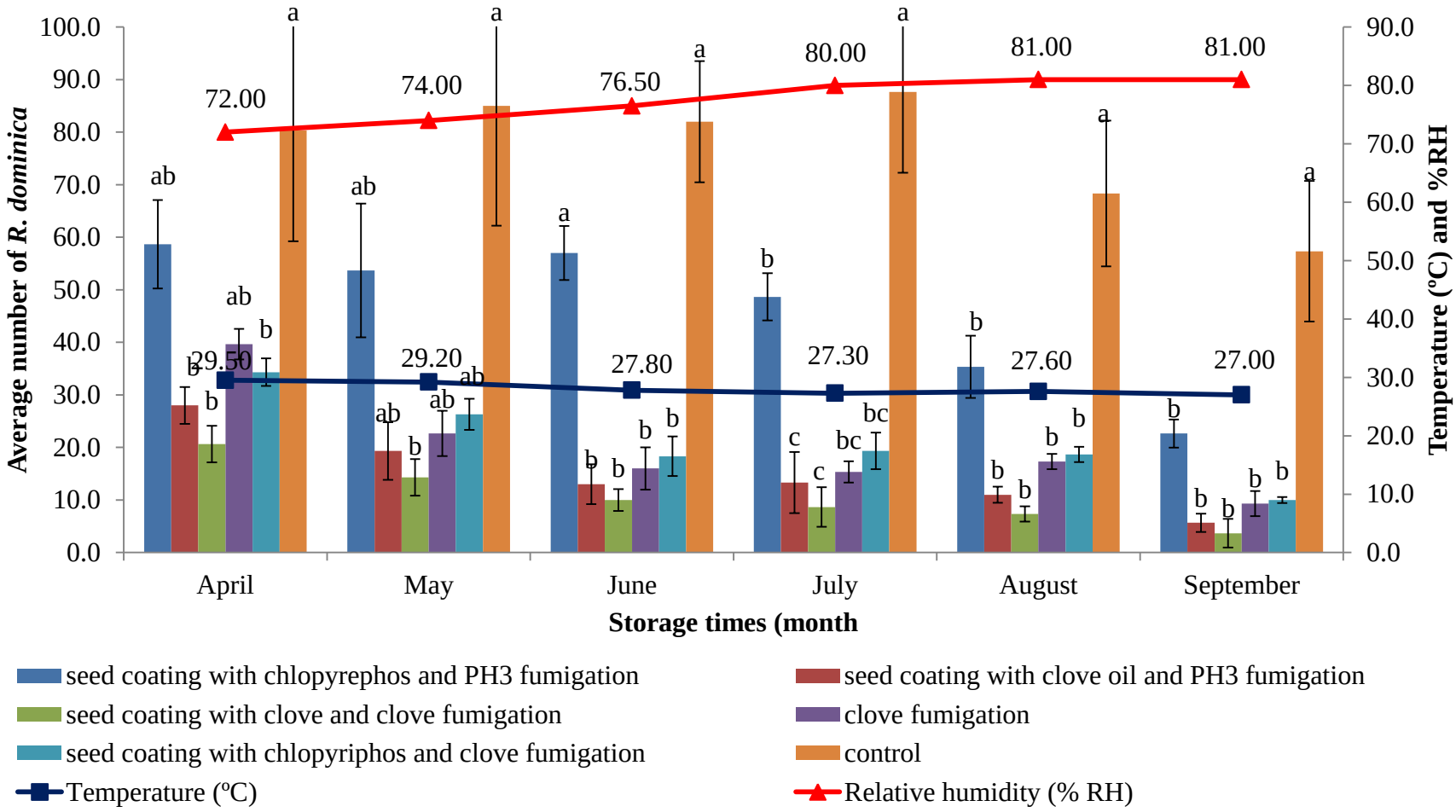

Figure 23 Average number of *Rhyzopertha dominica* in different treatments under different temperature (ºC) and relative humidity (%RH) at Suratthani Rice Seed Center during April to September 2016.

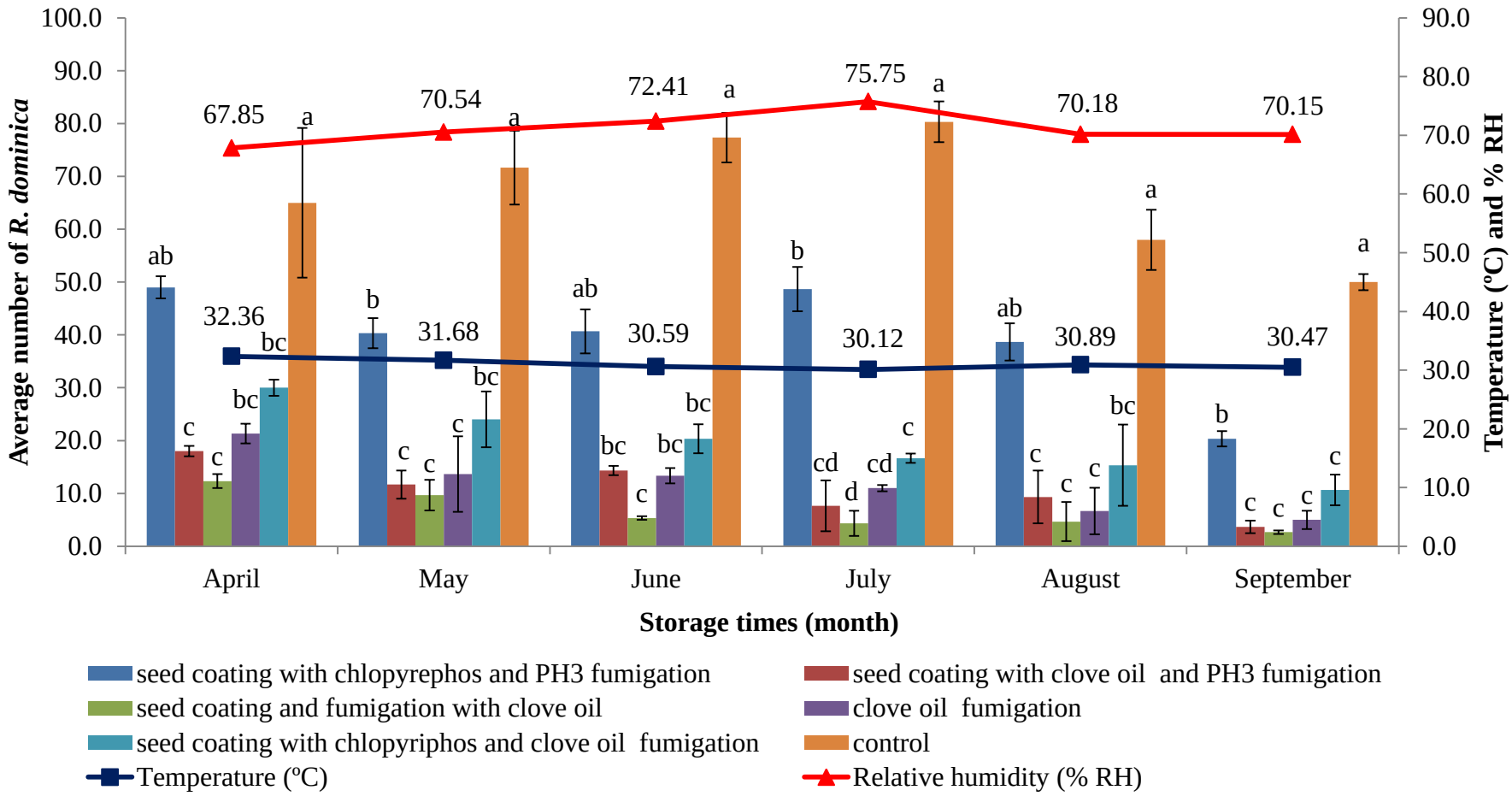

Figure 24 Average number adults of *Rhyzopertha dominica* in different treatments under different temperature (ºC) and relative humidity (%RH) at Pattalung Rice Seed Center during April to September 2016.

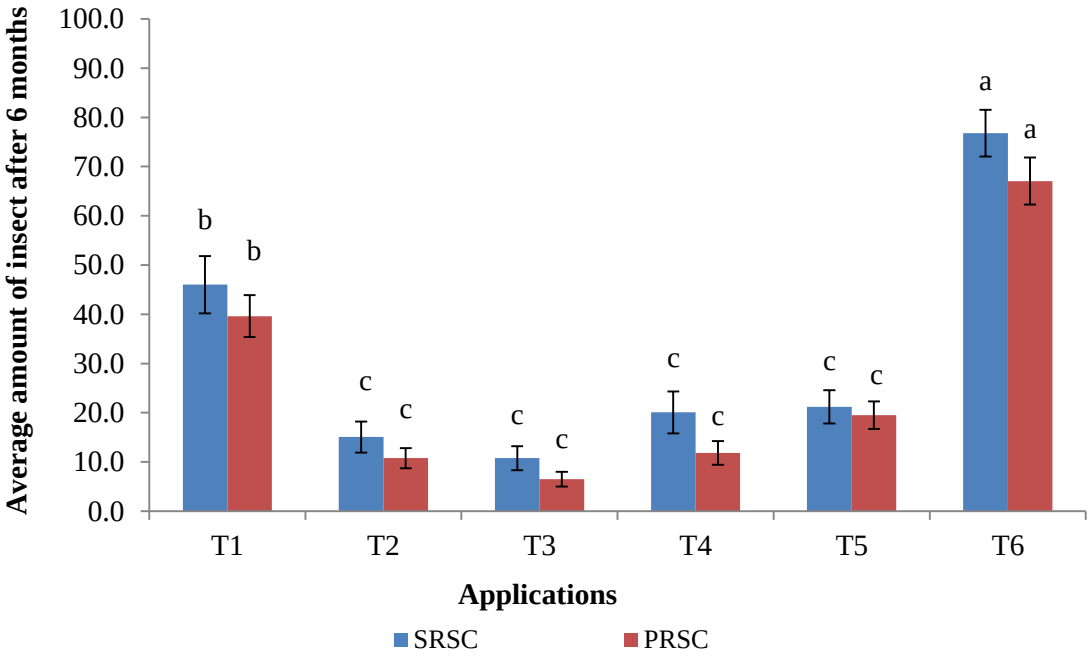

Figure 25 Average numbers of *Rhyzopertha dominica* after application of different treatments during April to September 2016 of storage at Suratthanee Rice Seed Center and Pattalung Rice Seed Center, Thailand

(Note: T1 = Seed coating with chlorpyriphos and PH_3 fumigation

T2 = Seed coating with *S. aromaticum* oil and PH_3 fumigation

T3 = Seed coating and fumigation with *S. aromaticum* oil

T4 = *S. aromaticum* oil fumigation only

T5 = Seed coating with chlorpyriphos and *S. aromaticum* oil fumigation

T6 = Control

Table 29 The moisture content percentages of rice seed after application with different treatments during April to September 2016 of storage at Suratthanee Rice Seed Center, Thailand.

Treatment	% Moisture content (Means±SE)[1/]					
	Apr. 16	**May 16**	**June 16**	**July 16**	**Aug. 16**	**Sep. 16**
Seed coating with chlorpyriphos and PH_3 fumigation	11.30±0.06	11.33±0.07	11.47±0.12b[2/]	12.17±0.09	12.40±0.12bc	12.43±0.12ab
Seed coating with *S. aromaticum* oil and PH_3 fumigation	11.53±0.09	11.57±0.12	12.07±0.13a	12.13±0.12	12.17±0.07bc	12.20±0.17b
Seed coating and fumigation with *S. aromaticum* oil	11.27±0.13	11.73±0.09	12.13±0.03a	11.57±0.43	12.67±0.24ab	12.97±0.29ab
S. aromaticum oil fumigation only	11.43±0.12	11.63±0.07	12.03±0.09a	12.03±0.09	12.00±0.06c	12.17±0.12b
Seed coating with chlorpyriphos and *S. aromaticum* oil fumigation	11.20±0.23	11.20±0.23	11.17±0.20b	11.50±0.10	13.70±0.07a	13.17±0.32a
Control	11.60±0.10	11.67±0.03	12.03±0.03a	12.10±0.06	12.10±0.13c	12.13±0.03b
F-test	ns	ns	**	ns	**	*

[1/] average from 3 replications, [2/] means within a column followed by the same letters are not significantly different (P > 0.05) by Turkey's multiple range tests, * significantly at P < 0.05, ** significantly at P < 0.01, ns: non-significantly at P>0.05.

Table 30 The moisture content percentages of rice seed after application with different treatments during April to September 2016 of storage at Pattalung Rice Seed Center, Thailand.

Treatment	% Moisture content (Means±SE)[1/]					
	Apr. 16	**May 16**	**June 16**	**July 16**	**Aug. 16**	**Sep. 16**
Seed coating with chlorpyriphos and PH_3 fumigation	10.97±0.20	11.17±0.27	11.43±0.07	11.60±0.25	12.33±0.32	12.47±0.24
Seed coating with *S. aromaticum* oil and PH_3 fumigation	10.60±0.06	10.87±0.09	11.10±0.06	11.43±0.34	12.00±0.15	12.67±0.18
Seed coating and fumigation with *S. aromaticum* oil	10.80±0.20	11.23±0.09	11.23±0.15	12.00±0.42	12.37±0.07	12.70±0.15
S. aromaticum oil fumigation only	10.53±0.28	10.80±0.29	11.20±0.55	11.70±0.21	11.93±0.07	12.17±0.09
Seed coating with chlorpyriphos and *S. aromaticum* oil fumigation	10.63±0.32	11.60±0.06	11.67±0.03	11.67±0.03	12.47±0.24	12.77±0.15
Control	10.60±0.06	11.17±0.20	11.50±0.10	11.83±0.03	12.00±0.17	12.10±0.26
F-test	ns	ns	ns	ns	ns	ns

[1/] average from 3 replications, [2/] means within a column followed by the same letters are not significantly different (P > 0.05) by Turkey's multiple range tests, ns: non-significantly at P>0.05.

Table 31 The germination percentages of rice seed after application with different treatments during April to September 2016 of storage at Suratthanee Rice Seed Center, Thailand.

Treatment	% Seed germination (Means±SE)[1/]					
	Apr. 16	**May 16**	**June 16**	**July 16**	**Aug. 16**	**Sep. 16**
Seed coating with chlorpyriphos and PH_3 fumigation	97.83±0.36	97.00±0.52	94.00±1.28	91.83±1.17	92.58±0.30a	90.83±0.88a
Seed coating with *S. aromaticum* oil and PH_3 fumigation	98.00±0.25	97.92±0.30	95.17±0.79	91.67±2.47	89.83±0.44ab	83.50±1.76b
Seed coating and fumigation with *S. aromaticum* oil	97.83±0.33	96.42±0.58	94.08±0.22	94.50±0.29	91.00±1.00ab	89.92±0.46b
S. aromaticum oil fumigation only	97.33±0.08	97.67±0.42	94.25±1.91	93.83±0.65	92.50±1.15a	90.33±0.88a
Seed coating with chlorpyriphos and *S. aromaticum* oil fumigation	97.67±0.08	97.25±0.14	96.33±0.17	94.92±0.73	93.33±0.33a	90.25±0.66a
Control	97.67±0.08	96.83±0.46	93.25±1.13	88.67±3.66	87.33±0.93c	80.83±1.36b
F-test	ns	ns	ns	ns	**	**

[1/] average from 3 replications, [2/] means within a column followed by the same letters are not significantly different (P > 0.05) by Turkey's multiple range tests, ** significantly at P < 0.01, ns: non-significantly at P>0.05.

Table 32 The germination percentages of rice seed after application with different treatments during April to September 2016 of storage at Pattalung Rice Seed Center, Thailand.

Treatment	% Seed germination (Means±SE)[1/]					
	Apr. 16	**May 16**	**June 16**	**July 16**	**Aug. 16**	**Sep. 16**
Seed coating with chlorpyriphos and PH_3 fumigation	97.33±0.22b	98.33±0.71	96.83±0.46	96.83±0.51	91.17±1.34	90.08±0.44
Seed coating with *S. aromaticum* oil and PH_3 fumigation	99.25±0.25a	97.50±0.72	97.92±0.17	95.25±0.76	94.33±1.52	88.67±3.66
Seed coating and fumigation with *S. aromaticum* oil	99.42±0.17a	99.00±0.25	97.75±0.14	96.33±0.44	89.25±2.08	88.42±1.60
S. aromaticum oil fumigation only	98.92±0.08a	98.33±0.22	98.00±0.43	91.25±7.64	91.08±1.59	90.00±3.13
Seed coating with chlorpyriphos and *S. aromaticum* oil fumigation	97.25±0.52b	97.17±0.22	97.08±0.51	96.67±0.73	95.33±0.79	94.92±1.34
Control	98.92±0.08a	97.17±9.22	97.50±0.72	96.67±0.73	95.92±0.82	92.50±0.43
F-test	*	ns	ns	ns	ns	ns

[1/] average from 3 replications, [2/] means within a column followed by the same letters are not significantly different (P > 0.05) by Turkey's multiple range tests, * significantly at P < 0.05, ns: non-significantly at P>0.05.

6. Cost comparison between clove oil and synthetic insecticide applications for controlling *R. dominica* and *S. zeamais* under storage conditions

Cost of chlorpyrifos and phosphine application in a comparison with clove oil application for six months of storage is shown in Table 34. The application by seed coating with chlorpyrifos and PH_3 fumigation was the cheapest; even it exhibited the lowest effective to control *R. dominica*. On the other hand, seed coating and fumigation with clove oil were the most expensive, even it was the best effective to control *R. dominica* (Table 28 and Figure 24). The cost of seed coating and fumigation with clove oils were 2,568.00 Bath/tons and 1,945.97 Bath/ton, respectively, while seed coating with chlorpyrifos and fumigation with PH_3 were 6.90 Bath/ton and 7.50 Bath/ton, respectively.

Based on the control efficiency of different application methods as shown in Table 25 it suggests that the application of seed coating with clove oil and PH_3 fumigation is a possible alternative method for controlling *R. dominica*. This method provided the control efficiency of *R. dominica* > 80% with the cost of 2,590.5 Baht/ton. As compared to other methods presenting clove oil, the cost ranged from 5,844.81-8,405.91 Baht/ton, but the control efficiency of *R. dominica* ranged from 70.92%-90.31% (Table 34 and Table 28). Although the seed coating with chlorpyrifos and PH_3 fumigation was the cheapest application, however, it gave the lowest control efficiency of *R. dominica* by approximately 40%. Currently, this method still remains in the application for rice storage in Thailand. In terms of a high efficiency for controlling stored insects, a lower resistance development rather than the synthetic insecticide and the more safety to human and the environment, the cost reduction of clove oil application should be concerned and focused for further research. Clove oil usually is imported to Thailand. It should be promoted to grow as a medicinal plant in Thailand to reduce the cost of import from abroad in the future.

As mentioned above the application cost of clove oil is higher than that of the synthetic insecticides. It is possible to use clove oil in rice nich market such as organic rice because of its high price. As shown in Table 34, the prices of organic paddy rice and organic milled rice are 1.4 to 1.5 folds and 2.3 to 2.5 folds of normal paddy rice and normal milled rice, respectively (Yotkaew, 2017). The price of organic rice in the foreign market is 25-30% higher than that of normal rice especially organic jasmine rice, which is close to Basmati rice variety of India (Kasikorn Research Center in Thailand, 2007). The main organic rice markets of Thailand are the EU countries which the demand increased by 15-20% per annum (Yotkaew, 2017; Ministry of Commerce, 2015). In addition, there is a tendency for Thai organic rice exporters to expand in the US, Japan, and Australia (Yotkaew, 2017). The organic rice especially jasmine rice which is popularly exported worldwide has rapidly increased in development and marketing value. The largest market share of 96% organic jasmine rice was reported for foreign markets (Yotkaew, 2017). One of the important reasons was driven by the rise in customer awareness that is a health benefit and environmental–friendly (Priyanga and Venkataraman, 2017). Furthermore, the storage of organic rice is important to avoid the use of chemicals. The plant extracts especially clove oil has an environmentally safe, non-residue in yield, and as insect poisoning. Therefore, the use of clove oil is an alternatives for protection insects in organic rice management and can increase the value and marketing channel of organic rice in the global market in the future.

Table 33 The price of paddy and milled of Jasmin rice between normal and organic rice during 2013-2014

Rice	Type	Price (Bath/Ton)
Paddy rice	Normal	13,200-15000
	Organic	20,000-21,000
Milled rice	Normal	28,000-30,000
	Organic	> 70,000

Source: http://www.thairicemillers.com; Ministry of Commerce, 2015

Table 34 Cost application of synthetic insecticide (chlorpyrifos and phosphine (PH_3)) compared with *S. aromaticum* oil and total cost of cost during March – September 2016 in rice storage

Treatment	Cost (Bath/Ton)							Total cost (Bath/Ton)
	Mar	Apr	May	Jun	Jul	Aug	Sep	
Seed coating with chlorpyrifos and PH_3 fumigation	6.90	7.50	7.50	7.50	7.50	7.50	7.50	51.90
Seed coating with *S. aromaticum* oil and PH_3 fumigation	2,568.00	7.50	-	7.50	-	7.50	-	2,590.50
Seed coating and fumigation with *S. aromaticum* oil	2,568.00	1,945.97	-	1,945.97	-	1,945.97	-	8,405.91
S. aromaticum oil fumigation	1,945.97	1,945.97	-	1,945.97	-	1,945.97	-	7,783.88
Seed coating with chlorpyriphos and *S. aromaticum* oil fumigation	6.90	1,945.97	-	1,945.97	-	1,945.97	-	5,844.81

CHAPTER 4

Conclusions and Recommendations

Bioassay tests of repelling, killing and anti-feeding actions of six plant oils against *Rhyzopertha dominica* and *Sitophilus zeamais* were investigated in a laboratory. Pepper oil was the most effective, while oil from *C. viscose* was the least effective to repel both insect species. The descending order of plant oils to repel these insect species were *P. nigrum*> *C. nadus*> *S. aromaticum*> *C. longa*> *A. excelsa*> *C. viscose.* Clove oil showed highly contact, stomach, inhalation toxicity, and effectively inhibited feeding of *R. dominica* and *S. zeamais*. Therefore, clove oil was selected to analyze chemical composition and to conduct further investigation. The major chemical compounds found in clove oil were eugenol, *β*–caryophyllene and eugenol acetate.

Three application methods of seed coating, fumigation and sack coating with clove oil in rice seeds were assessed to control *R. dominica* and *S. zeamais* as compared to chlorpyrifos and phosphine in the laboratory. Seed coating and fumigation methods showed high effectiveness, whereas sack coating was low effective. *R. dominica* was more tolerant to chlorpyrifos and phosphine than *S. zeamais*. However, the effectiveness of chlorpyrifos and phosphine were lower effective to kill *R. dominica* than clove oil, but all of them were still effective to kill *S. zeamais.*

Clove oil applications by seed coating and fumigation were evaluated in a comparison with chlorpyrifos and phosphine under warehouse storage conditions for six months at Suratthani Rice Seed Center and Phatthalung Rice Seed Center for controlling *Rhyzopertha dominica* and *Shitophilus zeamais* and seed germination was assessed as well. In addition, cost of application was compared between using clove oil and using both insecticides. Seed coating and fumigation with clove oil showed high effectiveness beyond seed coating with chlorpyrifos and fumigation with

phosphine. However, the cost of clove oil application was highly greater than that of chlorpyrifos and phosphine application. All application methods with clove oil, chlorpyrifos and phosphine exhibited no effect on rice seed germination.

In conclusions, chlorpyrifos and phosphine were low effective to control *R. dominica*, but still effective to control *S. zeamais*. In order to avoid detrimental effect following synthetic insecticide application, clove oil is, therefore, an alternative method for controlling *R. dominica* under a reasonable cost of application.

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VITAE

Name Miss Kanok-on Wuttiwong

Student ID 5510630012

Education Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Science (Agricultural)	Prince of Songkla University	2004
Master of Science (Entomology)	Prince of Songkla University	2008

Scholarship Award during Enrolment

1. A scholarship from the Agricultural Research Development Agency (public organization) for Ph.D. Program
2. Graduate School support the research funding for Thesis, Prince of Songkla University
3. Thesis research was supported by the Center of Excellence in Agricultural and Natural Resources Biotechnology (CoE-ANRB), Prince of Songkla University

Work Experience

2004–2005	Agricultural researcher (monthly employee) at Department of Agricultural (DOA), Bangkok, Thailand.
2008–2010	Agricultural researcher (monthly employee) at Bureau of Agricultural Research and Development Region 8, Songkhla, Thailand.
Feb–June 2010	Public health researcher at The Office of Disease Prevention and Control 6 Chonburi, Chonburi, Thailand.
2010– a present	Agricultural researcher at Surat Thani Rice Seed Center, Surat Thani, Department of Rice, Thailand.

List of Publications and Proceeding

Wuttiwong, K., Ngampongsai, A. and Chanbang, Y. 2015. Repellent activity against maize weevil (*Sitophilus zeamais Motschulsky*) of certain plant oils. Khon Kaen Agr. J. 43 Suppl. 1: 145–150.

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