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*Full Length Research Paper*

# Chemical composition and toxicities of the essential oil derived from *Kadsura heteroclita* stems against *Sitophilus zeamais* and *Meloidogyne incognita*

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**In our screening program for new agrochemicals from Chinese medicinal herbs and wild plants, essential oil of *Kadsura heteroclite* stems was found to possess strong toxicities against the root-knot nematode, *Meloidogyne incognita* and the maize weevil, *Sitophilus zeamais*. The essential oil of *K. heteroclita* was extracted via hydrodistillation and analyzed by GC-FID and GC-MS. A total of 46 components of the essential oil were identified. The main components of the essential oil were α-eudesmol (17.56%), 4-terpineol (9.74%), δ-cadinene (9.27%), and δ-cadinol (6.32%) followed by δ-4-carene (4.78%) and calarene (4.01%). The essential oil exhibited strong nematicidal activity against *M. incognita* with an $LC_{50}$ value of 122.94 µg/ml. The essential oil possessed contact toxicity against *S. zeamais* adults with an $LD_{50}$ value of 25.57 µg/adult and also showed pronounced fumigant toxicity against *S. zeamais* ($LC_{50}$ = 14.04 mg/L air). The essential oil of *K. heteroclite* shows potential to be developed as a possible natural insecticide/nematicide for control of stored product insects/nematodes.**

**Key words:** *Kadsura heteroclita*, *Sitophilus zeamais*, *Meloidogyne incognita*, nematicidal activity, contact toxicity, fumigant, essential oil composition.

## INTRODUCTION

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 our mass screening program for new agrochemicals from Chinese medicinal herbs and wild plants, essential oil of *Kadsura heteroclita* (Roxb.) Craib (Family: Schisandraceae) stems was found to possess strong toxicities against the root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood and the maize weevil, *Sitophilus zeamais* (Motsch). The maize weevil 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). However, repeated use of those fumigants for decades has disrupted biological control by natural enemies and led to resurgence of stored-product insect pests, sometimes resulted in the development of resistance, and had undesirable effects on non-target organisms (Isman, 2006). These problems have highlighted the need to

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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, 2006; Rajendran and Srianjini, 2008).

*M. incognita* is the most economically important and widely distributed nematode throughout China and a considerable crop loss is caused by this nematode. Nematode management is generally based upon chemical treatments (soil fumigation), but environmental concerns and governmental regulations (United Nations Environment Programme, 2000) are now resulting in a strong interest in nematicides of natural origin (Chitwood, 2002). Many plant constituents and metabolites including essential oils and monoterpenoids have been investigated for activity against plant-parasitic nematodes (Kim et al., 2008; Echeverrigaray et al., 2010; Ntalli et al., 2010). The results suggest that some of the essential oils tested and selected monoterpenoids are potential natural pesticides in the control of nematodes.

*K. heteroclite* is a climbing species distributed in the southwest part of China (Hubei, Guangdong, Guangxi, Hainan, Guizhou and Yunnan province) (Committee of Flora of China, 1996) and has been used for the treatment of gastric and duodenal ulcers, acute and chronic gastroenteritis, dysmenorrheal, postpartum abdominal pain, trauma and hepatic diseases in traditional Chinese medicine (Jiangsu New Medical College, 1977). Previous phytochemical studies on *K. heteroclite* resulted in the identification of flavonoids, sesquiterpenoids, triterpenoids, and lignans (especially dibenzocyclooctene-type lignans) and triterpenoids (Chen et al., 1992, 2006; Han et al., 1992; Li et al., 1989; Pu et al., 2008; Wang et al., 2006a, b, c, d, 2007; Xu et al., 2007, 2008, 2010; Yang et al., 1992). The chemical composition of *K. heteroclita* essential oil was also studied previously (Liu and Luo, 2002; Li et al., 2007). However, a literature survey has shown that there is no report on toxicities of *K. heteroclita* essential oil against insects and nematodes; thus we decided to determined chemical composition and toxicities of the essential oil against nematodes and insects.

## MATERIALS AND METHODS

### Insect and nematode

The maize weevils (*S. zeamais*) were obtained from laboratory cultures maintained for the last 15 years in the dark in incubators at 29 to 30°C 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) at 29 to 30°C and 70 to 80% relative humidity. Unsexed adult weevils and beetles used in all the experiments were about 2 weeks old.

Second stage juveniles (J2) of *M. incognita* were obtained from a pure culture that was previously initiated by egg masses and propagated on tomato (*Solanum lycopersicum*) in the glasshouse. Egg masses were handpicked using sterilized forceps from heavily infected roots (40 days after incubation). These egg masses were washed in distilled water, placed in 15 mesh sieves (8 cm in diameter) containing crossed layers of tissue paper in Petri-dishes with water just deep enough to contact the egg masses and incubated 25 to 26°C to obtain freshly hatched second stage juveniles (J2). Only juveniles collected within 48 h were used.

### Plant material

Dried stems (10 kg) of *K. heteroclita* (cultivated and harvested from Yulin City, Guangxi Zhuang Autonomous Region, 22.64°N latitude and 110.14°E longitude) were purchased from Puning Chinese Medicinal herbs Market (Guangdong 515300, China). The medicinal herb was identified by Dr. Liu, Q.R. (College of Life Sciences, Beijing Normal University, Beijing 100875, China) and a voucher specimen (CMH-GuagdongHaifengteng-2010-07) was deposited in the Department of Entomology, China Agricultural University. The dried stems were ground to a powder using a grinding mill (Retsch Mühle, Germany). Each 600 g portion of powder ground 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 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.

### Gas chromatography-mass spectrometry

The essential oil of *K. heteroclite* stems was subjected to gas chromatography-mass spectrometry (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 $min^{-1}$. 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 (Li and Luo, 2002; Li et al., 2007) 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

Range-finding studies were run to determine the appropriate testing concentrations of the essential oil. A serial dilution (six concentrations) of the essential oil was prepared in *n*-hexane. Aliquots of 0.5 µl per insect were topically applied dorsally to the thorax of insects, 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 treated and control insects 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. It was observed daily for determination of end-point mortality, which was reached after one week. Results from all replicates were subjected to probit analysis using the PriProbit Program V1.6.3 to determine $LD_{50}$ values (Sakuma, 1998).

#### Fumigant Bioassay

Range-finding studies were run to determine the appropriate testing concentrations of the essential oil. A serial dilution of the essential oil (six concentrations) was prepared in *n*-hexane. A Whatman filter paper (diameter 2.0 cm) were each impregnated with 10 µl dilution, and then placed on the underside of the screw cap of a glass vial (diameter 2.5 cm, height 5.5 cm, volume 25 ml). The solvent was allowed to evaporate for 20 s before the cap was placed tightly on the glass vial, each of which contained 10 insects inside to form a sealed chamber. Preliminary experiments demonstrated that 20 s was sufficient for the evaporation of solvents. n-Hexane was used as a control. Six replicates were carried out for all treatments and controls, and they were incubated for 24 h. The insects were then transferred to clean vials with some culture media and returned to the incubator and observed daily for determination of end-point mortality, which was reached after one week. The experiments were repeated three times. The $LC_{50}$ values were calculated by using Probit analysis (Sakuma, 1998).

#### Nematicidal toxicity bioassay

Range-finding studies were run to determine the appropriate testing concentrations. A serial dilution of the essential oil (five concentrations, dissolved first in 10 µl ethanol) was prepared in $H_2O$ solution with 2% DMSO. Aliquots of $H_2O$ (20 µl) containing ca. 100 juveniles (J2) were transferred to vials to which 980 µl of the solution containing ethanol extract or pure compounds was added. The vials were kept on a hood at 25°C. The inactive nematodes were counted every 24 h for 72 h. After the last count, the inactive juveniles were maintained in distilled $H_2O$ for 24 h to observe their revival. Six repetitions for each treatment were performed using $H_2O$ and a 2% DMSO in $H_2O$ solution as well as a 2% DMSO in $H_2O$ solution containing 10 µl ethanol as control. The experiments were repeated three times. Results from all replicates were subjected to probit analysis using the PriProbit Program V1.6.3 to determine $LC_{50}$ values (Sakuma, 1998). Carbofuran was purchased from National Center of Pesticide Standards (8 Shenliao West Road, Tiexi District, Shenyang 110021, China) and used as a positive control.

## RESULTS AND DISCUSSION

The steam distillation for 6 h of *K. heteroclite* stems afforded essential oils with a yield of 0.53% and the density of the concentrated essential oil was determined to be 0.86 g/ml. GC–MS analysis of the essential oil of *K. heteroclite* led to the identification and quantification of a total of 46 major components accounting for 93.43% of the total components present (Table 1). The main components of the essential oil were α-eudesmol (17.56%), 4-terpineol (9.74%), δ-cadinene (9.27%), and δ-cadinol (6.32%) followed by δ-4-carene (4.78%) and calarene (4.01%). However, δ-cadinene (22.59%), δ-cadinol (17.64%), calarene (7.63%), germacrene D (5.24%) and α-muurolene (5.8%) were the main constituents in the essential oil of *K. heteroclite* (Li and Luo, 2002). Li et al. (2007) demonstrated that the essential oil of *K. heteroclite* from supercritical carbon dioxide fluid extraction contained δ-cadinene (14.42%), δ-cadinol (9.94%), and calarene (6.50%) while that from steam distillation possessed δ-cadinene (19.46%), δ-cadinol (11.14%) and calarene (8.00%). It suggested that there are some variations in chemical composition of the essential oil of *K. heteroclite*. Further studies on plant cultivation and essential oil standardization are needed.

The essential oil of *K. heteroclite* possessed strong nematicidal activity against *M. incognita* with an $LC_{50}$ value of 122.94 µg/ml (Table 2). Compared with a synthetic insecticide, carbofuran ($LC_{50}$ = 72.29 µg/ml), the essential oil exhibited the same level of toxicity against *M. incognita* and shows potential to be developed as a possible natural nematicide for control of the root-knot nematodes.

The essential oil of *K. heteroclite* showed strong contact toxicity against *S. zeamais* adults with an $LD_{50}$ value of 25.57 µg/adult (Table 2). However, compared with the famous botanical (pyrethrum extract, 25% pyrethrine I and pyrethrine II), the essential oil was 6 times less active against the insects because of pyrethrum extract ($LD_{50}$ = 4.29 µg/adult against *S. zeamais* adults) (Liu et al., 2010). The essential oil of *K. heteroclite* also possessed pronounced fumigant toxicity against *S. zeamais* adults ($LC_{50}$ = 14.04 mg/L air). The currently used 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 air (Liu and Ho, 1999). Compared with MeBr, the essential oil was 21 times less active against the insects. Considering the currently used fumigants are synthetic insecticides, fumigant activity of the essential oil of *K. heteroclite* is quite promising and it shows potential to be developed as a possible natural fumigant for 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.

In previous studies, some of the main components of the essential oil were found to possess bioactivities against insects. For example, α-eudesmol was found to have toxicity against rice weevil (*Sitophilus oryzae*) and house dust and stored food mites (*Dermatophagoides pteronyssinus* and *Tyrophagus putrescentiae*) (Rai et al., 1993; Lee et al., 2009). 4-Terpineol has been found to have fumigant toxicity against several insects, such as granary weevil (*Sitophilus granarius*) (Kordali et al., 2006), *Lycoriella ingénue* (Park et al., 2008), adult mosquitoes (*Aedes aegypti*) (Lucia et al., 2009) and the German cockroach (*Blattella germanica*) (Jang et al.,

**Table 1.** Chemical constituents of essential oil derived from *Kadsura heteroclite* stems.

| RI* | Compounds | Peak Area (%) |
|---|---|---|
| 931 | α-Pinene | 3.69 |
| 981 | β-Pinene | 1.32 |
| 1005 | α-Phellandrene | 0.05 |
| 1022 | δ-4-Carene | 4.78 |
| 1025 | ρ-Cymol | 0.16 |
| 1029 | *d*-Limonene | 1.54 |
| 1032 | 1,8-Cineol | 3.25 |
| 1037 | (*Z*)-β-Ocimene | 0.84 |
| 1057 | γ-Terpinene | 0.54 |
| 1094 | Linalool | 2.52 |
| 1126 | *cis*-ρ-Menth-2-en-1-ol | 0.05 |
| 1146 | Camphor | 0.75 |
| 1169 | Borneol | 0.42 |
| 1179 | 4-Terpineol | 9.74 |
| 1191 | α-Terpineol | 1.32 |
| 1195 | Estragole | 0.07 |
| 1238 | Carvone | 2.82 |
| 1283 | Safrol | 0.73 |
| 1350 | α-Cubebene | 2.11 |
| 1372 | β-Maaliene | 0.36 |
| 1374 | Copaene | 1.01 |
| 1382 | β-Patchoulene | 0.28 |
| 1391 | β-Elemene | 0.53 |
| 1403 | Methyl eugenol | 1.05 |
| 1420 | Caryophyllene | 2.05 |
| 1444 | Calarene | 4.01 |
| 1454 | α-Caryophyllene | 1.73 |
| 1470 | γ-Selinene | 0.23 |
| 1473 | γ-Muurolene | 1.01 |
| 1485 | Germacrene D | 0.78 |
| 1492 | δ-Selinene | 0.33 |
| 1500 | α-Muunorol | 3.32 |
| 1506 | 1ξ,6ξ,7ξ-Cadina-4,9-diene | 0.58 |
| 1511 | β-Bisabolene | 0.91 |
| 1523 | δ-Cadinene | 9.27 |
| 1546 | α-Calacorene | 0.51 |
| 1547 | α-Elemol | 0.79 |
| 1562 | Ledol | 0.29 |
| 1578 | Spathulenol | 0.82 |
| 1583 | Caryophyllene oxide | 0.12 |
| 1596 | Ledene | 0.20 |
| 1643 | Cubenol | 0.45 |
| 1653 | α-Eudesmol | 17.56 |
| 1659 | δ-Cadinol | 6.32 |
| 1662 | *ar*-Tumerone | 0.83 |
| 1673 | α-Bisabolol | 1.38 |
| | Total | 93.42 |

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

**Table 2.** Toxicities of *Kadsura heteroclite* essential oil against *Sitophilus zeamais* adults and *Meloidogyne incognita* larvae.

| Toxicities | Treatment | $LC_{50}$(mg/L air) (µg/ml) (95%FL) | $LD_{50}$ (µg /adult) (95%FL) | Slope ± SE | Chi square ($\chi^2$) |
|---|---|---|---|---|---|
| Fumigant | Essential oil | 14.04(12.48-15.63) | - | 3.52 ± 0.49 | 13.45 |
| | MeBr* | 0.67 | - | - | - |
| Contact | Essential oil | - | 25.57(23.38-27.70) | 4.23 ± 0.47 | 12.39 |
| | Pyrethrum extract** | - | 4.29 (3.86-4.72) | 0.73 ± 0.02 | 13.51 |
| Nematocidal | Essential oil | 122.94(115.56-134.19) | - | 3.16 ± 0.32 | 15.69 |
| | Carbofuran | 72.29 (37.86-117.97) | - | 6.23± 0.51 | 13.57 |

*Data from Liu and Ho (1999); **data from Liu et al. (2010).

2005) and 4-terpineol also shows strong contact toxicity against 4th instars of *Spodoptera littoralis* and adults of *Aphis fabae* (Abbassy et al., 2009). The isolation and identification of the bioactive compounds in the essential oil of *K. heteroclite* are of utmost importance so that their potential application in controlling stored-product pests/nematodes can be fully exploited.

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