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Full Length Research Paper

Essential oil composition and insecticidal activity of *Evodia lepta* (Spreng) Merr. root barks from China against two grain storage insects

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In our screening program for new agrochemicals from local wild plants and Chinese medicinal herbs, the essential oil of *Evodia lepta* (Rutaceae) root barks was found to possess strong insecticidal activity against the maize weevil, *Sitophilus zeamais* Motsch. and red flour beetle, *Tribolium castaneum* Herbst. Essential oil of *E. lepta* root barks was obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 35 components of the essential oil were identified. The main components of the essential oil were α-pinene (26.68%), borneol (7.24%) and *trans*-pinocarveol (6.82%) followed by evodionol (4.71%), α-terpineol (4.56%) and α-campholenal (4.15%). The essential oil of *E. lepta* possessed strong fumigant toxicity against *S. zeamais* and *T. castaneum* adults with LC_{50} values of 25.05 and 12.09 mg/L air, respectively. The essential oil also showed contact toxicity against *S. zeamais* and *T. castaneum* adults with LD_{50} values of 125.21 and 166.94 µg/adult, respectively.

Key words: *Evodia lepta*, *Sitophilus zeamais*, *Tribolium castaneum,* contact toxicity, fumigant, essential oil composition.

INTRODUCTION

The maize weevil, *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) and red flour beetles, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) are two serious pest species of stored grains worldwide (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 is still one of the most effective methods for the protection of stored food, feedstuffs and other agricultural commodities from insect infestation 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). However, repeated use of synthetic fumigants for decades has led to resurgence of stored-product insect pests, sometimes resulted in the development of resistance, and had undesirable effects on non-target organisms (Zettler and Arthur, 2000). 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 the advantage over conventional fumigants in terms of low mammalian toxicity, rapid degradation and local availability (Isman, 2000, 2006). The toxicity of a large number of essential oils and their constituents has been also evaluated against a number of stored-product insects (Rajendran and Srianjini, 2008). Botanical pesticides have the

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advantage of providing novel modes of action against insects that can reduce the risk of cross-resistance as well as offering new leads for design of target-specific molecule. During the screening program for new agrochemicals from local wild plants and Chinese medicinal herbs, essential oil of *E. lepta* (Spreng) Merr. root barks were found to possess strong insecticidal activity against *S. zeamais* and *T. castaneum* adults. *E. lepta* belong to family Rutaceae (Huang, 1997). Its roots were used in traditional Chinese medicines as an anti-inflammatory, and analgesic, and externally it is used for trauma, abscesses, wound infections, eczema, dermatitis and hemorrhoides Jiangsu New Medical College (1977).

Several studies on chemical constituents of *E. lepta* have been reported and a number of chromenes, dichromenes, 2,2-dimethylchromenes and derivates, coumarins, and alkaloids have been isolated (Gunawardana et al., 1987; Li et al., 1997a,b; 2003; Li and Zhu, 1998a,b; 1999; Diao et al., 2004, 2006; Gao et al., 2009). Chemical composition of the essential oil derived from *E. lepta* leaves has previously been determined (Diao and Gao, 2008; Bi et al., 2005). However, a literature survey has shown that there is no report on the volatile constituents of essential oil derived from *E. lepta* root barks and insecticidal activity of essential oil of *E. lepta* against stored product insects was not measured. The present investigation consisted of two parts: determination of chemical composition of the essential oil of *E. lepta* root barks and evaluation of the essential oil as insecticide/fumigant for the control of two stored-product insect pests.

MATERIALS AND METHODS

Plant material

Dried root barks (10 kg) of *E. lepta* were purchased from Anguo Chinese Medicinal Herbs Market (Anguo 071200, Hebei Province, China) and ground to a powder using a grinding mill (Retsch Mühle, Germany). The herb was identified by Dr. Liu, Q.R. and the voucher specimen (BNU-CMH-zhilongliu-2010-07-29-002) was deposited at the Department of Entomology, China Agricultural University (Beijing 100193). Each 600 g portion of 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. Volatile 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℃) for subsequent experiments.

Insects

The maize weevils (*S. zeamais*) and red flour beetles (*T. castaneum*) were obtained from laboratory cultures maintained in the dark in incubators at 29 to 30℃ and 70 to 80% r elative humidity (r.h.). The red flour beetles were reared on wheat flour mixed with yeast (10:1, w/w) while maize weevils were reared on whole wheat at 12 to 13% moisture content in glass jars (diameter 85 mm, height 130 mm). Mixed-sex adult weevils/beetles used in all the experiments were about one week old. All the containers housing insects and the Petri dishes used in the experiments were made escape proof against insects with a coating of polytetrafluoro-ethylene (Fluon, Blades Biological, UK).

Gas chromatography-mass spectrometry

The essential oil of *E. lepta* was subjected to GC-MS analysis on an Agilent system consisting of a model 6890N GC, a model 5973N mass selective detector (EIMS, electron energy, 70 eV) and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a 5% phenyl-methylpolysiloxane stationary phase, film thickness of 0.25 µm, a length of 30 m, and an internal diameter of 0.25 mm. The GC settings were as follows: the initial oven temperature was held at 60℃ for 1 m in and ramped at 10℃ min^{-1} to 180℃ held for 1 min, and then ramped at 20℃ min^{-1} to 280℃ and held for 15 min. The injector temperature was maintained at 270℃. The sample (1 µl) was injected neat, with a split ratio of 1: 10. The carrier gas was helium at 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 GC by comparison of their retention indices with those of the literature (Diao and Gao, 2008; Bi et al., 2005) 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 08 and Wiley 275 libraries or with mass spectra from literature (Adams, 2007). Component relative percentages were calculated based on normalization method without using correction factors.

Contact toxicity by topical application

The contact toxicity of the essential oil of *E. lepta* root barks against *S. zeamais* and *T. castaneum* adults was measured as described by Liu and Ho (1999). Range-finding studies were run to determine the appropriate testing concentrations of the essential oil of *E. lepta*. A serial dilution of the essential oil (6 concentrations) was prepared in *n*-hexane. Aliquots of 0.5 µl per insect were topically applied dorsally to the thorax of the beetles, using a Burkard Arnold microapplicator. Controls were determined using 0.5 µl *n*-hexane per insect. Ten mixed-sex insects were used for each concentration and control, and the experiment was replicated six times. Both the treated and control weevils were then transferred to glass vials (10 insects/vial) with culture media and kept in incubators at 29 to 30℃ and 70 to 80% relative humidity. Mortality was observed after 24 h. The insects that have not presented any reaction when touched with a brush were considered as dead. Results from all replicates were subjected to probit analysis using the PriProbit Program V1.6.3 to determine LD_{50} values (Sakuma, 1998).

Fumigant toxicity bioassay

Range-finding studies were run to determine the appropriate testing concentrations of *Ancathia igniaria* essential oil. The fumigant toxicity of *E. lepta* essential oil was determined by the method of Liu and Ho (1999). A Whatman filter paper (diameter 2.0 cm) was placed on the underside of the screw cap of a glass vial (diameter 2.5 cm, height 5.5 cm, volume 24 ml). Ten microliters of the essential oil (6 concentrations) was added to the filter paper. The solvent was allowed to evaporate for 15 s before the cap was placed tightly on the glass vial (with 10 mixed-sex insects) to form a sealed chamber. They were incubated at 27 to 29℃ a nd 70 to 80%

Table 1. Chemical constituents of the essential oil derived from *E. lepta* root barks.

Compound	RI*	Peak area (%)
α-Pinene	939	26.68
Limonene	1028	2.58
Linalool oxide	1076	2.97
Linalool	1094	1.91
α-Fenchol	1098	3.87
α-Campholenal	1126	4.15
trans-Pinocarveol	1138	6.82
Borneol	1167	7.24
cis-ρ-Menth-2-en-1-ol	1126	1.57
α-Terpineol	1189	4.56
Myrtenol	1196	2.94
(S)-Verbenone	1204	0.76
cis-Carveol	1226	0.47
d-Carvone	1242	1.06
Geraniol	1252	0.34
Nonanoic acid	1264	0.81
Bornyl acetate	1285	1.55
α-Cubebene	1350	0.30
β-Elemene	1391	1.34
10s,11s-Himachala-3(12),4-diene	1399	1.53
Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1-methyl-6-methylene-4-(1-methylethyl)-	1401	1.14
α-Bergamotene	1413	0.65
α-Santalene	1422	1.22
(-)-Alloaromadendrene	1458	1.50
Curcumene	1474	0.53
β-Selinene	1483	0.45
Eremophilene	1486	1.49
1ξ,6ξ,7ξ-Cadina-4,9-diene	1502	1.28
β-Bisabolene	1506	0.77
δ-Cadinene	1523	2.35
epi-Globulol	1629	0.83
α-Cadinol	1653	2.95
α-Bisabolol	1683	2.72
Evodionol	1719	4.71
Phytol	2119	0.53
Total		96.16
Monoterpenoids		50.13
Sesquiterpenoids		41.93
Others		4.10

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

relative humidity for 24 h. Mortality of insects was observed and results from all replicates were subjected to probit analysis using the PriProbit Program V1.6.3 to determine LC_{50} values (Sakuma, 1998).

Table 2. Contact toxicity of essential oil of *E. lepta* against *S. zeamais* and *T. castaneum* adults.

Insect	Treatment	Concentration (%)	LC_{50} (mg/L air)	95% FL	Slope ± SE	Chi square (χ^2)
S. zeamais	**Essential oil**	**10.77-40.00**	**125.21**	**114.76-135.37**	**5.89 ± 0.86**	**23.8**
	Pyrethrum extract*	-	4.29	3.86-4.72	-	-
T. castaneum	Essential oil	20.90-52.00	166.94	153.09-183.22	4.99 ± 0.71	17.36
	Pyrethrum extract*	-	0.36	0.32-0.41	-	-

** Data from Liu et al. (2010).

RESULTS AND DISCUSSION

The yellow essential oil yield of *E. lepta* root barks was 0.24% (V/W) and the density of the concentrated essential oil was determined as 0.87 g/ml. A total of 35 components of the essential oil were identified, accounting for 96.57% of the total oil. The principal compounds in the essential oil of *E. lepta* were α-pinene (26.68%), borneol (7.24%), and *trans*-pinocarveol (6.82%) followed by evodionol (4.71%), α-terpineol (4.56%), and α-campholenal (4.15%) (Table 1). Monoterpenoids represented 16 of the 35 compounds, corresponding to 69.47% of the whole oil while 16 of the 35 constituents were sesquiterpenoids (21.05% of the crude essential oil). The composition of the essential oil derived from root barks was quite different from that of the stem and leaves reported in the previous studies. For example, the main constituents of essential oil of *E. lepta* leaves were caryophyllene oxide (7.73%) and caryophyllene (5.38%) (Bi et al., 2005) while the essential oil of *E. lepta* stem mainly contained hexadecanoic acid, δ-cadinene, octadecyl ester and neophytadiene (Liang and Gao, 2009).

The above findings suggest that there were great variations in chemical composition of the essential oil of *E. lepta* collected from different areas or obtained from different parts of the plants and further studies on plant cultivation and essential oil standardization are needed. The essential oil of *E. lepta* root barks possessed contact toxicity against *S. zeamais* and *T. castaneum* adults with LD_{50} values of 125.21 and 166.94 μg/adult, respectively (Table 2). However, when compared with the positive control pyrethrum extract (pyrethrum extract, 25% pyrethrine I and pyrethrine II), the essential oil of *E. lepta* root barks demonstrated only 29 and 463 times less acute toxicity against the two species of grain storage insects because the pyrethrum extract has acute toxicity to *S. zeamais* and *T. castaneum* with LD_{50} value of 4.29 and 0.36 μg/adult, respectively (Liu et al., 2010a). The essential oil of *E. lepta* root barks also exhibited stronger fumigant toxicity against *T. castaneum* adults (LC_{50} = 12.09 mg/L) than *S. zeamais* adults (LC_{50} = 25.05 mg/L) (Table 3). The commercial grain fumigant, methyl bromide (MeBr) was reported to have fumigant activity against *S. zeamais* and *T. castaneum* adults with LC_{50} values of 0.67 mg/L and 1.75 μg/L air, respectively (Liu and Ho, 1999). Compared with the commercial fumigant MeBr, the essential oil of *E. lepta* root barks was 37 and 7 times less toxic to *S. zeamais* and T. *castaneum*, respectively. However, considering the commercial fumigants are synthetic insecticides, fumigant activity of the essential oil of *E. lepta* is quite promising. Compared with the other essential oils in the literature, the essential oil of *E. lepta* possessed the same level of fumigant toxicity against *T. castaneum* adults, for example, essential oils of *Murraya exotica* (LC_{50} = 6.84 mg/L, Li et al., 2010), *Citrus reticulata* (LC_{50} = 19.47 μL/L), *Schinus terebenthifolius* (LC_{50} = 20.50 μl/L) (Mohamed and Abdelgaleil, 2008), *Perovskia abrotanoides* (LC_{50} = 11.39 μL/L, Arabi et al., 2008), and *Drimys winteri* (LC_{50} = 9.0-10.5 μL/L, Zapata and Smagghe, 2010), but lesser toxic than the essential oil of *Laurelia sempervirens* (LC_{50} = 1.6-1.7 μL/L, Zapata and Smagghe, 2010). Moreover, compared with the other essential oils in the previous studies, the essential oil of *E. lepta* also exhibited the same level of fumigant toxicity against the maize weevils, for example, essential oils of *M. exotica* (LC_{50} = 8.29 mg/L, Li et al., 2010), *Artemisia lavandulaefolia* (LC_{50} = 11.2 mg/L, Liu et al., 2010a), *Artemisia vestita* (LC_{50} = 13.42 mg/L, Chu et al., 2010a), *Illicium simonsii* (LC_{50} = 14.95 mg/L, Chu et al., 2010b), *A. sieversiana* (LC_{50} = 15.0 mg/L, Liu et al., 2010a) and *Kadsura heteroclite* (LC_{50} = 14.01 mg/L, Li et al., 20111).

In some previous reports, α-pinene, the main constituent of the essential oil had been reported to be toxic to several insects/mites, for example, *T. castaneum* (Kim et al., 2010), *Callosobruchus chinensis*, *Sitophilus oryzae* (Park et al., 2003), house flies, *Musca domestica* (Palacios et al., 2009), German cockroaches, *Blattella germanica* (Jung et al., 2007), larvae of *Pseudaletia unipuncta* and *Trichoplusia ni* (Isman et al., 2008), larva of the western corn rootworm, *Diabrotica virgifera* and the twospotted spider mite, *Tetranychus urticae* adult (Lee et al., 1997), and mosquito *Culex pipiens molestus* (Traboulsi et al., 2002). The isolation and identification of the bioactive compounds in the essential oil of *E. lepta* root barks are of utmost importance so that their potential application in controlling stored-product pests can be fully exploited. The above findings suggest that the essential oil of *E. lepta* can play an important role in stored grain protection and reduce the risks associated with of

Table 3. Fumigant toxicity of essential oil of *E. lepta* against *S. zeamais* and *T. castaneum* adults.

Insect	Treatment	Concentration (%)	LD50 µg/adult	95% FL	Slope ± SE	Chi square (χ^2)
S. zeamais	Essential oil	2.37-18.00	25.05	21.99-28.69	2.87 ± 0.31	15.36
	MeBr*	-	0.67	-	-	-
T. castaneum	Essential oil	1.91-20.00	12.09	17.27-20.08	2.48 ± 0.26	8.12
	MeBr*	-	1.75	-	-	-

* From Liu and Ho (1999).

synthetic insecticides. However, for the practical application of the essential oil as novel insecticide/fumigant, further studies on its safety to humans and on development of formulations are necessary to improve its efficacy and stability and to reduce cost.

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