

# Toxicity of Aromatic Plants and Their Constituents Against Coleopteran Stored Products Insect Pests

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## 1. Introduction

Many insecticides have been used for managing stored products insect pests, especially coleopteran insects such as beetles and weevils because most of them have cosmopolitan distribution and are destructive insects damaging various stored cereals, legumes and food stuffs. Approximately one-third of the worldwide food production has been economically affected, valued annually at more than 100 billion USD, by more than 20,000 species of field and storage insect pests, which can cause serious post-harvest losses from up to 9% in developed countries to 43% of the highest losses occur in developing African and Asian countries (Jacobson, 1982; Pimentel, 1991). Among the most serious economic insect pests of grains, internal feeders such as *Rhyzopertha dominica* and *Sitophilus oryzae* are primary insect pests (Phillips & Throne, 2010). The former lays eggs outside the kernel and hatching larvae intrude into it to complete development to the adult stage, and the latter lays eggs directly inside the kernel. The other commonly found insects in shelled kernel are *Sitophilus zeamais* and *Sitotroga cerealella*. In addition, external feeders such as *Tribolium castaneum*, *Cryptolestes ferrugineus*, and *Oryzaephilus surinamensis* are commonly found insect pests in wheat or maize. Especially, *R. dominica*, *S. oryzae*, and *S. cerealella* are major internal feeding pests of rice. Most of them belong to the order of Coleoptera.

Although the dependence on the liquid insecticides like organophosphates and pyrethroids and gaseous insecticides such as methyl bromide and phosphine are effective means in controlling the coleopteran pests, negative effects owing to their repeated use for decades have fostered environmental and human health concerns (Champ & Dyte, 1977; Subramanyam & Hagstrum, 1995; White and Leesch, 1996). Therefore, many studies have been focused on the development of alternatives to these synthetic chemicals and many plant extracts including essential oils have been raised as appropriate sources.

Plants constitute a rich source of bioactive chemicals, are largely free from adverse effects and have been used as traditional medicines in many Asian countries. We have been

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focusing on plant-derived materials as potential sources of commercial insect control agents and found the usefulness of several aromatic plant extracts and their active compounds or constituents. In spite of widespread public concern for the side effects of synthetic pesticides, the market share of biopesticides including botanical and microbial pesticides is less than 2.2% of the global pesticides market. However, the potential in market growth of botanical pesticides is very high because the use of many conventional insecticides has been restricted by lots of countries and these botanical pesticides as alternatives are likely to occupy the needs.

This chapter briefly describes resistance to insecticides used for control economically important stored products insect pests, and the insecticidal and antifeeding activities of several plant extracts obtained by lots of laboratory studies. Although promising activities of various aromatic plant extracts could be presented, mainly discussed plants in this review are *Acorus gramineus* including several oriental medicinal plants, *Cochlearia armoracia*, and *Origanum vulgare* and targeted insects are *S. zeamais*, *Callosobruchus chinensis*, *Lasioderma serricornis*, and *Attagenus unicolor japonicus*. Based on these results, these plant extracts including essential oils and their active components could be potential candidates to be used in management programmes as naturally occurring insect-control agents.

## 2. Resistance to insecticides

Chemical insecticides to manage stored products insect pests have been used extensively in grain storage facilities. Methyl bromide, phosphine, and sulfuryl fluoride as fumigants showing rapidly killing effect have been being used in a food stuff or a storage house. In addition, malathion, chlorpyrifos-methyl, dichlorvos, diazinon and deltamethrin plus piperonyl butoxide as contact poisoners have been sprayed directly on contaminated grains or structures and provided protection from the infestation of insect pests for several months (Hargreaves et al., 2000). Why are people looking for alternatives to these effective chemicals? Among many reasons, the most important issue will be responsible for the widely developed resistance in a target insect population.

Fumigation plays an important role in insect pest management in various stored products and currently, phosphine and methyl bromide are the two common fumigants used for protection world-widely (Rajendran & Sriranjini, 2008). Due to the internationally limited use of methyl bromide, the importance of phosphine in controlling coleopteran stored insects has relatively grown (Zettler & Arthur, 2000). This situation increased the frequency of its applications and resulted in higher selection pressure for phosphine resistance (Benhalima et al., 2004; Collins et al., 2002). Consequently, since FAO (Food and Agriculture Organization) carried out globally phosphine resistance between 1972 and 1973 years, there is a general increase in the frequency of resistant strains to phosphine over time (Table 1, Mills, 2001). Although the resistant level of these coleopteran insect pests to phosphine is different depending on both surveyed regions and targeted insect species, all the reports focusing on resistant problems showed that the resistant development is increasing. This indicates that the management strategies of resistance to phosphine must be developed. Most of all, we have to understand the phosphine resistance mechanism in these coleopteran insects to achieve the aim. Price (1984) suggested that the mechanism is the reduced uptake of phosphine and it is likely to be accepted because respiration is a good factor observing a physiological response of an

insect to the environmental changes (Chaudhry et al., 2004). Pimentel et al. (2008) also reported that phosphine resistance in four coleopteran insect pests (*T. castaneum*, *R. dominica*, *S. zeamais*, and *O. surinamensis*) collected from 36 locations over seven Brazilian states is related to the reduced production of carbon dioxide. Comparing with the respiration rates between the most resistant and the most susceptible populations to the fumigant, the carbon dioxide production of the former is significantly ( $P < 0.05$ ) lower than that of the latter. Similar results were obtained using *R. dominica* (Price, 1984), *L. serricornis* (Chaudhry et al., 2004), and some populations consisted of *R. dominica*, *S. oryzae*, and *T. castaneum* (Benhalima et al., 2004). Interestingly, uptake of a susceptible strain of *T. castaneum* exposed to 0.7 g/m<sup>3</sup> of phosphine for 5 hours at 25 °C was seven times more gas per gram than a resistant strain. These results strongly suggest that the lower phosphine uptake in resistant populations of coleopteran insects may be occurred and it may have been derived from the reduced respiration rate. However, to understand the fuller genetics of the resistance, it is important to study the most resistance strains available and also to develop and refine rapid resistance tests. These approaches are very useful for identifying resistance and allowing recognition of a problem or failure with a fumigation method.

Survey	Country	% of resistant strains					Refs.
		<i>R. dominica</i>	<i>T. castaneum</i>	<i>S. oryzae</i>	<i>S. granarius</i>	<i>S. zeamais</i>	
'72-73	Global	23.4	5.6	5.9	9.4	-	Champ & Dyte, 1976
'83-85	Developing countries	77.3	48.1	75.0	-	-	Taylor & Halliday, 1986
'86-88	Sa Paulo State, Brazil	90.0	90.0	100	-	-	Pacheco et al., 1990
'97-98	Poland	9.8	-	-	21.2	-	Ignatowicz, 2000
'99-01	Morocco	100	100	100	-	-	Benhalima et al., 2004
'04-07	Brazil	100	81.3	-	-	22.2	Pimentel et al., 2008

<sup>a</sup> Some of the listed data have been combined with those of Mills's report (2001)

Table 1. Phosphine resistance survey results over time<sup>a</sup>

Resistance to malathion, the other important contact insecticide, is widespread in USA, Canada, Australia, and Pakistan (Irshad & Gillani, 1992; Subramanyam & Hagstrum, 1995). In Pakistan, the development of resistance in *S. oryzae* to malathion was more prevalent in public sector storage than farm level, and furthermore, abamectin, spinosad, and buprofezin were more toxic to larvae of the malathion resistance strain (Irshad & Gillani, 1992). In case of Australia, resistance to the protectant was detected in *T. castaneum* in 1968 and *R. dominica* in 1972 (van Graver & Winks, 1994). The resistance became so widespread that the effectiveness of malathion began to be useless and the grain industry had to abandon it.

Therefore, the other organophosphorotionate chemicals to replace it such as fenitrothion, chlorpyrifos-methyl and pirimiphos-methyl were introduced into the control environments because most species did not extend to these potential protectants. However, the resistance to malathion of *R. dominica* was so strong that chemically similar insecticides could not be applied to this insect. The only alternative chemical group, pyrethroids played an important role in managing the pest. Especially, bioresmethrin was used successfully for about 12 years in Australia but in 1990, resistance also was first detected (Collins et al., 1993). Since detection to pyrethroids including deltamethrin, the frequency of resistance increased more than 50%. To overcome this difficult resistant problem, the juvenile hormone analogue methoprene was introduced and successfully controlled *T. castaneum* and *O. surinamensis*. Unfortunately, resistance to this chemical was detected in *R. dominica* in about 1996. According to a simple linear trend analysis, the resistance to pyrethroids and methoprene will reach about 85% in 2015 in Australia (Collins, 2005). Insect resistance to the methoprene might be due to its degradation before reaching the target sites of an insect or reduced affinity of juvenile hormone binding proteins resulted from point mutations or amplification of detoxification genes (Wilson & Ashok, 1998). Although methoprene resistance has also been detected in Australian populations of the lesser grain borer (Collins, 1998), the resistance to hydroxyurea, one of another juvenile hormone mimics is not reported in literatures. The protectant has been primarily used to control urban and stored-product pests in the US, but it is used in other countries to control field crop insect pests.

It is very encouraging that malathion-resistant strains of *T. castaneum* and *T. confusum* did not induce any cross-resistance to hydroxyurea (Amos et al., 1975). Thus, hydroxyurea can be considered as an alternative to conventional insecticides because of its specific activity against immature insect stages, low persistence in the environment, and virtually non-toxic effects on mammals (Mohandass et al., 2006). However, behavioral adaptations of target insects can play an important role in developing resistance to treated contact insecticides including hydroxyurea. Several studies using a strain of the sawtoothed grain beetle, *O. surinamensis* showed that the adults avoided surfaces or grains treated with permethrin and pirimiphos-methyl (Collins et al., 1988; Mason, 1996; Watson & Barson, 1996). Therefore, the highlighted problem, insect resistance, on conventional synthetic insecticides drove many researchers to study alternative methods including botanical insecticides from plants.

### 3. Plant essential oils as alternative insecticides

Many plant essential oils defined as any volatile oils and usually obtained by steam distillation may be an alternative source for insect control because they constitute a rich source of bioactive chemicals. Commercially plant essential oils have been primarily used as pharmaceutical agents, flavor enhancers or additives in lots of food products, flavors in fragrances, and insecticides or acaricides. These have strong aromatic components giving unique odor, flavor or scent to a plant and are by-products of plant secondary metabolites. Essential oils found in glandular hairs of plant cell wall mainly exist in various plant parts such as flowers, leaves, stems, bark or fruits. Most plant essential oils are found in the amount of 1-2% and can be sometimes contained in the amount of 0.01-10%. Today essential oils represent a market estimated at \$700 million and a total world production of 45,000 tons. Nearly 90% of this production is focused on 15 products, particularly mints (*Mentha piperata*, *Mentha arvensis*, and *Mentha spicata*) and citrus (orange, lemon, and lime). Among

the other important products are *Eucalyptus globulus*, *Litsea cubeba*, clove, cedar, and patchouli (Regnault-Roger, 1997).

Plant essential oils also have neurotoxic, cytotoxic, phototoxic, and mutagenic actions to a variety of organisms and act at multiple levels in the insects, indicating that the potential of causing resistance is little probable (Isman, 2000; Bakkali et al., 2008; Gutiérrez et al., 2009), and essential oils themselves or products are largely nontoxic to mammals, birds, and fish (Stroh et al., 1998). These properties of plant essential oils are worthy of consideration as a natural alternative in the control of stored grains insects. Most of all, they are suitable alternatives to control coleopteran insect pests generating economic damage in stored products. These oils could act as contact poisoners, fumigants, repellents, antifeedants or oviposition inhibitors (Table 2). Many plant essential oils are produced commercially from several natural sources, many of which are members of the Lamiaceae family. Especially, the plant essential oils from the Lamiaceae family were also the most known for these biological activities against coleopteran stored products insect pests (Table 2). The plant contain mainly aromatic monoterpenoids such as thymol, carvacrol, *p*-cymene, 1,8-cineole, borneol, linalool, pulegone, etc. as active ingredients. In our studies, the origanum oil (*O. vulgare*) belonging to the Lamiaceae as well as horseradish oil (*C. armoracia*) showed strong fumigant activity to the adults of *T. castaneum*, *S. zeamais*, *C. chinensis* and *L. serricornis* and the larvae of *A. unicolor japonicas* (Kim et al., 2003; Han et al., 2006).

Insect	Essential oil	Activity	Active ingredient	Refs.
<i>R. dominica</i>	<i>Mentha spicata</i> (Lamiaceae)	Insecticide (Fumigant)	Corvine & 1,8-cineole	Khalfi et al., 2006
	<i>Origanum glandulosum</i> (Lamiaceae)	Insecticide	Thymol, carvacrol, <i>p</i> -cymene, & $\gamma$ -terpinene	Khalfi et al., 2008
	<i>Aframomum melegueta</i> (Zingiberaceae)	Repellent	-	Ukeh, 2008
	<i>Mentha</i> sp. & <i>M. piperita</i> (Lamiaceae)	Fumigant	-	Michaelraj et al., 2007; Michaelraj et al., 2008
	<i>Lavandula angustifolia</i> , <i>Lavandula nobilis</i> , <i>Rosmarinus officinalis</i> , & <i>Thymus vulgaris</i> (Lamiaceae)	Fumigant	Camphor & linalool	Rozman et al., 2007
<i>C. chinensis</i>	<i>Artemisia selengensis</i> (Asteraceae)	Fumigant & contact	-	Yuan et al., 2007
	<i>Carum copticum</i> (Apiaceae) & <i>Cymbopogon narudus</i> (Poaceae)	Insecticide	-	Upadhyay et al. (2007)
	<i>Vitex negundo</i> (Lamiaceae)	Antifeedant	Agnuside & viridiflorol	Rana et al., 2005
	<i>Cymbopogon martini</i> (Poaceae)	Repellent	-	Rajesh et al., 2007

Insect	Essential oil	Activity	Active ingredient	Refs.
C. <i>maculatus</i>	<i>Cymbopogon martini</i> (Poaceae), <i>Piper aduncum</i> (Piperaceae), & <i>Lippia gracilis</i> (Verbenaceae)	Insecticide	-	Pereira et al., 2008
	<i>Carum copticum</i> (Apiaceae) & <i>Vitex pseudo-negundo</i> (Lamiaceae)	Fumigant	-	Sahaf and Moharramipour, 2008
	<i>Melaleuca quinquenervia</i> (Myrtaceae)	Fumigant	-	Nondenot et al., 2010
	<i>Simmondasia chinensis</i> (Simmondasiaceae)	Repellent	-	Kheradmand et al., 2010
	<i>Tagetes minuta</i> & <i>Tagetes patula</i> (Asteraceae)	Fumigant & contact	-	Alok et al., 2005
	<i>Artemisia sieberi</i> (Asteraceae)	Insecticidal & repellent	-	Negahban et al., 2006
	S. <i>granarius</i>	<i>Lavandula angustifolia</i> , <i>Laurus nobilis</i> , <i>Rosmarinus officinalis</i> , & <i>Tylenchorhynchus vulgaris</i> (Lamiaceae)	Fumigant	1,8-cineole, camphor, eugenol, linalool, carvacrol, thymol, borneol, bornyl acetate, & linalyl acetate
<i>S.oryzae</i>				
<i>S.oryzae</i>	<i>Acorus calamus</i> (Acoraceae) & <i>Zyzygium aromaticum</i> (Myrtaceae)	Inhibition of F1 progeny	-	Sharma and Meshram, 2006
	<i>Ocimum canum</i> (Lamiaceae)	Insecticide	-	Ngassoum et al., 2007
	<i>Hyptis spicigera</i> & <i>Ocimum canum</i> (Lamiaceae)	Repellent	-	Ngassoum et al., 2007
	<i>Hyptis spicigera</i> , <i>Ocimum canum</i> , <i>Plectranthus glandulosus</i> (Lamiaceae), & <i>Vepris heterophylla</i> (Rutaceae)	Insecticide	-	Ngamo et al., 2007
	<i>Vitex negundo</i> (Lamiaceae)	Antifeedant	Agnuside & viridiflorol	Rana et al., 2005
	<i>Artemisia princeps</i> (Asteraceae) & <i>Cinnamomum camphora</i> (Lauraceae)	Repellent & insecticide	-	Liu et al., 2006
	<i>Mentha</i> sp. & <i>M. piperita</i> (Lamiaceae)	Fumigant	-	Michaelraj et al., 2007; Michaelraj et al., 2008
	<i>Perovskia abrotanoides</i> (Lamiaceae)	Fumigant	Camphor & 1,8-cineole	Arabi et al., 2008
<i>Tagetes minuta</i> & <i>Tagetes patula</i> (Asteraceae)	Fumigant & contact	-	Alok et al., 2005	

Insect	Essential oil	Activity	Active ingredient	Refs.
S. zeamais	<i>Artemisia sieberi</i> (Asteraceae)	Insecticide and repellent	-	Negahban et al., 2006
	<i>Lavandula angustifolia</i> , L. <i>nobilis</i> , <i>R. officinalis</i> , & <i>Thymus vulgaris</i> (Lamiaceae)	Fumigant	1,8-cineole, borneol, & thymol	Rozman et al., 2007
	<i>Eucalyptus camaldulensis</i> , <i>Eucalyptus intertexta</i> & <i>Eucalyptus sargentii</i> (Myrtaceae)	Fumigation	-	Negahban and Moharramipour, 2007
	<i>Cymbopogon citratus</i> & <i>Elyonurus muticus</i> (Poaceae)	Contact	-	Stefanazzi et al., 2011
	<i>Schizonpeta multifida</i> (Lamiaceae)	Fumigant	Pulegone & menthone	Liu et al., 2011
	<i>Ocimum gratissimum</i> (Lamiaceae) & <i>Xylopiya aethiopica</i> (Annonaceae)	Knock down effect	$\beta$ -pinene & terpinen-4-ol	Jirovetz et al., 2005
	<i>Tanacetium nocturnum</i> (Bignoneaceae)	Fumigant & contact	-	Fazolin et al., 2007
	<i>Piper guineense</i> (Piperaceae)	Contact toxicity	$\alpha$ -pinene & $\beta$ -pinene	Tchoumboungang et al., 2009
	<i>Alpinia conchigera</i> , <i>Zingiber zerumbet</i> , & <i>Curcuma zedoaria</i> (Zingiberaceae)	Contact & antifeednat	-	Suthisut et al., 2011
	T. castaneum	<i>Baccharis salicifolia</i> (Asteraceae)	Insecticide & repellent	$\alpha$ -pinene & $\beta$ -pinene
<i>Tagetes terniflora</i> (Asteraceae)		Feeding deterrent	-	Stefanazzi et al., 2006
<i>Artemisia vulgaris</i> (Asteraceae)		Repellent & fumigant	-	Wang et al. (2006)
<i>Piper nigrum</i> (Piperaceae)		Repellent	-	Upadhyay and Jaiswal, 2007
<i>Vepris heterophylla</i> (Rutaceae)		Insecticide	-	Ngamo et al., 2007
<i>Trachyspermum ammi</i> , <i>Anethum graveolens</i> (Apiaceae), & <i>Nigella sativa</i> (Ranunculaceae)		Fumigant & repellent	-	Chaubey, 2007
<i>Cinnamomum cassia</i> (Lutaciae) & <i>Eugenia caryophyllata</i> (Myrtaceae)		Contact & fumigant	Cinnamaldehyde & eugenol	Mondal and Khalequzzaman, 2010
<i>Laurus nobilis</i> (Lauraceae) & <i>R. officinalis</i> (Lamiaceae)	Fumigant	1,8-cineole	Isikber et al., 2006	
<i>Anacyclus cyrtolepidioides</i> (Asteraceae)	Contact	-	Zardi-Bergaoui et al., 2008	
<i>Cymbopogon martini</i> (Poaceae)	Repellent	-	Rajesh et al., 2007	

Insect	Essential oil	Activity	Active ingredient	Refs.
	<i>Cymbopogon winterianus</i> (Poaceae) & <i>Prunus amygdalus</i> (Rosaceae)	Insecticide	-	Al-Jabr, 2006
	<i>Matricaria chamomile</i> (Asteraceae)	Repellent	-	Al-Jabr, 2006
	<i>Perovskia abrotanoides</i> (Lamiaceae)	Fumigant	Camphor & 1,8-cineole	Arabi et al., 2008
	<i>Tagetes minuta</i> & <i>Tagetes patula</i> (Asteraceae)	Fumigant & contact	-	Alok et al., 2005
	<i>Artemisia sieberi</i> (Asteraceae)	Insecticide & repellent	-	Negahban et al., 2006
	<i>Schizopeta multifida</i> (Lamiaceae)	Fumigant	Pulegone & menthone	Liu et al., 2011
	<i>Alpinia conchigera</i> , <i>Zingiber zerumbet</i> , & <i>Curcuma zedoaria</i> (Zingiberaceae)	Contact & antifeednat	Terpinen-4-ol	Suthisut et al., 2011
	<i>Cymbopogon distans</i> (Lamiaceae)	Repellent	Geraniol & citronellol	Zhang et al., 2011
<i>L. serricornis</i>	<i>Origanum acutidens</i> (Lamiaceae)	Fumigant	-	Caglar et al., 2007
	<i>Perilla frutescens</i> , <i>Satureja montana</i> , <i>Thymus vulgaris</i> , & <i>Mentha piperita</i> (Lamiaceae) & <i>Cinnamomum cassia</i> & <i>Litsea cubeba</i> (Lauraceae)	Repellent	$\alpha$ -Terpineol, linalool, & (-)-perillaldehyde	Hori, 2003; 2004
	<i>Nepeta racemosa</i> (Lamiaceae)	Fumigant	-	Aslan et al., 2005
	<i>Pistacia lentiscus</i> (Anacardiaceae)	Fumigant	-	Bachrouch et al., 2010
	<i>Mentha piperita</i> (Lamiaceae)	Fumigant	-	Bakr et al., 2010

<sup>a</sup>This table was summarized using a review paper written by Pérez et al. (2010) and was modified through the addition of several recent data

Table 2. Toxicity of plant essential oils against major economic stored products insect pests<sup>a</sup>

### 3.1 Fumigant toxicity of origanum oil (*O. vulgare*)

Several origanum plant species are used as food additives, sedatives, diuretics, antiseptics, and sweeteners in the treatment of gastrointestinal diseases. They are also rich in bitter substances (Baytop, 1999; Esen et al., 2007). In vapor-phase toxicity bioassay using both closed and open container methods, the insecticidal activity of origanum oil against *T. castaneum* adults was higher in closed containers than in open containers indicating that the activity be exerted by fumigant action (Table 3). In addition, 10 constituents of the origanum oil were *a*-pinene, camphene, myrcene, *p*-cymene,  $\gamma$ -terpinene, linalool, thymol, carvacrol, *a*-thujene, and caryophyllene oxide (Fig. 1) and major components among them were monoterpenes such as carvacrol (67.2%), *p*-cymene (16.2%),  $\gamma$ -terpinene (5.5%), thymol (4.9%), and linalool (2.1%). Daferera et al. (2003) also reported that *O. vulgare* oil from Greece contains thymol (63.7%), *p*-cymene (13.0%) and carvacrol (8.6%) as main components. These results indicate that



*Origanum* plants are largely plentiful sources of thymol, carvacrol,  $\gamma$ -terpinene, and *p*-cymene (Esen et al., 2007; Kordali et al., 2008). Plant essential oils contain very complex natural mixtures or compounds at different concentrations and two or three components at fairly high concentrations (20 to 70%) as main components. These main components generally have a biological property of the essential oil and they are composed of terpenoids and aromatic constituents characterized by low molecular weight (Bakkali et al., 2008). The most frequently found components in most plant essential oils which had strong insecticidal activity to various coleopteran stored products insect pests were monoterpenes such as *a*- or  $\beta$ -pinenes, camphor, 1,8-cineole, and terpinen-4-ol (Table 2). Especially, Lee et al. (2004) showed that *Eucalyptus nicholii*, *E. codonocarpa*, *E. blakelyi*, *Callistemon sieberi*, *Melaleuca fulgens* and *M. armillaris* belonging to the family Myrtaceae found in Australia had potent fumigant toxicity against *S. oryzae*, *T. castaneum*, and *R. dominica*. and the oils contained plentifully 1,8-cineole.

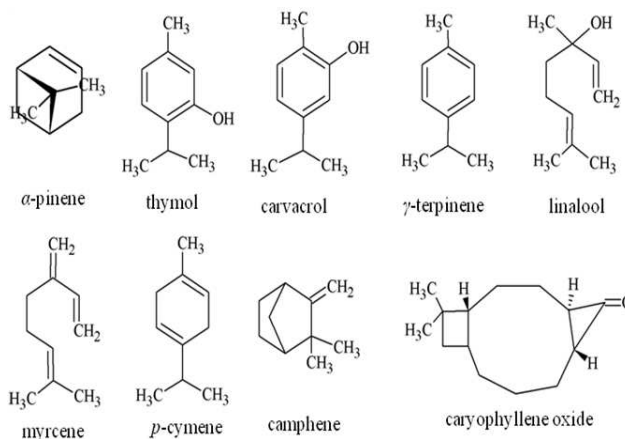


Fig. 1. Chemical structures of terpenoids for toxicity and repellent tests against *Tribolium castaneum* adults.

Method <sup>a</sup>	LD <sub>50</sub> (mg/cm <sup>3</sup> )	Slope ( $\pm$ SE)	95% CL
A	0.055	2.0 ( $\pm$ 0.22)	0.0432-0.0694
B	> 0.353	-	-

<sup>a</sup> A, vapour in closed container; B, vapour in open container.

Table 3. Fumigant toxicity of origanum essential oil against *Tribolium castaneum* adults 24 h after treatment

Carvacrol, *p*-cymene (16.2%),  $\gamma$ -terpinene (5.5%), and thymol derived from *Origanum* genus plants exerted both contact and fumigant toxicities to *T. castaneum* (Prates et al., 1998; Garcia et al., 2005) and similar results were obtained from our study (Kim et al., 2010). In the tests to evaluate the fumigant activities of *a*-pinene, camphene, myrcene, *p*-cymene,  $\gamma$ -terpinene, linalool, thymol, carvacrol, and caryophyllene oxide against *T. acastaneum* adults, the toxicity of caryophyllene oxide (LC<sub>50</sub>, 0.00018 mg/cm<sup>3</sup>) was comparable with that of dichlorvos (LC<sub>50</sub>, 0.00007 mg/cm<sup>3</sup>), and thymol, camphene, *a*-pinene, *p*-cymene, and  $\gamma$ -terpinene showed highly effective activity (LC<sub>50</sub>, 0.012-0.195 mg/cm<sup>3</sup>) [Table 4].

Interestingly, *T. castaneum* adults exposed to higher doses (0.18-0.353 mg/cm<sup>3</sup>) of the organum oil responded retarded behaviors such as random walking and wandering and then the exposed adults with body color of much darker brown died after 6 hours. This tendency was observed in tests with the active constituents from the *O. vulgare* essential oil. The strong toxicity of this origaum oil and its components with high volatility may result from the change of respiration rate of *T. castaneum* adults by the inhibition of the mitochondrial electron transport system. Emekci et al. (2002) reported that changes in the concentration of oxygen or carbon dioxide may elicit fumigant action by affecting respiration rate of immature stages of *T. castaneum*.

Material	Retention time, min	Relative composition ratio, %	LD <sub>50</sub> (mg/cm <sup>3</sup> )	Slope (±SE)	95% CL <sup>a</sup>
<i>a</i> -pinene	7.675	1.58	0.114	7.6 (±1.33)	0.0998-0.1274
Camphene	8.046	0.47	0.072	3.9 (±0.48)	0.0619-0.0833
<i>β</i> -myrcene	9.093	1.14	> 0.353	-	-
<i>p</i> -cymene	9.957	16.16	0.140	8.5 (±1.86)	0.1218-0.1563
<i>γ</i> -terpinene	10.828	5.52	0.195	6.7 (±0.94)	0.1784-0.2133
Linalool	11.845	2.13	> 0.353	-	-
Thymol	16.480	4.85	0.0012	2.7 (±0.41)	0.0009-0.0016
Carvacrol	16.733	67.23	> 0.353	-	-
Caryophyllene oxide	22.745	0.37	0.00018	1.9 (±0.39)	0.00007-0.00028
Dichlorvos	-	-	0.00007	3.5 (±0.59)	0.00004-0.00008

<sup>a</sup> CL, confident limit.

Table 4. Contact and fumigant toxicity of constituents identified from organum essential oil by gas chromatography coupled with mass spectroscopy (GC-MS) against *Tribolium castaneum* adults 24 h after treatment

In another test to determine the repellency of the organum oil and its constituents by using an area preference method against *T. castaneum* adults, the oil showed strong activity (98%) at 0.03 and 0.006 mg/cm<sup>2</sup> but was decreased rapidly at 0.001 mg/cm<sup>2</sup> (Fig. 2A to F). In addition, caryophyllene oxide and *a*-pinene gave 85 and 82% at 0.001 mg/cm<sup>2</sup>, respectively and hydrogenated monoterpenoids such as thymol, carvacrol, and myrcene also showed more than 77% at 0.03 and 0.006 mg/cm<sup>2</sup> (Fig. 2A to E). These results suggest that the organum essential oil exerting strong toxic effect also show high repellency against *T. castaneum* adults and the repellency of compounds such as sesquiterpene oxide (caryophyllene oxide) or monoterpene phenols (thymol & carvacrol) is much stronger than monoterpenes except for *a*-pinene and myrcene (Fig. 2E). Wang et al. (2009) also observed that *β*-pinene had both the strongest toxicity and the highest repellency against *T. castaneum* adults. However, the toxicity and repellency of the origaum oil were significantly decreased by concentration and exposed time. Especially, the activities of caryophyllene oxide that gave the strongest fumigant toxicity and repellency were depended on concentration (F=17.02, P = 0.0001), time (F=6.49, P = 0.0023), and concentration-time factors (F=2.88, P = 0.0292).

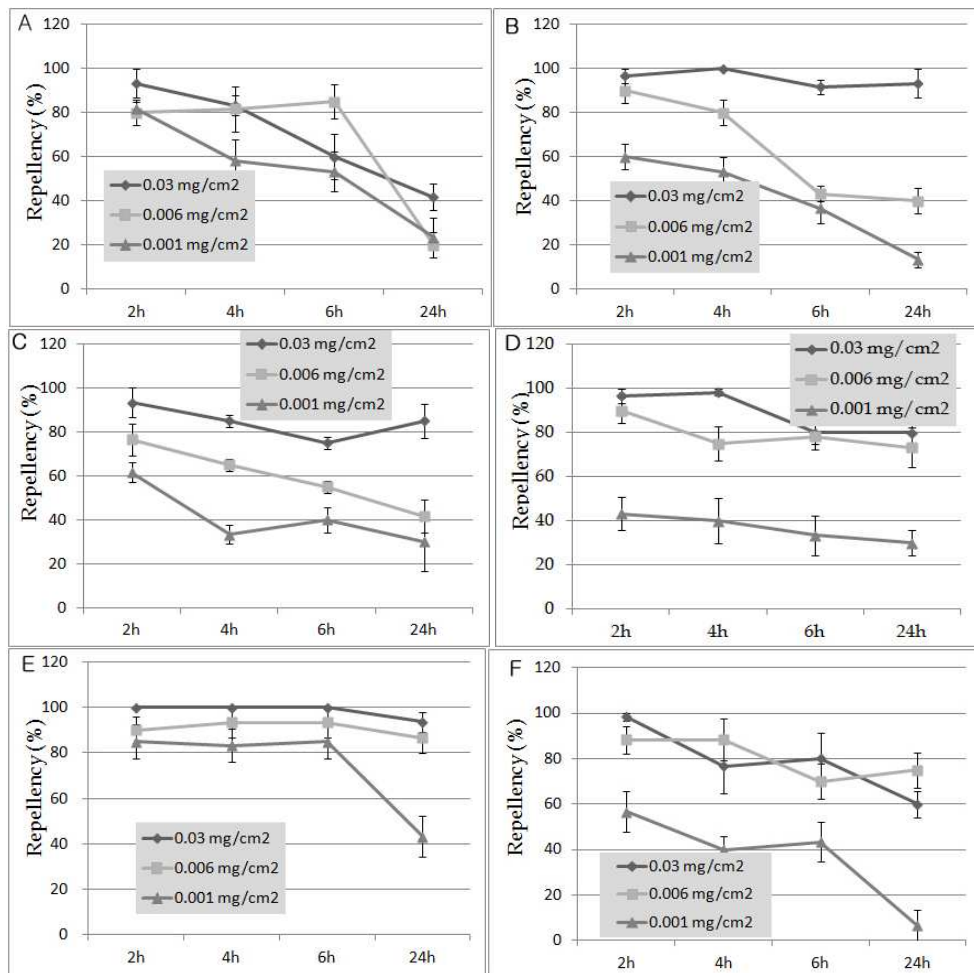


Fig. 2. The repellency of main constituents, *a*-pinene (A), myrcene (B), carvacrol (C), thymol (D), caryophyllene oxide (E), and the origanum essential oil (F) was observed 2, 4, 6, and 24 h after treatment at three concentrations against *Tribolium castaneum* adults. Repellency was calculated as follows: repellency, % = ((C-T)/(C+T))×100. The activity depended on concentration (F=17.02, P = 0.0001), time (F=6.49, P = 0.0023), and concentration-time factors (F=2.88, P = 0.0292). Twenty adults were used per replicate; 3 replicates per treatment (n = 60).

### 3.2 Fumigant toxicity of horseradish oil (*C. armoracia*)

Horseradish, *C. armoracia* (Brassicaceae), has been exclusively used in Japanese and Korean raw fish to provide the pungent property of its edible root. This plant species contains various volatile compounds such as allyl and butyl isothiocyanates (Ina et al., 1981). Allyl isothiocyanate is utilized as a spice and a preserver by the food industry and

is classified as generally regarded as safe (GRAS) by the Food and Drug Administration (FDA) of the United States (Isshiki et al., 1992). The distillate contained approximately 90% allyl isothiocyanate which completely inhibited the growth of *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Salmonella typhimurium*, *Listeria monocytogenes*, and *Serratia grimesii* on agar for seven days in aerobic storage at 12 °C. However, little work has been done to investigate their ability to control stored product insects, although insecticidal activity of the *Cochlearia* essential oil against *L. serricornis* adults was noted (Kim et al., 2003). When *C. armoracia* essential oil was tested to the adults of *S. zeamais*, *C. chinensis*, and *L. serricornis* and the larvae of *A. unicolor japonicus* using direct contact application, significant differences were observed in the mortality of the insects ( $n = 100$ ) (Table 5). A high mortality of 100% was occurred when *S. zeamais* adults were dosed at a rate of 0.35 mg/cm<sup>2</sup> for 24 h. Against *C. chinensis* adults, the essential oil gave 100% mortality when dosed at a rate of 0.18 mg/cm<sup>2</sup> for 24 h. A high mortality of 100% was occurred when *L. serricornis* adults were dosed at a rate of 0.35 mg/cm<sup>2</sup> for 24 h. Against *A. unicolor japonicus* larvae, the oil caused 93% mortality when dosed at a rate of 1.05 mg/cm<sup>2</sup> for 24 h. These results showed that *C. chinensis* adults were the most susceptible and *A. unicolor japonicus* larvae were the most tolerant to the oil.

Dose, mg/cm <sup>2</sup>	Mortality (%) mean±SE <sup>a</sup>			
	<i>S. zeamais</i>	<i>C. chinensis</i>	<i>L. serricornis</i>	<i>A. unicolor japonicus</i>
1.05	-. <sup>b</sup>	-	-	93±7a
0.70	-	-	-	10±2b
0.35	100±0a	-	100±0a	
0.18	27±7b	100±0a	40±6b	
0.09	0±0c	33±3b	0±0c	

<sup>a</sup> Means within a column followed by the same letter are not significantly different at  $P = 0.05$  (Scheffe's test).

<sup>b</sup> -, not determined.

Table 5. Insecticidal activity of *Cochlearia armoracia* essential oil against four coleopteran stored product insects ( $n = 100$ ) exposed for 24 h, using filter paper diffusion method

In another test using allyl and butyl isothiocyanates, the toxicity of these isothiocyanate against *S. zeamais* adults, *C. chinensis* adults, *L. serricornis* adults, and *A. unicolor japonicus* larvae varied according to dose and insect species (Table. 6). The two compounds caused 100% mortality of *S. zeamais* and *C. chinensis* adults at 0.07 mg/cm<sup>2</sup>. Against *L. serricornis* adults, the two isothiocyanates caused 100 and at least 85% mortality at 0.14 and 0.07 mg/cm<sup>2</sup> respectively, but the mortality was significantly reduced at 0.04 mg/cm<sup>2</sup>. Against *A. unicolor japonicus* larvae, allyl and butyl isothiocyanates both showed >90% mortality at 0.35 mg/cm<sup>2</sup> but 53% mortality at 0.21 mg/cm<sup>2</sup>, respectively.

Compound	Dose, mg/cm <sup>2</sup>	Mortality (%) mean±SE <sup>a</sup>			
		<i>S. zeamais</i>	<i>C. chinensis</i>	<i>L. serricornis</i>	<i>A. unicolor japonicus</i>
Allyl isothiocyanate	0.35	- <sup>b</sup>	-	-	97±2a
	0.21	-	-	-	53±1b
	0.14	-	-	100±0a	17±2cd
	0.07	100±0a	100±0a	85±3b	3±1e
	0.04	27±3b	92±1b	18±2c	0±0e
	0.02	7±2c	25±3c	0±0d	-
Butyl isothiocyanate	0.35	-	-	-	93±3a
	0.21	-	-	-	73±3b
	0.14	-	-	100±0.0a	50±6b
	0.07	100±0.0a	100±0.0a	88±1.9b	23±3c
	0.04	38±1.9b	98±1.9ab	12±2.0c	7±2de
	0.02	6±2.7c	19±2.8c	0±0.0d	0±0e

<sup>a</sup> Means within a column followed by the same letter are not significantly different at  $P = 0.05$  (Scheffe's test).

<sup>b</sup> -, not determined.

Table 6. Insecticidal activity of allyl isothiocyanate and butyl isothiocyanate against four coleopteran stored product insect pests ( $n = 100$ ) exposed for 24h, using filter paper diffusion method

In a fumigation test, susceptibility of *C. cautella* larvae, *S. oryzae* adults, *L. serricornis* adults, and *A. unicolor japonicus* larvae to fumigant action of two isothiocyanates from horseradish oil was evaluated using a bioassay system. Briefly, groups of twenty beetle adults or larvae were placed in diet cups (3.6 cm diameter × 4 cm) covered with 60-mesh cloth. Each filter paper (Whatman No. 2, 4.7 cm diameter) treated with each component of horseradish oil in 100 ul of methanol was placed in the bottom of the polyethylene cup (4.7 cm diameter × 8.4 cm), and then the diet cup was put into the polyethylene cup with a lid (method A), or without a lid (method B) in order to prevent direct contact of the tested insects with each materials. In the experiment for direct contact of the insects with test material, each filter paper treated with each test component in 100 ul of methanol was placed in the bottom of the polyethylene cup, and then test insects were placed in each cup either with a lid (method C), or without a lid (method D). Controls received 100 ul of methanol (Fig. 3).



Fig. 3. Bioassay system used for fumigant test of allyl and butyl isothiocyanates against four stored products insect pests.

Responses of each insect species varied with both treatment method and insect species (Table 3). There was significant difference ( $P = 0.05$ ) in insecticidal activity of the two components between with lids (A) and without lids (B) when there was no contact of the insects with filter paper treated with them. In above systems, four insects showed 100% mortality with method A but produced less than 40% mortality with method B. In case of direct contact, significant difference in insecticidal activity of the components between with lids (C, 100% mortality) and without lids (D) was also observed. In these systems, all tested insects showed similar results: allyl and butyl isothiocyanates were much more effective in closed containers with lids (A & C) than in open ones without lids (B & D), indicating that the mode of delivery of these compounds was largely due to action in the vapour phase, as for fumigants (Table 7). Additionally, the adults died by the oil and active components showed specific symptoms: they have trembled their legs or folded their forelegs toward thorax, and even *C. chinensis* adults have unfolded their inner wings.

Method <sup>b</sup>	Mortality (mean±SE, %)					
	<i>S. oryzae</i> <sup>a</sup>		<i>C. chinensis</i> <sup>a</sup>		<i>A. unicolor japonicus</i> <sup>b</sup>	
	AITC	BITC	AITC	BITC	AITC	BITC
A	100a	100a	100a	100a	100a	98±2.0a
B	30±7.1b	6±2.5b	60±2.5b	10±3.2b	40±2.4bc	8±3.7c
C	100a	100a	100a	100a	100a	100a
D	34±11.4b	2±2.0b	24±2.5b	22±3.7b	12±3.7b	62±3.7b

<sup>a, b</sup> Exposed to 0.21 and 0.70 mg/cm<sup>2</sup> of allyl isothiocyanate and butyl isothiocyanate.

Table 7. Fumigant activity of allyl isothiocyanate (AITC) and butyl isothiocyanate (BITC) against four stored product insects ( $n = 100$ ) exposed for 24h

Many studies have carried out to utilize allyl isothiocyanate as a fumigant, and it showed effectiveness when was applied to control coleopteran stored insect pests such as *L. serricornis* and *T. confusum* (Worfel et al., 1997), *Ryzopertha dominica* (Tsao et al., 2002), *S. oryzae* (Dilawari et al., 1991), and *S. zeamais* and *L. entomophila* (Wu et al., 2009). Besides

insecticidal activity, allyl isothiocyanate is found to possess attractant effect to *Delia brassicae* (Wallbank & Wheatley, 1979), repellent effect against *Culicoides impunctatus* (Blackwell et al., 1997), and antimicrobial activity to infectious bacteria and fungi (Mayton et al., 1996; Delaquis & Sholberg, 1997). Additionally, allyl isothiocyanate has low acute toxicity to mammals (Budavari et al., 1989), although this compound is known to have a mutagenic effect on *Salmonella typhimurium* (Azizan & Blevins, 1995). Allyl isothiocyanate from horseradish oil have been received global attention due to their pesticidal properties and potential to protect several food commodities, but there are few results about its insecticidal activity. Results of this and earlier studies indicate that the *Cochlearia* essential oil-derived materials might be useful for managing adults of *S. zeamais*, *C. chinensis*, and *L. serricornis*, and larvae of *A. unicolor japonicus* in enclosed spaces such as storage bins, glasshouses or buildings because of their fumigant action, provided that a carrier giving a slow release of active material can be selected or developed.

#### 4. Plant extracts as alternative insecticides

The practical use of plant extracts, derivatives or powders as insecticides can be traced back at least 4,000 years (Thacker, 2002). Ancient Indians and Egyptians in 2,000 and 1,000 BC, recognized plants as sources of poisoners and insecticidal compounds for pest control, respectively. Modern plant insecticides, a powder obtained from the dry flowers of pyrethrum plant, was used for control of head lice in children (Addor, 1995). Although a few plant extracts including the pyrethrum had shown promising effects after the Second World War, they were replaced by the introduction of synthetic chemical insecticides. However, due to biodegradable and relatively safe properties of plant insecticides, it leads to revival of growing interest in the use of plant extracts in modern agrochemical researches. Especially, many studies to find and identify insecticidal activity of oriental medicinal plant extracts have been done and the limited regional use of several plant extracts like *Capsicum* oleoresin became the limited regional exists in organic cultivated crops. In accordance with this trend, we evaluated the insecticidal activity of *A. gramineus* against *S. zeamais*, *C. chinensis* and *L. serricornis* adults and the antifeedant activities of some plant extracts against *A. unicolor japonicus* larvae.

##### 4.1 The insecticidal activity of *A. gramineus* materials

The rhizome from *A. gramineus* (Araceae) has long been considered to have medicinal properties such as a digestant, an expectorant, and a stimulant against digestive disorders, diarrhea, and epilepsy (Balakumbahan et al., 2010). It contains  $\beta$ -Asarone or (Z)-asarone which was the major constituent in the leaves (27.4 to 45.5%), whereas acorenone was dominant in the rhizomes (20.9%) followed by isocalamendiol (12.75%) (Venskutonsis et al., 2003). (Z)- and (E)-asarones identified in *A. gramineus* rhizome showed strong insecticidal activities to *Nilaparvata lugens* females and *Plutella xylostella* larvae, although the activity of (Z)-asarone was higher than that of (E)-asarone (Lee et al., 2002). Besides these compounds, (E)-asarone (8-14%), caryophyllene (1-4%), isoasarone (0.8-3.4%), (Z)-methyl isoeugenol (0.3-6.8%) and safrol (0.1-1.2%) were also identified in East Asia (Tang & Eisenbrand, 1992; Namba, 1993).

In a direct contact application using fractions from the methanol extract of *A. gramineus* rhizome, hexane fraction at 0.51 mg/cm<sup>2</sup> showed 100% mortality against adults of *S. zeamais*

and *C. chinensis* but 57% mortality against *L. serricornis* adults (Park, 2000). The insecticidal activities of methanol extract of the *Acorus* rhizome against adults of *S. zeamais* and *C. chinensis* were reported (Hill & Schoonhoven, 1981). The insecticidal activities of the *Acorus* rhizome-derived active constituents, which were characterized as the phenylpropenes (*Z*- and (*E*)-asarones, against *S. zeamais*, *C. chinensis*, and *L. serricornis* adults were tested using direct contact bioassay under laboratory conditions (Table 8). Responses depended on both the compound and exposure time, but there was no significant difference in the toxicity among the doses. Namely, in a filter paper diffusion test, (*Z*)-asarone showed strong toxicity (60-100% mortality) against *S. zeamais* adults at all the tested doses at 3 to 7 days after treatment (Table 8), but (*E*)-asarone at 0.255 mg/cm<sup>2</sup> caused only 37% activity even at 7 days after treatment. In a case of *C. chinensis* adults at a rate of 0.064 mg/cm<sup>2</sup>, (*Z*)- and (*E*)-asarones gave 100% mortality at 3 and 7 days after treatment, respectively. At 0.255 and 0.064 mg/cm<sup>2</sup> of (*Z*)-asarone against *L. serricornis* adults, it showed 90 and 83% mortality at 7 days after treatment, whereas (*E*)-asarone at 0.255 mg/cm<sup>2</sup> had weak insecticidal activity even at 7 days after treatment. These results showed that the toxicity of (*Z*)-asarone against *S. zeamais*, *C. chinensis*, and *L. serricornis* adults was much higher activity than (*E*)-asarone and the responses of *C. chinensis* and *L. serricornis* adults to the asarones were the most susceptible tolerant, respectively (Table 8). Thus, the differences in toxicity of asarones to the coleopteran insect pests might be due to the *cis* and *trans* configuration. Similar results were obtained from a study using ethanol extract of *A. calamus* (Yao et al., 2008). The ethanol

Compo und	Dose, mg/cm <sup>2</sup>	Mortality (%) (±SE) <sup>a</sup>								
		3 DAT <sup>b</sup>			4 DAT			7 DAT		
		<i>S.</i> <i>zeamais</i>	<i>C.</i> <i>chinensi</i> <i>s</i>	<i>L.</i> <i>serricor</i> <i>ne</i>	<i>S.</i> <i>zeamai</i> <i>s</i>	<i>C.</i> <i>chinens</i> <i>is</i>	<i>L.</i> <i>serricor</i> <i>ne</i>	<i>S.</i> <i>zeamais</i>	<i>C.</i> <i>chinens</i> <i>is</i>	<i>L.</i> <i>serricorn</i> <i>e</i>
(Z)- asarone	0.255	60±0.0a	100a	20±0.0a	90±2.9 a		40±0.0a	100a		90±0.0a
	0.127	60±0.0a	100a	17±3.3a b	83±3.3 a		43±3.3a	100a		87±1.7a
	0.064	60±0.0a	100a	10±0.0 b	70±2.9 a		40±0.0a	100a		83±3.3a
(E)- asarone	0.255	0b	0b	0c	7±3.3b	47±3.3a	0b	37±3.3 b	100a	33±3.3b
	0.127	0b	0b	0c	3±3.3b	50±0.0a	0b	30±0.0 b	100a	27±3.3b
	0.064	0b	0b	0c	7±3.3b	33±1.6 b	0b	33±3.3 b	100a	30±0.0b

<sup>a</sup> Means within a column followed by the same letter are not significantly different ( $P = 0.05$ , Scheffe's test) (20 adults per replicate; 3 replicates per treatment:  $n = 60$ ). Mortalities were transformed to arcsine square-root before ANOVA. Means (±SE) of untransformed data are reported.

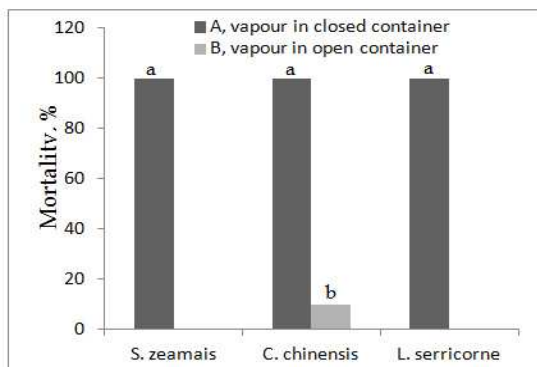
<sup>b</sup> Days after treatment.

Table 8. Insecticidal activity of *Acorus gramineus* rhizome-derived compounds against *Sitophilus zeamais*, *Callosobruchus chinensis*, and *Lasioderma serricornis* adults using direct contact application



extract strong repellency and contact effect to *S. zeamais* and the active constituent, (Z)-asarone also showed 100% mortality and 85% repellency at 40.89 and 314.54  $\mu\text{g}/\text{cm}^2$  at 12 h after treatment, respectively. (Z)-asarone from hexane extract of *Daucus carota* seed possesses significant weight reductions of *Helicoverpa zea*, *Heliothis virescens*, and *Manduca sexta* larvae (Momin & Nair, 2002), insecticidal activities to adults of *Nilaparvata lugens* and larvae of *Plutella xylostella*, and attractant effect for the oriental fruit flies (Jacobson et al., 1976), while (E)-asarone has antifeeding activity against *Peridroma saucia* (Koul et al., 1990) and oviposition-stimulating effect for *Psila rosae* (Städler & Buser, 1984).

In a fumigation test to determine whether the adulticidal activity of (Z)-asarone at 0.577  $\text{mg}/\text{cm}^2$  against *S. zeamais* was attributable to fumigant action or not, it was much more effective in closed cups than in open ones (Fig. 4). Similar results were obtained with adults of *C. chinensis* and *L. serricorne*, indicating that the insecticidal activity of the compound was largely attributable to fumigant action via vapor phase. The fumigant action of the phenylpropenes (E)-anethole, estragole, and asarones against adults of *S. zeamais*, *C. chinensis*, and *L. serricorne* has been early known (Ahn et al., 1998; Park, et al., 2000). Ahn et al. (1998) reported that carvacrol is highly toxic to adults of three coleopteran insect pests (*S. oryzae*, *C. chinensis*, and *L. serricorne*) and nymphs of the termite (*Reticulitermes speratus*) and exhibits insecticidal activity in the vapor phase. In terms of these results, *A. gramineus* rhizome-derived materials as naturally occurring insect-control agents could be useful for managing *S. zeamais*, *C. chinensis*, and *L. serricorne* adults.



Means within a column followed by the same letter are not significantly different ( $P = 0.05$ , Scheffe's test) (10 adults per replicate; 3 replicates per treatment;  $n = 30$ ).

Fig. 4. Fumigant activity of (Z)-asarone derived from *Acorus gramineus* rhizome against adults of three coleopteran stored products insect pests

#### 4.2 Antifeedant activity of aromatic plant extracts

In many literatures, antifeedant term has been frequently found but we use the term only when a plant source inhibits feeding initiation or continuation of a target insect, or the insect is killed by no feeding in this review. Many lines of research on antifeedants have been carried out almost in laboratory conditions using two-choice tests against usually one species. Recently, Du et al. (2011) reported that the ethanol extract of *Cerriops tagal* stems and

twigs possessed significant feeding deterrent against the red flour beetle, *T. castaneum*. In addition, most experimental insect species are lepidopteran larvae such as armyworms (*Spodoptera* spp.), budworms (*Heliothis* spp.), cabbage white butterflies (*Pieris* spp.), or locusts (*Locusta migratoria*). The monoterpene, camphor and 1,8-cineole are well known feeding deterrents to budworms (*Heliothis virescens* & *Anthonomus grandis*) and *T. castaneum* (Tripathi et al., 2001) and capsaicin derived from *Capsicum* spp. is a good repellent to *S. zeamais* (Ho & Ma, 1995). In another study with four enantiomeric pairs of  $\gamma$ -lactones, a terpenoid lactone exhibited antifeedant activity toward grain storage pests: the granary weevil beetle (*S. granarius*), the khapra beetle (*Trogoderma granarium*), and the confused flour beetle (*T. confusum*). Muzigadial like ugnadensidial and warburganal, and polygodial are a sesquiterpene from *Warburgia* spp. and originally isolated from the waterpepper plant (*Polygonum hydropiper*), respectively, showed potent antifeedant activity against the Australian carpet beetle (*Anthrenocerus australis*) at a 0.04% wool weight (Gerard et al., 1992). In addition, a neem extract containing azadirachtin as well as several other neem compounds (e.g. nimbin and azadirone) had antifeedant activity against the *A. australis* at as low as 0.01% wool weight for at least 14 days after treatment (Gerard et al., 1992). Although many studies on antifeedants have been carried out using various plant species, there are few researches on antifeedant activity of oriental medicinal plants against *A. unicolor japonicus*.

We determined the antifeedant activity of oriental aromatic plant extracts against the larvae of *A. unicolor japonicus* exposed to 5.2 mg/cm<sup>2</sup> for 28 days after treatment using a fabric impregnated application (Table 9). In this test, many plant extracts showed good antifeedant activity to the Japanese black carpet beetle (Han et al., 2006). Especially, the methanol extracts of *Angelica dahurica* root, *Cnidium officinale* rhizome, *Dryobalanops aromatica* resin, *Pterocarpus indicus* heart wood, *Allium sativum* rhizome, *Illicium verum* fruit, *Eugenia caryophyllata* flower bud, *Lysimachia davurica* whole plant, *Zanthoxylum schinifolium* fruit, *Nardostachys chinensis* rhizome, and *Kaempferia galanga* whole plant revealed complete antifeedant activity. These plants also kept 100% protection effect by antifeedant at a rate of 2.6 mg/cm<sup>2</sup> and five active plant extracts (*A. dahurica*, *E. caryophyllata*, *L. davurica*, *N. chinensis*, and *K. galanga*) showed complete protection activity even at 1.04 mg/cm<sup>2</sup> (Table 9). As expected, several plants such as *E. caryophyllata*, *D. aromatica*, *A. sativum*, and *I. verum* keeping potent antifeedant activity also gave 77-100% mortality at 1.04-5.2 mg/cm<sup>2</sup> within 28 days exposure periods. Although plants showing potent insecticidal activity give good antifeedant activity, it is unlikely to have a linear relationship between insecticidal and antifeedant activity of aromatic plants, because plants such as *A. dahurica*, *L. davurica*, *N. chinensis*, and *K. galanga* showing 100% antifeedant activity at 1.04 mg/cm<sup>2</sup> showed weak or a little insecticidal activity (Table 9).

Because several plants are complete antifeedants, we designed another test to determine the mixture or synergic effect of binary mixtures composed of *A. dahurica*, *I. verum*, *A. sativum*, and *D. aromatica*. Prepared binary mixtures were as follows: *A. dahurica* + *I. verum*, *A. dahurica* + *A. sativum*, *A. sativum* + *I. verum*, and *D. aromatica* + *A. sativum* (1:1, w/w; final dose 1.04 mg/cm<sup>2</sup>). Antifeedant activities of the binary mixtures against the carpet beetle larvae at 31 days were more than 95% except for the mixture of *A. sativum* + *I. verum* (Table 10). Additionally, the binary mixture of *D. aromatica* + *A. sativum* gave 77% mortality at 28 days after treatment but the other mixtures did not show good mortality (Table 10).

Plant	Dose, mg/cm <sup>2</sup>					
	5.2 <sup>a</sup>		2.6		1.04	
	DA, mg	AI, %	DA, mg	AI, %	DA, mg	AI, %
<i>Angelica dahurica</i>	0±0.0a	100	0±0.0a	100	0±0.0a	100
<i>Cnidium officinale</i>	0±0.0a	100	0±0.0a	100	4.6±1.5abc	93
<i>Foeniculum vulgare</i>	3.0±1.0ab	95	19.7±2.4b	67	19.7±2.4b	68
<i>Acorus calamus</i> var. <i>angustatus</i>	4.2±0.5ab	93	1.2±0.6ab	98	8.4±4.3abc	86
<i>Acorus gramineus</i>	3.6±0.2abc	94	3.5±0.2ab	94	55.2±4.2d	11
<i>Boswellia carterii</i>	9.0±1.4b-e	85				
<i>Artemisia princeps</i> var. <i>orientalis</i>	0.7±1.1abc	92	4.3±1.3bc	93	16.5±6.4abc d	73
<i>Inula helenium</i>	4.8±1.7abc	93	8.7±5.1bc	85	30.8±7.5bcd	50
<i>Brassica juncea</i>	0±0.0a	100	0±0.0a	100	0.1±0.1a	100
<i>Dioscorea batatas</i>	19.7±1.5d-f	67				
<i>Dryobalanops aromatica</i>	10.8±1.1b-e	82				
<i>Agastache rugosa</i>	4.7±0.6a-d	92	6.5±0.8ab	89	20.5±2.5abc d	67
<i>Schizonepeta tenuifolia</i>	10.9±0.2b-e	82				
<i>Pterocarpus indicus</i>	0±0.0a	100	0±0.0a	100	1.7±1.4cd	97
<i>Allium sativum</i>	0±0.0a	100	0.5±0.4a	99	33.8±11.3cd	46
<i>Illicium verum</i>	0±0.0a	100	44.4±8.2bc	24	38.7±10.1ab	38
<i>Eugenia caryophyllata</i>	0±0.0a	100	0±0.0a	100	0±0.0a	100
<i>Paeonia suffruticosa</i>	14.4±1.2b-e	76				
<i>Rheum coreanum</i>	22.1±2.3de	63				
<i>Lysimachia davurica</i>	0±0.0a	100	0±0.0a	100	0±0.0a	100
<i>Chaenomeles sinensis</i>	8.4±1.4efg	86				
<i>Evodia rutaecarpa</i>	12.0±1.2b-e	80				
<i>Zanthoxylum piperitum</i>	10.2±1.2abc	83				
<i>Zanthoxylum schinifolium</i>	0±0.0a	100	0±0.0a	100	5.3±1.0bcd	91
<i>Capsicum annuum</i>	11.9±2.4b-e	80				
<i>Stemona japonica</i>	17.3±2.2c-f	71				
<i>Aquillaria agallocha</i>	4.2±1.2abc	93	5.9±2.3ab	90	9.1±4.8bcd	85
<i>Nardostachys chinensis</i>	0±0.0a	100	0±0.0a	100	0±0.0a	100
<i>Kaempferia galanga</i>	0±0.0a	100	0±0.0a	100	0±0.0a	100
Control	59.8±1.5h	0	58.5±2.9c	0	62.0±1.4d	0

<sup>a</sup> DA, amount damaged by the Japanese black carpet beetle; AI (antifeedant index, %) = feeding weight of control - feeding weight of treatment/feeding weight of control ×100.

Table 9. Antifeeding activities of aromatic plant extracts against *A. unicolor japonicus* larvae at 28 days after treatment, using fabric impregnated application

Mixture <sup>a</sup>	Mortality (%) Mean±SE				DA, mg	AI, %
	7DAT	14DAT	21DAT	28DAT		
<i>A.dahurica</i> + <i>I. verum</i>	0±0.0b	0±0.0b	0±0.0b	0±0.0b	1±0.3a b	98
<i>A.dahurica</i> + <i>A. sativum</i>	0±0.0b	0±0.0b	7±3.3b	7±3.3b	3±0.18 b	95
<i>A. sativum</i> + <i>I. verum</i>	0±0.0b	0±0.0b	3±3.3b	3.3±3.3b	29±5.1 c	51
<i>D. aromatica</i> + <i>A. sativum</i>	50±10.0a	73±8.8a	77±8.8a	77±8.8a	0±0.0a	100
Control	0±0.0b	0±0.0b	0±0.0b	0±0.0b	59±3.5 d	0

<sup>a</sup> Exposed to 1.04 mg/cm<sup>2</sup>.

Table 10. Insecticidal and antifeedant activities of mixtures composed of 4 plant extracts against *A. unicolor japonicus* larvae using fabric impregnated application, exposed for 31 days

Results of this and earlier studies indicate that some plant extracts might be useful control or antifeedant agents for managing *A. unicolor japonicus* in appropriately enclosed systems such as storage bins, glasshouses or buildings. It needs to be carried out much further study on the investigation of insecticidal constituents against the carpet beetle from methanol extracts of the active plants, insecticidal mode of action of the constituents and appropriate formulation types for their utilization in grain stores or enclosed spaces. However, considering to commercially utilize a plant extract as insect antifeedant as follows, we have to recognize that plant extracts may exert different activities according to extracted or used solvents. For example, the methanol extract of *Cyperus articulatus* rhizome, which an insect repellent plant commonly found in Northern Nigeria and used traditionally in pest control, showed more antifeedant property than the light petroleum extract against *T. castaneum* (Abubakar et al., 2000).

## 5. Conclusions and future perspectives

We are facing challenges associated with the increasing global human population and also with the control of insect pests. This problem occurred by insect pests is not new because their appearance period is much prior to the appearance of human beings on the earth, 500 million vs 100,000 years. Thus, it should be reasonable results that we have not been able to manage completely the problems that a variety of insect pests caused. To control stored products insect pests, our ancestors have been anecdotally using a variety of plant species. Based on this knowhow and knowledge, farmers in developing countries often use backyard-grown or naturally occurring plant materials for insect management practices but the use on the commercial scale of plant extracts or whole plant materials in insect control field is not succeeded. The most difficult barriers in the commercialization of these botanical insecticides or plant extracts including essential oils are the lack of consistent efficacy, the difficulty in mass security of a targeted natural resource and in establishment of chemical standardization and quality control, sometimes odor and safety concerns, and difficulties in preparing lots of documents for registration. In spite of these problems, neem from *A. indica*

and pyrethrum from *Chrysanthemum cinerariifolium* are commercially available plant extracts and in some parts of the world, some formulations have been used to control stored products insect pests (Koul et al., 1990).

Some plant extracts, essential oils or their constituents described in this review have demonstrated high efficacy against coleopteran stored products insect pests responsible for post-harvest damage. As such, they may have considerable potential as fumigants for pest management from killing effect to antifeeding activity. According to currently accumulated information, these plant extracts are safe to the farmer as well as the environment. However, the efficacy duration of these plant derived-materials generally falls short comparing with that of conventional pesticides and then causes frequent application or greater application rate, although they produce comparative effects to some specific pests under controlled conditions such as glass houses. In addition, there are several problems including mass production of a plant source, quality control, etc. to overcome for commercialization based on plant extracts or essential oils. Firstly, it is necessary to develop technically and economically sound formulation methods to solve the barriers in the path of commercialization.

Nevertheless, pesticides from aromatic oriental plant extracts have several benefits. Their high volatility not only provides relatively much lower level of risk to applied environment than synthetic pesticides, but also gives less impact to non-target insects like predators and pollinators due to the minimum residual effect. Additionally, insect resistance to botanical based insecticides will develop more slowly or not because they contain complex mixtures or constituents. Ultimately, plant based insecticides will find their commercial position in the management of stored products considering their mode of action, fumigation. Of course, they will be most useful for public health, urban pest control, greenhouse crops, organic food production fields, etc. To promote the potential of plant materials to reduce the use of synthetic insecticides in current agricultural practices, it needs to be studied on their selectivity to various insect pests and non-target invertebrates as well as mode of action using molecular biological or biochemical techniques.

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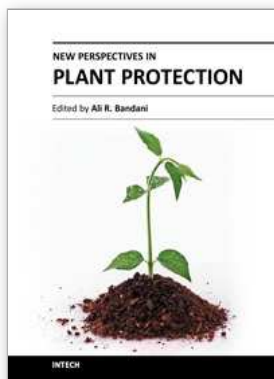


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## **New Perspectives in Plant Protection**

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Crop losses by pests (insects, diseases and weeds) are as old as plant themselves but as agriculture are intensified and cropping patterns including the cultivation of high yielding varieties and hybrids are changing over time the impact of the pests becoming increasingly important. Approximately less than 1000 insect species (roughly 600-800 species), 1500 -2000 plant species, numerous fungal, bacterial and nematode species as well as viruses are considered serious pests in agriculture. If these pests were not properly controlled, crop yields and their quality would drop, considerably. In addition production costs as well as food and fiber prices are increased. The current book is going to put Plant Protection approaches in perspective.

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