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THESIS

INSECTICIDAL ACTIVITIES OF THREE ESSENTIAL OILS AGAINST *Sitophilus zeamais* Motschulsky AND *Tribolium castaneum* (Herbst)

KO KO

A Thesis Submitted in Partial Fulfillment of
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Ko Ko 2009: Insecticidal Activities of Three Essential Oils against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst). Master of Science (Tropical Agriculture), Major Field: Tropical Agriculture, Interdisciplinary Graduate Program. Thesis Advisor: Professor Angsumarn Chandrapatya, Ph.D. 118 pages.

The insecticidal activities of essential oils extracted from the leaves of *Melaleuca cajuputi,* the mature fruits of *Litsea cubeba* and *L. salicifolia* were investigated under laboratory conditions. *Litsea cubeba* and *L. salicifolia* oils showed high repellency effects on *Sitophilus zeamais* and *Tribolium castaneum*, whereas *M. cajuputi* exhibited moderate repellency to *S. zeamais* and high repellency to *T. castaneum*. Generally, repellency effect increased with concentration of the essential oils. *Melaleuca cajuputi* evoked the highest fumigant toxicity on *T. castaneum* (213.17 µL/L) whereas *L. salicifolia* showed the highest fumigant toxicity to *S. zeamais* (4.435 µL/L), but its fumigant effect on *T. castaneum* was negligible. Meanwhile, *L. cubeba* had moderate fumigant effect on both species. Complete mortality of *S. zeamais* was detected when *L. cubeba* oil was applied at the rate of 370 µL/L 24 h after treatment. However, both *M. cajuputi* and *L. salicifolia* could only show the complete mortality of *S. zeamais* at the highest (556 µL/L) and the second highest (444 µL/L) application rates, 24 h after treatment.

Melaleuca cajuputi showed the highest contact toxicity to *S. zeamais* at the rate of 20% compared to *L. cubeba* and *L. salicifolia*. However, *M. cajuputi* oil was less effective against *T. castaneum* when compared *to S. zeamais* at all application rates. Meanwhile, *M. cajuputi* and *L. salicifolia* exhibited moderate contact toxicity against *T. castaneum* whereas *L. cubeba* had little contact toxicity to that species. Although *L. cubeba* oil could induce 100% mortality of *S. zeamais*, only 48% mortality of *T. castaneum* could be detected at the highest application rate and at the same duration. The complete mortality of *S. zeamais* was caused by 30% concentration of *L. salicifolia* at 3 d, whereas only 68% mortality of *T. castaneum* could be observed at the highest application rate (40%) at 7 d. In addition, *M. cajuputi* showed the highest antifeedant effect on *S. zeamais* than the other two oils whereas *M. cajuputi* and *L. salicifolia* had the highest antifeedant toxicity to *T. castaneum*. Up to 70% mortality of *S. zeamais* was caused by 8-10% concentrations of *M. cajuputi*, but only 5% mortality of *T. castaneum* could be detected at the highest concentration rate. In addition, only 24 and 12% mortality of *S. zeamais* and *T. castaneum* could be observed at the highest application rate of *L. cubeba* whereas only 4% mortality of *S. zeamais* and 10% mortality of *T. castaneum* could be found at the highest application rate of *L. salicifolia* oil.

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Student's signature Thesis Advisor's signature

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LIST OF ABBREVIATIONS

µg	=	microgram
µL	=	micriliter
ANOVA	=	Analysis of Variance
cm	=	centimeter
d	=	day
diam	=	diameter
E	=	East
FDI	=	Feeding Deterrence Index
Fig	=	Figure
g	=	gram
GC	=	Gas Chromatography
h	=	hour
L	=	Liter
LC50	=	Lethal Concentration 50%
LC95	=	Lethal Concentration 95%
LD50	=	Lethal Dose 50%
Lsd	=	Least Significant Difference Test
Ltd.	=	Limited
m	=	meter
mg	=	milligram
min	=	minute
mL	=	milliliter
mm	=	millimeter
MS	=	Mass Spectrometry
N	=	North
PR	=	Percent Repellency
RH	=	Relative Humidity
SPSS	=	Statistical Package of Social Science
USA	=	United States of America

INSECTICIDAL ACTIVITIES OF THREE ESSENTIAL OILS AGAINST *Sitophilus zeamais* Motschulsky AND *Tribolium castaneum* (Herbst)

INTRODUCTION

The major problem in agriculture nowadays is to produce enough food for world population whose number is in permanent augmentation. One of the most important constraints of having everyday sufficient food is the post harvest preservation of good quality and quantity. Stored products are the end result of a sequence of husbandry operations in the field, starting from land preparation to harvesting. After harvesting, it is necessary to store the products for some periods before consumption or use for a variety of purposes. However, during storage, stored crop products are usually liable to depreciation by pest organisms especially insects. Most of the stored pests are Coleopterans, especially those belong to *Sitophilus* and *Tribolium* genera (Dal Bello *et al*., 2001). These stored grain insect pests cause reductions in weight, quality, commercial value and seed viability of the stored products (Hou and Fields, 2003).

Post-harvest sector has been conspicuously neglected as compared to pre-harvest. However, in the recent past, trend has changed due to demand of good quality products. The quality of stored products decreases due to large numbers of dead insect bodies, cast skins, faecal remnants and also excretions. These insect pests often cause extensive damage to stored grains and their products and this may amount to 0.5-10% in the temperate zone and 20-30% in the tropical zone (Haque *et al.,* 2000).

The efficient and effective control of storage insects like *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst) mainly depended on the use of synthetic insecticides (Isikber *et al*., 2006; Mondal and Khalequzzaman, 2006). Pest control in many storage systems depends on fumigation with either methyl bromide or

phosphine. However, under the Clean Air Act and Montreal Protocol, the use of methyl bromide has been prohibited in developed countries since 2005 and will soon be restricted in developing countries in 2010 because of its potential to damage the ozone layer. Moreover, some stored-product insects are found to develop resistance to phosphine in many countries, thus the further use of phosphine could be threatened by further development of resistance strains (Bell and Wilson, 1995; Collins *et al*., 2001). Therefore, this wide role of methyl bromide and phosphine is likely to be more restricted in the future. Moreover, increased public concern over the residual toxicity of insecticides applied to stored insect strains and the necessary precautions to work with traditional chemical insecticides call for new approaches to control stored product insect pests (Yilsrim *et al.,* 2001).

Plants are rich sources of natural substances or secondary metabolites that can be utilized in the development of environmentally safe methods for stored insect pest control. An alternative to synthetic pesticides is the use of natural compounds such as essential oils resulted from secondary metabolism in plants. These compounds are volatiles with high insecticidal efficiency and very low persistence. Most of the active compounds of essential oils are specific to particular insect groups (Huang *et al*., 1997) and not to mammals (Isman, 2000), many of them are not dangerous to humans. Hence, they should be considered in pest management strategies. The toxicity of a large number of essential oils and their constituents has been evaluated against a number of stored-product insects (Paranagama *et al*., 2003). The deleterious effects of essential oils and crude plant extracts on stored insect pests are manifested in several ways including toxicity (Tapondjou *et al*., 2005; Bittner *et al*., 2008), growth retardation (Anwar *et al*., 2005), feeding inhibition (Isman, 2006), oviposition deterrence (Tunc *et al*., 2000), repellence (Wong *et al*., 2005) and fumigant (Tumaalii, 2002).

In the past, the use of locally available plant materials to limit insect damage in stored grains is a common practice in traditional farm storage in developing countries (Talukdar and Howse, 1995). Nowadays, with the development of phytopharmaceutical industries and liberalisation of chemical insecticide markets this

traditional know-how popular is disappearing. To date, many researchers investigated the property of several plants used to protect crops from insect pests. Many studies have documented plants used to protect crops from pest and insect attacks. Many of these botanicals are aromatic plants producing essential oils where main compounds are highly volatiles with low persistence (Papachristos and Stamopoulos, 2002; Park *et al*., 2003a). Essential oils with these properties are good alternatives tools to replace highly persistent chemical insecticides in the control of stored grain insect pests (Shaaya *et al*., 1997).

In the current scenario, it is an urgent need to develop new alternatives that must be ecologically sound with no residual activity and adverse effect on other non-target animals. In this regard, many plant products including essential oils have been evaluated for their toxic properties against different stored grain pests (Channoo *et al*., 2002; Kim *et al.*, 2003a; Chaubey, 2007; Ngamo *et al*., 2007a).

Cajuputi (*Melaleuca cajuputi* (Myrtaceae)) occurs naturally in Myanmar (Burma) and Thailand through Southeast East Asia to northern Australia (Weiss, 1997). *Litsea cubeba* (Lour.) Persoon occurs wildly from the eastern Himalayas to continental South-East Malaysia, Indonesia (Java, Kalimantan and Sumatra), southern China (up to the Yangze river), Taiwan (Nor Azah and Susiarti, 1999) and in Doi Inthanon, Thailand (Smitinand and Phengklai, 1986). *Litsea salicifolia* Roxb. ex Wall**.** is distributed in India, Nepal, Sikkim, Bangladesh, Myanmar, South China, North Vietnam. (Li and Li, 2005) and in Doi Suthep, Chiang Mai Province, Thailand (Craib, 1912). According to Doran (1999), the leaves of *M. cajuputi* possess antibacterial, anti-inflammatory and anodyne properties and are reputed to have insect-repellent properties. It is also used as flavor in cooking and as a fragrance and freshening agent in the soaps, cosmetics, detergents and perfumes. Moreover, *L. cubeba* oil has vitro antifungal properties against several pathogens such as *Alternaria alternata* (Fr.) Keissl., *Aspergillus niger* van Tieghem, *Candida albicans* (C. P. Robin), *Fusarium* spp. and *Helminthosporium* spp. (Nor Azah and Susiarti, 1999). *Litsea salicifolia* is one of the many plants used as phytopesticide, traditionally by various tribes of Assam (Phukan and Kalita, 2005).

As there are no information on the insecticidal activities of these plants against stored-product insects, their bioactivities were investigated as alternatives to control these insect pests. Moreover, tropical medicinal flora is abundant in developing countries like Myanmar and Thailand. Therefore, it might be very useful for farmers in developing countries if some of such plant essential oils and/or extracts are found to have insecticidal effects against insects such as *S. zeamais* and *T. castaneum*. Moreover, as these essential oils have been used as traditional medicines or cosmetics, fragrance and in cooking, they are considered to be safe for consumers when applied on stored grains and also can reduce pollution as they are highly volatile oils.

OBJECTIVES

The objective of these studies is to evaluate the insecticidal activities of *M. cajuputi, L. cubeba* and *L. salicifolia* essential oils on two stored pests, *S. zeamais* and *T. castaneum.*

LITERATURE REVIEW

Economic Importance of Stored-product Insect Pests

Stored products of agricultural and animal origins are attacked by more than 600 species of beetles, 70 species of moths and 355 species of mites causing severe quantitative and qualitative losses (Rajendran, 2002). Insect contamination in food commodities is also considered as an important quality control problem of concern for food industries. In industrialized countries like Canada and Australia there is zero tolerance for insect excrements in food grains (White, 1995; Pheloung and Macbeth, 2002).

Insect pest damage to stored grain causes major economic losses to warehouse keepers, the milling industry and small scale farmers throughout the world. This problem is greatest in developing countries. The global post-harvest grain losses caused by insect damage and other bio-agents vary from 10% to 40% (Raja *et al*., 2001; Papachristos and Stamopoulos, 2002). To reduce losses in postharvest systems from insect infestation, synthetic insecticides including fumigants are commonly used, despite known undesirable side effects such as ozone depletion, environmental pollution (World Meteorological Organization (WMO) 1995), toxicity to non-target organisms, pest resistance (Mohan and Fields, 2002) and pesticide residues (Kostyukovsky *et al*., 2002; Ogendo *et al*., 2003).

In the post-harvest period, stored crop products are usually liable to depreciation by pest organisms especially insects. Among insect species, coleopterans are the most important storage pests (Ogunleye and Adefemi, 2007). *Sitophilus* and *Tribolium* species are major pests of stored grains and grain products in the tropics (Howe, 1965).

Chemical Control of Stored-product Insect Pests and its Adverse Affects

In many storage systems, fumigants are the most economical and convenient tools for managing stored-grain insect pests, not only because of their ability to kill a broad spectrum of pests but also because of their easy penetration into the commodity while leaving minimal residues (Mueller, 1990). Currently, methyl bromide and phosphine fumigants are widely used for insect pest control in stored products. However, because of its ozone depletion potential, methyl bromide is being phased out (Shaaya and Kostyukovsky, 2006). Additionally, it has been reported that some stored-product insects develop resistance to phosphine in many countries (Bell and Wilson, 1995; Subramanyam and Hagstrum, 1995; Savvidou *et al*., 2002). In addition, there have been some arguments about the genotoxicity of phosphine (Garry *et al*., 1989; Meaklim, 1998). Moreover, unbalanced and extensive uses of broad-spectrum pesticides have caused development of pesticide resistance, vast destruction of beneficial organisms, uncontrolled outbreak of secondary pests and undesirable environmental effect (Negahban *et al*., 2006). Hence, there is a need to develop alternative methods with low adverse effects on consumers and less persistent in the environment. In fact, management of stored product pests, using substances of natural origin, is the subject of many studies nowadays (Isman, 2006).

The use of natural compounds, such as essential oils resulted from secondary metabolism in plants received more attention as the alternatives to synthetic pesticides. Alternative strategies included the search for new kind of insecticides, and the re-evaluation and use of traditional botanical pest control agents (Huang *et al*., 1999). Thus, the toxicity of a large number of essential oils and their constituents has been evaluated against a number of stored-product insects (Ho *et al*., 1995; Paranagama *et al*., 2003). In addition, a large number of plant species have been reported to have several effects on stored-product insects. It is well accepted that natural products from plants may constitute new sources of insect pest control.

Biologically active substances of plant origin may affect stored-product insects. Some secondary metabolites of plants are toxic to the pests (pyrethrum,

nicotine, rotenone), while the others are repellents, antifeedants (azadirachtin, rape seed extract), and sterilants (extracts of *Acorus calamus* L.) (Ignatowicz and Wesolowska, 1994).

Chemicals derived from plants are an important source of insecticides and various plant extracts have been used by humans for control of insects since the time of ancient Romans. During the 20th century, a few of the natural compounds such as nicotine, rotenone and pyrethrine have been used commercially as insecticides (Arnason *et al*., 1981). In many areas of Africa and Asia, locally available plants are being widely used as an alternative to synthetic pesticides to protect stored products from insect infestation (Golob and Webley, 1980; Su *et al*., 1982; Zehrer, 1984; Ahmed and Koppel 1985; Khalique *et al*., 1988). Hence, use of plants for pest control on stored grain seems to offer desirable solutions, especially in developing tropical countries where plants are found in abundance everywhere throughout the year. Moreover, there has been growing interest in the use of both plant extracts and their essential oils since it exhibits low mammalian toxicity and low persistence in the environment (Raja *et al*., 2001; Papachristos and Stamopoulos, 2002).

Sitophilus zeamais **(Maize Weevil)**

Distribution

This pest is virtually cosmopolitan throughout the warmer parts of the world, extending as far north as Japan and southern Europe. It has been recorded from Europe (Spain, Italy, Turkey); Africa (Angola, Arabia, South Africa, East Africa, Ethiopia, Ghana, Madagascar, Madeira, Mauritius, Morocco, Mozambique, Nigeria); Asia (India, Tibet, Malaysia, Molucca Isles, Borneo, Japan, Thailand, Myanmar); Australasia (Australia-New South Wales, Queensland, West Australia, New Guinea, New Zealand, Pacific Islands); USA (Texas, Florida); Central America (Costa Rica, Mexico, West Indies); South America (Argentina, Brazil, Guyana, Honduras, Chile, Equador, Guatemala, Nicaragua, Venezuela) (Dennis, 1983). As this pest is strong

and can fly very far, it can distribute rapidly and is found in all warm and tropical parts of the world where maize and other cereals are growing (Dobie *et al*., 1984).

Biology

One *S. zeamais* female usually lays about 150-300 eggs. The eggs are white and oval. These eggs hatch into tiny grubs which stay and feed inside the grain and are responsible for most of the damage. The incubation period of the egg is about 6 days. After hatching, the larva begins to feed inside the grain, excavating a tunnel as it develops. There are 4 larval instars. Mature larvae are plump, legless and white, about 4 mm long. Larval development takes 25 days at 27°C. Pupation takes place inside the grain and newly developed adult chews its way out leaving a large characteristic emergence hole. The adults also feed within the grain, but unlike the larvae, they move from one grain to another. The life cycle is about 5 weeks at 30°C and 70% RH; optimum conditions for development are 27–31˚C and more than 60% RH; below 17°C development ceases. The actual length of life-cycle depends on the quality of grain being infested such as different varieties of maize. The adults live 4-12 months (Pury, 1968; Dennis, 1983; Dobie *et al*., 1984; Hayashi *et al*., 2004).

Damage

Sitophilus zeamais is a small insect belonging to order Coleoptera and family Curculionidae. It is one of the most serious pests of maize by internal feeding. This beetle is associated primarily with maize although it is capable of developing on all cereal grains such as sorghum, rice and cereal products (Walgenback and Burkholder, 1986; Tipping *et al*., 1987). Initial infestations of maize grain occur in the field just before harvest and insects are carried into the store where the population builds up rapidly (Appert, 1987; Adedire and Lajide, 2003). The huge post harvest losses and quality deterioration caused by this pest is a major obstacle to achieve food security in developing countries (Rouanet, 1992). This insect is capable of infesting all cereal grains but recorded as favoring wheat, rice and other small grains.

Direct damage is caused by feeding on the cereal grains; one larva hollows out one small grain during its development (Hayashi *et al*., 2004). Damage caused by larval feeding is distinctive. A thin tunnel is bored by the larva from the surface of the grain kernel. Circular exit holes on the surface of the grain kernel are characteristic (Dennis, 1983). Adults cause further damage by feeding, mainly by attacking previously damaged grain. Infestation by this pest produces heat and moisture encouraging extensive quality loss, mould growth and growth of populations of other insect pests (Rees, 2004). The most destructive strains are able to cause losses of up to 90% of the stock after 5 months of storage (Nukenine *et al*., 2002).

Tribolium castaneum **(Red Flour Beetle)**

Distribution

Tribolium castaneum has a world-wide distribution and is among the most economically important stored-product pests (Garcia *et al*., 2005). This species is a serious secondary pest throughout the warmer parts of the world in food stores (Dennis, 1983). It has been recorded in USA, Canada, Central and South America, Europe, North Asia, Mediterranean basin, Africa, South and Southeast Asia, Australia and Oceania (Rees, 2004). This species is also found throughout the tropics and is regarded as a major pest of shelled groundnuts (Dick, 1987).

Biology

Each female lays about 150-600 eggs, scattering in the produce. The eggs are small, cylindrical and white. The larvae are yellowish white, cylindrical in shape about 6 mm long when fully grown, with two prominent horns on the last abdominal segment. The head is pale brown. The larvae live and develop inside the grain till pupation. The damage to the stored grains is done by the larvae. The pupa is yellowish-white, later becoming brown, the dorsum hairy, and the tip of the abdomen having two spine-like processes. The adult is rather flat, oblong, reddish-brown in color, and about 3-4 mm long. The life period from egg to adult is 35 days at 30°.

Adults fly in large numbers in the late afternoon. The adults are long-lived, under some circumstances living for a year or more (Rees, 2004; Dennis, 1983).

Damage

Tribolium castaneum is a very common pest infesting many flour mills, warehouses and grocery stores. It belongs to order Coleoptera and family Tenebrionidae. It can be a major pest in storage of grain-based products. This species has had a long association with human stored food and has been found in association with wide range of commodities including grain, flour, peas, beans, cacao, nuts, dried fruits and spices, but milled grain products such as flour appear to be their preferred food (Good, 1936; Campbell and Runnion, 2003). Infestation is apparently by the appearance of adults on the surface of the grain. There is an extensive damage to previously holed or broken grains, or grain damage to previously damaged by other pests. Damage to the stored grains is done by both larvae and adults.

Control Measures for Stored-product Pests

Chemical Insecticides

Chemical insecticide application is normally performed to control stored-product pests. Buildings or warehouses infested with such pests should be thoroughly cleaned and sprayed with BHC (Benzene Hexa Chloride) or malathion, and any parts of the building which cannot be reached with sprays should be fumigated with the use of recommended fumigants. Infested grains can be mixed with malathion WP (wetable powder) which is generally successful in achieving control.

Fumigation of infested grain with methyl bromide, or an ethylene dichloride and carbon tetrachloride mixture is effective but should only be carried out by approved operators because of the toxicity hazards (Dennis, 1983).

Plant-Based Insecticides (PBIs)

Plant-based insecticides (PBIs) as described by Rosenthal (1986) can be less toxic to man, readily biodegradable, suitable for use by small scale farmers and capable of protecting crops from attack by a wide range of insect pests. As a logical consequence of the undesirable side effects of chemical pesticides, there is a growing awareness of the toxicological and environmental problems involved in the use of synthetic pesticides in industrialized and also in developing countries. This awareness has led to a steadily increasing movement towards a more environmental – oriented, sustainable agriculture with low or no input of toxic synthetic pesticides and other agricultural chemicals in an attempt to preserve and protect the environment as well as human health.

Apart from those natural products in current use such as pyrethrins, rotenone, nicotine, ryania, sabadilla and neem oil, several substances of plant origin have been identified as having toxic, repellent, antifeedant, and/or growth and development inhibiting-potential on arthropod pests (Coats, 1994).

The use of neem leaves in protecting stored grain and other commodities is as age-old practice in India. Pruthi (1937) and Pruthi and Singh (1950) reported the efficacy of neem leaves in protecting stored grain from insect attack. Jotwani and Sircar (1965) showed that the neem kernel was effective as a grain protectant against stored grain insect pests. Yadav (1983) found that neem kernel protects leguminous seeds from attack by *Callosobruchus maculatus* (F.) and *C. chinensis* (L.). Margosan-O®, neem-based insecticide, had antifeedant activity against *T. castaneum.* (Xie *et al*., 1996). Tapondjou *et al*. (2002) showed that the dosage of 6.4% (w/w) of *Chenopodium ambrosioides* (L.) dry leaf powder induced total mortality of *S. zeamais* within 2 days and inhibited F1 progeny production and adult emergence. In 2000, Haque *et al*. found that seeds of *Basella alba* L. and leaves of *Operculina turpethum* L. and *Calotropis gigantea* L. were potent in delaying development and reducing adult emergence of *S. zeamais.* Pretheep-Kumar *et al*. (2004) demonstrated that

paddy grains treated with a protein-rich fraction derived from peas var. *bonneville* at 1% concentration repelled *T. castaneum.*

Pandey *et al*. (1976) observed that 1 to 3 parts of neem oil per 100 parts of seed effectively protects Bengal gram (*Cicer arietinum* L.) seeds from damage by the bruchid *C. chinensis* for at least 135 days without any adverse effects of treatment on germination. Ketkar (1976) reported the efficacy of neem kernel powder and neem oil in controlling the grain pests, under existing godong (warehouse) conditions. Powders prepared from parts of *Vernonia amygdalina* Del, *Ocimum grattissimum* L., *Piper guineense* Shum and Thonn, *Xylopia aetiopica* (Dunal) A. Rich, *Chromolaena odorata* (L.) King & H.E. Robins., *Afromomum melegueta* Shum, *Nicotiana tabacum* L., *Capsicum frutescens* L. which are indigenous to Nigeria had insecticidal activity against *S. zeamais* (Asawalam *et al*., 2007). Haryadi and Rahayu (2002) also observed that the mixtures of the acetone extracts of black pepper and nutmeg seeds had significant effects on the development of *S. zeamais.* The crude seed extracts of *Aphanamixis polystachya* Wall and Parker (pithraj) were strong repellents and moderate feeding deterrents and its ground leaves, barks and seeds reduced F1 progeny to *T. castaneum* (Talukder and Howse, 1995).

Essential Oils and Plant Extracts

An alternative to synthetic pesticides is the use of natural compounds such as essential oils resulted from secondary metabolism in plants. Essential oils would have the advantage over synthetic chemicals in being more acceptable both environmentally and to the consumer (Markham, 1999). The toxicity of a large number of essential oils and their constituents has been evaluated against a number of stored-product insects (Paranagama *et al*., 2003).

Essential oils are commercially used in four primary aspects: as aromas in fragrances and perfumes, as flavoring food additives, as pharmaceuticals and as insecticides. They recently have received much attention due to their multi-functions as antimicrobial, antifungal, antitumor and insecticidal agents (de Souza *et al*., 2005).

Essential oils and especially their main compounds monoterpenoids are volatile. They offer promising alternatives to classical fumigants (Lee *et al*., 2001; 2002a,b; 2004; Peterson and Ems-Wilson, 2003; Aslan *et al*., 2004), contact insecticides (Tapondjou *et al*., 2002; Peterson and Ems-Wilson, 2003), antifeedent or repellent effects (Kim *et al*., 2003a,b; Park *et al*., 2003a,b; Garcia *et al*., 2005) and may also affect some biological parameters such as growth rate, life span and reproduction of stored-product insects (Tunc *et al*., 2000; Kathuria and Kaushik, 2005; Rahmat *et al*., 2006).

For several years, many researches had been performed to study the insecticidal activities of many plant crude extracts and their essential oils. Ajayi (2007) indicated that the application of benniseed (*Sesamum indicum* L.), olive (*Olea europaea* L.) and horseradish (*Moringa oleifera* Lam.) edible oils significantly reduced infestation of stored pearl millet grains by *T. castaneum.* Owusu *et al*. (2007) observed that methanol extract of candlewood, *Zanthoxylum xanthoxyloides* (Lam.) caused significant mortalities in *S. zeamais* whereas methanol, hexane, isopropyl alcohol, ethanol and hexane: isopropyl (4:1) extracts evoked strong to moderate repellent actions against this insect pest. Moreover, Ogunleye and Adefemi (2007) demonstrated that the methanol extracts of *Garcinia kolae* Heckel had rapid lethal effects on *S. zeamais*. Moreira *et al*. (2007) reported that hexane and coumarin extracts of *Ageratum conyzoides* L. showed toxicity to *S. zeamais*. Dichloromethane extracts of *Piper nigrum* L. had pesticidal potency on *S. zeamais* (Awoyinka *et al*., 2006). Asawalam (2006) also observed that the acetonic oil extracts of black pepper, *P.guineense* had strong repellency effect on *S. zeamais*. Huang and Ho (1998) also proved that methylene chloride extract of cinnamon, *Cinnamomum aromaticum* Nees had contact, fumigant and antifeedant against *S. zeamais* and *T. castaneum.* Topical applications of *Trigonellu foenum-graecum* extracts also produced a high degree of mortality in *T. castaneum* (Pemonge *et al*., 1997).

Methanol extracts from four medicinal plants, *Peganum harmala* L. (Zygophyllaceae), *Ajugaiva iva* (L.) (Labiateae), *Aristolochia baetica* L. (Aristolochiaceae) and *Raphanus raphanistrum* L. (Brassicaceae) inhibited F1

progeny production of *T. castaneum* (Jbilou *et al*., 2006). The essential oils of *X. aethiopica* and *O. gratissimum* acted as insect growth regulator by contact and ingestion on adults *S. zeamais* and *T. castaneum* (Kouninki *et al*., 2007b) and essential oil of *X. aetiopica* gave good protection to the stored maize grains by suppressing reproduction (F1 progeny emergence) of *S. zeamais* (Asawalam *et al*., 2006; Kouninki *et al*., 2007a). Jbilou *et al*. (2008) also showed that *Centaurium erythraea* (Rafin.), *P. harmala*, *A. iva*, *A. baetica*, *Pteridium aquilinum* (L.) Kuhn and *R. raphanistrum* extracts inhibited larval growth of *T. castaneum*. Moreover, the crude extracts and essential oil of *A. polystachya* (pithraj seeds) had strong repellent effects and moderate feeding deterrent and insecticidal (direct-contact) effects on adult *T. castaneum* (Talukder and Howse, 1993; 1994).

Liu *et al*. (2007) studied 30 Chinese medicinal herbs and found that those herbs exhibited insecticidal or feeding-deterrent activities against *S. zeamais* and n-hexane extracts of 6 Chinese medicinal herbs, namely *Artemisia argyi* Levl. Et., *Evodia rutaecarpa* Hook f. et., *Polygonum aviculare* L., *Quisqualis indica* L., *Alangium chinense* (Lour.) Harms and *Daphne genkea* Siebold et Zuccarini possessed fumigant toxicity to *T. castaneum* where n-hexane extracts of 17 Chinese medicinal herbs, namely *Dictamnus dasycarpus* Turcz., *E. rutaecarpa*, *Litsea cubeba* (Lour.) Persoon, *Narcissus tazetta* L. var. *chinensis*, *Pharbitis nil* Choisy, *A. chinense*, *Alpinia galanga* (L.) Willd., *A. argyi*, *Clausena lansium* Skeels, *Cyperus rotundus* L., *Punica granatum* L., *Q. indica*, *Ricinus communis* L., *Sophora flavescens* Aiton, *Stellera chamaejasme* L., *Tripterygium wilfordii* Hook. f., and *Torreya grandis* Fortune ex Lindl. exhibited contact toxicity to *T. castaneum.* Moreover, both n-hexane and methanol extracts of 11 Chinese medicinal herbs: *A. argyi*, *D. dasycarpus*, *L. cubeba*, *Melia toosendan* Siebold & Zucc., *N. tazetta*, *P. aviculare*, *P. granatum*, *Rhododendron molle* (Blume) G. Don, *S. flavescens*, *Stemona sessilifolia* Franch. & Sav., and *T. wilfordii* were shown to have feeding-deterrent activity to *T. castaneum.* Methanol extracts of 4 Chinese medicinal herbs: *A. galanga, Brucea javanica* (L.), *Clematis armandii* Franch. and *Scutellaria baicalensis* Gerogi were shown to possess feeding deterrent activity against *T. castaneum.* The n-hexane extracts of *Dryopteris crassirhizoma* Nakai, *Momordica charantia* L., *Q. indica* and *T. grandis*

demonstrated feeding-deterrent activity against *T. castaneum*. Moreover, Farhana *et al*. (2006) observed that the chloroform extracts of coriander (*Coriandrum sativum* L.), ajowain (*Trachyspermum ammi* (L.) Link.) and fenugreek (*Trigonella foenum-grecum* L.) had toxicity and repellent effects on *T. castaneum*. Shaaya *et al*. (1997) found that *Labiatae* sp. oil ZP51 had fumigant toxicity against *T. castaneum*. Asawalam and Hassanali (2006) observed that 0.3% of the essential oil of *V. amygdalina* induced the highest mortality in the *S. zeamais* after 7 days.

Jayasekara *et al*. (2005) proved that *Securidaca longepedunculata* Fres. root powder, methanol extract and the main volatile component, methyl salicylate exhibited repellent and fumigant toxicity to *S. zeamais* adults. Tapondjou *et al*. (2005) showed essential oils from *Eucalyptus saligna* Sm. and *Cupressus sempervirens* L. had repellent and contact toxicity against *S. zeamais*. Huang *et al*. (2002) also demonstrated that eugenol significantly reduced food consumption in the adults of *S. zeamais* at a concentration of 13.2 mg/g food and eugenol, isoeugenol and methyleugenol showed contact toxicity to *S. zeamais* (LD_{50} values = 30 µg/mg insect). Hassanali (2001) found that essential oil from bekele, *Ocimum kilimandscharicum* Baker ex Gürke caused 100% mortality of *S. zeamais*. Boudaa *et al*. (2001) also proved that the essential oils extracted from leaves of *Ageratum conyzoides* L., *C. odorata* and *Lantana camara* L. had insecticidal activity against *S. zeamais*.

Fazolin *et al*. (2007) proved that cyanidric acid, liberated from the essential oil of *Tanaecium nocturnum* (Barb. Rodr.) Bureau & K. Schum. possessed fumigant insecticidal effect on *S. zeamais*. Ngamo *et al*. (2007a) showed that the essential oil of *Lippia rugosa* A Chev. had insecticidal effect on *S. zeamais.* The essential oil derived from both the whole fruit and/or from the fibers of the fruit of the local aromatic plants *X. aethiopica* (Annonaceae) led to 100% mortality of *S. zeamais* (Kouninki *et al*., 2007a).

Huang *et al*. (2000a) demonstrated that two of the major constituents of garlic, *Allium sativum* L. essential oil, methyl allyl disulfide and diallyl trisufide had contact,

fumigant and antifeedant effects against *S. zeamais*. Huang *et al*. (2000b) also showed that the essential oil of cardamom, *Elletaria cardamomum* (L.) Maton had contact, fumigant and antifeedant activities against *S. zeamais*.

The essential oil of *E. rutaecarpa* showed a moderate repellent effect on *S. zeamais* and was toxic to *T. castaneum* adults when applied topically to the insects and also had fumigant toxicity, repellency and antifeedant activities (Liu and Ho, 1999). Don-Pedro (1996) showed that (+)-Limonene, citruspeel oil components was toxic to *S. zeamais*. The hexane extract from star anise, *Illicium verum* Hook f. at 0.96 g/ml caused 55% mortality in *S. zeamais* adults and non-polar crude extracts of dried fruits of star anise at 0.01 g/ml killed all *T. castaneum* eggs and high mortality (70%) of *T. castaneum* adults occurred in rice treated with the non-polar extract where F1 adult emergence was completely suppressed (Ho *et al*., 1995). The essential oils of *Annona senegalensis* Pers. (Annonaceae), *Hyptis spicigera* L. (Lamiaceae) and *L. rugosa* reduced the oviposition of *S. zeamais* (Ngamo *et al*., 2007b).

The essential oil extracted from nutmeg seeds may be useful as a grain protectant with contact, fumigant and antifeedant activities against *T. castaneum* (Huang *et al*., 1997). This essential oil significantly affected the hatching of *T. castaneum* eggs and the subsequent survival of the larvae at the concentration range 1.4-3.2 mg/cm^2. F1 progeny production was totally suppressed at nutmeg oil concentrations of 1.05 g/l00 g rice for *T. castaneum*. Essential oils of *Artemisia aucheri* Boiss and *Artemisia scoparia* Waldst and Kit. had fumigant activity against *T. castaneum* (Shakarami *et al*., 2004a, b, c; Negahban *et al*., 2004). Wang *et al*. (2006) also demonstrated that *Artemisia vulgaris* L. oil repelled *T. castaneum* adults at concentrations of 0.6 mL/mL (v/v) and above and was highly significant at 1.0 mL/mL and 100% repellency was achieved at 1.0 mL/mL concentration. Ngamo *et al*. (2007c) found that the essential oil of *H. spicigera* and *L. rugosa* had insecticidal effects on *T. castaneum* as well.

Obeng-Ofori and Reichmuth (1997) reported that eugenol of *Ocimum suave* (Wild.) papers, was highly toxic to *T. castaneum* when whole grains or glass pebbles

were applied topically or impregnated on filter. Shukla *et al*. (2008) observed that essential oils from *Myristica fragrans* Gronov. and *I. verum* reduced oviposition potential of *T. castaneum*. Sahaf *et al*. (2007) also showed that *Carum copticum* C. B. Clarke had fumigant activity against *T. castaneum*. Krishna *et al*. (2005) demonstrated that essential oils of genotypes of *Tagetes minuta* (Khakibush) TM-1 and TP-2 induced 100% adult mortality for *T. castaneum* at dosages of 50,000 ppm and 500 mg/insect in fumigant and contact toxicity bioassays. Moreover, Mondal and Khalequzzaman (2006) also observed that cardamom (*E. cardamomum*), cinnamon (*C. aromaticum*), and clove (*Syzygium aromaticum* (L.) Merr. and Petry) essential oils had contact and fumigant toxicity against *T. castaneum*.

Shaaya *et al*. (1991) and Tripathi *et al.* (2001) observed that 1,8-cineole and the essential oils of anise and peppermint had contact, fumigant, antifeedant toxicities and reduced progeny production of *T. castaneum.* Turmeric oil and sweetflag oil at 200, 400 or 800 µg/cm^2 repelled *T. castaneum* during the first 2 weeks (Jilani *et al*., 1988). In addition, essential oil from *Artemisia annua* L. was largely responsible for both repellent (behavioral) and toxic (physiological) actions of *T. castaneum* (Tripathi *et al*., 2000).

Tripathi *et al.* (2002) observed that essential oil extracted from the leaves of turmeric, *Curcuma longa* L. had contact and fumigant toxicities and reduced progeny production of *T. castaneum*. Moreover, Tripathi *et al.* (2003) showed that l-Carvone, d-Carvone and Dihydrocarvone, three chemical constituents of essential oil, exhibited contact and fumigant activities against *T. castaneum*. Head space volatiles, including 73% di-*n*-propyl disulfide, were collected from freshly crushed neem seeds. This compound along with previously reported diallyl disulfide (di-2-propenyl disulfide) was toxic when applied topically or as a fumigant to *T. castaneum* adults and 8-, 12-, and 16-d-old larvae (Koul, 2004).

Cineole in *Eucalyptus sp*. essential oil and limonene in *Citrus sp.* had fumigant, contact and ingestion activities against *T. castaneum* (Prates *et al*., 1998). Hexane + isopropyl alcohol extract of leaves of *Ocimum viride* Willd. proved

effective in the control of *T. castaneum* (Owusu, 2001). Three monoterpenoids, the ketones pulegone, l-fenchone and the aldehyde perillaldehyde purchased from Aldrich (Milwaukee, WI), Sigma (St. Louis, MO), or Pfaltz and Bauer (Waterbury, CT) were effective against *T. castaneum* in the fumigation assay (Lee *et al*., 2003).

The 1,8-cineole, camphor and linalool in the essential oils of the aromatic plants *Lavandula angustifolia* Mill., *Raphanus officinalis* Crantz , *Thymus vulgaris* L. and *Laurus nobilis* L. had fumigant toxicity against *T. castaneum* (Rozman *et al*., 2007). *Tribolium castaneum* egg mortality increased with garlic oil concentration, complete kill of eggs being achieved at 4.4 mg/cm^2 using the filter paper impregnation bioassay and F1 progeny production was reduced when rice were treated with garlic oil (Ho *et al*., 1996). Leaf essential oils of *Vepris heterophylla* (Engl.) Letouzey also demonstrated contact and fumigant activities against *T. castaneum* (Ngamo *et al.*, 2007d).

Pungitore *et al.* (2005) observed that triterpenes from *Junellia aspera* (Gill. & Hook.) Moldenke acted as toxic compounds to *T. castaneum* when applied topically and/or incorporated into the food. Al-Jabr (2006) also found that *Matricaria chamomilla* L. exhibited high repellency (84.73%) at 1% concentration against *T. castaneum* and 1% of *Prunus Amygdalus* (Mill.) D.A.Webb and *Cymbopogon winterianus* Jowitt. gave complete mortality of *T. castaneum* after two weeks of exposure. Andronikashvili and Reichmuth (2002) also demonstrated that *O. gratissimum* (Lamiaceae) and *L. nobilis* (Lauraceae) had repellency and contact toxicity against *T. castaneum.*

***Melaleuca cajuputi* Powell**

Melaleuca cajuputi (Myrtaceae) is a tropical pioneer tree species that frequently forms populations on degraded land in southern Thailand (Miwa *et al*., 2001). Weiss (1997) stated that cajuputi occurs naturally in Myanmar (Burma) and Thailand through Southeast East Asia to northern Australia. The most important use of cajuputi is as a source of oil obtained by steam and hot distillation of its leaves and

terminal twigs (Sakasegawa *et al*., 2003). The leaves of *M. cajuputi* possess antibacterial, anti-inflammatory and anodyne properties and are reputed to have insect-repellent properties. It is also used as flavor in cooking and as a fragrance and freshening agent in the soaps, cosmetics, detergents and perfumes (Doran, 1999). Moreover, cajuputi oil, which has a camphor-like odor, is used as an insect repellent and as a painkiller for headache, toothache, rheumatism and convulsions in the form of applied plaster (Ogata, 1969). Potential applications include control agents of plant pathogens, insect repellants, antifeedants and insecticides. Whilst direct contact was more effective, the oil also demonstrated significant antifungal activity in the vapor phase, a characteristic which suggests the possibility of its use as a fumigant for stored crops (Markham, 1999).

In a small published trial (Goodwin and Hardiman, 2000), tea tree oil was reported as an efficacious treatment for tinea infection. However, this trial used undiluted essential oil, with serious consequences (lower limb dermatitis) for some participants. It is known that certain *Melaleuca* species exhibit activity against mites (Yatagai *et al.*, 1998).

***Litsea cubeba* (Lour.) Persoon**

May Chang, *Litsea cubeba* (Lour.) Persoon (Lauraceae) is an evergreen shrub or small tree with lemon-scented leaves and small, pepper-like fruit. The flowers, leaves and fruits are used as medicine and for extracting an essential oil. *Litsea* species are evergreen, rarely deciduous trees or shrubs (Hooker, 1885; Li, 1963). *Litsea cubeba* occurs in the wild from the eastern Himalayas to continental South-East Malaysia, Indonesia (Java, Kalimantan, Sumatra), southern China (up to the Yangze river) and Taiwan. It is growing wildly in many parts of Thailand (Ngernsaengsaruay, 2005). This plant is cultivated for its essential oil mainly in Japan, China and Taiwan. The essential oil is applied in cases of fever, stomachache, chest pain and as a tonic. *Litsea cubeba* oil has in vitro antifungal properties against several pathogens such as *Alternaria alternate* (Fr.) Keissl., *Aspergillus niger* van

Tieghem, *Candida albicans* (C. P. Robin), *Fusarium* spp. and *Helminthosporium* spp. All parts of *L. cubeba* contain essential oil, but only the essential oil steam distilled from the fruit is of major commercial importance (Nor Azah and Susiarti, 1999).

Litsea essential oils are steam-distilled from the fruits and from the leaves. The essential oils obtained from *Litsea* fruits are called 'May Chang oil'. Because of its pleasant citrus-like smell and taste, it is a modifier for lemon and lime flavors and general freshener in fruit flavors. In perfumery, May Chang oil is used as an alternative for verbena oil and lemongrass oil in colognes, household sprays, soaps and air fresheners. The oil produced in West Java is called trawas oil where from Central Java krangean oil. Both oils are used medicinally and in soap perfumes. In China, *Litsea* is planted as a windbreak in tea plantations. It has antiseptic, astringent, deodorant, disinfectant, insect repellant and sedative properties. In addition, it has been used as traditionally for atopic eczema (Anderson *et al*., 2000).

The roots of *Litsea* are an ingredient of a medicine given after childbirth. The bark of the roots, branches and leaves are also employed against athlete's foot and other skin diseases. The fruits are carminative, stomachic, expectorant and a treatment for hernia, bronchitis and dyspepsia. In Indo-China, a decoction is useful in cases of vertigo, hysterical affections, paralysis, melancholy or forgetfulness (Perry, 1980).

In India and China, the fruit is edible, aromatic and carminative and is reported to be used for headache, dizziness, hysteria, paralysis and loss of memory (Satyavati and Gupta, 1987). All plant parts of *L. cubeba* are applied medicinally and have antiparalytic, anticephalalgic, antihysteric, carminative, spasmolytic and diuretic properties. The fruit is used in decoctions for the treatment of vertigo, paralysis and in post-partum preparations; the leaves for treating skin diseases. Traditionally the Dayak Kenyah people of East Kalimantan use the fruits and bark as oral and topical medicine for babies as well as for adults. It is applied in cases of fever, stomachache, chest pain, and as a tonic. It is also an antidote to treat drunkenness. In aromatherapy, the oil is applied as a cooling agent against acne and dermatitis and to relieve anxiety

and stress. Recent studies found that the essential oil may be useful in the treatment of cardiac arrhythmia. Liu *et al.* (2007) showed that hexane and methanol leaf extracts of *L. cubeba* had contact, fumigant toxicity and feeding deterrent effect against *S. zeamais* Motschulsky and *T. castaneum* (Herbst).

***Litsea salicifolia* Roxb. ex Wall.**

Litsea salicifolia, Roxb. Ex. Wall (Lauraceae) is distributed in India, Nepal, Sikkim, Bangladesh, Myanmar, South China and North Vietnam (Li and Li, 2005). Craib (1912) reported that there are 5 species of *Litsea* on Doi Suthep, Chiang Mai Province including *L. salicifolia*.

Litsea salicifolia is one of the many plants used as phytopesticide, traditionally by various tribes of Assam (Phukan and Kalita, 2005). Phukan and Kalita (2005) showed that the hexane extract (2000 ppm) of *L. salicifolia* exhibited 70% repellent activity for 3 h against *Aedes aegypti* (L.) and 46% activity for 3 h against *Culex quinquefasciatus* where the LC_{50} value of *L. salicifolia* against *A. aegypti* was 0.72% (Phukan and Kalita, 2004).

MATERIALS AND METHODS

Insects

Sitophilus zeamais Motschulsky and *Tribolium castaneum* (Herbst) from Department of Agriculture, Ministry of Agriculture and Co-operatives, Thailand were used in this study. *Sitophilus zeamais* was reared on rice 12–13% moisture content while *T. castaneum* was reared on rice bran. The cultures were maintained in the laboratory at 29-32˚C and 70–80% RH (Fig. 1).

Extraction of the Essential Oils

Fresh leaves of *M. cajuputi* were collected at Kasetsart University, Bangkhen campus, (13º98'N, 48º18'E) in June 2007 and mature fruits of *L. cubeba* and *L. salisifolia* were collected at Doi Ang-khang (19º54'N, 99º2'E), Fang District, Chiang Mai Province in June 2007 (Fig. 2). All voucher plant specimens (#CHKU 00028, #CHKU 00022, #CHKU 00023, respectively) were deposited at the Bangkok Herbarium, Botanical Research Unit, Department of Agriculture, Bangkok, Thailand.

The essential oils were extracted by water-distillation using a Clevenger-type apparatus for 6 h (Fig. 3). The superior phase was collected from the condenser, dried over anhydrous sodium sulphate and stored in amber-colored vials at 10 - 12 ºC for further experiments.

Repellency Test

Petri-dishes of 9 cm in diameter were used to confine insects during experiment. Essential oils of *M. cajuputi*, *L. cubeba* and *L. salicifolia* were diluted in ethanol to different concentrations (0.5%, 1%, 1.5% and 2%) and ethanol absolute was used as control. Filter papers (9 cm diam) were cut in half. One ml of

each essential oil was applied separately to one half of the filter paper treatment as uniformly as possible with a micropipette. The other half (control) was treated with 1 ml of ethanol absolute. Both treated half and control sides were then air–dried to evaporate the solvent completely. Full disc was carefully remade by attaching tested halves to control halves with sellotape. Precautions should be taken so that attachment was not prevent the free movement of insects from one half to another, but the distance between the filter-paper halves remained sufficient to prevent seepage of test samples from one half to another. Each remade filter paper was placed in a petri-dish with the seam oriented in one of four randomly selected different directions to avoid any stimuli affecting the distribution of insects (Figs. 4).

Ten insects were released at the center of each filter-paper disc and a cover was placed on the Petri-dish. For each essential oil, five replicates were used and the experiment was repeated once. Counts of the insects present on each strip were made after 1 h and at hourly interval up to the fifth hour. These numbers were then calculated to know percent repellency of each volatile oil by using the formula PR (%) = $[(N_c – N_t) / (N_c + N_t)] \times 100$ where N_c is the number of insects present in the control half and N_t is the number of insects present in the treated half.

Fumigant Toxicity

To determine the fumigant toxicity, Whatman No. 1 filter papers were cut into pieces of 2 cm in diameter and impregnated with oil at doses calculated to give equivalent fumigant concentrations of 37, 56, 94, 130, 185, 296, 370, 444 and 556 µL/L in air. The impregnated filter paper was then attached to the under surface of the screw cap of a glass vial (27 mL). The caps were screwed tightly on the vials containing 10 adults (1-7 days old) of either *S. zeamais* or *T. castaneum* (Figs. 5). Each concentration and control was replicated five times. Mortality was determined after 3, 6, 9, 12 and 24 h from commencement of exposure. When no

leg or antennal movements were observed, insects were considered dead. Percentage insect mortality was calculated using the Abbott's formula for natural mortality in untreated controls (Abbott, 1925). Probit analysis was used to estimate LC_{50} and LC_{95} values. The experiment was arranged by complete randomized design and ANOVA was computed using SPSS 16.0 software package.

Contact Toxicity

Aliquots of 0.5 µL of the dilutions (10%, 20%, 30% and 40%) of each oil sample were applied topically to the thorax of the *S. zeamais* and *T. castaneum* using a Burkard Arnold microapplicator (Burkard Manufacturing Company Ltd. England) (Fig. 6). Controls were prepared using ethanol. Both treated and control insects were then transferred to glass vials (10 insects/vial) (2 cm diam and 5.5 cm height with plastic cap) and kept in incubators set at 27°C and 60% RH. Culture media were added to each treatment after 24 h. Mortality of insects was observed daily until end-point mortality (when the number of dead insects no longer increased with time) was reached 1 week after treatment.

Antifeedant Test

Aliquots of 200 µL of a suspension of wheat flour in water (10 g in 50 ml) were dropped onto a clean plastic placed in a tray (Fig. 7). The discs were left in the fume hood 24 h to dry, after which they were put in an oven at 60°C for 1 h. Each essential oil was diluted in ethanol to get different concentrations (4% 6%, 8% and 10%), ethanol absolute and clean wheat flour were used as controls. After evaporation of the solvent, 2 discs for *S. zeamais* and 1 disc for *T. castaneum* were placed in glass vials (diameter 2.5 cm, height 3 cm). All the insects were starved for 24 h before use. Then, ten group-weighed, unsexed adults were added to each preweighed vial containing the disc. Glass vials containing flour discs but without insects were also prepared to determine if any

decrease in weight had occurred due to evaporation. A 5 μL of different concentrations was dropped onto each flour disc and ethanol was used as control. Five replicates were prepared for this experiment.

After three days, the glass vials with flour discs were weighed again and mortality of insects, if any, was recorded. For antifeedant action, the formula described by Isman *et al.* (1990) was modified in calculating the feeding deterrence index FDI (%) = (C-T)/C x 100, where C = the consumption of control discs and T = the consumption of treated discs. The following criteria were adopted to categorize the essential oils:

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

Figure 1 Colonies of *Sitophilus zeamais* (above) and *Tribolium castaneum* (below) maintained under laboratory condition at 29-32˚C and 70-80% RH.

Figure 2 Plants used in this study: a) *Melaleuca cajuputi*, b) *Litsea cubeba* and c) *Litsea salicifolia*

Figure 3 Clevenger-type apparatus for extracting essential oil by water distillation

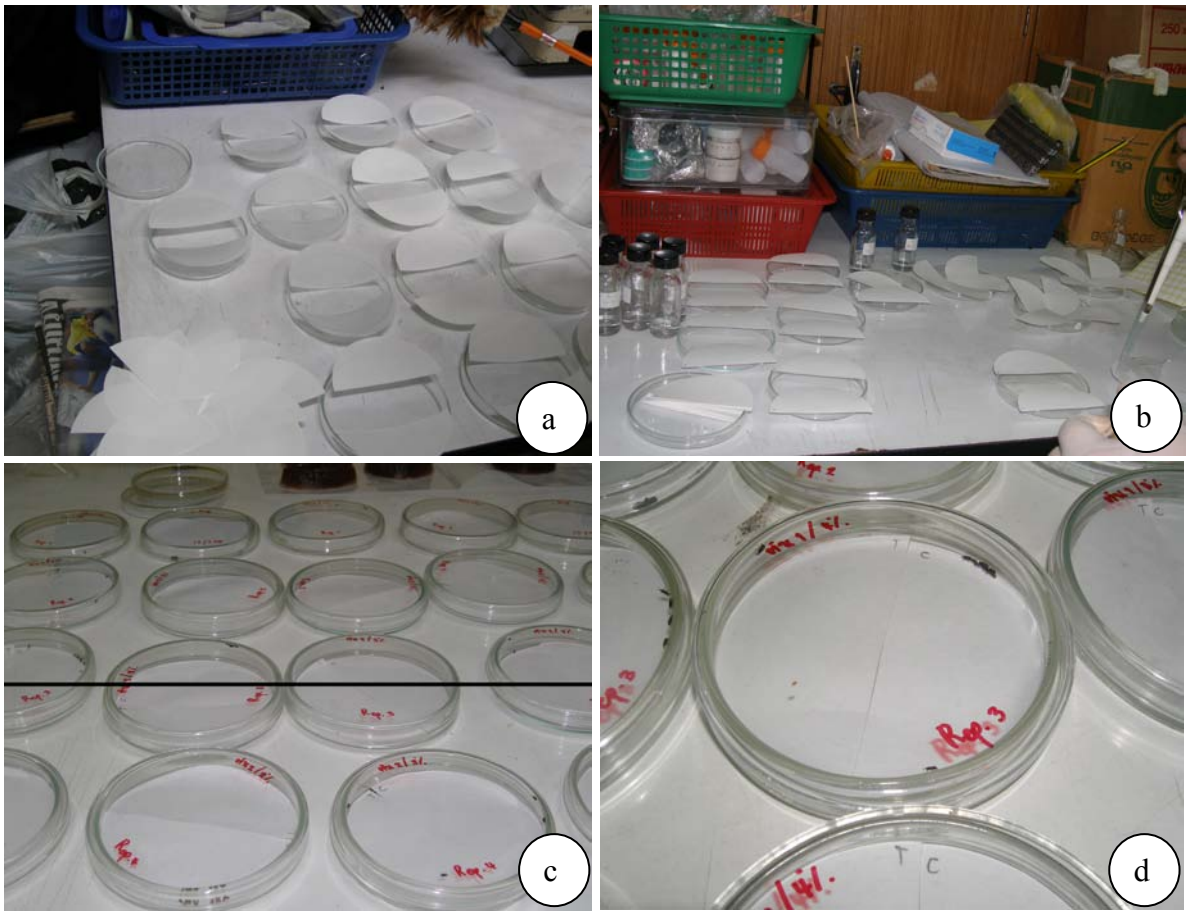

Figure 4 Repellent bioassay: a) filter papers were cut in half, b) 1 mL of each essential oil was dropped onto the treated side, c) treated and control sides were attached to each other with sellotape and d) 10 insects were placed in each Petri-dish.

Figure 5 Fumigation bioassay: a) dropping essential oils on filter papers, b) filter papers impregnated with essential oil are air-dried at room temperature, c) filter papers ready to be placed under the screw cap and d) tested vial for fumigation activity.

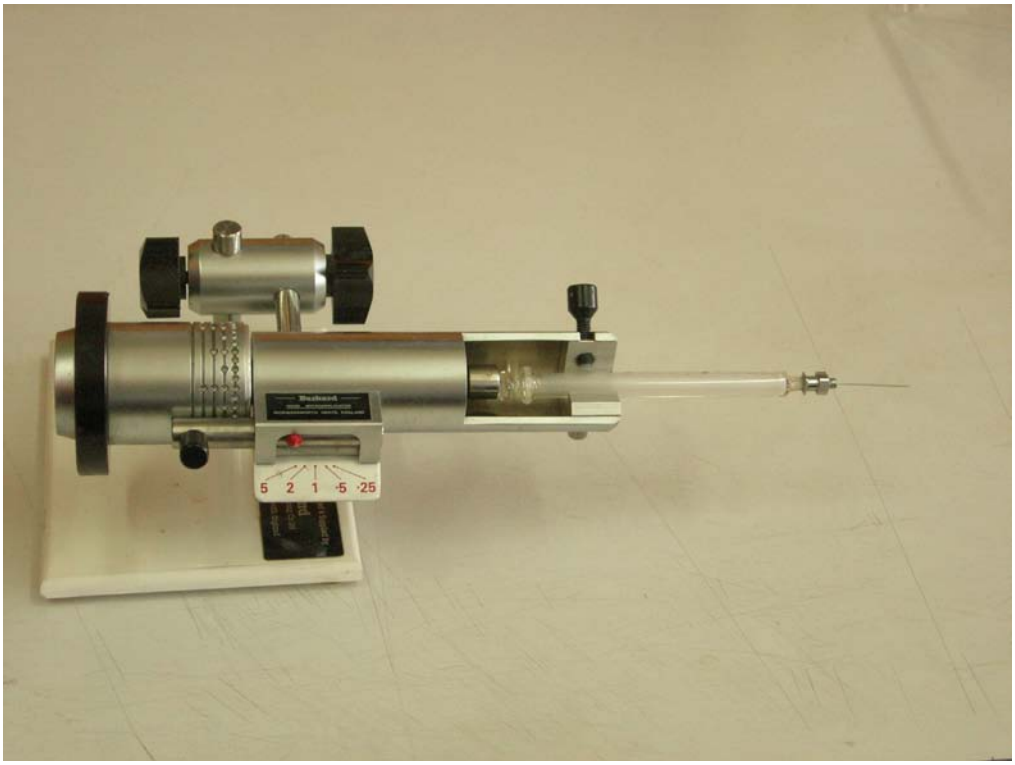

Figure 6 Microapplicator (Burkard Manufacturing Company Ltd., England) used to drop the essential oil onto the thorax of each insect.

Figure 7 Antifeedant bioassay: a) flour discs made from wheat flour in water (10 gm wheat flour in 50 mL water) served as tested diet and b) rearing unit for antifeedant test.

RESULTS AND DISCUSSION

Analysis of Chemical Constituents of Essential Oils by GC/MS

All three essential oils were analyzed by GC/MS (Shimadzu QP 5050A) equipped with a DB-5 capillary column. Essential oil components were identified by comparing their GC retention times and mass spectra with those presented in the MS library. The chemical constituents of essential oils extracted from leaves of *M. cajuputi* and mature fruits of *L. cubeba* and *L. salicifolia* are shown in Tables 1-3.

The results revealed that terpineolene, γ-terpinene and ρ-cymene were major components of *M. cajuputi* leaf essential oil. Other minor constituents were α-thujene, (Z)-β-famesene, α-terpinene, α-pinene, terpinen-4-ol, limonene and α-terpineol (Table 1). The major constituents of *L. cubeba* essential oil extracted from mature fruits were (E)-citral and (Z)-citral, and other components were 1,8-cineole (eucalyptol), limonene, camphor, linalool, β-pinene and terpinen-4-ol (Table 2). The major chemical constituents contained in *L. salicifolia* were (E)-citral and (Z)-citral where limonene, linalool, terpinen-4-ol and α-terpineol were minor components (Table 3).

The limonene, β-myrcene and terpinen-4-ol were commonly found in all tested plant species (Tables 1-3). The common chemical components found in both *L. cubeba* and *L. salicifolia* were β-myrcene, limonene, terpinen-4-ol, methylheptenone, linalool, citronellal, (Z)-geraniol, (Z)-citral, (E)-geraniol and (E)-citral (Tables 2-3). Brophy *et al*. (2002) studied the chemical compounds of volatile leaf essential oil of *M. cajuputi* in Narathivas Province, Thailand and their major chemical constituents were not much differed from this result. However, some constituents were not presented in their findings as these findings and vice versa. For example, α – thujene and β – myrcene could be found in this study, but these constituents were not presented in their findings. Similarly, although their results showed that linalool could be found in *M. cajuputi*, this constituent was not detected in this study. Unfortunately, reports on chemical constituents of both *L. cubeba* and *L. salicifolia* were not available

in the literature even though *L. cubeba* oil was commonly used as medicine in many countries.

Repellency Test

The results regarding the repellency of *M. cajuputi*, *L. cubeba* and *L. salicifolia* to *S. zeamais* and *T. castaneum* at different application rates during first, second, third, fourth and fifth hour are presented in Tables 4-6.

Melaleuca cajuputi moderately repelled *S. zeamais* and *T. castaneum* except at the highest concentration where 100% repellency could be detected for *T. castaneum* at the second hour after treatment. Repellency effect of *M. cajuputi* against *S. zeamais* increased gradually from the first to the fifth hour at the lowest application rate (0.16 μL/cm^2). When the concentration was increased to 0.31 μL/cm^2, the repellency effect slightly varied during the 5 h periods. After the first hour, repellency of *M. cajuputi* decreased from 52% to 48% and, its repellency effect decreased from 68% to 56% after the fourth hour. At the application rate of 0.47 μL/cm^2, the repellency was gradually increased from the first till the third hour, and then gradually decreased afterward till the end of experiment. Moreover, the repellency at the application rate of 0.63 μL/cm^2 was also increased from the first to the second hour and remained constant from the third till the fourth hour (Table 4).

The repellency effect of *M. cajuputi* against *T. castaneum* at 0.16 μL/cm^2 was increased from the first to the second hour, but stayed lower than 50% afterward. At the application rate of 0.31 μL/cm^2, its repellency was increased from the first to the second hour, but decreased again in the third hour. During the third and the fourth hours, its repellency remained constant and then decreased at the fifth hour. When 0.47 μL/cm^2 application rate was tested for repellency against *T. castaneum*, its repellency effect was increased from the first to the second hour and then remained constant from the second till the fourth hour before decreasing at the fifth hour. At the highest application rate of 0.63 μL/cm^2, its repellency effect increased from 88 to 100% in the second hour, but slightly decreased to 96% in the third hour and remained

constant in the fourth hour. The 100% repellency was again occurred at the fifth hour. According to these results, it can generally be assumed that *M. cajuputi* had more repellency effect against *T. castaneum* than *S. zeamais*, especially at the highest concentration (Table 4).

Litsea cubeba essential oil strongly repelled *S. zeamais* and *T. castaneum*, but the repellency was more marked against *T. castaneum*. At the lowest application rate of 0.16 μL/cm^2, repellency effect of *L. cubeba* against *S. zeamais* decreased from 76% at the first hour to 60% and 48% in the second and the third hour, but slightly increased in the fourth hour and then decreased again at the fifth hour. When the concentration was increased to 0.31 μL/cm^2, its repellency effect increased from 68% at the first hour to 92% at the second hour and then remained constant at 96% repellency. The 100% repellency was occurred at the first hour when applied with 0.47 μL/cm^2. However, the repellency effect was slightly decreased at the second hour to the end of the experiment where 92% were recorded. At the highest application rate, the 100% repellency was recorded at the second hour and again at the fourth hour and the fifth hour (Table 5).

When repellency of *L. cubeba* to *T. castaneum* was examined, its repellency remained constant (96%) at the first and the second hour when the lowest application rate was applied. Then, the 100% repellencies were occurred at the third and fifth hours although its repellency slightly decreased at the fourth hour. At the application rate of 0.31 μL/cm^2, the repellency started with 92% at the first hour and then gradually increased to 96-100% at the second hour till the fifth hour. At the application rate of 0.47 μL/cm^2, *L. cubeba* showed 100% repellency in the second, third and fifth hours although it slightly decreased in the fourth hour. When the highest rate (0.63 μL/cm^2) was applied, 96% repellency was detected in the first and the second hours and gradually increased to 100% in the third hour (Table 5).

The essential oil of *L. salicifolia* also strongly repelled *S. zeamais* and *T. castaneum* even at the lowest concentration and the repellency effect was more obvious on *T. castaneum*. The 100% repellency to both species was occurred in all

application rates except at 0.47 μL/cm^2 in *S. zeamais*. Moreover, the 100% repellency could be detected in the second hour at 0.16 μL/cm^2, in the first and the second hours at 0.31 μL/cm^2 and in the second hour at the highest application rate. In addition, the 100% repellency to *T. castaneum* occurred in all application rates of *L. salicifolia* essential oil. The repellency of *L. salicifolia* essential oil was persistent on *T. castaneum* where 76-100% was recorded at the lowest application rate during 5 hour period. Moreover, 90-100% repellency was found at the second lowest dose when 96-100% repellency was detected at the second highest to the highest application rates during the experiment.

Litsea cubeba and *L. salicifolia* essential oils showed the highest average repellency to *S. zeamais* at all application rates compared to those of *M. cajuputi* (Fig. 8). Average repellency effects of *L. cubeba* and *L. salicifolia* to *S. zeamais* and *T. castaneum* are not significantly differed but they are different from those caused by *M. cajuputi* (Fig. 9). At the lower rates (0.16 and 0.31 μL/cm^2), *L. salicifolia* showed slightly more repellency effects on *S. zeamais* than *L. cubeba*. In contrast, *L. cubeba* showed slightly more repellency effects on *T. castaneum* than *L. salicifolia* at the 0.16 and 0.31 μL/cm^2. When the concentration was increased to 0.47 and 0.63 μL/cm^2, *L. cubeba* showed more repellency effect to *S. zeamais* than *L. salicifolia*. However, *L. salicifolia* exhibited slightly more repellency effect on *T. castaneum* at the same application rate of 0.47 and 0.63 μL/cm^2. The high repellency effect (90-100%) to *T. castaneum* was occurred at all application rates when *L. cubeba* and *L. salicifolia* essential oils were applied. Meanwhile, *M. cajuputi* demonstrated less repellency effects on *S. zeamais* and *T. castaneum* according to the average PR values of 65.9% and 65% respectively (Figs. 8 and 9) except for *T. castaneum* where 100% PR was occurred at 5 h after treated with the highest dose of 0.63 μL/cm^2.

The highest persistence of repellency to *S. zeamais* was shown by *L. salicifolia* and *L. cubeba*. Generally, repellency effect increased with concentration of the essential oils. However, significant differences could not be found in each essential oil when the concentrations were increased. Average repellency effect shown by *L. cubeba* against *S. zeamais* at the rate of 0.63 μL/cm^2 was 95.2% followed by 90.4%

and 69.6% when *L. salicifolia* and *M. cajuputi* were applied at the same rate, respectively. Generally, average repellency effect at lower application rates of 0.16, 0.31 and 0.47 μL/cm^2 was lower than those of 0.63 μL/cm^2 in each essential oil, but the difference was not significant (Tables 4-6).

In conclusion, it was clear that *M. cajuputi*, *L. cubeba* and *L. salicifolia* had repellency activities on *S. zeamais* and *T. castaneum* where *M. cajuputi* exhibited the least repellency effect to *S. zeamais* and *T. castaneum*. Prates *et al*. (1998) showed insecticidal activity of limonene against *T. castaneum*. The limonene was found in all tested plant species among which *L. salicifolia* contained more limonene than *L. cubeba* and *M. cajuputi* (Tables 1-3). So, it can be concluded that more persistent repellency to *T. castaneum* and *S. zeamais* detected in *L. salicifolia* than *L. cubeba* and *M. cajuputi* was caused by limonene. In addition, the most repellent compound in *Baccharis salicifolia* (Ruiz & Pav.) Pers. essential oil against *T. castaneum* was α-terpineol (Garcia *et al*., 2005). The α-terpineol was presented in *M. cajuputi* and *L. salicifolia* (Tables 1 and 3). According to the results, it can be concluded that limonene and α-terpineol repelled not only *T. castaneum* but also *S. zeamais*. However, more repellency could be observed in *T. castaneum* than *S. zeamais* in both *M. cajuputi* and *L. cubeba* treatments. Obeng-Ofori *et al*. (1997) found 1,8-cineole (eucalyptol) highly repelled *S. zeamais* and other three stored pests. Since there was 1,8-cineole in *L. cubeba*, it can be concluded that 1,8-cineole repelled not only *S. zeamais* but *T. castaneum* as well. Ojimelukwe and Adler (1999) reported that α-pinene had potent repellent and toxic effects on *T. confusum*. The α-pinene was found in *L. salicifolia* and *M. cajuputi*, hence, it could be seen that this compound repelled *S. zeamais* and *T. castaneum*. In 2007, Stamapoulos *et al*. proved that terpinen-4-ol and linalool had insecticidal activity against *T. confusum*. The linalool and terpinen-4-ol were also found in *L. cubeba* and *L. salicifolia* oils and *M. cajuputi* also had terpinen-4-ol. Therefore, linalool and terpinen-4-ol could be assumed to have repellency effect on *S. zeamais* and *T. castaneum*.

Table 1 Chemical constituents of the essential oil from *Melaleuca cajuputi* Powell leaves collected from Kasetsart University, Bangkhen campus, Thailand.

Compound	Retention Time (RT)	% Composition
α - thujene	13.485	5.92
α-pinene	13.959	4.26
β - myrcene	18.266	1.38
α - phellandrene	19.414	3.76
α -terpinene	20.520	4.44
ρ - cymene	21.273	8.39
limonene	21.661	2.91
γ - terpinene	24.740	25.25
terpineolene	27.519	29.77
terpinen-4-ol	33.654	4.06
α - terpineol	34.444	1.09
β - elemene	43.362	1.88
Z) β - famesene	44.452	5.00
α - caryopphyllene	45.647	1.63
germacrene B	46.761	0.25

Table 2 Chemical constituents of the essential oil from mature fruits of *Litsea cubeba* (Lour.) Persoon collected from Doi Angkang, Chiangmai Province, Thailand.

Compound	Retention Time (RT)	% Composition
β - myrcene	18.357	1.47
limonene	21.758	2.75
terpinen-4-ol	33.718	0.31
β - pinene	16.934	1.51
methylheptenone	18.058	5.56
1, 8- cineole	22.052	3.19
fenchone	27.479	1.80
linalool	28.589	2.16
camphor	31.617	2.18
citronellal	32.217	1.78
(Z) - geraniol	36.526	0.92
(Z) - citral	37.150	30.08
(E) - geraniol	37.777	1.37
(E) - citral	38.543	41.31
Linalool acetate	42.944	0.61

Table 3 Chemical constituents of the essential oils from mature fruits of *Litsea salicifolia* Roxb. ex Wall. collected from Doi Angkang, Chiangmai Province, Thailand.

Compound	Retention Time (RT)	% Composition
α - pinene	13.968	0.29
methylheptenone	17.995	11.20
β - myrcene	18.286	1.74
limonene	21.686	5.59
citronellal	32.159	2.26
terpinen-4-ol	33.672	0.32
linalool	28.589	2.16
α - terpineol	34.462	0.19
(Z)-geraniol	36.478	2.01
(Z) - citral	37.099	29.10
(E) - geraniol	37.731	2.19
(E) - citral	38.491	40.88
(Z)- β - farnesene	44.455	0.19

Table 4 Percent repellency (PR) of the leaf essential oil of *Melaleuca cajuputi* Powell to *Sitopilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst) using treated filter paper test.*

Insect	Oil (μL/cm^2)	PR (Mean% ± *SD*)[a] hours after insect release*					PR (Mean %)[b]
		1	2	3	4	5	
S. zeamais	0.16	52 ± 36 a	60 ± 42 a	68 ± 18 a	68 ± 3 a	72 ± 39 a	64.0
	0.31	52 ± 22 a	48 ± 22 a	68 ± 23 a	68 ± 33 a	56 ± 33 a	58.4
	0.47	56 ± 43 a	80 ± 28 a	90 ± 33 a	72 ± 32 a	60 ± 32 a	71.6
	0.63	56 ± 45 a	72 ± 22 a	72 ± 23 a	72 ± 33 a	78 ± 33 a	69.6
$F_{(3, 16)}$		0.018	1.077	0.120	0.053	0.387	
P		0.996	0.387	0.947	0.983	0.764	
T. castaneum	0.16	40 ± 57 a	64 ± 41 a	44 ± 77 a	48 ± 69 a	44 ± 77 a	48.0
	0.31	44 ± 43 a	64 ± 38 a	56 ± 54 a	56 ± 52 a	48 ± 64 a	53.6
	0.47	52 ± 18 a	68 ± 11 a	68 ± 18 a	68 ± 18 a	56 ± 33 a	62.4
	0.63	88 ± 18 a	100 ± 0 a	96 ± 9 a	96 ± 9 a	100 ± 0 a	96.0
$F_{(3, 16)}$		1.678	1.854	1.083	1.132	1.203	
P		0.212	0.178	0.385	0.366	0.34	

[a] Values were based on 4 levels of content (0.16,0.31, 0.47 and 0.63 μL/cm^2), five replicates of 10 insects in each replication.
[b] Values were means of 4 levels of content (0.16,0.31, 0.47 and 0.63 μL/cm^2) over the 5 h duration (at 1, 2, 3, 4, 5 hours after insects were released).
* For each insect species, means in the same column followed by the same letters do not differ significantly ($P > 0.05$) as determined by Lsd test.

Table 5 Percent repellency (PR) of the fruit essential oil of *Litsea cubeba* (Lour.) Persoon to *Sitopilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst) using treated filter paper test.*

Insect	Oil (μL/cm^2)	PR (Mean% ± *SD*)[a] hours after insect release*					PR (Mean %)[b]
		1	2	3	4	5	
S. zeamais	0.16	76 ± 13.2 a	60 ± 11 a	48 ± 13 b	96 ± 3.5 a	88 ± 4 a	75.0
	0.31	68 ± 13.2 a	92 ± 11 a	96 ± 13 a	96 ± 3.5 a	96 ± 4 a	90.0
	0.47	100 ± 13.2 a	96 ± 11 a	84 ± 13 ab	96 ± 3.5 a	92 ± 4 a	94.0
	0.63	84 ± 13.2 a	100 ± 11 a	92 ± 13 a	100 ± 0.08 a	100 ± 4 a	95.2
$F_{(3, 16)}$		1.073	2.789	2.824	0.333	1.667	
P		0.388	0.074	0.072	0.801	0.214	
T. castaneum	0.16	96 ± 9 a	96 ± 9 a	100 ± 0 a	92 ± 11 a	100 ± 0 a	96.8
	0.31	92 ± 11 a	96 ± 9 a	100 ± 0 a	100 ± 0 a	100 ± 0 a	97.6
	0.47	80 ± 45 a	100 ± 0 a	100 ± 0 a	96 ± 9 a	100 ± 0 a	95.2
	0.63	96 ± 9 a	96 ± 9 a	100 ± 0 a	100 ± 0 a	100 ± 0 a	98.4
$F_{(3, 16)}$		0.503	0.333	-	1.467	-	
P		0.686	0.801	-	0.261	-	

[a] Values were based on 4 levels of content (0.16,0.31, 0.47 and 0.63 μL/cm^2), five replicates of 10 insects in each replication.
[b] Values were means of 4 levels of content (0.16,0.31, 0.47 and 0.63 μL/cm^2) over the 5 h duration (at 1, 2, 3, 4, 5 hours after insects were released).
* For each insect species, means in the same column followed by the same letters do not differ significantly ($P > 0.05$) as determined by Lsd test.

Table 6 Percent repellency (PR) of the fruit essential oil of *Litsea salicifolia* Roxb. ex Wall**.** to *Sitopilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst) using treated filter paper test.*

Insect	Oil (μL/cm^2)	PR (Mean% ± *SD*)[a] hours after insect release*					PR (Mean %)[b]
		1	2	3	4	5	
S. zeamais	0.16	92 ± 11 a	100 ± 0 a	72 ± 33 a	88 ± 18 a	88 ± 18 a	88.0
	0.31	100 ± 0 a	100 ± 0 a	92 ± 11 a	96 ± 9 a	80 ± 28 a	93.6
	0.47	92 ± 11 a	96 ± 9 a	80 ± 14 a	88 ± 11 a	92 ± 11 a	89.6
	0.63	84 ± 17 a	100 ± 0 a	92 ± 18 a	88 ± 18 a	88 ± 18 a	90.4
$F_{(3, 16)}$		0.917	1.000	1.636	0.381	2.794	
P		0.455	0.418	0.221	0.768	0.074	
T. castaneum	0.16	92 ± 11 a	100 ± 0 a	88 ± 18 a	100 ± 0 a	76 ± 26 a	91.2
	0.31	92 ± 11 a	100 ± 0 a	90 ± 11 a	100 ± 0 a	96 ± 8 a	95.6
	0.47	100 ± 0 a	96 ± 9 a	100 ± 0 a	100 ± 0 a	96 ± 9 a	98.4
	0.63	96 ± 9 a	100 ± 0 a	100 ± 0 a	100 ± 0 a	100 ± 6.5 a	99.2
$F_{(3, 16)}$		0.917	7.111	1.636	-	2.794	
P		0.455	0.418	0.221	-	0.074	

[a] Values were based on 4 levels of content (0.16,0.31, 0.47 and 0.63 μL/cm^2), five replicates of 10 insects in each replication.
[b] Values were means of 4 levels of content (0.16,0.31, 0.47 and 0.63 μL/cm^2) over the 5 h duration (at 1, 2, 3, 4, 5 hours after insects were released).
[*] For each insect species, means in the same column followed by the same letters do not differ significantly ($P > 0.05$) as determined by Lsd test.

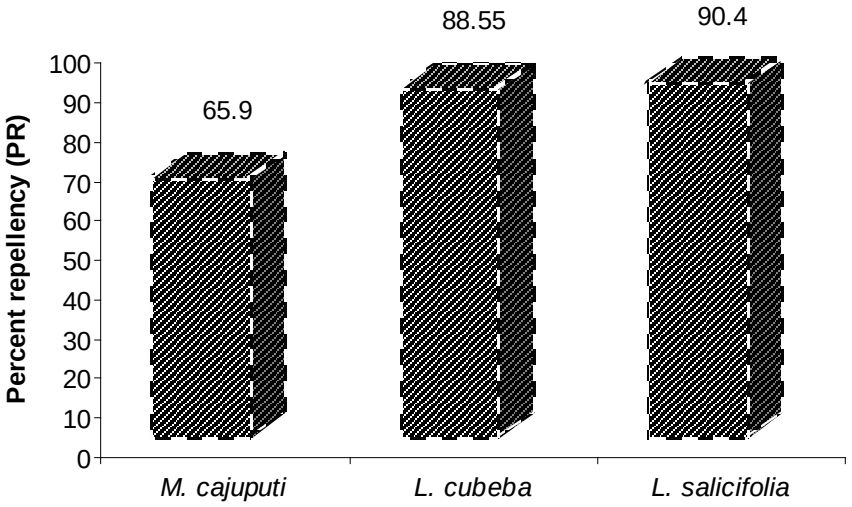

Figure 8 Mean repellency of three essential oils against *Sitophilus zeamais* Motschulsky

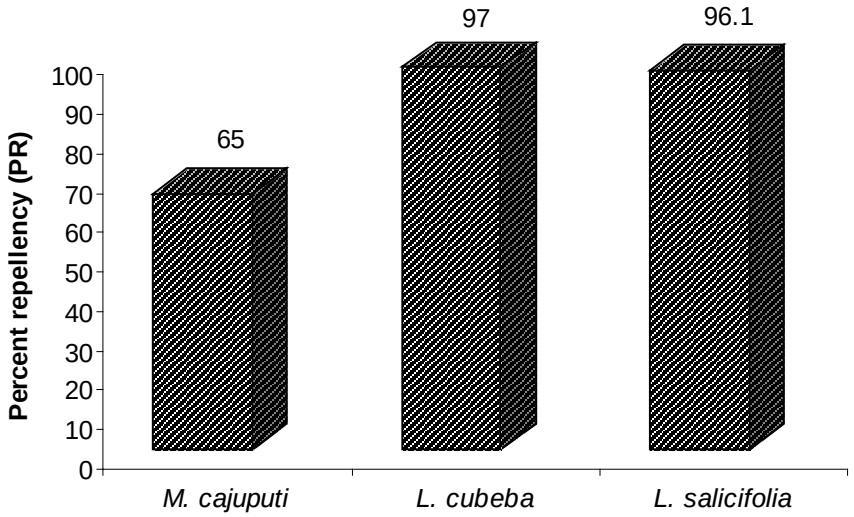

Figure 9 Mean repellency of three essential oils against *Tribolium castaneu*m (Herbst)

Fumigant Toxicity

Fumigant Toxicity of *M. cajuputi* to *S. zeamais* and *T. castaneum*

The results regarding the fumigant toxicity of *M. cajuputi* against *S. zeamais* and *T. castaneum* was shown in Tables 7-9. When *M. cajuputi* was applied at the application rates of 37-130 µL/L, only lower than 50% mortality was detected for both species. Then, the mortality rate increased tremendously when the concentration increased. However, significant different could not be detected for the mortality rates of *S. zeamais* when treated with *M. cajuputi* oil at the rates of 185-556 µL/L. In addition, 52% mortality rate of *S. zeamais* was detected at the highest application rate 9 h after treatment. Meanwhile, the 60% mortality of *S. zeamais* could be observed at 12 h after treatment at the application rates of 185 and 296 µL/L. Consequently, these mortality rates went up to 86% and 82% at 24 h after treatment (Table 7). However, statistical different was not found when *S. zeamais* was treated with *M. cajuputi* at the rates of 185 and 296 µL/L, 12 h and 24 h after treatment. Moreover, 82-100% mortality of *S. zeamais* could be found at 24 h after treatment at the application rates of 185-556 µL/L and their mortality among these application rates were not significant. The 100% mortality rate could only be detected at 24 h after the highest concentration rate was applied (Table 7).

Melaleuca cajuputi induced only 2-10% mortality of *T. castaneum* at the concentrations of 37-130 µL/L over 24 h periods. When the concentration was increased to higher doses, higher mortality rates were recorded (Table 8). The mortality rates of 70-100% of *T. castaneum* could be detected at the application rates of 185-556 µL/L (Table 8). Interestingly, *M. cajuputi* applied at 556 µL/L could cause 80% mortality of *T. castaneum* 3 h after treatment and gradually increased with time where 100% mortality was recorded 24 h after application. In contrast, *M. cajuputi* at the rate of 556 µL/L failed to kill *S. zeamais* after applied for 3 h and only 12% mortality was detected 6 h after application. The effect of *M. cajuputi* started to increase to 52, 82 and 100% at the highest application rate 9, 12 and 24 h after treatment, respectively.

When the mortality rates of *S. zeamais* caused by the fumigant toxicity of *M. cajuputi* was examined, the application rates of 37-94 µL/L could only induce the mortality rates of 14-18% at 24 h. Then, when the application rate was increased to 130 µL/L, the mortality rate went up to 40% but did not statistically differed from 18% caused by 94 µL/L. Consequently, when the application rate was increased to 185 µL/L, up to 2 folds of mortality rate was recorded and steadily increased until 444 µL/L was used. Finally, 100% mortality was recorded at the highest application rate 24 h after treatment. However, there were non-significant among the mortality of *S. zeamais* caused by the application rates of 185-556 µL/L (Table 9).

Meanwhile, when the mortality rates of *T. castaneum* caused by fumigant toxicity of *M. cajuputi* was observed, low mortality of *T. castaneum* could be detected at the application rates of 37-130 µL/L which was comparatively lower than mortality rate of *S. zeamais* caused by the same application rates. When the application rate was increased to 185 µL/L, 6-7 folds mortality of *T. castaneum* could be recorded (Table 8). After that, the mortality was increased to 84% when the application rate was increased to 296 µL/L, its mortality was statistically different from those of 185 µL/L. Finally, 92-100% mortality could be found at the application rates of 370-556 µL/L. However, non-significant was found among these application rates (Table 9).

The 50% mortality of *T. castaneum* could be observed at the second highest application rates, 6 h after treatment. Surprisingly, although no mortality of *S. zeamais* was found at the highest application rate 3 h after treatment, 80% mortality of *T. castaneum* was investigated at the same rate and same duration. Moreover, although higher mortality of *T. castaneum* could be detected at the highest and second highest application rates at 3 and 6 h, only 12-22% mortality rate of *S. zeamais* were recorded at the same application rates and durations. Hence, it could be seen that *M. cajuputi* showed more fumigant effect on *T. castaneum* than *S. zeamais* at the application rates of 444-556 µL/L at 3 and 6 h durations. However,

according to the LC_{50} values, *M. cajuputi* showed high fumigant effect on *S. zeamais* (LC_{50} = 178.23 µL/L) than *T. castaneum* (LC_{50} = 213.17 µL/L) (Table 16).

Fumigant Toxicity of *L. cubeba* to *S. zeamais* and *T. castaneum*

The results regarding the fumigant toxicity of *L. cubeba* against *S. zeamais* and *T. castaneum* were shown in Tables 10-12. When *L. cubeba* essential oil was applied at the rates of 37-56 µL/L, only lower than 50% mortality of *S. zeamais* was detected at 24 h duration. Then, its mortality went up slightly to 58-62% at the application rates of 94-130 µL/L at 24 h periods. When the application rates of *L. cubeba* was increased to 185 µL/L, its mortality went up to 88% and then steadily increased with concentrations to100% mortality where 370-556 µL/L was applied for 24 h duration.

Although the 100% mortality of *S. zeamais* was detected at the application rates of 370 µL/L 24 h after treatment, this application rate only induced 30% mortality of *T. castaneum* at the same duration and only 48% mortality of *T. castaneum* could be found at the highest application rate during 24 h experimental periods. Hence, it could be concluded that although *L. cubeba* had good fumigant effect on *S. zeamais*, its effect on *T. castaneum* was not so good. So, *L. cubeba* oil could not be recommended to use as fumigant against *T. castaneum* (Tables 10-12).

Moreover, when 185-556 µL/L of *L. cubeba* oil were tested for fumigant toxicity against *S. zeamais*, all mortality rates were not statistically differed among them. In contrast, the mortality rates of *T. castaneum* caused by 370-556 µL/L *L. cubeba* oil was not statistically different at 24 h after treatment where only 48% mortality of *T. castaneum* could be detected at the highest application rate of 556 µL/L 24 h after treatment. When the lowest application rate of *L. cubeba* oil was applied to *S. zeamais*, 30% mortality could be observed, whereas only 18% mortality was detected when *M. cajuputi* was tested at the same rate and the same duration (Table 9). So, *L. cubeba* oil had more fumigant effect on *S. zeamais* than *M. cajuputi* oil at the lowest application rate. The highest application rate of *L.*

cubeba oil recommended for *S. zeamais* was 370 µL/L where 66% mortality could be seen 6 h after treatment and then steadily went up to 100% mortality 24 h after treatment. According to the LC_{50} values, *L. cubeba* had much more fumigant effect on *S. zeamais* (LC_{50} = 92.46 µL/L) than *T. castaneum* (LC_{50} = 549.57 µL/L) (Table 17).

Fumigant Toxicity of *L. salicifolia* to *S. zeamais* and *T. castaneum*

The results regarding the fumigant toxicity of *L. salicifolia* against *S. zeamais* and *T. castaneum* were shown in Tables 13-15. Up to 84% mortality of *S. zeamais* could be detected at the lowest application rate of 37 µL/L and 100% mortality could be found at 444-556 µL/L 24 h after treatment (Table 13). The 60% mortality of *S. zeamais* could be found 9 h after treatment at the lowest application rate. All application rates to *S. zeamais* showed no statistically different in mortality rate 24 h after application (Table 15).

Surprisingly, the fumigant toxicity of *L. salicifolia* could be seen since the lowest application rate of 37 µL/L where 84% mortality of *S. zeamais* could be occurred, but only 14% mortality of *T. castaneum* was detected at the highest application rate 24 h treatment (Table 14). All application rates of *L. salicifolia* to *T. castaneum* exhibited no statistically different in mortality rate. In addition, all application rates of *L. salicifolia* showed more fumigant toxicity to *S. zeamais* than *T. castaneum* (Table 15). According to the LC_{50} values, *L. salicifolia* showed much more fumigant effect on *S. zeamais* (LC_{50} = 4.435 µL/L) than *T. castaneum* (LC_{50} = 845.16 µL/L) (Table 18). Hence, it could generally be concluded that although *L. salicifolia* had good fumigant effect on *S. zeamais*, it should not be recommended to use as fumigant on *T. castaneum*.

Generally, all three essential oils evoked fumigant effect to *S. zeamais*. Among all three essential oils, *M. cajuputi* had fumigant toxicity on both insect species. Although *L. cubeba* and *L. salicifolia* had fumigant effect on *S. zeamais*, their fumigant toxicity to *T. castaneum* was not obvious. Hence, it can be

concluded that *L. c*ubeba and *L. salicifolia* essential oils had the best fumigant toxicity on *S. zeamais* whereas *M. cajuputi* leaf essential oil could be assumed as the highest fumigant on *T. castaneum.* Moreover, according to the LC_{50} values, *L. salicifolia* had the highest fumigant effect on *S. zeamais*, followed by *L. cubeba* and *M. cajuputi*. In contrast, *M. cajuputi* had the highest fumigant effect on *T. castaneum*, followed by *L. cubeba* and *L. salicifolia* (Tables 16-18).

Litsea cubeba showed the highest fumigant toxicity to *S. zeamais* where 100% mortality could be detected at the application rate of 370 µL/L 24 h after treatment (Table 10). However, both *M. cajuputi* and *L. salicifolia* could only show the 100% mortality of *S. zeamais* at the highest (556 µL/L) and the second highest application rates (444 µL/L) 24 h after treatment, respectively (Tables 7 and 13).

Although *L. salicifolia* evoked the 100% mortality of *S. zeamais*, only 14% mortality of *T. castaneum* could be detected at the highest application rate 24 h after treatment (Table 9). Meanwhile, *M. cajuputi* exhibited the highest fumigant toxicity to *T. castaneum* where 100% mortality could be found at the highest application rate 24 h after treatment (Table 7). In contrast, *L. cubeba* showed moderate fumigant toxicity against *T. castaneum* since only 48% mortality was recorded with the highest application rate 24 h after treatment (Table 11).

Lee *et al*. (2001), Papachristos *et al*. (2004), Erler (2005) and Stamopoulos *et al*. (2007) proved that terpinen-4-ol showed fumigant toxicity on some stored-product insects. As terpinen-4-ol was detected in all three essential oils, it could be concluded that terpinen-4-ol also had fumigant effect on *S. zeamais* and *T. castaneum*. Tripathi *et al*. (2001), Lee *et al*. (2004) and Rozman *et al*. (2007) showed that 1,8-cineole had fumigant effect on some stored-product insects. Since 1,8-cineole was found in *L. cubeba*, it could also be assumed that 1,8-cineole also had fumigant effect on *S. zeamais* and *T. castaneum*. In addition, Rozman *et al*. (2007) demonstrated that linalool had fumigant effect on some stored-product insects. It could be stated that linalool had fumigant effect on *S. zeamais* and *T. castaneum* as it could be found in *L. cubeba* and *L. salicifolia*.

Liu *et al.* (2007) showed that hexane and methanol leaf extracts of *L. cubeba* had fumigant toxicity against these two species. Therefore, not only crude leaf extracts but also fruit essential oil of *L. cubeba* exhibited fumigant toxicity against *S. zeamais* and *T. castaneum*.

Table 7 Accumulate mortality rate of *Sitophilus zeamais* Motschulsky caused by fumigant toxicity of *Melaleuca cajuputi* Powell leaf essential oil at different concentrations and different intervals.

Application rate (µL/L)	**Mortality (%)**				
	3 h	**6 h**	**9 h**	**12 h**	**24 h**
37	0 a	0 b	2 cd	8 de	18 bc
56	0 a	0 b	0 d	4 e	14 c
94	0aa	2 ab	10 bcd	12 de	18 b
130	0 a	2 ab	22 abc	32 cd	40 b
185	2 a	8 ab	24 abc	60 ab	86 a
296	0 a	18 ab	22 abc	60 ab	82 a
370	0 a	8 ab	30 abc	44 bc	90 a
444	0 a	22 a	38 ab	52 bc	88 a
556	0 a	12 ab	52 a	82 a	100 a

[a]Values were means of five replicates of 10 insects in each replication over 3, 6, 9, 12 and 24 h duration.
Means in the same column by the same letters do not differ significantly ($P > 0.05$) as determined by one way ANOVA and Lsd test.

Table 8 Accumulate mortality rate of *Tribolium castaneum* (Herbst) caused by fumigant toxicity of *Melaleuca cajuputi* Powell leaf essential oil at different concentrations and different intervals.

Application rate (µL/L)	Mortality (%)				
	3 h	6 h	9 h	12 h	24 h
37	0 c	2 c	2 d	4 c	4 d
56	2 c	2 c	2 d	2 c	2 d
94	6 c	6 c	6 d	6 c	6 d
130	8 c	8 c	10 c	10 c	10 d
185	12 c	30 bc	30 bcd	60 b	70 c
296	12 c	32 bc	32 bcd	66 b	84 b
370	18 c	34 bc	38 bc	60 b	92 ab
444	42 b	50 b	50 b	74 b	94 ab
556	80 a	90 a	92 a	92 a	100 a

[a]Values were means of five replicates of 10 insects in each replication over 3, 6, 9, 12 and 24 h duration.
Means in the same column by the same letters do not differ significantly ($P > 0.05$) as determined by one way ANOVA and Lsd test.

Table 9 Mortality of *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst) caused by fumigant toxicity of *Melaleuca cajuputi* Powell leaf essential oil at different concentrations over 24 hour.

Application rate	**Mortality (%)**[a]	
(µL/L)	*Sitophilus zeamais*	*Tribolium castaneum*
37	18 bc	4 d
56	14 c	2 d
94	18 bc	6 d
130	40 b	10 d
185	86 a	70 c
296	82 a	84 b
370	90 a	92 ab
444	88 a	94 ab
556	100 a	100 a

[a] Values were means of five replicates of 10 insects in each replication over 24 h duration.
For each species, means in the same column by the same letters do not differ significantly ($P > 0.05$) as determined by one way ANOVA and Lsd test.

Table 10 Accumulate mortality rate of *Sitophilus zeamais* Motschulsky caused by fumigant toxicity of essential oil extracted from mature fruits of *Litsea cubeba* (Lour.) Persoon at different concentrations and different intervals.

Application rate (µL/L)	**Mortality (%)**				
	3 h	**6 h**	**9 h**	**12 h**	**24 h**
37	0 c	24 b	24 b	28 b	30 c
56	10 bc	24 b	26 b	32 b	42 bc
94	6 bc	26 b	34 b	40 b	58 b
130	0 c	26 b	34 b	40 b	62 b
185	38 a	62 a	78 a	80 a	88 a
296	40 a	74 a	78 a	84 a	98 a
370	26 ab	66 a	88 a	94 a	100 a
444	46 a	70 a	78 a	84 a	100 a
556	22 abc	52 ab	72 a	82 a	100 a

[a]Values were means of five replicates of 10 insects in each replication over 3, 6, 9, 12 and 24 h duration.
Means in the same column by the same letters do not differ significantly ($P > 0.05$) as determined by one way ANOVA and Lsd test.

Table 11 Accumulate mortality rate of *Tribolium castaneum* (Herbst) caused by fumigant toxicity of essential oil extracted from mature fruits of *Litsea cubeba* (Lour.) Persoon at different concentrations and different intervals.

Application rate (µL/L)	**Mortality (%)**				
	3 h	**6 h**	**9 h**	**12 h**	**24 h**
37	0 b	0 a	0 a	2 b	4 d
56	0 b	0 a	2 a	2 b	14 cd
94	2 b	2 a	2 a	10 b	16 cd
130	0 b	0 a	6 a	12 b	18 bcd
185	0 b	0 a	0 a	22 ab	22 bcd
296	0 b	4 a	10 a	20 ab	24 bcd
370	0 b	2 a	14	22 ab	30 abc
444	6 a	6 a	6 a	24 ab	40 ab
556	2 b	6 a	12 a	38 a	48 a

[a]Values were means of five replicates of 10 insects in each replication over 3, 6, 9, 12 and 24 h duration.
Means in the same column by the same letters do not differ significantly ($P > 0.05$) as determined by one way ANOVA and Lsd test.

Table 12 Mortality of *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst) caused by fumigant toxicity of essential oil extracted from mature fruits of *Litsea cubeba* (Lour.) Persoon at different concentrations over 24 hour.

Application rate	**Mortality (%)[a]**	
(µL/L)	*Sitophilus zeamais*	*Tribolium castaneum*
37	30 c	4 de
56	42 bc	14 cde
94	58 b	16 cde
130	62 b	18 cde
185	88 a	22 bcd
296	98 a	24 bcd
370	100 a	30 ab
444	100 a	40 ab
556	100 a	48 a

[a] Values were means of five replicates of 10 insects in each replication over 24 h duration.
For each species, means in the same column by the same letters do not differ significantly ($P > 0.05$) as determined by one way ANOVA and Lsd test.

Table 13 Accumulate mortality rate of *Sitophilus zeamais* Motschulsky caused by fumigant toxicity of essential oil extracted from *Litsea salicifolia* Roxb. ex Wall**.** at different concentrations and different intervals.

Application rate (µL/L)	**Mortality (%)**				
	3 h	**6 h**	**9 h**	**12 h**	**24 h**
37	34 cd	38 c	60 c	68 b	84 b
56	18 d	52 bc	66 c	78 ab	88 ab
94	46 bcd	58 bc	68 c	70 b	92 ab
130	20 d	36 c	52 c	64 b	94 ab
185	38 bcd	58 bc	72 bc	82 ab	96 ab
296	44 bcd	58 bc	72 bc	76 ab	96 ab
370	64 abc	78 ab	92 ab	98 a	98 a
444	78 a	90 a	96 a	98 a	100 a
556	70 ab	96 a	100 a	100 a	100 a

[a]Values were means of five replicates of 10 insects in each replication over 3, 6, 9, 12 and 24 h duration.
Means in the same column by the same letters do not differ significantly ($P > 0.05$) as determined by one way ANOVA and Lsd test.

Table 14 Accumulate mortality rate of *Tribolium castaneum* (Herbst) caused by fumigant toxicity of essential oil extracted from mature fruits of *Litsea salicifolia* Roxb. ex Wall**.** at different concentrations and different intervals.

Application rate (µL/L)	**Mortality (%)**				
	3 h	**6 h**	**9 h**	**12 h**	**24 h**
37	0 a	0 a	0 a	0 a	0 a
56	0 a	0 a	0 a	0 a	0 a
94	0 a	0 a	0 a	0 a	0 a
130	0 a	0 a	0 a	0 a	2 a
185	0 a	0 a	0 a	0 a	2 a
296	0 a	4 a	4 a	4 a	4 a
370	2 a	2 a	4 a	4 a	6 a
444	0 a	0 a	4 a	6 a	12 a
556	0 a	0 a	6 a	6 a	14 a

[a]Values were means of five replicates of 10 insects in each replication over 3, 6, 9, 12 and 24 h duration.
Means in the same column by the same letters do not differ significantly ($P > 0.05$) as determined by one way ANOVA and Lsd test.

Table 15 Mortality of *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst) caused by fumigant toxicity of essential oil extracted from mature fruits of *Litsea salicifolia* Roxb. ex Wall**.** at different concentrations over 24 hour.

Application rate	**Mortality (%)**[a]	
(µL/L)	*Sitophilus zeamais*	*Tribolium castaneum*
37	84 b	0 a
56	88 ab	0 a
94	92 ab	0 a
130	94 ab	2 a
185	96 a	2 a
296	96 a	4 a
370	98 a	6 a
444	100 a	12 a
556	100 a	14 a

[a] Values were means of five replicates of 10 insects in each replication over 24 h duration.
For each species, means in the same column by the same letters do not differ significantly ($P > 0.05$) as determined by one way ANOVA and Lsd test.

Table 16 Fumigant toxicity of *Melaleuca cajuputi* Powell leaf essential oil against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst).

Insect species	LC_{50} [a, b]	LC_{95} [a, b]	Slope ± SE	Degrees of freedom	Chi-square (x^2)
S. zeamais	178.23 (119.23-243.04)	408.54 (321.52-604.84)	0.007 ± 0.00	8	95.41
T. castaneum	213.17 (168.95-266.33)	376.1 (311.67-503.37)	0.010 ± 0.001	8	82..04

[a]Units LC_{50} and LC_{95} = µL/L air, applied for 24 h at 27˚C.
[b]95% lower and upper fiducial limits are shown in parenthesis.

Table 17 Fumigant toxicity of mature fruits of *Litsea cubeba* (Lour.) Persoon essential oil against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst).

Insect species	LC_{50} [a, b]	LC_{95} [a, b]	Slope ± SE	Degrees of freedom	Chi-square (x^2)
S. zeamais	92.46 (72.49 – 113.16)	224.53 (188.09 – 290.22)	0.012 ± 0.001	8	27.509
T. castaneum	549.57 (457.43 – 720.31)	1147.000 (935.99 – 1673.11)	0.003 ± 0	8	18.96

[a]Units LC_{50} and LC_{95} = µL/L air, applied for 24 h at 27˚C.
[b]95% lower and upper fiducial limits are shown in parenthesis.

Table 18 Fumigant toxicity of mature fruits of *Litsea salicifolia* Roxb. ex Wall. against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst).

Insect species	LC_{50} [a, b]	LC_{95} [a, b]	Slope ± SE	Degrees of freedom	Chi-square (x^2)
S. zeamais	4.435 (–)	174.63 (–)	0.10 ± 0.001	8	390.511
T. castaneum	845.16 (729.81-1052.01)	1345.000 (1118.69-1760.79)	0.003 ± 001	8	4.8

[a]Units LC_{50} and LC_{95} = µL/L air, applied for 24 h at 27˚C.
[b]95% lower and upper fiducial limits are shown in parenthesis.

Contact Toxicity

Contact toxicity of *M. cajuputi* against *S. zeamais* and *T. castaneum*

The results regarding the contact toxicity of *M. cajuputi* against *S. zeamais* and *T. castaneum* were shown in Tables 19-22. From this study, 10% of *M. cajuputi* oil could only kill 26% *S. zeamais* at day 1 and went up to 36% after 5 days. Once the concentration was increased to 20%, the mortality increased to 92% at the first day after application. At higher concentration rates (30-40%), there were no significant different in mortality rate, however only 62% mortality of *T. castaneum* was recorded at the same application rate 7 d after treatment (Tables 19-20).

Melaleuca cajuputi oil demonstrated low contact toxicity on *T. castaneum* compared to *S. zeamais*. The result clearly revealed that 10% of *M. cajuputi* oil induced only 16% mortality of *T. castaneum* whereas 36% mortality could be detected for *S. zeamais* at 7 d after application (Tables 19-20). Then, nearly 3 folds of mortality rate of *T. castaneum* could be observed when the application rate was doubled, 7 d after treatment. The mortality slightly increased to 60-62% when the application rates were raised to 30-40%. However, no statistically different in mortality of *T. castaneum* were detected among the application rates of 20-40% at day 7 (Table 21). Moreover, the mortality rate did not change obviously at all application rates during 1-7 d after treatment. So, it could be concluded that *M. cajuputi* oil was less effective against *T. castaneum* as compared to *S. zeamais* at all application rates. The LD_{50} values also supported this conclusion since *M. cajuputi* showed LD_{50} value of 12.40% where 28.64% was reported for *T. castaneum* (Table 22).

Contact toxicity of *L. cubeba* against *S. zeamais* and *T. castaneum*

The results regarding the contact toxicity of *L. cubeba* against *S. zeamais* and *T. castaneum* were shown in Tables 23-26. The results indicated that only 10% mortality of *S. zeamais* could be detected after treated with 10% application rate for

7 d. Mortality increased while concentration increased. As the concentration rate was increased to 30%, the mortality rate was gone up to 86% at 1 d after application and increased to 88% mortality afterwards. The 100% mortality could be investigated at the highest application rate (40%) since the first day period. However, mortality of *S. zeamais* induced by *L. cubeba* did not significantly differ when applied at 30-40% application rates where 88-100% mortalities were reported (Table 25).

Litsea cubeba only caused 20-26% mortality of *T. castaneum* where 10-30% *L. cubeba* oil were applied and 48% mortality could be examined at the highest concentration rate 7 d after treatment (Table 24). However, there were no statistically different in the mortality of *T. castaneum* treated with 10-30% *L. cubeba* oil (Table 25).

Although *L. cubeba* could induce 100% mortality of *S. zeamais* at the highest application rate, only 48% mortality of *T. castaneum* could be detected at the same rate and duration. Hence, *L. cubeba* oil could be recommended for *S. zeamais* at 30-40% concentrations where 86-100% mortality rates could be noticed at 1 d period. In contrast, it could not be recommended for *T. castaneum*. According to the LD_{50} values, *L. cubeba* oil was more toxic to *S. zeamais* (21.450%) than *T. castaneum* (42.302%) (Table 26).

Contact toxicity of *L. salicifolia* against *S. zeamais* and *T. castaneum*

The results on the contact toxicity of *L. salicifolia* against *S. zeamais* and *T. castaneum* were shown in Tables 27-30. The lowest application rate could only induce 26% mortality of *S. zeamais* 1 d after treatment and gradually increased to 28% at day 4. Then, when the application rate was increased to 20%, nearly 2.5 folds of mortality rate of *S. zeamais* could be detected 7 d after treatment. Once the concentration rate was raised to 30-40%, mortality rates of *S. zeamais* went up to 98-100%. In addition, no significant different was found between the highest and the second highest application rates. Hence, it could be recommended that *L.*

salicifolia oil at the rate of 30% could be used for *S. zeamais* as 100% mortality could be detected at 3 d period (Table 27).

When *L. salicifolia* was applied to *T. castaneum* at 10% concentration, only 26% mortality was noted on day 1 after application. As the time progressed, the mortality rate gradually went up to 46% at day 4. Its mortality rate slightly increased to 60-62% when the application rates of 20-30% applied. In addition, only 68% mortality of *T. castaneum* could be detected at 7 d after treatment at the highest application rate. However, no statistically different was found among all application rates (Table 29).

Hence, it could be concluded that *L. salicifolia* oil should be recommended to apply for *S. zeamais* at 30% concentration since 98% mortality was detected at 1 d period. In addition, higher than 40% is required to decrease the population of *T. castaneum* since only 68% of *T. castaneum* were killed at the highest dose of *L. salicifolia* oil (Table 28). LD_{50} values of *L. salicifolia* against *S. zeamais* was 15.743% where that of *T. castaneum* was 22.263% indicating that this oil was more toxic to *S. zeamais* as compared to *T. castaneum* (Table 30).

Generally, all three essential oils showed contact toxicity against *S. zeamais*. *Melaleuca cajuputi* oil at the concentration of 20% could kill 92% of *S. zeamais* adult at 1 d period. In addition, *L. cubeba* induced 86% mortality of *S. zeamais* at the application rate of 30% at 1 d period. Meanwhile, 30% *L. salicifolia* oil caused 98% mortality of *S. zeamais* at 1 d after treatment. Hence, it could be concluded that *M. cajuputi* had the best contact toxicity against *S. zeamais* followed by *L. cubeba* and *L. salicifolia*.

Meanwhile, *M. cajuputi* oil induced 62% mortality of *T. castaneum* at the highest application rate, 7 d after treatment where 68% mortality of this insect could be found at the highest application rate of *L. salicifolia* oil at the same duration. In contrast, only 48% *T. castaneum* mortality could be detected at the highest application rate of *L. cubeba* oil at the same duration. Hence, *M. cajuputi* and *L.*

salicifolia showed moderate contact toxicity to *T. castaneum* where *L. cubeba* oil had considerably the lowest contact toxicity to *T. castaneum*.

Melaleuca cajuputi oil exhibited high contact toxicity against *S. zeamais* whereas it only showed moderate contact toxicity to *T. castaneum*. In contrast, although *L. cubeba* oil evoked high toxicity to *S. zeamais*, its toxicity to *T. castaneum* was slightly low (48%). Meanwhile, *L. salicifolia* oil had high contact toxicity to *S. zeamais* and moderate toxicity to *T. castaneum*.

Chiam *et al*. (1999) showed that allyl disulfide compound from garlic contributed to contact toxicity to *S. zeamais* and *T. castaneum*. Huang *et al*. (2002) also demonstrated the contact toxicity of eugenol, isoeugenol and methyleugenol on these two insect species. Moreover, Huang *et al*. (1997) showed the contact toxicity of nutmeg oil to these insect species. Ngamo *et al*. (2007b) also revealed that *Lippia rugosa* L. essential oil had contact toxicity to these insect species. Some authors proved that plant species from Myrtaceae family had contact toxicity to stored-product insects. For example, Tapondjou *et al*. (2005) showed that *Eucalyptus saligna* Sm. had contact toxicity to *S. zeamais* and *T. confusum* du Val. Kim *et al.* (2003b) proved that *Eugenia caryophyllata* Thunberg had contact toxicity to *S. oryzae* and *C. chinensis*. Moreover, some authors showed the contact toxicity of plant species from Lauraceae family. For instance, Huang and Ho (1998) proved that *C. aromaticum* had contact toxicity to *S. zeamais* and *T. castaneum*. Liu *et al*. (2007) also proved that hexane leaf extract of *L. cubeba* showed contact toxicity to these two species.

Many researchers have shown the toxicity of some chemical constituents against stored-product insects. For example, terpinen-4-ol was toxic to some stored-product insects (Lee *et al*., 2001; Papachristos *et al*., 2004; Erler, 2005; Stamopoulos *et al*., 2007). The 1,8-cineol had contact toxicity effects to *S. zeamais* and other stored-product insects (Obeng-Ofori *et al*., 1997; Tripathi *et al*., 2001; Lee, 2003; Papachristos *et al*., 2004; Stamopoulos *et al*., 2007). Papachristos *et al*. (2004) and Stamopoulos *et al*. (2007) indicated that linalool had contact toxicity

against some stored-product insects. In addition, limonene, α-pinene, β-pinene and camphor were also known to induce contact toxicity against stored-product insects (Prates *et al*., 1998; Garcia *et al*., 2005). Hence, as these constituents were presented in the treated oil, it could be concluded that these chemicals also had contact toxicity to *S. zeamais* and *T. castaneum*.

Table 19 Accumulate mortality rate of *Sitophilus zeamais* Motschulsky caused by contact toxicity of *Melaleuca cajuputi* Powell leaf essential oil at different concentrations and different intervals.

Concentration (%)	Mortality (%)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
10	26	30	32	34	36	36	36
20	92	92	92	94	94	94	94
30	98	98	98	98	98	98	98
40	100	100	100	100	100	100	100

Table 20 Accumulate mortality rate of *Tribolium castaneum* (Herbst) caused by contact toxicity of *Melaleuca cajuputi* Powell leaf essential oil at different concentrations and different intervals.

Concentration (%)	Mortality (%)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
10	8	10	10	12	12	14	16
20	40	44	44	44	44	44	44
30	54	56	58	58	60	60	60
40	60	62	62	62	62	62	62

Table 21 Mortality of *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst) caused by contact toxicity of *Melaleuca cajuputi* Powell leaf essential oil at different concentrations over 7 days.

Concentration (%)	Mortality (%)	
	Sitophilus zeamais[a]	*Tribolium castaneum*[a]
10	36 b	16 b
20	94 a	44 a
30	98 a	60 a
40	100 a	62 a

[a] Values were means of five replicates of 10 insects in each replication over 7 d duration.
For each species, means in the same column by the same letters do not differ significantly ($P > 0.05$) as determined by independent sample t test.

Table 22 Contact toxicity of *Melaleuca cajuputi* Powell leaf essential oil applied topically to *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst).

Insect	LD_{50} (95% fiducial limit) (%)	LD_{95} (95% fiducial limit) (%)	Slope ± S. E	Y-intercept ± S. E
S. zeamais	12.401 (1.496-21.748)	22.117 (15.948-71.228)	0.169 ± 0.015	- 2.099 ± 0.015
T. castaneum	28.643 (17.528-56.307)	59.185 (41.769-196.373)	0.054 ± 0.005	-1.543 ± 0.005

Table 23 Accumulate mortality rate of *Sitophilus zeamais* Motschulsky caused by contact toxicity of essential oil extracted from mature fruits of *Litsea cubeba* (Lour.) Persoon at different concentrations and different intervals.

Concentration (%)	Mortality (%)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
10	8	10	10	10	10	10	10
20	32	32	34	36	36	36	36
30	86	88	88	88	88	88	88
40	100	100	100	100	100	100	100

Table 24 Accumulate mortality rate of *Tribolium castaneum* (Herbst) caused by contact toxicity of essential oil extracted from mature fruits of *Litsea cubeba* (Lour.) Persoon at different concentrations and different intervals.

Concentration (%)	Mortality (%)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
10	10	12	18	20	20	20	20
20	12	14	18	20	20	20	20
30	26	26	26	26	26	26	26
40	38	38	44	46	48	48	48

Table 25 Mortality of *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst) caused by contact toxicity of essential oil extracted from mature fruits of *Litsea cubeba* (Lour.) Persoon at different concentrations over 7 days.

Concentration (%)	Mortality (%)	
	Sitophilus zeamais[a]	*Tribolium castaneum*[a]
10	10 c	20 bc
20	36 b	20 bc
30	88 a	26 b
40	100 a	48 a

[a] Values were means of five replicates of 10 insects in each replication over 7 d duration.
For each species, means in the same column by small letters do not differ significantly ($P > 0.05$) as determined by independent sample t test.

Table 26 Contact toxicity of mature fruits of *Litsea cubeba* (Lour.) Persoon essential oil applied topically to *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst).

Insect	LD_{50} (95% fiducial limit) (%)	LD_{95} (95% fiducial limit) (%)	Slope ± S. E	Y-intercept ± S. E
S. zeamais	21.450 (20.172-22.740)	33.908 (31.840-36.590)	0.132 ± 0.010	- 2.832 ± 0.010
T. castaneum	42.302 (28.928-1001.717)	85.180 (53.802-3349.235)	0.038 ± 0.005	-1.623 ± 0.005

Table 27 Accumulate mortality rate of *Sitophilus zeamais* Motschulsky caused by contact toxicity of essential oil extracted from mature fruits of *Litsea salicifolia* Roxb. ex Wall**.** at different concentrations and different intervals.

Concentration (%)	Mortality (%)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
10	26	26	26	28	28	28	28
20	58	64	66	66	68	68	68
30	98	98	100	100	100	100	100
40	98	98	98	98	98	98	98

Table 28 Accumulate mortality rate of *Tribolium castaneum* (Herbst) caused by contact toxicity of essential oil extracted from mature fruits of *Litsea salicifolia* Roxb. ex Wall**.** at different concentrations and different intervals.

Concentration (%)	Mortality (%)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
10	26	40	44	46	46	46	46
20	50	50	50	60	60	60	60
30	36	36	44	50	54	60	62
40	60	62	66	66	66	66	68

Table 29 Mortality of *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst) caused by contact toxicity of essential oil extracted from mature fruits of *Litsea salicifolia* Roxb. ex Wall**.** at different concentrations over 7 days.

Concentration (%)	Mortality (%)	
	Sitophilus zeamais[a]	*Tribolium castaneum*[a]
10	28 c	46 a
20	68 b	60 a
30	100 a	62 a
40	98 a	68 a

[a] Values were means of five replicates of 10 insects in each replication over 7 d duration.
For each species, means in the same column by small letters do not differ significantly ($P > 0.05$) as determined by independent sample t test.

Table 30 Contact toxicity of mature fruits of *Litsea salicifolia* Roxb. ex Wall**.** essential oil applied topically to *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst).

Insect	LD_{50} (95% fiducial limit) (%)	LD_{95} (95% fiducial limit) (%)	Slope ± S. E	Y-intercept ± S. E
S. zeamais	15.743 (-0.552-29.995)	28.865 (20.430-116.090)	0.125 ± 0.010	- 1.973 ± 0.010
T. castaneum	22.263 (-)	60.714 (–)	0.043 ± 0.005	-0.952 ± 0.005

Antifeedant Toxicity

Antifeedant toxicity of *M. cajuputi* to *S. zeamais* and *T. castaneum*

Antifeedant toxicity of *M. cajuputi* to *S. zeamais* as indicated by % mortality and % FDI was shown in Table 31. The highest concentration showed 70% antifeedant toxicity to *S. zeamais* where 78.57 FDI was recorded. However, % FDI induced by 6-10% *M. cajuputi* oil on *S. zeamais* was not significantly differed. Meanwhile, % FDI induced by 4% concentration was significant lower from 8-10% but not significantly differed from 6% concentration. The 54% mortality was detected in treatment with 4-6% concentrations, while 70% mortality was found at 8-10% concentrations. However, there were no significant different on % mortality of *S. zeamais* in all treatments (Table 31).

Melaleuca cajuputi oil exhibited antifeedant to *T. castaneum* approximately at the same rate as in *S. zeamais* (Table 32). The highest concentration showed the highest antifeedant to *T. castaneum* where 76.32% FDI was detected. The % FDI induced by 4 and 6% concentrations was not statistically different, but was statistically lower than 76.32% induced by 10% concentration. *Melaleuca cajuputi* oil exhibited very low antifeedant toxicity to *S. zeamais* as only 5% mortality was occurred at the highest concentration rate, whereas no mortality was detected at all lower concentrations. In contrast, this oil was toxic to *T. castaneum* as 54-70% mortality rate were recorded at 4-10% concentrations.

When the antifeedant of *M. cajuputi* to *S. zeamais* and *T. castaneum* were compared, 52.38% FDI and 50% FDI were detected at 4% concentration, respectively. Meanwhile, 71.43-78.57% FDI for *S. zeamais* and 60.53-76.32% FDI for *T. castaneum* were recorded when 6-10% *M. cajuputi* oil were applied. No significant different was occurred between %FDI of *S. zeamais* and *T. castaneum* at 6-10% and 8-10% concentrations respectively (Tables 31 and 32). In conclusion, *M. cajuputi* oil at 6-10% showed strongly feeding deterrence to *S. zeamais* while 10% concentration was required to induce strongly feeding

deterrence to *T. castaneum*. Moreover, this particular oil also exhibited strong oral toxicity to *S. zeamais* since 70% mortality was observed when the insects were treated with 8% oil.

Antifeedant toxicity of *L. cubeba* to *S. zeamais* and *T. castaneum*

Antifeedant toxicity of *L. cubeba* as described by % FDI and % mortality was shown in Table 33. Only 34.83% FDI was occurred at the highest concentration rate for *S. zeamais*. The % FDIs of *S. zeamais* treated with 6-10% *L. cubeba* oil were not significantly different, however, those insects fed with flour disc containing 4% oil showed considerably low antifeedant (FDI = 6.74%). In addition, only 10-24% mortality was detected in all concentration rates.

Litsea cubeba oil evoked antifeedant to *T. castaneum* (Table 34). The result revealed that *L. cubeba* deterred feeding of *T. castaneum* by 30-77% at 4-10% concentrations. The % FDIs significantly increased with the concentrations. However, no significant different in % FDI values when the oil was applied at the highest and the second highest concentrations. Moreover, there was no statistically different in % FDIs when *T. castaneum* was treated with 4% and 6% *L. cubeba* oil. In addition, 10% *L. cubeba* oil induced 24 and 12% mortality of *S. zeamais* and *T. castaneum* respectively. Hence, it can be concluded that *L. cubeba* was able to strongly deter feeding of *T. castaneum* (% FDI= 76.92) more than S. *zeamais* (% FDI =34.83%), but causing more mortality of *S. zeamais* (24%) than *T. castaneum* (12%) at the highest application rate. When the % FDIs of all application rates were compared to *T. castaneum*, % FDI induced by 8% was statistically differed from those of 4% and 6%, but not statistically different from 10%. Only 12% mortality of *T. castaneum* could be detected at 10% (Table 34). When the % FDI values of all application rates of *S. zeamais* were compared, the % FDI caused by 6%, 8% and 10% oil were statistically different from that of 4%. In addition, 24% mortality could be observed at the highest concentration rate (Table 33)

Antifeedant toxicity of *L. salicifolia* to *S. zeamais* and *T. castaneum*

Antifeedant toxicity of *L. salicifolia* against *S. zeamais* was shown in Table 35. *Litsea salicifolia* oil showed no or weak feeding deterrence against *S. zeamais*, since only 29.63 % FDI was detected at the highest concentration rates (10%). Moreover, it was relatively no oral toxic to *S. zeamais* according to no mortality was detected when the insects were treated with 4% concentration and only 2% mortality was found at 6-8% concentrations and finally 4% mortality was observed at the highest concentration rate.

Litsea salicifolia oil showed weaking feeding deterrence to *T. castaneum* when applied at 4% concentration. The result clearly revealed that more than 50% of flour disc were not fed by *T. castaneum* at the lowest concentration rate (FDI =53.85%). The % FDI values increased considerably with concentrations where 84.62% FDI was recorded at the highest dose. In addition, there was not statistically different among the % FDI values when treated with all concentrations except 4%. Moreover, only 4-10% mortality was observed in all concentration rates (Table 36) which indicated that this oil had relatively low oral toxicity to this insect.

Kongkathip *et al*. (2004) showed the antifeedant toxicity (FDI = 89%) of 5% and 10% hexane crude extracts of *Melaleuca leucadendron* L. against *Spodoptera litura* (F.) larvae in choice bioassay. Huang *et al*. (1997) demonstrated the antifeedant toxicity of 84 g/100 ml of nutmeg oil on *S. zeamais* (FDI = 62.3%) and *T. castaneum* (FDI = 33.4%). In addition, Huang and Ho (1998) also showed the antifeedant activity of of methylene chloride extracts of *C. aromaticum* to *S. zeamais* adult (FDI = 34.87%) and 54.5 mg/g food of that extracts deterred *T. castaneum* larvae with 54.24% FDI. Liu and Ho (1999) stated that 4.49 mg/disc of *E. rutaecarpa* had low feeding deterrence to *S. zeamais* adult (FDI = 20.46%) and *T. castaneum* larvae (FDI =34.56%). Huang *et al*. (2000b) also showed the antifeedant activity of 1.44 x 10^4 ppm of *E. cardamomum* to *S. zeamais* adult (FDI

= 26.9%) and *T. castaneum* larvae (FDI =18.2%). Liu *et al*. (2007) reported that 30 Chinese medicinal herbs had feeding deterrence to these two species, among which hexane and methanol leaf extracts of *L. cubeba* exhibited 50-70% FDI to both insect species.

According to the result, *L. salicifolia* oil showed more feeding deterrence to *T. castaneum* than *S. zeamais* adults which was in agreement with Liu and Ho (1999) who showed that 4.49 mg/disc of *E. rutaecarpa* showed more feeding deterrence to *T. castaneum* (34.56%) than *S. zeamais* (20.46%).

Table 31 Antifeedant toxicity of *Melaleuca cajuputi* Powell leaf essential oil against *Sitophilus zeamais* Motschulsky.

Concentration (%)	Mortality (%)*	FDI (%)*
4	54 a	52.38 b
6	54 a	71.43 ab
8	70 a	76.19 a
10	70 a	78.57 a

*Values were means of five replicates of 10 insects in each replication over 3 d duration.
Means in the same column with the same letters do not differ significantly ($P > 0.05$) as determined by one way ANOVA and Lsd.

Table 32 Antifeedant toxicity of *Melaleuca cajuputi* Powell leaf essential oil against *Tribolium castaneum* (Herbst).

Concentration (%)	Mortality (%)*	FDI (%)*
4	0 b	50.00 c
6	0 b	60.53 bc
8	0 b	66.79 ab
10	5 a	76.32 a

*Values were means of five replicates of 10 insects in each replication over 3 d duration.
Means in the same column with the same letters do not differ significantly ($P > 0.05$) as determined by one way ANOVA and Lsd.

Table 33 Antifeedant toxicity of essential oil extracted from mature fruits of *Litsea cubeba* (Lour.) Persoon against *Sitophilus zeamais* Motschulsky.

Concentration (%)	Mortality (%)*	FDI (%)*
4	10 ab	6.74 b
6	18 a	20.22 ab
8	22 a	30.34 a
10	24 a	34.83 a

*Values were means of five replicates of 10 insects in each replication over 3 d duration.
Means in the same column with the same letters do not differ significantly ($P > 0.05$) as determined by one way ANOVA and Lsd.

Table 34 Antifeedant toxicity of essential oil extracted from mature fruits of *Litsea cubeba* (Lour.) Persoon against *Tribolium castaneum* (Herbst).

Concentration (%)	Mortality (%)*	FDI (%)*
4	2 b	30.77 c
6	6 ab	46.15 bc
8	8 ab	61.54 ab
10	12 a	76.92 a

*Values were means of five replicates of 10 insects in each replication over 3 d duration.
Means in the same column with the same letters do not differ significantly ($P > 0.05$) as determined by one way ANOVA and Lsd.

Table 35 Antifeedant toxicity of essential oil extracted from mature fruits of *Litsea salicifolia* Roxb. ex Wall**.** against *Sitophilus zeamais* Motschulsk*y.*

Concentration (%)	Mortality (%)*	FDI (%)*
4	0 a	6.17 c
6	2 a	7.41 c
8	2 a	19.75 b
10	4 a	29.63 a

*Values were means of five replicates of 10 insects in each replication over 3 d duration.
Means in the same column with the same letters do not differ significantly ($P > 0.05$) as determined by one way ANOVA and Lsd.

Table 36 Antifeedant toxicity of essential oil extracted from mature fruits of *Litsea salicifolia* Roxb. ex Wall**.** against *Tribolium castaneum* (Herbst).

Concentration (%)	Mortality (%)*	FDI (%)*
4	4 a	53.85 b
6	8 a	69.23 ab
8	10 a	76.92 ab
10	10 a	84.62 a

*Values were means of five replicates of 10 insects in each replication over 3 d duration.
Means in the same column with the same letters do not differ significantly ($P > 0.05$) as determined by one way ANOVA and Lsd.

CONCLUSION

The essential oils extracted from the leaves of *M. cajuputi*, the mature fruits of *L. cubeba* and *L. salicifolia* could be used as repellents, fumigant, contact poison and feeding deterrence against *S. zeamais* and *T. castaneum*. Among the tested oils, *L. cubeba* and *L. salicifolia* oils show the highest repellency effect on *S. zeamais* and *T. castaneum* compared to *M. cajuputi* oil. Meanwhile, *L. salicifolia* oil possesses the highest fumigant effect on *S. zeamais*, followed by *L. cubeba* and *M. cajuputi.* However, *M. cajuputi* evokes the highest fumigant effect on *T. castaneum*, followed by *L. cubeba* and *L. salicifolia*. It was noted that although *L. salicifolia* exhibits the highest fumigant toxicity to *S. zeamais*, its fumigant effect on *T. castaneum* is the least among three essential oils.

Moreover, *M. cajuputi* oil evokes its highest contact toxicity and *L. salicifolia* oil shows second best contact toxicity while *L. cubeba* oil exhibits the least contact toxicity on *S. zeamais*. In addition, *M. cajuputi* and *L. salicifolia* oils possess moderate contact toxicity against *T. castaneum* whereas *L. cubeba* has considerably less contact toxicity to this species. When the antifeedant toxicity of three essential oils is compared, *M. cajuputi* shows the highest antifeedant effect on *S. zeamais*. Although the antifeedant effect of *L. cubeba* oil on *S. zeamais* is not so good as *M. cajuputi* oil, this essential oil shows better antifeedant effect than the *L. salicifolia* oil. In contrast, *L. salicifolia* oils show the higher antifeedant effect on *T. castaneum* than *L. cubeba* and *M. cajuputi* oils. Generally, these three essential oils show more repellent, fumigant, contact and antifeedant toxicity on *S. zeamais* than *T. castaneum*.

This research provides a scientific basis on applying phytochemicals from the tested plant species against stored-product pests. Moreover, essential oils may be exploited against insect infestation at small scale farmer's level as they may be more effective and less cumbersome than the application of dried foliage. Essential oils are not particularly dangerous to consumers since they are commonly used in many pharmaceutical preparations and easily evaporated during cooking of the

foodstuff. However, the essential oils of these tested plant species had strong odor, as did many other essential oils. Further studies should be done for the bioactivity of these plant species and their constituents against other stored-product insects before considering commercial application.

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