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TITLE: Insecticidal Activities of Essential Oils from Three Thai Plants (Zingiberaceae) and Their Major Compounds Against *Sitophilus zeamais* Motschulsky, *Tribolium castaneum* (Herbst) and Two Parasitiods

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THESIS

INSECTICIDAL ACTIVITIES OF ESSENTIAL OILS FROM THREE THAI PLANTS (ZINGIBERACEAE) AND THEIR MAJOR COMPOUNDS AGAINST *SITOPHILUS ZEAMAIS* MOTSCHULSKY, *TRIBOLIUM CASTANEUM* (HERBST) AND TWO PARASITIODS

DUANGSAMORN SUTHISUT

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The essential oils from rhizomes of *Alpinia conchigera*, *Zingiber zerumbet*, *Curcuma zedoaria*; their major compounds (camphene, camphor, 1,8-cineole, α-humulene, isoborneol, α-pinene, β-pinene and terpinen-4-ol) and synthetic essential oils were evaluated under laboratory conditions. In fumigation bioassay, *A. conchigera* oils were toxic to *Sitophilus zeamais*, *Tribolium castaneum* and *Trichogramma deion*. *Zingiber zerumbet* oils (LD_{50}: 26 µL/L in air) and *C. zedoaria* oils (25 µL/L in air) were significantly more toxic to adults of *Anisopteromalus calandrae* than *A. conchigera* oils (37 µL/L in air). *Sitophilus zeamais* and *T. castaneum* adults were more susceptible to *A. conchigera* oils than their eggs, larvae or pupae. Synthetic essential oils were more toxic than the extracted essential oils to *S. zeamais* and *T. castaneum*. *Tribolium castaneum* was more susceptible than *S. zeamais* to the eight pure compounds. Terpinen-4-ol was highly toxic to both insects.

In contact bioassay, *S. zeamais* was more sensitive to *C. zedoaria* oils (LD_{50}: 18 µL/L) than *Z. zerumbet* (21 µL/L) and *A. conchigera* oils (24 µL/L), respectively. The LD_{50} values of synthetic *A. conchigera* and synthetic *Z. zerumbet* oils were similar to those of the extracted essential oils. The synthetic *C. zedoaria* oils showed lower contact toxicity than the extracted *C. zedoaria* oils to both insects. *Sitophilus zeamais* and *T. castaneum* were sensitive to terpinen-4-ol and isoborneol. In feeding bioassay, the three extracted oils were able to decrease the consumption of flour disks, especially *Z. zerumbet* oils whereas, both insect species could feed on the flour disks treated with three synthetic essential oils. Only terpinen-4-ol deterred feeding of both insects. In repellency bioassay, *A. conchigera* oils at highest concentration repelled *S. zeamais* and *T. castaneum*. All synthetic essential oils could not repel *S. zeamais* and *T. castaneum* and only terpinen-4-ol showed repellent activity to both insect species.

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LIST OF ABBREVIATIONS

µg	=	microgram
µL	=	microliter
^{o}C	=	degree Celsius
ANOVA	=	Analysis of Variance
cm	=	centimeter
CT	=	concentration-time
d	=	day
Fig.	=	Figure
g	=	gram
GC	=	Gas Chromatography
h	=	hour
L	=	Liter
LC_{50}	=	Lethal Concentration 50%
LC_{90}	=	Lethal Concentration 90%
LD_{50}	=	Lethal Dose 50%
LD_{90}	=	Lethal Dose 90%
mg	=	milligram
mL	=	milliliter
mm	=	millimeter
MS	=	Mass Spectrometry
RH	=	relative humidity

INSECTICIDAL ACTIVITIES OF ESSENTIAL OILS FROM THREE THAI PLANTS (ZINGIBERACEAE) AND THEIR MAJOR COMPOUNDS AGAINST *SITOPHILUS ZEAMAIS* MOTSCHULSKY, *TRIBOLIUM CASTANEUM* (HERBST) AND TWO PARASITIODS

INTRODUCTION

Thailand is located in the tropics. Therefore, stored commodities are infested with several insects and mites because of the high temperature and humidity. Infestation by these pests results loss in nutrition and considerable physical damage. Two of the most serious pests of stored products in Thailand and the world are *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst) (Hayashi *et al*., 2004; Rees, 2004).

Sitophilus zeamais feeds on all cereal grains such as wheat, rice and other small grains, and it can cause 90% damaged to grain after 5 months of storage (Nukenine *et al*., 2002). *Tribolium castaneum* causes damage to stored grain and processed products by reducing dry weight and nutritional value (Rees, 2004). Generally, *T. castaneum* feeds on grain previously damage by other pests or broken kernels, or processed cereal products. Larvae have a preference for germ of seeds and feed on many stored products including cereals, cereal products, nuts, coffee, cocoa, dried fruits and museum specimens (Via, 1999; Weston and Rattlingourd, 2000). Therefore, these two serious pests can cause heavy losses of both quantity and quality of stored products (Rees, 2004).

Control of these insect populations depends in large part on the application of chemical insecticides, either as residual insecticides or as fumigants (Subramanyam and Hagstrum, 1996). The most commonly used fumigants are methyl bromide, phosphine and sulfuryl fluoride. Fumigants have a broad spectrum of activity, penetrate deep into the grain and leave few residues (Mueller, 1990; Emekci, 2010).

However, fumigation has some drawbacks; such as no long-term protection, dangerous to apply, insect become resistant insect populations and damage to the environment (Bell and Wilson, 1995; Emekci, 2010). In addition, various groups of insecticides such as organochlorines (lindane), organophosphates (malathion), carbamates (carbaryl) and pyrethriods (deltamethrin) are currently in use or have been used as residual insecticides to control stored-product insects. These insecticides are applied to the empty granaries and warehouses, to bags or directly to grain (Snelson, 1987; White and Leesch 1996). Malathion has been used extensively to control stored-product insects, but there is now wide-spread resistance around the world to this insecticide and other insecticides in stored-product insect populations (Subramanyam and Hagstrum, 1996; Perez-Mendoza, 1999). In general, parasitoids are more susceptible to contact insecticides than pest insects (Schöller and Flinn, 2000), although there are a few cases where a parasitoid has developed resistance to an insecticide (Baker *et al*., 1998). Therefore, alternatives to chemical insecticides are needed to control pests in stored products.

Research with secondary metabolites from plant products is another option for stored-product protection. Essential oils are secondary metabolites that plants produce for their own needs other than for nutrition. Researchers have been concentrated on the search for essential oils derived from plants as an alternative to conventional insecticides for insect control. The essential oils compose of bioactive chemicals which are low toxicity to warm-blooded animals, high volatility, and toxic to stored-grain insect pests (Shaaya *et al*., 1991, 1997; Regnault-Roger *et al*., 1993). Furthermore, several essential oils possess antiparasitical, bactericidal, fungicidal, virucidal and insecticidal properties (Bakkali *et al*., 2008; Rajendran and Sriranjini, 2008).

The essential oils are rich in monoterpenes and cause death of insects by inhibiting acetylcholinesterase activity on nervous system (Houghton *et al*., 2006). Essential oils of many plant species exhibit insecticidal activities to stored-product insects (Huang *et al*., 2000; Negahban and Moharramipour, 2007; Rajendran and Sriranjini, 2008). These bioactive compounds can control stored-product pests in

many ways, such as repellency (Talukder and Howse, 1995; Fields *et al*., 2001; Liu *et al*., 2006), contact toxicity (Shaaya *et al*., 1997; Kim *et al*., 2003b) fumigant (Lee, 2002; Lee *et al*., 2003; Rajendran and Sriranjini, 2008) and antifeedant (Huang *et al*., 1997; Huang and Ho, 1988; Hough-Goldstein, 1990; Isman *et al*., 1990). This method can reduce environmental contamination and reduce the risk to humans (Grainge and Ahmed, 1988).

Some researchers found that certain plants in the family Zingiberaceae produce secondary metabolites which are toxic to stored-product insects (Stoll, 2000; Ukeh *et al*., 2009) for example; *Alpinia* spp, *Zingiber officinale* Rosoe and *Curcuma domestica* Val. (Wattanasombat, 1995; Stoll, 2000). Therefore, three Thai plants: *Alpnia conchigera* Griff, *Zingiber zerumbet* Smitt and *Curcuma zedoaria* (Berg.) Roscoe, were investigated for their toxicities against *S. zeamais* and *T. castaneum* as fumigant, contact insecticide, antifeedant and repellent. Furthermore, pesticides either synthetic or natural in origin may be toxic to parasitoids of stored-product insects (Hou *et al*., 2004). Hence, the fumigation toxicity effects of essential oils on *Anisopteromalus calandrae* (Howard) and *Trichogramma deion* Pinto & Oatman were also examined. In addition, the insecticidal activity of major pure compounds and mixture of the pure compounds in the same proportions as the extracted natural essential oils was also determined with *S. zeamais* and *T. castaneum*.

OBJECTIVES

1. To determine major compounds and chemical constituents of essential oils from *Alpinia conchigera*, *Zingiber zerembet* and *Curcuma zedoaria.*

2. To investigate the efficiency of the natural essential oils from *Alpinia conchigera*, *Zingiber zerembet* and *Curcuma zedoaria* against *Sitophilus zeamais* and *Tribolium castaneum* and parasitiods.

3. To determine the insecticidal activities of major pure compounds and synthetic essential oils against *Sitophilus zeamais* and *Tribolium castaneum*.

LITERATURE REVIEW

1. Importance of stored-product pests

Cereal grains, oilseeds and legumes are a major food for humans and feed for animals. Normally, these crops are stored in the warehouse for long periods and the loss in both quantity and quality can occur during storage (Madrid *et al*., 1990; Rajendran, 2002). The stored grains are attacked by insects, mites, rodents, birds and microorganisms (Rees, 2004). Generally, feeding by insects causes lower germination and reduced visual appeal (Snelson, 1987). Losses due to insect infestations are under 5% in developed countries, but can be above 30% in developing countries and in some cases crop are lost (Rees, 2004). Several species of insects and mites are associated with stored grain and human living for a long time. The insect orders of pests associated with stored products are Coleoptera, Lepidoptera and Psocoptera (Rees, 2004). Among these pests, Coleopteran pests are the most abundant, with over 600 species reported (Rajendran, 2002). *Sitophilus zeamais* and *T. castaneum* are considered serious pests of stored products in Thailand (Hayashi *et al*., 2004) and throughout the world (Rees, 2004).

1.1 *Sitophilus zeamais* (Coleloptera: Curculionidae)

Sitophilus zeamais (maize weevil) is a primary pest that feeds directly on stored cereals, especially rice and maize. This species can be secondary pest of sorghum, rice and other stored grains produced in the tropical and subtropical areas as well as in southern Europe (Stoll, 2000).

1.1.1 Biology

Sitophilus zeamais start to mate after emergence 3 days (Walgenbach and Burkholder, 1987). The female inserts her eggs individually into each grain, up to 150-300 eggs are laid during her lifespan (about 4-12 mouths). The eggs are laid at temperature between 15-35 ^{o}C and moisture content above 19% RH (Howe, 1952).

The eggs hatch within 6 days. The white, legless larvae feed inside the grain and develops within the grain cavity. The larva completes its development in 25 days at 25 ^{o}C (Hayashi *et al*., 2004).

Larvae are not able to migrate between grains (Danho *et al*., 2002). Smith and Lessells (1985) concluded that *Sitophilus* female attempts to reduce competition faced by its offspring by killing eggs already present, also larvae within a grain can kill other larvae, which increase their fitness. However, 1-5 adult weevils can emerge from a single maize kernel (Adams, 1976). The pupal stage lasts 3-7 days, within the cavity made by the larva.

Adults have circular-shaped puncture on pronotum and the body length is 3.5-4.5 mm (Rees, 2004). *Sitophilus zeamais* males have shorter and thicker snouts than those of female (Dobie *et al*., 1984). The life cycle under favorable condition can be completed within 35 days (Hayashi *et al.*, 2004). Danho *et al.* (2002) reported that the mean weight of the emerged adults is not significantly influenced by grain quantity, but females are heavier than males.

1.1.2 Damage

Sitophilus zeamais is a serious pest of maize and rice. Direct damage is caused by its feeding on the cereal grain (Hayashi *et al*., 2004; Rees, 2004). This insect destroys maize by boring small tunnels to the center of the maize. The round exit holes on the grain surface are quite characteristic and specific for *Sitophilus* spp. (Stoll, 2000). *Sitophilus zeamais* infests all cereal grains such as wheat, rice, and other small grains. Adults can fly long distances, at least 402 meter (Giles, 1969). Unlike most stored-product insect pests, *S. zeamais* is capable of infesting a crop while it is still in the field. Taylor (1971) found that flight periodicity curve of *S. zeamais* in the field was bell-sheped and very little flight occurred during the night, whereas the flight periodicity curve in warehouse was flat-topped and flight activity was prolonged far into the night. Therefore, the flight activities depend on the temperature. For this reason, *S. zeamais* population can rapidly increase and

distribute thoughout a storage facility, creating up to 90% damage after 5 months of storage (Nukenine *et al*., 2002).

1.1.3 Natural enemies

The immature stage of *S. zeamais* is attacked by some ectoparasites such as *A. calandrae, Theocolax elegans* (Westwood) and *Lariophagus distinguendus* (Förster) (Hayashi *et al*., 2004; Rees, 2004) whereas the parasites of adults are unknown.

1.2 *Tribolium castaneum* (Coleoptera: Tenebrionidae)

Tribolium castaneum (red flour beetle) is the major pest of stored product worldwide, especially in grain stored and mills. However, it is commonly found in the tropics to warm temperate regions (Rees, 2004). Dennis (1983) reported that this species is a serious secondary pest in food stored because they cannot feed on undamaged, dry seed with less than 12% moisture content, and limit its attack to grain dust, broken grain and milled stocks. This particular species is very tolerant to low humidity (Rees, 2004).

1.2.1 Biology

Female of *T. castaneum* lays their eggs randomly amongst the commodity. The eggs are small, generally kidney-shaped and white to cream color. Females of this species can lay up to 1,000 eggs (2-10 eggs/day) over their lifetime (Rees, 2004). The larvae are yellowish-white in color and move through the food. Both larvae and adult are cannibalism. They will also prey on eggs, young larvae and pupae of other stored-product insects. The last larval instar is about 10 mm long. The larval stage lasts about 15 days. The pupal stage requires 3.7 days on average (Hayashi *et al*., 2004). Normally, they are surrounded by the food. The adult is flatten, elongate and reddish-brown in color, 3-4 mm long (Rees, 2004). The adults are long lived, under some circumstances live for a year or more, and they are strong

fliers especially in the afternoon. Furthermore, adults can survive up to 35 days without food (Daglish, 2006). When larvae and adult infest the commodity, they can lead to persistent disagreeable odors in the goods caused by the secretion of benzoquinones from the abdominal gland. The life cycle is completed within about 35 days at 30 ^{o}C (Rees, 2004).

1.2.2 Damage

Tribolium castaneum damages to stored grain and processed products by reducing dry weight and nutritional value (Rees, 2004). It is considered the most important pest of stored products in cereal processing factories, grocery stores and homes. They destroy many stored product including cereals, cereal products, nuts, coffee, cocoa and dried fruits (Via 1999; Weston and Rattlingourd, 2000). Generally, *T. castaneum* destroys the grains previously damaged by other pests. Larvae prefer the germ of grains, while adults feed on surface of the grain. The grains with high moisture content are preferred. The beetles release a displeasing odor, and their presence encourages mold growth in grain. Moreover, feeding by *T. castaneum* also causes a grey tint to grain.

1.2.3 Natural enemies

Larvae of *T. castaneum* are parasitizied by *Proconura caryobori* (Hanna) and *Holepyris sylvanidis* (Brethes) while *Xylocoris flavipes* (Reuter), *Amphibolus venator* (Klug) and *Peregrinator biannulipes* (Montrouzier and Signoret) are recorded as valuable predators of *T. castaneum* larvae and adults (Sokoloff, 1974; Hayashi *et al*., 2004; Rees, 2004).

2. Using parasitoids for stored-product insect control

Several solitary ectoparasitoids are reported as biological agent to control stored-product insects. These are *A. calandrae* (Lucas and Riudavets, 2002; Hansen and Steenberg, 2007), *Habrobracon hebetor* Say (Ghimire and Phillips, 2010; Mbata

and Shapiro-Ilan, 2010), *T. elegans* (Flinn and Hagstrum, 2002; Flinn *et al*., 2006) and *Trichogramma* sp. (Steidle *et al*., 2001; Hayashi *et al*. 2004; Rees 2004; Grieshop *et al*., 2006). These parasitoids have been found associated with insect pests in stored grain. In Thailand, a total of 21 species of parasitoids were recorded during the survey of insects and their parasitoids (Hayashi *et al*., 2004).

The parasitic wasps attack the immature stages of *Sitophilus* and moths (Rees, 2004). For example, *A. calandrae* parasitized both larval and pupal stages of several stored-product insects. The hosts of *A. calandrae* consist of *Sitophilus oryzae* (L.) (Lucas and Riudavets, 2002*Sitophilus granarius* (L.) (Ghani and Sweetman, 1955), *S. zeamais* (Wen and Brower, 1994), *Rhyzopertha dominica* (F.) (Ahmed, 1996). *Trichogramma* sp. parasitizes eggs of moth such as *Plodia interpunctella* (Hübner) (Grieshop *et al*., 2006), *Ephestia kuehniella* Zeller and *Ephestia cautella* (Walker) (Steidle *et al*., 2001). Both parasitoids have been used commercially to control stored-product insects (Ahmed, 1996; Jalali *et al*., 2007)

Parasitoids are normally found near the windows or the lights or on the surface of grain where the hosts are founded (Rees, 2004). Hence, chemical application would certainly affect parasitoids when the insecticide is used to control insect pests. Parasitoids are often more susceptible to insecticides than their hosts (Schöller and Flinn, 2000). For example, Baker and Weaver (1993) showed that *A. calandrae* is sensitive to chlorpyrifos-methyl, pirimipos-methyl and malathion. On the other hand, Hou *et al*. (2004) indicated that protein rich pea flour is not toxic and did not reduce the offspring of *A. calandrae*, which is a parasitoid of *S. oryzae* and *Cephalonomia waterstoni* (Gahan), a parasitoid of *Cryptolestes ferrugineus* (Stephens).

3. Chemical control of stored-product insects

Sitophilus zeamais and *T. castaneum* are key pests of stored-product insects. Control of these insect populations in stored food, feed stuffs and other agriculture commodities around the world is primarily depended upon the application of residual organophosphorus and pyrethroid insecticides and the fumigants such as methyl

bromide and phosphine (Subramanyam and Hagstrum, 1996). Insecticide application has been widely used to protect grains from insect infestation. However alternatives to synthetic insecticide are sought because of the health hazards, risks of environmental contamination, increasing costs of application, pest resurgence and insect resistance to pesticides (Shaaya *et al*., 1977; Mondal and Khalequzzaman, 2006).

The 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 their easy penetration into the commodity while leaving minimal residues (Mueller, 1990; van Someren Graver, 2004). Currently, phosphine and methyl bromide are the products most widely used (Bond, 1984; Fields and White, 2002; Lee *et al*., 2004; Emekci, 2010). Carbon dioxide and sulfuryl fluoride are also registered for fumigation of stored grain in several countries.

However, the fumigation method also causes some problems such as chemical residues in the environment, pest resistance and lethal effects to non-target organisms in addition to direct toxicity to users. According to the April 2000 decision of Montreal Protocol, methyl bromide was phased out in the 2005 in developed countries and will be phased out by 2015 in developing countries, since it was proven that this compound causes ozone depletion in the Stratosphere (TEAP, 2000; Fields and White, 2002). Furthermore, phosphine is not effective against some insect populations in India, Australia and Brazil, because of resistance (Bell and Wilson, 1995; Benhalima *et al*., 2004; Collins *et al*., 2005; Pimentel *et al*., 2009).

More than 504 species of insects and mites are resistant to insecticides and there is still a steady increase in resistance to specific chemicals, with many species now resistant to several groups of insecticides (Georghiou, 1990; Herron, 1990; Pimentel *et al.*, 2009). In addition, carbon dioxide requires high temperatures and high concentrations to control insect populations (Soderstrom *et al*., 1992). Sulfuryl fluoride is being used as a replacement for methyl bromide, but eggs require high doses or long durations to be effective (Kenaga, 1957; Baltaci *et al*., 2009). However,

for small subsistent farmers in developing countries these fumigants are not available or too costly to use.

4. Plant secondary metabolites for controlling stored-product insects

Secondary metabolites, also known as natural products, are chemical compounds produced by metabolism process that are not essential for normal growth, development or reproduction of an organism (Schoonhoven *et al*., 2005). It has been estimated that more than 100,000 compounds are already known from numbers of secondary metabolites and new chemical structure from plant secondary metabolites are discovered all the time (Dewick, 2002). In addition, several plant secondary metabolites have been formulated as botanical pesticides for plant protection (Coats, 1994).

Plant secondary metabolites play an important role in insect interactions because they constitute a rich source of bioactive chemical compounds for controlling insects (Wink, 1993). In the past, natural products such as nicotine, rotenone and pyrethrin from plants have been used to control the insects. Both plant extracts and essential oils serve as alternative sources to control stored-product insects due to their low toxicity to warm-blooded mammals (Shaaya *et al*., 1991, 1997; Kim *et al*., 2003b).

Essential oils are secondary metabolites that plants produce for their own needs other than for nutrition. In general, they are the complex mixtures of organic compounds that give characteristic odor and flavor to the plant parts such as leaves, flowers, fruits, seeds, barks and rhizomes (Bakkali *et al*., 2008). Generally, essential oils have a broad spectrum of active ingredients that work through several modes of action against insects (Liu *et al*., 2006). The toxicity of essential oils to stored-product insects is influenced by the chemical composition of the oil, which in turn depends on the source, season and ecological conditions, method of extraction, time of extraction and plant part used (Lee *et al*., 2001).

To date, both crude extracts and essential oils of many plant species exhibit insecticidal activities against stored-product insects. Stoll (2000) revealed that crude extracts are toxic to egg, larva prior to or directly after entering a grain and to adults of stored-product pests. In recent years, several studies have showed the antifeedant potential of plant essential oils against post-harvest pests, aphids, thrips, moths, termites and mites pests (Hori, 1999; Koschier *et al*., 2002; Maistrello *et al*., 2003).

Plant volatile oils and crude extracts can control the adults of major stored pests such as the rice weevil, *S. oryzae* (Shaaya *et al*., 1997; Lee *et al*., 2003; Liu *et al*., 2006; Rajendran and Sriranjini, 2008); the maize weevil, *S. zeamais* (Huang *et al*., 1997, 2000; Liu and Ho, 1999; Bouda *et al*., 2001; Tapondjou *et al*., 2005; Rajendran and Sriranjini, 2008); the red flour beetle, *T. castaneum* (Huang *et al*., 1997, 2000; Liu and Ho, 1999; Lee *et al*., 2003; Wang *et al*., 2006; Rajendran and Sriranjini, 2008); the lesser grain borer, *R. dominica* (Shaaya *et al*., 1997; Rajendran and Sriranjini, 2008); the dried bean beetle, *Acanthoscelides obtectus* (Say) (Papachristos and Stamopoulos, 2002; Rajendran and Sriranjini, 2008); the cowpea beetle, *Callosobruchus maculatus* (F.) (Rajendran and Sriranjini, 2008); the adzuki bean weevil, *Callosobruchus chinensis* (L.) (Kim *et al*., 2003a; Salunke *et al*., 2005; Rajendran and Sriranjini, 2008) and the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Shaaya *et al*., 1997; Lee *et al*., 2003; Rajendran and Sriranjini, 2008). Immature stages of important stored-product insects such as *T. castaneum* (Huang *et al*., 1997, 2000; Wang *et al*., 2006; Rajendran and Sriranjini, 2008), *R. dominica* and *Sitophilus* spp. (Rajendran and Sriranjini, 2008) are also controlled by various secondary metabolites. The effectiveness of secondary metabolites depends on species, developmental stage and sex (Rajendran and Sriranjini, 2008).

Rajendran and Sriranjini (2008) reported that secondary metabolites from more than 75 plant species were evaluated for insect control. The efficacy of essential oils of several plants (mainly belong to Apiaceae, Lamiaceae, Lauraceae and Myrtaceae families) and their components (cyanohydrins, monoterpenoids, sulphur compounds, thiocyanates and others) are also tested for control beetle pests such as *T. castaneum*, *R. dominica*, *S. oryzae* and *S. zeamais*, but little attention has been paid

towards moths such as *Corcyra cephalonica* (Stainton) and *Sitotroga cerealella* (Oliv.) (Rajendran and Sriranjini, 2008).

Isman (2000) summarized that toxicity of secondary metabolites to insect often shows differential effects depending on the mode of action and insect species. The effects of monoterpenoids from essential oils on control stored-product insects have been extensively researched (Rajendran and Sriranjini, 2008). Such compounds have been considered as potential pest control agents because of their acute toxic and repellent (Watanabe *et al*., 1993), antifeedant (Hough-Goldstein, 1990) and fumigant activities (Lee *et al*., 2003). The monoterpenoids inhibit acetyl-cholinesterase enzyme (AChE) activity and causing death of insects (Houghton *et al*., 2006).

Neem oil is probably the most well known essential oil. It is extracted from *Azadirachta indica* A. Juss. (F. Meliaceae) and has been used for control of field and storage insects for hundred years (Pruthi, 1937). The major active ingredient with insecticidal activity is azadiractin, Neem oil also has a number of other chemical substances such as salannin and meliantriol, which have primarily repellent effect, and nimbin, which possess antiviral activity (Dennis, 1983). Generally, neem oil kills *S. zeamais* eggs which are laid on the seed coat, but it has no effect on the larvae, which develope inside the seed (Stoll, 2000). Neem oil is also effective in controlling the rice moth, *C. cephalonica* (Senguttuvan *et al.*, 1995).

Pyrethrum is another widely used botanical insecticide (Snelson, 1987). It is extracted from chrysanthemum (*Tanacetum cinerariaefolium* (Trevir.) Sch. Bip.: Asteraceae) flowers, and has been used to control stored-product insects since 1950. In addition to these two plants, there are hundreds of plants that are repellent or insecticidal to stored-product insectss (Golob *et al*., 1999). For many of these plants, the active compounds have been described (Shaaya, 1991; Coats, 1994; Negahban and Moharramipour, 2007; Cosimi, 2009; Kim *et al*., 2010; Zoubiri and Baaliouamer, 2010). For example, the essential oils from clove are highly toxic to *S. oryzae* and *R. dominica* (Sighamony *et al*., 1986). Huang *et al*. (1997) stated that the essential oil from nutmeg fruit (*Myristica fragrans* Houtt: Myristicaceae) can protect the seed

from *S. zeamais* and *T. castaneum* due to its contact, fumigation or antifeedant activities.

The major component of garlic oil is allyl disulfide. This chemical is well known to have repellent effects on *S. zeamais* adults. In addition, it acts as an antifeedant with *T. castaneum* adults, and less so against larvae or adult *S. zeamais* (Wan *et al*., 1999). Furthermore, garlic oils show higher contact toxicity against *T. castaneum* than *S. zeamais* adults (Ho *et al*., 1996).

Liu *et al*. (2006) reported that essential oils extracted from leaves of *Artemisia princeps* Pamp (Asteraceae) show repellent activity against *S. oryzae* and *Bruchus rugimanus* Bohem. In addition, Wang *et al*. (2006) found that the essential oil of *Artemisia vulgaris* L. had a very strong repellent activity at 600 µL/L (v/v) and high fumigant activity against larvae of *T. castaneum*, where as adults are much more susceptible than larvae. The adult mortality reached 100% at 800 µL/L, but only 49%, 53% and 52%, larval mortality was observed 12, 14 and 16-day post-treatment, respectively. Furthermore, Kordali *et al*. (2006) found that essential oils of three *Artemisia* species (*Artemisia absinthium* L., *Artemisia santonicum* L. and *Artemisia spicigera* C. Koch.) were toxic to adult of granary weevil, *S. granarius*. The oils caused 80–90% mortality of *S. granarius* at 9 µL/L air after 48 h of exposure. All pure compounds were toxic against *S. granaries*, where 1, 8-cineole and terpinen-4-ol were the most toxic, among the tested pure compounds. These major components caused 100% mortality at 0.5, 0.75 and 1µL/L air 12 h after exposure.

Huang and Ho (1998) found that *Cinnamomum aromaticum* Nees bark oil is toxic to *S. zeamais* and *T. castaneum*, while the essential oils of *Cinnamomum sieboldii* Meissn. root bark and *Cinnamomum cassia* (L.) Presl. bark were also highly effective against *S. oryzae*, *C. chinenesis* and *Lasioderma serricorne* (F.) (Kim *et al*., 2003a, b). In addition, seed extract of *C. camphora* (L.) Sieb. was reported against *S. oryzae* and *B. rugimanus* (Liu *et al*., 2006).

The essential oils of eucalyptus have a broad spectrum activity including anti-microbial, fungicidal, insecticidal, repellent, herbicidal, acaricidal and nematicidal (Batish *et al.*, 2008). Several species of eucalyptus were investigated for their biological activity. These are *Eucalyptus cinerea* F. Muell. ex Benth., *Eucalyptus camaldulensis* Dehnh, *Eucalyptus globutus* Labill., *Eucalyptus saligna* Sm. and *Eucalyptus viminalis* Hook. These *Eucalyptus* species have 1, 8-cineole as the major component (Yang *et al*., 2004; Tapondjou *et al*., 2005; Ceferino *et al*., 2006; Su *et al*., 2006; Lucia *et al*., 2007). *Eucalyptus globutus* was reported to show repellent toxicity, reduced fecundity and decreased egg hatchability of *A. obtectus* (Papachristos and Stamopoulos, 2002).

The 1, 8-cineole was also highly toxic to stored-product insects (Batish *et al*., 2008). In contrast, less mortality occurred when this compound was tested on *T. castaneum* (Rozman *et al*., 2007). Tapondjou *et al.* (2005) found that essential oils from *E. saligna* are repellent and toxic to *S. zeamais* and *T. confusum*. In addition, this oil can reduce the F1 progeny production and grain weight loss as well. Besides, Negahban and Moharramipour (2007) pointed out that essential oils extracted from *Eucalyptus intertexta* R.T. Baker, *Eucalyptus sargentii* Maiden and *E. camaldulensis* had potent fumigant toxicity against *C. maculatus, S. oryzae* and *T. castaneum*. Sahaf *et al*. (2007) studied the fumigant toxicity of *Carum copticum* C. B. Clarke (Apiaceae) essential oil against *S. oryzae* and *T. castaneum* adults and observed that *S. oryzae* was significantly susceptible to the oils (LC_{50}: 0.9 μL/L) than *T. castaneum* (LC_{50}: 33.1 μL/L). The mortality of both insects reached 100% at concentration higher than 185.2 μL/L, 12 h after exposure. The findings indicate strong insecticidal activity of *C. copticum* oil and its potential role as a fumigant for control stored-product insects.

5. The efficiency of secondary metabolites from Zingiberaceae to control the insects

Thailand has a long and rich tradition of using plants for pest control (Chuwit and Pracha, 1995; Wongtong and Nawanich, 2001). Zingiberaceae or wild ginger consists of several plants that are used for food, medicinal, perfumes, antibacterial and

antifungal purposes (Jantan *et al*., 2003; Tushar *et al*., 2010). For example, *A. conchigera* or lesser alpinia, commonly known as "Kha Ling" in Thailand, is found throughout Southeast Asia (Pongpiriyadacha *et al*., 2008). Secondary metabolites from *Alpinia* spp. are toxic to diamondback moth, *Plutella xylostella* (L.) (Wattanasombat, 1995) and tropical cattle tick, *Boophilus microplus* (Canestrini) (Chungsamarnyart *et al*., 1991).

Zingiber zerumbet or wild ginger is native to southern Thailand and has been widely cultivated in tropical and subtropical areas. Secondary metabolites from the ginger family are toxic to stored-product pests and other insects. For example, ginger extracts from *Zingiber officinale* Rosoe are toxic to *C. chinensis* (Owolabi *et al*., 2009), and are repellent to *S. zeamais* (Ukeh *et al*., 2009). In addition, an alcohol extract of *Z. zerumbet* rhizomes was toxic to larvae and pupae of mosquito (Tewtrakul *et al*., 1998).

Several plants from the *Curcuma* genus possess insecticidal activity. Turmeric oil from *C. domestica* is also used for control many stored-product pests such as *C. maculatus*, *C. chinensis*, *R. dominica*, *Tribolium* spp., *S. granarius* and *S. oryzae* (Stoll, 2000).

MATERIALS AND METHODS

1. Insect culture

Sitophilus zeamais (Fig. 1) and *T. castaneum* (Fig. 2) were obtained from Department of Agriculture, Ministry of Agriculture and Co-operatives, Thailand. *Sitophilus zeamais* was reared on wheat (hard red spring, 14% moisture content) and *T. castaneum* was cultured on wheat flour mixed with 5% brewers' yeast (ICN Biomedicals, Inc., Aurora, Ohio, USA).

Anisopteromalus calandrae and *T. deion* were obtained from Cereal Research Centre, Agriculture and Agri-Food, Winnipeg, Manitoba, Canada. *Anisopteromalus calandrae* was reared on thrid instar larva of *S. zeamais* fed on wheat and *T. deion* was reared on sterile eggs of *E. cautella*. All colonies and all experiments were kept in incubator at 30$\pm$1 ^{0}C and 70$\pm$10 % RH in complete darkness.

2. Insect preparation for bioassay studies

Eggs (0-24 h-old), larvae (14 d-old), pupae (25 d-old) or unsexed adults (0-14 d-old) of *S. zeamais* were prepared in fumigation experiment while only adult (0-14 d-old) were used in contact, feeding and repellent experiments (Fig. 1). A total of 6,600 *S. zeamais* adults were released on 1,100 g wheat for 24 h to obtain infested wheat kernels. All adults were sieved out and grains were gently mixed before 10 g of grain was placed into each glass vial (27 mL). Larval and pupal stages of *S. zeamais* were prepared as above, and glass vials containing infested grains were kept for 14 and 25 days before use.

Eggs (0-24 h-old), larvae (12 d-old), pupae (21 d-old) or unsexed adults (0-14 d-old) of *T. castaneum* were used in fumigation experiments while only adult (0-14 d-old) were used in contact antifeedant and repellent experiments (Figs. 2-3). A total of 500 adults were held for 24 h on 250 g flour that had been previously sieved through a 150 µm aperture sieve. Eggs were collected by sieving the flour with 180 µm

aperture sieve. Larvae and pupae were sieved out after 12 and 21 d using a 425 µm aperture sieve, respectively.

Anisopteromalus calandrae (Fig. 4A) was reared on immature stages of *S. zeamais* in wheat and 0-3 day-old adults were used in fumigation experiment. *Trichogramma deion* (Fig. 4B) was reared on *E. cautella* eggs. Immature stages of *T. deion* were prepared by sprinkling 68-100 *E. cautella* eggs on a cardboard (1.5 x 1.5 cm) with fresh mucilage glue (Reeves and Poole Group, Toronto, Canada). *Trichogramma deion* adults were allowed to parasitize the moth eggs for 24 h. All cards were removed from the vials and kept for 48 h before starting the fumigation experiment.

3. Extracted essential oils

Rhizomes of *A. conchigera* (Fig. 5) were collected from Nakhon Si Thammarat province, whereas *Z. zerumbet* (Fig. 6) and *C. zedoaria* (Fig. 7) were collected from Phang-Nga province, Thailand during 2008-2009. The voucher specimens (BK 64163, BK 64164 and BK 64166 respectively) were deposited at the Bangkok Herbarium, Botanical Research Unit, Department of Agriculture, Bangkok, Thailand. The fresh rhizomes were machine-cut into small pieces (Fig. 8A) before placing in a flask and sterile water was added at the ratio of 1:3 (weight/volume). The essential oils were extracted by water-distillation using a Clevenger-type apparatus for 8 h (Fig. 8B) and anhydrous sodium sulphate was used to remove water after extraction (Dharmagadda *et al*., 2005). All samples were stored in a refrigerator at 8-10^{o}C for future study.

Chemical compounds of the essential oils were determined using GC-MS (Shimadzu QP 5050A) equipped with a DB-5 capillary column (60 m, 0.25 mm, 0.25 µm film thickness) (J&W Scientific). The column temperature was programmed at 60°C for 3 min, then increased at 4°C/min until the final temperature of 220°C was reached, where it was held for 10 min. The injector and detector temperatures were 250°C, using helium as the carrier gas, at a flow rate of 1.2 mL/min. The injection

volume was 1 µL with a split ratio of 1:7. GC retention time and their mass spectra that presented in MS library were used for identify the essential oil components (Fig. 9).

4. Pure compounds and synthetic essential oils

Eight major compounds found in the three extracted essential oils were purchased for bioassay study (Table 1). Camphene, camphor, 1,8-cineole, isoborneol, α-pinene, β-pinene and terpinen-4-ol were purchased from Sigma-Aldrich Canada Ltd. (Toronto, Canada), while α-humulene was purchased from MP Biomeicals (Solon, Ohio, USA). Camphene, camphor and isoborneol were solid at room temperature, and were dissolved with ethanol to prepare the 20% stock solution.

The synthetic essential oils were prepared by combining the major compounds in the ratios determined by GC-MS (Tables 2-4). *Alpinia conchigera* synthetic essential oils composed of 1,8-cineole (62.2%), β-pinene (24.3%), α-pinene (8.1%) and terpinen-4-ol (5.4%). *Zingiber zerumbet* synthetic essential oils composed of camphene (44.3%), α-humulene (22.9%), camphor (19.7%) and 1,8-cineole (13.1%). *Curcuma zedoaria* synthetic essential oils composed of camphor (67.9%), camphene (18.9%) and isoborneol (13.2%). Both individual compounds and synthetic essential oils were tested with adults of *S. zeamais* and *T. castaneum*.

5. Fumigant toxicity

Different amounts of essential oils, pure compounds, or synthetic essential oils (mixture of pure compounds) at the doses of 0, 1, 2, 4, 8, 12, 16 µL corresponding to 0, 37, 74, 148, 296, 444, 593 µL/L in air were placed onto Whatman No.1 filter paper disks of 2 cm diam. Each filter paper disk was then air dried for 2 min and placed on the underside of the screw cap of a glass vial (27 mL). Ten adults, *S. zeamais* or *T. castaneum* or *A. calandrae*, were placed into each vial (5 replicates/dose) without food before the cap was screwed tightly and the lid was sealed with parafilm. Insect mortality was checked after 12, 24 and 48 h (Fig. 10).

One card of *E. cautella* eggs parasitized with *T. deion* was placed into each vial and exposed to various doses of extracted essential oils as above-mentioned for 24 h (1 card/replicate, 3 replicates/dose). Then, each card was transferred to new vial. The number of emerged *T. deion* adults was recorded after 7 d.

The most effective essential oil, *A. conchigera*, was selected to test with egg, larval and pupal stages of *S. zeamais* and *T. castaneum*. For *S. zeamais*, 10 g of infested grain containing eggs (0-24 h-old), larvae (14 d-old) or pupae (25 d-old) were placed in each vial (5 vials/replicate) before exposing to various doses of *A. conchigera* oils for 12, 24 and 48 h. All grain that contained eggs, larvae and pupae were transferred to the clean glass vials after exposure to essential oils. The number of adults that emerged from the wheat was recorded after 6 and 7 weeks for eggs, 4 and 5 weeks for larvae and 2 and 3 weeks for pupae.

For *T. castaneum*, eggs (0-24 h-old), larvae (12 d-old), pupae (21 d-old) or male and female (0-14 d-old) and adults unsexed (0-14 d-old) were tested as above. After fumigation, the eggs and pupae of *T. castaneum* were transferred to empty multiwell tissue culture plates (Falcon 3046, 6-well-plates, Becton Dickenson, Lincoln Park, NJ, USA), and the percentage of egg hatch was noted for 3 days while adults emerging from pupae was noted daily for 10 days. For larvae and adults, individuals were checked for mortality 12, 24 and 48 h after fumigation. Individuals that showed no movement of legs or antenna were considered dead.

6. Contact toxicity

Aliquots of 0.5 µL of three essential oils, eight pure compounds or three synthetic oils at the different concentrations (0, 10, 20, 30, 40 or 50% of oils or compounds diluted with ethanol) were applied topically onto the thorax of *S. zeamais* (average weight of insect, 2.305 mg) or *T. castaneum* (average weight of insect, 2.004 mg) adults using a microapplicator (Custom made, Cereal Research Centre, Winnipeg, Canada modified from Buchanan, 1965) (25 insects/replicate, 3 replicates/dose). The solvents were allowed to evaporate and treated insects were

transferred to glass vials (2.5 cm diameter, 6.5 cm height with screened plastic cap). Insects treated with ethanol were used as a control. Culture media was added to vials 24 h after treatment (Fig. 11). Mortality of insect was noted 7 days after treatment.

7. Feeding

Flour disks (Xie *et al*., 1996) were prepared for feeding deterrent bioassay by mixing 10 g of wheat flour with 50 mL of water until completely dissolved. Then, wheat flour suspension was pipetted (200 µL) onto a plastic sheet (Glad Cling Wrap, Clorox Company, Toronto, Canada), holding for 24 h at room temperature before drying in an oven at 60^{o}C for 1 h. Each flour disk was treated with 5 µL of different concentration of essential oils (0, 0.4, 4, 8, 16 and 32%, or 0 to 21.1 µL/g). Control disks received only ethanol. The disks were held at room temperature for at least 5 min for solvent evaporation. Then, disks were weighed and placed in plastic Petri dish (diam 9 cm, height 1.5 cm) (5 disks/Petri dish, 5 replicates/dose) (Fig. 12). Twenty-five unsexed *S. zeamais* and *T. castaneum* adults were added to each Petri dish. The insects were allowed to feed for 3 days, then the insects mortality were counted and the flour disks were reweighed.

8. Repellency

The repellency of three essential oil, eight pure compounds and three synthetic oils against *S. zeamais* and *T. castaneum* were tested by using Petri dish choice bioassay (Ko *et al*., 2009a, 2009b, 2010). The essential oils were diluted with absolute ethanol to prepare different concentrations (0.001, 0.01, 0.1 and 1% equivalent to 0.31 x 10^{-3} to 314.56 x 10^{-3} µL/cm^2 for *S. zeamais* whereas 0.0001, 0.001, 0.01 and 0.1% equivalent to 0.03 x 10^{-3}, to 31.46 x 10^{-3} µL/cm^2 for *T. castaneum*). Filter papers (9 cm diam) were cut in half. One mL of treated solution was placed on one half of the filter paper and allowed to dry for 2 min. Other half was treated with absolute ethanol. Treated side was then joined with control side by sellotape and placed in glass Petri dishes (9 cm diameter). The sides of the Petri dishes were painted with Fluon® (AGC Chemicals Americas, Inc., Bayonne, New

Jersey) to retain insects on the filter paper. Twenty unsexed *S. zeamais* and *T. castaneum* adults were released at the center of each filter-paper disk and the Petri dish cover was replaced (10 replicates/dose) (Fig. 13). The number of insects in the control side was noted after 2 and 4 h. The experiments were run in the dark, the temperature varied from 18-20 °C and the relative humidity varied between 55-65 % RH.

9. Data analysis

All data were corrected using Abbott's formula (Abbott, 1925). The lethal concentrations (LC_{50} and LC_{90} values), the lethal dose (LD_{50} and LD_{90} values) were calculated, using the POLO-PLUS program version 2.0 for fumigation and contact toxicities (LeOra Software, Petaluma, CA, USA). For contact toxicity data, the weights of compounds were converted from volumes using the purity of the solutions and their specific gravity. The data for feeding and repellency were analysed and Dunnett's test was used to compare treatment means (Sigma Stat, 2006). For data reported as a percentage data, was transformed using the square root of the arcsin transformation brfore analysis.

Place and Duration

1) Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand (December 2008-October 2009)

2) Cereal Research Centre, Agriculture and Agri-Food, Winnipeg, Manitoba, Canada (October 2009-October 2010)

Study Support

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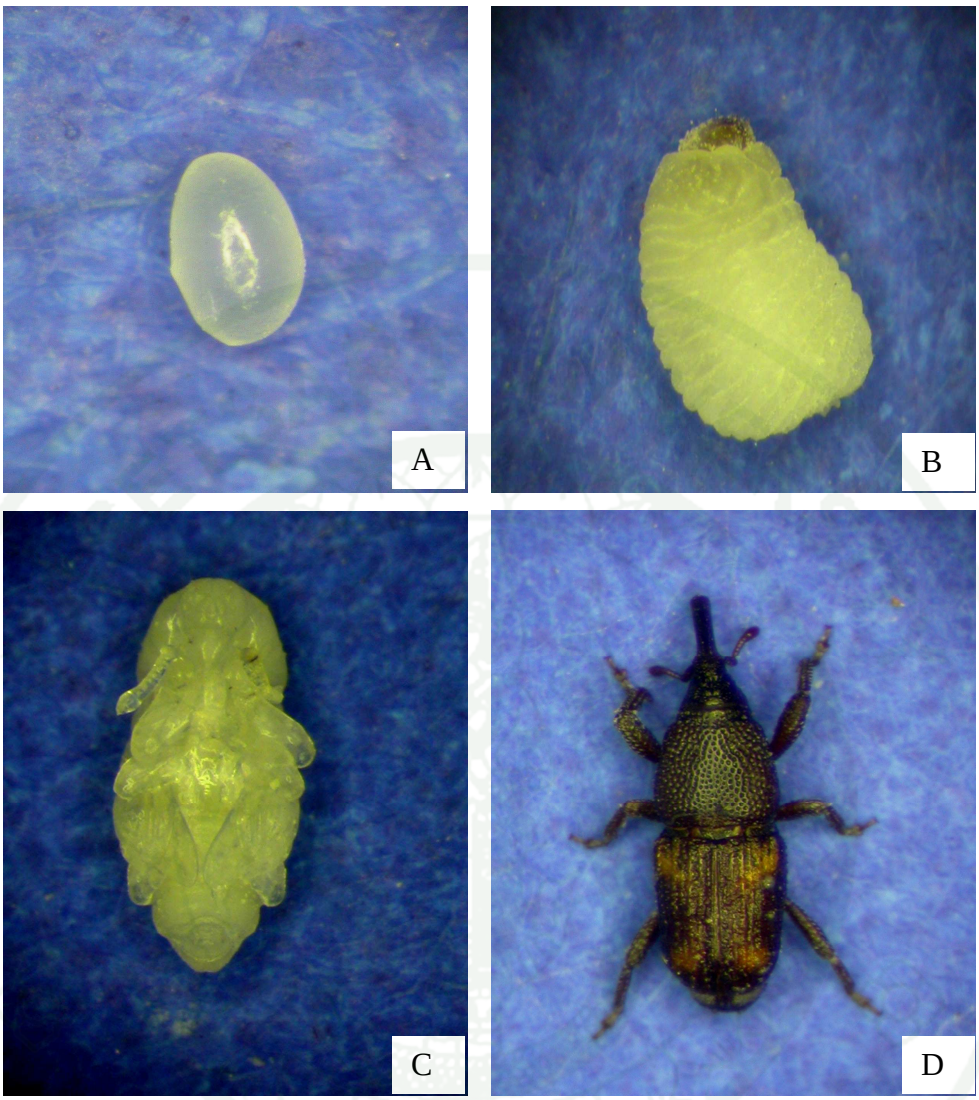


Figure 1 *Sitophilus zeamais* A) egg, B) larva, C) pupa and D) adult

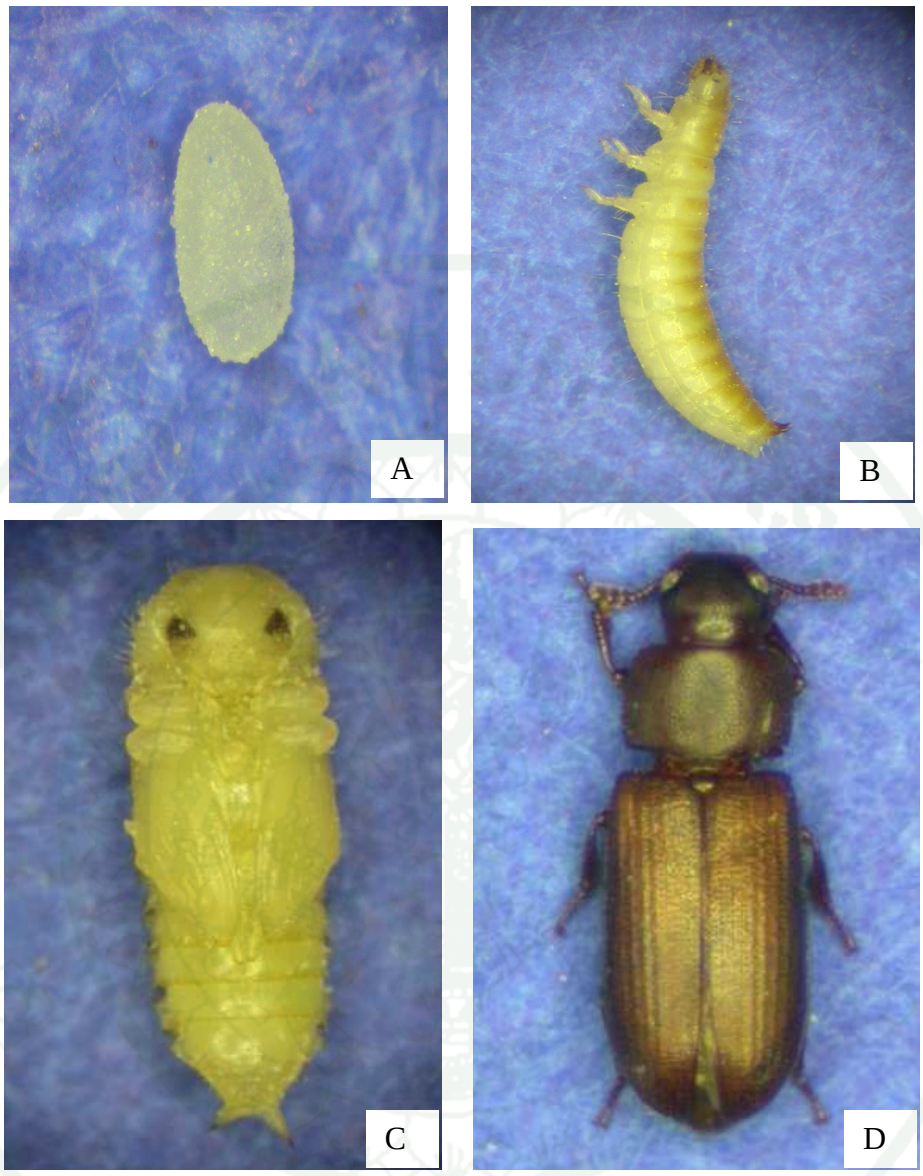


Figure 2 *Tribolium castaneum* A) egg, B) larva, C) pupa and D) adult

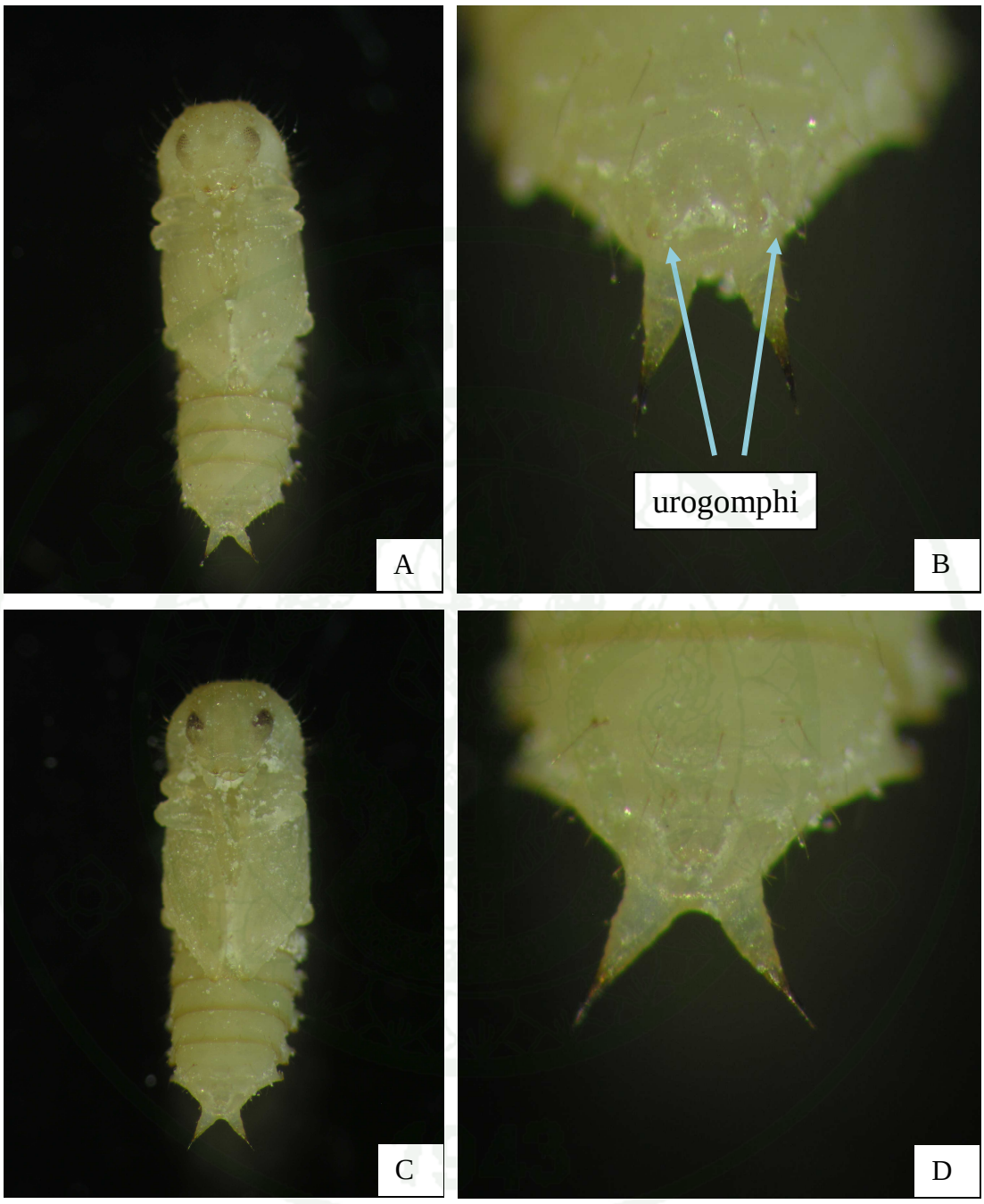


Figure 3 *Tribolium castaneum* pupae A, B) female and C, D) male

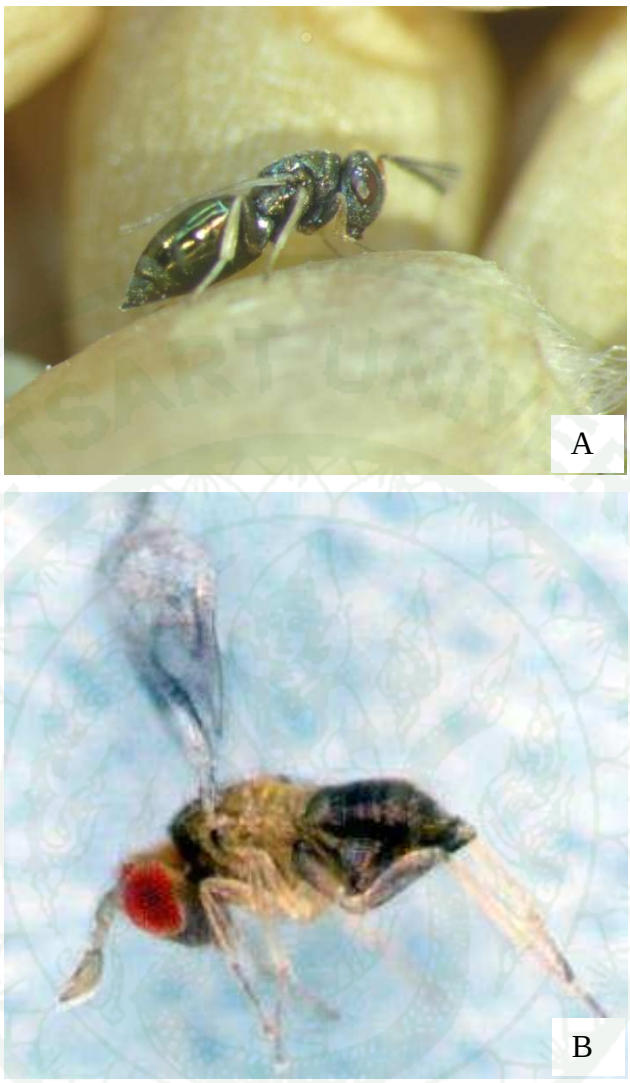


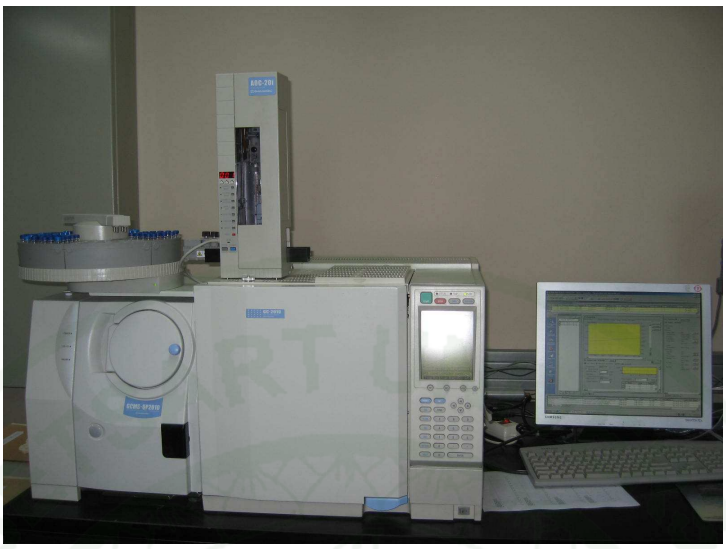
Figure 4 Parasitoids A) *Anisopteromalus calandrae* and B) *Trichogramma deion***,** Source: Peabody (2005)

Figure 5 *Alpinia conchigera* A) the whole plant, B) rhizomes and C) the essential oils

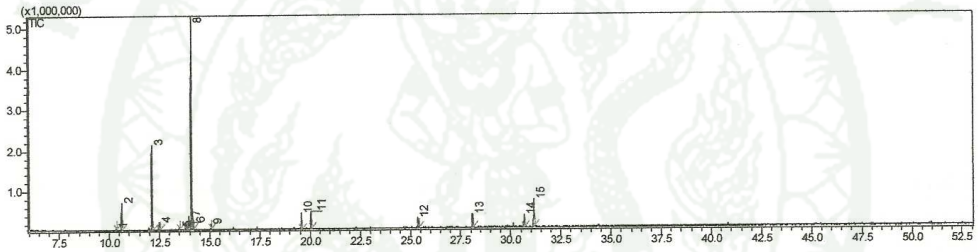
Figure 6 *Zingiber zerumbet* A) the whole plant, B) rhizomes and C) the essential oils

Figure 7 *Curcuma zedoaria* A) the whole plant, B) rhizomes and C) the essential oils

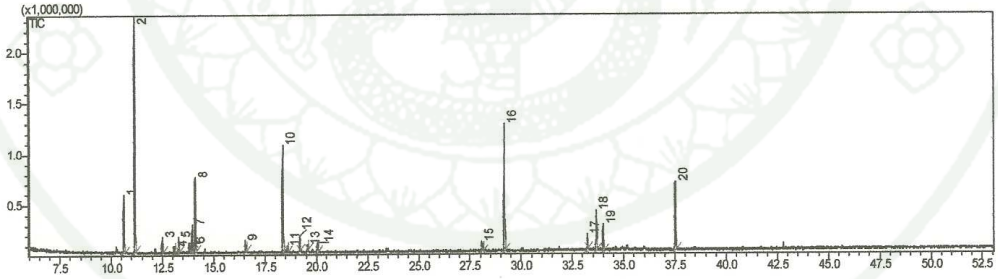
Figure 8 A) The rhizomes were cut into small pieces and B) a Clevenger-type apparatus



1. *Alpinia conchigera*



2. *Zingiber zerumbet*



3. *Curcuma zedoaria*

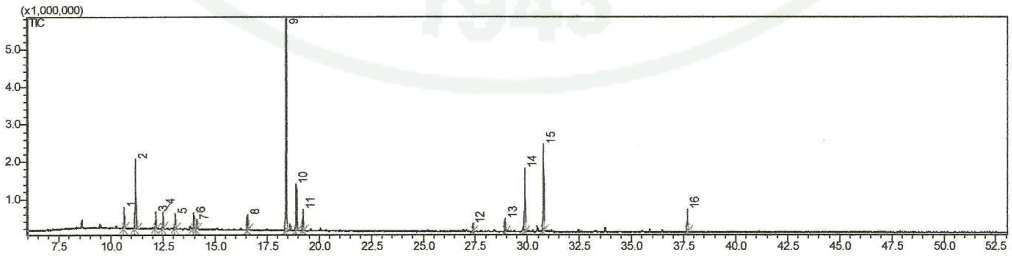


Figure 9 Gas chromatography-Mass spectrometry (GC-MS) was used for analyzing compounds in essentail oils

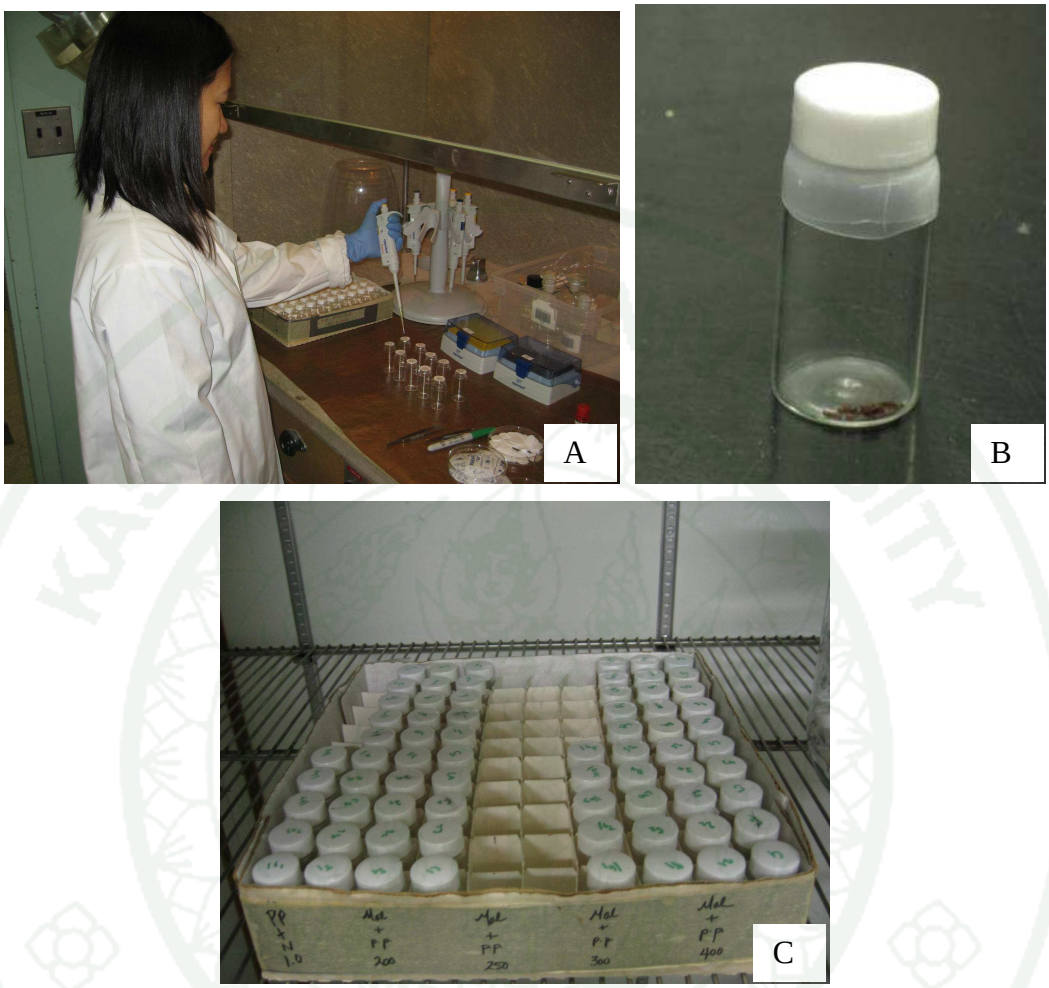


Figure 10 Fumigation bioassay: A) essential oils were placed on small filter paper, B) the vial with treated-filter paper on the underside of the lid and tested insects and C) all units were kept in the incubator

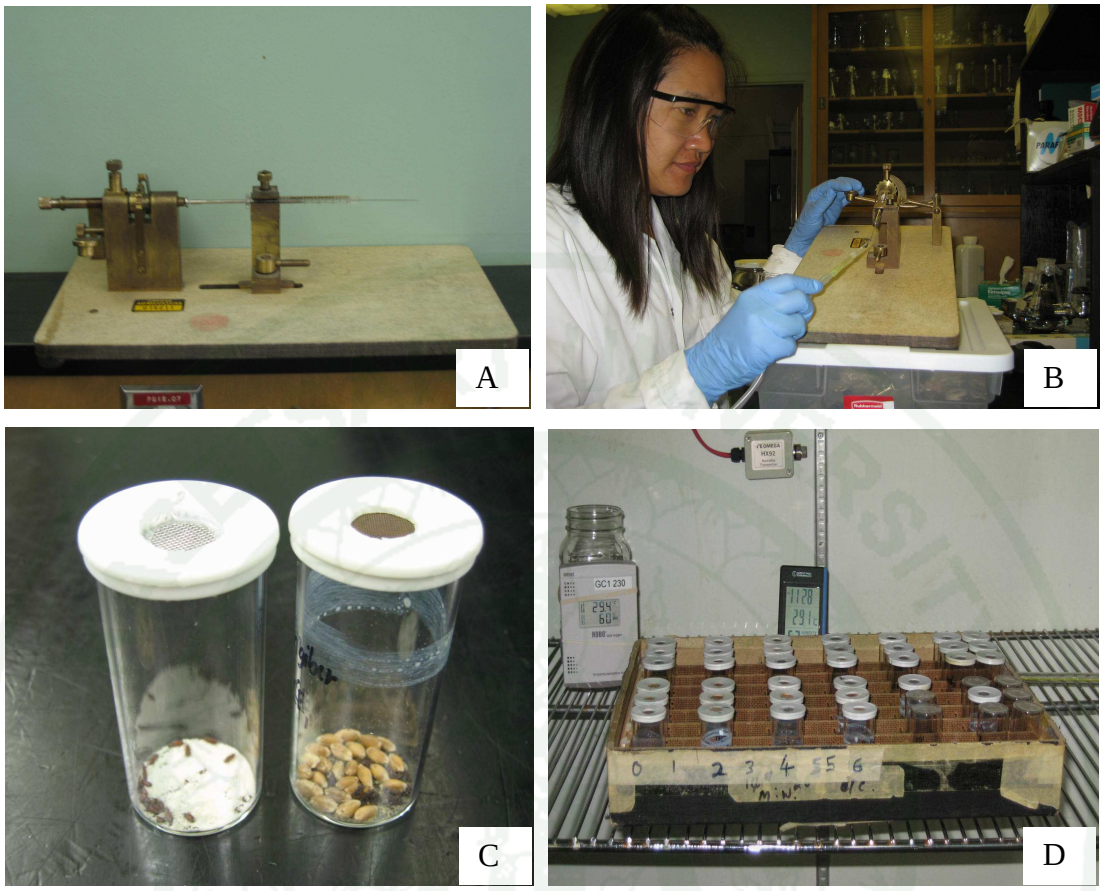


Figure 11 Contact bioassay: A) microapplicator, B) the solutions were applied topically onto the thorax of adults, C) the insects were transferred to vial containing food and D) all units were kept in the incubator

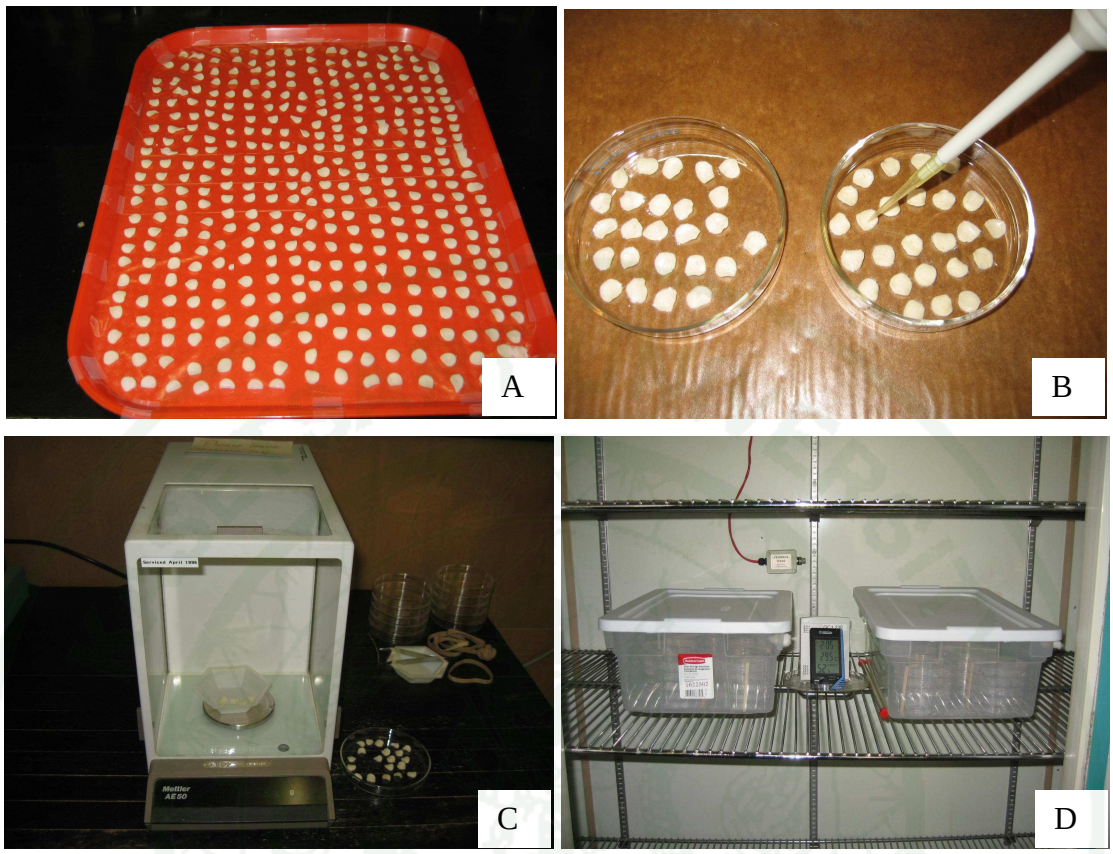


Figure 12 Feeding bioassay: A) the flour disks were prepared B) the solutions were dropped on each flour disk, C) the flour disks were weighted and D) the insect were kept in the incubator for 3 days

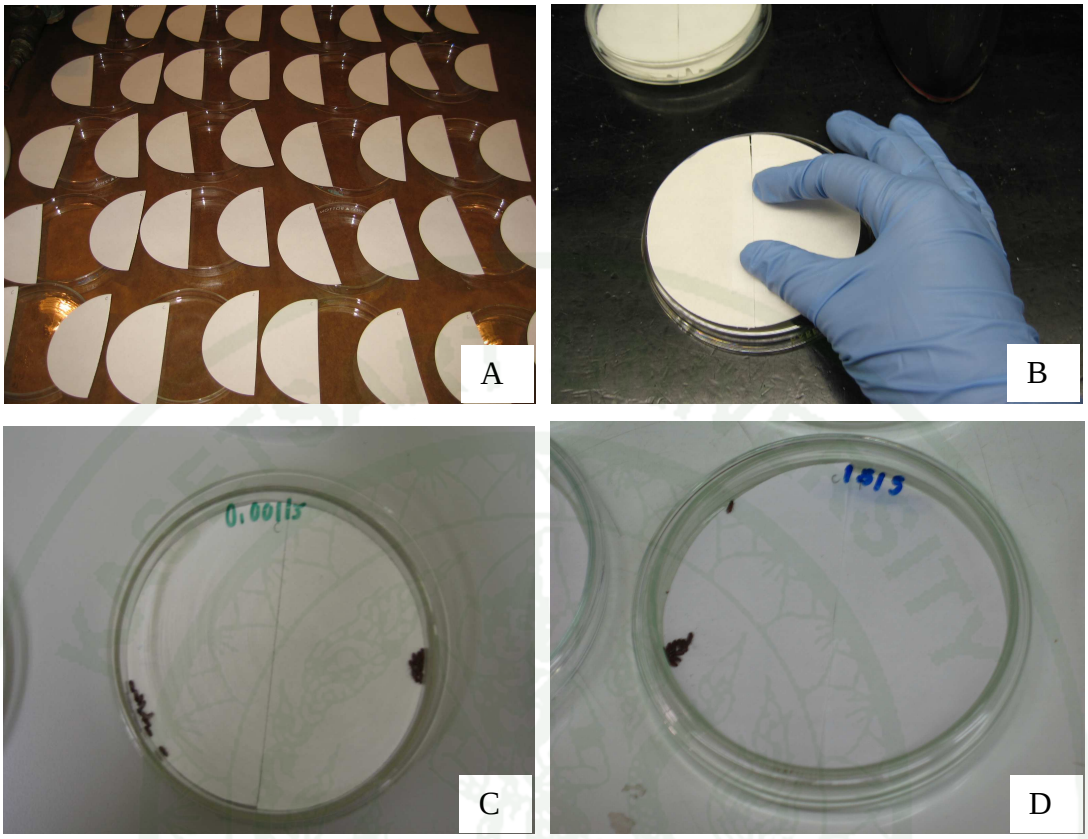


Figure 13 Repellency bioassay: A) the filter papers were treated with solutions, B) the treated side and control side were joined by sellotape, C) the insects found on both sides indicating no repellency and D) the insects found on control side indicating repellency

Table 1 Pure compounds used in bioassay.

Product name	Formula	Purity	Brand	Product number
1-8 cineole	$C_{10}H_{18}O$	99%	Aldrich	C 80601
α-pinene	$C_{10}H_{16}$	98%	Aldrich	P 45680
β-pinene	$C_{10}H_{16}$	99%	Aldrich	112089
terpinen-4-ol	$C_{10}H_{18}O$	≥ 98.5%	Fluka	86477
camphene	$C_{10}H_{16}$	95%	Aldrich	456055
camphor	$C_{10}H_{16}O$	98%	Aldrich	857300
α-humulene	$C_{15}H_{24}$	99%	MB Biomedicals	157391
isoborneol	$C_{10}H_{18}O$	95%	Aldrich	I 13901

RESULTS AND DISCUSSION

1. Chemical analysis of extracted essential oils by GC-MS

Water-distillation of 1 kg of *A. conchigera*, *Z. zerumbet* and *C. zedoaria* rhizomes using a Clevenger-type apparatus for 8 h yielded 3, 2 and 4 mL essential oils, respectively. The main compounds in the essential oils extracted from *A. conchigera*, *Z. zerumbet* and *C. zedoaria* rhizomes as determined and quantified by GC-MS were presented in Tables 2-4. A total of 15, 20 and 16 major compounds were identified from *A. conchigera*, *Z. zerumbet* and *C. zedoaria* essential oils, respectively. The five major compounds of essential oils extracted from *A. conchigera* rhizomes were 1,8-cineole (46%), β-pinene (18%), α-pinene (6%), β -sesquiphellandrene (6%) and α-terpineol (4%). The major compounds of *Z. zerumbet* oils were camphene (27%), α-humulene (14%), camphor (12%), 1,8-cineole (8%) and zerumbone (8%), while those of *C. zedoaria* oils were camphor (36%), α-zingiberene (13%), camphene (10%), α-curcumene (9%) and isoborneol (7%).

The 1,8-cineole could be detected in all essential oils in varying amounts. This compound was more abundant (46%) in *A. conchigera* oils as compared to *Z. zerumbet* (8%) and *C. zedoaria* (1%) oils. Alpha-pinene, limonene and β–myrcene were also detected in all three oils. Camphene and camphor were detected in *Z. zerumbet* and *C. zedoaria* oils, but were not present in *A. conchigera* oils (Tables 2-4). Camphene was present in higher amounts in *Z. zerumbet* oils (27%) than in *C. zedoaria* oils (10%), whereas camphor was present in lower amounts in *Z. zerumbet* oils (12%) than in *C. zedoaria* oils (36%).

The essential oils from *A. conchigera* (Athamaprasangsa *et al*., 1994; Sirat and Nordin, 1995; Wong *et al*., 2005; Ibrahim *et al*., 2009), *Z. zerumbet* (Tewtrakul *et al*., 1997; Bhuiyan *et al*., 2009) *and C. zedoaria* (Singh *et al*., 2002), have been extensively studied. Athamaprasangsa *et al*. (1994) found 12 compounds with a yield of 0.15% from *A. conchigera*, the major ones being; α -pinene, β-pinene, ρ-cymene, cineol, terpinen-4-ol, but percent composition for each compound was not reported.

Sirat and Nordin (1995) found 34 compounds in *A. conchigera* rhizome from southern Peninsular Malaysia, the main ones being; β–sesquiphellandrene (21%), β-bisabolene (12%) and 1,8-cineole (12%). Wong *et al*. (2005) found 50 compounds from *A. conchigera* collected in the Penang Botanical Garden, Malaysia where the main ones being β-bisabolene (29%), 1,8-cineole (15%) and β-caryophyllene (10%), with a yield of 0.14%. Ibrahim *et al*. (2009) detected 39 compounds from *A. conchigera* rhizomes harvested from Jeli Province of Kelantan, Malaysia, and the major chemical compounds are 1,8-cineole (18%), β-bisabolene (14%), β–sesquiphellandrene (7%) and β–elemene (4%). In this study, we identified 15 components and the major ones being 1,8-cineole (46%), β-pinene (18%), α-pinene (6%), β-sesquiphellandrene (6%), α-terpineol (4%), and terpinen-4-ol (4%) which was quite different from previous studies.

Tewtrakul *et al*. (1997) found that zerumbone (57%), humulene epoxide II (2%) and humulene epoxide I (1%) were major components in *Z. zerumbet* essential oils collected from Songkhla province, Thailand. Furthermore, Bhuiyan *et al*. (2009) identified compounds in *Z. zerumbet* essential oils from Bangladesh, and concluded that zerumbone (37%) was the most common compound. In contrast, camphene (27%) was the most abundant in *Z. zerumbet* rhizome collected from Phang-Nga province, Thailand followed by α–humulene (14%), camphor (12%), 1,8- cineole (8%) and zerumbone (8%).

The chemical makeup of several *Curcuma* oils have been determined, for example, Usman *et al*. (2009) found β-bisabolene (14%), trans-ocimene (10%), myrcene (8%), 1,8-cineole (7%) and α–thujene (7%) in *Curcuma longa* L. rhizomes from Nigeria. The major compounds of *Curcuma caesia* Roxb. from India were camphor (28%), ar-turmerone (12%), (Z)-β-ocimene (8%), ar-curcumene (7%) (Pandey and Chowdhury, 2003). In this study, *C. zedoaria* extracted essential oils contained camphor (36%), α-zingiberene (13%), camphene (10%), α-curcumene (9%) and isoborneol (7%) as major compounds.

For a given plant, the number of compounds, their relative amounts and the yield of essential oils vary considerably from different studies. The variation in the makeup of essential oils from the same plant could be attributed to differences in method of isolation and equipment being used. Moreover, the variation between studies may be due to differences in weather, location, plant varieties or time of harvest (Wong *et al*., 2005; Bakkali *et al*., 2008).

Table 2 Chemical constituents of essential oils from *Alpinia conchigera* rhizomes collected from Nakhon Si Thammarat province, Thailand.

Compound	Retention time (min)	Composition (%)
1,8-cineole	14.11	46.2
β-pinene	12.13	18.0
α-pinene	10.62	6.2
β-sesquiphellandrene	31.17	6.0
α-terpineol	20.04	3.8
terpinen-4-ol	19.57	3.6
limonene	13.97	3.1
caryophyllene	28.10	3.0
β-bisabolene	30.67	2.6
chavicol	25.38	2.2
p-cymene	13.79	1.7
β-myrcene	12.49	1.6
γ-terpinene	15.06	1.2
α-terpinene	13.51	0.6
α-thujene	10.35	0.5

Table 3 Chemical constituents of essential oils from *Zingiber zerumbet* rhizomes collected from Phang-Nga province, Thailand.

Compound	Retention time (min)	Composition (%)
camphene	11.15	27.0
α-humulene	29.19	13.9
camphor	18.38	11.9
1,8-cineole	14.10	8.1
zerumbone	37.52	8.1
α-pinene	10.61	6.6
caryophyllene oxide	33.67	4.5
humulene oxide	34.00	3.2
limonene	13.96	3.1
Δ-3-carene	13.29	1.7
isoborneol	19.20	1.7
(Z)-neroldol	33.23	1.6
linalool	16.54	1.6
β-myrcene	12.48	1.6
p-cymene	13.80	1.2
α-terpineol	20.05	1.0
camphene hydrate	18.57	0.9
α-phellandrene	13.07	0.8
caryophyllene	28.09	0.7
terpinen-4-ol	19.57	0.7

Table 4 Chemical constituents of essential oils from *Curcuma zedoaria* rhizomes collected from Phang-Nga province, Thailand.

Compound	Retention time (min)	Composition (%)
camphor	18.42	36.3
α -zingiberene	30.78	12.5
camphene	11.17	10.0
α -curcumene	29.89	9.4
isoborneol	18.90	7.2
germacrone	37.69	3.4
borneol	19.22	3.2
α-pinene	10.64	3.0
β-pinene	12.15	2.4
2-nonanol	16.56	2.3
limonene	13.98	2.2
α-phellandrene	13.09	2.1
β-myrcene	12.50	2.1
(E)-β-farnesene	28.94	1.9
α-bergamotene	27.40	1.1
1,8-cineole	14.13	1.1

2. Fumigant toxicity

Fumigation toxicity of essential oils from rhizomes of *A. conchigera*, *Z. zerumbet*, *C. zedoaria* and their major compounds; camphene, camphor, 1,8-cineole, α-humulene, isoborneol, α-pinene, β-pinene and terpinen-4-ol were investigated with adults of *S. zeamais*, *T. castaneum*, *A. calandrae* and *T. deion* larvae.

2.1 Fumigant toxicity of extracted essential oils

Alpinia conchigera essential oils were more toxic to *S. zeamais* and *T. castaneum* than the other two extracted essential oils. *Sitophilus zeamais* treated with 593 µL/L in air of *Z. zerumbet* and *C. zedoaria* oils showed only 4% and 6% mortality after 48 h exposure, respectively. Furthermore, *Z. zerumbet* and *C. zedoaria* oils at this dose failed to cause mortality in *T. castaneum* eggs and adults (Table 5).

The essential oils were more toxic at longer durations. The LC_{50} with confidence intervals for *S. zeamais* adults exposed to *A. conchigera* oils at 12, 24 and 48 h were; 625, 484-934; 109, 98-121 and 85, 78-94 µL/L in air, and for *T. castaneum* the values were; 140, 105-178; 97, 81-116 and 73, 64-82 µL/L in air. The LC_{50} of *S. zeamais* pupae at 24 and 48 h were 294, 191-459 and 278, 202-328 µL/L in air while, the LC_{50} of *T. castaneum* pupae at 48 h was 414, 331-557 µL/L in air. The LC_{50} of *S. zeamais* larvae at 24 and 48 h were 2452, 1096-28915 and 437, 256-606 µL/L in air while the LC_{50} of *T. castaneum* larvae at 12, 24 and 48 h were 235, 187-286; 219, 143-289 and 196, 120-268 µL/L in air, respectively. For the other immature stages, mortality was not high enough to estimate the LC_{50}. Furthermore, *T. castaneum* adult males, with the LC_{50} and confidence intervals at 12, 24 and 48 h equaled to 274, 225-334; 108, 97-119 and 92, 74-114 µL/L in air were slightly more susceptible to this oil than adult females (367, 326-416; 147, 129-165 and 123, 105-143 µL/L in air) (Tables 6-8).

In general, the parasitoids were more sensitive to extracted essential oils than *S. zeamais* and *T. castaneum* (Table 9). After 48 h exposure, the LC_{50} values of

A. calandrae fumigated with *Z. zerumbet* and *C. zedoaria* oils were 26, 10-37 and 25, 12-34 µL/L in air, respectively, while those treated with *A. conchigera* oils had the LC_{50} of 37, 31-41 µL/L in air (Table 9). *Alpinia conchigera* oils was quite effective against *T. deion* larvae with LC_{50} of 62, 49-74 µL/L in air after 24 h of exposure. This insect was tolerant to *Z. zerumbet* and *C. zedoaria* oils, since *T. deion* adult emerged from moth eggs that were treated with *Z. zerumbet* oils and *C. zedoaria* oils at the highest dose tested (593 µL/L in air) (Table 10).

2.2 Fumigant toxicity of individual compounds

In general, *T. castaneum* and *S. zeamais* showed similar sensitivity to pure compounds, with the exception of isoborneol and β-pinene that were more toxic to *T. castaneum* than *S. zeamais* (Tables 11-16). The toxicity of pure compounds to *S. zeamais* can be divided into four groups after 48 h, from most to least toxic: terpinen-4-ol and 1,8-cineole (Group 1, LC_{50} = 45-48 µL/L in air); isoborneol, camphor and camphene (Group 2, LC_{50} = 73-95 µL/L in air); α-pinene and β-pinene (Group 3, LC_{50} = 120-172 µL/L in air) and α-humulene (Group 4, LC_{50} > 593 µL/L in air). The toxicity of pure compounds to *T. castaneum* can be divided into three groups, similar to the groupings for *S. zemais*, from most to least toxic: terpinen-4-ol, camphor, 1,8-cineole and isoborneol (Group 1, LC_{50} = 36-45 µL/L in air); β-pinene, α-pinene and camphene (Group 2, LC_{50} = 88-118 µL/L in air) and α-humulene (Group 3, LC_{50} >593 µL/L in air) (Tables 13 and 16).

The LC_{50} progressively decreased with increasing exposure time for several of the pure compounds (Figs. 14 and 15). The greatest drop occurred from 12 to 24 h. However, there are several compounds that did not decrease greatly with time; β-pinene, isoborneol for *S. zeamais* (Fig. 14); camphene, isoborneol for *T. castaneum* (Fig. 15). Finally, the LC_{50} did not reduce much for all compounds from 24 to 48 h. For the corresponding CT products for 50% mortality for camphor for *S. zeamais*, an example of LC_{50} declining with time, were 1750, 2476 and 3715 g-h/m^3 at 12, 24 and 48 h. Whereas for an example of LC_{50} remaining constant with time, β-

pinene for *S. zeamais* the CT products were 1823, 3646 and 7166 g-h/m^3 at 12, 24 and 48 h.

2.3 Fumigant toxicity of synthetic essential oils

The synthetic essential oils or mixtures of the three to four major components were more toxic than respective essential oils extracted from the rhizomes (Tables 17-19). For example, oils extracted from *Z. zerumbet* and *C. zedoaria* had almost no toxicity, but a combination of their major compounds did. In addition, *T. castaneum* adults were relatively more sensitive to essential oils and synthetic essential oils than *S. zeamais* adults (Tables 17-19). The synthetic *Z. zerumbet* oil was more effective than the synthetic *A. conchigera* oil, which was more toxic than the synthetic *C. zedoaria* oil.

Generally, the synthetic essential oils were more toxic at longer durations. The LC_{50} confidence intervals of *S. zeamais* adults at 12, 24 and 48h were 124, 51-249; 61, 55-67 and 57, 52-63 µL/L in air to the synthetic *A. conchigera* oil; 82, 65-100; 59, 49-71 and 53, 48-59 to the synthetic *Z. zerumbet* oil; 431, 397-468; 336, 299-370 and 261, 233-289 µL/L in air to the synthetic *C. zedoaria* oil, while the LC_{50} confidence intervals of *T. castaneum* adults were 70, 63-76; 58, 52-63 and 40, 36-45 µL/L in air to the synthetic *A. conchigera* oil; 48, 43-54; 32, 22-39 and 34, 26-38 µL/L in air to the synthetic *Z. zerumbet* oil; 180, 146-220; 114, 87-146 and 90, 68-116 µL/L in air to the synthetic *C. zedoaria* oil (Tables 17-19).

Essential oils and the individual compounds that make up the essential oils act as fumigants against stored-product insects (Tripathi *et al*., 2001; Lee *et al*., 2004; Rozman *et al*., 2007; Rajendran and Sriranlini, 2008). In this study, the synthetic essential oils were always more effective than the respective essential oil extracted from rhizomes. These differences were small with *A. conchigera*, indicating that the minor compounds that were not added to the synthetic essential oil had no role in the fumigant toxicity of the extracted essential oil.

However, for the other two plant rhizomes, *Z. zerumbet* and *C. zedoaria*, the synthetic essential oils were much more effective than the extracted essential oils. One possible explanation for these differences is that some of minor compounds in the extracted essential oil but not in the synthetic essential oils acted as antagonist against the fumigant toxicity of the major compounds. Some possible ways that this could occur are; degradation of the major compounds on the filter paper, inhibition of the release of the major compounds on the filter paper, or some interaction of these compounds within the insect.

Susceptibility of *S. zeamais* and *T. castaneum* varied between species and stages. In general, this result found that *T. castaneum* was more sensitive than *S. zeamais* to the essential oils, although in some of the tests there were no differences. Previous studies show similar trends (Huang and Ho, 1998; Liu and Ho, 1999), while other studies show that *T. castaneum* is less sensitive than *S. zeamais* by fumigation with essential oils (Huang *et al*., 1997, 2000). In general, eggs are the most resistant stage and adults are the most susceptible stage to fumigation (Bond, 1984). This is true for the commercial fumigants sulfuryl fluoride (Kenaga, 1957) and phosphine (Lindgren and Vincent, 1966), as well as the essential oils (Wang *et al*., 2006) and this study.

Basically, the longer insects are exposed to a fumigant, the lower the dose that is required to control insects. The rate of decline is described by the CT product; concentration, usually the LD_{50} or the LD_{95}, multiplied by time. For some fumigants, such as methyl bromide, the CT is constant with time, in other words the dose can be reduced by half if the duration is doubled (Estes, 1965; Bell and Glanville, 1973; Bond, 1984). Some fumigants, such as phosphine, are much more effective at longer durations (Bell and Glanville, 1973). For example, the CT of phosphine reduces by 50% when the exposure goes from 2h to 24h (Lindgren and Vincent, 1966). Whereas other fumigants, such as sulfuryl fluoride, have higher CT with longer durations (Kenaga, 1961). For many of the pure products that tested the LD_{50} remained constant with time, causing the CT to increase with time, even the products, such as camphor that the LD_{50} declined with time, the CT increased with time.

Understanding the factors that affect CT of essential oils will be important in predicting mortality under field conditions, where concentrations vary due to loss of gas from leakage and absorption by commodities (Bond, 1984).

The amount of fumigant needed to control insects depends upon a number of factors: stage, species, duration, temperature and commodity (Bond 1984, Table 20). For the purposes of comparison, the concentration x time product (CT_{95}) was listed for adults for three commercial fumigants, for several constituents of the essential oils and several essential oils from this study and others (Table 20). This study choosed to give the adult CT product as a measure of gas toxicity to compare between studies with different durations. One drawback to this approach, is the CT values are not constant with time for phosphine (Bond, 1984), nor for the pure compounds tested. Also the eggs (Kenaga, 1957; Baltaci *et al*., 2009) and pupae (Lindgren and Vincent, 1966) are often more tolerant of fumigants, than the more commonly tested adult. All the essential oils and the compounds that make up the essential oils were less toxic than the commercial fumigants (Table 20), in some cases tens of thousands of times less toxic. The calculations presume that all the chemical applied volatized. This is true for the fumigants that have a very high vapour pressure (sulfuryl fluoride, 520 kPa, Anonymous 2010a), but may not be true for the essential oils and their constituents compounds that have a low vapour pressure (eg α-pinene, 0.53 kPa, Annoymous 2006). However, essential oils are also be toxic to insects via ingestion or contact with the cuticle, which would reduce the amount of product needed to apply to control insects (Bakkali *et al*., 2008).

Low toxicity to insects could be overlooked if a product is inexpensive, has low toxicity to non target organisms, such as predators and parasites or presents fewer concerns for workers or consumers. This study has no data on the cost of the essential oils, but small farmers have extensively used botanical insecticides to protect their harvest when chemical insecticides are not available or are too expensive (Golob *et al*., 1999). This result shows that the parasite *A. calandrae* was more sensitive to the essentials oils than its host *S. zeamais*. Further work is required to determine if the other predators and parasites are also sensitive to the essential oils. Many of the

essential oils or the compounds that make up the essential oils have many uses (Bakkali *et al*., 2008), for example 1,8-cineole is widely used as a food additive (Ash and Ash, 1995), perfume (Chisvert and Salvador, 2007) and cosmetic industry (Tripathi *et al*., 2001). However, some essential oils or their constituent compounds are toxic to mammals either via ingestion, skin or inhalation (Elvin-Lewis, 2005, Table 20). The ratio of the toxicity to insects (*T. castaneum* CT) to the toxicity to mammals (inhalation rat) was used to calculate a risk factor in order to compare the various fumigants. Low values for this risk factor would be desirable, showing less product is needed to control insects and more products are tolerated by mammals. By this metric, the pure compounds that make up the essential oils are riskier than the commercial fumigants. However, there are a few flaws with this approach. The actual amounts of gas and durations needed to control should be calculated with the most tolerant stage; egg for phosphine and sulfuryl fluoride and the essential oils and pupal for methyl bromide, rather than the adults. Also, the mammalian toxicity data for inhalation is lacking or inaccurate for several of the pure compounds and the extracted essential oils.

Although the essential oils and their constituent compounds tested as fumigants were not as active as commercial fumigants, they, unlike commercial fumigants, do act as and have contact toxicity (Bakkali *et al*., 2008). Further testing is required to determine if the essential oils can be applied to grain, provide initial mortality acting as a fumigant and then provide long-term protection as a repellent and a contact insecticide. Larger scale and longer term studies would be required to determine if these essential oils are practical for insect control in grain stores. Finally, the effects on end-use quality, lingering off-odors or taste and risk to humans would need to be determined before commercialization.

Table 5 Adult mortality rate of *Sitophilus zeamais* and *Tribolium castaneum* caused by fumigant toxicity of 3 extracted essential oils at different concentrations over 48 h.

Application rate (µL/L)	Mortality (%)					
	A. conchigera		*Z. zerumbet*		*C. zedoaria*	
	S. zeamais	*T. castaneum*	*S. zeamais*	*T. castaneum*	*S. zeamais*	*T. castaneum*
0 (control)	0	0	0	0	0	0
37	2	0	0	0	0	0
74	44	32	0	0	0	0
148	98	98	0	0	8	0
296	98	100	0	0	4	0
444	100	100	2	6	0	0
593	100	100	4	0	6	0

Table 6 Fumigant toxicity of extracted essential oils from *Alpinia conchigera* rhizomes against all stages of *Sitophilus zeamais* and *Tribolium castaneum*, 12 h after exposure.

Insect	Plant species	Stage	LC_{50} (µL/L)	95% confidence interval (µL/L)	LC_{90} (µL/L)	95% confidence interval (µL/L)	Degrees of freedom	Chi-square	Slope ±SE	Intercept ±SE
S. zeamais	*A. conchigera*	Egg	>593	-	-	-	-	-	-	-
		Larva	>593	-	-	-	-	-	-	-
		Pupa	>593	-	-	-	-	-	-	-
		Adult	625	484-934	2894	1658-7984	28	25	1.9±0.3	-5.3±0.7
T. castaneum	*A. conchigera*	Egg	>593	-	-	-	-	-	-	-
		Larva	235	187-286	536	423-774	28	54	3.5±0.4	-8.5±0.9
		Pupa	>593	-	-	-	-	-	-	-
		Adult	140	105-178	270	208-420	28	100	4.5±0.4	-9.6±1.0
		-Male	274	225-334	783	589-1237	28	44	2.8±0.3	-6.8±0.7
		-Female	367	326-416	795	657-1061	28	32	3.8±0.5	-9.8±1.2

Table 7 Fumigant toxicity of extracted essential oils from *Alpinia conchigera* rhizomes against all stages of *Sitophilus zeamais* and *Tribolium castaneum*, 24 h after exposure.

Insect	Plant species	Stage	LC_{50} (µL/L)	95% confidence interval (µL/L)	LC_{90} (µL/L)	95% confidence interval (µL/L)	Degrees of freedom	Chi-square	Slope ±SE	Intercept ±SE
S. zeamais	*A. conchigera*	Egg	>593	-	-	-	-	-	-	-
		Larva	2452	1096-28915	>593	-	28	59	0.8±0.2	-2.7±0.4
		Pupa	294	191-459	5687	2328-37934	28	170	1.0±0.1	-2.5±0.2
		Adult	109	98-121	193	166-244	28	3	1.6±0.9	-13.5±1.8
T. castaneum	*A. conchigera*	Egg	>593	-	-	-	-	-	-	-
		Larva	219	143-289	441	333-716	28	78	4.2±0.5	-9.9±1.4
		Pupa	>593	-		-	-	-	-	-
		Adult	97	81-116	166	136-233	28	56	5.5±0.7	-11.0±1.3
		-Male	108	97-119	166	146-201	28	35	6.8±0.9	-13.8±1.8
		-Female	147	129-165	282	245-338	28	32	4.5±0.4	-9.8±1.0

Table 8 Fumigant toxicity of extracted essential oils from *Alpinia conchigera* rhizomes against all stages of *Sitophilus zeamais* and *Tribolium castaneum*, 48 h after exposure.

Insect	Plant species	Stage	LC_{50} (µL/L)	95% confidence interval (µL/L)	LC_{90} (µL/L)	95% confidence interval (µL/L)	Degrees of freedom	Chi-square	Slope ±SE	Intercept ±SE
S. zeamais	*A. conchigera*	Egg	>593	-	-	-	-	-	-	-
		Larva	437	256-606	2892	1428-50719	28	97	1.6±0.3	-4.1±0.7
		Pupa	278	202-328	627	533-863	28	111	3.6±0.4	-8.9±0.9
		Adult	85	78-94	120	106-205	28	7	8.7±1.4	-16.7±2.7
T. castaneum	*A. conchigera*	Egg	>593	-	-	-	-	-	-	-
		Larva	196	120-268	366	269-623	28	88	4.7±0.6	-10.8±1.6
		Pupa	414	331-557	1472	955-3260	28	41	2.3±0.3	-6.0±0.7
		Adult	73	64-82	116	100-149	28	35	1.3±0.7	-11.6±1.5
		-Male	92	74-114	158	124-249	28	77	5.4±0.6	-10.6±1.2
		-Female	123	105-143	203	169-276	28	47	5.9±0.7	-12.3±1.5

Table 9 Fumigant toxicity of extracted essential oils from rhizomes of *Alpinia conchigera*, *Zingiber zerumbet* and *C. zedoaria* against the parasitoids, *Anisopteromalus calandrae* and *Trichogramma deion*.

Insect	Plant species	Duration (h)	LC_{50} (µL/L)	95% confidence interval (µL/L)	LC_{90} (µL/L)	95% confidence interval (µL/L)	Degrees of freedom	Chi-square	Slope ±SE	Intercept ±SE
A. calandrae	*A. conchigera*	12	44	40-48	61	54-75	28	13	8.9±1.5	-14.7±2.4
(Adults)		24	38	33-42	58	51-75	28	13	7.1±1.5	-11.3±2.4
		48	37	31-41	57	50-75	28	14	6.7±1.5	-10.5±2.4
	Z. zerumbet	12	43	38-47	64	56-80	28	22	7.2±1.2	-11.8±2.0
		24	34	27-39	56	48-76	28	27	6.0±1.5	-9.3±2.4
		48	26	10-37	50	42-78	28	29	4.5±1.4	-6.5±2.3
	C. zedoaria	12	141	86-211	1383	695-6542	28	55	1.3±0.2	-2.8±0.4
		24	46	31-58	105	80-175	28	59	3.5±0.5	-5.8±0.9
		48	25	12-34	59	48-86	28	20	3.5±0.9	-4.9±1.6
T. deion	*A. conchigera*	24	62	49-74	181	152-224	16	37	2.7±0.2	-4.9±0.3
(Larvae)	*Z. zerumbet*	24	>593	-	-	-	-	-	-	-
	C. zedoaria	24	>593	-	-	-	-	-	-	-

Table 10 Percentage of *Trichogramma deion* adult emergence after fumigation with three essential oils at 24 h.

Application rate (µL/L)	Adult emergence (%)		
	Alpinia conchigera	*Zingiber zerumbet*	*Curcuma zedoaria*
0	77	67	68
37	59	63	72
74	29	71	75
148	11	68	62
296	4	75	58
444	0	64	60
593	0	66	69

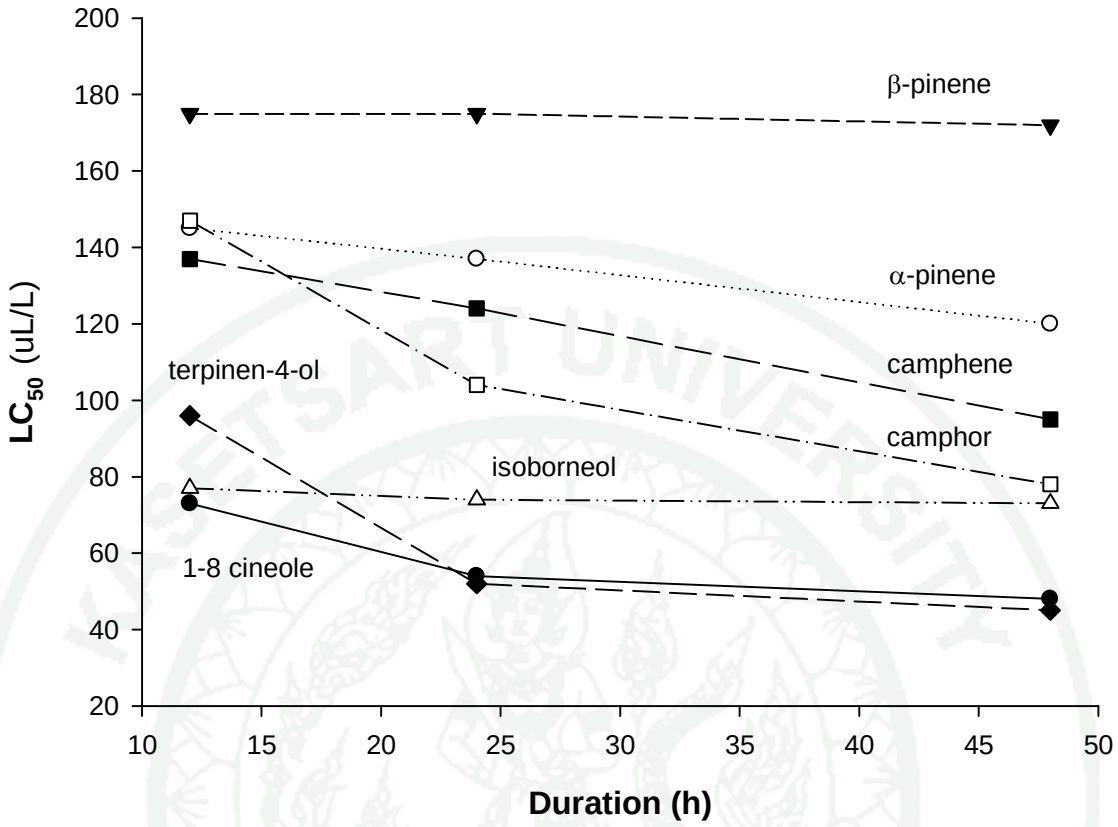


Figure 14 Lethal concentration for 50% (LC_{50}) of *Sitophilus zeamais* adults after different durations of exposure to various pure compounds found in the essential oils

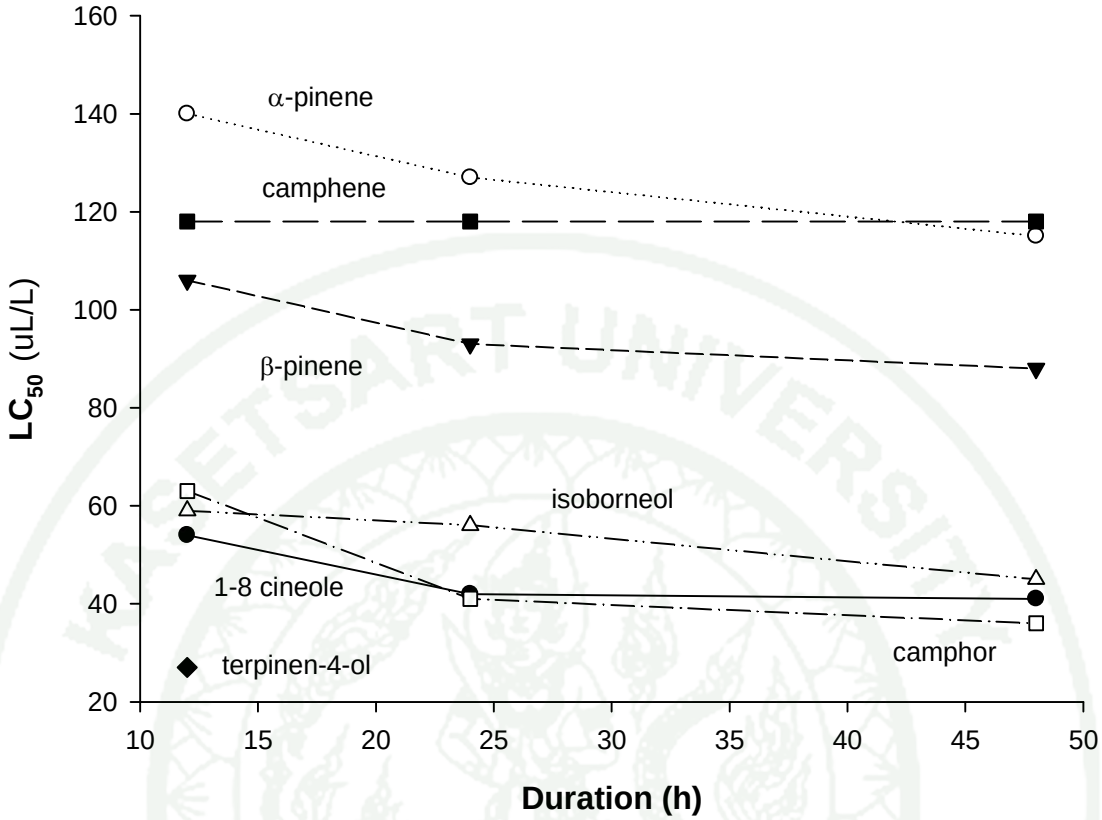


Figure 15 Lethal concentration for 50% (LC_{50}) of *Tribolium castaneum* adults after different durations of exposure to various pure compounds found in the essential oils

Table 11 Fumigant toxicity of pure compounds against *Sitophilus zeamais* adults, 12 h after exposure.

Compound	LC_{50} (µL/L air)	95% confidence interval (µL/L)	LC_{90} (µL/L)	95% confidence interval (µL/L)	Degrees of freedom	Chi-square	Slope±SE	Intercept±SE
terpinen-4-ol	96	39-158	1207	549-11183	28	96	1.2±0.2	-2.3±0.4
1,8-cineole	73	65-81	116	101-144	28	18	6.3±0.8	-11.7±1.6
isoborneol	77	61-94	149	118-224	28	62	4.5±0.5	-8.4±1.0
camphor	147	94-234	250	175-851	28	243	5.6±0.6	-12.0±1.3
camphene	133	92-178	288	211-489	28	128	3.8±0.4	-8.1±0.8
α-pinene	145	127-164	239	207-297	28	35	5.9±0.7	-12.8±1.6
β-pinene	175	147-213	262	215-388	28	76	7.3±0.9	-16.4±2.0
α-humulene	>593	-	-	-	-	-	-	-

Table 12 Fumigant toxicity of pure compounds against *Sitophilus zeamais* adults, 24 h after exposure.

Compound	LC_{50} (µL/L air)	95% confidence interval (µL/L)	LC_{90} (µL/L)	95% confidence interval (µL/L)	Degrees of freedom	Chi-square	Slope±SE	Intercept±SE
terpinen-4-ol	52	38-68	99	74-235	28	122	5.8±0.8	-9.9±1.4
1,8-cineole	54	49-59	78	70-73	28	13	7.9±1.1	-13.6±1.9
isoborneol	74	57-93	134	105-221	28	86	5.0±0.6	-9.3±1.2
camphor	104	79-139	199	146-440	28	125	5.8±0.7	-11.8±1.4
camphene	124	80-176	271	189-663	28	186	4.9±5.0	-10.2±1.0
α-pinene	137	116-158	226	111-295	28	48	5.9±0.7	-12.7±1.5
β-pinene	172	143-209	306	243-479	28	75	6.6±0.8	-14.7±1.7
α-humulene	>593	-	-	-	-	-	-	-

Table 13 Fumigant toxicity of pure compounds against *Sitophilus zeamais* adults, 48 h after exposure.

Compound	LC_{50} (µL/L air)	95% confidence interval (µL/L)	LC_{90} (µL/L)	95% confidence interval (µL/L)	Degrees of freedom	Chi-square	Slope±SE	Intercept±SE
terpinen-4-ol	45	41-49	61	54-75	28	12	9.4±1.5	-15.5±2.5
1,8-cineole	48	44-53	63	56-75	28	13	11.0±1.6	-18.6±2.6
isoborneol	73	56-92	133	103-221	28	88	4.9±0.6	-9.2±1.2
camphor	78	62-100	153	117-259	28	78	4.4±0.5	-8.3±0.9
camphene	95	87-105	125	112-149	28	10	10.7±1.5	-21.2±3.0
α-pinene	120	109-131	170	152-200	28	17	8.4±1.2	-17.5±2.5
β-pinene	172	143-209	262	219-533	28	75	6.5±0.7	-14.7±1.7
α-humulene	>593	-	-	-	-	-	-	-

Table 14 Fumigant toxicity of pure compounds against *Tribolium castaneum* adults, 12 h after exposure.

Compound	LC_{50} (µL/L)	95% confidence interval (µL/L)	LC_{90} (µL/L)	95% confidence interval (µL/L)	Degrees of freedom	Chi-square	Slope±SE	Intercept±SE
terpinen-4-ol	27	7-48	356	224-863	28	36	1.1±0.2	-1.6±0.4
camphor	63	49-79	136	103-229	28	64	3.9±0.5	-6.9±0.9
1,8-cineole	54	38-69	101	77-185	28	97	4.7±0.6	-8.1±1.1
isoborneol	59	53-65	83	74-100	28	32	8.6±1.2	-15.3±2.2
β-pinene	106	75-155	161	121-436	28	187	7.1±0.9	-14.4±1.8
α-pinene	140	127-154	203	180-249	28	20	7.9±1.3	-17.0±2.8
camphene	118	107-129	166	149-195	28	23	8.6±1.2	-17.9±2.6
α-humulene	>593	-	-	-	-	-	-	-

Table 15 Fumigant toxicity of pure compounds against *Tribolium castaneum* adults, 24 h after exposure.

Compound	LC_{50} (µL/L)	95% confidence interval (µL/L)	LC_{90} (µL/L)	95% confidence interval (µL/L)	Degrees of freedom	Chi-square	Slope±SE	Intercept±SE
terpinen-4-ol	< 37		-	-	-		-	-
camphor	41	28-51	95	74-154	28	46	3.5±0.6	-5.7±1.0
1,8-cineole	42	29-54	74	57-149	28	91	5.3±0.9	-8.7±1.5
isoborneol	56	49-64	82	71-105	28	43	7.8±1.1	-13.6±1.9
β-pinene	93	71-130	134	104-311	28	153	8.0±1.0	-15.8±2.1
α-pinene	127	115-139	177	159-211	28	10	8.8±1.4	-18.6±3.1
camphene	118	107-129	166	149-195	28	23	8.6±1.2	-17.9±2.6
α-humulene	>593	-	-	-	-	-	-	-

Table 16 Fumigant toxicity of pure compounds against *Tribolium castaneum* adults, 48 h after exposure.

Compound	LC_{50} (µL/L)	95% confidence interval (µL/L)	LC_{90} (µL/L)	95% confidence interval (µL/L)	Degrees of freedom	Chi-square	Slope±SE	Intercept±SE
terpinen-4-ol	< 37		-	-	-		-	-
camphor	36	24-45	74	60-120	28	43	4.1±0.8	-6.5±1.3
1,8-cineole	41	36-47	60	51-82	28	39	8.0±1.5	-12.9±2.4
isoborneol	45	41-49	61	54-75	28	16	9.4±1.5	-15.5±2.5
β-pinene	88	68-129	124	97-345	28	148	8.5±1.2	-16.5±2.4
α-pinene	115	105-126	159	144-184	28	15	9.1±1.2	-18.8±2.6
amphene	118	107-129	166	149-195	28	23	8.6±1.2	-17.9±2.6
α-humulene	>593	-	-	-	-	-	-	-

Table 17 Fumigant toxicity of 3 essential oils and synthetic essential oils against adults of *Sitophilus zeamais* and *Tribolium castaneum*, 12 h after exposure.

Insect	Source	LC_{50} (µL/L)	95% confidence interval (µL/L)	LC_{90} (µL/L)	95% confidence interval (µL/L)	Degrees of freedom	Chi- square	Slope±SE	Intercept±SE
S. zeamais	*A. conchigera* oils	470	379-632	2104	1326-4566	28	23	2.0±0.3	-5.3±0.7
T. castaneum		199	163-238	404	327-555	28	60	4.1±0.4	-9.5±0.9
S. zeamais	Synthetic *A. conchigera* oil [a]	124	51-249	200	136-4853	28	340	6.2±0.8	-12.9±1.6
T. castaneum		70	63-76	97	86-123	28	5	8.9±1.8	-16.4±3.4
S. zeamais	*Z. zerumbet* oils	> 593							
T. castaneum		> 593							
S. zeamais	Synthetic *Z. zerumbet* oil [b]	82	65-100	196	154-282	28	29	3.4±3.0	-6.5±0.7
T. castaneum		48	43-54	82	71-103	28	25	5.6±0.8	-9.5±1.5
S. zeamais	*C. zedoaria* oils	> 593							
T. castaneum		> 593							
S. zeamais	Synthetic *C. zedoaria* oil [c]	431	397-468	672	596-819	28	18	6.6±0.9	-17.5±2.5
T. castaneum		180	146-220	379	300-545	28	67	4.0±0.4	-8.9±0.8

[a] 1,8-cineole (62.2%), β-pinene (24.3%), α-pinene (8.1%) and terpinen-4-ol (5.4%).

[b] camphene (44.3%), α-humulene (22.9%), camphor (19.7%) and 1,8-cineole (13.1%).

[c] camphor (67.9%), camphene (18.9%) and isoborneol (13.2)

Table 18 Fumigant toxicity of 3 essential oils and synthetic essential oils against adults of *Sitophilus zeamais* and *Tribolium castaneum*, 24 h after exposure.

Insect	Source	LC_{50} (µL/L)	95% confidence interval (µL/L)	LC_{90} (µL/L)	95% confidence interval (µL/L)	Degrees of freedom	Chi- square	Slope±SE	Intercept±SE
S. zeamais	*A. conchigera* oils	128	114-144	218	190-265	28	30	5.5±0.6	-12.0±1.4
T. castaneum		78	31-129	245	147-944	28	220	2.6±0.3	-4.9±0.6
S. zeamais	Synthetic *A. conchigera* oil [a]	61	55-67	84	76-98	28	12	9.1±1.4	-16.3±2.6
T. castaneum		58	52-63	80	72-93	28	6	9.2±1.4	-16.2±2.5
S. zeamais	*Z. zerumbet* oils	> 593							
T. castaneum		> 593							
S. zeamais	Synthetic *Z. zerumbet* oil [b]	59	49-71	89	75-127	28	69	7.2±1.0	-12.8±1.7
T. castaneum		32	22-39	71	59-102	28	17	3.7±0.8	-5.5±1.4
S. zeamais	*C. zedoaria* oils	> 593							
T. castaneum		> 593							
S. zeamais	Synthetic *C. zedoaria* oil [c]	336	299-370	523	465-626	28	36	6.6±0.8	-16.8±2.0
T. castaneum		144	87-146	259	195-421	28	84	3.6±0.4	-7.4±0.7

[a] 1,8-cineole (62.2%), β-pinene (24.3%), α-pinene (8.1%) and terpinen-4-ol (5.4%).

[b] camphene (44.3%), α-humulene (22.9%), camphor (19.7%) and 1,8-cineole (13.1%).

[c] camphor (67.9%), camphene (18.9%) and isoborneol (13.2)

Table 19 Fumigant toxicity of 3 essential oils and synthetic essential oils against adults of *Sitophilus zeamais* and *Tribolium castaneum*, 48 h after exposure.

Insect	Source	LC_{50} (µL/L)	95% confidence interval (µL/L)	LC_{90} (µL/L)	95% confidence interval (µL/L)	Degrees of freedom	Chi-square	Slope±SE	Intercept±SE
S. zeamais	*A. conchigera* oils	87	79-97	133	117-161	28	8	7.0±1.0	-13.7±1.9
T. castaneum		67	54-81	111	90-164	28	73	5.8±0.7	-10.6±1.4
S. zeamais	Synthetic *A. conchigera* oil [a]	57	52-63	76	69-86	28	22	10.7±1.5	-18.9±2.8
T. castaneum		40	36-45	59	52-75	28	25	7.8±1.5	-12.6±2.4
S. zeamais	*Z. zerumbet* oils	> 593							
T. castaneum		> 593							
S. zeamais	Synthetic *Z. zerumbet* oil [b]	53	48-59	74	67-87	28	22	8.9±1.3	-15.3±2.3
T. castaneum		34	26-38	55	48-75	28	10	6.0±1.5	-9.1±2.4
S. zeamais	*C. zedoaria* oils	> 593							
T. castaneum		> 593							
S. zeamais	Synthetic *C. zedoariaa* oil [c]	261	233-289	414	366-490	28	34	6.4±0.7	-15.5±1.8
T. castaneum		90	68-116	192	145-327	28	87	3.9±0.4	-7.6±0.8

[a] 1,8-cineole (62.2%), β-pinene (24.3%), α-pinene (8.1%) and terpinen-4-ol (5.4%).

[b] camphene (44.3%), α-humulene (22.9%), camphor (19.7%) and 1,8-cineole (13.1%).

[c] camphor (67.9%), camphene (18.9%) and isoborneol (13.2)

Table 20 The concentration-time (CT) need to kill 95% of adult stored product insects, rat inhalation toxicity and relative risk factor for commercial fumigants, pure compounds from essential oils and essential oils.

Gas / Essential oils	Plant Family	Plant part	CT to kill 95% of adults (g-h/m^3)[a]					References for CT	Rat LC_{50} (mg/L 4 h)	Risk Factor (*T. castaneum* CT /Rat LC_{50})	References for rat LC_{50}
			S. granarius	*S. oryzae*	*S. zeamais*	*T. castaneum*	*T. confusum*				
methyl bromide	-	-	-	60	-	46	27 / 54	Heseltine and Thompson (1974); Lindgren and Vincent (1965)	3.03	15	Kato *et al*. (1986)
phosphine	-	-	0.18	-	-	0.29	0.24	Lindgren and Vincent (1966); Nakakita and Winks (1981)	0.015	19	Waritz and Brown (1975)
sulfuryl fluoride	-	-	55	-	-	-	15	Kenaga (1957)	4.8	3[b]	Anonymous (2010a)
camphor	-	-	-	-	4303	2595	-	This study	0.500	5190	Anonymous (2010b)
terpinen-4-ol	-	-	-	-	2141	<778	-	This study			
1,8-cineole	-	-	-	-	1881	1859	-	This study			
isoborneol	-	-	-	-	3438	1989	-	This study			
β-pinene	-	-	-	-	6616	3200	-	This study			
α-pinene	-	-	-	-	5622	4216	-	This study	13.7[c]	308	Anonymous (2006)

Table 20 (Continued)

Gas / Essential oils	Plant Family	Plant part	CT to kill 95% of adults (g-h/m^3)[a]					References for CT	Rat LC_{50} (mg/L 4 h)	Risk Factor (*T. castaneum* CT /Rat LC_{50})	References for rat LC_{50}
			S. granarius	*S. oryzae*	*S. zeamais*	*T. castaneum*	*T. confusum*				
camphene	-	-	-	-	5859	3957	-	This study	17.1	231	Anonymous (2010c)
α-humulene	-	-	-	-	>12822	>12822	-	This study			
Alpinia conchigera	Zingiberaceae	Rhizome	-	-	4173	3546	-	This study			
Artemisia sieberi	Asteracae	Aerial parts	-	336	-	-	1239	Negahban *et al*. (2007)			
Drimys winteri	Winteraceae	Leaf	-	-	-	3162	-	Zapata and Smagghe (2010)			
Eucalyptus codonocarpa	Myrtaceae	-	-	124	-	-	-	Lee *et al.* (2004)			
Laurelia sempervirens	Monimiaceae	Leaf	-	-	-	1194	-	Zapata and Smagghe (2010)			
Litsea cubeba	Lauraceae	Mature fruits	-	-	4855	24800	-	Ko Ko *et al.* (2009a)			
Melaleuca cajuputi	Mytaceae	Leaf	-	-	8843	8130	-	Ko Ko *et al.* (2009b)			

a. Duration of exposure was mostly 24 h.

b. CT for *T. confusum* used.

c. This value is for turpentine oil which is 54% α-pinene, 24% β-pinene and 5% dipentene.

3. Contact toxicity

Three essential oils from *A. conchigera*, *Z. zerumbet* and *C. zedoaria* rhizomes, their major components and synthetic essential oils were tested with *S. zeamais* and *T. castaneum* adults using micro-applicator by topically method.

3.1 Contact toxicity of extracted essential oils

The LD_{50} of *S. zeamais* and *T. castaneum* adults after exposed to tested meterials for 7 days were shown in Table 21. The result indicated that *T. castaneum* was more tolerant to all extracted essential oils than *S. zeamais.* In addition, *S. zeamais* was more sensitive to *C. zedoaria* oils (LD_{50}, confidence intervals: 18, 13-21µg/mg) than *Z. zerumbet* (21, 18-23µg/mg) and *A. conchigera* oils (24, 20-27µg/mg). *Tribolium castaneum* showed similar trends, but the differences were not statistically significant.

3.2 Contact toxicity of pure compounds

The toxicity of pure compounds to *S. zeamais* can be divided into four groups, from most to least toxic: terpinen-4-ol and α-humulene (Group 1, LD_{50} 10-20 µg/mg); 1,8-cineole (Group 2, LD_{50} 48 µg/mg); β-pinene, camphor and α-pinene (Group 3, LD_{50} 113-227 µg/mg); camphene and isoborneol (Group 4, LD_{50} > 76 µg/mg; (Table 22). The toxicity (LD_{50}) of pure compounds to *T. castaneum* ranges from < 18 µg/mg for isoborneol to a high of 70 µg/mg for camphor (Table 22). It was not possible to make distinct groups, as with *S. zeamais*, because of significant overlap between confidence intervals. For both insects, terpinen-4-ol, α-humulene and 1,8-cineole were the most toxic compounds. Isoborneol was the least toxic compound for *S. zeamais*, yet the most toxic compound for *T. castaneum.* There was a tendency for *S. zeamais* adults to be more tolerant to pure compounds than *T. castaneum*, with β-pinene and α-pinene showing significant differences. This is the opposite of what was seen with the mixtures, where *S. zeamais* was less tolerant than

T. castaneum (Table 21). Only for α-humulene, *S. zeamais* (20, 18-22 µg/mg) was less tolerant than *T. castaneum* (31, 26-36 µg/mg).

3.3 Contact toxicity of synthetic oils

The LD_{50} of *S. zeamais* and *T. castaneum* adults treated with synthetic oils for 7 d were shown in Table 21. There were no differences in toxicity between the synthetic and extracted oils from *A. conchigera* and *Z. zerumbet*. In contrast, the synthetic *C. zedoaria* oils were significantly less effective than extracted *C. zedoaria* oils to *S. zeamais* and *T. castaneum*.

Essential oils from *A. conchigera*, *Z. zerumbet* and *C. zedoaria* were toxic when applied topically to *S. zeamais* and *T. castaneum*. Essential oils from these plants also act as fumigants (Table 5). However, the mortality due to this mode of action would be minor in the contact bioassay, because in the contact bioassay the solvent was allowed to evaporate before insects were placed in unsealed vials with the highest doses being 7 µL/L, compared to fumigant bioassay which had sealed vials with LD_{50} being above 36 µL/L. Essential oils from other plants from the Zingiberaceae are toxic to several insect pests. The essential oils from *C. longa* leaves were toxic to *R. dominica* (LD_{50}: 37 µg/mg weight of insect), *T. castaneum* (52 µg/mg) and *S. oryzae* (96 µg/mg) (Tripathi *et al*., 2002), which is similar to the level of toxicity reported in this study.

In this experiment, *S. zeamais* was more susceptible than *T. castaneum*. Similar trends are seen with the essential oil of nutmeg, *M. fragrans* (Huang *et al*., 1997). However, *T. castaneum* adults are more sensitive to *C. longa* oils than *S. oryzae* (Tripathi *et al*., 2002), where *S. zeamais* and *T. castaneum* have the same susceptibility to essential oils of *E. cardamomum* (Huang *et al*., 2000). The individual compounds, such as camphor, camphene, 1,8-cineole, eugeneol, isoeugenol, methyl-eugenol, α-pinene and limonene, that make up the essential oils are also toxic to stored-product insects, (Obeng-Ofori and Reichmuth 1997; Prates *et al*., 1998; Huang *et al*., 2002; Park *et al*., 2003; Abdelgaleil *et al*., 2009), or other

insects (Pandji *et al*., 1993). In this study, terpinen-4-ol and isoborneol were most toxic of the eight compounds tested against *S. zeamais* and *T. castaneum* with the LD_{90} values between 21 and 32 µg/mg and the next most toxic was 1,8-cineole with a LD_{90} between 91 and 123 µg/mg. These values are similar to previous studies on compounds found in essential oils. Obeng-Ofori *et al*. (1997) found that 100% mortality of *S. zeamais, T. castaneum, S. granarius* and *Prostephanus truncatus* (Horn) occurred at approximately 6 µg 1,8-cineole/mg, whereas Tripathi *et al*. (2001) showed that the LD_{95} for 1,8-cineole isolated from *Artemisia annua* L. against *T. castaneum* adults was 230 µg/mg. Other compounds isolated from essential oils, eugenol, iso-eugenol and methyl-eugenol, had LD_{95} values ranging from 47 to 116 µg/mg for *S. zeamais* and *T. castaneum* (Huang *et al*., 2002).

Several researches demonstrated that some essential oils are toxic to stored- product insects by contact method. For example; the essential oils from *M. fragrans* and *Elletaria cardamomum* (L.) Maton were toxic to *S. zeamais* and *T. castaneum* by contact bioassay (Huang *et al*., 1997; Huang and Ho, 2000). The essential oil from *Mentha longifilia* L. subsp. *capensis* was highly toxic to *S. zeamais* (Odeyemi *et al*., 2009). The oil extracted from *Callistemon viminalis* (Gaertn.) G. Don leaves were toxic to *A. obtectus* and *C. maculatus* (Ndomo *et al*., 2010). Furthermore, the essential oils from Family Zingiberaceae were proved to have the contact toxicity on various insect pests. For example, Pandji *et al*. (1993) revealed that ten compounds from rhizomes of *Curcuma xanthorrhiza* Roxb., *C. zedoaria, Kaempferia galanga* L. and *K. pandurata* Roxb. had contact toxicity to *Spodoptera littoralis* Boisduval larvae. Tripathi *et al*. (2002) indicated that the essential oils from *C. longa* L. leaves were toxic to *R. dominica, T. castaneum* and *S. oryzae* adults.

The contact toxicity of extracted essential oils was very similar to the synthetic essential oils for *A. conchigera* and *Z. zerumbet* oils, but not for *C. zedoaria* oil. This indicates that the four compounds that used to make the synthetic essential oils for *A. conchigera* and *Z. zerumbet* oils are likely the only ones responsible for their toxicity. *Curcuma zedoaria* essential oils contained α–zingiberene (12.5%), α–curcumene (9.4%) and 11 other minor compounds, but these compounds were not

tested in this study. Further research is required to determine which of these other compounds is responsible for the toxicity seen in the extracted *C. zedoaria* essential oil.

The toxicity of contact commercial insecticide, essential oils and pure compounds toward mammals and insects were shown in Table 24. The risk factor values of the contact insecticides are considerably lower than those of neem oil and pure compounds in this study because the contact insecticides are more toxic to the tested insects than neem and pure compounds. The LD_{50} values in *T. castaneum* of chemical insecticide (actellic 50 EC and deltamethrin) were 1000 times lower than neem and pure compounds. However, the essential oils and their pure compounds can be used as alternative way for controlling the insects.

Ideally, insecticides are very toxic to target insects, yet are not toxic to non-target organisms such as plants, insects (parasites, predators, pollinators) and other animals, such as fish and birds (Tomlin, 2003). Above all, the risk to workers and consumers must be very low. There is some information on the toxicity to mammals of the individual compounds that make up the essential oils, but no information on the essential oils themselves, neem oil extracted from *A. indica* being the exception (Table 24). Although the mammalian toxicity to the pure compounds is low, large doses are needed to control stored-product insects. This study calculated a modified mammalian selectivity ratio using the rat acute oral LC_{50} divided by the LD_{50} of *T. castaneum* (Table 24), that is better adapted to insecticides that are to be used to protect stored products. By this measure, the pure compounds and neem oil are 100 to 4000 times riskier to use than the current stored-product chemical insecticides. There are several factors that determine the risk to using insecticides; mode of exposure, degradation in the environment, chronic toxicity, that this simplistic mammalian selectivity ratio does not take into account. As the essential oils are used in medicines and in foods, rough estimates could be made of the concentrations that are safe for humans. Further research is required to determine toxicity of the essential oils to mammals, the mammalian selectivity ratio and to determine if these compounds can be used commercially without effecting end-use quality while providing effective control of stored-product insect populations.

Table 21 Toxicity of extracted or synthetic essential oils applied topically to *Sitophilus zeamais* and *Tribolium castaneum* adults, 7 days after exposure.

Insect	Source	LD_{50} (µg/mg)	95% confidence intervals (µg/mg)	LD_{90} (µg/mg)	95% confidence intervals (µg/mg)	Degrees of freedom	Chi-square	Slope±SE	Intercept±SE
S. zeamais	*A. conchigera* oils	24	20-27	37	33-46	20	20.7	6.5±0.8	-8.9±1.1
T. castaneum		45	15-57	82	67-129	29	28.6	4.8±1.0	-8.0±1.9
S. zeamais	Synthetic *A. conchigera* oil [a]	25	19-30	54	45-70	25	25.4	3.8±0.4	-5.4±0.6
T. castaneum		37	20-50	128	90-314	37	37.0	2.4±0.3	-3.7±0.6
S. zeamais	*Z. zerumbet* oils	21	18-23	30	26-38	13	15.6	8.0±1.4	-10.6±1.9
T. castaneum		58	43-79	319	176-1638	13	25.8	1.7±0.3	-3.0±0.5
S. zeamais	Synthetic *Z. zerumbet* oil [b]	28	20-34	53	45-66	13	30.4	4.4±0.4	-6.3±0.7
T. castaneum		43	22-60	226	130-1377	13	31.9	1.8±0.3	-2.9±0.5
S. zeamais	*C. zedoaria* oils	18	13-21-	31	27-42-30	13	22.1	4.7±1.3	-5.2±1.8
T. castaneum		35	21-47	122	89-238	13	26.8	2.4±0.3	-3.7±0.6
S. zeamais	Synthetic *C. zedoaria* oil [c]	>98	-	-	-	-	8.7	-	-
T. castaneum		108	73-263	1100	372-80342	13	15.2	1.3±0.3	-2.6±0.7

[a] 1,8-cineole (62.2%), β-pinene (24.3%), α-pinene (8.1%) and terpinen-4-ol (5.4%). [b] camphene (44.3%), α-humulene (22.9%), camphor (19.7%) and 1,8-cineole (13.1%). [c] camphor (67.9%), camphene (18.9%) and isoborneol (13.2)

Table 22 Toxicity of pure compounds found in essential oils applied topically to *Sitophilus zeamais* adults, 7 days after exposure.

Compound	LD_{50} (µg/mg)	95% confidence intervals (µg/mg)	LD_{90} (µg/mg)	95% confidence intervals (µg/mg)	Degrees of freedom	Chi-square	Slope±SE	Intercept±SE
terpinen-4-ol	10	3-14	21	16-26	13	12.6	3.8±1.2	-3.9±1.6
α-humulene	20	18-22	29	26-33	13	4.2	8.2±1.3	-10.7±1.7
1,8-cineole	48	37-60	123	90-243	13	47.0	3.1±0.3	-5.3±0.5
β-pinene	113	81-225	853	335-12024	13	15.7	1.5±0.3	-3.0±0.5
camphor	137	87-725	565	218-26421	13	26.7	2.0±0.4	-4.4±0.7
α-pinene	227	140-872	1223	443-23748	13	13.0	1.7±0.4	-4.1±0.8
camphene	> 76	-	-	-	-	15.2	-	-
isoborneol	> 76	-	-	-	-	16.9	-	-

Table 23 Toxicity of pure compounds found in essential oils applied topically to *Tribolium castaneum* adults, 7 days after exposure.

Compound	LD_{50} (µg/mg)	95% confidence intervals (µg/mg)	LD_{90} (µg/mg)	95% confidence intervals (µg/mg)	Degrees of freedom	Chi-square	Slope±SE	Intercept±SE
isoborneol	< 18	-	-	-	-	-	-	-
terpinen-4-ol	19	14-22	32	29-40	13	5	5.1±1.0	-6.5±1.4
1,8-cineole	24	7-35	91	63-245	13	43	2.2±0.3	-3.0±0.6
α-hemulene	31	26-36	70	61-83	13	11	3.7±0.4	-5.6±0.8
β-pinene	37	30-43	91	77-117	13	16	3.2±0.3	-5.0±0.6
α-pinene	44	37-51	87	73-114	13	31	4.3±0.4	-7.1±0.7
camphene	62	41-130	370	158-2479	13	37	1.6±0.3	-3.0±0.5
camphor	70	53-112	887	341-11433	13	10	1.2±0.	-2.1±0.5

Table 24 Lethal dose need to kill 50% of adult stored product insects, rat oral toxicity and mammalian selectivity ratio (modified from Metcalf, 1972) for commercial contact insecticides, neem oil, essential oils and pure compounds from essential oils.

Insecticide / Essential oils	Chemical or Plant Family	Plant part	LD_{50} of adults (µg/mg)		References for LD_{50}	Rat acute oral LD_{50} (mg/kg)	Mammalian selectivity ratio (Rat LC_{50} / *T. castaneum* LD_{50}x 10^{-3})	References for rat LC_{50}
			S. zeamais	*T. castaneum*				
actellic 50 EC® (pirimiphos-methyl)	Organophosphate	-	-	0.0091	Andrić *et al.* (2010)	1414	155	Tomlin (2003)
deltamethrin	Pyrethriod	-	-	0.0069	Andrić *et al.* (2010)	>2000	290	Tomlin (2003)
malathion	Organophosphate	-	-	0.28	Andrić *et al.* (2010)	3438	12	Tomlin (2003)
pyrethrum				0.36 [a]	Li *et al.* (2010)	1500	4	Casida and Quistad, (1995)
isoborneol	-	-	>76 [a]	<18 [a]	This study	5200	2.9	Anonymous (2010d)
terpinen-4-ol	-	-	10 [a]	19 [a]	This study	1300	0.2	Golob *et al.* (1999)
1,8-cineole	-	-	48 [a]	24 [a]	This study	2480	0.1	Regnault-Roger (1997)
α-humulene	-	-	20 [a]	31 [a]	This study	-	-	-
β-pinene	-	-	113 [a]	37 [a]	This study	3388	0.09	Anonymous (2010e)
α-pinene	-	-	227 [a]	44 [a]	This study	3700	0.08	Anonymous (2010e)
camphene	-	-	>76 [a]	62 [a]	This study	>5000	0.08	Anonymous (2010f)
camphor	-	-	137 [a]	70 [a]	This study	-	-	-

Table 24 (Continued)

Insecticide / Essential oils	Chemical or Plant Family	Plant part	LD_{50} of adults (µg/mg)		References for LD_{50}	Rat acute oral LD_{50} (mg/kg)	Mammalian selectivity ratio (Rat LC_{50} / *T. castaneum* LD_{50}x 10^{-3})	References for rat LC_{50}
			S. zeamais	*T. castaneum*				
Azadiracta indica or Neem oil	Meliaceae	seed	-	74.27 [a]*	Islam and Talukder (2005)	>5000	0.07	Anonymous (2010g)
Alpinia conchigera	Zingiberaceae	Rhizome	24 [a]	25 [a]	This study	-	-	-
Zingiber zerumbet	Zingiberaceae	Rhizome	21 [a]	58 [a]	This study	-	-	-
Curcuma zedoaria	Zingiberaceae	Rhizome	13 [a]	35 [a]	This study	-	-	-
Curcuma longa	Zingiberaceae	Leaf	-	51.49 [a]	Tripathi *et al*. (2002)	-	-	-
Laurelia sempervirens	Monimiaceae	Leaf	-	44.05 [a]	Zapata and Smagghe (2010)	-	-	-

* at 72 h after exposure

[a] topically applied onto thorax

4. Feeding

Three essential oils, eight pure compounds and three synthetic oils were dropped on the flour disk at different concentrations. The insect mortality was checked and the flour disks were re-weighted. Antifeedant activity in this study was determined by comparing the consumption of flour disks in control with those of treated disks.

4.1 Feeding deterrence of extracted essential oil

The essential oils from *A. conchigera*, *Z. zerumbet* and *C. zedoaria* rhizomes did not increase insect mortality. In addition, *C. zedoaria* caused 16.8% mortality of *S. zeamais* at the highest concentration (21.1 µL/g), but the other two essential oils did not (Table 25).

The extracted essential oils from all three Thai plants decreased consumption of flour disks by *S. zeamais*, whereas only two plants decreased feeding by *T. castaneum* (Table 27). *Zingiber zerumbet* and *C. zedoaria* oils were most effective, causing a significant reduction in feeding by *S. zeamais* at 0.5 µL/g, whereas *A. conchigera* oils caused a significant reduction only at much higher doses (10.5 µL/g). *Alphinia conchigera* oils did not reduce feeding in *T. castaneum*, even at the highest concentration tested, 21.1 µL/g, but *Z. zerumbet* and *C. zedoaria* oils did decrease feeding at 15.8 µL/g.

4.2 Feeding deterrence of pure compounds

Percentage mortality of *S. zeamais* and *T. castaneum* treated with 8 pure compounds are shown in Table 26. Most pure compounds did not cause insect mortality, except terpinen-4-ol. *Sitophilus zeamais* adults were more sensitive to terpinen-4-ol than *T. castaneum*, where the mortality rate was 54.4% at 15.8 µL/g and 94.4 % at highest application rate (21.1 µL/g). Meanwhile, the mortality rate of *T. castaneum* adults at 21.1 µL/g was only 7.2%.

Most pure compounds did not suppress feeding by *S. zeamais* and *T. castaneum* (Table 28). The exception was terpinen-4-ol. It reduced feeding at the highest concentration tested. In contrast, camphor and α-humulene at the highest concentration, 21.1 µL/g, significantly increased feeding of *T. castaneum* by approximately 50% (Table 28).

4.3 Feeding deterrence of synthetic essential oils

Only 0.8-2.4% mortality of *S. zeamais* and 0.8-4.8% mortality of *T. castaneum* were observed in all concentration rates of synthetic essential oils (Table 25). All three synthetic essential oils failed to decrease feeding of flour disks in both species (Tables 27). In addition, this experiment found that the synthetic *C. zedoaria* oil increased *S. zeamais* feeding at the concentration of 5.3 µL/g while camphor and α-humulene (21.1 µL/g) appeared to be increase to *T. castaneum* feeding.

Sitophilus zeamais feeding was reduced by all three essential oils, whereas *T. castaneum* feeding was reduced only at high concentrations of *Z. zerumbet* and *C. zeadoaria* essential oils. *Tribolium castaneum* may be more sensitive to the oils than *S. zeamais*, because *T. castaneum* feeds on a wider variety of food than does *S. zeamais* (Rees, 2004). Similar findings are seen with essential oils from nutmeg, *M. fragrans* (Huang *et al*., 1997), *Evodia rutaecarpa* (Jussieu) Benth (Liu and Ho, 1999), and *Litsea cubeba* (Lour.) Persoon and *Litsea salicifolia* Roxb. Ex Wall (Ko *et al*., 2009a, 2010).

This study showed that terpinen-4-ol reduced feeding in both insects at 15.8 µL/g, but the other compounds used alone did not. Tripathi *et al*., (2001) showed that 1,8-cineole isolated from *A. annua* had antifeedant activity to *T. castaneum* adults at 122 µL/g, doses higher than this study.

Furthermore, the synthetic *C. zedoaria* oil increased *S. zeamais* feeding as did camphor and α-humulene increased *T. castaneum* feeding. This is similar to Arakaki *et al*., (2009), who showed that camphor is an attractant for *Protaetia pryeri*

pryeri (Janson) in the field. In addition, cinnamaldehyde is a major compound of *C. aromaticum*, did not reduce feeding by *T. castaneum* at 14 mg/g, but did reduce feeding by *S. zeamais* at 7 mg/g (Huang and Ho, 1998).

Table 25 Mortality of *Sitophilus zeamais* and *Tribolium castaneum* treated with extracted essential oil and synthetic essential oils by antifeedant toxicity.

Insect	Concentration (µL/g)	Mortality (%)					
		A.conchigera oil	*Z. zerumbet* oil	*C. zedoaria* oil	Synthetic *A. conchigera* oils	Synthetic *Z. zerumbet* oils	Synthetic *C. zedoaria* oils
S. zeamais	0	0	0	0	0	0	0
	0.5	0	0	0	0.8	0	0
	5.3	1.6	0	0	0	2.4	0.8
	10.5	0.8	0	0	0.8	0	0
	15.8	1.6	0	3.2	1.6	0	0
	21.1	0	2.4	16.8	1.6	0	0
T. castaneum	0	0	0	0	0	0	0
	0.5	1.6	0.8	0.8	0.8	4	0
	5.3	0.8	1.6	0	1.6	2.4	0.8
	10.5	3.2	0.8	2.4	1.6	0.8	0
	15.8	0.8	1.6	0	1.6	1.6	2.4
	21.1	2.4	2.4	4	4	4.8	4.8

Table 26 Mortality of *Sitophilus zeamais* and *Tribolium castaneum* treated with eight pure compounds by antifeedant toxicity.

Insect	Concentration	Mortality (%)							
	(µL/g)	camphene	camphor	1-8 cineole	α-humulene	isoborneol	α-pinene	β-pinene	terpinen-4-ol
S. zeamais	0	0	0	0	0	0	0	0	0
	0.5	0	0	0.8	0	0.8	1.6	0	0
	5.3	0	2.4	0	0	0	0	0	0
	10.5	0	0.8	0	0	0	0	0	0.8
	15.8	0	0	0	0	0	0	0.8	54.4
	21.1	0.8	1.6	3.2	8	0.8	0	0	94.4
T. castaneum	0	0	0	0	0	0	0	0	0
	0.5	1.6	1.6	1.6	0	1.6	0	1.6	1.6
	5.3	0.8	3.2	4.8	0	3.2	1.6	2.4	0.8
	10.5	2.4	3.2	2.4	0.8	0	2.4	0	4
	15.8	2.4	3.2	2.4	0	0.8	2.4	0	0.8
	21.1	7.2	6.4	4.8	6.4	0.8	2.4	0.8	7.2

Table 27 Feeding of flour disks by *Sitophilus zeamais* and *Tribolium castaneum* over 3 days. Flour disks were treated with extracted or synthetic essential oils.

Insect	Concentrations (µL/g)	Consumption of flour disks (mg±SE)					
		A. conchigera oil	*Z. zerumbet* oil	*C. zedoaria* oil	Synthetic *A. conchigera* oils	Synthetic *Z. zerumbet* oils	Synthetic *C. zedoaria* oils
S. zeamais	0	29.4±1.1	26.0±0.9	25.1±3.4	27.4±2.3	24.6±0.8	19.3±1.2
	0.5	26.7±1.2	20.6±2.5*	14.2±2.3*	23.1±1.7	24.4±0.6	24.2±2.3
	5.3	25.1±1.5	12.0±2.1*	11.6±2.0*	22.9±2.0	27.0±1.5	28.7±2.0*
	10.5	21.7±1.3*	8.5±0.9*	10.5±1.0*	23.5±1.7	24.5±0.8	31.5±2.9*
	15.8	19.6±1.8*	7.6±1.3*	10.7±1.2*	23.6±1.9	26.1±1.4	29.6±1.1*
	21.1	19.0±1.0*	2.5±1.8*	9.7±3.0*	22.1±2.7	23.6±0.6	27.9±2.3*
T. castaneum	0	14.8±2.0	12.9±0.4	14.8±1.4	15.6±1.2	9.2±0.9	13.3±1.1
	0.5	16.3±1.3	13.5±1.6	18.5±2.4	20.3±0.8	11.7±1.3	19.6±2.9
	5.3	15.2±0.7	13.5±1.1	18.9±1.9	19.4±2.0	11.7±1.2	19.0±1.1
	10.5	16.4±1.0	13.4±1.2	13.6±1.8	18.8±1.6	11.7±1.4	20.5±1.4
	15.8	14.9±2.7	6.9±1.5*	7.0±1.4*	18.5±1.1	14.6±1.1	20.7±1.5
	21.1	11.3±3.1	2.8±1.2*	6.2±1.7*	18.6±0.8	13.7±1.9	20.6±2.9

***** For a given oil, a treatment is significantly different from the control (µL/g) as determined by Dunnett's test (P=0.05, n=5)

Table 28 Feeding of flour disks by *Sitophilus zeamais* and *Tribolium castaneum* over 3 days. Flour disks were treated with pure compounds found in essential oils.

Insects	Concentrations (µL/g)	Consumption of flour disks (mg±SE)							
		camphene	camphor	1-8 cineole	α -humulene	α -pinene	β -pinene	isoborneol	terpinen-4-ol
S. zeamais	0	23.0±0.9	32.3±1.8	21.3±1.1	44.5±2.2	24.8±3.9	29.9±2.2	26.5±1.4	36.9±1.9
	0.5	27.5±1.5	32.8±1.1	23.8±3.3	41.0±1.7	27.4±1.7	29.4±0.7	28.8±3.6	33.0±2.0
	5.3	26.1±3.0	33.0±1.2	22.9±1.6	40.3±2.0	26.5±0.5	28.8±1.3	29.7±1.3	32.1±1.8
	10.5	26.5±2.1	33.2±1.9	23.1±0.6	40.0±2.2	25.3±1.9	28.2±1.5	27.2±0.7	32.3±2.4
	15.8	26.8±0.7	34.9±1.9	22.6±1.6	37.3±3.5	25.1±1.6	28.2±2.4	23.8±1.6	14.9±3.9 *****
	21.1	28.5±2.0	37.1±1.2	22.4±0.3	35.6±2.3	25.1±1.3	27.9±1.1	22.3±4.0	0.9±0.5 *****
T. castaneum	0	13.5±1.3	13.0±1.1	17.0±0.9	18.6±0.9	11.0±1.0	16.9±2.4	8.7±2.1	13.8±0.8
	0.5	16.1±0.9	12.2±1.1	17.3±0.6	17.5±0.9	9.8±1.1	21.4±1.5	9.4±1.9	13.0±1.3
	5.3	16.1±1.3	12.0±1.3	16.9±0.3	16.9±3.0	12.0±1.1	20.9±0.9	9.4±2.2	13.0±1.2
	10.5	15.5±0.9	12.1±2.2	18.3±0.7	22.3±1.0	11.9±0.8	20.7±0.8	9.5±2.0	13.0±1.1
	15.8	15.2±1.2	14.6±1.4	16.6±2.0	23.7±2.1	11.0±0.5	20.7±1.1	17.9±2.3	7.7±1.3*
	21.1	15.1±1.8	19.7±0.8*	15.6±1.4	27.9±2.3*	11.5±1.7	20.6±0.8	18.4±1.6	3.8±0.9*

***** For a given oil, a treatment is significantly different from the control (µL/g) as determined by Dunnett's test (*P*=0.05, n=5)

5. Repellency

The repellency of three essential oil, eight pure compounds and three synthetic oils against *S. zeamais* and *T. castaneum* adults were tested at different concentrations using Petri dish choice bioassay.

5.1 Repellency of extracted essential oils

The result in this study demonstrated that *A. conchigera* and *C. zedoaria* extracted essential oils at the highest concentration (314.56 x 10^{-3} µL/cm^2) significantly repelled *S. zeamais* adults, whereas only *A. conchigera* (31.46 x 10^{-3} µL/cm^2) could repel *T. castaneum* adults. In addition, *Z. zerumbet* oils could not repel *S. zeamais* and *T. castaneum*, even at the highest concentration (Table 29). The same results were observed at 4 h after exposure (Table 30).

5.2 Repellency of pure compounds

Terpinen-4-ol at the concentration of 0.31 x 10^{-3} µL/cm^2 repelled *T. castaneum*, whereas 31.46 x 10^{-3} µL/cm^2 was required to repel *S. zeamais* at the same rate where 77.5 and 77.0% of *T. castaneum* and *S. zeamais* were seen on untreated side of filter paper. The other seven pure compounds failed to repel both insects. However, α-pinene and isoborneol tended to attract *T. castaneum* at 2 and 4 h (Tables 31-32).

5.3 Repellency of synthetic essential oils

All synthetic essential oils did not repel either *S. zeamais* or *T. castaneum* both 2 and 4 h after exposure (Tables 29-30).

This study showed that *A. conchigera* oils repelled *S. zeamais* and *T. castaneum*, while *C. zedoaria* oil repelled only *S. zeamais*. Several other studies have shown that essential oils are repellent to a wide variety of insects: *S. zeamais*

(Tapondjou *et al*., 2005; Ko *et al*., 2009a, 2009b, 2010), *T. castanaeum* (Chander *et al*., 1994; Ko *et al*., 2009a, 2009b, 2010; Zapata and Smagghe, 2010), *Tribolium confusum* Jacquelin du Val (Tapondjou *et al*., 2005), *Periplaneta americana* (L.) (Paranagama and Ekanayake, 2004), *Anopheles stephensi* Liston, *Aedes aegypti* (L.) and *Culex quinquefasciatus* Say (Prajapati *et al*., 2005). For example, Tapondjou *et al*. (2005), Ko *et al*. (2009a, 2010), Zapta and Smagghe (2010) reported that essential oils from *Cupressus sempervirens* L., *Drimys winteri* J.R. Forst. & G. Forst., *E. saligna, Laurelia sempervirens* (Ruiz and Pavón), *L. cubeba* and *L. salicifolia* could repel *S. zeamais, T. castaneum* and *T. confusum.* Paranagama and Ekanayake (2004) stated that *A. calcarata* Rosc oils repelled *P. americana*. Moreover, Chander *et al*. (1994) found that *C. longa* oils repelled *T. castaneum* and Prajapati *et al*. (2005) concluded that the essential oils from *C. longa* rhizome repelled *A. stephensi, A. aegypti, C. quinquefasciatus*, but *Z. officinale* oils did not.

Of the pure compounds tested, only terpinen-4-ol repelled *S. zeamais* and *T. castaneum* in this study. The repellency of pure compounds that make up essential oils has been shown for several insects. The 1,8-cineole repels *T. castaneum*, 66% in untreated side at 21 µL/cm^2 (Obeng-Ofori *et al*., 1997), which is very similar to the numbers we observed. Obeng-Ofori *et al*. (1997) also showed that 85% of *S. zeamais* were on the control side at 210 x 10^{-3} µL/cm^2, which is more than we observed (63% at 315 x 10^{-3}µL/cm^2). Obeng-Ofori *et al*. (1997) also determined that 1,8-cineole repels *S. granarius* and *P. truncatus.* García *et al*. (2005) found that α-terpineol, α-pinene and camphene repel *T. castaneum*, but β -pinene did not. Whereas we found camphene was not repellent to *T. castaneum* at the 31.5 x 10^{-3}µL/cm^2 and α-pinene was attractive at this concentration. Hence, it can be summarized that the efficacy of *A. conchigera* and *C. zedoaria* oils occurred from 1,8-cineole and camphene, respectively. In addition, minor compounds in *A. conchigera* and *C. zedoaria* oils probably served as synergist for repellency activity. Further research is needed to determine if these differences are due to differences in insect strains, or methodology.

Table 29 Percent of *Sitophilus zeamais* and *Tribolium castaneum* adults (n=20) on the untreated side of Petri dish after 2 h. Half the filter paper was treated with ethanol (untreated) and the other half was treated with different concentrations of extracted or synthetic essential oils.

Insect	Concentration ($\mu L/cm^2$) x 10^{-3}	Insects found on untreated side (% mean±SE)					
		A. conchigera oil	*Z. zerumbet* oil	*C. zedoaria* oil	Synthetic *A. conchigera* oils	Synthetic *Z. zerumbet* oils	Synthetic *C. zedoaria* oils
S. zeamais	0	59.5±8.6	65.0±7.1	55.0±6.9	43.5±6.9	42.5±4.5	55.0±7.0
	0.31	50.5±7.9	38.5±6.8	49.5±7.4	30.0±8.0	34.5±8.1	42.0±8.2
	3.15	66.0±9.3	51.0±11.2	53.0±5.7	34.0±8.0	45.5±8.5	37.5±9.5
	31.46	72.0±6.7	51.5± 4.4	58.5±6.7	45.0±8.8	44.0±6.1	51.5±6.7
	314.56	90.0±4.9*	76.0±5.8	87.5±3.8*	55.5±10.8	55.0±7.3	46.0±8.8
T. castaneum	0	53.5±11.5	70.5±11.3	48.5±9.6	69.0±9.0	48.5±9.6	35.0±10.3
	0.03	68.0±9.5	81.0±4.6	52.5±12.3	54.5±11.0	52.5±12.3	46.5±12.8
	0.31	76.0±7.7	84.0±4.8	56.0±11.2	56.0±12.2	56.0±11.2	22.0±6.9
	3.15	76.5±6.0	84.0±6.2	58.5±11.7	55.5±12.7	58.5±11.7	33.5±13.5
	31.46	94.5±3.4*	84.5±6.3	75.5±10.1	49.0±10.4	75.5±10.1	39.5±15.0

***** For a given oil, a treatment is significantly different than the control ($\mu L/cm^2$) as determined by Dunnett's test (P=0.05, n=10)

Table 30 Percent of *Sitophilus zeamais* and *Tribolium castaneum* adults (n=20) on the untreated side of Petri dish after 4 h. Half the filter paper was treated with ethanol (untreated) and the other half was treated with different concentrations of extracted or synthetic essential oils.

Insect	Concentration ($\mu L/cm^2$) x 10^{-3}	Insects found on untreated side (% mean±SE)					
		A. conchigera oil	*Z. zerumbet* oil	*C. zedoaria* oil	Synthetic *A. conchigera* oils	Synthetic *Z. zerumbet* oils	Synthetic *C. zedoaria* oils
S. zeamais	0	56.5±9.0	62.5±7.5	62.0±8.6	51.0±9.6	47.0±5.8	47.0±8.2
	0.31	58.5±9.7	39.5±7.1	35.0±8.0*	28.5±8.9	35.5±9.0	50.0±9.1
	3.15	62.5±9.7	40.5±9.5	58.5±8.0	42.0±10.0	45.5±9.2	39.0±10.8
	31.46	72.5±6.5	54.0±4.5	60.5±7.9	44.5±8.8	43.5±8.7	42.0±8.4
	314.56	93.0±3.9*	68.0±8.6	90.5±3.4*	56.5±11.8	56.0±6.3	37.0±9.3
T. castaneum	0	58.5 ±10.1	71.5±10.9	51.0±11.5	68.5±10.8	51.0±11.5	34.5±11.7
	0.03	66.0±11.3	85.5±4.3	63.5±14.5	54.5±12.1	59.5±13.6	46.0±13.2
	0.31	70.5±9.1	80.0± 8.3	58.5±12.8	56.5±13.6	58.5±12.8	22.0±8.8
	3.15	70.5±10.8	82.0±8.6	43.0±13.1	58.0±11.5	62.0±12.7	32.5±14.1
	31.46	95.5±2.5*	80.0±9.1	80.0±10.6	52.5±10.3	80.0±10.6	28.0±14.0

***** For a given oil, a treatment is significantly different than the control ($\mu L/cm^2$) as determined by Dunnett's test (P=0.05, n=1)

Table 31 Percent of *Sitophilus zeamais* and *Tribolium castaneum* adults (n=20) on the untreated side of Petri dish after 2 h. Half the filter paper was treated with ethanol (untreated) and the other half was treated with different concentrations of pure compounds found in essential oils.

Insect	Concentration ($\mu L/cm^2$) x 10^{-3}	Insects found on untreated side (% mean±SE)							
		camphene	camphor	1,8-cineole	α-humulene	α-pinene	β-pinene	isoborneol	terpinen-4-ol
S. zeamais	0	42.0±8.0	40.5±8.5	60.5±8.4	49.5±7.9	55.5±7.7	56.0±10.1	44.5±4.2	46.0±8.3
	0.31	29.5±8.4	41.0±7.1	41.5±6.9	58.5±5.0	45.5±11.5	50.0±7.8	50.0±5.7	55.5±6.3
	3.15	42.5±8.2	28.0±5.9	61.5±6.9	57.0±7.6	51.0±10.8	56.0±7.3	53.0±7.5	72.0±6.3
	31.46	39.5±8.1	40.0±6.7	61.5±7.1	59.0±6.7	54.5±8.7	67.0±9.3	43.5±9.0	77.0±8.0*
	314.56	36.5±7.5	58.0±7.9	63.0±9.1	72.5±8.7	65.0±6.0	73.5±5.8	34.5±8.1	80.0±5.0*
T. castaneum	0	37.5±11.5	54.0±8.4	50.5±11.7	62.0±10.9	78.5±9.3	45.0±12.3	53.5±13.4	41.0±11.0
	0.03	41.5±10.9	54.5±8.8	41.5±10.5	57.5±9.7	39.0±12.6*	63.5±10.8	27.0±6.9	51.0±10.3
	0.31	22.5±6.6	45.0±13.0	32.5±11.3	61.5±8.8	43.0±12.0	56.0±12.0	14.5±7.4*	77.5±6.5*
	3.15	20.0±10.5	51.0±11.5	54.5±11.5	52.5±12.1	49.0±9.3	65.5±10.7	20.0±7.1*	83.5±8.5*
	31.46	27.0±9.0	50.5±11.1	67.5±8.4	66.0±10.3	24.5±7.2*	67.5±8.9	20.0±7.2*	94.0±3.0*

***** For a given oil, a treatment is significantly different than the control ($\mu L/cm^2$) as determined by Dunnett's test (P=0.05, n=10)

Table 32 Percent of *Sitophilus zeamais* and *Tribolium castaneum* adults (n=20) on the untreated side of Petri dish after 4 h. Half the filter paper was treated with ethanol (untreated) and the other half was treated with different concentrations of pure compounds found in essential oils.

Insect	Concentration ($\mu L/cm^2$) x 10^{-3}	Insects found on untreated side (% mean±SE)							
		camphene	camphor	1,8-cineole	α-humulene	α-pinene	β-pinene	isoborneol	terpinen-4-ol
S. zeamais	0	48.5±8.7	45.5±9.3	66.5±8.4	47.5±8.1	37.0±9.9	52.5±10.7	41.5±4.9	44.5±10.0
	0.31	31.0±9.8	45.5±4.7	44.0 ±7.6	57.0±5.3	45.5±12.2	44.5±6.9	51.5±9.6	58.5±7.9
	3.15	38.5±9.2	34.0±9.0	60.0±7.5	63.0±9.1	52.5±11.9	63.5±8.0	52.0±8.8	65.0±8.3
	31.46	39.5±8.5	33.0±9.2	60.5±6.9	63.5±8.8	54.5±10.5	66.0±9.8	50.5±8.5	76.5±7.5*
	314.56	27.0±7.7	55.5±9.1	68.0±9.4	78.5±7.6	74.0±5.9	75.0± 6.4	36.5±7.0	81.0±4.9*
T. castaneum	0	38.0±11.8	47.5±8.3	51.0 ±12.5	65.5± 11.4	78.0±9.6	48.0±12.3	54.0±13.5	44.5±11.2
	0.03	42.5±11.6	59.5±9.4	46.0±12.0	57.5±10.0	33.5±12.0*	61.5±13.0	24.5±7.3	51.5±11.7
	0.31	20.0±7.7	42.5±11.9	29.5±11.8	59.0±8.7	41.0±11.9*	58.5±12.7	7.5±5.0*	81.0±7.2*
	3.15	20.5±12.1	49.5±12.2	54.0±12.3	52.5±13.5	48.5±8.7	69.5±10.9	20.5±8.3*	83.5±10.2*
	31.46	19.5±9.4	51.0±11.0	70.0± 9.7	68.5±10.4	24.0±6.9*	69.5±8.6	18.5±7.2*	94.5±2.9*

***** For a given oil, a treatment is significantly different than the control ($\mu L/cm^2$) as determined by Dunnett's test (P=0.05, n=1)

CONCLUSIONS AND RECOMMENDATIONS

The three essential oils from Zingiberaceae were extracted from rhizomes of *A. conchigera*, *Z. zerumbet*, *C. zedoaria*. Their major compounds (camphene, camphor, 1,8-cineole, α-humulene, isoborneol, α-pinene, β-pinene and terpinen-4-ol) and three synthetic essential oils were investigated for the efficiency to control the stored-product insects. Generally, all extracted essential oils could be used as botanical insecticides to control *S. zeamais* and *T. castaneum* by fumigant, contact, feeding and repellency.

In fumigant toxicity, the *A. conchigera* oils showed the highest toxicity against *S. zeamais*, *T. castaneum* and *T. deion*, while the other two essential oils did not. Adults of *S. zeamais* and *T. castaneum* were more susceptible to *A. conchigera* oils than their eggs, larvae or pupae. However, all extracted essential oils were toxic to *A. calandrae* by the fumigation method. With topical applications, the three extracted oils had similar toxicity against *S. zeamais* and *T. castaneum*. In addition, all extracted essential oils reduced feeding and repelled to both insect species.

Eight individual compounds were tested with *S. zeamais* and *T. castaneum* adults. Terpinen-4-ol was highly toxic to both insects in all bioassays. Furthermore, *S. zeamais* and *T. castaneum* were susceptible to isoborneol by contact toxicity.

The synthetic essential oils or mixture of compounds in the same ratio of as the extracted essential oils were more toxic to *S. zeamais* and *T. castaneum* adults by fumigant toxicity. In contact toxicity trials, synthetic *A. conchigera* and synthetic *Z. zerumbet* oils showed similar effects to those of the extracted essential oils. On the other hand, the synthetic *C. zedoaria* oils showed lower contact toxicity than the extracted *C. zedoaria* oils to both insects. The synthetic essential oils did not act as antifeedants and failed to repel both insects.

The toxicity of each essential oil depended on the bioassay technique being used. Therefore, further research is required to determine which of the compounds in

these complex mixtures is responsible for activity. This task is further complicated by the possibility that some of the compounds in these mixtures may act as synergists (Fields *et al*., 2010) or as antagonists (Kordali *et al*., 2006). This work is needed especially if essential oils are to be used commercially to control stored-product insects. As any natural product, the amounts of these compounds in plants vary during the year and from one year to the next (Weaver and Subramanyam, 2000). Manufacturers need to know which of the components are necessary for activity to deliver an insecticide that can reliably control stored-product insect populations.

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APPENDIX

Appendix Table 1 Accumulative mortality of *Sitophilus zeamais* caused by contact toxicity of *Alpinia conchigera* oil and synthetic *A. conchigera* oil at different concentrations and different intervals.

Source	Concentration	Mortality (%)						
	(µg/mg)	1d	2d	3d	4d	5d	6d	7d
A. conchigera	Control	0	0	0	0	0	0	0
oils	Control							
	Ethanol	0	0	1	1	1	1	1
	20	9	20	29	29	29	31	33
	39	67	77	80	88	88	89	91
	59	100	100	100	100	100	100	100
	78	100	100	100	100	100	100	100
	98	100	100	100	100	100	100	100
Synthetic	Control	0	0	0	0	0	0	0
A. conchigera	Control							
oil	Ethanol	0	0	0	0	0	0	0
	22	0	20	36	45	45	45	45
	45	12	37	49	52	53	57	59
	67	47	75	83	89	93	95	96
	90	81	95	97	100	100	100	100
	112	93	100	100	100	100	100	100

Appendix Table 2 Accumulative mortality of *Tribolium castaneum* caused by contact toxicity of *Alpinia conchigera* oil and synthetic *A. conchigera* oil at different concentrations and different intervals.

Source	Concentration	Mortality (%)						
	(µg/mg)	1d	2d	3d	4d	5d	6d	7d
A. conchigera	Control	0	0	0	1	0	0	0
oils	Control							
	Ethanol	3	3	7	12	12	20	23
	20	21	27	31	33	35	35	37
	39	44	51	53	55	55	57	57
	59	85	88	89	89	89	91	91
	78	81	87	91	91	91	91	91
	98	100	100	100	100	100	100	100
Synthetic	Control	0	1	1	1	1	1	1
A. conchigera	Control							
oils	Ethanol	1	1	3	4	5	5	5
	22	8	23	33	36	37	39	40
	45	35	47	53	55	41	55	56
	67	48	57	60	61	63	63	63
	90	80	83	83	84	84	85	85
	112	87	88	89	91	92	95	95

Appendix Table 3 Accumulative mortality of *Sitophilus zeamais* caused by contact toxicity of *Zingiber zerumbet* oil and synthetic *Z. zerumbet* oil at different concentrations and different intervals.

Source	Concentration	Mortality (%)						
	(µg/mg)	1d	2d	3d	4d	5d	6d	7d
Z. zerumbet	Control	0	0	0	0	0	0	0
oils	Control							
	Ethanol	0	0	0	0	0	0	3
	20	31	35	37	40	43	45	47
	39	87	89	93	93	96	97	99
	59	97	99	100	100	100	100	100
	78	100	100	100	100	100	100	100
	98	100	100	100	100	100	100	100
synthetic	Control	0	0	0	0	0	1	1
Z. zerumbet oil	Control							
	Ethanol	0	0	0	0	0	0	0
	22	1	7	21	27	32	33	36
	45	5	25	44	52	60	61	65
	67	65	91	95	97	97	97	99
	90	69	91	95	95	99	99	99
	112	99	100	100	100	100	100	100

Appendix Table 4 Accumulative mortality of *Tribolium castaneum* caused by contact toxicity of *Zingiber zerumbet* oil and synthetic *Z. zerumbet* oil at different concentrations and different intervals.

Source	Concentration	Mortality (%)						
	(µg/mg)	1d	2d	3d	4d	5d	6d	7d
Z. zerumbet	Control	0	0	0	0	0	0	0
oils	Control							
	Ethanol	0	0	0	0	0	0	0
	20	28	28	28	28	28	28	28
	39	32	32	32	32	32	32	32
	59	55	56	56	56	56	57	59
	78	56	56	56	56	56	56	56
	98	72	72	72	72	75	76	76
Synthetic	Control	0	0	0	0	1	1	1
Z. zerumbet oil	Control							
	Ethanol	1	1	1	3	3	4	4
	22	29	31	32	33	33	37	39
	45	45	45	45	47	47	47	48
	67	59	63	63	64	64	64	64
	90	60	60	61	61	61	61	61
	112	91	91	91	91	91	91	91

Appendix Table 5 Accumulative mortality of *Sitophilus zeamais* caused by contact toxicity of *Curcuma zeadoaria* oil and synthetic *C. zedoaria* oil at different concentrations and different intervals.

Source	Concentration (µg/mg)	Mortality (%)						
		1d	2d	3d	4d	5d	6d	7d
C. zedoaria oils	Control	0	0	0	0	0	0	0
	Control Ethanol	0	0	0	0	3	3	4
	20	75	83	83	83	83	83	83
	39	92	93	93	95	97	99	99
	59	100	100	100	100	100	100	100
	78	100	100	100	100	100	100	100
	98	100	100	100	100	100	100	100
Synthetic *C.zedoaria* oil	Control	0	0	0	0	0	0	0
	Control Ethanol	0	3	3	4	4	4	4
	22	0	4	8	9	11	11	11
	45	0	0	3	4	5	5	5
	67	0	3	4	5	5	5	5
	90	3	3	3	5	7	7	7
	112	3	8	12	12	12	13	13

Appendix Table 6 Accumulative mortality of *Tribolium castaneum* caused by contact toxicity of *Curcuma zedoaria* oil and synthetic *C. zedoaria* oil at different concentrations and different intervals.

Source	Concentration	Mortality (%)						
	(µg/mg)	1d	2d	3d	4d	5d	6d	7d
C. zedoaria	Control	0	0	0	0	0	0	0
oils	Control Ethanol	4	5	5	5	5	5	8
	20	37	43	43	43	43	43	43
	39	52	52	52	52	52	53	55
	59	72	76	76	76	77	77	77
	78	80	80	80	81	81	81	81
	98	95	95	95	95	95	95	95
Synthetic *C.*	Control	0	1	1	4	4	4	5
zedoaria oil	Control Ethanol	7	7	7	7	7	7	7
	22	21	24	24	24	24	24	25
	45	35	35	35	36	36	37	37
	67	32	33	36	37	37	41	41
	90	44	44	44	44	44	44	44
	112	53	53	53	53	53	53	60

Appendix Table 7 Accumulative mortality of *Sitophilus zeamais* and *Tribolium castaneum* caused by contact toxicity of camphene at different concentrations and different intervals.

Insect	Concentration	Mortality (%)						
	(µg/mg)	1d	2d	3d	4d	5d	6d	7d
S. zeamais	Control	0	0	0	0	0	0	0
	Control							
	Ethanol	0	0	0	0	0	0	0
	15	0	0	0	4	5	5	7
	30	0	0	4	9	9	9	12
	46	0	3	11	15	15	15	15
	61	0	1	5	9	12	15	15
	76	1	1	5	11	11	13	16
T. castaneum	Control	0	0	0	1	1	1	1
	Control							
	Ethanol	1	1	1	1	1	3	3
	18	15	15	20	20	20	24	24
	35	21	24	29	29	29	32	32
	53	29	32	33	37	37	40	43
	70	43	52	55	56	56	56	56
	88	52	55	57	61	61	64	64

Appendix Table 8 Accumulative mortality of *Sitophilus zeamais* and *Tribolium castaneum* caused by contact toxicity of camphor rhizome at different concentrations and different intervals.

Insect	Concentration	Mortality (%)						
	(µg/mg)	1d	2d	3d	4d	5d	6d	7d
S. zeamais	Control	0	0	0	1	1	4	4
	Control							
	Ethanol	0	0	0	0	0	0	0
	15	1	3	7	5	5	5	5
	30	4	1	1	3	5	7	7
	45	4	4	4	4	9	9	11
	60	4	4	7	8	11	12	13
	75	15	27	32	43	43	43	43
T. castaneum	Control	0	0	0	0	0	0	0
	Control							
	Ethanol	31	33	33	35	35	35	35
	17	73	75	75	75	76	76	76
	35	77	80	80	80	80	80	80
	52	80	81	81	81	81	81	81
	69	81	88	88	88	88	88	88
	87	88	92	92	93	93	93	93

Appendix Table 9 Accumulative mortality of *Sitophilus zeamais* and *Tribolium castaneum* caused by contact toxicity of 1-8 cineole at different concentrations and different intervals.

Insect	Concentration	Mortality (%)						
	(µg/mg)	1d	2d	3d	4d	5d	6d	7d
S. zeamais	Control	0	0	0	0	0	0	0
	Control							
	Ethanol	0	0	0	0	0	0	0
	20	0	9	15	20	21	21	21
	40	0	12	20	23	27	27	27
	59	1	20	40	43	49	51	51
	79	25	57	67	68	69	71	57
	99	69	89	97	99	99	99	99
T. castaneum	Control	0	0	0	0	0	0	0
	Control							
	Ethanol	0	0	1	1	3	4	4
	23	60	60	60	60	60	60	60
	45	53	53	56	57	60	60	61
	68	76	79	79	79	80	80	80
	91	89	89	89	68	91	91	91
	105	99	99	99	99	100	100	100

Appendix Table 10 Accumulative mortality of *Sitophilus zeamais* and *Tribolium castaneum* caused by contact toxicity of α-humulene at different concentrations and different intervals.

Insect	Concentration	Mortality (%)						
	(µg/mg)	1d	2d	3d	4d	5d	6d	7d
S. zeamais	Control	0	0	0	0	0	0	1
	Control							
	Ethanol	0	0	0	0	3	3	4
	18	15	15	21	32	36	36	39
	37	93	97	99	99	99	99	99
	55	100	100	100	100	100	100	100
	73	100	100	100	100	100	100	100
	92	100	100	100	100	100	100	100
T. castaneum	Control	0	1	1	1	1	1	1
	Control							
	Ethanol	11	11	11	11	11	11	12
	21	35	35	35	37	37	37	37
	42	65	67	67	67	67	68	68
	63	77	83	85	87	87	87	92
	84	91	95	96	96	97	97	97
	105	87	93	96	96	96	97	97

Appendix Table 11 Accumulative mortality of *Sitophilus zeamais* and *Tribolium castaneum* caused by contact toxicity of isoborneol at different concentrations and different intervals.

Insect	Concentration	Mortality (%)						
	(µg/mg)	1d	2d	3d	4d	5d	6d	7d
S. zeamais	Control	0	0	0	0	0	0	0
	Control							
	Ethanol	0	0	0	0	0	0	0
	15	0	0	0	0	1	1	1
	30	1	1	1	1	1	1	3
	46	0	0	0	0	0	0	3
	61	0	0	0	0	0	0	1
	76	1	0	0	0	1	1	1
T. castaneum	Control	0	0	0	0	0	0	0
	Control							
	Ethanol	0	0	0	0	0	0	0
	18	61	65	65	65	65	65	65
	35	67	69	69	69	69	69	69
	53	67	68	68	68	68	68	71
	70	72	72	73	73	73	73	73
	88	68	72	73	73	75	75	75

Appendix Table 12 Accumulative mortality of *Sitophilus zeamais* and *Tribolium castaneum* caused by contact toxicity of α-pinene at different concentrations and different intervals.

Insect	Concentration	Mortality (%)						
	(µg/mg)	1d	2d	3d	4d	5d	6d	7d
S. zeamais	Control	0	0	0	0	0	0	0
	Control							
	Ethanol	0	0	0	0	0	1	1
	18	0	0	7	0	0	0	0
	36	3	9	12	13	15	15	15
	55	0	5	9	11	11	15	16
	73	0	5	9	13	13	15	19
	91	0	5	15	19	20	23	23
T. castaneum	Control	0	0	0	0	0	0	0
	Control							
	Ethanol	0	0	0	0	0	0	0
	21	1	5	7	7	7	7	7
	42	45	51	51	52	53	53	53
	63	65	68	68	68	68	69	69
	84	84	87	84	87	87	87	87
	105	97	97	97	97	97	97	97

Appendix Table 13 Accumulative mortality of *Sitophilus zeamais* and *Tribolium castaneum* caused by contact toxicity of β-pinene at different concentrations and different intervals.

Insect	Concentration	Mortality (%)						
	(µg/mg)	1d	2d	3d	4d	5d	6d	7d
S. zeamais	Control	0	0	0	0	0	0	0
	Control							
	Ethanol	0	0	0	0	0	0	0
	18	0	5	12	13	13	16	16
	37	0	7	17	20	20	20	21
	55	4	13	19	20	21	21	21
	74	8	24	36	43	40	43	43
	92	15	25	40	47	47	51	51
T. castaneum	Control	0	0	0	0	0	0	0
	Control							
	Ethanol	1	1	1	3	3	3	4
	21	15	17	20	21	24	24	25
	42	49	52	56	56	57	60	60
	64	76	76	76	77	77	77	77
	85	83	83	83	84	85	85	88
	106	93	93	93	93	93	95	95

Appendix Table 14 Accumulative mortality of *Sitophilus zeamais* and *Tribolium castaneum* caused by contact toxicity of terpinen-4-ol at different concentrations and different intervals.

Insect	Concentration	Mortality (%)						
	(µg/mg)	1d	2d	3d	4d	5d	6d	7d
S. zeamais	Control	0	0	0	0	0	0	0
	Control							
	Ethanol	0	0	0	0	0	0	0
	20	65	75	80	84	84	84	88
	40	99	99	99	99	99	99	99
	60	100	100	100	100	100	100	100
	80	100	100	100	100	100	100	100
	100	100	100	100	100	100	100	100
T. castaneum	Control	0	0	0	0	0	0	0
	Control							
	Ethanol	0	0	0	0	0	0	0
	23	61	65	67	67	68	68	68
	46	95	97	97	97	97	97	97
	69	100	100	100	100	100	100	100
	92	100	100	100	100	100	100	100
	115	100	100	100	100	100	100	100

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