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Full Length Research Paper

Insecticidal activity of the essential oil of *Lonicera japonica* flower buds and its main constituent compounds against two grain storage insects

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The aim of this research was to determine acute toxicity of the essential oil of *Lonicera japonica* Thunb. (Caprifoliaceae) flower buds against the booklouse (*Liposcelis bostrychophila* Badonnel) and the maize weevils (*Sitophilus zeamais* Motschulsky). Essential oil of *L. japonica* flower buds was obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 25 components of the essential oil were identified. The principal compounds in the essential oil were estragole (80.17%) and linalool (6.05%). The essential oil exhibited strong contact toxicity against *S. zeamais* and *L. bostrychophila* with LD_{50} values of 21.54 µg/adult and 64.04 µg/cm^2, respectively. The constituent compounds, estragole (LD_{50} = 49.95 µg/cm^2) and linalool (LD_{50} = 172.54 µg/cm^2) also possessed contact toxicity against *L. bostrychophila*. *L. japonica* essential oil and its constituent compounds (estragole and linalool) exhibited fumigant toxicity against *S. zeamais* with LC_{50} values of 13.36, 14.10 and 10.46 mg/L, respectively. The essential oil of *L. japonica* (LC_{50} = 0.20 mg/L) and its constituent compounds, estragole (LC_{50} = 0.16 mg/L) and linalool (LC_{50} = 0.41 mg/L) possessed fumigant toxicity against *L. bostrychophila*. The results indicated that the essential oil of *L. japonica* and its constituent compounds showed potential in terms of contact and fumigant toxicity against grain storage insects.

Key words: *Lonicera japonica*, *Liposcelis bostrychophila*, *Sitophilus zeamais*, contact toxicity, fumigant, essential oil composition, estragole, linalool.

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 stored-product grains, often in extremely high numbers, in amylaceous products (Nayak et al., 2005). Currently, psocids are perhaps the most important category of emerging pests in stored grains and related commodities (Turner, 1999). Infestations of stored product insects could be controlled by fumigation or insecticidal treatment of commodities and surfaces, which has led to problems such as disturbances of the environment, increasing costs of application, pest resurgence, pest resistance to pesticides and lethal effects on non-target organisms in addition to direct toxicity to users (Zettler and Arthur, 2000).

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Essential oils or their constituents may provide an alternative to currently used fumigants/pesticides to control stored-food insects (Isman, 2000). Investigations in several countries confirm that some plant essential oils not only repel insects, but possess 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). The toxicity of a large number of essential oils and their constituents has been evaluated against a number of 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 design of target-specific molecules (Isman, 2006). During the screening program for new agrochemicals from Chinese medicinal herbs, the essential oil of *Lonicera japonica* Thunb. (Family: Caprifoliaceae) flower buds was found to possess strong insecticidal toxicity against the two grain storage insects, *S. zeamais* and *L. bostrychophila*.

L. japonica is a medicinal plant widely used in China. As a traditional Chinese medicine with a wide spectrum of biological and pharmacological properties, the dried flowers of *L. japonica* have been used in clinical practice for thousands of years for their antibacterial, antiviral and antioxidant activities and in the treatment of exopathogenic wind-heat, epidemic febrile diseases, sores, carbuncles and furuncles (Jiangsu New Medical College, 1977; Peng et al., 2000). The essential oil and chlorogenic acid are reported to be effective components of *L. japonica* (Li et al., 2006). The essential oil of *L. japonica* is an edible natural perfume that is often used in foods, cigarettes and cosmetics (Schlotzhauer et al., 1996, Shang et al., 2011). Previous phytochemical studies on *L. japonica* resulted in the identification of several saponins, triterpenoid saponins, cerebrosides, caffeoylquinic acids and esters, flavoids, alkaloids and iridoid glucosides (Kawai et al., 1988a, b; Son et al., 1992; 1994; Kakuda et al. 2000; Teng et al., 2000; Kumar et al., 2005; 2006; Lin et al., 2008; et al., 2008).

The chemical composition of *L. japonica* essential oil was also studied previously (Ikeda et al., 1994; Schlotzhauer et al., 1996; Rahman and Kang, 2009; wang et al., 2009). The methanol extract of leaves and twigs of *L. japonica* possessed insecticidal and acaricidal activity against the cotton aphid (*Aphis gossypii*), the green peach aphid (*Myzus persicae*), the greenhouse whitefly (*Trialeurodes vaporariorum*), the two-spotted spider mite (*Tetranychus urticae*), and the citrus red mite (*Panonychus citri*) (Kim et al., 2005) while repellency of the *n*-hexane, ethyl acetate, *n*-butanol and water extracts of *L. japonica* were observed against the Asian tiger mosquito, *Aedes albopitus* (Yoon and Kyung, 2002).

However, insecticidal activity of *L. japonica* essential oil against grain storage insects was not determined. This study analyses the chemical composition and toxicity of essential oil of *L. japonica* flower buds against the two grain storage insects.

MATERIALS AND METHODS

Plant material

Three kilogram of fresh flower buds of *L. japonica* were collected from Pingyi Country (35.35° N latitude and 117.37° E longitude), Shangdong Province, China in May 2011. The samples were air-dried and identified by Dr. Liu, Q.R. (College of Life Sciences, Beijing Normal University, Beijing 100875, China) and a voucher specimen ((BNU-DuShushan-Jinyinghua-shangdong-2011-05) was deposited in the State Key Laboratory of Earth Surface Processes and Resource Ecology, Beijing Normal University. 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℃) for subsequent experiments. Estragole (98%) and linalool (98%) were purchased from Aladdin-Reagent (China) Co. (Shanghai 201206, China).

Insects

The maize weevils (*S. zeamais*) and the booklouse, *L. bostrychophila* were obtained from laboratory cultures in the dark in incubators at 29 to 30℃ and 70 to 80% relative humidity. The maize weevils were reared on whole wheat at 12 to 13% moisture content in glass jars (diameter 85 mm, height 130 mm) and the booklouse were reared on a 1: 1: 1 mixture, by mass, of milk powder, active yeast, and flour. Unsexed adult weevils and booklous 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).

Gas chromatography-mass spectrometry (GC-MS)

The essential oil of *L. japonica* was subjected to GC-MS analysis on an Agilent system consisting of a model 6890N gas chromatograph, a model 5973 N mass selective detector (EIMS, electron energy, 70 eV), and an Agilent ChemStation data system. The GC column was an HP-5 ms 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 min 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 gas chromatography by comparison of their retention indices with those of the literature (Ikeda et al., 1994; Schlotzhauer et al., 1996; Rahman and Kang, 2009) 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

Range-finding studies were run to determine the appropriate testing concentrations of the essential oil of *L. japonica* flower buds. A serial dilution of the essential oil 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℃ and 70 to 80% relative humidity. Mortality was observed after 24 h. Results from all 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

Range-finding studies were run to determine the appropriate testing concentrations of *L. japonica* flower buds essential oil and the constituent compounds. A 3.5 cm diameter filter paper was treated with 150 µl of the solution of the essential oil/compounds. The filter paper after treated with solid glue (Glue Stick, Jong Ie Nara Co., Ltd. Hong Kong) was placed in a 3.5 cm diameter petri dish and 10 booklice were put on the filter paper. A cover was put and all the peteri dishes were kept in incubators at 27 to 29℃, 70 to 80% relative humidity. Acetone was used as a negative control and pyrethrum extract was used as a positive control. Five concentrations (in acetone) and five replicates of each concentration were used. Mortality of insects was observed after 24 h and 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 the pure compounds and *L. japonica* flower buds essential oil. A filter paper strip (3.5 ´ 1.5 cm) treated with 10 µl of an appropriate concentration of the test essential oil/compounds. The impregnated filter paper was then placed in the bottom cover of a 250 ml volume of glass bottle. Ten unsexed adults of the booklouse in a small glass bottle (8 ml) were put into the glass bottle and exposed for 24 h. Five concentrations of the oil/compounds were used in the experiments and each concentration with five replicates. Acetone was used as a negative control and dichlorvos was used as a positive control. The LC_{50} values were calculated by using Probit analysis (Sakuma, 1998). Dichlorvos (99.9%) was purchased from Aladdin-reagent Co. (Shanghai, China).

The fumigant toxicity of *L. japonica* flower buds essential oil/constituent compounds against the maize weevils was determined by used the method of Liu and Ho (1999) with some modifications. 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/ compounds 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℃ and 70 to 80% 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).

RESULTS AND DISCUSSION

The yellow essential oil yield of *L. japonica* flower buds was 0.07% (V/W) and the density of the concentrated essential oil was determined to be 0.96 g/ml. A total of 25 components of the essential oil were identified, accounting for 94.35% of the total oil. The principal compounds in *L. japonica* flower buds essential oil were estragole (80.17%) and linalool (6.05%) followed by germacrene D (3.17%) (Table 1). Monoterpenoids represented 10 of the 25 compounds, corresponding to 82.62% of the whole oil while also 10 of the 25 constituents were sesquiterpenoids (5.88% of the crude essential oil). The result is quite different from the previous report. For example, the major compounds detected in the oil of *L. japonica* floral parts harvested from Korean were *trans*-nerolidol (16.31%), caryophyllene oxide (11.15%), linalool (8.61%), ρ-cymene (7.43%), hexadecanoic acid (6.39%), eugenol (6.13%), and geraniol (5.01%) (Rahman and Kang, 2009).

Twenty-seven compounds were identified among the three developmental stages of *L. japonica* flowers. Germacrene D was a major component at all stages; linalool and α-farnesene appeared in high concentrations in fresh and 24 h flowers but were greatly reduced in overnight flowers (Schlotzhauer et al., 1996). Moreover, Wang et al. (2009) demonstrated that the primary components of the volatile oil were linalool (0.15 to 15.35%), linalool oxide (0.09 to 6.53%), geraniol (0.24 to 8.17%) and α-terpineol (0 to 10.57%) and obvious variations in components of the volatile oil were observed at six different developmental stages. The foregoing findings suggest that there are great variations in chemical composition of the essential oil of different population and even of different development stage of the same population. Further studies on plant cultivation and essential oil standardization are needed.

The essential oil of *L. japonica* flower buds exhibited contact toxicity against *S. zeamais* adults with a LD_{50} value of 21.54 mg/adult (Table 2). When compared with the positive control pyrethrum extract, the essential oil demonstrated 5 times less toxic against *S. zeamais*. *L. japonica* essential oil also possessed contact toxicity (LD_{50} = 64.04 mg/cm^2) against the booklouse and the two constituent compounds, estragole and linalool acting against the booklouse with LD_{50} values of 49.95 and 172.54 mg/cm^2, respectively (Table 3). When compared with the positive control, pyrethrum extract, the essential oil and its constituent compounds, estragole and linalool, showed 3, 2.5 and 9 times less active against the booklouse (Table 3). However, compared with the other

Table 1. Chemical constituents of essential oil derived from *Lonicera japonica*.

Compounds	RI*	Peak area (%)
1-Octen-3-ol	978	0.04
β-Pinene	981	0.03
(d)-Limonene	1029	0.17
1, 8-Cineole	1032	0.31
Benzyl alcohol	1034	0.08
Acetophenone	1065	0.34
Linalool	1094	6.05
Phenylethyl alcohol	1116	0.25
α-Terpineol	1189	0.12
Estragole	1197	80.17
Linalool acetate	1248	0.28
(Z)-β-Damascenone	1347	0.16
α-Cubebene	1350	0.12
Eugenol	1361	0.23
β-Geranyl acetate	1379	0.12
β-Caryophyllene	1420	0.25
β-Gurjenene	1435	0.36
Germacrene D	1480	3.17
α-Selinene	1492	0.31
α-Farnesene	1508	1.01
β-Cadinene	1519	0.12
Elemol	1551	0.22
Caryophyllene oxide	1583	0.15
α-Cadinol	1654	0.11
Junipher camphor	1695	0.18
Total		94.35
Monoterpenoids		87.62
Sesquiterpenoids		5.88
Others		0.85

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

Table 2. Contact toxicity of *Lonicera japonica* essential oil and its main constituent compounds against *Sitophilus zeamais* adults.

Treatment	LD_{50} (μg/adult)	95% FL	Slope ± SE	Chi square (χ^2)
L. japonica	21.54	19.56-23.42	4.11 ± 0.42	20.72
Estragole*	17.63	15.56-19.97	3.09 ± 0.32	12.04
Linalool*	13.90	13.05-14.83	5.86 ± 0.55	9.80
Pyrethrum extract*	4.29	3.86-4.72	-	-

***** from Wang et al. (2011).

essential oils in the literature, the essential oil of *L. japonica* possessed stronger contact toxicity against *S. zeamais* adults, for example, 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, 2010b; 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 *L. japonica* and its constituent compounds, estragole and linalool exhibited fumigant toxicity against the maize weevils with LC_{50} values of 13.36, 14.10 and 10.46 mg/L, respectively (Table 4). The commercial grain fumigant, methyl bromide (MeBr) was reported to have fumigant activity against *S. zeamais* adults with a LC_{50} value of 0.67 mg/L (Liu and Ho, 1999), thus the essential oil and its constituent compounds were 15 to 21 times less toxic to *S. zeamais* adults compared with MeBr. Moreover, the essential oil of *L. japonica* (LC_{50} = 0.20 mg/L) and its constituent compounds, estragole (LC_{50} = 0.16 mg/L) and linalool (LC_{50} = 0.41 mg/L) possessed fumigant toxicity against the booklouse.

Compared with the positive control, dichlorvos (LC_{50} =

Table 3. Contact toxicity of *Lonicera japonica* essential oil and its main constituent compounds against *Liposcelis bostrychophila*.

Treatment	LD_{50} (μg/cm^2)	95% FL	Slope ± SE	Chi square (χ^2)
L. japonica	64.04	60.44-69.85	14.98 ± 1.42	27.42
Estragole	49.95	48.04-52.14	19.65 ± 2.01	30.36
Linalool	172.54	157.71-186.94	27.86 ± 2.53	9.43
Pyrethrum extract	18.99	17.56-20.06	7.64 ± 1.05	22.96

Table 4. Fumigant toxicity of essential oil of *Lonicera japonica* and its main constituent compounds against *Sitophilus zeamais* (SZ) and *Liposcelis bostrychophila* (LB) adults.

Insects	Treatment	LC_{50} (mg/L air)	95% FL	Slope ± SE	Chi square (χ^2)
SZ	*L. japonica*	13.36	11.63-14.96	2.44 ± 0.30	21.23
	Estragole*	14.10	12.45-15.97	3.09 ± 0.32	12.04
	Linalool*	10.46	9.58-11.55	4.20 ± 0.47	10.36
	MeBr**	0.67	-	-	-
LB	*L. japonica*	0.20	0.18-0.22	4.97 ± 0.46	33.59
	Estragole	0.16	0.13-0.19	5.01 ± 0.45	67.39
	Linalool	0.41	0.37-0.43	8.39 ± 0.93	22.54
	Dichlorvos	1.35×10^{-3}	1.25×10^{-3}-1.40×10^{-3}	6.87 ± 0.77	19.78

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

1.35 μg/L), the essential oil and its constituent compounds show 100 to 300 times less fumigant toxicity against the booklouse. However, by considering the currently used fumigants are synthetic insecticides, the foregoing findings suggest that fumigant activity of the essential oil of *L. japonica* and its constituent compounds are quite promising and they show potential to be developed as possible natural fumigants/insecticides for control of stored product insects. For the practical application of the essential oil and its constituent compounds as novel insecticides/fumigants, further studies on the safety of the essential oil/compounds to humans and on development of formulations are necessary to improve the efficacy and stability and to reduce cost.

Conclusion

The composition of the essential oil derived from *L. japonica* flower buds was determined by GC-FID and GC-MS. The essential oil and its constituent compounds exhibited strong contact and fumigant toxicity against the two grain storage insects.

These findings suggest that the essential oil of *L. japonica* flower buds and its constituent compounds possess potential for development as novel natural insecticides/fumigants for stored products. Further studies are required to evaluate the safety of the essential oil/compounds to humans.

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