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Full Length Research Paper

Composition and insecticidal activity of the essential oil of *Cananga odorata* leaves against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

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The aim of this research was to determine chemical composition and insecticidal activity of the essential oil of *Cananga odorata* (Lam.) Hook. f. and Thomson leaves against maize weevils (*Sitophilus zeamais* Motschulsky). Essential oil of *C. odorata* leaves was obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 23 components of the essential oil were identified. The principal compounds in the essential oil were linalool (21.08%), linalool acetate (16.14%), α-pinene (12.73%), eugenol (8.86%) and α-terpineol acetate (7.71%) followed by isobornyl acetate (3.56%), α-terpineol (3.46%), and camphor (3.23%). The essential oil of *C. odorata* leaves exhibited strong contact toxicity against *S. zeamais* with an LD_{50} value of 33.14 µg/adult. The essential oil also possessed fumigant toxicity against *S. zeamais* with an LC_{50} value of 14.77 mg/L. The results indicated that the essential oil of *C. odorata* leaves showed potential in terms of contact and fumigant toxicity against grain storage insect.

Key words: *Cananga odorata*, *Sitophilus zeamais*, contact toxicity, fumigant, essential oil composition.

INTRODUCTION

The maize weevil (*Sitophilus zeamais* Motschulsky) is associated with corn storage, where it can attack the whole kernel. It 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). Currently, control of stored product insects relies heavily on the use of synthetic insecticides and fumigants, which has led to problems such as disturbance of the environment, increasing application costs, pest resurgence, pest resistance to pesticides and lethal effects on non-target organisms in addition to direct toxicity to the users (Zettler and Arthur, 2000). Thus, there is a considerable interest in developing natural products that are relatively less damaging to mammalian health and the environment than existing conventional pesticides, as alternatives to non-selective synthetic pesticides to control the pests of medical and economic importance (Isman, 2000, 2006). The use of plant derived insecticides has played important role in traditional method of storage pest control in Africa and Asia (Bekele and Hassanali, 2001; Liu et al., 2007). In view of the potential of plant products as alternative eco-friendly insect pest control agents, in China, there has been a growing interest in evaluating their efficacies and in elucidating the basis of their protective action (Chu et al., 2011a, b; Li et al., 2011; Liu et al., 2011b; Yang et al., 2011; Zhang et al., 2011). 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;

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Rajendran and Srianjini, 2008). During the screening program for new agrochemicals from Chinese medicinal herbs and wild plants, the essential oil of *Cananga odorata* (Lam.) Hook. f. and Thomson (Family: Annonaceae) leaves was found to possess strong insecticidal toxicity against the grain storage insect, *S. zeamais*. *C. odorata*, commonly called Ylang-Ylang, is a fast-growing tree and native to the Philippines, Union of Myanmar, Malaysia and Indonesia. It has been cultivated in Taiwan, Fujian, Guangdong, Guangxi, Yunnan and Sichuan Province, China (Tsiang and Li, 1979). The bark of *C. odorata* is used in Tonga and Samoa to treat stomach ailments and sometimes as a laxative. In Java, the dried flowers are used against malaria, and the fresh flowers are pounded into a paste to treat asthma (Manner and Elevitch, 2006). Several monoterpene glucosides, terpenoid spirolactones, sesquiterpenoids, and alkaloids were isolated from the methanol extract of this plant (Rao et al., 1986; Yang and Huang, 1988; Hsieh et al., 2001; Caloprisco et al., 2002; Rahman et al., 2005; Nagashima et al., 2010; Matsunami et al., 2010). The essential oil of ylang-ylang flower is used in aromatherapy. It is believed to relieve high blood pressure, normalize sebum secretion for skin problems, and is considered to be an aphrodisiac (Manner and Elevitch, 2006). The essential oil of *C. odorata* flowers has been shown to possess repellency against mosquito bites (*Aedes aegypti*) and two grain storage insects, *S. zeamias* and *Tribolium castaneum* (Trongtokit et al., 2005; Nerio et al., 2009; Caballero-Gallardo et al., 2011). The essential oil was also evaluated insecticidal toxicity against *Cadra cautella* and *Trialeurodes vaporariorum* (Choi et al., 2003 Sim et al., 2006). However, a literature survey has shown that there is no report on insecticidal activity of the essential oil of *C. odorata* leaves, thus we decided to investigate the chemical constituents and insecticidal activities of the essential oil derived from *C. odorata* leave against *S. zeamais* Motschulsky (Coleoptera: Curculionidae).

MATERIALS AND METHODS

Plant Material

The leaves of *C. odorata* were collected in May 2011 from Baoshan city, Yuannan Province (25.12° N latitude and 99.17° E longitude). 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 (CLSBUA-Annonaceae-10043) was deposited at the Key Laboratory of Urban Agriculture (North), Ministry of Agriculture, Beijing University of Agriculture (Beijing 102206). The samples were ground to powder using a grinding mill (Retsch Mühle, Germany). Each 600 g portion of powder was mixed in 1800 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*) were obtained from laboratory cultures in the dark in incubators at 29 to 30℃ 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).

Gas chromatography-mass spectrometry

The essential oil of *C. odorata* leaves was subjected to GC-MS analysis on an Agilent system consisting of a model 6890 N 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-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 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 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 *C. odorata*. A serial dilution of the essential oil (2.23 to 12.00%, 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℃ 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).

Fumigant toxicity bioassay

Range-finding studies were run to determine the appropriate testing concentrations of *C. odorata* essential oil. The fumigant toxicity of *C. odorata* essential oil was determined using 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

Table 1. Chemical constituents of essential oil derived from *Cananga odorata* leaves.

Compounds	RI*	Peak area (%)
α-Pinene	931	12.73
Camphene	953	2.20
β-Pinene	974	1.48
2-Octanone	1009	0.56
d-Limonene	1027	0.78
1,8-Cineole	1032	2.04
Dihydrolinalool	1095	1.49
Linalool	1097	21.08
β-Terpineol	1143	0.52
Camphor	1146	3.23
4-Terpineol	1179	0.58
α-Terpineol	1191	3.46
γ-Terpineol	1202	0.98
Linalool acetate	1248	16.14
trans-Geraniol	1253	2.06
Geranial	1266	0.88
Isobornyl acetate	1275	3.56
Dihydrocarveol acetate	1285	0.53
α-Terpineol acetate	1350	7.71
Eugenol	1356	8.86
α-Copaene	1375	2.63
Caryophyllene	1420	1.78
Diisobutyl phthalate	1868	2.40
Total		97.65
Monoterpenoids		81.45
Sesquiterpenoids		6.78
Others		9.42

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

microliters of the essential oil (2.63 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℃ and 70 to 80% relative humidity for 24 h and the experiment was replicated six times. 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 *C. odorata* leaves was 0.12% (V/W) and the density of the concentrated essential oil was determined as 0.85 g/ml. A total of 23 components of the essential oil were identified, accounting for 97.65% of the total oil. The principal compounds in the essential oil were linalool (21.08%), linalool acetate (16.14%), α-pinene (12.73%), eugenol (8.86%) and α-terpineol acetate (7.71%) followed by isobornyl acetate (3.56%), α-terpineol (3.46%), and camphor (3.23%) (Table 1). Monoterpenoids represented 18 of the 23 compounds, corresponding to 81.45% of the whole oil while only 3 of the 23 constituents were sesquiterpenoids (6.78% of the crude essential oil). There are some variations in the essential oils of *C. odorata* derived from different parts. For example, Sun et al. (1985) found that the essential oil of of *C. odorata* flowers contained β-caryophyllene (33.0%), γ-muurolene (19.8%), humulene (7.7%), geranyl acetate (6.2%), bergamotene (5.4%) and benzyl benzoate (5.3%). However, benzyl acetate (18.2%), linalool (14.1%), methyl benzoate (10.0%), ρ-methylanisole (8.9%) and gernacrene D (7.8%) were main constituents of the essential oils of *C. odorata* leaves and flowers (Caballero-Gallardo et al., 2011).

The essential oil of *C. odorata* leaves exhibited contact toxicity against *S. zeamais* adults with an LD_{50} value of 33.14 µg/adult (Table 2). When compared with the positive control pyrethrum extract, the essential oil demonstrated 6 times less toxic against *S. zeamais*. However, compared with the other essential oils in the literature, the essential oil of *C. odorata* leaves 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 *C. odorata* leaves possessed fumigant toxicity against the maize weevils with an LC_{50} value of 14.77 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 21 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 *Ancathia igniaria* exhibited the same level or stronger fumigant toxicity against *S. zeamais* adults, e.g. essential oils of *Silene multifida* (Liu et al., 2011a), *Murraya exotica* (Li et al., 2010), and several essential oils from Genus *Artemisa* (Chu et al., 2010a; Liu et al., 2010a, b). In the previous reports, one of the main constituent compounds, linalool was found to have fumigant toxicity against the triatomine bug (*Rhodnius prolixus*) (Sfara et al., 2009) and houseflies with a 24 h LC_{50} value of 13.6 mg/L air (Palacios et al., 2009). Moreover, linalool possessed both contact and fumigant toxicity against human head louse (*Pediculus humanus capitis*) (Yang et al., 2009) and *S. zeamais* (Wang et al., 2011) and showed a high acaricidal activity by vapor action against mobile stages of *Tyrophagus putrescentiae* (Sanchez-Ramos and Castanera, 2001). Linalool was found to be a competitive inhibitor of acetyl-cholinesterase (AchE) (Ryan and Byrne, 1998). It is suggested that the insecticidal activity of *C. odorata*

Table 2. Contact (CT) and fumigant toxicity (FT) of *Cananga odorata* essential oil against *Sitophilus zeamais* adults.

Variable	Treatment	LD_{50} (µg/adult) LC_{50} (mg/L air)	95% FL	Slope ± SE	Chi square (χ^2)
CT	*C. odorata*	33.14	29.76 - 37.61	4.22 ± 0.45	30.80
	Pyrethrum extract*	4.29	3.86 - 4.72	-	-
FT	*C. odorata*	14.77	13.06 - 16.33	2.43 ± 0.30	23.52
	MeBr**	0.67	-	-	-

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

essential oil maybe attributed to linalool.

The aforementioned findings suggest that fumigant activity of the essential oil of *C. odorata* leaves is quite promising by considering some synthetic fumigants and it shows potential to be developed as possible natural fumigant/insecticide for control of stored product insects.

Conclusion

The composition of the essential oil derived from *C. odorata* leaves was determined by GC-MS for the first time. The essential oil was demonstrated to exhibit strong contact and fumigant toxicity against *S. zeamais* adults. These findings suggest that the essential oil of *C. odorata* leaves possessed potential for development as novel natural insecticide/fumigant for stored products. 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.

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