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CHEMICAL COMPOSITION AND FUMIGANT TOXICITY OF ESSENTIAL OILS ISOLATED FROM EGYPTIAN PLANTS AGAINST STORED PRODUCT INSECTS *SITOPHILUS ORYZAE* (L.) AND *TRIBOLIUM CASTANEUM* (HERBST)

Samir A.M. Abdelgaleil[1*] Mohamed E.I. Badawy[1], Magdy I.E. Mohamed[2], Mohamed S. Shawir[1]

[1]Department of Pesticide Chemistry and Technology, Faculty of Agriculture, 21545-El-Shatby, Alexandria University, Alexandria, Egypt
[2]Department of Stored Product Pests, Plant Protection Research Institute, Agricultural Research Center, Sabahia, Alexandria, Egypt
*Corresponding author's e-mail: *samir1969us@yahoo.com*

ABSTRACT

The essential oils from six Egyptian plants, namely *Citrus paradisi*, *Artemisia judaica*, *Astoma seselifolium*, *Citrus limon*, *Pituranthos tortuosus* and *Citrus sinensis* were isolated by hydrodistillation. The chemical composition of the isolated oils was identified by gas chromatography/mass spectrometry (GC-MS). The major constituents of the isolated oils were *dl*-limonene (74.29%) in *C. paradise*, *β*–thujone (49.83%) and chrysanthenone (10.88%) in *A. judaica*, sabinene (23.02%) and 4-terpineol (17.83%) in *A. seselifolium*, *dl*-limonene (56.30%) in *C. limon*, sabinene (32.09%) and 4-terpineol (20.31%) in *P. tortuosus* and *dl*-limonene (89.23%) in *C. sinensis*. The isolated oils were tested for their fumigant toxicity against two of the most destructive stored product insects *Sitophilus oryzae* and *Tribolium castaneum*. The oil of *C. limon* showed the highest toxicity against *S. oryzae* followed by the oil of *C. sinensis* with LC_{50} values of 9.89 and 19.67 mg/L respectively, while the oil of *A. seselifolium* revealed the lowest toxicity. When tested against *T. castaneum*, the oils *C. sinensis* and *C. limon* were again the most potent toxicants, whereas the oil of *A. judaica* showed the weakest insecticidal activity. In general, the isolated oils were more toxic against *S. oryzae* than *T. castaneum*. The results of the present study suggested that the isolated oils, particularly of *C. limon* and *C. sinensis* could be used as potential natural products for control of *S. oryzae* and *T. castaneum*.

Key Words: Egyptian plants, essential oils, fumigant toxicity, *Sitophilus oryzae*, *Tribolium castaneum*

INTRODUCTION

The stored grain losses caused by insect damage and other organisms vary from 10% to 40% in countries where modern storage technologies are yet to be fully adopted (Raja et al., 2001; Ogendo et al., 2003). The quantity of loss is dependent upon the insect species involved, storage duration and pest control methods among other factors. The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), and the rust red flour beetle, *Tribolium castaneum*

(Herbst) (Coleoptera:Tenebrionidae), are among the most widespread and destructive stored product insects throughout the world. They cause significant losses of stored products, particularly in tropical and warm temperate regions (Hill, 1990).

Control of stored product insects around the world relies heavily on use of organophosphorus and pyrethroid insecticides, and fumigants (i.e. methyl bromide and phosphine). However, continuous and heavy use of these synthetic pesticides has created serious problems such as ozone depletion and environmental pollution (World Meteorological Organization, 1995), toxicity to non-target organisms such as parasitoids, predators, pollinators and fish, pest resistance (Mohan and Fields, 2002) and pesticide residues (Ogendo et al., 2003). Therefore, there is an urgent need to develop new, convenient and safer alternatives to synthetic pesticides. The extracts and secondary metabolites of plants are among the most promising alternatives. These botanical pesticides have the advantage of providing novel modes of action against insects that can reduce the risk of cross-resistance as well as offering new leads for the design of target-specific molecules (Isman, 2008).

Essential oils and their major constituents, mainly monoterpenoids, attracted research attention in recent years as potential alternatives to synthetic insecticides (Aslan et al., 2004). The present study describes the isolation and chemical analysis of essential oils of six Egyptian plants. The fumigant toxicity of the isolated oils was evaluated against two major stored product insects *S. oryzae* and *T. castaneum*.

MATERIALS AND METHODS

Plant materials

The fruits of the three *Citrus* plants: *Citrus paradisi* Macfad., *Citrus limon* (L.) Burm. f. and *Citrus sinensis* (L.) Osbeck were purchased from Alexandria Main Market for Vegetables and Fruits in February, 2011. The fruit peels were used as the source for essential oils. The aerial parts of three other plants: *Artemisia judaica* L., *Pituranthos tortuosus* (Desf.) Benth and *Astoma seselifolium* DC. were collected from Alhamam (west Alexandria) and Edko (east Alexandria) regions, Egypt, in April and May 2011. The plant materials were identified with guidance from the Student's Flora of Egypt by Tackholm (1974) and confirmed by Prof. FathAllah Zaitoon of the Faculty of Agriculture, Alexandria University. Voucher specimens have been deposited in the Department of Pesticide Chemistry, Faculty of Agriculture, Alexandria University.

Test insects

Colonies of the rice weevil, *Sitophilus oryzae* (L.), and the rust red flour beetle, *Tribolium castaneum* (Herbst), were reared in our laboratory over 10 years without exposure to insecticides on sterilized whole wheat and wheat flour mixed with yeast (10 : 1, w/w, respectively). Insect rearing and all experimental procedures were carried out at 26±1°C and 65±5% rh and in a 12: 12 light: dark photoperiod. Adults used in fumigant toxicity studies were 2 weeks post-eclosion.

Isolation of essential oils

The aerial plant parts were partially dried at room temperature (26±1°C) for five days and the fruit peels were used fresh. Essential oils were extracted by hydrodistillation in a Clevenger-type apparatus for 3 h. The oils were dried over anhydrous sodium sulfate, and stored at 4°C until used for biological activity tests and GC-MS analysis.

Analysis of essential oils

Essential oils were diluted in diethyl ether and 0.5 µl was injected into the gas chromatography (Hewlwett Packard 5890)/mass spectrometry (Hewlwett Packard 5989B) (GC-MS) apparatus. The GC column was a 30 m (0.25 mm i.d., film thickness 0.25 µm) HP-5MS (5% diphenyl) dimethylpolysiloxane capillary column. The GC conditions were as follows: injector temperature, 240°C; column temperature, isothermal at 70°C for 2 min, then programmed to 280°C at 6°C/ min and held at this temperature for 2 min; ion source temperature, 200°C; detector temperature, 300°C. Helium was used as the carrier gas at the rate of 1 ml/min. The effluent of the GC column was introduced directly into the ion source of the MS. Spectra were obtained in the EI mode with 70 eV ionization energy. The sector mass analyzer was set to scan from 40 to 400 amu for 5 s.

Fumigant toxicity assay

The toxicity of essential oil vapors was tested against *S. oryzae* and *T. castaneum* adults by using a modified fumigant toxicity assay as described by Huang et al. (2000). Glass jars (1 L) were used as fumigation chambers. Essential oils were applied to filter paper pieces (2×3 cm) attached to the undersurface of the screw caps of jars at: 1, 2.5, 5, 10, 20, 30, 40, 60, 80 and 100 mg. The inner side of the jar's neck was painted with vaseline to prevent direct contact of insects with essential oils. Caps were screwed tightly on the jars after adding 20 adults of *S. oryzae* or *T. castaneum* to each. Control insects were kept under the same conditions without application of essential oils. Three replicates of each treatment and control were set up. The number of dead insects was counted after 24 h of treatment, and the mortality percentages and median lethal concentrations (LC_{50} values) were calculated according to Finney (1971).

RESULTS AND DISCUSSION

Chemical composition of the isolated essential oils

The major components of essential oils identified from the 6 plants are given in Table 1 and Fig. 1. The major constituents of the essential oils were *β*- thujone (49.83%), chrysanthenone (10.88%) and *α*-thujone (8.21%) in *Artemisia judaica*, sabinene (23.02%), 4-terpineol (17.83%), *γ*-terpinene (8.97%) and germacrene D (8.27%) in *Astoma seselifolium*, dl-limonene (56.30%), *β*-pinene (8.81%) and *γ*-terpinene (6.42%) in *Citrus limon*, dl-limonene (74.29%), L-linalool (4.61%) and linalool oxide (4.18%) in *C. paradisi*, dl-limonene (89.23%) and linalool (2.98%) in *C. sinensis*, sabinene (32.09%) and 4-terpineol (20.31%) in *Pituranthos tortuosus*. Some major components were found in more than one plant, such as dl-limonene, sabinene, 4-terpineol and *γ*-terpinene but others were specific to the plant species. The major constituents of the essential oils mainly belonged to three groups: oxygenated monoterpenes (*α*-thujone, chrysanthenone, 4-terpineol, L-linalool and linalool oxide), monoterpene hydrocarbons (dl-limonene, sabinene, *γ*-terpinene, *β*-pinene) and sesquiterpene hydrocarbons (germacrene D).

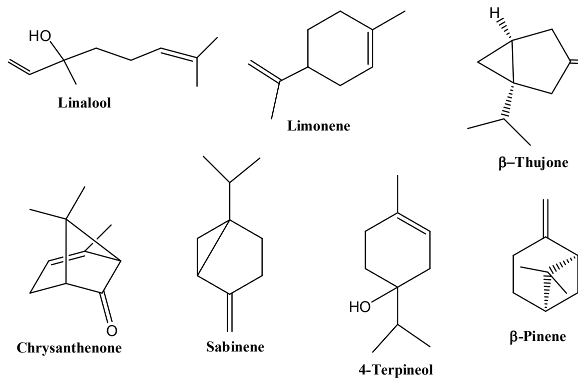

Fig. 1- Chemical structures of major compounds of the isolated oils.

Table 1. Major constituents of the essential oils isolated from Egyptian plants

Plant oil	Major constituents (%)
Artemisia judaica	*β* –Thujone (49.83)
	Chrysanthenone (10.88)
	α- Thujone (8.21)
	1,8-Cineole (4.91)
	L-Camphor (3.0)
Astoma seselifolium	Sabinene (23.02)
	4-Terpineol (17.83)
	γ-Terpinene (8.97)
	Germacrene D (8.27)
	α-Pinene (6.20)
	β-Myrcene (3.64)
Citrus limon	dl-Limonene (56.30)
	β-Pinene (8.81)
	γ-Terpinene (6.42)
	α-Citral (4.96)
	β-Citral (3.83)
	α-Terpineol (3.38)
Citrus paradisi	dl-Limonene (74.29)
	L-Linalool (4.61)
	Linalool oxide (4.18)
	β-Citral (2.66)
Citrus sinensis	dl-Limonene (89.23)
	Linalool (2.98)
	β-Myrcene (1.77)
	Octanal (1.28)
Pituranthos tortuosus	Sabinene (32.09)
	4-Terpineol (20.31)
	Myristicine (6.84)
	Dillapiole (5.72)
	γ-Terpinene (4.16)
	α-Pinene (3.25)

The oil composition of *A. judaica* from Alhamam in this study differed from that isolated from *A. judaica* growing in Sinai Peninsula, Egypt (Mohamed and Abdelgaleil, 2008) and the oil isolated from *A. judaica* growing in Algeria (Charchari, 2002). Interestingly, piperitone, a major constituent of many *A. judaica* oil samples, was absent in the isolated oil while *β*-thujone, the major compound isolated, had not been previously reported.

The oil of *A. seselifolium* was analysed for the first time in this study. The chemical compositions of the isolated essential oils from the three *Citrus* species are in accordance with those previously reported (Lota et al., 2001; Ahmed et al., 2006; Viuda-Martos et al., 2009). Some of the major constituents of the essential oil of *P. tortuosus* were similar to those previously reported for the oil isolated from plants growing in Egypt (Singab, 2003). However, the percentages of constituents differed. The differences in essential oil compositions could be due to several factors, such as geographical location, season, environmental conditions, nutritional status of the plants and other factors (Perry et al., 1999).

Insecticidal activity of essential oils against *Sitophilus oryzae*

The six isolated oils showed pronounced fumigant toxicity against the adults of *S. oryzae*. The values of LC_{50}, 95% confidence limits and other parameters generated from the concentration-mortality regression lines are shown in Table 2. The oil of *C. limon* revealed the strongest fumigant toxicity, followed by *C. sinensis* and C. *paradise*. The LC_{50} values of these oils were 9.89, 19.67 and 24.13 mg/L, respectively. In contrast, the oils of *A. seselifolium* and *P. tortuosus* were the less effective among the tested oils.

Many essential oils have been reported to possess fumigant toxicity against *S. oryzae*. Of the many oils tested only those of *Mentha microphylla*, *Asiasarum sieboldi* and *Carum copticum* showed higher activity than those tested here (Sahaf, et al., 2007; Mohamed and Abdelgaleil, 2008; Kim and Park, 2008; Chaubey, 2011).

Table 2. Fumigant toxicity of essential oils against the adults of *Sitophilus oryzae*

Compound	LC_{50}[a] (mg/L)	95% confidence limits (mg/L)		Slope[b] ± SE	Intercept[c] ± SE	(χ^2)[d]
		Lower	Upper			
Artemisia judaica	29.97	25.21	36.14	1.72±0.25	-2.54±0.37	3.34
Astoma seselifolium	44.43	36.36	59.77	1.99±0.30	-3.29±0.45	1.26
Citrus limon	9.89	7.23	13.85	3.74±0.28	-3.72±0.28	9.57
Citrus paradisi	24.13	19.23	29.59	7.17±0.59	-9.92±0.83	13.97
Citrus sinensis	19.67	13.60	33.93	5.39±0.44	-6.97±0.57	26.31
Pituranthos tortuosus	41.01	38.49	44.36	4.97±0.60	-8.01±0.94	1.97

[a]The concentration causing 50% mortality.
[b]Slope of the concentration-mortality regression line ± standard error.
[c]Intercept of the regression line ± standard error.
[d]Chi square value.

Insecticidal activity of essential oils against *Tribolium castaneum*

Fumigant toxicity of the isolated oils against the adults of *T. castaneum* in terms of LC_{50} values is summarized in Table 3. The essential oils of *C. sinensis* (LC_{50} = 24.57 mg/L) and *C. paradisi* (LC_{50} = 25.52 mg/L) were the most potent toxicants. The oil of *A. judaica* showed the lowest toxicity. The value of LC_{50} for this oil was greater than 50 mg/L. The oils of *P. tortuosus* and *A. seselifolium* revealed similar insecticidal activity against *T. castaneum*.

All of the tested oils were more effective fumigants against *S. oryzae* than *T. castaneum*. This finding is in agreement with our previous studies on the fumigant toxicity of some essential oils and monoterpenes in which *S. oryzae* was more susceptible than *T. castaneum* (Mohamed and Abdelgaleil, 2008; Abdelgaleil et al., 2009).

Table 3. Fumigant toxicity of essential oils against the adults of *Tribolium castaneum*

Compound	LC_{50}[a] (mg/L)	95% confidence limits (mg/L)		Slope[b] ± SE	Intercept[c] ± SE	(χ^2)[d]
		Lower	Upper			
Artemisia judaica	>50	-	-	-	-	-
Astoma seselifolium	46.55	42.17	53.95	4.29±0.63	-7.15±0.97	0.11
Citrus limon	25.52	20.17	35.64	3.55±0.31	-4.99±0.42	9.33
Citrus paradisi	30.06	20.16	63.70	5.38±0.43	-7.95±0.63	29.63
Citrus sinensis	24.57	14.76	97.93	4.84±0.37	-6.73±0.50	39.01
Pituranthos tortuosus	45.31	34.02	158.70	3.50±0.42	-5.80±0.64	13.16

[a]The concentration causing 50% mortality.
[b]Slope of the concentration-mortality regression line ± standard error.
[c]Intercept of the regression line ± standard error.
[d]Chi square value.

The essential oils of the three *Citrus* species showed strong insecticidal activity against the adults of *S. oryzae* and *T. castaneum*. This potent toxicity could be attributed to the major constituent, *dl*-limonene. In fact, limonene showed relatively less insecticidal activity than these three oils. The LC_{50} values of limonine on *S. oryzae* and *T. castaneum* were 29.92 and 33.37 mg/L, respectively (Abdelgaleil et al., 2009). It has often been found that some essential oils have a greater insecticidal activity than their isolated major constituents as observed with the essential oil of *Asiasarum sieboldi* against *S. oryzae* (Kim and Park, 2008), indicating the beneficial effect of combined action of the different components.

It is well recognized that the insecticidal activity of essential oils are mainly attributed to their monoterpenoidal contents (Huang et al., 1998; Garcìa et al., 2005; Abdelgaleil et al., 2009). Monoterpenes act as neurotoxicants against different insect species (Coats et al., 1991). They have been shown to inhibit acetylcholinesterase (AChE) isolated from different insect species (Ryan and Byrne, 1988; Abdelgaleil et al., 2009).

In conclusion, this study shows that the essential oils tested, especially those obtained from *Citrus* species, have remarkable fumigant toxicity against the adults of *S. oryzae* and *T. castaneum*. The huge consumption of the *Citrus* fruits generates tons of waste peels. Converting these waste products to safer natural insecticides is highly recommended. Based on the insecticidal activity of the *Citrus* oils demonstrated in this study, these oils could be used in integrated pest management (IPM) programs of *S. oryzae* and *T. castaneum*.

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REFERENCES

Abdelgaleil SAM, Mohamed MIE, Badawy MEI, El-Arami SAA (2009) Fumigant and contact toxicities of monoterpenes to *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) and their inhibitory effects on acetylcholinesterase activity. J Chem Ecol 35: 225-232.

Aslan İ, Özbek H, Çalmaşur Ö, Şahİn F (2004) Toxicity of essential oil vapours to two greenhouse pests, *Tetranychus urticae* Koch and *Bemisia tabaci* Genn. Indust Crops Prod 19: 167–173.

Charchari S (2002) The essential oil of *Artemisia judaica* L. from Algeria. J Essential Oil Res 14: 16-17.

Chaubey MK (2011). Fumigant toxicity of essential oils against the rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae), J Biol Sci 11, 411-416.

Coats JR, Karr LL, Drewes CD (1991) Toxicity and neurotoxic effects of monoterpenoids in insects and earthworms. In: Hedin PA (ed) Naturally Occurring Pest Bioregulators. Am Chem Soc, Washington DC, pp 305–316.

Finney DJ (1971) *Probit Analysis*. 3rd ed. Cambridge University Press, London.

Garcìa M, Donadel OJ, Ardanaz CE, Tonn CE, Sosa, ME (2005) Toxic and repellent effects of *Baccharis salicifolia* essential oil on *Tribolium castaneum*. Pest Manag Sci 61: 612–618.

Hill DS (1990) Pests of Stored Products and Their Control. Belhaven Press, London, pp. 8–55.

Huang Y, Hee SK, Ho, SH (1998) Antifeedant and growth inhibitory effects of a -pinene on the stored-product insects, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. Int Pest Control 40: 18–20.

Huang Y, Lam SL, Ho SH (2000) Bioactivities of essential oil from *Elletaria cardamomum* (L.) Maton. to *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst). J Stored Prod Res 36: 107–117.

Isman MB (2008) Prospective botanical insecticides: for richer, for poorer. Pest Manag Sci 64: 8-11.

Kim J, Park K (2008) Fumigant toxicity of Korean medicinal plant essential oils and components from *Asiasarum sieboldi* root against *Sitophilus oryzae* L. Flavour Fragr J 23: 79–83.

Lota M-L, de Rocca Serra D, Jacquemon C, Tomi F, Casanova J (2001) Chemical variability of peel and leaf essential oils of sour orange. Biochem System Ecol 16: 89 – 96.

Mohamed MIE, Abdelgaleil SAM (2008) Chemical composition and insecticidal potential of the essential oils from Egyptian plants against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). J App Ent Zool 43: 599-607.

Mohan S, Fields PG (2002) A simple technique to assess compounds that are repellents or attractive to stored product insects. J Stored Prod Res 33: 289–298.

Ogendo JO, Belmain SR, Deng AL, Walker DJ (2003) Comparison of toxic and repellent effects of *Lantana camara* L. with *Tephrosia vogelii* Hook and a synthetic pesticide against *Sitophilus zeamais* Motschulsky in maize grain storage. Insect Sci Appl 23: 127-135.

Perry NB, Anderson RE, Brennan NJ, Douglas MH, Heaney AJ, McGrimpsey JA, Smallfield BM (1999) Essential oil from Dalmation sage (*Salvia officinalis* L.), variations among individuals, plant parts, seasons and sites. J Agric Food Chem 47: 2048–2054.

Raja N, Albert S, Ignacimuthu S, Dorn S (2001) Effect of plant volatile oils in protecting stored cowpea *Vigna unguiculata* (L.) Walpers against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) infestation. J Stored Prod Res 37: 127-132.

Ryan M F, Byrne O (1988) Plant-insect coevolution and inhibition of acetylcholine-esterase. J Chem Ecol 14: 1965–1975.

Sahaf BZ, Moharramipour S, Hadi M (2007) Chemical constituents and fumigant toxicity of essential oil from *Carum copticum* against two stored product beetles. Insect Sci 14: 213–218.

Singab A B (2003) Essential oils and lipids content of *Pituranthos* species growing in Egypt. Bull Fac Pharm Cairo Univ 41: 213-217.

Tackholm V (1974) Student Flora of Egypt. 2nd ed, Cairo University Press, Beirrut.

Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Álvarez, JA (2009) Chemical composition of mandarin (*C. reticulata* L.), grapefruit (*C. paradisi* L.), lemon (*C. limon* L.) and orange (*C. sinensis* L.) essential oils. J Essent Oil-Bearing Plants 12: 236-243.

World Meteorological Organization (1995) Scientific Assessment Of Ozone Depletion: World Meteorological Organization Global Ozone Research and Monitoring Project. Report No. 37, WMO, Geneva, Switzerland.