

African Journal of Biotechnology Vol. 10(42), pp. 8476-8480, 8 August, 2011  
Available online at <http://www.academicjournals.org/AJB>  
DOI: 10.5897/AJB11.1003  
ISSN 1684-5315 © 2011 Academic Journals

## Full Length Research Paper

# Chemical composition and toxic activity of essential oil of *Caryopteris incana* against *Sitophilus zeamais*

Sha Sha Chu<sup>1</sup>, Qi Zhi Liu<sup>1</sup>, Ligang Zhou<sup>2</sup>, Shu Shan Du<sup>3</sup> and Zhi Long Liu<sup>1\*</sup>

<sup>1</sup>Department of Entomology, China Agricultural University, 2 Yuanmingyuan West Road, Haidian District, Beijing 100094, P. R. China.

<sup>2</sup>Department of Plant Pathology, China Agricultural University, 2 Yuanmingyuan West Road, Haidian District, Beijing 100094, P. R. China.

<sup>3</sup>Protection and Utilization of Traditional Chinese Medicine of Beijing Area Major Laboratory, Beijing Normal University, Haidian District, Beijing 100875, P. R. China.

Accepted 29 June, 2011

During our screening program for new agrochemicals from Chinese medicinal herbs, essential oil of *Caryopteris incana* aerial parts was found to possess strong insecticidal activities against the maize weevil, *Sitophilus zeamais*. A total of 37 components of the essential oil were identified by GC and GC/MS. Estragole (24.8%) and linalool (14.0%) are the two main components of the essential oil followed by 1,8-cineol (5.2%) and  $\delta$ -guaiene (4.1%). The essential oil possesses strong fumigant toxicity against *S. zeamais* adults with an LC<sub>50</sub> value of 10.05 mg/L air. The essential oil of *C. incana* also showed contact toxicity against *S. zeamais* adults with an LD<sub>50</sub> value of 122.65  $\mu$ g/adult. The essential oil *C. incana* may have potential to be developed as a new natural fumigant/insecticide for the control of stored product insects.

**Key words:** *Caryopteris incana*, *Sitophilus zeamais*, fumigant, contact toxicity, essential oil composition, estragole, linalool.

## INTRODUCTION

The maize weevil, *Sitophilus zeamais* (Motschulsky) is one of the most widespread and destructive primary insect pests of stored cereals (Liu and Ho, 1999). Infestations not only cause significant losses due to the consumption of grains; they also result in elevated temperature and moisture conditions that lead to an accelerated growth of molds, including toxigenic species (Magan et al., 2003). Fumigation plays a very important role in insect pest elimination in stored products not only because of their ability to kill a broad spectrum of pests but because of their easy penetration into the commodity while leaving minimal residues (Zettler and Arthur, 2000). The currently used synthetic fumigant is still the most effective means for the protection of stored food, feedstuffs and other agricultural commodities from insect infestation. However, repeated use of those synthetic

fumigants for decades has disrupted biological control by natural enemies and led to resurgence of stored-product insect pests, which sometimes resulted in the development of resistance, and had undesirable effects on non-target organisms. Moreover, the use of methyl bromide (MeBr) will be prohibited in the near future because of its ozone depletion potential (United States Environmental Protection Agency, 1993). These problems have highlighted the need to develop new types of selective insect-control alternatives with fumigant action. Plant essential oils and their components have been shown to possess potential to be developed as new fumigants and they may have advantage over conventional fumigants in terms of low mammalian toxicity, rapid degradation and local availability (Isman, 2006, 2008). Essential oils derived from many plant species, so far have been evaluated for fumigant toxicity against stored product insects (Rajendran and Srianjini, 2008).

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

\*Corresponding author. E-mail: zhilongliu@cau.edu.cn Tel/Fax: 86-10-62732800.

de-sign of target-specific molecules (Isman, 2006). During the screening program for new agrochemicals from Chinese medicinal herbs, the essential oil of *Caryopteris incana* (Thunb. ex Hout.) Miq. (Family: *Verbenaceae*) aerial parts was found to possess strong insecticidal toxicity against *S. zeamais*.

Aerial parts of *C. incana* are used in traditional Chinese medicine to treat coughs, colds and rheumatic pains (Jiangsu New Medical College, 1977). Previous phytochemical studies on *C. incana* resulted in the identification of phenolic acids, abietane diterpenoids, triterpenoids, iridoid glucoside, phenylethanoid, phenylethanoid glycosides and phenylpropanoid glycosides (El-Hela et al., 1998; 1999; Gao et al., 1996a, b, 2000; Gao and Han, 1997, 1998; Zhao et al., 2009). The chemical composition of *C. incana* essential oil was also studied previously (Pu et al., 1984; Sun et al., 2004; Kim, 2008). However, no reports on insecticidal activity of *C. incana* essential oil against stored product insects are available so far. This study analyzed the chemical composition and toxicity against the maize weevil.

## MATERIALS AND METHODS

*S. zeamais* were obtained from laboratory cultures maintained for the last 10 years in the dark in incubators at 27 to 29°C and 70 to 80% relative humidity. *S. zeamais* adults were reared on whole wheat at 12 to 13% moisture content. Unsexed adults of the maize weevils used in all the experiments was about 2 weeks old.

Ten kilograms of aerial parts of *C. incana* were purchased from Puning Chinese Medicinal herbs Market (Guangdong 515300, China). The species was identified, and the voucher specimens (BNU-Liuzhilong-Verb-09-302) were deposited at the Herbarium (BNU) of College of Life Sciences, Beijing Normal University. The herb was first ground to powder using a grinding mill (Retsch Muhle, Germany). Each 600 g portion of ground powder was mixed in 1,800 ml of distilled water and soaked for 3 h. The mixture was then boiled in a round-bottom flask, and steam distilled for 6 to 8 h. Essential oil from distillation was collected in a flask. Separation of the essential oil from the aqueous layer was done in a separatory funnel, using the non-polar solvent, *n*-hexane. The solvent was evaporated using a vacuum rotary evaporator (BUCHI Rotavapor R-124, Switzerland). The sample was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and kept in a refrigerator (4°C) for subsequent experiments.

### Analysis of the essential oils

Components of the essential oil were separated and identified by gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) Agilent 6890N gas chromatography hooked to Agilent 5973N mass selective detector. The same column and analysis conditions were used for both GC and GC/MS. They were equipped with a flame ionization detector and capillary column with HP-5MS (30 m x 0.25 mm x 0.25 μm). The GC settings were as follows: the initial oven temperature was held at 60°C for 1 min and ramped at 10°C min<sup>-1</sup> to 180°C for 1 min, and then ramped at 20°C min<sup>-1</sup> to 280°C for 15 min. The injector temperature was maintained at 270°C. The samples (1 μl) were injected neat, with a split ratio of 1:10. The carrier gas was helium at the flow rate of 1.0 ml min<sup>-1</sup>. Spectra were scanned from 20 to 550 m/z at 2 scans s<sup>-1</sup>. Most constituents were identified by gas chromatography by comparison of their retention indices with

those of the literature (Pu et al., 1984; Sun et al., 2004; Kim, 2008) or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of *n*-alkanes (C<sub>8</sub>-C<sub>24</sub>) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature (Adams, 2007). Component relative percentages were calculated based on GC peak areas without using correction factors.

### Fumigant toxicity

A Whatman filter paper (diameter 2.0 cm, CAT No. 1001020) was placed on the underside of the screw cap of a glass vial (diameter 2.5 cm, height 5.5 cm, volume 24 ml). 20 μl of 4.0 to 25.0% of the essential oil (V:V, 6 concentrations) was added to the filter paper. The solvent was allowed to evaporate for 30 s before the cap was placed tightly on the glass vial (with 10 unsexed insects) to form a sealed chamber. Fluon (ICI America Inc) was used inside glass vial to prevent insects from the treated filter paper. *n*-Hexane was used as the controls. Six replicates were used in all the treatments and controls and they were incubated at 27 to 29°C and 70 to 80% relative humidity for 24 h. The insects were then transferred to clean vials with some culture media and kept in an incubator. Mortality of insects was observed daily until end-point mortality was reached one week after treatment. Results from all replicates were subjected to probit analysis using the PriProbit Program V1.6.3 to determine LC<sub>50</sub> values (Sakuma, 1998).

### Contact toxicity using topical application

The contact toxicity of essential oil against *S. zeamais* adults was measured as described by Liu and Ho (1999). A serial dilution of the essential oil (2.0 to 30.0%, 6 concentrations) was prepared in *n*-hexane. Aliquots of 0.5 μl of the dilutions were applied topically to the dorsal thorax of the insects. Controls were determined using *n*-hexane. Six replicates were used in all the treatments and controls. Both treated and control insects were then transferred to glass vials (10 insects/vial) with culture media and kept in incubators. Mortality of insects was observed daily until end-point mortality was reached one week after treatment. The LD<sub>50</sub> values were calculated by using probit analysis (Sakuma, 1998).

## RESULTS AND DISCUSSION

### Essential oil

The steam distillation for 3 h of the aerial parts of *C. incana* produced essential oil (yellow) with a yield of 0.21% (v/w) and the density of the concentrated essential oil was found to be 0.89 g/ml. The GC and GC-MS analysis of the essential oils of the aerial parts of *C. incana* led to the identification and quantification of a total of 37 major components accounting for 96.36% of the total components present (Table 1). Estragole (24.8%) and linalool (14.0%) were the two main components of the essential oil followed by bicyclosquiphellandrene (7.7%), eucalyptol (5.2%), α-bulnesene (4.1%) and β-elemene (3.7%). Chemical composition of the essential oil of *C. incana* was different from that reported in other studies. For example, the essential oil of *C. incana* collec-

**Table 1.** Chemical constituents of the volatile oil from *C. incana* aerial parts.

Compound	RI*	Peak area (%)
$\alpha$ -Pinene	931	0.54
$\beta$ -Pinene	981	0.56
1,8-Cineol	1032	5.18
Linalool oxide	1078	0.53
Fenchone	1087	1.20
Linalool	1094	14.04
$\alpha$ -Fenchyl acetate	1122	1.03
4-Acetyl-1-methyl-cyclohexene	1135	0.33
Camphor	1143	0.87
4-Terpinenol	1179	0.32
Estragole	1195	24.80
Chavicol	1254	0.64
Bornyl acetate	1285	1.97
$\rho$ -Methoxyacetophenone	1304	0.28
Ylangene	1372	0.61
$\alpha$ -Copaene	1374	0.55
$\beta$ -Bourbonene	1385	0.53
$\beta$ -Elemene	1391	3.65
Methyleugenol	1401	0.96
$\beta$ -Caryophyllene	1420	0.99
$\alpha$ -Bergamotene	1435	2.68
$\alpha$ -Guaiene	1438	2.61
$\alpha$ -Caryophyllene	1454	0.74
4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene	1468	0.55
$\gamma$ -Muurolene	1473	1.52
$\beta$ -Selinene	1478	1.56
Bicyclosesquiphellandrene	1491	7.70
$\alpha$ -Selinene	1492	2.05
$\delta$ -Guaiene	1503	4.09
$\gamma$ -Maaliene	1513	0.61
$\delta$ -Cadinene	1523	1.49
Cadina-4,9-diene	1546	3.10
(+)-Nerolidol	1567	2.82
Spathulenol	1578	1.49
$\tau$ -Cadinol	1642	0.94
$\beta$ -Eudesmol	1648	2.11
Alloaromadendrene oxide	1687	0.38
Total		96.36
Monoterpenoids		51.04
Sesquiterpenoids		43.75
Other		1.57

\*RI, retention index as determined on a HP-5MS column using the homologous series of *n*-hydrocarbons.

ted from Sichuan Province, China contains limonene (38.5%),  $\alpha$ -terpinene (117.3%),  $\beta$ -pinene (12.9%) and  $\rho$ -cymene (12.6%) (Pu et al., 1984). The essential oil of *C. incana* harvested from Jiangxi Province, China possesses linalool (16.3%), perillalcohol (15.3%), carvone (14.7%) and orthodene (9.7%) (Sun et al., 2004).

However, 4,6,6-tri-Me [1S-(1 $\alpha$ ,2 $\beta$ ,5 $\alpha$ )]-bicyclo[3.1.1]hept-3-en-2-ol (11.8%),  $\tau$ -cadinol (9.4%), myrtenyl acetate (9.2%), pinocarvone (7.0%), 1-hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene (6.3%) and  $\delta$ -3-carene (6.2%) were the main components of essential oil of *C. incana* aerial parts collected from Korea (Kim, 2008).

**Table 2.** Insecticidal activity of *C. incana* essential oil against *S. zeamais* adults.

Essential oil	Contact toxicity		Fumigant toxicity	
	LD <sub>50</sub> (µg/adult)	95% fiducial limits	LC <sub>50</sub> (mg/L air)	95% fiducial limits
<i>Caryopteris incana</i>	122.65	118.65-126.79	10.05	8.99-11.36
MeBr	-	-	0.67*	-
Phosphine	-	-	0.01*	-
Pyrethrum extract	4.29**	-	-	-

\*Data from Liu and Ho (1999); \*\* data from Liu et al. (2010).

These results suggest that great variations in chemical composition of essential oil of *C. incana* aerial parts may be due to harvest time and local, climatic and seasonal factors as well as storage duration of the medicinal herbs.

### Insecticidal activities

The essential oil of *C. incana* aerial parts showed contact toxicity against *S. zeamais* adults with an LD<sub>50</sub> value of 122.65 µg/adult (Table 2). However, the essential oil demonstrated weak acute toxicity against the weevil when compared with the control (pyrethrum extract, 25% pyrethrin I and pyrethrin II) because the pyrethrum extract had acute toxicity to the maize weevils with an LD<sub>50</sub> value of 4.3 µg/adult (Liu et al., 2010a). In previous studies, the two main components of the essential oil (estragole and linalool) demonstrated toxicity against several species of insects and mites (Sanchez-Ramos and Castanera, 2001; Sampson et al., 2005; Chang et al., 2009; Yang et al., 2009).

The essential oil of *C. incana* aerial parts also possessed strong fumigant activity against *S. zeamais* adults with an LC<sub>50</sub> value of 10.05 mg/L air (Table 2). The currently used grain fumigants, MeBr and phosphine were reported to have fumigant activity (24 h) against *S. zeamais* adults with LC<sub>50</sub> values of 0.67 mg/L and 6 µg/L air, respectively (Liu and Ho, 1999). The essential oil of *C. incana* was 15 times less toxic to the maize weevil when compared with the commercial fumigant MeBr. In comparison with the other essential oils in the literature, the essential oil of *C. incana* aerial parts possessed the same level or stronger fumigant toxicity against *S. zeamais* adults; essential oils of *Murraya exotica* (LC<sub>50</sub> = 8.29 mg/L) (Li et al., 2010), *Artemisia lavandulaefolia* (LC<sub>50</sub> = 11.2 mg/L) and *Artemisia sieversiana* (LC<sub>50</sub> = 15.0 mg/L) (Liu et al., 2010a), *Artemisia vestita* (LC<sub>50</sub> = 13.42 mg/L) (Chu et al., 2010a), *Artemisia capillaris* (LC<sub>50</sub> = 5.31 mg/L), *Artemisia mongolica* (LC<sub>50</sub> = 7.35 mg/L) (Liu et al., 2010b) and *Illicium simonsii* (LC<sub>50</sub> = 14.95 mg/L) (Chu et al., 2010b).

The earlier mentioned findings suggest that fumigant activity of the essential oil of *C. incana* aerial parts is quite promising by considering the currently used fumigants which are synthetic insecticides, and it shows

potential to be developed as a possible natural fumigant for the control of stored product insects. However, for the practical application of the essential oil as novel fumigant, further studies on the safety of the essential oil to humans and on development of formulations are necessary to improve the efficacy and stability and to reduce cost. The isolation and identification of the bioactive compounds in the essential oil of *C. incana* aerial parts are of utmost importance so that their potential application in controlling stored-product pests can be fully exploited. Moreover, further studies on plant cultivation and essential oil standardization is necessary because chemical composition of the essential oil varies greatly with the plant population.

### ACKNOWLEDGEMENTS

This work was funded by the Hi-Tech Research and Development of China (2011AA10A202 and 2006-AA10A209). We thank Dr. Q.R Liu from the College of Life Sciences, Beijing Normal University, Beijing 100875, for the identification of the investigated medicinal herb.

### REFERENCES

- Adams RP (2007). Identification of essential oil components by GC-MS, 4<sup>th</sup> ed. Allured Publ. Corp., Carol Stream, IL.
- United States Environmental Protection Agency (1993). Regulatory action under the clean air act on methyl bromide. United States Environ. Protection Agency, Office of Air Radiation Stratospheric Protection Division, Washington, DC.
- Chang CL, Cho IK, Li QX (2009). Insecticidal activity of basil oil, *trans*-anethole, estragole, and linalool to adult fruit flies of *Ceratitis capitata*, *Bactrocera dorsalis*, and *Bactrocera cucurbitae*. J. Econ. Entomol. 102: 203-209.
- Chu SS, Liu QR, Liu ZL (2010a). Insecticidal activity and chemical composition of the essential oil of *Artemisia vestita* from China against *Sitophilus zeamais*. Biochem. Syst. Ecol. 38: 489-492.
- Chu SS, Liu SL, Jiang GH, Liu ZL (2010b). Composition and toxicity of essential oil of *Illicium simonsii* Maxim (Illiciaceae) fruit against the maize weevils. Rec. Nat. Prod. 4: 205-210.
- El-Hela AA, Cisowski W, Krauze-Baranowska M (1998). Phytochemical investigations of *Caryopteris incana* Miq. Herba Polonica, 44: 121-125.
- El-Hela AA, Luczkiewicz M, Cisowski W (1999). Free phenolic acids from *Caryopteris incana* (Thunb.) Miq. qualitative analysis. Herba Polonica, 45: 73-79.
- Gao JJ, Han GQ, Yang L (1996a). Two new phenylpropanoid glycosides from *Caryopteris incana* (Thumb.) Miq. Chin. Chem. Lett. 7: 445-448.

- Gao JJ, Han GQ, Yan JF, Wu Y (1996b). A new rearranged abietane diterpenoid from *Caryopteris incana* (Thunb.) Miq. *Chin. Chem. Lett.* 7: 621-622.
- Gao JJ, Han GQ (1997). Cytotoxic abietane diterpenoids from *Caryopteris incana*. *Phytochemistry*, 44: 759-761.
- Gao JJ, Han GQ (1998). Phenylpropanoid and iridoid glycosides from *Caryopteris incana* (Thunb.) Miq. *Indian J. Chem.* 37B: 1157-1160.
- Gao JJ, Igarashi K, Nukina M (2000). Three new phenylethanoid glycosides from *Caryopteris incana* and their antioxidative activity. *Chem. Pharm. Bull.* 48: 1075-1078.
- Isman MB (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Ann. Rev. Entomol.* 51: 45-66.
- Isman MB (2008). Perspective botanical insecticides: for richer, for poorer. *Pest Manage. Sci.* 64: 8-11.
- Jiangsu New Medical College (1977). *Dictionary of Chinese Herbal Medicine*. Shanghai Sci. Technol. Press, Shanghai, China, pp. 774-775.
- Kim SM (2008) Composition and cell cytotoxicity of essential oil from *Caryopteris incana* Miq. in Korea. *Han'guk Eungyong Sangmyong Hwahakhoeji*, 51: 238-244.
- Li WQ, Jiang CH, Chu SS, Zuo MX, Liu ZL (2010). Chemical composition and toxicity against *Sitophilus zeamais* and *Tribolium castaneum* of the essential oil of *Murraya exotica* aerial parts. *Molecules* 15: 5831-5839.
- Liu ZL, Ho SH (1999). Bioactivity of the essential oil extracted from *Evodia rutaecarpa* Hook f. et Thomas against the grain storage insects, *Sitophilus zeamais* Motsch. and *Tribolium castaneum* (Herbst). *J. Stored Prod. Res.* 35: 317-328.
- Liu ZL, Liu QR, Chu SS, Jiang GH (2010a). Insecticidal activity and chemical composition of the essential oils of *Artemisia lavandulaefolia* and *Artemisia sieversiana* from China. *Chem. Biodiv.* 7: 2040-2045.
- Liu ZL, Chu SS, Liu QR (2010b). Chemical composition and insecticidal activity against *Sitophilus zeamais* of the essential oils of *Artemisia capillaris* and *Artemisia mongolica*. *Molecules*, 15: 2600-2608.
- Magan N, Hope R, Cairns V, Aldred D (2003). Postharvest fungal ecology: impact of fungal growth and mycotoxin accumulation in stored grain. *Eur. J. Plant Pathol.* 109: 723-730.
- Pu ZL, Shi Y, Yang YC, Zhang J, Lu YC (1984). Chemical constituents of the essential oils of Chinese *Caryopteris* Bunge. I. GC/MS analyses of the hydrocarbon fraction of *Caryopteris incana*, *C. trichosphaera*, *C. forrestii* and *C. forrestii* var. *minor*. *Acta Chimica Sinica* 42: 1103-1105 (in Chinese with English abstract).
- Rajendran S, Srianjini V (2008). Plant products as fumigants for stored-product insects control. *J. Stored Prod. Res.* 44: 126-135.
- Sakuma M (1998). Probit analysis of preference data. *Appl. Entomol. Zool.* 33: 339-347.
- Sampson BJ, Tabanca N, Kirimer N, Demirci B, Baser KHC, Khan IA, Spiers JM, Wedge DE (2005). Insecticidal activity of 23 essential oils and their major compounds against adult *Lipaphis pseudobrassicae* (Davis) (Aphididae: Homoptera). *Pest Manage. Sci.* 61: 1122-1128.
- Sanchez-Ramos I, Castanera P (2001). Acaricidal activity of natural monoterpenes on *Tyrophagus putrescentiae* (Schrank), a mite of stored food. *J. Stored Prod. Res.* 37: 93-101.
- Sun LF, Ye WF, Chen HM (2004). Studies on chemical constituents of the volatile oil from *Caryopteris incana* (Thunb.)Miq. *J. Jiangxi Normal Univ.* 28: 196-199 (in Chinese with English abstract).
- Yang YC, Lee SH, Clark JM, Ahn YJ (2009). Ovicidal and adulticidal activities of *Origanum majorana* essential oil constituents against insecticide-susceptible and pyrethroid/malathion-resistant *Pediculus humanus capitis* (Anoplura: Pediculidae). *J. Agric. Food Chem.* 57: 2282-2287.
- Zettler JL, Arthur FH (2000). Chemical control of stored product insects with fumigants and residual treatments. *Crop Prot.* 19: 577-582.
- Zhao DP, Matsunami K, Otsuka H (2009) Iridoid glucoside, (3R)-oct-1-en-3-ol glycosides, and phenylethanoid from the aerial parts of *Caryopteris incana*. *J. Nat. Med.* 63: 241-247.