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Composition and insecticidal activity of the essential oil of *Pelargonium hortorum* flowering aerial parts from China against two grain storage insects

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The aim of this research was to determine chemical composition and insecticidal activity of the essential oil of *Pelargonium hortorum* Bailey (Geraniaceae) flowering aerial parts against the booklouse (*Liposcelis bostrychophila* Badonnel) and maize weevils (*Sitophilus zeamais* Motschulsky). The essential oil of *P. hortorum* flowering aerial parts was obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 36 components of the essential oil were identified. The principal compounds in the essential oil were 1,8-cineole (23.01%), α-terpineol (13.22%), α-pinene (8.13%) and camphor (8.12%) followed by 4-terpineol (4.63%), β-myrcene (4.56%) and β-caryophyllene (4.08%). The essential oil exhibited contact toxicity against *L. bostrychophila* and *S. zeamais* with LD_{50} values of 149.78 µg/cm^2 and 14.41 µg/adult, respectively. The essential oil also possessed fumigant toxicity against *L. bostrychophila* and *S. zeamais* adults with LC_{50} values of 1.31 mg/L air and 7.22 mg/L air, respectively. The results indicated that the essential oil of *P. hortorum* showed potential in terms of contact and fumigant toxicity against grain storage insects.

Key words: *Pelargonium hortorum*, *Liposcelis bostrychophila*, *Sitophilus zeamais*, contact toxicity, fumigant, essential oil composition

INTRODUCTION

The maize weevil (*Sitophilus zeamais* Motschulsky) is one of the major pests of stored grains and grain products in the tropics and subtropics (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). The booklouse (*Liposcelis bostrychophila* Badonnel) is frequently found in extremely high numbers in amylaceous products. Currently, psocids are one of the most important categories of emerging pests in stored grains and related commodities (Turner, 1999). Infestations of stored product insects are typically controlled by fumigation or insecticidal treatment of commodities and surfaces, which has led to problems such as pest resurgence and increasing costs of application originate from resistance to insecticides as well as lethal effects of these synthetic insecticides on non-target organisms in addition to direct toxicity to users (Zettler and Arthur, 2000). Fortunately, essential oils or their constituents may provide an alternative to currently used fumigants/pesticides to control stored-food insects/mites. Investigations in several countries confirm that some plant essential oils not only repel insects, but possess

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contact and fumigant toxicity against stored product pests as well as exhibited feeding inhibition or harmful effects on the reproductive system of insects (Isman, 2006; Phillips and Throne, 2010). Many essential oils from plants including medicinal herbs, spices and fruits have been evaluated and shown to be effective as pesticides against stored product insects (Liu et al., 2007; Rajendran and Srianjini, 2008; Chu et al., 2011; Liu and Du, 2011). During our mass screening program for new agrochemicals from Chinese medicinal herbs and wild plants, the essential oil of *Pelargonium hortorum* Bailey (Geraniaceae) flowering aerial parts was found to possess insecticidal activity against the two grain storage insects. *P. hortorum* is annual or perennial herbaceous plant, sometimes woody, and originated from Southern Africa and has been widely cultivated in China now (Xu and Huang, 1998). Several triterpenoids, sterols, flavonoids and anacardic acids were isolated from this plant (Aexel et al., 1972; Gerhold et al., 1985). Chemical composition of the essential oil derived from *P. hortorum* has been also determined (Carro and Retamar, 1971; Deng et al., 2004; Wang et al., 2010). This plant has been shown to secrete anacardic acids in the form of a viscous sticky exudate from tall glandular trichomes, and this exudate provides a sticky trap defense against small pest species (Gerhold et al., 1984; Grazzini et al., 1997; Schultz et al., 2006). However, a literature survey has shown that there is no report on insecticidal activity of the essential oil of *P. hortorum* flowering aerial parts, thus we decided to investigate the chemical constituents and insecticidal activities of the essential oil derived from *P. hortorum* flowering aerial parts against the two grain storage insects for the first time.

MATERIALS AND METHODS

Plant

The flowering aerial parts of *P. hortorum* were collected in August 2011 from the campus of Bejing University of Agriculture (Beijing 102206). The samples (20 kg) were air-dried for two weeks and identified by Dr. Liu Q. R. (College of Life Sciences, Beijing Normal University, Beijing 100875, China) and a voucher specimen (ENTCAU-Geraniaceae -10045) was deposited at the Department of Entomology, China Agricultural University (Beijing 100193). The samples were ground to a powder using a grinding mill (Retsch Mühle, Germany). 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°C) for subsequent experiments.

Insects

The maize weevils (*S. zeamais*) were obtained from laboratory cultures in the dark in incubators at 29 to 30°C and 70 to 80% relative humidity and were reared on whole wheat at 12 to 13% moisture content in glass jars (diameter 85 mm, height 130 mm). Unsexed adult weevils used in all the experiments were about one week old. All containers housing insects and the petri dishes used in experiments were made escape proof with a coating of polytetrafluoroethylene (Fluon, Blades Biological, UK). Booklice, *L. bostrychophila* were obtained from laboratory cultures that had been reared in the dark in incubators at 28 to 30°C and 70 to 80% relative humidity and on a 1: 1: 1 mixture (by mass) of milk powder, active yeast, and flour. All the containers housing insects and the petri dishes used in experiments were made escape proof with a coating of polytetrafluoroethylene (Fluon, Blades Biological, UK). Unsexed adult insects used in all the experiments were approximately one week old.

Gas chromatography-mass spectrometry

The essential oil of *P. hortorum* flowering aerial parts was subjected to GC-MS analysis on an Agilent system consisting of a model 6890N gas chromatograph, 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°C for 1 min and ramped at 10°C min^{-1} to 180°C held for 1 min, and then ramped at 20°C min^{-1} to 280°C and held for 15 min. The injector temperature was maintained at 270°C. 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. 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 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 to 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

Range-finding studies were run to determine the appropriate testing concentrations of the essential oil of *P. hortorum* flowering aerial parts. A serial dilution of the essential oil (8.00 to 25.00%, 6 concentrations) was prepared in *n*-hexane. Aliquots of 0.5 µl per insect were topically applied dorsally to the thorax of the weevils, using a Burkard Arnold microapplicator. Controls were determined using 0.5 µl *n*-hexane per insect. Ten 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°C and 70 to 80% relative humidity. Mortality was observed after 24 h. Results from all the replicates were subjected to probit analysis using the PriProbit Program V1.6.3 to determine LD_{50} values (Sakuma, 1998).

Contact toxicity by filter paper impregnation

Contact toxicity bioassays were conducted using filter paper with a diameter of 3.5 cm and treated with 150 µl of the essential oil. The filter papers were attached to the Petri dish (3.5 cm in diameter) using a glue stick (Jong Ie Nara Co., Ltd. Hong Kong) which was

placed in a Petri dish and 10 booklice were put on the filter paper.

The Petri dishes were covered by using glass covers with small holes and placed in incubators at 27 to 29°C, 70 to 80% relative humidity. Range-finding studies were run to determine the appropriate testing concentrations of *P. hortorum* essential oil. Five concentrations (8,500 to 30,000 ppm, in acetone) and five replicates of each concentration were used to determine LC_{50} values. All the treatments were replicated three times. Acetone was used as a negative control and pyrethrum extract was used as a positive control. Mortality of insects was observed after 24 h. The observed mortality data were corrected for control mortality using Abbott's formula. Results from all replicates were subjected to probit analysis using the PriProbit Program V1.6.3 to determine LC_{50} values (Sakuma, 1998). Pyrethrum extract (25% pyrethrine I and pyrethrine II) was purchased from Fluka Chemie.

Fumigant toxicity bioassay

Range-finding studies were run to determine the appropriate testing concentrations of *P. hortorum* essential oil against the weevils. 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 (2.00 to 20.00%, 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 unsexed insects) to form a sealed chamber. They were incubated at 27 to 29°C and 70 to 80% relative humidity for 24 h. Mortality of insects was observed and results from all the replicates were subjected to probit analysis using the PriProbit Program V1.6.3 to determine LC_{50} values (Sakuma, 1998).

For fumigant toxicity bioassays against the booklice, a filter paper strip (3.5 × 1.5 cm) was treated with 10 µl of an appropriate concentration of the essential oil. The impregnated filter paper was then placed on the underside of the lid of a 250 ml glass bottle. A small glass bottle (8 ml, containing ten unsexed adults booklice) were put into the glass bottle and exposed for 24 h and each concentration with five replicates. All the treatments were replicated three times. Acetone was used as a negative control and dichlorvos was used as a positive control. The observed mortality data were corrected for control mortality using Abbott's formula. The LC_{50} values were calculated using Probit analysis (Sakuma, 1998). Dichlorvos (99.9%) was purchased from Aladdin-reagent Company (Shanghai).

RESULTS AND DISCUSSION

The yellow essential oil yield of *P. hortorum* flowering aerial parts was 0.11% (V/W) and the density of the concentrated essential oil was determined as 0.87 g/ml. A total of 36 components of the essential oil were identified, accounting for 97.68% of the total oil. The principal compounds in the essential oil of *P. hortorum* flowering aerial parts were 1,8-cineole (23.01%), α-terpineol (13.22%), α-pinene (8.13%) and camphor (8.12%) followed by 4-terpineol (4.63%), β-myrcene(4.56%) and β-caryophyllene (4.08%) (Table 1). Monoterpenoids represented 24 of the 36 compounds, corresponding to 83.54% of the whole oil while 10 of the 36 constituents were sesquiterpenoids (11.93% of the crude essential oil).

The essential oil of *P. hortorum* flowering aerial parts possessed contact toxicity against *L. bostrychophila* with an LD_{50} value of 149. 78 µg/cm^2 (Table 2). The positive control, pyrethrum extract has contact activity with an LD_{50} value of 18.99 µg/cm^2, thus the essential oil shows 7 times less active against *L. bostrychophila* adults. The essential oil also exhibited contact toxicity against *S. zeamais* adults with an LD_{50} value of 14.41 µg/adult (Table 3). When compared with the positive control pyrethrum extract, the essential oil demonstrated 5 times less toxic against *S. zeamais* (Wang et al., 2011). However, compared with the other essential oils in the literature, the essential oil of *P. hortorum* flowering aerial parts possessed stronger contact toxicity against *S. zeamais* adults, e.g. essential oils of *Artemisia lavandulaefolia*, *Artemisia sieversiana*, *Artemisia capillaries*, *Artemisia mongolica*, and *Artemisia vestita* (LD_{50} = 55.2, 113.0, 106.0, 87.9, and 50.6 µg/adult, respectively) (Liu et al., 2010a, b; Chu et al., 2010a), essential oil of *Schizonpeta multifida* (30.2 µg/adult) (Liu et al., 2011), essential oil of *Illicium simonsii* fruits (LD_{50} = 112.7 µg/adult) (Chu et al., 2010b).

The essential oil of *P. hortorum* flowering aerial parts exhibited fumigant toxicity against *L. bostrychophila* adults with an LC_{50} value of 1.31 mg/L air (Table 2). However, the positive control, dichlorvos has fumigant activity with an LC_{50} value of 1.35 µg/L air, thus the essential oil demonstrated 970 times less toxic against *L. bostrychophila* (Table 2). The essential oil of *P. hortorum* flowering aerial parts also possessed fumigant toxicity against the maize weevils with an LC_{50} value of 7.22 mg/L (Table 2). The commercial grain fumigant, methyl bromide (MeBr) was reported to have fumigant activity against *S. zeamais* adults with an LC_{50} value of 0.67 mg/L (Liu and Ho, 1999), thus the essential oil was 11 times less toxic to *S. zeamais* adults compared with MeBr. However, compared with the other essential oils in the previous studies, the essential oil of *P. hortorum* exhibited stronger fumigant toxicity against *S. zeamais* adults, e.g. essential oils of *S. multifida* (Liu et al., 2011), *Murraya exotica* (Li et al., 2010), and several essential oils from Genus *Artemisa* (Chu et al., 2010a; Liu et al., 2010a, b). Moreover, in the previous reports, those main constituent monoterpenoids of the essential oil, 1,8-cineole, α-terpineol, α-pinene and camphor have been found to possess fumigant toxicity against several stored product insects, such as *Sitophilus granarius*, *Sitophilus oryzae*, *Tribolium castaneum, Tribolium confusum*, and *Rhyzopertha dominica* (Aggarwal et al., 2001; Lee et al., 2004; Kordali et al., 2006; Rozman et al., 2007; Abdelgaleil et al., 2009; Suthisut et al., 2011).

The earlier findings suggest that fumigant activity of the essential oil of *P. hortorum* flowering aerial parts is quite promising by considering the currently used fumigants are synthetic insecticides and it shows potential to be developed as possible natural fumigant/insecticide for control of stored product insects. However, for the practical application of the essential oil as novel insecticide/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.

Table 1. Chemical constituents of the essential oil derived from *Pelargonium hortorum* flowering aerial parts.

Peak No.	Compound	RI*	Peak area (%)
1	α-Pinene	931	8.13
2	Camphene	953	0.50
3	β-Pinene	974	3.31
4	β-Myrcene	991	4.56
5	Yomogi alcohol	998	0.83
6	δ-4-Carene	1002	0.73
7	o-Cymene	1020	1.38
8	D-Limonene	1027	0.70
9	β-Phellandrene	1030	2.51
10	1,8-Cineole	1032	23.01
11	γ-Terpinene	1059	0.15
12	Artemisia ketone	1062	3.02
13	Artemesia alcohol	1083	1.65
14	Myrcenol	1126	1.32
15	*trans*-Pinocarveol	1139	3.23
16	Camphor	1146	8.12
17	β-Terpineol	1149	0.02
18	(-)-Borneol	1152	0.48
19	4-Terpineol	1179	4.63
20	α-Terpineol	1191	13.22
21	2,6-Dimethyl-3(E),5(E),7-octatriene-2-ol	1208	1.36
22	Carvone	1238	0.12
23	Bornyl acetate	1287	0.34
24	Carvacrol	1303	0.23
25	Triacetin	1344	1.81
26	Eugenol	1356	0.39
27	Copaene	1375	0.95
28	β-Caryophyllene	1430	4.08
29	(Z)-β-Farnesene	1438	0.73
30	*allo*-Aromadendren	1458	1.43
31	Germacrene D	1485	2.66
32	δ-Selinene	1492	0.22
33	δ-Cadinene	1523	0.17
34	Spathulenol	1578	0.21
35	α-Cedrol	1598	0.17
36	β-Eudesmol	1648	1.31
	Total	-	97.68
	Monoterpenoids	24	83.54
	Sesquiterpenoids	10	11.93
	Others	2	2.20

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

Table 2. Contact (CT) and Fumigant toxicity (FT) of the essential oil of *Pelargonium hortorum* flowering aerial parts against *Liposcelis bostrychophila* adults.

Parameter	Treatment	LD_{50} (µg/cm^2)/LC_{50} (mg/L air)	95% FL	Slope ± SE	Chi square (χ^2)
CT	*P. hortorum*	149.78	143.33-154.96	14.80±1.90	6.31
	Pyrethrum extract	18.99	17.56-20.06	7.64±1.05	9.78
FT	*P. hortorum*	1.31	1.19-1.44	4.82±0.51	13.34
	Dichlorvos	1.35×10^{-3}	1.25×10^{-3}- 1.47×10^{-3}	6.87±0.77	12.13

Table 3. Contact (CT) and Fumigant toxicity (FT) of the essential oil *Pelargonium hortorum* flowering aerial parts against *Sitophilus zeamais* adults.

Parameter	Treatment	LD_{50} (μg/adult)/LC_{50} (mg/L air)	95% FL	Slope ± SE	Chi square (χ^2)
CT	*P. hortorum*	23.56	21.56-25.78	4.19±0.46	15.96
	Pyrethrum extract*	4.29	3.86-4.72	-	-
FT	*P. hortorum*	7.01	6.32-7.66	4.38±0.50	14.56
	MeBr**	0.67	-	-	-

*Wang et al. (2011). ** Liu and Ho (1999).

Conclusion

The essential oil of *P. hortorum* flowering aerial parts was demonstrated to exhibit contact and fumigant toxicity against the two grain storage insects, *L. bostrychophila* and *S. zeamais* adults. These findings suggest that the essential oil of *P. hortorum* flowering aerial parts possessed potential for development as novel natural insecticide/fumigant for stored products.

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