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Full Length Research Paper

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

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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 δ-guaiene (4.1%). The essential oil possesses strong fumigant toxicity against *S. zeamais* adults with an LC_{50} value of 10.05 mg/L air. The essential oil of *C. incana* also showed contact toxicity against *S. zeamais* adults with an LD_{50} value of 122.65 µg/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 eva-luated 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

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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 phyto-chemical studies on *C. incana* resulted in the iden-tification of phenolic acids, abietane diterpenoids, triterpenoids, iridoid glucoside, phenylethanoid, pheny-letha-noid 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 chemi-cal 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 compo-sition 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_2SO_4 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^{-1} to 180°C for 1 min, and then ramped at 20°C min^{-1} 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^{-1}. Spectra were scanned from 20 to 550 m/z at 2 scans s^{-1}. 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_8–C_{24}) 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_{50} 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_{50} 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 bicyclosesquiphellandrene (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 (%)
α-Pinene	931	0.54
β-Pinene	981	0.56
1,8-Cineol	1032	5.18
Linalool oxide	1078	0.53
Fenchone	1087	1.20
Linalool	1094	14.04
α-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
ρ-Methoxyacetophenone	1304	0.28
Ylangene	1372	0.61
α-Copaene	1374	0.55
β-Bourbonene	1385	0.53
β-Elemene	1391	3.65
Methyleugenol	1401	0.96
β-Caryophyllene	1420	0.99
α-Bergamotene	1435	2.68
α-Guaiene	1438	2.61
α-Caryophyllene	1454	0.74
4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene	1468	0.55
γ-Muurolene	1473	1.52
β-Selinene	1478	1.56
Bicyclosesquiphellandrene	1491	7.70
α-Selinene	1492	2.05
δ-Guaiene	1503	4.09
γ-Maaliene	1513	0.61
δ-Cadinene	1523	1.49
Cadina-4,9-diene	1546	3.10
(+)-Nerolidol	1567	2.82
Spathulenol	1578	1.49
τ-Cadinol	1642	0.94
β-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%), α-terpenene (117.3%), β-pinene (12.9%) and ρ-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-(1α,2β,5α)]-bicyclo[3.1.1]hept-3-en-2-ol (11.8%), T-cadinol (9.4%), myrtenyl acetate (9.2%), pinocarvone (7.0%), 1-hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene (6.3%) and δ-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_{50} (µg/adult)	95% fiducial limits	LC_{50} (mg/L air)	95% fiducial limits
Caryopteris incana	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_{50} 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_{50} 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_{50} 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_{50} 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_{50} = 8.29 mg/L) (Li et al., 2010), *Artemisia lavandulaefolia* (LC_{50} = 11.2 mg/L) and *Artemisia sieversiana* (LC_{50} = 15.0 mg/L) (Liu et al., 2010a), *Artemisia vestita* (LC_{50} = 13.42 mg/L) (Chu et al., 2010a), *Artemisia capillaris* (LC_{50} = 5.31 mg/L), *Artemisia mongolica* (LC_{50} = 7.35 mg/L) (Liu et al., 2010b) and *Illicium simonsii* (LC_{50} = 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.

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